A DYNAMIC MODEL

FOR THE

ACTIVATED SLUDGE TREATMENT

OF

COKE OVEN EFFLUENTS

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SUMMARY

This is an Inter-Disciplinary Higher Degree (IHD) thesis about Water Pollution Control in the Iron and Steel Industry.

After examining the compositions, and various treatment methods, for the major effluent streams from a typical Integrated Iron and Steelworks, it was decided to concentrate investigative work on the activated-sludge treatment of coke-oven effluents. A mathematical model of this process was developed in an attempt to provide a tool for plant management that would enable improved performance, and enhanced control of Works Units.

The model differs from conventional models in that allowance is made for the presence of two genera of micro-organisms, each of which utilises a particular type of substrate as its energy source. Allowance is also made for the inhibitive effect of phenol on thiocyanate biodegradation, and for the self-toxicity of the bacteria when present in a high substrate concentration environment.

The enumeration of the kinetic characteristics of the two groups of micro-organisms was shown to be of major importance. Laboratory experiments were instigated in an attempt to determine accurate values of these coefficients. The use of the Suspended Solids concentration was found to be too insensitive a measure of viable active mass. Other measures were investigated, and Adenosine Triphosphate concentration was chosen as the most effective measure of bacterial populations.

Using this measure, a model was developed for phenol biodegradation from experimental results which indicated the possibility of storage of substate prior to metabolism. A model for thiocyanate biodegradation was also developed, although the experimental results indicate that much work is still required in this area.

KEY WORDS

Modelling, Phenol, Thiocyanate, ATP, Activated-Sludge

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CHAPTER 1. INTRODUCTION

1.1 The problem in its perspective

This is an Inter-Disciplinary Higher Degree (IHD) thesis about water pollution in the Iron and Steel Industry. Steel production is a major user of water, and invariably a certain amount of pollution is involved when used-waters are discharged to the environment.

The origins of this work come from a desire by the British Steel Corporation to understand more about the total process of water use and discharge of effluent to the environment.

It is well known that used-waters in rivers, estuaries and streams are, to a greater or lesser extent, self-purified. By understanding the total process of pollution, treatment, and passage of water through the works and into the environment, it was hoped that economics of treatment and benefits to the environment could be obtained. The work started out with the concept of looking at modelling this complete system. It was soon established however, that models of the various processes were available and could be connected to investigate interactions of policies at different stages, both inside the works and in the environment. In certain areas however, the models were not felt to be adequate representations of observed behaviour. In particular, the treatment of a major effluent, coke-oven effluent, by the most popular process, activated-sludge, was thought to be poorly understood on a quantitative basis.

It has been difficult to control these plants, and frequent failures in treatment of coke-oven effluents have occurred. Research effort became concentrated on this effluent and on this process. It was decided to try to develop mathematical models for the process so that operational strategies could be developed by simulation. The search for values to give to the model's parameters quickly forced the scene of the action into the laboratory, where batch reactors on a bench-scale were monitored. These investigations produced results which did not fit existing concepts of behaviour of the organisms involved, and new experimental techniques had to be derived to measure the

kinetic parameters. Models of the processes involved were then developed and tested against the laboratory data. In the case of the biodegradation of phenolic coke-oven effluents, a model was developed which is substantially different from existing beliefs. Analysis of the kinetics of such a model shows the importance of the complete treatment of these effluents, and indicates why activated-sludge plants treating coke-oven effluents are susceptible to frequent breakdowns. Further work is required in this field to formulate control strategies to prevent such collapses of treatment, and it is a measure of the success of the project that the British Steel Corporation is planning to continue this work.

It is interesting to note how attention became focused on an ever smaller area which formed a key link in the understanding of the total system which is required to achieve the global aims of the BSC in this sector. It is believed that this is the correct approach for industrial problem solving. In this way, one is only expending effort on necessary research to achieve the global objective. This detailed study is seen as a small brick in the fabric of the design; its place and importance is clearly seen. The objectives of the laboratory effort are clearly defined. Sight of the real problem need never be lost.

The water pollution problems facing the BSC can be seen in terms of water resources and demand throughout the country. The following section deals with this perspective, and elaborates in more detail on the course of research.

1.2 Water Resources. Demand and the need for Conservation

Fortunately, fresh water is a natural resource that can be re-used and, with the aid of solar energy, is in fact self-renewing. As long as solar energy is available, water will be lifted up from the earth's surface, stored temporarily in the atmosphere, and then precipitated back to the surface in the form of rain, snow, hail etc. As the water is lifted from the surface, it gains two valuable properties. Sea-water for example, when evaporated, loses its salinity and becomes purified. It also gains a considerable increase in potential energy. As the water is precipitated back to the surface, both purity and potential

energy begin to be lost.

The average precipitation in England and Wales is of the order of 400 million cubic meters per day[1]. Of this, about half is lost by evaporation and by transpiration through vegetation, leaving a theoretical residual rainfall of approximately 200 million cubic meters per day to replenish streams, rivers and underground watercourses. Unfortunately, variations due to geographical and seasonal distribution cause the actual replenishment available to be normally very much less than this theoretical figure. The distribution of the rainfall over the country is not uniform. Large areas of North and West England and Wales, where the population and industrial density is low, have very high average rainfall, whereas in the more populated Southern and Eastern areas, average rainfall may be as little as one third of the national average[2].

Seasonal variations can affect the availability of fresh water even more. The total amount flowing in the rivers in a dry season can be as low as 10% of the average of 200 million cubic meters per day[3].

The demand for water in England and Wales, excluding that needed for cooling at CEGB power stations, much of which is estuarial saline water, is approximately 23 million cubic meters per day[4]. Industrial demand is approximately half of this figure, the remainder being for domestic, agricultural and other miscellaneous usage. The total demand for all purposes is increasing and is expected to approach 45 million cubic meters daily by 2000 AD[5].

It can be seen then that when taking into account season fluctuations and geographical distribution, there are at present deficiencies between available fresh water and demand, and that such deficiencies are expected to worsen considerably in the forseeable future.

These difficulties are presently met by providing artificial storage (reservoirs), in areas where rainfall is plentiful, or by drawing on the natural underground storage

(aquifers). However, with a large expected increase in demand over the next twenty five years, the social and economic consequences of further developing inland reservoirs would be considerable. It is for this reason that there is today so much interest in developing other methods of augmenting existing water supplies, measures which include desalination processes, economics in domestic and industrial water usage and reclamation of sewage and industrial effluents.

1.3 Water Conservation and Effluent Disposal in the Iron and Steel Industry

A survey of water usage throughout the works of the British Steel Corporation carried out between April 1966 and March 1969 showed that total intake of water in that period amounted to 2.4 million m^3/day[6]. Of this, nearly 1 million m^3/day was due to fresh water intake, the remainder being dominated by abstractions from tidal sources. Thus, fresh water intake to BSC works in this period amounted to about 10% of total industrial demand.

The rate of charge for this water is governed by the revenue required to implement the necessary work to maintain and augment resources where necessary. The total revenue required, and hence the rate of charge, vary considerably from area to area. The actual charge made in any one area is dependent upon several factors, namely the type of source, quality of water, the purpose for which the water is to be used and the proportion returned to source. For example, the charge for water from public water undertakings is very high compared to the cost of river, canal or borehole water. This is because it includes in the water cost the cost of treatment, pumping and delivery to the works, whereas charges for river water etc. are for the water alone.

In the period 1968-69, the average cost to the British Steel Corporation of water from public undertakings was 1.8p/m^3. By 1973, the average cost of such water had doubled to 3.5p/m^3 [7]. In spite of an overall cut in demand for water between 1968 and 1976 of 37% caused by more efficient use of water resources, the amount of water from public water undertakings has remained fairly constant.

It would be unwise to attempt to tabulate average water usage and costs for the whole of the BSC as the nature and amount of water abstracted, and the costs of such water, vary greatly from one works to another. Table 1.1 shows potable water usage and cost figures for the years 1972 to 1978 for the Scunthorpe Area Works of the BSC.

TABLE 1.1 POTABLE WATER CHARGES AT SCUNTHORPE WORKS - ANGLIAN WATER AUTHORITY

Year Ending March 31st	Usage x 10^6 m^3	Cost pence/m^3
1972	1.146	-
1973	1.238	3.51
1974	1.561	3.51
1975	1.660	4.26
1976	1.874	7.33
1977	-	10.40
1978	-	13.20*

*The most recent water charge, that of the current financial year 1977-78, is an estimated figure, as the actual charge to be levied is still under negotiation. However, it is believed that the figure shown is a conservative one, and that the final levy may well be higher.

Table 1.1 shows a threefold increase in potable water charges for the Scunthorpe Works between 1974 and 1977. An increase in water usage is also indicated, but this is allied to a steady increase in steel production. The actual usage of potable water in terms of steel production has remained fairly steady over the period shown, at a figure of approximately 0.5 m^3 potable water/tonne steel.

The corresponding figures for total freshwater usage and costs at the Scunthorpe Works are shown in Table 1.2.

TABLE 1.2 TOTAL FRESHWATER CHARGES AT SCUNTHORPE WORKS

Year Ending March 31st	Usage x 10^6 m^3	Cost pence/m^3
1972	8.8	1.22
1973	9.7	1.15
1974	9.9	1.41
1975	10.6	1.75
1976	9.9	2.7
1977	10.1	4.23

As with potable water charges there is a threefold increase in the cost of total freshwater charges between 1974 and 1977. An increase in water usage due to increased steel production is also indicated. Between 1972 and 1976 steel output increased by 70%, whereas there has been only a 12% increase in water intake. This is due to the water conservation practices currently in practice at this, and at other works of the BSC.

However, in spite of the meritorious attempt to conserve water, total water charges at the Scunthorpe Works have increased from £107 000 in 1972 to £427 000 in 1977. In terms of steel output, the water costs have increased from about 4p/tonne of steel produced in 1972 to approximately 10p/tonne steel in 1977.

It can be seen from the very brief review above that the economics of water conservation and re-use is becoming increasingly important to the Iron and Steel Industry.

In recent years, this need has been recognised and much is being done within the British Steel Corporation both to reduce overall intake of water, and to treat works effluents so that they may either be re-used or discharged to inland watercourses. Although about 200 tonnes of water are required for every tonne of steel produced in an integrated iron and steelworks[8], most of this is not consumed in the sense of being destroyed or evaporated. The actual consumption amounts to no more than 5 tonnes/tonne of steel produced, the bulk of which is lost as vapour into the atmosphere. Thus, efficient recirculation and cascade re-use practices can greatly lower the requirement for water intake. Cook[8] reported that fresh intake of water at Appleby-Frodingham

Steelworks was reduced from 5.10 tonnes/tonne of steel in 1962 to 3.41 tonnes/tonne of steel in 1969 by the adoption of such practices.

The requirement for improved effluent treatment within the iron and steel industry is also growing alongside the need for water conservation practices. In the past, the stringency of trade effluent control has often been uneven throughout the country. Even though the legal powers for control were usually available, they were not always used to the fullest extent, particularly by local authorities conscious of their obligations to local industry. For example, where industry has been charged for its effluent to be received into the local sewers, such charges have varied from one authority to another, and in many cases the charges have not adequately met the costs of receiving and treating the effluent.

On the 1st April 1974, the Water Act 1973 came into effect, which concentrated the responsibility of water resources management and development into 10 Regional Water Authorities (RWA). As a central authority, and to link the 10 RWA's, the National Water Council (NWC) was also established at that time, composed of the chairman of each of the 10 RWA's and 10 others appointed for their special fields of interest and competence. The NWC has since become the focus for decisions concerning programs to implement national water policy. Because of this major re-organisation, it seems likely that the new authorities will impose realistic charges for reception and treatment of industrial effluents, and it is also likely that similar systems of charging will be adopted by individual authorities.

The Water Act (1973) obliges Water Authorities to meet their costs from charges levied, and informs them that they should not expect government subsidies to meet differences between costs incurred, and charges levied.

Under the Control of Pollution Act 1974, firms that had previously paid no charges for effluents discharged to sewers - because they were discharging before the Public Health (Trade Effluents) Act of 1937, or because they were exempted in order to

bring industry to the area, had to notify their RWA before 31 January 1975 in order to obtain consent to discharge.

However, the RWA is now empowered to review both the consents to discharge and the question of making charges. Such reviews are subject to right of appeal by industry.

As an example, it was recently reported[9] that the Severn-Trent Water Authority had announced that the present cost (in 1975) of treating trade effluent discharge to sewers was £7.7 millions, compared with revenues collected from industry of only £2.8 millions. They proposed that their charges to industry should be calculated according to a standard formula which would take into account the volume and strength of the effluent, and that charges should be equalised over the area. It was further recommended that there should not be an immediate increase in charge to industry to cover the difference, but that discussions should take place with the Confederation of British Industry (CBI) in order to phase the increases over an acceptable period of time.

Waste treatment can be undertaken wholly by the industrialist, with discharge of the treated waste to the receiving watercourse, or wholly by the regional water authority; or the industrialist may partially treat the waste before discharge to the public sewer. The reasons for choosing one way or another differ greatly, but include economic evaluations, geographical location, and strength, volume and toxicity of the waste. In general, an industry with low effluent loadings will often prefer to discharge directly to the public sewer, paying the costs of reception and treatment directly to the RWA. In the iron and steel industry however, as has been pointed out, water usage is very high, and corresponding to this effluent volumes are also very high. Furthermore, the toxicity and strength of many of the effluents from an integrated iron and steelworks are such that they would not be permitted into the sewerage system, as the possibility of their interfering with normal sewage treatment is often much too great.

The necessity of treating its own used waters has been

recognised by the British Steel Corporation for many years, and existing works have been equipped with effluent treatment systems with the aims of reducing nett intake of fresh water, and of reducing pollution loads of discharges to receiving watercourses. The planning of new works now takes into account 'state-of-the-art' recirculation processes and treatment plant in order to reduce, as much as possible, the total cost of water and the environmental impact of effluent discharges.

1.4 Steel Industry Effluents and their Treatment

Iron and Steel industry processes give rise to a number of effluents that require special treatment to avoid polluting the receiving watercourses. The main effluent streams from an integrated iron and steelworks originate from the four basic operations i.e. cokemaking, ironmaking, steelmaking and rolling processes. Each of these effluents are discussed briefly below.

1.4.1 Coke-oven and by-product wastes

In any integrated steelworks, coke is produced from coal for the purpose of iron manufacture.

The coal is heated in the absence of air to produce coke, but in so doing evolves a by-product gas. Before this gas can be utilised as an energy source, water vapour and other impurities such as tar, phenols, ammonia, H_2S etc., must be removed. The mechanisms by which these impurities are removed are shown diagramatically in Figure 1.1. It is not proposed to describe these methods any more fully at this stage, as such information is readily available in the literature.

The waste liquor produced by cleaning the gas is called "Ammoniacal liquor". This is a strong liquor containing much ammonia - both free and fixed, and various toxic substances such as cyanides, sulphides, phenols, thiosulphates and thiocyanates in significant quantities. The ammoniacal liquor is further processed to remove much of the free and fixed ammonia as ammonium sulphate, and "Devil Gases" containing H_2S, CO_2, HCN and C_6H_5OH; see Figure 1.2.

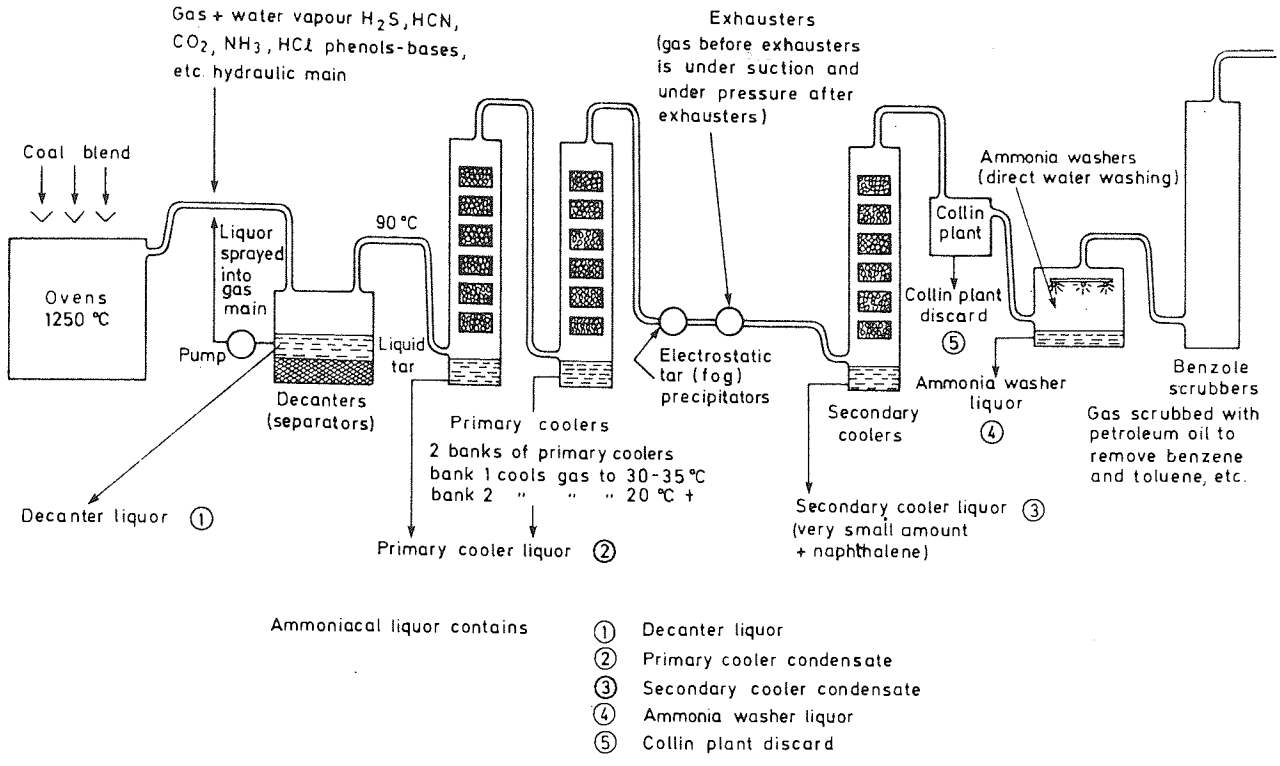

FIG. 1.1 COKING PROCESS SHOWING THE COMPOSITION OF AMMONIACAL LIQUOR

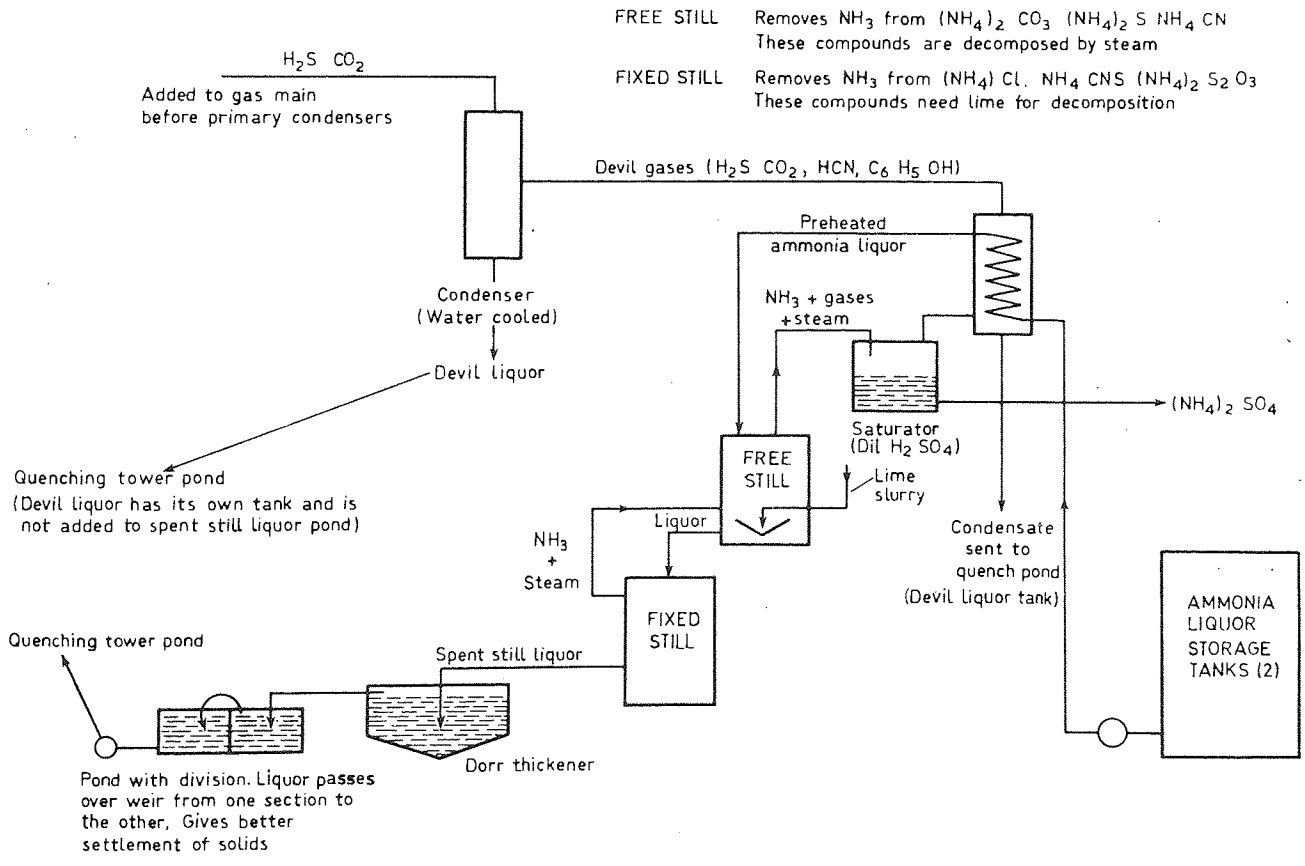

FIG.1.2 AMMONIA STILLS

The remaining liquor, termed "Spent liquor", constitutes the major effluent from coking processes.

Two other effluent streams - namely "Devil Liquor" and "Benzole-Still Condensate" are normally added to the spent liquor before treatment. The devil liquor is that effluent obtained as the condensate from hot devil gases, whilst the benzole-still condensate is obtained by stream distillation of the benzole solution.

One other effluent stream is normally added to the above waste waters prior to treatment;this is the liquor remaining after quenching the hot coke. Assuming that quench water is either river water or is the treated effluent from another source, the only pollutants transferred by the quench process will be:-

(1) High concentration of suspended solids
(2) Elevated temperature.

Most of the suspended solids will be due to coke dust, or "breeze".

Typical compositions of the combined wastes are given in Table 1.3, together with a typical discharge consent[10]. The normal range of volumetric flows of such effluent is between 130 and 225 litres per tonne of coal carbonised[6,11].

TABLE 1.3 TYPICAL COMPOSITIONS AND CONSENT CONDITIONS FOR A COKE-OVEN EFFLUENT

Constituent gm/m^3	Untreated Effluent - Works A		Untreated Effluent - Works B		Consent Condition Works B
	Average	Range	Average	Range	
PV	2100	1800-2500	1900	1400-2700	100
Mono-Phenols	610	480-830	650	450-800	5
Total Phenols	1270	980-1450	900	750-1200	20
Thiocyanate	420	340-530	120	90-170	5
Free Cyanide	6.0	0.5-15	13	5-20	0.5
Ammonia (as N)	700	300-1600	100	80-200	100

Treatment processes for coke oven effluent

Treatment of coke-oven effluent is liable to be the most difficult, and most costly, of the effluent streams under

consideration. This is due to the high toxicity and varied nature of the spent liquor. Most of the more common methods are described below, together with possible future methods.

Distillation

This is now generally recognised as a necessary stage of ammoniacal liquor treatment. Referring to Figure 1.2, free ammonia in the ammoniacal liquor is distilled off, and is dissolved in dilute sulphuric acid in a Saturator. The fixed ammonia remaining in the liquor is then liberated by adding lime, the ammonia again being distilled off and dissolved in sulphuric acid.

One problem that arises due to the addition of lime is that the effluent then becomes strongly discoloured due to alkaline oxidation and polymerisation of dihydric phenols.

Such treatment has been shown in practice to reduce ammonia concentrations in spent liquor (the liquor remaining after ammonia distillation) from about 2000 gm/m^3 to 100-200 gm/m^3.

Solvent Extraction

The phenol content of the spent liquor may be reduced by solvent extraction in anaerobic conditions. Examples of possible solvents are:-

(a) Benzole - to reduce concentration of monohydric phenols
(b) Butyl acetate or methyl isobutyl ketone - to reduce the concentrations of polyhydric phenols.

This method is not normally used however, as normal treatment of spent liquor usually involves some sort of biological treatment, which easily deals with the monohydric phenols. As for the polyhydric phenols, they are in a very low concentration to begin with, and their removal by solvent extraction methods would be uneconomic.

Coke - Quenching

Spent ammoniacal liquor has in the past been disposed of by using it as a quench liquor for the hot coke. In most works, this practice has now ceased because of:-

(a) Air pollution effects - the quench liquor is vaporised by the hot coke, causing toxic materials to be carried with the water vapour into the atmosphere[12].

(b) Enhanced plant corrosion - chlorides present in the spent liquor tend to corrode the coke-carrying cars. Studies have shown that costs of fresh water, or of treated effluent, are more than offset by savings gained from reduced down-time, repair or renewal costs of coke-cars and associated equipment[12,13,14].

(c) Deleterious effects on the coke - this is mentioned only as a possibility in the literature, but no real evidence has been found to support it[12,14].

Chemical Treatment

(a) Oxidation with ozone - laboratory studies[15,16] have shown that although it is technically possible to oxidise the constituents of spent ammoniacal liquor with ozone, the process is very expensive. This process may however prove valuable as a finishing process to reduce any residual chemical oxygen demand.

(b) Ion exchange - thiocyanates and thiosulphates can be selectively removed from spent liquor by an ion-exchange resin, followed by the adsorption of phenols onto active carbon. Practically all the noxious constituents can be removed by this process, leaving an effluent consisting mainly of a waste solution of calcium chloride[17].

However, the process is very expensive both as regards capital and operating costs, and one is still left with the problem of disposal of large

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Physical/Chemical Treatment (PCT)

Pilot studies are at present under way at a NATO sponsored project at Coleshill, West Midlands, to evaluate PCT processes to replace or supplement existing conventional physical/biological methods of sewage treatment. A second pilot project is also being studied at the North West Water Authority Davyhulme Works[19].

The use of activated carbon to remove dissolved organics provides the first alternative to space consuming biological filters. Also, toxic substances do not affect PC treatment, whereas biological processes are often liable to be inhibited by some industrial discharges.

This is mentioned as a possible future alternative for treatment of coke-oven liquors. No information on their reaction to PC treatment has yet appeared in the literature.

1.4.2 Effluents originating from ironmaking processes

The major liquid effluent from the ironmaking process is that produced from the blast furnace when the evolved gas is scrubbed with water - see Figure 1.3.

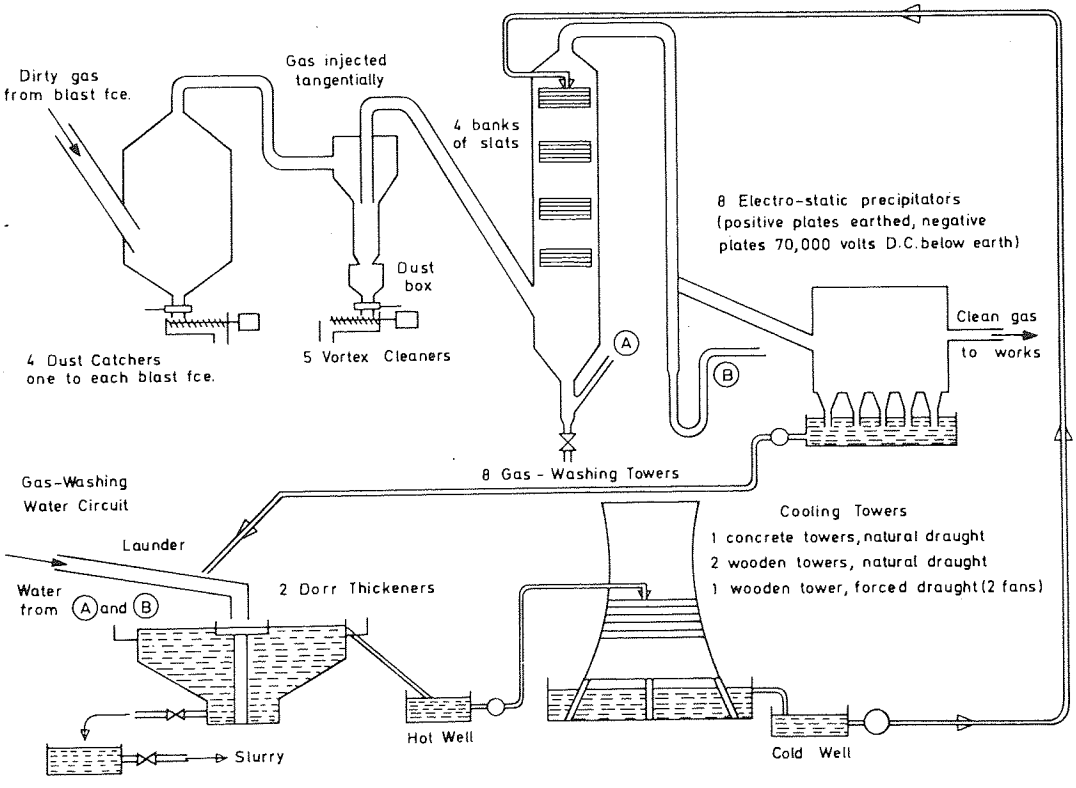


FIG. 1.3 BLAST FURNACE GAS - WASHING PLANT

This gas first enters a dust catcher, where heavy solids simply drop out. It then enters a vortex cleaner, where its velocity is increased and a vortex flow set up. This removes some of the lighter particles of dust in the gas. The next stage in the cleaning operation is a series of towers where the gas is scrubbed with water. This removes the majority of remaining solids, the remainder being removed in the next stage - a series of electrostatic precipitators. These are washed down periodically, the wash water being added to the effluent from the scrubbing towers.

The pollutants normally encountered in a gas wash-water are suspended solids, oxygen absorbers, cyanide, zinc, oil and, to a lesser extent phenols, thiocyanates and toxic metals. Suspended solids generally consist of insoluble particles of burden material, mostly coke, iron ore or sinter.

The suspended solids content of the water leaving the gas plants generally varies from about 400-4500 gm/m^3, the water flow being of the order of 4 - 5 cubic metres for each 1000 m^3 of gas cleaned[13,20].

This flow is taken to a clarifier, see Figure 1.3 where the suspended solids content of the overflow is reduced to about 20-40 gm/m^3. This supernatent liquor is then cooled down either by lagooning, or by the use of cooling towers. A major proportion of the overflow from the thickeners is recycled to be reused as gas wash-water, the remainder being bled off as discharge to the sewer or to secondary treatment. The proportion of this bleed controls the build up of contaminants in the recirculating flow, and therefore has a significant effect on the composition and concentration of the discharge from primary treatment.

Coagulants such as chlorinated copperas are often used in the primary thickeners to improve their efficiency. Tests at one such works[21] have reported zinc removal due to the primary thickeners from 5 - 35 gm/m^3 in the wash water to 0.75 - 5 gm/m^3 in the effluent. The actual concentrations and compositions of the wash waters vary greatly from works to works, and depend on the quality of the iron ore, plant practice and coke quality.

The effluent from the primary thickeners is rich in suspended solids, and also contains many toxic substances which are to be removed before the effluent can enter a watercourse. The actual composition of this effluent will depend greatly upon the nature of the ore being used in the blast furnace, upon the type and condition of the gas washing plant, and upon many other parameters. Often though, this effluent contains appreciable quantities of zinc, cyanides, chlorides and sulphate, and may even contain traces of heavy metals such as lead and cadmium. Before any secondary treatment can be instigated at a works, the types of pollutants and their ranges of concentration must first be determined.

1.4.3 Fume cleaning from oxygen steelmaking

Wet methods such as Venturi scrubbers or wet plate electrostatic precipitators are frequently used to collect the fine iron oxide fume evolved from oxygen steelmaking processes. The other main pollutant in the waste gas is temperature, and so any effluent treatment must satisfy the following requirements:

(i) Removal of iron oxide dust from gas.
(ii) Lowering of the gas temperature. This also lowers the risk of an explosion.

The process currently favoured by the British Steel Corporation for gas cleaning is the OG Gas Cleaning System, as developed by the Yawata Iron and Steel Company Limited - now part of Nippon Steel. The reasons for this are as follows:

(i) It does not inhibit steelmaking.
(ii) It's physical size is relatively small, and it is light in weight - low capital costs.
(iii) Running costs are relatively low.
(iv) An indication of the progress of the blow is continuously available by analysis of the waste gas, because it is a closed, suppressed combustion system.
(v) It permits recovery of the iron oxide.
(vi) No deleterious effects upon the environment.

However, it should not be assumed that all Basic Oxygen Steelworks (BOS) plants in the UK utilise the OG. system. Indeed this is not at all the case, but it does seem likely that as new plants are built, and older ones reconditioned, the OG. system for gas cleaning will be used.

Figure 1.4 shows a diagrammatic arrangement of the OG. system, and also the spray and cooling water systems. The waste gas produced during the blow changes direction at the spark box and is carried downwards. Water is sprayed into the gas and, by evaporation, its temperature is reduced from about 900 ^{0}C to virtually ambient by the time it reaches the first saturation venturi. These two venturis rough clean the gas.

Below the saturation venturi is an elbow separator which removes from the ductwork the majority of the excess water remaining from the sprays and saturation venturis.

The gas next reaches the second venturi where the cleaning process is completed. This is a Pease-Anthony (PA.) venturi and has a variable throat which is used for controlling the pressure in the base of the converter hoods by regulating the gas flow.

Below the PA. venturi, there is a second elbow separator which extracts the water from the ductwork. The gas now passes through water-mist separators to the induced-draught fan, from which the clean exhaust gases pass up through the stack, at the top of which is a burner with coke-oven pilot flames to ignite the gas.

The water used in cleaning the gas is polluted by dust - mainly as iron oxide, and by an elevated temperature. This water is directed to an effluent treatment system as shown in Figure 1.4, consisting simply of a conditioning tank and two thickeners. In the conditioning tank caustic soda is added as necessary to maintain pH. The two settlers each have a residence time of about seven hours, and a slurry is continuously withdrawn to a rotary vacuum filter where the moisture content of the sludge is reduced from about 90% to 40%. This cake is further dried before being

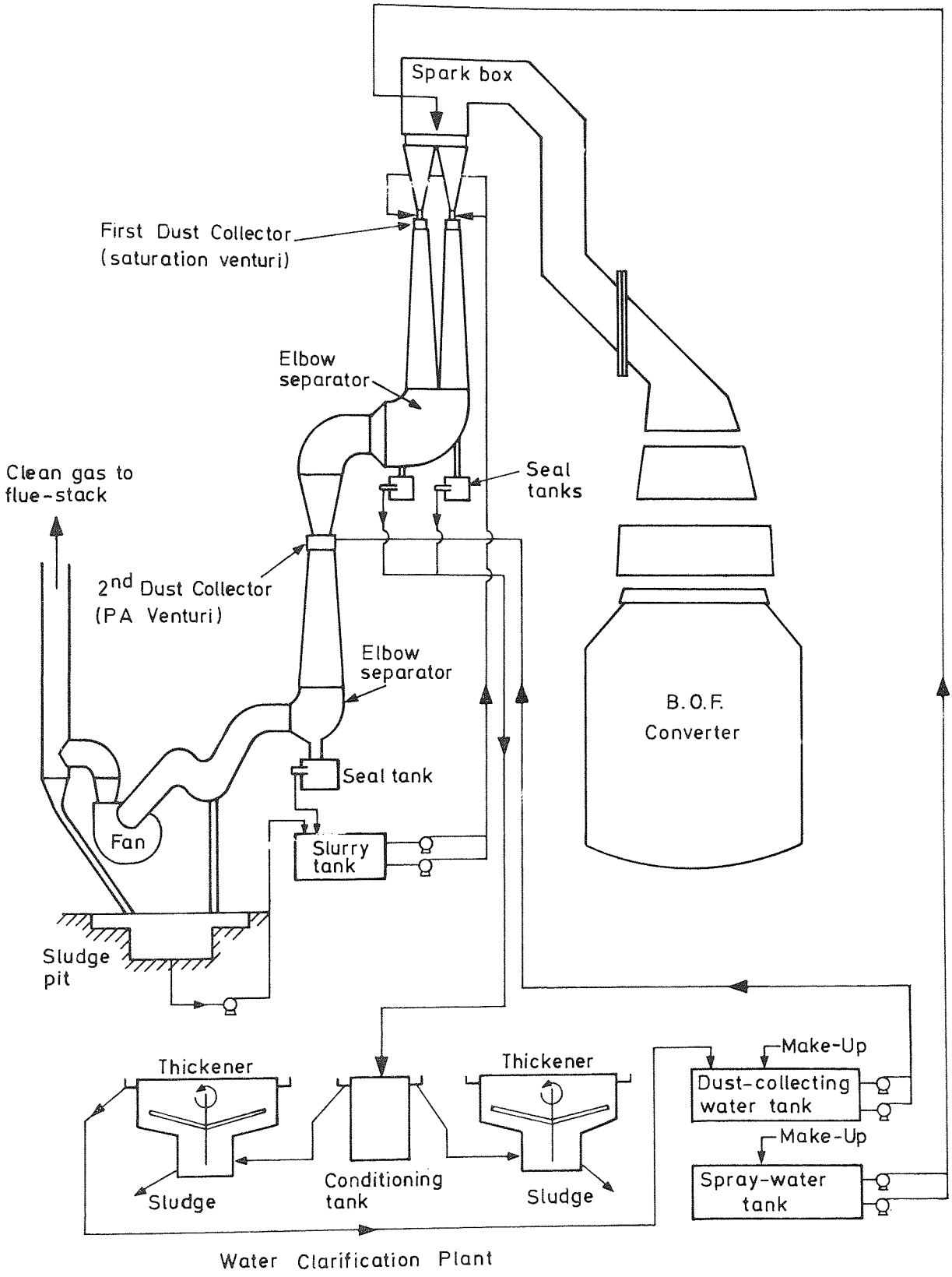


FIG. 1.4 SPRAY-AND DUST-COLLECTING WATER SYSTEMS; O.G. WASTE GAS CLEANING

sent to the sinter plants.

The thickeners reduce the suspended solids content of the gas-wash water from about 10 000 gms/m^3 to less than 100 gms/m^3, and their surface area is such that the temperature of the liquor is lowered to ambient by natural evaporation[22].

The overflow from the thickeners is re-used as process water on the PA. venturi scrubber, the only discharge effluent being that remaining after filtration of the slurry.

1.4.4 Effluents from rolling mills

Water is used in rolling mills mainly for scale removal and for cooling both the rolls and the rolled product. Although the water requirements for rolling processes are very high, the actual fresh intake required is relatively low due to the recirculatory systems that are employed.

The main contaminants of the effluent from a hot rolling mill are millscale, oils, greases and sludges. Scale pits, settling ponds and oil-skimming facilities are normally available to reduce the concentration of coarser materials. The effluent from these rolling mills is then normally treated in clarification and filtration units before being returned to the plant. A purge discharge from this secondary treatment controls the build-up of dissolved salts so that the circulating water is usually only lightly contaminated.

Liquid wastes from cold rolling mills are usually contaminated with rolling oils, detergents and metallic particles. After preliminary settlement and skimming such effluents are acidified, usually with spent pickle liquor or an acidic rinse water, to break down the emulsions. The free oil is then skimmed, and the remaining liquor neutralised with lime or an alkaline-waste cleaning solution, and then clarified. Further filtration may be necessary before final discharge to waterway or sewer.

1.5 Choice of Project

At the outset of the project, there was no commitment to a firm structure to be rigorously followed. The BSC required

expansion of their research into iron and steelworks liquid effluent treatment and control, with the emphasis placed on control techniques for treatment systems currently in use within the major works of the BSC, or for those systems expected to be employed in the near future. The rationalisation of the steel industry in the UK since nationalisation in 1967 has meant that most of the iron and steel manufacture is now centred in seven or eight integrated iron and steelworks. An integrated iron and steelworks is one in which all four of the basic operations are undertaken, i.e. coke-making, ironmaking, steelmaking and rolling processes. Thus, although individual effluents vary from works to works, the effluent treatment systems required in each integrated works will have basic similarities. The main objective from this research was therefore to further knowledge of some aspect of a treatment system which would be applicable to any one of the BSC's integrated works.

It was initially decided that the project should attempt to model a 'typical' effluent treatment system that could be found at any of the integrated iron and steelworks. At this stage, it was decided that effluents from rolling mills and from finishing operations such as cleaning, annealing, galvanising, tinning etc., should not be included in the model, as such processes vary greatly from one works to another. It was further decided to confine the study of effluents from the steel manufacturing process to those arising from a Basic Oxygen Steelworks, as other steel manufacturing processes such as the open-hearth and electic-arc furnaces are now normally being replaced where possible by BOS plant.

The initial aim of the project was therefore to produce a comprehensive mathematical modelling system for determining the performance of plant for treating liquid effluents from coke-making, ironmaking and steelmaking (as BOS) plants, and predicting the effect upon the environment of final effluents from such plant. The purpose of this aim was to enable the British Steel Corporation to improve the design and operation of treatment plant, and obtain realistic consents for the quality of final effluent. A schematic of the monitoring and control system as originally envisaged is shown in Figure 1.5. The limits of the system are shown by

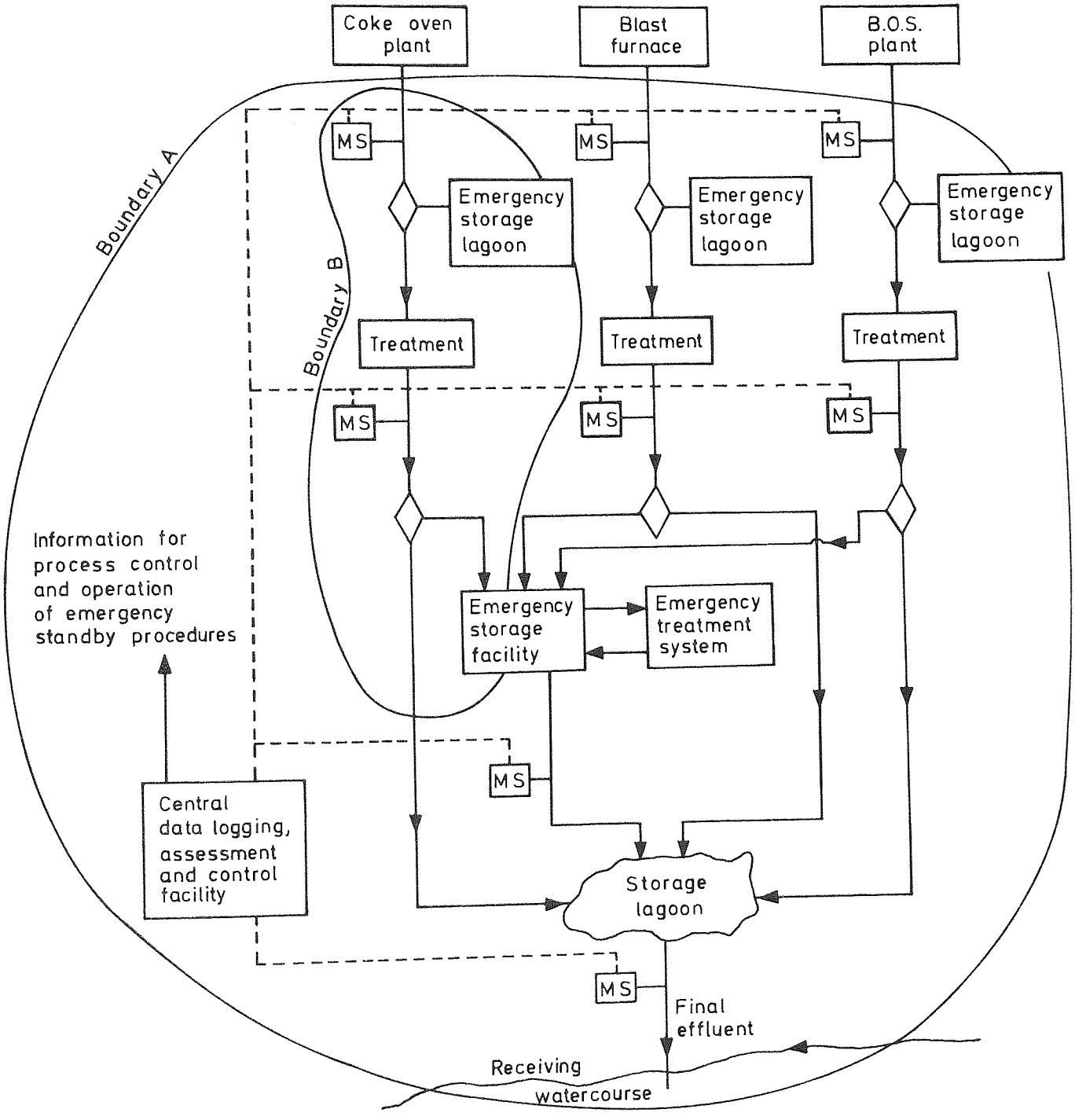


FIG. 1.5 A TOTAL SYSTEMS APPROACH TO IRON AND STEELWORKS EFFLUENT CONTROL

Effluent flow paths

Information flow to central control facility

Effluent flow control point

MS Monitoring station

boundary A.

The work began by studying the nature of steelworks effluent, and various plant to treat these effluents. These have been outlined in the preceding sections. A report based on the findings of this study was submitted to the supervisory group at the end of 1974. It was decided at that time that future progress should take in the quantitative aspect of modelling, with particular emphasis to be placed on the characteristics of the Activated Sludge Process. This was instead of continuing with the initial aim of building an overall model of the entire treatment system based on performance relationships derived from the results of published results, or from data extracted from existing records of BSC's effluent treatment plant.

The reasons for this first change in concept of the project are outlined below. Firstly, although treatment processes for the three effluent streams under consideration are reasonably well established in the BSC, namely activated-sludge for coke-oven effluents, and sedimentation processes for ironmaking and steelmaking effluent, performance data for such processes is not readily available, particularly in the case of the activated sludge treatment of coke oven effluents. Until recently water treatment expertise has been lacking in the iron and steel industry, as there has previously been little or no legislative or economic reason to concern management with effluent problems. Because of this, historical data on treatment plant performance is negligible. Although much research has been undertaken in the past on the performance of activated sludge processes, nearly all of this work concerned itself with the treatment of mixed domestic and industrial effluent. The little work that had been published on activated sludge treatment of coke-oven liquors has mostly been undertaken by the British Carbonisation Research Association and the Gas Industry in the 1950's and 1960's. Much of this work was found to be out of date, or inapplicable to coke-oven effluents from coking processes at a modern, integrated iron and steelworks. There was therefore a major problem of data on which to base the model.

The second reason for abandoning the concept of a 'total package' approach to the model was that there are numerous

packages presently available which would forecast the effect of a particular effluent on the receiving watercourse (see for example Ref. 23). Depending upon the geographical site (i.e. estuarial or river discharge etc.), and the nature of the model required, such a model can be readily obtained and built on to the model of the treatment system.

Research then continued into modelling techniques for the cokemaking, ironmaking and steelmaking effluent treatment processes. In mid 1975 the Supervisory team decided on a second change in concept of the project. Research into modelling techniques for Blast Furnace and Basic Oxygen Steelworks effluent had shown that the physical and chemical treatment of such an effluent was reasonably well understood, and that modelling of such treatment systems would be simple compared to the modelling of a biological system such as the activated sludge treatment of coke oven liquors. Furthermore, many models were available on the oxidative and settlement effects of lagoons, and suitable lagoon models could be obtained and incorporated into the treatment system model.

It was therefore decided that activated sludge treatment of coke oven effluents was the weak link in the overall treatment system model, and that more research into modelling this process was necessary in order to eventually enable the construction of a model of the total effluent treatment system of an integrated iron and steelworks. The limits of the work were thus reduced, and are shown schematically in Figure 1.5 by boundary B.

Once effort had been concentrated into modelling of the activated sludge process for the treatment of coke oven effluents, that which was to become the major objective of the project quickly became apparent. As stated earlier, there are numerous models available for the activated sludge process, the vast majority of which being for sewage effluents. Nearly all of these models assume the influent to consist of a single index of pollution such as BOD, TOC, or PV, and that this 'single constituent' is treated by an homogenous sludge.

The two main constituents of a coke-oven effluent are

phenol and thiocyanate. Tests were instigated in the laboratory to determine whether these constituents would be degraded at the same rates, and by similar sludges. Theoretically, it was expected that both their oxidation rates, and the sludges which utilised them as substrate, would prove very different, and this was in fact shown to be so. Thus, neither could the influent to the plant be described by a single index of pollution, nor could the sludge be treated as an homogenous mass. In order to determine rate constants for the process, both inorganic and organic constituents in the influent had to be considered, and the sludge had to be described by two inter-related sub-systems.

The actual value of these rate constants was shown to be so significant, that without being able to determine them accurately, the activated sludge treatment of coke-oven effluents could not be adequately modelled, without which the overall model of the treatment system could not be constructed.

Many problems became apparent in the determination of the rate constants, so much so that their determination became the major aspect of the project. These problems, and their eventual solution, are described in later chapters.

CHAPTER 2. LITERATURE SURVEY

2.1 General

This chapter deals with the literature surveyed throughout the project. Although some of this work is not of significant relevance to the eventual outcome of the research, it is still worthy of note, as without investigating such concepts as those effluents arising from ironmaking or steel-rolling and finishing operations, the decision to concentrate on coke-oven effluent could not have been made.

Several papers have been published on pollution control technology for the iron and steel industry. A particularly useful publication in the formative stages of the project was the "Management of Water in the Iron and Steel Industry", ISI 1970[24]. This is a collection of papers on water supply and conservation in the steel industry, on plant practice, effluent treatment and disposal. The relevant papers in this publication have previously been discussed in the preceding chapter.

Darnell[25] discussed pollution problems in the iron and steel industry in relation to air and water pollution, solid wastes and noise. Major waste-water sources were cited as coke-oven gas washing and cooling, blast furnace gas washing, and steel-rolling and finishing operations. Principal sources of water pollution, types of water use and major waste water constituents were considered. Harrison[26] also reported on general waste-water sources and characteristics in the iron and steel industry. He reported typical levels of water usage as 100-200 tonnes per tonne of steel produced. Use of recirculating systems reduced water usage to 3 to 6 tonnes per tonne of steel. These figures are very similar to those reported by Cook[8]. Darnell[25] also agreed that with a recirculating system, water use of 5 tonnes/tonne steel is possible, and stated that of this quantity, 80% is lost by evaporation. Rates of use of recirculative water of 88% and 93% have been reported from the Keihim and Fukuyama (Japan) Works respectively[27]. Andoneu[28] reported average water re-use in USSR steel industries for 1972 as 80%.

Most of the water intake in a modern integrated iron and

steel works is used for cooling purposes. Of this, about 75% is used in indirect cooling systems, the remainder being used for direct cooling[29]. Generally speaking, bleed-off effluent from the recirculatory system of the indirect cooling water does not constitute a pollution problem, even if discharged to a fresh water stream. It is the water used for direct-cooling, in which the water comes into direct contact with the substance being cooled which, in addition to absorbing heat, invariably picks up contaminants in suspension and/or in solution. Table 2.1 shows the main direct cooling systems at one integrated iron and steel works, together with the major contaminants picked up by the water.

TABLE 2.1 MAIN DIRECT COOLING RECIRCULATING SYSTEMS AND MAJOR POLLUTANTS ENTERING THE WATER

Plant	Process	Major Pollutants
Rolling Mills	Roll cooling Product quenching Scale washdown	Scale particles, lubricating oils, hydraulic fluids
BOS Furnaces	Fume cooling and cleaning	Iron oxide particles, fluoride
Continuous Casting	Spray cooling of hot steel	Scale particles, lubricating oils, hydraulic fluids
Coke Ovens	Gas cooling and purification	Tar oils, ammonia, phenols, thiocyanate, cyanide, thiosulphate
Blast Furnaces	Gas cooling and cleaning	Dust particles, cyanide, fluoride, lead, zinc

It will be noted that with the exception of effluents from the Coke Ovens and the Blast Furnaces, the remaining effluents from direct cooling systems are mainly polluted by contaminants in suspension. Such effluents are most easily and satisfactorily treated by physical/chemical methods such as skimming, chemical flocculation and sedimentation. Effluents arising from the Coke Ovens and from the Blast Furnaces on the other hand contain contaminants in solution which are not so readily treated by such methods.

2.2 Modelling for Water Pollution Control

Andrews[30] explained very concisely, the benefits that could be obtained by sensible use of mathematical models in the analysis, design and operation of water pollution systems. He suggested that this technique would be useful in the following ways:

1. Looking at a system as an integrated whole, yet with recognition of the interactions between the elements in a system and between the system and its environment.
2. Recognition of the universality of characteristics among systems.
3. An increased awareness of the importance of dynamic behaviour, information handling needs, and an orderly examination of alternate ways of accomplishing objectives.
4. A team or interdisciplinary approach is needed for the analysis, design or operation of a system.

Superficially, effluent treatment systems have a kind of archaic simplicity. They have remained virtually unchanged for the last 20 years or more. If we consider the most complex form of effluent treatment i.e. that of biological treatment, we immediately notice the following:

1. GRAVITY is used as a prime mover wherever possible
2. BACTERIA and
3. SLOW PERCOLATION through natural media are widely employed
4. AIR is the most important additive.

These together make up the most important processes in biological effluent treatment. Although they are natural processes, the initial impression of simplicity is but an illusion. Closer inspection of the treatment process reveals:

1. A BIOPHYSICAL PROCESS of great complexity - the dynamics of which are so complex and variable that even today the true nature of what actually occurs within a biological plant is not fully understood.

2. FEEDSTOCK - whose variability is its only certainty. Even

with the amount of data available on, for example, coke-oven effluents, we are still unable to predict exactly what composition such an effluent will have at any given time.

3. OPERATIONAL TECHNIQUES - the actual operation of conventional effluent treatment plants still relies heavily on empirical knowledge and experience, and has little scientific basis.

Correct utilisation of mathematical models can help to dispel some facets of the above problems. Vast amounts of data on effluents can be obtained by modern instruments, but such data is meaningless without a means of analysing it. A mathematical model can sort and utilise the data. A model can help in the understanding of the dynamic inter-actions with a system, and can then optimise for the system as a whole. Given influent data, the model will describe the condition of the effluent at each stage of its treatment, and will also predict the site-effluent quality and its effect upon the receiving waterway.

An example of the possible benefits to be incurred from sensible use of a mathematical model was reported by Roesther[31]. By monitoring the dissolved oxygen (DO) concentrations of an activated-sludge reactor and controlling the DO using modelling techniques, he claimed plant efficiency to be improved from 85 to 96 per cent. Plant operators also found less problems with filamentous bulking and reported that the sludge was easier to settle and handle.

Using a mathematical model to investigate various control strategies for an activated sludge plant, Busby and Andrews[32] suggested that control of sludge recycle rate could lead to significant improvements in plant efficiency.

Models have been used to determine the least cost design of water-waste treatment systems, see for example Middleton and Lawrence[33], Parking and Dague[34], Smith[35].

Other potential benefits include increased productivity,

greater reliability, lower operational costs, more stable operation and faster start-ups, (Andrews[36]).

2.3 Coke-oven Effluent Treatment

This section surveys some of the major techniques for treating waste-waters from coal carbonisation plants and shows the necessity for efficient treatment of such liquors. The conclusions of this survey indicated that activated sludge treatment of these liquors has been, and is likely to remain the preferred treatment process in the foreseeable future.

Effluents from coking plants arise as a result of evaporation of the moisture in the coal, production of water vapour by pyrolysis of volatile matter and from washing operations in the processing of the by-products, e.g. gas, tar, benzole and ammonia.

Approximately 0.31 m^3 of effluent originate from the production of one tonne of coke. Typical compositions of coke-oven liquors are shown in Table 1.3. Pearce and Punt[37] recently reviewed treatment systems for coke-oven effluents, and they, along with many other workers in the field[26,38,39,40] have stated that the activated sludge process seems to be the optimal treatment system for reasons of economics, space and efficiency.

However, other types of treatment processes have been investigated. Wurm[41] reported on the use of centrifugal extractors for removal of phenols from coke-oven effluents, using benzole as the solvent. One horizontal Podbielniak extractor was used for dephenolation, and another for removal of phenols from the benzole by reaction with caustic soda. During a test with a throughput of 18 m^3 per hour of liquor containing 3,500 $gm.m^{-3}$ of phenols, he reported a recovery of 98%. Other solvents that have been used for dephenolation include butyl acetate and isopropyl ether, Grudzien[42], and coal tar wash oil, Korobchanski et al[43].

Grossman and Pasynkiewicz[44] carried out laboratory tests on the oxidation of phenols in aqueous solution by ozone. They found removal efficiencies of up to 100%. Hall and Nellist[45] tested ozination of carbonisation effluents, and found it to be both practical and economic for final purification after biological

treatment. Midland Tar Distillers Ltd[46] developed a method for treating liquors containing polyhydric phenols. To remove these constituents, the oxidation products of which would cause difficulties in the subsequent biological treatment of the effluent, the liquor is oxidised with air at 50 - 60 °C in the presence of 1 - 2% lime. Some of the oxidation products of the phenols are removed with the lime after settling or filtration. The oxidation process also destroys some of the monohydric phenols and thiocyanate.

Fisher[47] investigated the use of soil percolation as a means of disposal of coke-oven effluents. At a rate of 3500 gallons per day per acre he found a high degree of purification, but ponding and lack of aeration eventually reduced the efficiency of the process. It was suggested however that the discharge effluent from an activated-sludge plant might be disposed of by percolation through soil supporting vegetation, and ploughed periodically.

The British Carbonisation Research Association (BCRA) have investigated the use of packed towers for coke oven effluent treatment[48]. It was concluded that the packed tower process may be useful for treating large volumes of weak liquors, especially where partial treatment only is required. However, in most cases, the activated-sludge process is to be preferred as it almost always gives an effluent of superior quality.

Kostenbader and Flecksteiner[49] compared the use of percolating filters, chemical treatment and ion-exchange processes with the activated-sludge process. They concluded that the activated-sludge process is to be preferred, as percolating-filters necessitate excessive acreage, whilst chemical treatment and ion-exchange processes are uneconomic.

The BCRA have also investigated the use of activated carbon as a means of "polishing" coke-oven effluents after biological treatment. It was stated[50] that the operating costs of such secondary treatment would be 10-14 pence per m^3 of effluent, when the PV of the influent to the carbon stage is no greater than 100 gm m^{-3}. A stronger influent could be treated, but only at a

disproportionately higher cost. Considerable mechanical difficulties were encountered in operating the process, and the work indicated that it was virtually impossible to achieve a PV value of 20 $gm.m^{-3}$ in the final effluent by this process.

Ashmore[51] recently published a review and analysis of methods for treating liquid effluents arising from coal carbonisation. The processes considered included adsorption, ion-exchange, precipitation and coagulation, solvent extraction, ozonisation, electrolysis, incineration and biological oxidation. Limited information on treatment costs were also summarised. It was concluded that of the treatments that had been put into commercial practice, biological oxidation was the most common and most successful.

The standard of the treated effluent for discharge can vary considerably depending if the treated effluent goes to a river or to a sewer. Even within the various river authorities there may be considerable variation in consent conditions. Pearce and Punt[37] reported the figures reproduced in Table 2.2 for river authority consent conditions. This variation arises due to the volume of dilution water available and its flow, the degree of pollution already existing and the requirement for abstraction of river water for potable purposes. As stated in the preceding chapter, the necessity for industrial waste-water treatment is becoming increasingly more evident, both from legislative and economic points of view. Because the required degree of purification varies so much from one area to another, it would be very inadvisable to attempt to legislate for requisite degrees of purification for all industrial effluents. However, the Food and Agriculture Organisation of the United Nations has recently surveyed the lethal and sub-lethal effect of phenols on fisheries[52]. They proposed tentative maximum allowable concentrations of phenols in inland waters as:

For Salmonid fish - maximum of 1 $gm.m^{-3}$ of total phenols.
For Coarse fish - maximum of 2 $gm.m^{-3}$ of total phenols.

TABLE 2.2 RIVER AUTHORITY CONSENT CONDITIONS

Constituent	Range, gm m^{-3}
Free Ammonia	20 - 250
Mono phenols	0.2 - 15
thiocyanate	1 - 25
cyanide	0.1 - 2.0
sulphide	0.1 - 15
PV - permanganate value	30 - 200

2.4 Review of Activated Sludge Systems

The activated sludge process had its origin in the classical experiments on the aeration of Manchester sewage carried out by Ardern and Lockett in 1913-14[53]. Previous investigators had tried aeration, but had found that the periods of aeration required to bring about oxidation of sewage were so long that it was unlikely that the process would be economically feasible on a large scale. However, these earlier investigators had made the mistake of rejecting the insoluble deposits of "humus" that were formed. Ardern and Lockett on the other hand, returned the "humus" to the aeration basin after completion of oxidation of one batch of sewage, and found that by always retaining the accumulated deposits of sludge, they were able to reduce oxidation time from about five weeks to only a few hours. It was they who named this sludge "activated-sludge".

The work of Ardern and Lockett led to the development of the activated sludge process on a large scale, so that it has now taken its place alongside the percolating filter as one of the two outstanding modern aerobic biological sewage treatment processes. The activated sludge process has a considerable attraction for large cities and industrial sites where the land is scarce, since the plant occupies only a fraction of the area of a percolating filter installation.

The original method of introducing air to the oxidation basin was by air diffusers. In 1925, Dr. H.H. Kessener introduced the first important mechanical aeration system, the Kessener Brush system. Circulation and aeration in the aeration basin are achieved by rapidly revolving stainless steel brushes which are

partially submerged in the mixed liquor along one side of the aeration tank, and produce a spray of fine droplets as well as waves along the surface. This system was first introduced in the UK in Stockport in 1939[54]. In 1954, Ames, Crosta Mills and Co.Ltd. reported on the use of the Simplex surface areation system[55]. This inverted, rapidly rotating cone provided with steel blades draws the sewage up a steel pipe in a hopper-bottom aeration tank and sprays it across the liquid surface, thus causing intense aeration of the mixed liquor. This system of aeration and mixing has found much favour in modern sewage and industrial effluent treatment plants.

2.4.1 Modifications of the Conventional Activated-sludge Process

Tapered aeration - in order to secure better utilisation of air, this sytem was advanced in 1936[56]. More air is provided where the sewage is strong (near the inlet end) than where it is nearly purified (near the outlet end).

Step aeration - introduced by Gould[57], this system attempts to even up the initial sewage load to the mixed liquor by adding a portion (say ¼) of the influent to each of four inlets.

Extended aeration - Tapleshay[58] found that by use of prolonged aeration periods and a high rate of return sludge so as to give a high concentration of suspended solids in the mixed liquor, it is possible to achieve much oxidation of the activated sludge solids so that their rate of accumulation is very small. This, it was claimed, virtually eliminates the surplus sludge problem - particularly important for small plants.

Contact stabilisation - First developed in the USA by Ullrich and Smith[59], this system employs a high concentration of activated sludge and unsettled sewage, a very short aeration period so that much smaller aeration tanks are required, and re-aeration of the sludge in a separate tank for about 2-3 hours.

Use of pure oxygen - Budd and Lambeth[60] showed that it is possible to treat sewage by the activated-sludge process using oxygen instead of air, with significant savings in the size of plant needed. New techniques for the production of oxygen have

recently resulted in the lowering of cost, and there is now much interest in direct-oxygen activated-sludge plants for the treatment of domestic and industrial wastes[61,62,63,64].

2.5 The Activated-Sludge Treatment of Coke-Oven Effluents

Many authors have reported upon the use of activated-sludge plants for the treatment of effluents arising from coal-carbonisation plants. Within the term "activated-sludge plant", there are many modifications that have been investigated, and it is a discussion of such modifications that is presented in this Section.

Dewes[64] reported upon pilot-scale experiments on the purification of coke-oven phenolic effluents in admixture with domestic sewage. Using a Lurgi "Aero-Accelator" activated-sludge plant with mechanically clarified domestic sewage and an aeration time of two hours, he observed the rate of removal of phenol to be twice that of a similar plant without sewage addition. He concluded that most of the phenol was converted enzymatically into biological cell material, a process more rapid than oxidation to end-products of water and carbon dioxide. On the basis of these tests a scheme for the treatment of sewage from several communities together with the effluent from a coking plant at Brebach was drawn up.

Muller and Coventry[65] investigated a similar scheme at the Gary works of the US Steel Corporation. The effluent from this coke-oven plant was diluted with sewage at a ratio of 1 volume of effluent to 100 volumes of sewage. Some trouble occurred with calcium carbonate blockage due to calcium ions in lake water which reacted with the liquor in the sewer. This was overcome by pipework modifications and the addition of a scale-control agent. The activated-sludge units at the sewage plant reduced the phenol and thiocyanate concentrations to low levels, but had little effect on the ammonia. The experiment was eventually stopped because it was not possible with existing equipment to add enough chlorine to overcome the ammonia problem in the final effluent from the sewage plant.

Cooper and Catchpole[66] reported on tests undertaken by the

BCRA into factors affecting the biological treatability of coke-oven effluents. It was found in particular that the oxygen demand of an effluent was inadequate for predicting its behaviour during treatment. They concluded that the only safe-course was to examine individual liquors to determine their response to biological purification. In the same paper, they reported on investigations into the effects of interfering substances. They found cyanide, sulphide and oxidised phenols to be amongst the worst inhibitors, but stated that the effects of such inhibitors might be minimised by dilution of the liquor before treatment.

Fisher[67] also found dilution to be a vital part of biological treatment of coke-oven effluents. With ammonia liquor stripped of free ammonia and diluted threefold with clean water, he reported a reduction in BOD from 1000 to 5 $gm.m^{-3}$, phenol reduction from 300 to 0.04 $gm.m^{-3}$, and thiocyanate removal from 150 to 2 $gm.m^{-3}$. When dilution was carried out with treated effluent instead of clean water, there was an increase in the colour of the effluent, but no significant effect on treatment. Finally, he investigated the use of activated carbon and ozone to reduce the colour of the effluent, but concluded such methods would be uneconomic.

In another BCRA investigation into the effects of inhibitory compounds on activated sludge treatment of coke-oven effluents, Ashmore et al[67] used oxidised catechol to represent the "coloured inhibitory material" so often reported as being responsible for the residual PV of a treated liquor. They found that addition of 50 $gm.m^{-3}$ of this compound had a marked inhibitory effect on thiocyanate removal.

Ludberg and Nicks[68] reported upon a treatment plant built for Dominion Foundries and Steel Plant Ltd., Ontario. After reviewing alternative methods for coke-oven effluent treatment, it was decided to use the activated-sludge process. Using a retention time of 19 hours, they observed minimal reduction of ammonia contents, but phenol removals from about 300 $gm.m^{-3}$ to less than 1 $gm.m^{-3}$. Thiocyanate removal was reported as being very erratic.

Kostenbader and Flecksteiner[49] of the Bethlehem Steel

Corporation, USA., investigated the effects of various factors on the degree of purification of coke-oven liquors. They found high ammonia concentrations to adversely affect phenol removal. Under favourable conditions, over 99.8% phenol removals were observed. It was not found possible to correlate any of the variables investigated with the degree of thiocyanate removal, which varied from less than 10% to over 99% in pilot-scale tests. Cyanide concentrations could be reduced by storing the liquor, and overall cyanide removals of 70-90% were observed by a combination of storage and biological treatment, but only when the removal of thiocyanate was good. No reduction in ammonia concentration was observed as a result of biological treatment.

2.5.1 The Effect of Growth-Factors

The BCRA have undertaken much work into the effect of adding small concentrations of growth-inducing compounds either prior to, or during coke-oven effluent treatment. Such compounds are commonly termed "growth-factors".

Several organic chemicals have been investigated by the BCRA and patents have been issued on the use of p-aminobenzoic acid or p-hydroxybenzoic acid[69] and nicotinic acid, isonicotinic acid, pyruvic acid and certain amino acids[70] as growth factors for improving the biological treatment of liquid wastes.

Catchpole and Cooper[71] reported on the addition of small concentrations of p-aminobenzoic acid (PAB) to activated sludge. They found the retention time for bio-oxidation of ammonium thiocyanate to be half of that of a similar system without PAB addition. It was suggested that PAB itself is not the growth factor, but that it is modified by the bacteria to a fairly simple compound which is of benefit to enzymatic reactions suppressed by inhibitors. Theoretical considerations suggested this modified compound to be pyruvic acid, this suggestion being supported by the effectiveness of pyruvic acid itself as a growth factor and also by later experiments[74]. Glucose was also investigated as a growth factor, and found to be effective. This is further evidence of pyruvic acid being the active growth-inducing compound, as glucose is known to be converted to pyruvic acid during the fermentation process, Rose[72].

More recent work by BCRA indicates that once the growth factor has established a modified metabolic pathway, further addition of the growth factor might, in some cases, prove unnecessary[73].

BCRA have also reported on investigations into the effect of growth factors on nitrification[75]. For coke-oven waste-waters with an ammonia content of the order of 200 $gm.m^{-3}$, suitable adjustment of retention time enabled a growth factor to produce complete oxidation of ammonia to nitrate without any additional stage of treatment.

2.5.2 Costs

In 1971, the BCRA estimated the cost of adding glucose as a growth factor in the activated-sludge process at 0.04 - 0.08 pence per m^3 of influent[76]. Total costs of activated sludge treatment of coke-oven effluents have been given as 35 pence per m^3 of liquor[77] and 5.45 pence per tonne of coke produced[78]. Assuming the production of 0.23 m^3 of waste-water per tonne of coal carbonised as reported by Speight and Davies[79], 5.45 pence per tonne of coke is approximately 25 pence per m^3 of liquor.

2.5.3 Microbial aspects of the Biodegradation of Coke-Oven Effluents

Carbonisation liquors provide compounds that can be readily utilised by micro-organisms as sources of energy and carbon. Many workers have isolated and characterised micro-organisms present in the activated sludges of biological plants treating coke-oven effluents. In general, it has been found that the organic constituents of the effluent such as the mono-hydric phenols, together with some of the more easily biodegradable poly-hydric phenols, are oxidised by heterotrophic bacteria. These are micro-organisms which are unable to synthesise all of their carbon compounds from carbon dioxide and require, in addition, an organic source of carbon in the environment. Thiocyanate on the other hand, together with some other inorganic constituents of the coke oven effluent such as cyanides and thiosulphates are oxidised by autotrophic bacteria. These are "self-sustaining" micro-organisms which have the capacity to synthesise all of their carbon compounds from carbon dioxide.

The predominant bacteria involved in the activated-sludge treatment of carbonisation liquors have been shown to include Pseudomonads and Thiobacilli; the former group being involved in the heterotrophic oxidation of organics such as phenols and pyridines, with the reduced sulphur compounds autotrophically oxidised by Thiobacilli[80,81]. Jones[82] acclimated a mixed population of bacteria to a laboratory scale biological plant treating a synthetic liquor comprised of phenol and thiocyanate. After some months of operation, he identified three different genera of phenol tolerant bacteria; Vibrio, Actinomycetes and Pseudomonads, and five different species of thiocyanate tolerant bacteria within the single genus Thiobacillus. Stafford and Callely[83] reported the isolation of two strains of bacteria capable of growth on phenol, and a third which grew on thiocyanate. The two phenol tolerant organisms were Comanonas sp and Moraxella/Acinetobacter, whilst the thiocyanate organism was considered to be similar to Thiobacillus thioparus, which was able to utilise thiocyanate heterotrophically. The organism was strictly heterotrophic and utilised thiocyanate only in the presence of organic substrates. However, they found that thiocyanate oxidation by the organism was inhibited by ammonia concentrations of 72 $gm.m^{-3}$. Since ammoniacal liquor from coke ovens usually contains at least 100-200 $gm.m^{-3}$, it is unlikely that P.stutzeri will play a major role in the biological treatment of coke-oven effluents. Hiam[84] isolated six species of bacteria capable of destroying thiocyanate in coke-oven effluents. These included two species of Thiobacillus, Micrococcus sp, Achromobacter sp, and two isolates of fluorescent pseudomonads, both of which resembled Pseudomonas putida.

2.5.4 Inhibition of Autotrophic Metabolism by High Phenolic Concentrations

Because a mixed microbial population is present degrading a wide range of chemicals in carbonisation effluents, some of these constituents can affect the metabolism of some of the degradative bacteria. For example, an increase in the concentration of phenol can seriously affect the activity of Thiobacilli, with thiocyanate subsequently ceasing to be removed in the treatment plant. Apart from certain Pseudomonad-like bacteria, which are able to degrade phenol when present at a concentration of 1000 $gm.m^{-3}$, to many bacteria, including Thiobacilli and nitrifying

bacteria, phenol is a well known bacteriocide[85].

During a characterisation of an organism resembling Th. thiocyanoxidans, Happold et al.[86] observed that phenol levels of 0.1% w/v (= 1000 gm.m^{-3}) were very inhibitory to thiocyanate oxidation. Pankhurst[87] reported total inhibition of thiocyanate degradation during biological oxidation of spent gas liquor by phenolic concentrations of 300 gm.m^{-3}. Hutchinson[88] found that phenol levels of 100 gm.m^{-3} inhibited the autotrophic oxidation of the thiocyanate, whilst Lynn[89] stated phenolic levels of 50 gm.m^{-3} would totally inhibit thiocyanate oxidation. Finally McLee and Moore[90] recommended that the concentration of total phenols should be reduced to 10-20 gm.m^{-3} before thiocyanate removal could proceed satisfactorily.

It seems that there is some doubt as to the minimum concentration of phenolics that is inhibitive to thiocyanate degradation in a biological plant treating coke-oven effluents. There are probably two major reasons for this confusion.

Firstly, thiocyanate is degraded by more than one species of bacteria; the actual composition of the bacterial flocs may easily be very different from one works to another. Thiocyanate is often degraded by organisms of the genus Thiobacilli[37,81,82], but instances have been reported where thiocyanate has been found to be degraded by heterotrophic organisms such as Pseudomonas stutzeri (Stafford[83]) and Pseudomonas putida (Hiam[84]).

Secondly, it is not fully established what exactly causes the inhibition of thiocyanate when phenol is present in the mixed liquor at elevated concentrations. The authors mentioned earlier have quite correctly reported concentrations of phenolics at which thiocyanate degradation has been inhibited. However, Jones and Carrington[91] recently found that phenol itself is not inhibitory to the thiocyanate-degrading organism, but rather it is the presence of actively-growing masses of phenol-degrading bacteria that causes the inhibition. They concluded that in an activated-sludge plant oxidising phenol and thiocyanate concurrently, the growth-rate of the phenol-bacteria must be kept low in order to prevent thiocyanate inhibition. An obvious means of keeping the

growth rate low is to try and ensure low phenol concentrations.

Dagley et al.[92] postulated that the bacteriostatic action of phenol was a result of the inhibition of formation of essential metabolic intermediates. They found that the lag in growth of Escherichia coli caused by phenol could be alleviated by the provision of small amounts of glutamate, succinate and methionine. This is very similar to the BCRA's work on growth factors (see Section 2.5.1) where small amounts of known metabolic intermediates are added to the mixed liquor to improve process efficiency. Work undertaken by the BCRA on the phenolic inhibition of thiocyanate suggests that it is the partially oxidised polymeric phenols, such as oxidised catechol, that are responsible for the inhibition[67]. Baird et al.[93] also suggested that inhibitive effects are due to the formation of toxic oxidation intermediates rather than to the phenol itself.

2.6 Modelling for Design and Control of Activated Sludge Processes

In order to reduce the apparent complexities of activated sludge aerobic biological treatment to their simplest terms, some knowledge of the reactions that occur within the system is essential.

The basic biochemical reaction which results in conversion of organic waste to cellular matter and harmless inorganic end-products may be expressed thus.

$$\text{Food} + \text{Micro-organisms} + O_2 \xrightarrow{\text{Nutrients}} \text{Cellular Matter} + \text{Energy} + CO_2 + H_2O + NH_3$$

In designing or operating a plant to carry out this reaction, it is necessary to choose the relative quantities of reactants (waste or food; sludge or mixed liquor solids, and air) to be combined in the reaction (aeration) tank. Control of the reaction may then be effected by alteration of external influences (food to micro-organisms ratio, temperature, pH etc.) but the basic reaction cannot be changed; material and energy in and out must balance.

Lesperance[94] listed several factors of import to the

design and control of activated sludge plants, and included -

(a) Organic loading can be used to size an activated-sludge process.
(b) Toxicity and other environmental factors must be evaluated and, where necessary, adjusted.
(c) Organic loading will establish removal efficiency, oxygen requirement, excess sludge production, etc.
(d) Oxygen requirement as a function of organic loading and temperature is predictable.
(e) Mixing in aeration systems can be quantitatively evaluated with respect to its effect on the bio-chemical process.

In recent years, optimal design and operation of industrial activated sludge processes has not received the attention it perhaps deserves. The reasons for this are twofold. Firstly, water has until recently been regarded as a cheap and plentiful natural resource. As explained in the introductory chapter, this view has now changed, due mainly to the escalating demand and cost of fresh-water. The second reason lies in the fact that activated-sludge treatment is a biochemical reaction, and there were very few engineers willing to study the biological reactions involved with a view to optimising the process. As a result, recommended design has been based on current practice rather than on the design criteria which would require the least cost or the maximum benefit.

Fortunately, this trend is now being reversed, primarily because the economics of water treatment have become very significant, but also because the general availability of computers have enabled engineers to more easily model the biological processes involved.

Many models of the activated-sludge process have been advanced, but most are based on the early work by Monod[5,9,6]. In 1942 he proposed a kinetic model of micrcbial growth which depends on the cell and substrate concentrations. He expressed the growth rate of micro-organisms thus;

$$\frac{dX}{dt} = \mu X$$

where $\frac{dX}{dt}$ is the rate of microbial growth, μ is the specific growth rate and X is the concentration of bacterial cells. Monod found that the value of μ is not constant, but depends on the concentration of growth limiting substrate, S, according to the equation

$$\mu = \mu_{max} \frac{S}{K_s + S}$$

The maximum growth rate, μ_{max}, and the saturation constant, K_s, are kinetic parameters, and are assumed to be constant for a specific system.

Monod also suggested that the relationship between the growth of bacteria and utilisation of substrate be represented by

$$-\frac{dX}{dS} = \frac{\text{Weight of bacteria formed}}{\text{Weight of substrate used}} = Y$$

where Y is referred to as the yield factor which he assumed to be constant.

Armed with these relationships, several workers have since attempted to model the activated sludge process, with varying degrees of success. Downing et al.[97] found success in using the Monod Model to predict nitrifying activity in the activated-sludge process, and it was later used by the same workers to develop dynamic models of different population systems in biological treatment plant[98]. McKinney [99] developed models for various activated-sludge processes based on these equations, as did Herbert[100], Keshevan et al.[101], Reynolds and Yang[102] and Rananathan and Gaudy [103]. However, each of these workers used the model to predict the responses of a homogenous, single species system operating at steady state. If the model is to be used in practice for design and control purposes, it must be applicable to a works environment in which there are normally several substrates, each of which having at least one species of micro-organism associated with it.

Tench and Morton[104] tested the model on a mixed-substrate system, assuming a homogenous sludge, and relating the overall substrate availability by the Biochemical Oxygen Demand (BOD).

They found the model to give erroneous results under such circumstances. Tucek et al.[106] also observed the application of the Monod model to complex cultures to be questionnable, and proposed a model based on Dimensional Analysis. Tischler and Eckenfelder[105] found that in mixed cultures, the order of substrate removal is actually a summation of the zero order removals of each substrate, and that Monod kinetics can hold if the substrates are treated singularly and then summed.

Many workers have attempted to develop the Monod equations in order to make them more representative of particular observations. Jones[107] suggested that the mathematical expression for the cells, X, should be split into the concentration of viable cells, and the concentration of those cells that are able to metabolise the substrate without further growth. A similar model was proposed by Hultman[108], whilst Jacquart et al.[109] described a model which allowed for substrate being "stored" by the cells, as well as being metabolised. Finally, Lee et al.[110] developed a model based on Monod kinetics which allows for different kinetics for dispersed-phase and flocculent bacteria.

To summarise this section, whilst modelling of the activated-sludge process is deemed important in order to optimally design and control works plant, there seems to be some controversy as to the usefulness of the basic (Monod) equations. Whilst they have been found to adequately represent the kinetics of a single substrate, single-culture system, some workers have found them inapplicable to a heterogenous system.

Examination of the effluent from a coke-oven works shows two main contaminants, phenols and thiocyanate. Whilst both of these are biodegradable, it seems likely that each will be degraded by a different species of bacteria, each of which will have different kinetics. It is thus proposed to develop the Monod model to take account of such a two-component system, and to observe whether such a model will adequately represent the dynamics of a coke-oven works activated sludge plant.

CHAPTER 3. A MODEL OF THE COMPLETELY MIXED ACTIVATED SLUDGE PROCESS

3.1 Introduction - The Activated Sludge Process

The Activated Sludge Process has, for many years, been used for the treatment of both industrial and domestic wastes.

Many modifications have taken place since the original system was first developed, in order to try and overcome some of the operational difficulties. Such modifications include step-wise aeration, tapered aeration, completely mixed processes, recycle and by-pass systems.

The used water to be treated, usually containing colloidal and dissolved organic matter, is pretreated by screening - to remove coarse floating matter such as rags - and this is followed by settlement to remove as much as possible of the organic matter as sludge.

The supernatent liquor is then passed into the activated sludge plant, the first, and possibly only, stage of which, being a reaction vessel where the waste-liquors are aerated with a flocculent mass of micro-organisms. Such microbial matter is commonly termed Activated Sludge.

The character of the activated sludge depends to some extent upon the nature of the wastes being treated. In general however, it consists largely of a variety of bacteria in different stages of development, aggregated into flocs together with organic debris and various forms of protozoa[111].

The bacteria assimilate the organic wastes, but their ability to do so is dependent upon them oxidising the waste. Thus oxygen is a necessary ingredient for the successful operation of the process, and is usually supplied, in the form of dissolved oxygen, (DO), either by surface aerators, or by a series of air diffusers along the base of the reactor.

A high degree of agitation is also necessary in the process, to enable intimate contact between the individual cells

and the dissolved oxygen. Without this agitation, the negligible relative movement between floc and waste water would considerably reduce the rate of oxygen transfer between the entrained DO and the surface of the respiring cell, thus considerably reducing the removal of Biological Oxygen Demanding (BOD) material.

3.1.1 The Nature of Organic Waste Removal

The bacteria embed themselves in a common gelatinous matrix (commonly termed a floc), causing a solid: liquid interface at which organic wastes accumulate. These are then used by the bacteria for food, respiration and synthesis. Thus, the bacteria multiply, and the floc size increases. During development of the floc it becomes invaded by higher organisms such as ciliated protozoa and nematode worms. These organisms utilise the bacteria as a food substrate.Some of the ciliated protozoa appear to be swimming freely, and are not intimately associated with individual flocs. Such protozoa feed on bacteria present in the interstitial liquor.

In contrast, hypertrichous cilliates may be seen to be creeping over the surface of the floc, with which they are more closely associated. Peritrichous cilliates on the other hand, are actually attached to the floc.

3.1.2 Settlement of the Activated Sludge

The mixture of flocculent material and partially treated liquor is now normally directed to one or more settlement tanks, where the activated-sludge is then settled out. This is then usually recycled back to the reaction vessel (aeration tank), with a small amount being wasted to control the concentration and "age" of sludge in the system.

Supernatent liquor from the settlement tanks should now be clear, and have a low BOD and may finally be discharged to the receiving waterway.

3.2 Completely - Mixed Process

The Completely-Mixed Activated Sludge Process has been shown to have many advantages over other modifications of the system. Eidsness[112] was amongst the first to report the

combination of high effluent quality and low residence times possible in such plant.

Other workers[113,114] have shown that the continuous, complete-mixing system is able to handle higher organic loads than the conventional, plug-flow system.

Some of it's main advantages are outlined below:-

(1) Maximum Equalisation of the oxygen uptake rate

(2) Maximum Damping of Shock-Loads

(3) Maximum neutralisation of carbon dioxide produced during respiration

(4) Relatively constant environmental conditions for the biological mass.

The above advantages make the completely-mixed activated sludge process well suited for treatment of industrial wastes. Wastes high in oxygen demand may be effectively treated since the oxygen supplied is uniformally distributed throughout the aeration tank, and any shock load is immediately and intimately mixed with the whole of the contents of the vessel, thus minimising its toxicity.

3.2.1 Completely-Mixed Activated Sludge Process for the Treatment of Coke-oven Effluents

In Chapter 1, several methods of treating coke-oven effluents have been outlined. The use of the Activated Sludge system for treatment of such effluents was recommended.

Other workers have discussed the relative merits of the Activated Sludge System for treatment of such effluents, and in virtually all cases have reported in favour of that system. Pearce and Punt[37] discussed the possibility of using bacteria beds - or percolating filters - to treat carbonisation wastes, but reported that studies found that filter performance was adversely affected by an accumulation of biological film causing filter blockage, and resulting in the development of anaerobic conditions.

The use of plastic towers in pilot plant studies have

given encouraging results in terms of shock loadings, temperature variations and pH sensitivity[115]. However, they are reported to suffer from precipitation of lime on the plastic surface when treating limed ammoniacal liquors. Also the results for thiocyanate removal were disappointing.

The Completely Mixed modification of the process is particularly suitable for the treatment of such wastes, because of the instantaneous mixing that occurs throughout the entire aeration tank. Carbonisation effluents in particular have a high BOD and the instantaneous mixing effect lowers the initial organic loading. Also, and possibly more important, steelworks effluents suffer from a large variance in their composition making shock-loadings a common occurrence. In such cases, the aeration tank operates as a surge tank to level out variations in the organic strength of the raw wastes, so that shock-loads due to variations of organic concentrations do not have such a great shock-effect on the micro-organisms in the aeration tank.

3.3 System Representation

3.3.1 Modelling Techniques

A Model may be defined as being a representation of a system in a form suitable for demonstrating the way the system behaves.

When properly applied, modelling can result in considerable savings of both time and money. For example it is usually much less expensive to construct a pilot plant (physical model) and conduct experiments on a specific used-water stream than it is to build and experiment with full-scale plant.

There are many types of models, and the type selected depends primarily upon the purpose for which it is to be used. For example, the schematic diagram given in Figure 3.1 is adequate as a pictorial model to illustrate the components and describe some of the interactions in a used-water treatment system. However, pictorial models such as this tell nothing about the quantitative nature of the system's behaviour.

Mathematical models may be classified in many different ways, and one of the most important distinctions in used-water treatment is the difference between DYNAMIC and STEADY-STATE models.

Most models currently in use are based upon the assumption of steady-state, which is that state at which equilibrium is reached or, in mathematical terms, that state at which the system variables are independant of time such that any derivative terms in the system equations become equal to zero.

More recently, with the advent of computer simulation, it has become possible to more easily simulate dynamic conditions, which better relate to the actual conditions suffered by the plant. It is thus possible to study, by means of computer simulation, the effects of time-variant inputs on the model, and to analyse the resultant varying outputs.

Another important classification of mathematical models is as DETERMINISTIC OR STOCHASTIC. Deterministic models are those in which the inputs, outputs and system parameters can be assigned a definite fixed number, or series of fixed numbers,for any given set of conditions. In contrast, stochastic models have a degree of uncertainty, and statistical techniques are used to express the model in mathematical terms. Deterministic and stochastic features may both be used in a model. For example, in used-water treatment, a stochastic model may be used to determine the stream flow when evaluating the effect of a used-water treatment plant discharge, (deterministically modelled), on a stream.

3.3.2 Choice of Model for the Activated-Sludge Process

An activated-sludge plant for the treatment of iron-and-steel-works effluent is in a continuous state of variability. For example, one of the few statements one may make about the nature of a coke-oven effluent is that "it's variability is it's only certainty".

Thus, it can be immediately seen that a steady-state model would not represent the actual behaviour of the plant. A dynamic model is therefore chosen which, with present-day simulation techniques, should not prove to be insoluble.

As to a deterministic or stochastic model, the former is to be preferred - at least initially. The model is intended to show a "cause and effect" relationship, i.e. given a particular set of conditions, what effect will they have on the system variable (effluent quality, residence time ....)?

Were the model to show the probability of a certain value of the system variable, a stochastic model would be necessary.

3.4 Mathematical Representation of the Activated Sludge Process

3.4.1 Introduction - Theory

Many workers have developed sets of equations to represent the activities of various types of activated sludge plants. Most of them consider bacterial growth and substrate utilisation on the basis of the Monod equations as given in Section 2.6, i.e.:

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad \text{..... Eq. 3.1}$$

$$\frac{dS}{dt} = -\frac{X}{Y} \mu_{max} \frac{S}{K_s + S} \quad \text{..... Eq. 3.2}$$

These equations were analysed by Downing and Wheatland[115] and the effects of mixed populations and simultaneous metabolism were discussed. Reynolds and Yang[102] developed a steady-state model based on these equations, and showed them to be valid for a simulated organic waste under certain laboratory conditions. More recently, Curds[111] modelled the dynamics of various specific microbial organisms, and found that many of the predictions made by the model were observed in full-scale or experimental plants. Finally Jones[107] used the equations to develop a model for single substrates, and found correlation between the predictions of the model and practical observations.

3.4.2 Limitations of the Equations

Some workers[98,104] have reported that the Monod equations are unsuitable for predicting removal of carbonaceous matter from mixed substrates - concentrations of which are usually expressed in terms of BOD and COD. Their most successful application has been by Downing et al.[97] to the development and decline of

nitrifying activity in the activated-sludge process. It remains to be tested how successful a model, based on these equations, will be for iron- and steel-works effluents.

3.4.3 Single-Stage Activated Sludge Plant Model

Figure 3.1 is a schematic representation - or pictorial model - of a single-stage activated sludge unit. It comprises a single, completely mixed reactor of volume $V m^3$, which receives a continuous flow, $q\ m^3/h$. of works effluent. The contents of the reactor overflow into a settling tank at a rate $(1+a).q\ m^3/h$, which then concentrates flocculent microbial populations. Some of the concentrated active sludge is continuously wasted at a rate $wq\ m^3/h$, the remainder being recycled back to the reactor at a rate $aq\ m^3/h$.

3.4.4 Assumptions

Three assumptions form the basis of the model:

1. The rate of multiplication of bacterial populations is proportional to the cells already present - that is, each cell is dividing at the same rate

   Thus $\frac{dX}{dt} = \mu X$ where μ = growth rate. Eq. 3.3

2. The actual growth rate is a fraction of the maximum rate that could occur when all necessary nutrient is limiting, the growth rate being dependent upon this nutrient according to the equation.

   $$\mu = \mu_{max} \frac{S}{K_s+S} \quad \text{..... Eq. 3.1}$$

   as developed by Monod.

3. The removal of substrate is connected to the increase in cells (active mass) by the relationship:

   $$\frac{\text{Weight of bacteria formed}}{\text{weight of substrate used}} = \frac{-dX}{dS} = Y, \text{ yield coefficient.}$$

   Thus, over a small period of time, dt:

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \quad \text{..... Eq. 3.4}$$

These three equations may be combined to give the second Monod relationship (Eq. 3.2).

$$\text{i.e.} \quad -\frac{dS}{dt} = \mu_{max} \frac{X}{Y} \left(\frac{S}{(K_s+S)}\right)$$

Thus, the rate of removal of substrate is expressed in terms of the maximum specific growth rate, μ_{max}, the cell concentration, X, the yield coefficient, Y, and the saturation constant K_s. It should be noted that the assumptions 1-3 represent nothing more than expressions which reasonably fit experimental observations.

Care should be taken not to confuse equation 3.1 with the Michaelis-Menton equation for the rate of enzymatic reactions. Biological growth rates and enzymatic reaction rates are not identical.

Many workers incorporate a term for cell-death. It can be seen that Eq. 3.1 does not allow for death of bacteria unless the substrate concentration (S) becomes negative. Thus a fourth major assumption is to be made.

Bacteria require a small amount of energy to maintain normal functions such as motion and enzyme activation. This basal-energy requirement is known as endogenous respiration.

Endogenous respiration is a continuous activity that results in the metabolism of certain components of the protoplasm. As endogenous metabolism proceeds, the bacteria reach a point at which they can no longer sustain life and die. A portion of the dead bacteria undergoes lysing, releasing the remaining nutrients into the environment where other bacteria can utilise them as food. In the activated sludge, in which millions of bacteria exist in every millilitre of liquid, the endogenous reaction can be assumed to be a continuous reaction.

Change in active mass due to endogenous respiration is dependant upon the active mass present, and thus we may write:

$$\frac{dX}{dt} = k_e X \quad \text{..... Eq. 3.5}$$

where k_e is termed the endogenous respiration rate.

Basing the model on these four assumptions, it is now possible to obtain expressions to represent the dynamics of any activated-sludge plant.

3.5 Continuous Flow, Completely Mixed, Single Stage Activated Sludge Plant

It is assumed at this stage that all of the active mass leaving the reactor is settled out, making the concentration of active mass in the final effluent leaving the settler equal to zero. It is further assumed that the cell growth and endogenous changes that occur in the settler are negligible.

With these assumptions, the following mass balances may be taken around the reactor only, or around the plant as a whole, the same result occurring in each case.

Key

All concentrations shown in square brackets.

All other symbols relate to flows, unless otherwise stated.

See Appendix (1) for notation.

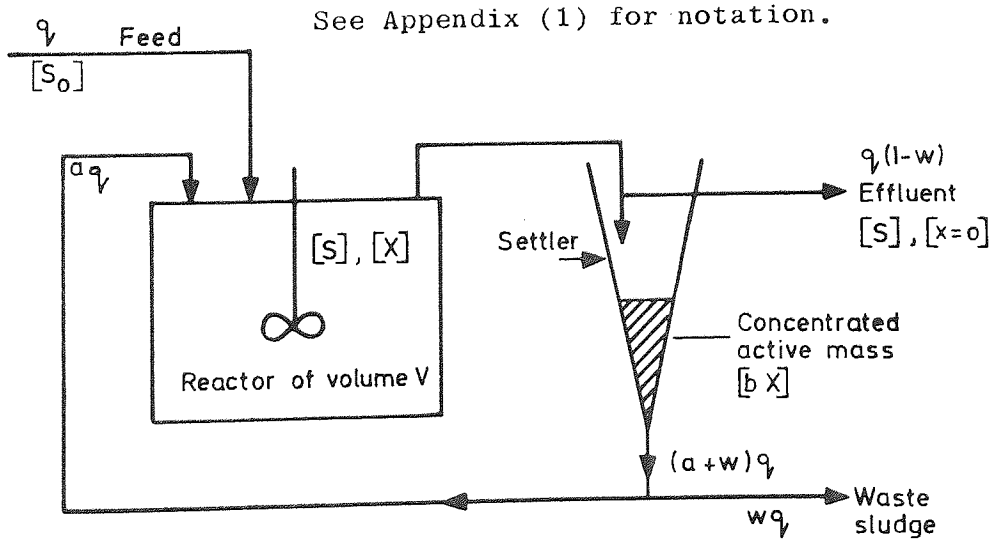


FIG. 3.1 FLOW DIAGRAM OF SINGLE-STAGE ACTIVATED-SLUDGE PLANT

Referring to Fig. 3.1, a material balance of the cells in the system gives:

$$\begin{bmatrix}\text{Nett}\\ \text{Accumulation}\\ \text{of}\\ \text{cells}\end{bmatrix} = \begin{bmatrix}\text{Increase}\\ \text{due to}\\ \text{recycled}\\ \text{flow}\end{bmatrix} + \begin{bmatrix}\text{Increase}\\ \text{due to}\\ \text{growth}\end{bmatrix} - \begin{bmatrix}\text{Decrease}\\ \text{due to}\\ \text{Endogenous}\\ \text{respiration}\end{bmatrix} - \begin{bmatrix}\text{Decrease}\\ \text{due to}\\ \text{flow from}\\ \text{reactor}\end{bmatrix}$$

which in terms designated (See Appendix 1) is:

$$V\ \frac{dX}{dt} = aq.bX + V\mu X - Vk_eX - q(1+a)\ X$$

Putting $q/V = D$, the dilution rate, re-arrangement gives

$$\frac{dX}{dt} = DabX + \mu X - k_eX - DX\ (1+a) \quad \ldots\ldots\ \text{Eq. 3.6}$$

Similarly, a material balance for the substrate gives:

$$\begin{bmatrix}\text{Nett}\\ \text{Decrease}\\ \text{of}\\ \text{substrate}\end{bmatrix} = \begin{bmatrix}\text{Increase}\\ \text{due to}\\ \text{influent}\\ \text{flow}\end{bmatrix} + \begin{bmatrix}\text{Increase}\\ \text{due to}\\ \text{recycled}\\ \text{flow}\end{bmatrix} - \begin{bmatrix}\text{Decrease}\\ \text{due to}\\ \text{cell}\\ \text{growth}\end{bmatrix} - \begin{bmatrix}\text{Decrease}\\ \text{due to}\\ \text{effluent}\\ \text{from}\\ \text{reactor}\end{bmatrix}$$

$$\text{or}\quad V.\ \frac{dS}{dt} = q.S_o + aq.S - V.\left(\frac{dS}{dt}\right)_{\text{growth}} - (1+a)\ qS$$

From equations 3.3 and 3.4,

$$\left(\frac{dS}{dt}\right)_{\text{growth}} = \frac{\mu X}{Y} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ \text{Eq. 3.7}$$

Substituting for $\left(\frac{dS}{dt}\right)_{\text{growth}}$, and putting $q/V = D$, we obtain:

$$\frac{dS}{dt} = D\ (S_o + aS) - \frac{\mu X}{Y} - DS(1+a) \quad \ldots\ldots\ \text{Eq. 3.8}$$

Equations 3.1, 3.6 and 3.8 now constitute the basis of the mathematical model to relate substrate loss, cell accumulation and time.

3.6 Simulation

Simulation is the solving of the equations which comprise the model, in order to predict the behaviour of the process. As mentioned in Section 3.1, present day simulation techniques permit the successful modelling of dynamic situations.

Prior to the advent of computers, efforts at mathematical modelling were frequently unsuccessful because the equations generated could not be used. However, the advent and availability of both computers, and of general purpose programs such as FORTRAN and ALGOL, has enabled much more useful work to be done in the field of mathematical modelling. In particular, the recent availability of continuous system modelling languages, relatively simple to use, has enabled the engineer to concentrate on model development rather than on computational details.

3.6.1 S.L.A.M. A Simulation Language for Analogue Modelling

Such a simulation language is SLAM (ICL 1972). This language may be regarded as being one level higher than general purpose languages such as FORTRAN, since SLAM statements simplify the programming of dynamic situations, and are then automatically translated into FORTRAN by the computer. Because the FORTRAN translation is available to the user, it is very easy to modify or augment a routine written in SLAM. Unlike most languages SLAM statements may be written, with a few exceptions, in any order, and are then automatically sorted into the corrected sequence by the computer. SLAM provides certain standard functions such as integrators, products, limiters, etc., which may be used in the mathematical model. These standard functions are augmented by FORTRAN statements such as Square root, Sine and Logarithms.

SLAM is particularly useful as a simulation language in that it offers a choice of several standard integration methods for the solution of non-linear differential equations. It also has the facility of function generation, which should prove useful for example, in observing the effects of shock loadings upon the activated sludge model.

The hardware used for the simulation was an I.C.L. 1905S computer, situated at the Corporate Engineering Laboratories

(C.E.L) of the B.S.C. in Battersea. SLAM was loaded on to the 1905S in early 1975, and macros to enable SLAM to be run from an inter-active terminal were developed. Two such macros are now available to users of SLAM at the C.E.L., see Appendix (2).

3.6.2 Test Simulation of the Model

The model was programmed using SLAM (see Section 3.6.1). Copies of the listings produced, and outputs obtained, are attached in Appendix (3).

3.6.3 Data

In order to de-bug the program, it was necessary to give values to the various constants, and to set the various initial conditions. This section outlines the values used, and the reasons for their choice.

A search of the literature yielded the following experimental values[102] for the kinetic constants for activated sludge treatment of a synthetic, industrial waste, containing both organic and inorganic constituents:

Y = 0.39 gm. M.L.V.S.S./gm. substrate removed

k_e = 0.007 h^{-1}

μ_{max} = 0.80 h^{-1}

K_s = 345 $gm.m^{-3}$

The remaining values to be found are the volume, flow-rate, recycle-ratio, and the wastage rate.

These were set, for trial purposes, at the following arbitrary,but realistic, values. The values given for aeration tank volume, and influent flow-rate are actual values, taken from the coke-oven effluent treatment plant, Appleby-Frodingham Steel works, Scunthorpe[29].

Volume of aeration tank	$V = 5000\ m^3$
Influent flow rate	$q = 227\ m^3/h$
Recycle ratio	$a = 0.35$
Wastage ratio	$w = 0.05$

It only remained then to set up the initial conditions. The program had been developed such that different values S_o, X_i, and S_i could be easily inputted, and their effects simulated.

S_o = influent substrate concentration $gm.m^{-3}$

X_i = cell concentration in reactor at time = 0

S_i = substrate concentration in reactor at time = 0

In a similar manner, the integration method, the duration of the simulation, and the iterative step could also very easily be changed from run to run, enabling different degrees of accuracy to be achieved.

The six initial conditions mentioned above were input into a separate data-file. Table 3.1 gives the values input to test the program. These values correspond to the simulation given in Appendix 3.

3.6.4 The Program

A listing of the program is given in Appendix 3. A listing of the SLAM translation of this program is given in Appendix 4.

The program is held under filename MIX 1

The program name is DYNAS 1

The initial values are held in data-file MIX DATA 1 and correspond to the values given in Table 3.1:

TABLE 3.1 MIXDATA 1 data-file

S_o	X_i	S_i	Iterative Step	Simulation Duration	Integration Method
$gm.m^{-3}$	$gm.m^{-3}$	$gm.m^{-3}$	h	h	
2000	100	1500	1	20	Simpsons Rule
2000	1000	1000	0.1	10	

From the initialisation data, it will be noticed that in the first run, each of the differential equations are to be integrated over a period of 20 hours, the iterative step being at 1 hour intervals. When this run is finished, the program will be

run again over a period of 10 hours, the integration interval this time being 0.1 hours.

Different values of X_i and S_i are input to the program for each run. The integration method used is SIMPSON's RULE, and the evaluation is done via SLAM programming.

As mentioned earlier, the facility is available for using more accurate integration methods, but until the model is tested against real data, this would be meaningless and wasteful in terms of computer time.

3.7 Simulation Experience

The simulation given in Appendix 3 illustrates the development of the model, at this stage. Much trouble had been experienced due to mathematical and programming errors, and to the "de-bugging" of SLAM on the I.C.L. 1905S. However, the exercise of simulating the model had resulted in enabling the operation of SLAM on the 1905S, and in teaching the author the basic programming techniques. Although it was fully realised that this model would not be representative of an activated-sludge plant treating coke-oven effluent, it was felt that the techniques mastered could now enable further development.

3.8 The Influence of Variable Growth Rates in a Heterogenous System

It is normal practice in modelling microbiological systems to relate the substrate removal to one parameter, usually BOD or TOC and to assume that the sludge properties can be modelled as though the sludge consists of one bacterial species only. This is certainly not a practical assumption in the case of coke-oven effluents and, to make the model more representative of actual conditions, the system is to be represented as two inter-related sub-systems. The two major groups of substrate present in coke-oven effluent are phenolics and the inorganics thiocyanate and thiosulphate. The nature of the micro-organisms which utilise each substrate group are very different. Monohydric phenols, together with some of the more easily biodregadable polyhydric phenols are oxidised by heterotrophic bacteria. These are micro-organisms which are unable to synthesise all of their carbon

compounds from carbon dioxide and require, in addition, an organic source of carbon in the environment.

Thiocyanate, together with some other inorganic constituents of the coke oven effluent such as cyanides and thiosulphates are normally oxidised by autotrophic bacteria. These are self-sustaining micro-organisms which have the ability to synthesise all of their carbon compounds from carbon dioxide. Normally the doubling time of heterotrophic bacteria is much shorter than that of the autotrophs.

The model of the coke oven effluent treatment system has therefore been modified to represent the bacterial population as being composed of two groups of micro-organisms, the heterotrophs and the autotrophs.

3.8.1 Theory

Let X_p represent the concentration of heterotrophic micro-organisms.

Let X_T represent the concentration of autotrophic micro-organisms.

For a given activated-sludge unit treating a coke oven liquor containing both phenols and thiocyanates, conditions of Steady-State will eventually be attained when the ratio:

$$\frac{X_p}{X_T} = \text{constant} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 3.9}$$

For any sudden change in either the dilution rate, D, or substrate influent concentration S_0, steady-state conditions will no longer apply, and the ratio $X_p:X_T$ will consequently change.

In order to simulate the effects of such a change, a model must be developed to account for the different metabolic activity of the heterotrophic and autotrophic bacteria.

Monod kinetics are to be used to model such activities. Equation 3.2 shows the Monod model relating bacterial growth and substrate utilisation for pure cultures [9 5].

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad \text{..... Eq. 3.2}$$

In order to allow for both heterotrophic and autotrophic micro-organisms, Eq. 3.2 may be developed to give:

$$\mu_p = \mu_{max_p} \frac{S_p}{K_{s_p} + S_p} \quad \text{..... Eq. 3.10}$$

and

$$\mu_T = \mu_{max_T} \frac{S_T}{K_{s_T} + S_T} \quad \text{..... Eq. 3.11}$$

where μ_p and μ_T are defined as the specific growth rates of heterotrophic and autotrophic bacteria respectively.

S_p and S_T are the phenol and thiocyanate substrate concentrations respectively.

K_{S_p} and K_{S_T} are the concentrations of phenols and thiocyanates at which the specific growth rate is half the maximum growth rate, μ_{max}.

3.8.2 Assumptions of the Model

It is assumed that for each model the concentration of phenols or thiocyanate constitutes the limiting substrate.

It is further assumed that as an organism grows, it utilises substrate at a constant rate such that

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \quad \text{..... Eq. 3.4}$$

where Y is the Yield Coefficient.

If Y_p and Y_T are the yield coefficients for the heterotrophic and autotrophic bacteria respectively, we can say that over a finite period of time:

$$Y_p = \frac{\text{weight of heterotrophic bacteria formed}}{\text{weight of organic substrate utilised}} \quad \text{.....Eq. 3.12}$$

$$Y_T = \frac{\text{weight of autotrophic bacteria formed}}{\text{weight of inorganic substrate utilised}} \quad \ldots\ldots \text{Eq. 3.13}$$

Equations 3.10 to 3.13 may now be incorporated into the model developed earlier, and the effects of different phenolic and thiocyanate concentrations in the influent to the plant may be simulated.

Equation 3.6, which describes the nett accumulation of bacteria in the system may be split for the two component sludge to separately describe the nett accumulation of autotrophic and heterotrophic bacteria.

i.e. $$\frac{dX_T}{dt} = DabX_T + \mu_T X_T - k_{e_T} X_T - DX_T(1+a) \qquad \text{Eq. 3.14}$$

$$\frac{dX_P}{dt} = DabX_P + \mu_P X_P - ke_P X_P - DX_P(1+a) \qquad \text{Eq. 3.15}$$

Similarly, equation 3.8 may be split into two equations describing the nett decrease of thiocyanate and phenol;

$$\frac{dS_T}{dt} = D(S_{o_T} + aS_T) - \frac{\mu_T X_T}{Y_T} - DS_T(1+a) \qquad \text{Eq. 3.16}$$

$$\frac{dS_P}{dt} = D(S_{o_P} + aS_P) - \frac{\mu_P X_P}{Y_P} - DS_P(1+a) \qquad \text{Eq. 3.17}$$

However, before simulation of this model, it was decided to incorporate an inhibition factor into the model, details of which are given below.

3.9 Inhibition of Autotrophic Metabolism by High Phenolic Concentration

In Section 2.5.4 of the Literature Survey (see pp 35-37), details have been given of some of the work that has been undertaken into phenolic inhibition of thiocyanate degradation. No conclusions can be drawn from these findings, as different workers have found thiocyanate inhibition at phenolic concentrations ranging from 10-1000 $gm.m^3$. Also, there have been several

different theories postulated for the mechanism of the inhibitive process.

Further work in this field seems to be necessary in order to determine a model of the inhibitive process. However, at this stage of the project, it was decided that a simple inhibition model should be incorporated into the model of the activated sludge system in order to make some allowance for inhibition and so gain further programming experience. Such an inhibition model could be changed, as necessary, when the relevant experimental information became available.

The only experimental work being undertaken by the CEL on coke-oven effluent treatment at this stage of the project was on the effect that increased DO levels had upon process efficiency[63]. Two continuous, laboratory-scale, completely-mixed activated sludge units treating an actual works effluent were being used for this study. Observation of the discharge composition from these units indicated that in a completely-mixed process, very small increases of total phenol concentration (5-10 $gm.m^{-3}$) in the treated effluent were often associated with much larger increases (100-200 $gm.m^{-3}$) of thiocyanate in the discharge.

These observations were made over a long period of time, of the order of twelve months, but due to pressure of other work it was impossible to gain enough analytical data to attempt to determine phenolic levels that would be associated with different degrees of thiocyanate inhibition.

However, it did seem that inhibition of thiocyanate oxidation invariably began as soon as phenol levels in the treated effluent rose above normal, steady-state, base level. Complete inhibition of thiocyanate, i.e. where the thiocyanate concentration in the treated effluent was the same as that in the influent was observed at various phenol levels between 30 and 80 $gm.m^{-3}$.

It must be emphasised that the information given above is not considered to be adequate data for the basis of an inhibition model. Such information should be obtained from a plant operating at steady-state, when a small upset (caused by a change in organic

or hydraulic loading) is known to be responsible for changes in effluent composition. The two units mentioned were rarely at steady-state, the phenol concentrations of influent and effluent were monitored only occasionally, mechanical breakdowns were usually responsible for the upsets, and most importantly the research program for the two units was centred on the effect of different DO levels, not on inhibition effects.

However, in the absence of reliable historical data, it was decided to incorporate a simple inhibitive model based on the assumption that inhibition is zero at base level phenol concentrations, and that the inhibitive effect is total at phenol concentrations of 50 $gm.m^{-3}$ above base level in the mixed liquor.

For simplicity, it was decided to linearise this model, and the inhibitive effect was therefore described by:

$$I.F. = \frac{S_p}{50.0} \quad \text{for} \quad S_p < 50.0 \quad \ldots\ldots\ldots\ldots \quad \text{Eq. 3.18}$$

and

$$I.F. = 1.0 \quad \text{for} \quad S_p > 50.0 \quad \ldots\ldots\ldots\ldots \quad \text{Eq. 3.19}$$

where I.F. is an Inhibition Factor.

The above inhibition model was then incorporated into the growth rate model for autotrophic bacteria (equation 3.11).

Thus

$$\mu_T = \mu max_T \frac{S_T}{K_{S_T} + S_T} - I.F. \left(\mu max_T \frac{S_T}{K_{S_T} + S_T}\right)$$

or

$$\mu_T = \mu max_T \frac{S_T}{K_{S_T} + S_T} (1-I.F.) \quad \ldots\ldots\ldots\ldots \quad \text{Eq. 3.20}$$

At this stage, a dynamic model of a continuous, completely mixed, single stage activated sludge plant with allowance for variable growth rates and inhibition caused by a two-component system had been developed.

This model comprises equations 3.10 and 3.12 to 3.20.

3.10 Simulation of the Two-State Inhibition Model

The model was programmed using SLAM (see section 3.6.1). Copies of the listings produced, and outputs obtained, are attached in Appendix (5).

3.10.1 Steady-State Values of Substrate and Cell Concentrations

In order to have a base from which the effects of transient loadings on the activated sludge system can be observed, the steady-state values of both substrate concentrations and cellular concentrations must first be determined.

The equations for these are developed below.

At the steady-state, the substrate and active-mass concentrations will have attained a constant value, and therefore

$$\left(\frac{dX}{dt}\right)_{P,T} = 0$$

and

$$\left(\frac{dS}{dt}\right)_{P,T} = 0$$

Re-arrangement of equation 3.14 then gives

$$\mu_T \ (S/S) = k_{e_T} + D(1+a) - Dab \ \ldots\ldots\ldots \text{Eq. } 3.21$$

This equation confirms mathematically that the growth rate of the autotrophic bacteria will be constant at the steady state.

Similarly, from equation 3.15,

$$\mu_P \ (S/S) = k_{e_P} + D(1+a) - Dab \ \ldots\ldots\ldots \text{Eq. } 3.22$$

From equations 3.10 and 3.20 we can now determine the steady-state values of residual substrate concentration,

i.e. $$S_P \ (S/S) = \frac{\mu_P \ K_{s_P}}{\mu_{max_P} - \mu_P} \ \ldots\ldots\ldots\ldots\ldots \text{Eq. } 3.23$$

and

$$S_T \ (S/S) = \frac{\mu_T \ K_{s_T}}{(1-I.F.)(\mu_{max_T} - \mu_T)} \ \ldots\ldots\ldots \text{Eq. } 3.24$$

The steady-state values of bacterial concentrations are determined from equations 3.16 and 3.17

Thus:

$$X_p \quad (S/S) = D(S_{o_p} - S_p) \frac{Y_p}{\mu_p} \quad \text{..... Eq. 3.25}$$

$$X_T \quad (S/S) = D(S_{o_T} - S_T) \frac{Y_T}{\mu_T} \quad \text{..... Eq. 3.26}$$

3.10.2 Data for Simulation

In order to de-bug the program it was necessary to give values to the various constants and set the initial conditions as explained in Section 3.6.3.

By the time the model had been developed to this stage, laboratory experiments on the heterotrophic sludge had yielded some values for the kinetic coefficients. These values were obtained by the curve-fitting technique, which is later explained in Section 4.11.

These values were thus input to the model, and in terms denoted in Appendix 1 are:

$\mu_{max_p} = 0.134\ h^{-1}$

$k_{e_p} = 0.09\ h^{-1}$

$K_{s_p} = 10\ gm.m^{-3}$

$Y_p = 1.8$ gm.MLSS per gm. substrate.

Unfortunately, no information was available on the kinetics of the autotrophic bacteria. However, observation of the two continuous laboratory units treating coke-oven effluents (see Section 3.9) had shown that when a plant upset caused an increase in phenol and thiocyanate levels in the discharge, phenol treatment would normally recover within a couple of days, whilst thiocyanate treatment would often take some weeks to recover.

Such a process indicates that the growth rate of autotrophic bacteria is much less than that of the heterotrophs.

The following arbitrary values for the autotrophic coefficients were therefore used to test the program

μ_{max_T} = 0.05 h^{-1}

k_{e_T} = 0.0023 h^{-1}

K_{s_T} = 10 $gm.m^{-3}$

Y_T = 0.4 gm. MLSS per gm. substrate

The same values of volume, flow-rate, recycle ratio and wastage ratio as used earlier (Section 3.6.3) were again taken, whilst the initial conditions were set at

S_{o_T} = influent thiocyanate concentration = 500 $gm.m^{-3}$

S_{o_P} = influent phenol concentration = 1000 $gm.m^{-3}$

The steady state values of S_T, S_P, X_T and S_T were then determined from equations 3.23 to 3.26, and these values were inputted to the model via a separate data-file.

3.10.3 Effect of Shock Loading

One further, major change was made to the program before simulation. When validated, the model will be used to observe the effects that increases in influent loading will have upon the discharge effluent concentrations.

An increase in phenol organic loading was therefore programmed into the model in order to observe the effect that this would have upon the discharge. As mentioned in Section 3.6.1 SLAM makes available to the user several standard functions by which effects such as shock loading may be simulated. One such function is SRAMP which is illustrated below.

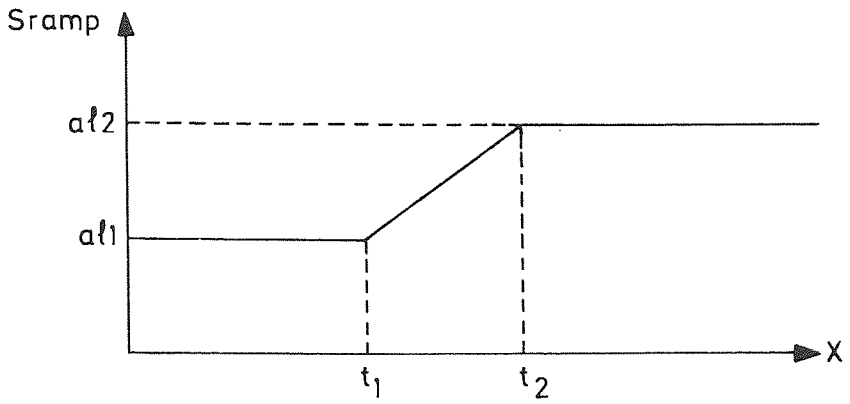
The form of the SRAMP function is:

SRAMP (x, t_1, t_2, a11, a12)

where:

x, t_1, t_2, a11, a12 are arithmetic expressions of type REAL.

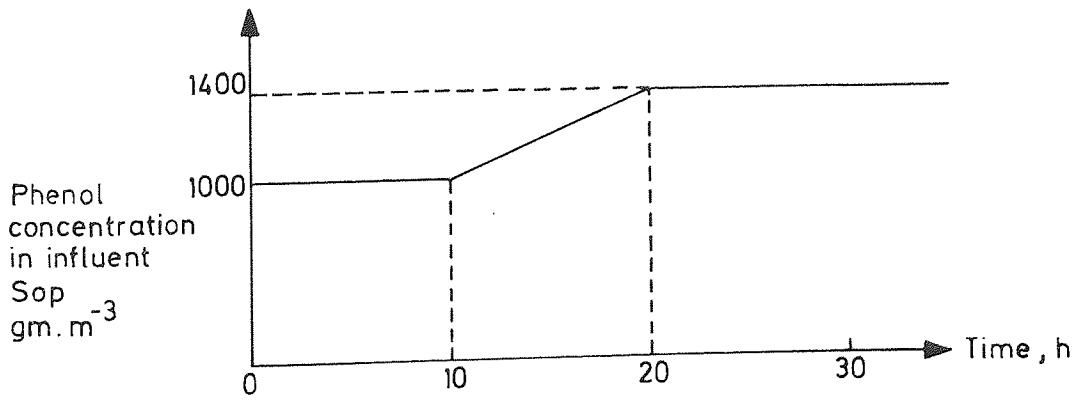
The value of the function depends on the values of the above arithmetic expressions as illustrated in the following diagram.



By using this function, a steady increase in phenol concentration in the influent between time t_1 and t_2 is easily programmed.

For the purposes of testing the model, the initial phenol concentration in the influent was set at 1000 $gm.m^{-3}$, this to remain steady until 10 hours after initialisation. During the next ten hours, the phenol concentration is to rise at a linear rate until it attains a value of 1400 $gm.m^{-3}$, after which time it is to remain steady at this value.

This is illustrated below, i.e.:



The SLAM function to describe this effect is then given as

So_P = SRAMP (TIME, 10.0, 20.0, 1000.0, 1400.0)

3.10.4 Results

A listing of the above program is given in Appendix 5.

The program is held under filename SOP7

The program name is BACTIN

The initialisation and data values are held in data-file SOP7DATA.

Figure 3.2 shows graphically the results output by the model. Variation of the coefficients of both heterotrophic and autotrophic bacteria was found to alter the output as would be expected, but the general shape of the discharge curves (given by S_P and S_T) remained the same.

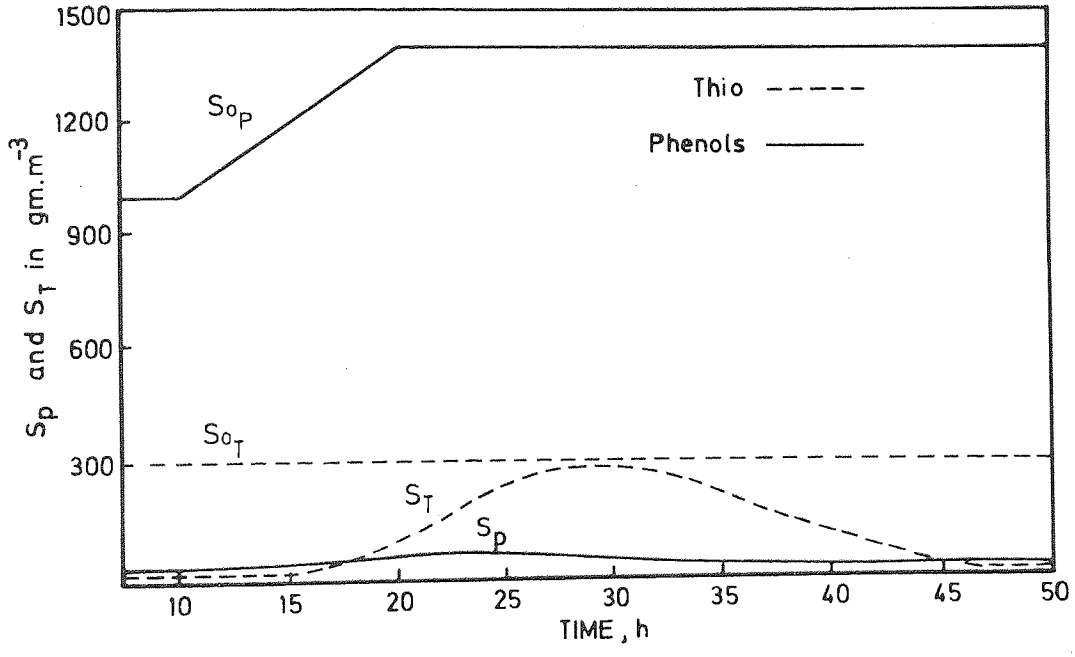


FIG. 3.2 A SIMULATION SHOWING THE EFFECTS OF A GRADUAL INCREASE IN PHENOL INFLUENT CONCENTRATION

This shape is caused by the inhibitive effect programmed into the model. As residual phenol concentration in the discharge (S_p) increases due to the increased organic loading (So_p), the growth rate of the autotrophic bacteria decreases due to inhibition.

Residual thiocyanate concentration (S_T) therefore increases at a high rate, until the residual phenol has fallen back to a minimal value. The autotrophic bacteria then begin to

grow faster as the inhibitive effect decreases, and eventually, residual thiocyanate re-attains a low steady-state value.

3.10.5 Discussion

This type of effect is similar to the effects that have been observed in practice. However, before the model may be used to investigate control strategies for works plant, the real values of the kinetic coefficients of the various bacteria must be determined.

CHAPTER 4. DETERMINATION OF KINETIC COEFFICIENTS USING THE CONCENTRATION OF SUSPENDED SOLIDS AS A MEASURE OF ACTIVE BIOMASS

4.1 Introduction

Before the model developed in Chapter 3 could be tested and used for design or control purposes, it was necessary to determine numerical values for the various sludge coefficients for both the heterotrophic and autotrophic bacteria. To re-cap, the coefficients under investigation were:-

- Y - the yield factor
- k_e - the basal metabolism rate
- μ_{max} - the maximum growth rate
- K_s - the saturation constant

Some of the methods that were available for determining the values of these parameters are discussed below:-

4.1.1 Curve-fitting techniques

If sufficient data is obtainable either from a laboratory activated-sludge unit, or from an actual works, curve-fitting techniques may be used to determine those values of the kinetic coefficients that best simulate the real data. Such an exercise may be undertaken either in the batch mode or in the continuous mode.

However, this technique has two major drawbacks. Firstly, it is likely that there will be several sets of values which will fit a particular set of data. For example, the rate of increase of active mass is dictated mainly by the difference between the growth rate and the death rate. For different data-sets, the determined values of μ_{max} and k_e may be very different, but if the difference between the two is similar, the data-sets may still be adequately fitted. This problem may be surmounted by determining at least one of the constants from other experiments before making any attempt at curve fitting.

The second major drawback to this technique is that too much information is required, information that is not readily available. For a pure culture, this method may be viable, but in

the activated sludge treatment of coke-oven liquors the sludge comprises many species of bacteria, each with different kinetics. The model developed to date has assumed only two genera of micro-organisms and that the species which make up each of these genera may be described by one set of kinetic values. Even with only two sets of values to be determined, accurate information on the numbers of autotrophic and heterotrophic cells would be required in order to fit curves to a data-set from a heterogenous system. To gain this information, microbiological plating techniques would have to be used. This is a difficult and time-consuming process, and neither the facilities for such work, nor the time required was available.

4.1.2 Graphical techniques

Graphical techniques for the determination of the kinetic coefficients of a sludge may also be used either in the batch or continuous mode.

The principle behind each of these methods is given below:-

(a) Continuous system

Using a continuous flow, laboratory activated sludge unit without recycle, coefficients for a homogenous system may be determined by graphical analysis of the Monod equations. Reynolds and Yang[102] used this technique to determine the kinetics of a sludge oxidising a synthetic industrial waste. They developed two equations for the above system operating at steady state.

$$\frac{S_o - S}{X} = \frac{k_e}{Y} \cdot \theta + \frac{1}{Y} \qquad \text{..... Eq. 4.1}$$

and $$\frac{\theta}{1 + k_e\theta} = \frac{K_s}{\mu_{max} S} + \frac{1}{\mu_{max}} \qquad \text{..... Eq. 4.2}$$

where $$\theta = \frac{1}{\mu - k_e} = \text{steady state detention time} \qquad \text{..... Eq. 4.3}$$

The laboratory model was operated at decreasing

detention times over a period of 1½ months, and the MLSS and MLVSS concentrations and COD concentration in the effluent were determined.

A plot of $(S_o - S)/X$ against θ then gave an intercept of $1/Y$ and gradient k_e/Y.

Using these values of Y and k_e, a second plot of $\theta/(1 + k_e\theta)$ against $1/S$ enabled determination of μ_{max} and k_s.

Although this method appears to give the absolute values of the coefficients, it is again only valid for a pure culture, or for determining the overall kinetics of a particular sludge. Without knowledge of the numbers of autotrophic and heterotrophic cells, this technique could not be used to determine the necessary parameters for the model previously developed.

(b) Batch system

In a batch culture, Equation 3.6 reduces to

$$\frac{dX}{dt} = \mu X - k_e X \qquad \text{..... Eq. 4.4}$$

The rate of removal of substrate in such a system is given by Equation 3.7

$$\frac{dS}{dt} = \mu \frac{X}{Y} \qquad \text{..... Eq. 3.7}$$

Removing the growth rate, μ, from Equations 4.4 and 3.7 we have that

$$\frac{dX}{dt} = Y\frac{dS}{dt} - k_e X \qquad \text{..... Eq. 4.5}$$

This equation may be linearised by dividing by X

$$\text{Thus} \quad \frac{\Delta X}{\Delta t . X_{av}} = Y\frac{\Delta S}{\Delta t . X_{av}} - k_e \qquad \text{..... Eq. 4.6}$$

where X_{av} is the average sludge concentration over the time period Δt. Obviously, Equation 4.6 is only

an approximation of Equation 4.5, but the shorter that Δt is made, the more accurate the equation will become.

Reynolds and Yang[102] used this equation to determine Y and k_e and found that a time period of one day gave sufficiently accurate results.

Y is determined from the slope of the straight line obtained by plotting $\Delta X/(\Delta t.X_{av})$ against $\Delta S/(\Delta t.X_{av})$, and k_e is given by the intercept.

This method of analysis can be expanded to determine μ_{max} and K_s by approximating equation 3.7 over a small period of time, and finding average values of the specific growth rate;

$$\text{i.e.} \quad \mu_{av} = \frac{Y}{X_{av}} \cdot \frac{\Delta S}{\Delta t} \qquad \text{..... Eq. 4.7}$$

Using the average specific growth rate over time Δt, K_s and μ_{max} may then be estimated graphically from the Monod expression for growth rate, equation 3.1

Again however, this method is applicable only for pure cultures, or for the determination of the overall kinetics of a particular heterogenous sludge.

4.2 Choice of Experimental Technique

Because of the lack of historical data from coke-oven effluent activated-sludge plants, it was deemed necessary to observe the kinetics of such a system in the laboratory.

As stated earlier in section 4.1.1, neither the facilities nor the time was available to enumerate individual cell populations by microbiological plating techniques. As this is the only reliable way of distinguishing between heterotrophic and autotrophic populations in the heterogenous system, it was therefore decided to sub-culture the two genera of bacteria separately, in order that their individual kinetics might be evaluated. The two cultures developed were therefore:-

(a) A heterotrophic culture - this was obtained by taking some of the mixed culture from a pilot-scale continuous treatment plant (see section 3.9) and selectively developing the heterotrophs by providing phenol as the only substrate.

(b) An autotrophic culture - this was obtained in a similar fashion, using potassium thiocyanate as the substrate.

A detailed explanation of how the two cultures were grown is given in sections 4.3.2 and 4.7.

It was initially decided to evaluate the coefficients of each of these two cultures by graphical means, as it was thought that this method would more easily determine the absolute values of the kinetic coefficients, rather than relative values as explained in section 4.1.1.

Tests were then instigated in the laboratory on each of these two cultures to determine their separate kinetic coefficients. Such tests were carried out in both the batch and continuous modes, but physical problems of sludge blockage soon established that batch tests were to be preferred.

No results are in fact available for those tests undertaken on continuous units without recycle. Every time such a test was instigated, it was found that the overflow from the reactor would gradually become blocked with sludge, and this blockage would tend to act as a filter, preventing sludge removal from the reactor. Such a state of affairs obviously prevented attainment of steady-state conditions. Various modifications to the reactor overflow were tried - these are illustrated in Figure 4.1 - but none solved the problem completely.

Added to this the fact that the available peristatic pumps were often needed for other work, it was decided to concentrate solely on determination of the coefficients by batch processes.

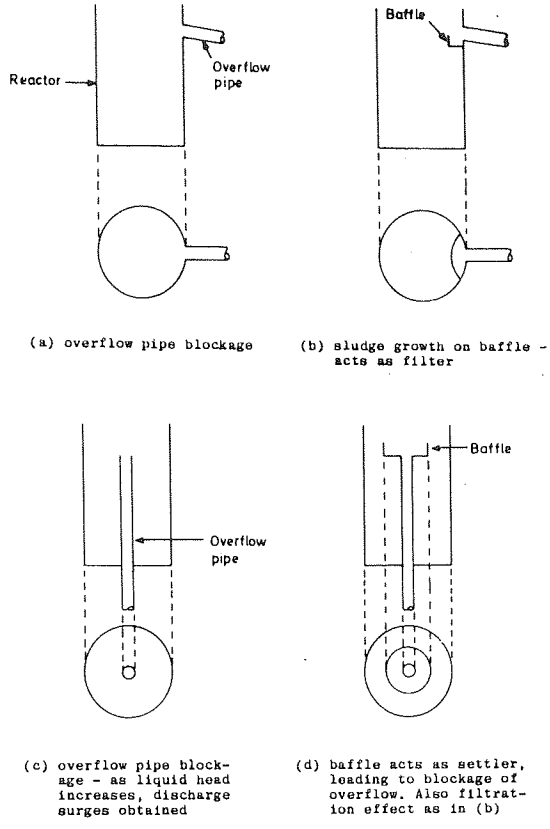


FIG. 4.1 CONTINUOUS LABORATORY REACTOR - OVERFLOW MODIFICATIONS

4.3 Experimental Methods

When experimental work first began, Pyrex beakers of 3 litre capacity were used to grow, and subsequently test, the cultures. Each beaker was fitted with two glass air-diffusers of "O" porosity connected to a compressed-air line pressurised at 70 p.s.i. Valves fitted to each air diffuser enabled regulation of the aeration pressure. The compressed air supply to the beakers was responsible for both aeration of the Mixed Liquor, and for its mixing pattern.

Each beaker was fitted with a small (40 watt) aquarium heater and thermostat. At this stage, no pH control was established. A strip of $\frac{1}{4}$ in perspex held the aerators, heater and thermostat in place. The above apparatus is illustrated in Figure 4.2.

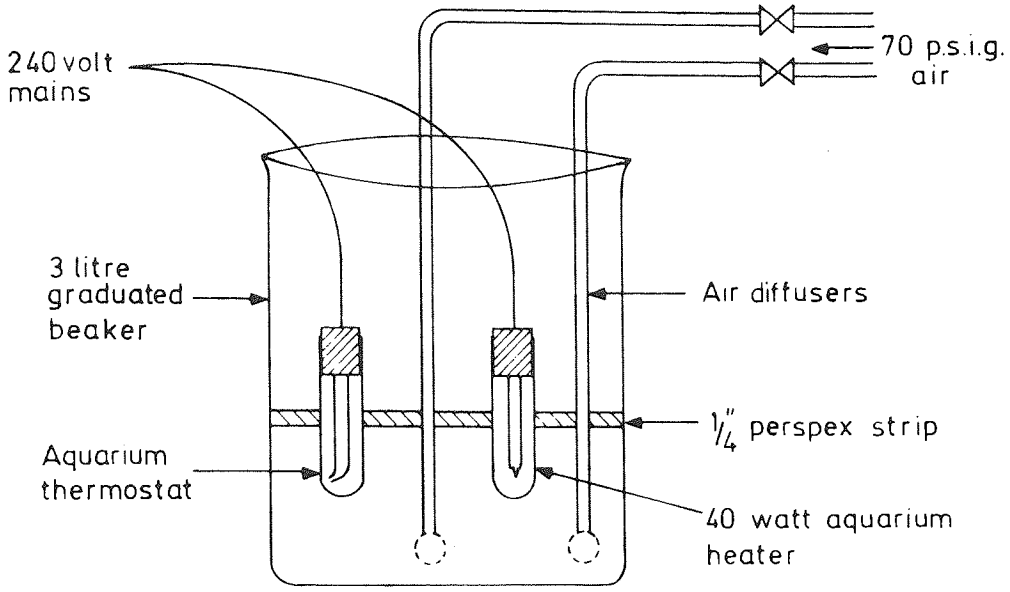


FIG. 4.2 INITIAL APPARATUS USED FOR BATCH CULTURE

4.3.1 Culture media

The medium used in developing each of the cultures is of prime importance as the species of micro-organisms that are developed will depend mainly upon the environment to which they must become accustomed.

Since the aim of the model is to represent the dynamics of actual works plant, it is necessary that the micro-organisms grown in the laboratory should, as far as possible, be cultured in the same way as those grown in full-scale plant.

Thus, great emphasis was placed on the culture media for both heterotrophic and autotrophic sludges.

Fresh coke-oven effluent from the Orgreave Coking Works was treated in two continuous pilot activated-sludge units (see Section 3.9) to remove mono-phenols and some of the more easily biodegradable poly-hydric phenols, together with thiocyanate and other degradable inorganics. Table 4.1 shows a typical analysis of the influent and effluent to this plant.

TABLE 4.1 INFLUENT AND EFFLUENT ANALYSES: LABORATORY-SCALE CONTINUOUS PLANT

Constituent	Influent Conc. $gm.m^{-3}$	Effluent Concentration in $gm.m^{-3}$	
		Unit "A"	Unit "B"
Total phenols	535.0	23.5	26.2
Mono phenols	425.0	3.35	3.4
Thiocyanate	327.4	5.0	4.8
Ammonia	348.0	424.0	428.0
Cyanide	2.3	0.42	0.52
P.V.	1249.5	97.0	95.0
Thiosulphate	47.1	15.7	16.8

As Table 4.1 shows, there is a substantial residual poly-hydric phenol concentration in the treated effluent. This is due to non-biodegradable phenolic substances which many authors consider may be inhibitive to thiocyanate oxidation (see Section 2.5.4). There is also a high concentration of ammonia in the treated effluent, which is also considered to be toxic to thiocyanate oxidation at high concentrations. Stafford et al.[83] found ammonia to be inhibitory to Pseudomonas stutzeri at concentrations of 72 $gm.m^{-3}$. Since the ammonia concentration in the untreated effluent from Orgreave was approximately 350 $gm.m^{-3}$ in the batch analysed in Table 4.1, it would appear very unlikely that organisms such as P. stutzeri would be grown either in the full-scale plant or in the laboratory units. More likely, autotrophs to which ammonia is not so toxic would be developed in both units.

For the reasons given above, the treated effluent from the continuous laboratory units was collected and used as the base-liquor on which the autotrophic and heterotrophic cultures were separately established. The inhibitory effect of some, if not all of the non-biodegradable constituents of a coke-oven effluent would then be incorporated into the kinetic coefficients, and the "correct" micro-organisms would be selected.

Phosphate is a necessary nutrient for bacterial growth, and since this is added "on-site", it was also added to the two separate cultures in the form of sodium hexametaphosphate, at

concentrations of approximately 10 $gm.m^{-3}$ as PO_4. Happold et al[86] found that a minimum concentration of phosphate of 0.4 $gm.m^{-3}$ was necessary for bacterial growth, whilst a concentration of 950 $gm.m^{-3}$ reduced growth.

Trace metals have often been reported to be necessary for growth[86,116,117,118]. In view of the nature and origin of coke-oven liquors, there should be sufficient concentrations of necessary trace elements already in the treated effluent. However, as a safeguard, a trace metals solution as used by Hiam[84] was made up, and added to the treated effluent in the ratio of 10 mls trace metals solution to each litre of effluent.

The temperature was also held constant at a value similar to that found "on-site". The temperature of the mixed-liquor in a works activated-sludge plant varies throughout the year according to the season, but normally lies within the range 10 °C to 30 °C. The temperature of the laboratory cultures was thus held constant at 20 °C ± 0.5 °C.

4.3.2 Establishment of a Phenol-tolerant sludge

Using the apparatus, methods and medium described above, attempts were made to establish a heterotrophic culture which would degrade phenols.

Phenol was added to a beaker containing two litres of the base medium, and a seed of mixed-sludge from the continuous laboratory units. The concentration of phenol initially added was low, of the order of 20 $gm.m^{-3}$, but as the sludge was able to degrade this amount of phenol very quickly (less than ½ hour), the phenol concentration in the mixed-liquor was increased to about 100 $gm.m^{-3}$. As soon as the sludge had completely degraded this amount of substrate, more phenol was added to bring the concentration back to 100 $gm.m^{-3}$.

Each day, 10% of the mixed liquor was wasted. This had the effect of removing the non-phenol degrading bacteria and of lessening the possible accumulation of toxic substances produced during the biodegradation of the substrate.

The contents of the beaker were allowed to settle twice a week, and the supernatent liquor withdrawn and discarded. Fresh treated coke-oven effluent, with phosphate and trace-metals solution added, was then mixed with the sludge, and aeration recommenced.

As the sludge volume increased, and the time for substrate degradation decreased, the initial concentration of phenol in the mixed liquor was stepped-up until a value of 1200 $gm.m^{-3}$ was attained. By this time, an appreciable amount of phenol-degrading sludge had been developed, and tests upon the characteristics of this sludge could be instigated.

Attempts were made to culture a thiocyanate degrading sludge in a similar way using potassium thiocyanate as substrate. Two problems immediately became apparent. Firstly, oxidation of thiocyanate caused an appreciable pH drop. For example, during the batch oxidation of 100 $gm.m^{-3}$ of thiocyanate, the pH fell from 7.4 to 5.2. Occasionally, the pH of the mixed liquor at the end of the batch was even lower than 5.0 - values of 4.4 and 4.6 were recorded. Happold et al[87] found that the optimum pH for thiocyanate degradation by T. thiocyanoxidans was 7.0 to 7.2, and that pH values substantially different to neutrality would be detrimental to bacterial activity. Symons et al[119] also reported that pH should be near neutral if bacterial activity is to proceed unimpeded. George and Gaudy[120] found that heterogenous cultures could become acclimatised to a substantial increase or decrease in pH, but that the nature of the biological solids would change from a predominantly bacterial-protozoan population to one consisting predominantly of filamentous fungi. The settlability of such a sludge was found to be very poor.

It was the settlability of the culture that caused the second problem. Although the thiocyanate was being degraded at reduced pH levels, it became virtually impossible to settle the sludge so that the supernatent liquor could be decanted. It is imperative that the supernatent is discarded often - at least 3-4 times per week. This is because ammonia concentration would otherwise accumulate in the mixed-liquor, caused by the complete degradation of thiocyanate according to equations 4.8 and 4.9:

$$CNS^- + H_2O \xrightarrow{\text{hydrolysis}} HCNO + SH^- \quad \text{..... Eq. 4.8}$$

$$HCNO + H_2O \xrightarrow{\text{hydrolysis}} CO_2 + NH_3 \quad \text{..... Eq. 4.9}$$

Table 4.1 shows this accumulation of ammonia in the continuous reactors, when the ammoniacal concentration has increased from 348 $gm.m^{-3}$ in the influent to 428 $gm.m^{-3}$ in the effluent.

Furthermore, if tests had continued at this stage to determine the coefficients of the thiocyanate degrading sludge, the coefficients would not have been applicable to a works continuous system as, in the works, the buffering ability of the plant is such that pH is normally near neutral. Observed kinetics of filamentous fungi at pH 5.0 would obviously be different to those of a bacterial population operating at pH 7.0.

It was obvious that some form of pH control was necessary for the thiocyanate tolerant cultures. Batch addition of dilute sodium hydroxide was tried over a period of several weeks, but this was found insufficient to overcome the very rapid pH changes observed. It was then decided that continuous pH controllers should be used to keep the cultures neutral, and work therefore had to cease on the kinetics of thiocyanate - tolerant bacteria whilst the necessary equipment was ordered and installed.

4.4 Initial Attempts at Establishing the Kinetics of a Heterotrophic System

Using several batch reactors, as illustrated by Figure 4.2, experiments were instigated to determine the kinetics of the heterotrophic culture developed by the method outlined in Section 4.3.2.

The Mixed Liquor Suspended Solids concentration (MLSS) was taken to represent the concentration of active cells in these primary tests.

The theory of the method is outlined in Section 4.1.2,

and the relevant equations are:

$$\frac{\Delta X}{\Delta t . X_{av}} = \frac{Y . \Delta S}{\Delta t . X_{av}} - k_e \qquad \text{..... Eq. 4.6}$$

$$\mu_{av} = \frac{Y}{X_{av}} \cdot \frac{\Delta S}{\Delta t} \qquad \text{..... Eq. 4.7}$$

4.5 Determination of Y and k_e

A total of seven batch cultures were investigated in two preliminary runs. Different ratios of substrate concentration (as phenol) to sludge (as Mixed Liquor Suspendid Solids - MLSS) were used in each beaker. The beakers were then aerated for a predetermined time period. The temperature during the reaction was held constant at 20 ^{0}C. Samples, each of 40 millilitres were periodically removed from the mixed liquor. Each of these samples was then analysed for residual phenol concentration and for MLSS concentration. Tables 4.2 and 4.3 give the results of these analyses.

4.5.1 Analytical Methods

As the samples were not analysed until several hours after sampling, one drop of saturated mercuric chloride was added to each sample in order to stop further phenol degradation. Mercuric chloride has the effect of "killing" the micro-organisms, thus freezing residual phenol concentration and bacterial population levels at the moment of sampling.

MLSS

Because of the large numbers of samples involved, it was decided to determine the MLSS concentration by centrifuging. It is fully realised that this method is not as accurate as could be desired, but it was felt that the ease of the method, and rapidity of results outweighed any slight loss of accuracy over the filtration method.

Pyrex centrifuge tubes of 50 ml nominal capacity were dried at 105 ^{0}C, cooled in a dessicator, and then weighed. Each sample was then centrifuged in one of these pre-weighed tubes for 20 minutes at 3000 rpm. The supernatent liquor was decanted to

be analysed for residual phenol content, whilst the sediment was washed twice with distilled water, recentrifuging after each wash. Finally, the tube and washed sediment was dried overnight in an oven at 105 ^{0}C, allowed to cool in a dessicator, and then re-weighed. The MLSS concentration was then determined according to the following equation:

$$MLSS = \frac{(\text{Wt. tube + dried solids - Wt. tube alone}) \times 10^{6} gm.m^{-3}}{40}$$

Mono-Phenols

The residual mono-phenol content of each sample was determined as per standard methods[121].

4.5.2 Results

TABLE 4.2 PHENOL DECAY-RUN-P1- AERATION PERIOD 5 HOURS

	Beaker 1		Beaker 2		Beaker 3	
Time Hours	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$
0	7420	785	*	*	7675	1080
1/3	7470	730	8040	575	7662	965
1	7410	705	7910	378	7693	900
2	7670	665	8970	31	7920	620
3	7785	465	8440	11.5	8228	136
4	*	*	*	*	*	*
5	8050	83	*	*	*	*

TABLE 4.3 PHENOL DECAY-RUN-P2- AERATION PERIOD 24 HOURS

	Beaker 1		Beaker 2		Beaker 3		Beaker 4	
Time Hours	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	Phenol $gm.m^{-3}$
0	2342	1095	915	1090	1002	920	1462	705
½	2470	1120	1107	1195	1030	905	1205	695
¾	2500	1100	1432	1195	1150	950	1462	710
1½	2480	1060	1725	1175	1277	950	1470	750
2½	2468	1000	1885	1130	*	975	1490	600
4½	3182	785	1987	1040	1655	760	1795	288
6	3200	334	2387	760	1790	540	2102	95
24	3982	23.7	2535	15.2	2222	24.9	2340	17

*Denotes centrifuge tube breakage

Because of the many tube breakages in the first Run, and the subsequent loss of analyses, the results cannot be compared over the five hour time period. The maximum time period over which these results are comparable is that between the 1/3 hour and 3 hour analyses. This time period of 2 2/3 hours is really too short a time to give realistic results, as only four samples from each beaker were taken, and any analytical inaccuracies will thus have a magnified effect.

The problem of centrifuge-tube breakage worsened as the tubes became older. Heat Treatment tests on polypropylene centrifuge tubes showed these tubes to be reasonably inert at temperatures of about 110 ^{0}C, once they had achieved a steady-state weight.

It was therefore decided to use polypropylene tubes for the determination of the suspended solids content of the sample instead of the pyrex ones previously used.

The results of the heat-treatment tests are given in Table 4.4. Two tubes were selected at random from a sample of 24 for the tests.

TABLE 4.4 HEAT TREATMENT TESTS ON 50 ml POLYPROPYLENE CENTRIFUGE TUBES

Test	Time Hours	Weight in Grams	
		Tube A	Tube B
New tube - unheated	0	12.1633	12.1971
Heat at 110 ^{0}C, cool in dessicator	1	12.1583	-
Heat at 110 ^{0}C, cool in dessicator	2	-	12.1920
Heat at 110 ^{0}C, cool in dessicator	4	12.1479	-
Wash, reheat, cool in dessicator	18	12.1412	12.1711
Heat at 110 ^{0}C, cool in dessicator	48	12.1313	12.1652
No heat, leave in open air	96	12.1313	12.1651
Heat at 110 ^{0}C, cool in dessicator	136	12.1310	12.1649

The reduced results for Runs P1 and P2 are shown in Table 4.5. These are the results from which the linear graph of equation 4.6 will be drawn.

TABLE 4.5 REDUCED RESULTS - INITIAL PHENOL DECAY CURVES

ALL CONCENTRATIONS IN $gm.m^{-3}$

Beaker	X init.	X fin.	ΔX	X av.	S init.	S fin.	ΔS	Δt hrs.	$\frac{\Delta S}{\Delta t.X_{av.}}$	$\frac{\Delta X}{\Delta t.X_{av.}}$
Run P1										
1	7470	7785	315	7584	730	465	265	2.67	.013	.016
2	8040	8440	400	8340	575	11.5	563.5	2.67	.025	.018
3	7662	8278	616	7888	965	136	829	2.67	.039	.029
Run P2										
1	2342	3982	1640	2820	1095	23.7	1071.3	24	.016	.024
2	915	2535	1620	1746	1090	15.2	1074.8	24	.025	.038
3	1002	2222	1220	1447	920	24.9	895.1	24	.026	.035
4	1462	2340	878	1666	605	17	588	24	.015	.022

These results are plotted in Figure 4.3 and the Yield coefficient Y, and the basal metabolism rate, k_e, for each run are then read off from the graph as the gradient and intercept respectively.

TABLE 4.6 REDUCED RESULTS FOR EQUATION 4.10 - RUN P2

Beaker	ALL CONCENTRATIONS IN $gm.m^{-3}$												
	S_i	S_f	ΔS	S_{av}	X_f	X_i	ΔX	X_{av}	Δt hours	μ_x hr^{-1}	μ_s hr^{-1}	μ_{av} hr^{-1}	$\frac{S_{av}}{\mu_{av}}$
1	1000	334	666	706	3200	2468	732	2950	3.75	0.068	0.090	0.079	8937
2	1130	760	370	976	2387	1885	502	2086	3.75	0.066	0.071	0.069	14145
3	760	540	220	650	1790	1655	135	1722	1.5	0.052	0.028	0.09	7222
4	600	95	505	327	2102	1490	612	1795	3.75	0.092	0.113	0.103	3175

4.5.3 Discussion

The seven points determined from the batch tests do not fit one curve. However reasonable linearity is shown for each set of results from the two trials.

Considerable doubt has already been expressed about the results from the first run because of the large number of centrifuge tube breakages. This doubt is further increased by the results obtained in Figure 4.3.

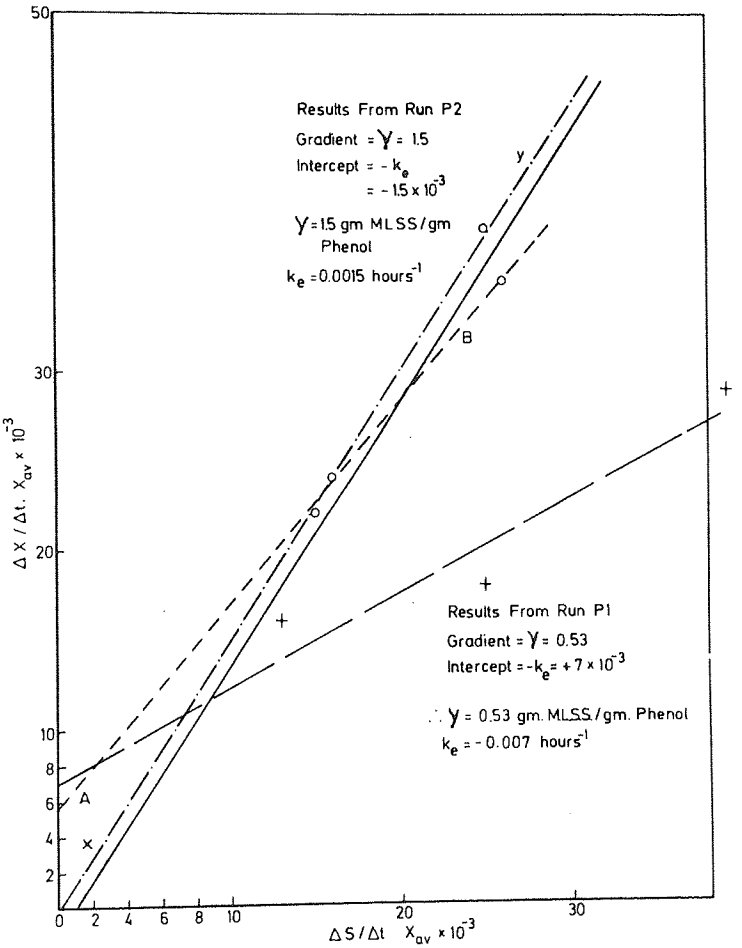


FIG. 4.3 INITIAL GRAPHICAL DETERMINATION OF Y AND Ke PHENOL-OXIDISING SLUDGE

The curve of these three points does not fit the data very well. More importantly, a negative value for k_e is shown - such a value would be impossible in practice.

If the values from this trial are discounted, the four points obtained from the second run may be seen to fit a linear curve reasonably well. Furthermore, a negative intercept is

obtained, giving a positive value of k_e if the curve is fitted to equation 4.6.

However, it should be pointed out that a very small change in the gradient of this line would greatly affect the value of k_e obtained. Curves AB and XY show two lines that could conceivably be drawn through the data, each assuming one of the points was inaccurate, and values of k_e of -0.0056 and 0.0 are respectively obtained. Obviously, far more data points are required to gain an accurate value for the basal metabolism rate.

To conclude, the following points appear to have been found from the initial tests:

1) The experimental technique used above to determine Y and k_e appears to be viable for a phenol; tolerant sludge.

2) The assumption of Monod Kinetics for such a sludge appears to be valid.

3) The sampling frequency needs to be shorter in order to minimise the effect of rogue results.

4) The number of individual batch tests need to be greater in order to gain more data points for curve fitting.

4.6 Determination of μ_{max} and K_s

4.6.1 Theory

From initial assumptions of the model, the rate of increase of bacterial mass is proportional to the mass of cells already present and to the rate of decay due to basal metabolism. This is expressed by equation 4.4

i.e. $$\frac{dX}{dt} = \mu X - k_e X$$

Over a small finite period of time, equation 4.4 may be re-arranged and expressed thus;

$$\mu_{av} = \left(\frac{\Delta X}{\Delta t} + k_e \cdot X_{av} \right) / X_{av} \qquad \text{..... Eq. 4.8}$$

Having previously determined the basal metabolism rate k_e, equation 4.8 may be used to determine average growth rates μ_{av} over time Δt for individual batch tests.

From Monod Kinetics, the specific growth rate is related to the maximum growth rate by equation 3.1

$$\mu = \frac{\mu_{max}\ S}{K_S + S}$$

Over a small finite period of time, equation 3.1 may be expressed in terms of average growth rates and concentrations, i.e.

$$\mu_{av} = \frac{\mu_{max}\ S_{av}}{K_S + S_{av}} \qquad \text{..... Eq. 4.7}$$

This equation transforms to a linear function thus:

$$S_{av} = \mu_{max} \frac{S_{av}}{\mu_{av}} - K_S \qquad \text{..... Eq. 4.9}$$

By plotting values of S_{av} against S_{av}/μ_{av}, μ_{max} and K_S can be read off from the curve as the gradient and intercept respectively.

Check

As a check on the validity of this method, the average growth rate may also be determined using the expression for rate of removal of substrate.

Again, from basic theory, the rate of decrease of substrate is related to the concentration of micro-organisms present by equation 3.7.

i.e. $$\frac{dS}{dt} = \frac{\mu X}{Y}$$

Over a small finite time period, equations 3.7 may be re-arranged and expressed thus:

$$\mu_{av} = \frac{\Delta S}{\Delta t} \cdot \frac{Y}{\bar{X}_{av}} \qquad \text{..... Eq. 4.10}$$

Having previously determined the yield coefficient Y, equation 4.10 may be used to determine the average growth rate over time period Δt.

If the method is acceptable, and the initial assumption of Monod kinetics is valid, then the values of μ_{av} obtained from equation 4.8 and 4.10 should equate.

4.6.2 Experimental

Values of substrate (as phenol) and MLSS concentrations are to be taken from the second Run and fitted to equations 4.8 and 4.10 to determine average growth rates over a small period of time.

Time Period

Since equations 4.8 and 4.10 are derived from expressions which relate specific growth rates to concentrations of substrate or active-mass, it is desirable to choose a time period over which the growth rate is reasonably constant.

Regarding Table 4.3, it can be seen that in all four beakers, the growth rate is initially inhibited due to the shock effect of the high phenol concentration. At the end of each batch, the growth rate will also be very small due to substrate deficiency.

In between these two limits, it is to be expected that the growth rate will remain fairly constant, as there is sufficient available substrate and no inhibitive effect.

Thus the time period chosen for analysis of μ_{av} is that between 2¼ and 6 hours. In all of the batches, with the exception of Beaker 3, noticeable increase of MLSS coincides with a large decrease of substrate during this period. For Beaker 3

unfortunately, a tube breakage prevented determination of the MLSS. If the time period for Beaker 3 was to be expanded to include the 1½ hour reading, the results show that substrate inhibition would affect the analysis. Therefore the period must in this case be contracted to only two readings, between 4½ and 6 hours.

4.6.3 Results

The reduced results for equations 4.8 and 4.10 are given in Table 4.6 as are the calculated values of the average growth rates over the given time period - μ_x signifying that value determined from equation 4.8 and μ_s being the value determined from equation 4.10.

Figure 4.4 shows the plot of equation 4.10 and the determined values of μ_{max} and K_s from the data of the second run.

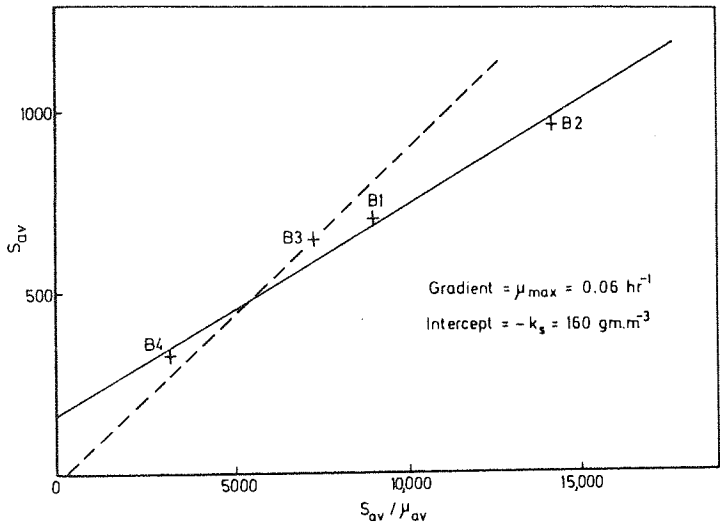


FIG.4.4

INITIAL GRAPHICAL DETERMINATION OF μ_{max} AND k_s: PHENOL-OXIDISING SLUDGE

4.6.4 Discussion

Table 4.6 shows that the values of μ_{av} over the indicated time period calculated from equations 4.8 and 4.10 do not equate. However, with the exception of the values obtained from Beaker 3, calculated values of μ_x and μ_s are reasonably close considering the small amount of data used in the analysis, and the high expected analytical error from each of the data points.

In the case of Beaker 3, only two analyses were used to determine this point, and any slight error in the determination of either MLSS or phenol content of either sample would be magnified out of all proportion in the subsequent mathematical analysis.

Plots of phenol decay and MLSS increase against time such as are given in Tables 4.2 and 4.3 invariably show a smooth curve fitted to most of the phenol data-points, whilst the curve fitted through the MLSS data shows scatter in the data. Such a plot is illustrated in Figure 4.5, and indicates that the major source of experimental error is in the determination of MLSS.

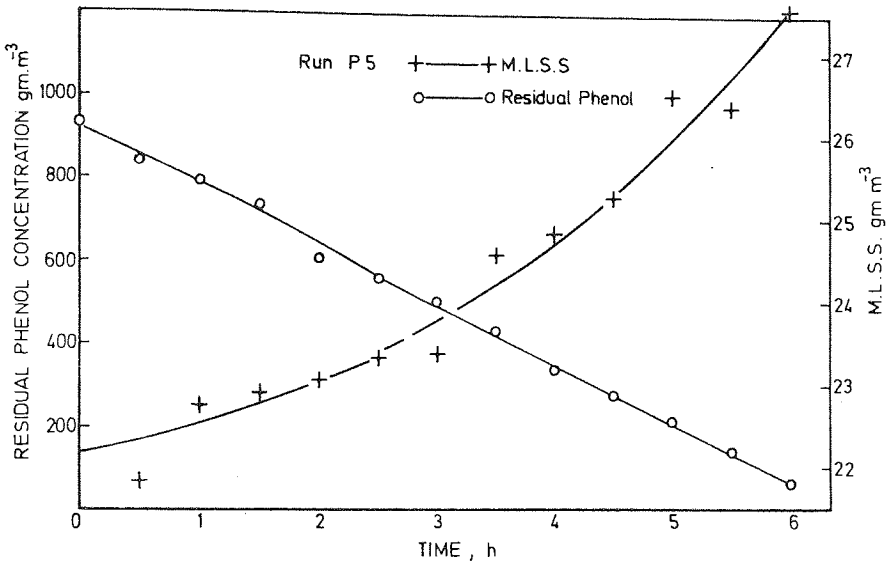


FIG.4.5 TYPICAL PHENOL DECAY AND MLSS CONCENTRATION AGAINST TIME

The scatter shown however is not as bad as might be expected from the results of the mathematical analysis above. It seems likely therefore that the greatest source of error has been in the very small numbers of samples used in the analysis.

If then the total error effect is due to the experimental technique rather than to the more basic mistake in assuming Monod kinetics, the average values of μ_x and μ_s should equate much more readily if the time period is enlarged to encompass as many values as possible. Table 4.7 shows the reduced results for Run P2 over the time period 0-24 hours, and also the calculated values of μ_x and μ_s.

TABLE 4.7 REDUCED RESULTS FOR RUN P2 - 24 HOUR TIME PERIOD
ALL CONCENTRATIONS IN $gm.m^{-3}$

Beaker	S_i	S_f	ΔS	S_{av}	X_f	X_i	ΔX	X_{av}	Δt hours	μ_s	μ_x
1	1095	23.7	1071.3	815	3982	2342	1640	2820	24	.024	.026
2	1090	15.2	1075	950	2535	915	1620	1746	24	.038	.040
3	920	24.9	895.1	753	2222	1002	1220	1447	24	.039	.037
4	605	17	588	483	2340	1462	878	1666	24	.022	.023

In this case, the calculated values of μ_s and μ_x are very much closer together. Unfortunately these results cannot be used to determine μ_{max} and K_s from equation 4.10 as the specific growth rate will have varied greatly over the 24 hours.

The results of Table 4.7 do however indicate that the method outlined for determination of μ_{max} and K_s is likely to be valid if sufficient samples are taken during a period of maximal growth rate. Credence is also given for the assumption of Monod kinetics.

Several points arise from the plot of Table 4.6 shown in Figure 4.4. Firstly, the points appear to lie on a straight line, giving further justification to both the method and the assumption of Monod kinetics. However the values of μ_{max} and K_s obtained from this graph are unacceptable. The maximum growth rate of 0.06 $hour^{-1}$ is less than the specific growth rates determined in Table 4.6. Furthermore, the observed value of K_s is negative. Since K_s is the substrate concentration at which the specific growth rate becomes half of the maximum growth rate, a negative value for K_s is meaningless.

If the data-point obtained from Beaker 2 is omitted, it would be possible to draw the dotted-line shown which would then give a positive value of K_s of 40 $gm.m^{-3}$, and a maximum growth rate of 0.1 $hour^{-1}$. Such values would be much more acceptable than those previously obtained.

What is apparent is that much more data is required to accurately draw this curve, and that such data must be determined as accurately as possible.

4.7 Establishment of a Thiocyanate - Tolerant Culture

As explained in Section 4.3.2, continuous pH controllers were found necessary firstly to cultivate the thiocyanate-tolerant sludge, and then to proceed with tests on the kinetics of the sludge. It was further decided at this stage to use larger reactors for the batch tests, in order to minimise the effect that sample extraction might have.

In Section 4.6.4 it was stated that much more data was required from each batch test in order to more accurately determine the kinetic coefficients of the heterotrophic sludge. Having decided to extract 40 ml samples each time in order to minimise the effect of sampling and analytical errors in the determination of MLSS content, a batch of say 20 samples from one of the Pyrex beakers would have reduced the volume of the Mixed Liquor by almost half at the end of the batch. Reductions in liquor volume of this magnitude would very likely have meant changes in DO concentrations and mixing patterns, and would also diminish the buffering effect of the liquors as regards temperature and pH control - i.e. the controllers would more easily start "hunting".

Therefore, six batch reactors of 5.5 litres nominal capacity were constructed as illustrated in Figure 4.6.

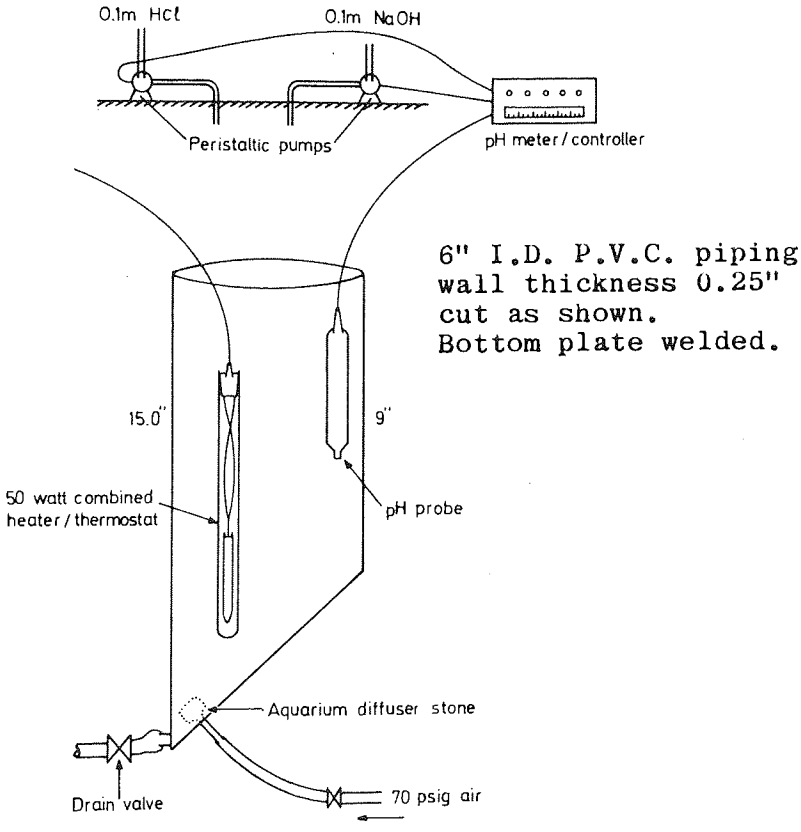


FIG. 4.6 BATCH REACTOR - 5.5 ℓ NOMINAL CAPACITY

Integrated heaters and thermostats were used to control the temperature of the Mixed Liquor, and four of the reactors were fitted with pH controllers. These controllers fed 0.1 M HCL or

0.1 M NaOH to the reactor via small peristaltic pumps whenever the pH of its contents varied from the set range.

The complete set-up of reactors, and control equipment is shown in Figure 4.7.

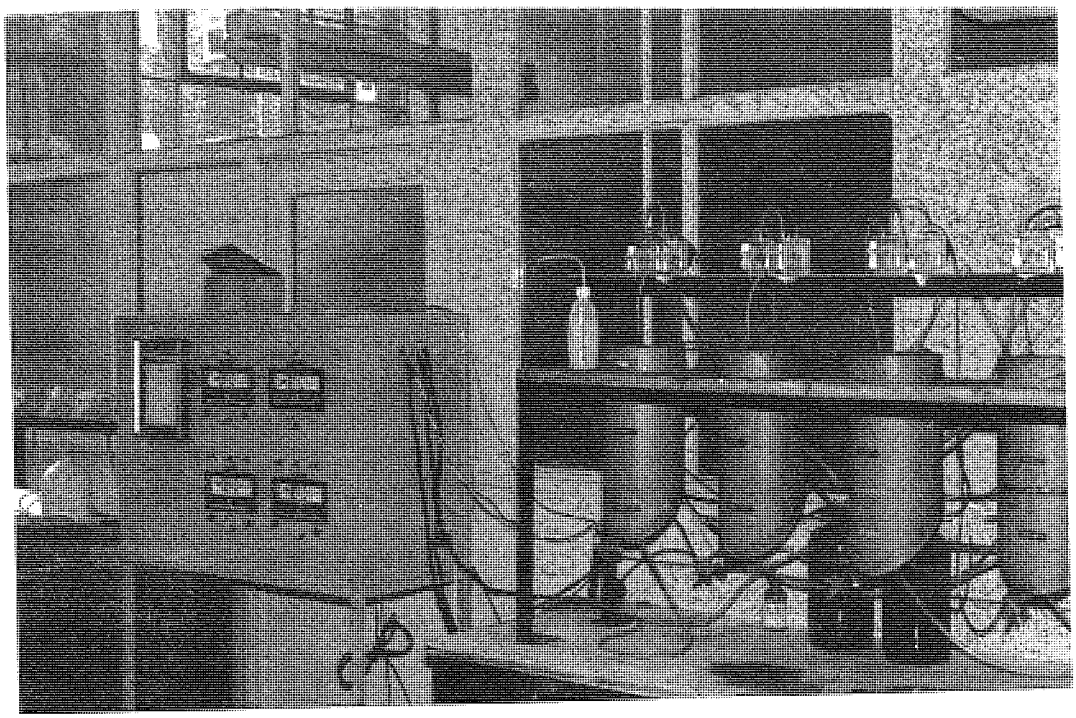


FIG. 4.7

Four of these reactors were initially used to establish the autotrophic culture - the four fitted with pH controllers. The culture was grown in a similar way to the hetetrophic culture (see Section 4.3.2), except that potassium thiocyanate was added at ever-increasing initial concentrations as substrate, and the pH of the Mixed Liquor was controlled between 6.8 and 7.3.

Initially, the contents of each reactor was settled daily, and the supernatent discarded to prevent accumulation of toxics such as ammonium ions. Fresh treated coke oven effluent, plus nutrients and substrate was then added to the sludge and aeration recommenced. In such a way the sludge volume slowly began to build up. Occasionally, some of the settled sludge would be wasted to lessen accumulation of inhibitors in the sludge. As the sludge volume increased, so too did the frequency

of wasting, until approximately 5% of the autotrophic sludge was being discarded daily. By this time, discard of supernatent had been reduced to 3 or 4 times per week, and sufficient sludge had been built up to commence tests on its kinetics.

4.8 Further Investigations into the Kinetics of a Phenol-Degrading Sludge

Using the larger reactors, illustrated in Figure 4.6 and described in Section 4.7, further batch experiments were undertaken to gain more data-points for the graphical determination of the kinetic coefficient of a phenol tolerant sludge.

The reduced results of these experiments are given in Table 4.8 and their graphical interpretation in Figures 4.8 and 4.9.

TABLE 4.8 REDUCED RESULTS - RUNS P3 TO P7

Test	ALL CONCENTRATIONS IN $gm.m^{-3}$										
	Δt hrs	ΔX	X_{av}	ΔS	S_{av}	$\frac{\Delta S}{\Delta t}.X_{av}$	$\frac{\Delta X}{\Delta T}.X_{av}$	μ_s	μ_x	μ_{av}	$\frac{S_{av}}{\mu_{av}}$
P3	5	548	3209	936	595	.058	.034	.047	.038	.042	14 167
P4	5	283	7350	515	406	.014	.008	.011	.012	.012	33 833
P5	5	483	2459	735	423	.060	.040	.048	.043	.045	9400
P5	5	622	1877	818	441	.087	.066	.07	.07	.07	6300
P5	4.5	455	1317	497	261	.084	.069	.067	.081	.074	3527
P6	5	322	3120	628	1226	.040	.021	.032	.025	.028	43 785
P6	4.5	335	1641	560	1217	.068	.045	.061	.049	.055	22 127

Note that in Figure 4.9 points have been included from Test P2, and are represented by a cross within a circle. These values have been recalculated with the updated values of Y and k_e from Figure 4.8. Also, two of the calculated points have not been given any weight in the determination of μ_{max} and K_s as they obviously do not lie on the straight line which runs through the rest of the data.

4.8.1 Discussion

Figure 4.8 illustrates the difficulty in establishing the kinetic coefficients of a sludge by the method used - there is considerable scatter through which several straight lines could be drawn. However the range of possible gradients to fit the

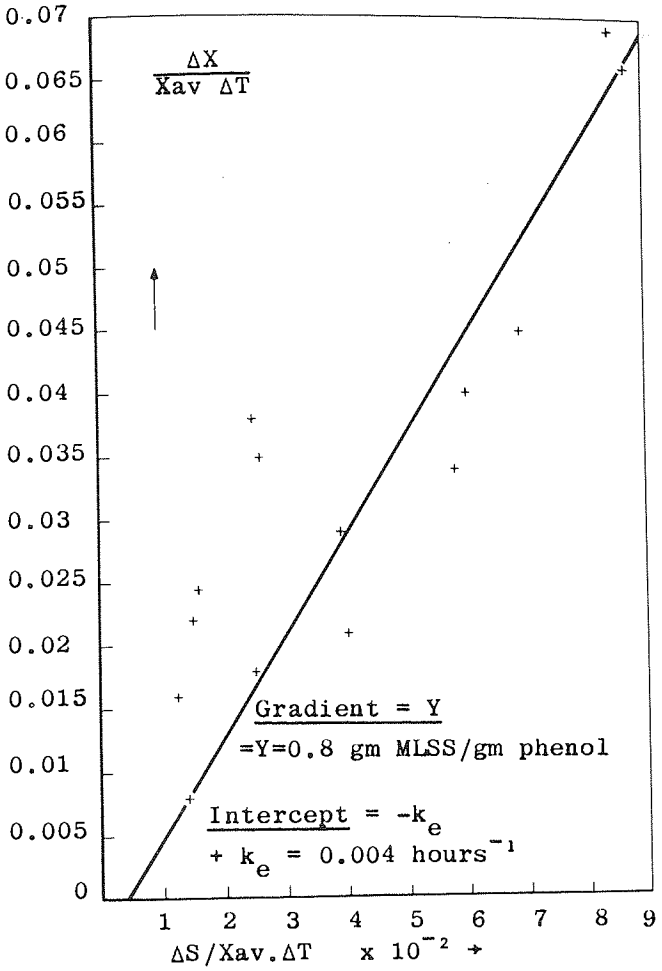


FIG. 4.8
GRAPHICAL DETERMINATION OF Y AND k_e RESULTS FROM 14 BATCH TESTS

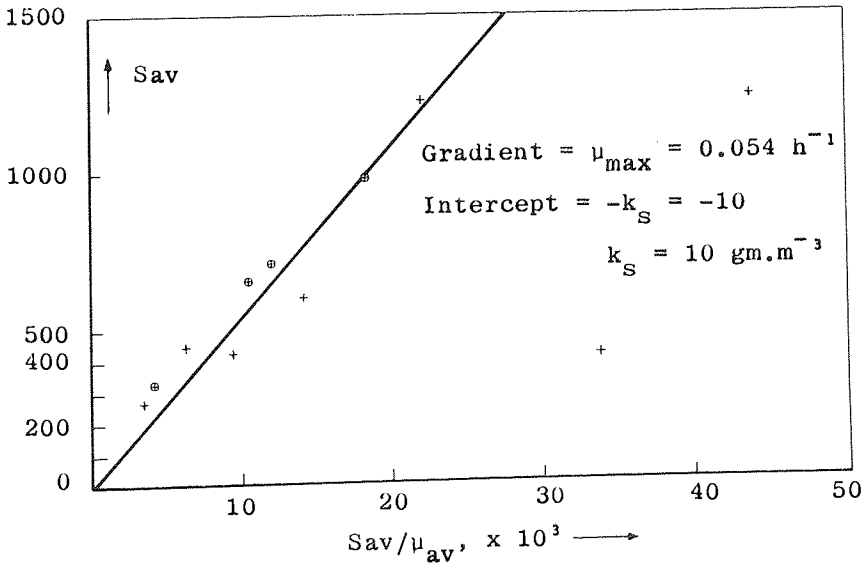


FIG. 4.9 GRAPHICAL DETERMINATION OF μ_{max} AND k_s - RESULTS FROM 11 BATCH TESTS

observed data is relatively narrow, giving a value for Y of between 0.5 and 1.0 gm MLSS per gram of phenol utilised. The main problem is the determination of the value of k_e - the intercept. Different lines drawn through the data could give values between $k_e = 0.01\ hr^{-1}$ and $k_e = -0.01\ hr^{-1}$.

A similar problem arises in Figure 4.9. Although the scatter is much reduced, and the range of possible gradients is small, it is impossible to establish an absolute value for K_s.

4.9 Initial Attempts to Determine the Kinetics of an Autotrophic Sludge

Using three of the batch reactors as illustrated in Figure 4.6, experiments were instigated to determine the kinetic coefficients of an autotrophic sludge by the method previously outlined in Section 4.1.2.

Different ratios of substrate (as potassium thiocyanate) to sludge were added to each reactor, and the mixed liquor was then aerated for four hours. 50 millilitre samples were taken periodically for subsequent analysis of thiocyanate and MLSS content.

A check on the residual thiocyanate concentration of the beaker contents was periodically made using the acidified ferric chloride test in a Lovibond Comparator. Appendix 6 gives details of this very brief test.

4.9.1 Analytical Methods

Thiocyanate content was measured as per standard methods[122].

MLSS concentration was determined by the centrifugal method as given in Section 4.5.1.

4.9.2 Results

The results of the first three tests are given in Table 4.9, and the thiocyanate decay and change in MLSS concentration of each batch is plotted against time in Figure 4.10.

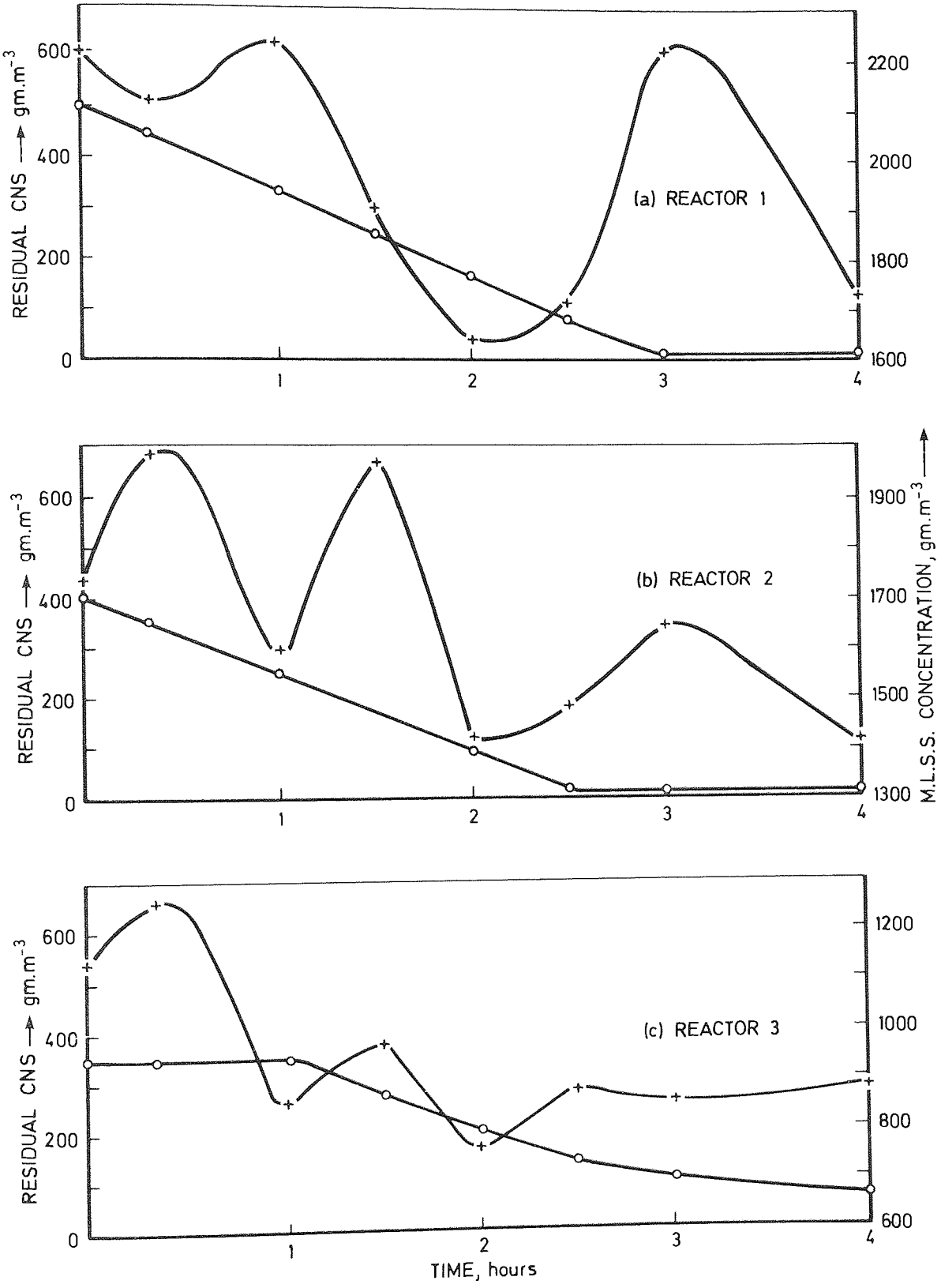


FIG.4.10 BATCH THIOCYANATE DELAY CURVES

TABLE 4.9 THIOCYANATE DECAY - RUN T1 - AERATION PERIOD 4 HOURS

Time hrs.	ALL CONCENTRATIONS IN $gm.m^{-3}$					
	Reactor 1		Reactor 2		Reactor 3	
	CNS	MLSS	CNS	MLSS	CNS	MLSS
0	500	2210	400	1727	350	1142
1/3	450	2115	357	1992	345	1255
1	335	2237	254	1600	350	863
1½	250	1904	173	1975	274	973
2	172	1642	100	1417	199	768
2½	83	1717	18	1487	132	878
3	8	2237	2.9	1645	100	858
4	1.7	1735	2.0	1410	63	880

The three graphs of Figure 4.10 show an irregular pattern of MLSS concentration during each batch test. Since the MLSS curve does not conform to the standard Monod curve as illustrated in Figure 4.11, the coefficients of this sludge cannot be determined from these results by the graphical technique of Section 4.1.2 used previously for the heterotrophic sludge.

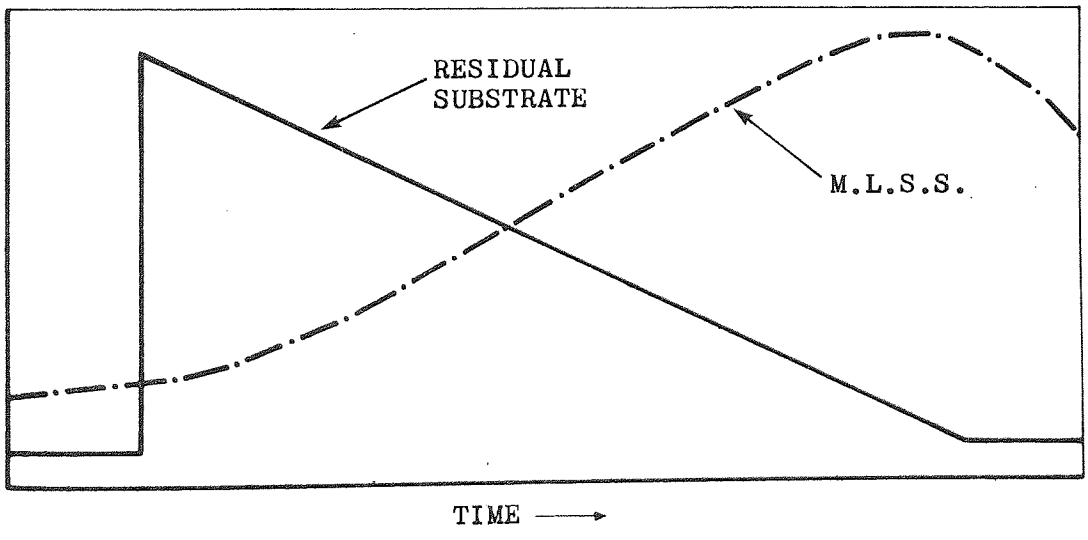


FIG. 4.11 STANDARD MONOD CURVE - BATCH CULTURE

4.9.3 Discussion

What then was the cause of the irregular pattern of the MLSS curve? There are "peaks" and "valleys" in each of the plots, suggesting that some necessary nutrient might have been limiting at certain stages of the batch, indicated by MLSS decline. If this was the case however, it would seem likely that thiocyanate removal would also have been affected, but the results do not indicate this. Furthermore, known necessary nutrients such as phosphate and trace elements were supplied in excess.

It has been stated that selective growth of the thiocyanate culture had proven to be very difficult in the laboratory. It was only when pH control was effected that some visible signs of growth had occurred, and even then it had taken several months to accumulate a small volume of the sludge (5% of the volume of each reactor after ½ hour settlement).

The phenol-tolerant sludge on the other hand grew very quickly, and large volumes of the sludge (20-30% by volume, ½ hour settlement) could be produced within a matter of days.

The nature of the two sludges was also very different. The heterotrophic sludge was lightly coloured, flocculant and fluffy, and settled very easily. The autotrophic sludge however was of a gritty and particulate nature, and only the heavier particles settled easily, the remainder staying in suspension in the supernatant.

Experience in culturing and handling of the two sludges promoted the belief that the heterotrophic sludge had comparatively very high growth rates, and was mostly made up of active cellular mass. The difficulty in growing the autotrophic sludge indicated very slow growth rates - or very high decay rates - and its nature gave cause for belief in a high inorganic debris content. Happold[66] and Hutchinson[88] found that elemental sulphur was released by actively growing cultures of thiobacilli. If the active content of the sludge - as cells able to metabolise the substrate - is sufficiently low, then there would be only a very small increase in dried solids due to cell growth. This increase could then be completely masked by inorganics such as sulphur, or by cellular intermediates, going in and out of solution during the course of the batch.

Whatever the reason for the variations in MLSS concentration, it is quite apparent that MLSS is not a sufficiently good enough indicator of viable active mass for an autotrophic sludge oxidising thiocyanate and without such a measure of the active mass, the kinetics of the system cannot be measured.

4.10 The Use of Mixed Liquor Volatile Suspended Solids (MLVSS) as a Measure of Active Biomass

In the previous section, it was shown that MLSS is not an adequate indicator of viable active mass for an autotrophic sludge growing on thiocyanate. In an attempt to improve on the measurement of cellular populations, the Mixed Liquor Volatile Suspended Solids (MLVSS) concentration was then measured during the batch oxidation of thiocyanate by an autotrophic sludge.

Measurement of MLVSS involves heating the dried suspended solids to a temperature of about 500 ^{0}C so that all volatile constituents of the sludge are driven off. The weight of the volatile solids is then determined and used to represent the concentration of cells present.

4.10.1 Analytical Methods

Thiocyanate content was measured as per standard methods[122].

MLVSS A 40.0 ml sample of the mixed liquor was centrifuged for 20 minutes at 3000 rpm. The supernatent liquor was decanted to be analysed for residual thiocyanate content, whilst the sediment was washed twice with distilled water, recentrifuging after each wash. The sediment was then washed into a pre-weighed crucible, and dried overnight in an oven at 105 ^{0}C. The crucible and dried sludge was allowed to cool in a dessicator, and re-weighed. It was then heated in a furnace at 540 ^{0}C for four hours. After cooling in a dessicator, it was again re-weighed. The concentration of Volatile Suspended Solids is given by:

$$\text{MLVSS} = \frac{(A - C) - (B - C)}{40} \times 10^{6} \text{ gm.m}^{-3}$$

where: A = Weight of crucible and dried solids
B = Weight of crucible and non-volatiles
C = Weight of crucible alone

For comparison, the MLSS was also determined from:

$$MLSS = \frac{A-C}{40} \times 10^6 \text{ gm.m}^{-3}$$

4.10.2 Results

Table 4.10 shows the results of three batch tests on the biodegradation of thiocyanate by an autotrophic sludge. The sludge concentration is represented by both MLSS and MLVSS content. Also given is the ratio of volatile suspended solids to total suspended solids in each case.

4.10.3 Discussion

In each test the ratio of MLVSS to MLSS remains constant, insofar as the accuracy of the measurement technique allows. This indicates that measurement of MLVSS will no better relate to the numbers of autotrophic cells in the system than did MLSS measurement.

Graphical interpretation of the results has not been included as the pattern followed in each case is similar to that observed in Figure 4.10, i.e. the MLVSS concentration shows no regular form that can be modelled.

TABLE 4.10 THIOCYANATE DECAY - RUN T2 - MEASUREMENT OF MLVSS

Time	Reactor 1				Reactor 2				Reactor 3			
(hrs)	CNS	MLSS	MLVSS	% VSS/ TSS	CNS	MLSS	MLVSS	% VSS/ TSS	CNS	MLSS	MLVSS	% VSS/ TSS
0	900	3130	2853	91	837	3085	2736	89	869	4020	2910	72
¼	852	3157	2874	91	786	3020	2749	91	874	3715	2832	76
½	767	3042	2749	90	715	3115	2708	87	818	3642	-	-
1	618	3212	2955	92	589	3072	2779	90	759	3657	2840	78
2	412	3010	2775	92	342	3247	2963	91	627	3422	2471	72
3	275	3105	-	-	64	3157	2916	92	445	3547	2635	74
3½	-	-	-	-	8.2	3158	2862	91	-	-	-	-
3 2/3	-	-	-	-	9.6	3148	2883	92	-	-	-	-
3 5/6	-	-	-	-	10.9	3117	2851	91	-	-	-	-
4	93	3182	2874	90	10.9	3157	2834	90	255	3715	2901	78
4½	-	-	-	-	10.9	3272	2964	91	-	-	-	-
5	4.1	3162	2877	91	6.9	3350	2990	89	8.2	3972	-	-
5⅛	8.2	3647	3220	88	-	-	-		10.9	3570	2786	78
5½	4.1	3165	2811	89	-	-	-		9.6	3890	2865	74

Further determinations of MLSS and MLVSS in both autotrophic and heterotrophic batch systems were made as will be seen in Chapters 6 and 7, and in every case the ratio of MLVSS to MLSS remains reasonably constant over the duration of the test.

In conclusion, it appears that neither MLSS nor MLVSS is a sufficiently good indicator of microbial population in an autotrophic system.

4.11 Curve Fitting - A Numerical Method for the Determination of the Kinetic Coefficients of a Heterotrophic Sludge

At the same time as tests were being undertaken on the autotrophic sludge, further investigations were being made into the kinetics of the heterotrophic sludge.

This time however, curve-fitting techniques were used to evaluate the coefficients.

In section 4.8.1 it was stated that the graphical technique appears to be too insensitive to accurately determine absolute values of k_e and K_s. It is believed that some of the inherent inaccuracies of the graphical method are caused by:-

(i) placing too much reliance on the analysed results, particularly those of the MLSS concentrations

(ii) compounding errors caused in (i) above by reducing the data to enable fitting of linear equations

(iii) using average values of the growth rate, substrate and MLSS content, whereas specific values are in fact required.

A more direct method is felt to be necessary in order to reduce these errors.

4.11.1 Theory

Curves are obtained from each of the batch tests by plotting substrate concentration against time, and cell concentration (as MLSS) against time. Inaccuracies in the determination of sludge concentration are reduced by "smoothing"

the curves. For each test, the two curves S/t and X/t are then represented by the following equations, assuming Monod kinetics to hold;

$$-\frac{dS}{dt} = \mu_{max}\frac{X}{Y}\left(\frac{S}{(K_s + S)}\right) \qquad \text{..... Eq. 3.2}$$

$$\frac{dX}{dt} = \frac{\mu_{max}\,S}{K_s + S} \cdot X - k_e X \qquad \text{..... Eq. 4.11}$$

Equation 4.11 is derived from equations 3.1 and 4.4 and represents the nett change in sludge concentration in a batch system.

A "guessed-value" for each of the constants was then inputted into equations 3.2 and 4.11 and an iterative technique set up to vary the values until the computed curves reasonably fitted the experimentally derived curves. This technique was repeated for each of the batch tests and ranges of values for each of the constants which gave a Best Fit to the experimentally derived data were thus obtained.

4.11.2 Curve fitting techniques, and simulation hardware

Two methods were used to fit the equations to the experimentally derived data. Firstly, programmes were written for a small programmable calculator (Hewlitt-Packard HP 65) and also for inter-active use from a MOP terminal on the CEL's ICL 1905S. The latter programmes were written in FORTRAN.

Using both of the computational aids, the above equations were integrated over small periods of time, dt, by approximating X and S to be X_i and S_i (i.e. X_i = X when $t = t_i$). This method is illustrated below for the determination of a new value for substrate concentration.

For each batch test, the initial substrate and cell concentrations are known from analysis. Guessed-values of the four coefficients are then inputted into a finite-time version of equation 3.2, i.e.:

$$\frac{S_i - S_1}{\Delta t} = \frac{\mu_{max} \; X}{Y} \cdot \frac{S}{K_s + S}$$

From which

$$S_n = S_{(n-1)} \frac{\mu_{max} \; X}{Y} \cdot \frac{S}{K_s + S} \cdot \Delta t$$

where S is approximated by $S = S_{(n-1)}$

X is approximated by $X = X_{(n-1)}$

For very small values of Δt, it was found that this approximate method of integration was adequate for fitting the equations to the experimental data. The program used to integrate these equations on the HP 65 is given in Appendix 7. Using this method, values of S and X were computed over the time period of the test, and the resultant curves were compared with the experimentally derived data. Each of the coefficients was then varied in turn, until a Best Fit was obtained.

A second numerical method was also used to determine the values of the coefficients. Using a program devised by the Mathematics Department of the Corporate Engineering Laboratories, $\frac{\partial S}{\partial t}$ and $\frac{\partial X}{\partial t}$ were evaluated from the experimentally derived data, and then a non-linear least squares calculation performed to find the best-values of the four coefficients given that

$$\frac{\partial S}{\partial t} = - \frac{\mu_{max} \; X}{Y} \cdot \frac{S}{K_s + S}$$

and

$$\frac{\partial X}{\partial t} = \frac{\mu_{max} \; X \; S}{K_s + S} - k_e X$$

The order of the polynomials fitted to X and S is increased until the percentage change in the values of the four parameters is small.

A copy of this program is unfortunately not available to the author.

4.11.3 Results

Table 4.11 gives the results of three batch tests on a heterotrophic (phenol-tolerant) sludge. The experimental technique is as established in Section 4.5 and the analytical methods as in Section 4.5.1. The results obtained previously, tests P_1 to P_6, were not used in this method, as it was felt that there were insufficient data points through which to draw "smoothed-curves".

Both methods outlined above were then used to determine the coefficients. It was found that the iterative technique, using either the HP 65 or the CEL computer was sufficiently accurate, providing that the time period was kept very small - no more than 0.05 hours. The non-linear least squares method computed values of S and X that reasonably fitted the data, but only if the original guesses of the coefficients were fairly close to the final computed values.

TABLE 4.11 PHENOL DECAY CURVES - RUN P7

Time hours	All Concentrations in $gm.m^{-3}$					
	Reactor 1		Reactor 2		Reactor 3	
	Phenol	MLSS	Phenol	MLSS	Phenol	MLSS
0	925	2227	565	1115	970	1612
0.5	840	2185	515	1108	905	1593
1.0	795	2275	485	1170	835	1618
1.5	735	2290	415	1255	730	1670
2.0	605	2305	370	1175	680	1653
2.5	560	2333	320	1270	630	1725
3.0	500	2338	260	1320	550	1842
3.5	430	2458	200	1357	475	1838
4.0	337	2482	132	1405	374	1913
4.5	275	2528	54	1487	293	1955
5.0	215	2652	18	1562	197	2050
5.5	140	2637	16	1505	75	2145
6.0	60	2757	16	1490	17	2240

The above results are plotted in Figure 4.12. Also plotted in each of these graphs are the computed values of S and X from equations 3.2 and 4.11. These values are illustrated by the continuous lines in each graph, and correspond to the Best Fit

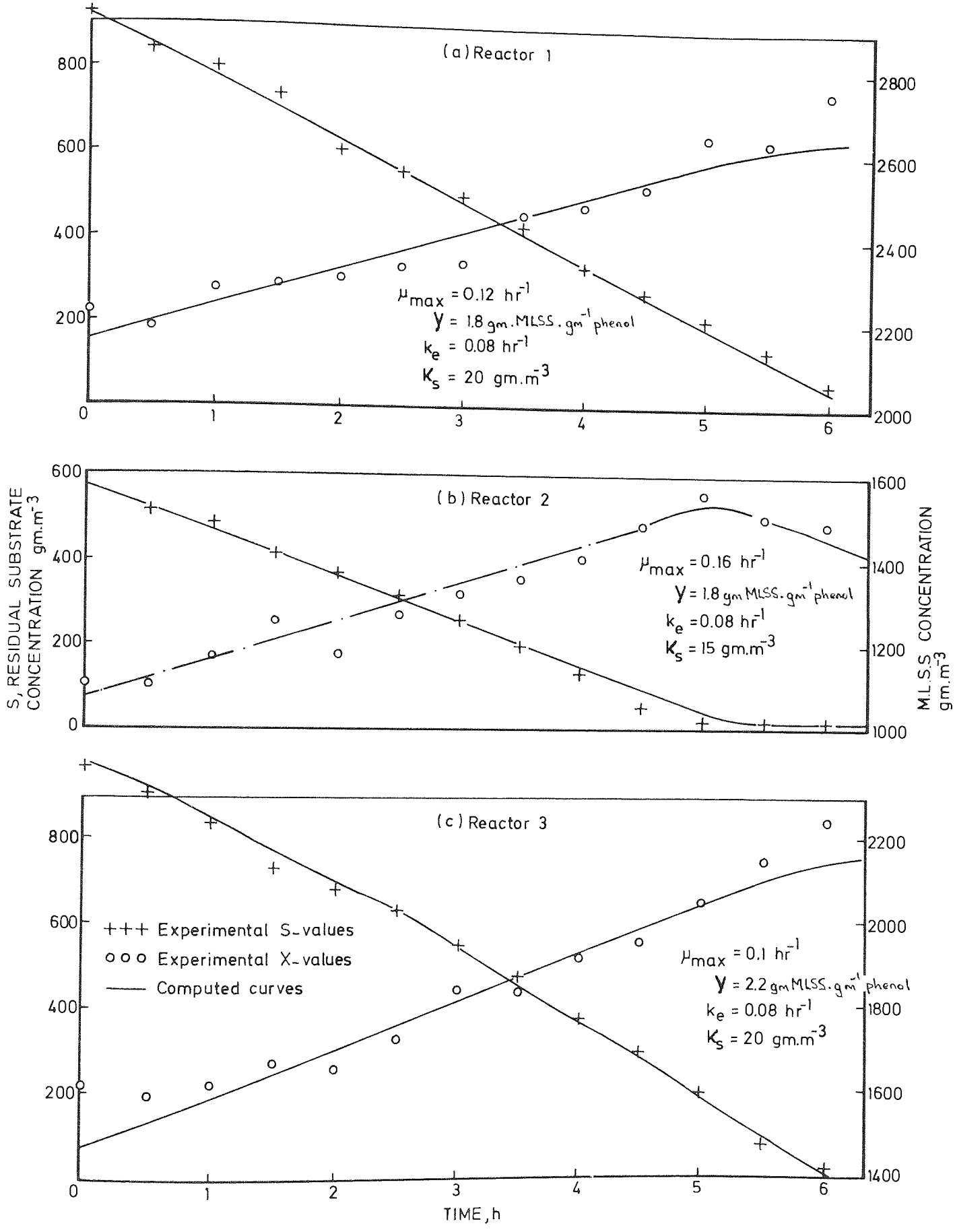


FIG. 4.12 COMPUTED AND EXPERIMENTAL CURVES HETEROTROPHIC SYSTEM

obtained to the experimental data. The values of the coefficients which gave these fits are also shown on each graph.

Figure 4.13 shows a typical "conversation" on the MOP terminal. The operative program is called BATC, and calculates values of X and S, together with the differences between the historical (as experimental) and the calculated values. The results shown are for Reactor 2 of Run P7.

Input to the program is underlined.

```
15.04.38 ← LØAD PRØGRAM BATC
15.04.53   JØB IS NOW FULLY STARTED
15.04.54 ← ØL *TRO
15.05.03 ← ØL *LPO
15.05.08 ← EN
15.05.12   0.01 CORE GIVEN 5248
← 7 - - - - - - - - - - - - - - - - - - - - - - -   Number of pairs of
← 570 1080                                          historical data
← 470 1150                                          points
← 360 1250   - - - - - - - - - - - - - - - - - -   Historical S and X
← 250 1350                                          data points
← 140 1450
←  40 1530
←  10 1460
← 100 0.01 0.16 0.08 15.0 1.8 - - - - - - - - -   Input iterative time
MUM = 0.160 KE = 0.080 KS = 15.0 Y = 1.80           difference and
  HX       CX       DX      HS      CS      DS      "guesstimates" of
1080.00 1080.00    0.00 570.00 570.00   0.00       coefficients
1150.00 1164.72 -14.72 470.00 473.07  -3.07 - -   Output historical
1250.00 1254.79 - 4.79 360.00 369.28  -9.28        and calculated values
1350.00 1349.36   0.64 250.00 258.89  -8.89        of S and X, and the
1450.00 1445.28   4.72 140.00 143.49  -3.49        difference
1530.00 1525.03   4.97  40.00  33.02   6.98
1460.00 1463.62 - 3.62  10.00   0.03   9.97
```

FIG. 4.13 ENUMERATION OF COEFFICIENTS - BEST FIT ITERATIVE METHOD

4.11.4 Discussion

Figure 4.12 shows that the computed curves fit the experimentally derived data reasonably well in each case. Also the values obtained for the coefficients that best fit each curve are very similar.

Once the technique of curve-fitting had been established, it was found relatively easy to speedily fit further curves by observation of curve-shape. This is further explained in the next section.

On the basis then of apparent ease of method, and repeatability of results, it was decided to continue investigative work into the kinetics of both autotrophic and heterotrophic sludges. using curve-fitting techniques to evaluate the coefficients.

However, the values obtained for the coefficients by the curve-fitting technique differ substantially from those obtained by the graphical method. The reason for this is not known, but as previously explained, the amount of scatter observed using graphical techniques could easily account for much of the difference.

Besides, it had by this time been decided to seek an alternative method of estimating biomass, one that would be far more accurate than determination of suspended solids, and which would be applicable to both autotrophic and heterotrophic mixed liquors. Any coefficients based on such a measure would obviously be different to those based on MLSS.

4.12 Curve Fitting Technique - The Relative Importance of Each of the Coefficients, and Methodology for Parameter Variation

Whilst the results of Table 4.11 were being fitted, it became apparent that each of the coefficients had an individual effect on the general shape of the X-and S-curves. It was noticed for example, that a small variation in μ_{max} had a much greater effect on both curves than did a much greater variation in K_s. It was therefore decided to attempt to illustrate the relative

importance of each of the coefficients and to establish a standard method of progressive parameter variation in order to most speedily determine the Best Fit.

4.12.1 Method

Having determined the values of the coefficients which give the Best Fit to a particular set of experimental results, one of the four parameters is then varied slightly from its "true" value. The curves obtained with this new value are then compared with the "true" curves. The percentage variation differs for each coefficient, being dependant upon the variation needed to effect a small but noticeable change in curve-shape.

4.12.2 Observations

Figures 4.14 to 4.17 show the effects of small changes in each of the coefficients. Each plot has a base-curve illustrated by the solid-line, this being the "true" curve, or plot of the Best Fit. The two other curves on each plot show the effect of a small increase or decrease in the value of the parameter under study.

From the graphs, two of the constants emerge as being most significant. μ_{max}, given as MUM in Figure 4.14 is shown to have a great effect on the gradient of both the X- and S-curves. Figure 4.15 shows that the Yield Coefficient most affects the final shape of the curve, i.e. the value of Y becomes very significant in the endogenous phase when S is low, and X is decaying. Figures 4.16 and 4.17 however suggest that the values of k_e and particularly K_s are much less significant than the other two coefficients.

4.12.3 Recommendations

When fitting X- and S-curves of this type, it is recommended that the following procedures are followed:

A) INITIAL FIT TO THE S-CURVE

(i) Assume values for k_e, K_s, Y, μ_{max}. These should be taken from the literature wherever possible, otherwise they should be estimated from previous experience.

(ii) Alter Y until the slope is approximately correct.

(iii) Alter μ_{max} for final shape.

(iv) Alter K_s for best fit.

(v) Go back to (ii).

When no significant improvement in fit is obtained, go to B.

B) INITIAL FIT TO X-CURVE

(i) Assume above values of Y, μ_{max} and K_s.

(ii) Alter k_e for required $X_{maximum}$.

(iii) Alter μ_{max} for required slope.

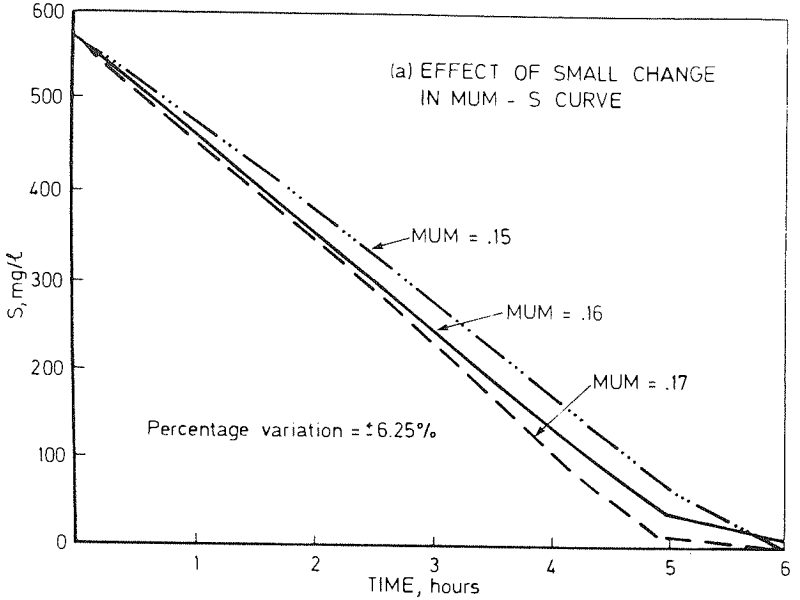
(iv) Alter Y for required final shape.

(v) Alter K_s for best fit.

(vii) Go back to (Bii).

When no improvement is found in the fit of the X-curve, go to C.

C) Check these values for fit with the S-curve. Go back to A if necessary.



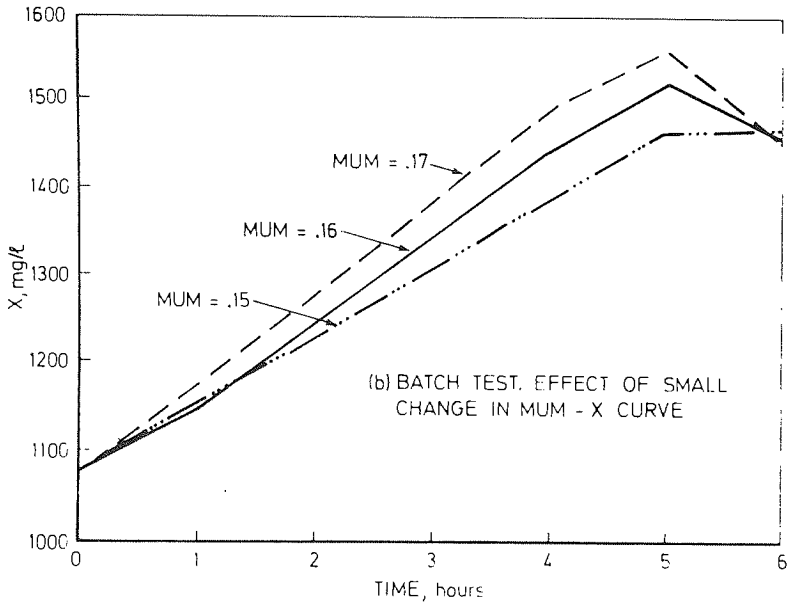
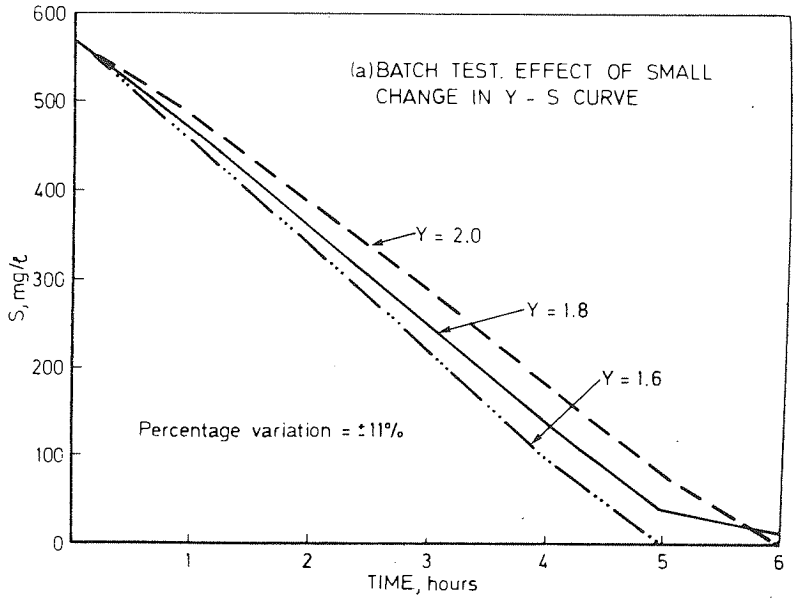


FIG 4.14



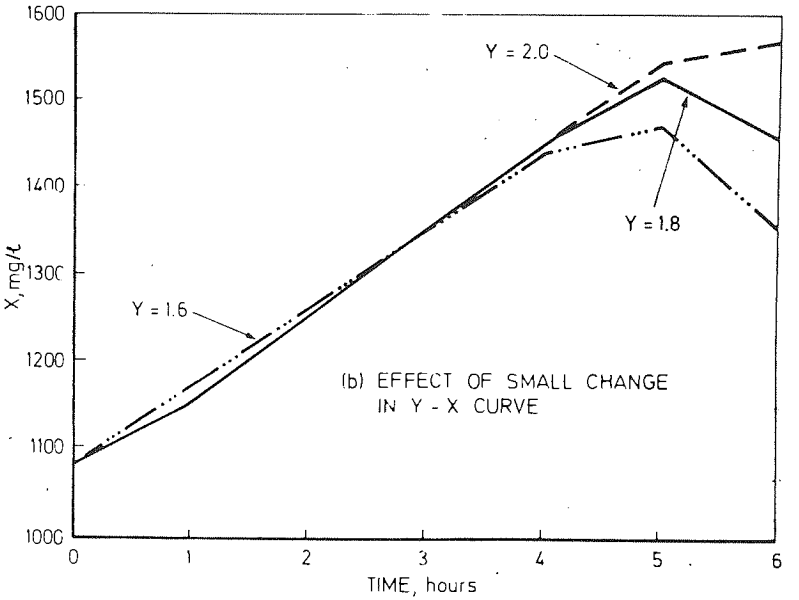
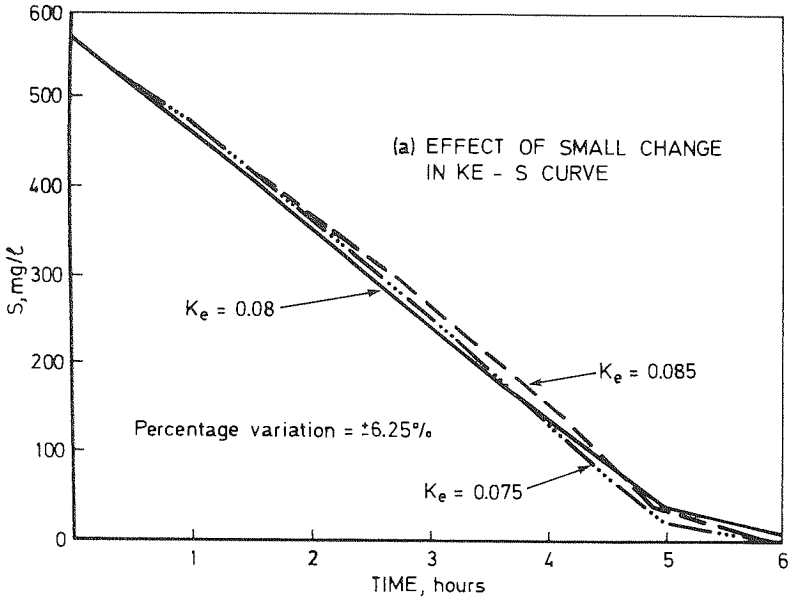


FIG.4.15



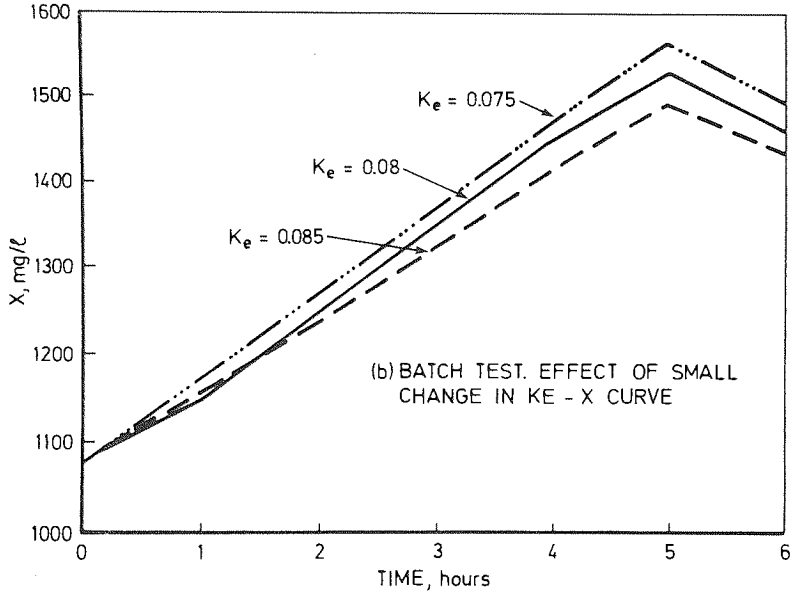
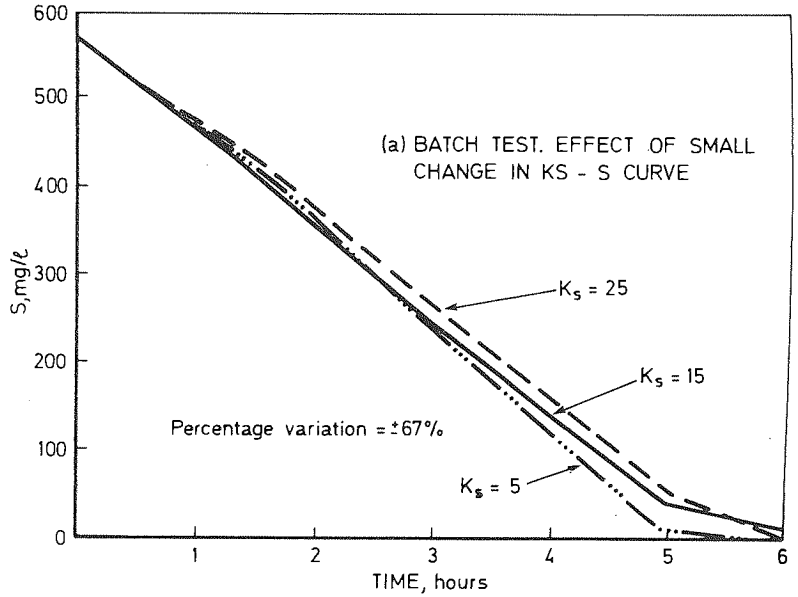


FIG.4.16



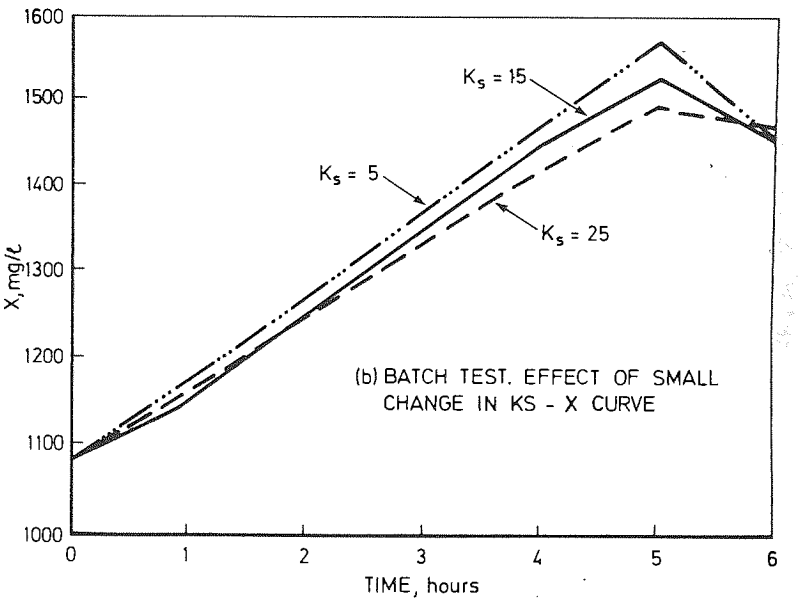


FIG.4.17

CHAPTER 5. ADENOSINE TRIPHOSPHATE (A.T.P.) AS A MEASURE OF ACTIVE BIOMASS

5.1 Introduction

It has been found that the normal methods of enumerating cell populations, namely relating the active biomass concentration to the M.L.S.S. or M.L.V.S.S., do not appear to be applicable to cultures actively growing on thiocyanate in the environment of a coke oven liquor. The reasons for this are not known, but it has been hypothesised that mineral salts may be constantly going in and out of solution during the course of autotrophic thiocyanate oxidation, and that the presence of such precipitated inorganics completely masks the increase in suspended solids concentration due to cellular growth.

The advantages and disadvantages of these methods of enumerating bacterial populations, as well as other methods reported in the literature, were studied in the early stages of the project. A technical note was prepared at that time discussing some of the various methods - see Appendix 8.

Briefly, the available methods are:

1. Determination of M.L.S.S. or M.L.V.S.S.
2. Measurement of oxygen utilisation
3. Measurement of carbon dioxide generation
4. Direct organism counts
5. Indirect organism counts

In order to determine the kinetic coefficients of a sludge by one of the methods previously outlined, there were certain limitations imposed on the choice of method for enumerating active biomass for this project:

(1) Speed of Analysis: The analytical facilities available for this work were very limited. In terms of manpower, there was partial availability - 2 to 3 hours daily - of one analytical chemist, and the author himself. The method chosen must therefore be relatively rapid.

(2) Simplicity: in view of the manpower problems, the method must be simple enough to enable the author - neither chemist nor microbiologist - to accurately and consistently determine cell populations.

(3) Repeatability and Accuracy: Since the tests envisaged place great reliance on a few results over a short time period, the results themselves must be accurate, and consequently repeatable.

Of the available methods of enumerating micro-organisms listed above, only the method of Indirect Organism Counts satisfies all of the three criteria given. The other four fail to satisfy one or more of the criteria. This is discussed more fully in Appendix 8.

There are various ways of measuring active biomass concentrations by Indirect Counts, but each method measures the concentration of a compound or reaction product which is common to all active cellular processes. Some of the available methods are discussed below.

5.2 Assessment of Microbiological Activity by Indirect Counts

In 1964, Levin et al[123] suggested the use of Adenosine Triphosphate (A.T.P.) as a measure of cellular activity. A.T.P. is found in all living cells and is the prime energy donor in cellular life processes. The presence of A.T.P. is only associated with living cells, and it has been found[124] that the amount of A.T.P. per cell remains fairly constant across all phases of the growth cycle.

Measurement of A.T.P. can thus be related to the population of active cells. Its analysis entails extraction of the A.T.P. from a sample of the sludge by chemical means, followed by reaction of the extract with a mixture of luciferin and luciferase. This results in a light-flash, the intensity of which is directly proportional to the amount of A.T.P. liberated, and hence to the number of living organisms present in the sample. An instrument is commercially available which measures the intensity of the light flash, and can be calibrated to output either the A.T.P.

content of the sample, or to the concentration of micro-organisms present.

The main disadvantage of this method is the high cost of the equipment.

Deoxyribonucleic acid (D.N.A.) has been suggested as another parameter of bacterial activity. The D.N.A. concentration of an active sludge appears to be a function of the quantity and types of organisms present[125], but the analysis of D.N.A. is less sensitive than that for A.T.P., and it does not indicate the difference between young and old cells.

Triphenyl tetrazolium chloride (T.T.C.) is an electron acceptor dye that has been used by a number of workers[124,126-128] to measure dehydrogenase activity. During cellular oxidation reactions, T.T.C. gives a visual indication of the rate of reduction by a colour change. The optical density of the solution may be measured and related to cell population. However, doubt has been expressed about this method because of uncertainty over the incubation times and temperatures necessary for accurate and repeatable results. Reported incubation times have ranged from 10 minutes to seventy-two hours at temperatures between 20 ^{0}C and 37 ^{0}C. The presence of oxygen during incubation was also reported to interfere with dehydrogenase results[129].

5.3 Choice of Measurement Technique

This was dictated mainly by the literature reports of other workers, and by the fact that an A.T.P. analyser became available for testing in the latter stages of the project.

Patterson et al[129] concluded that measurement of dehydrogenase activity with T.T.C. presents certain hazards in both technique and interpretation of results. They also discussed the use of D.N.A. as a specific chemical measure of biomass, but preferred the measurement of A.T.P. to any other biochemical parameter. Coakley et al[124] also mentioned A.T.P. in preference to D.N.A., but thought that the high cost of the necessary equipment would inhibit its use. Recently many workers[130-136] have experimented with A.T.P. and all have found it to be a fairly

reliable and quick method of assessing bacterial activity.

When the possibility of estimating active biomass by A.T.P. measurement in this project was first discussed, it was initially discounted because of economic reasons. There was no information available as to whether the A.T.P. content of a sludge from an activated sludge plant treating a coke-oven effluent could be satisfactorily measured. It was feared that some of the toxic constituents of the liquor, such as the mono or polyhydric phenols, would interfere with the analytical method and give meaningless measurements of A.T.P. Varnham[131] found that certain classes of compounds would quench the luminescent response by chemical interference with the enzymatic reaction, and later mentioned in a private discussion[137] that phenols could well prove to be such compounds.

With such doubts expressed as to the efficacy of A.T.P. for bacterial measurement in coke-oven liquors, it would have been foolhardy to have purchased the necessary equipment at that time. Work then continued using M.L.S.S. and later M.L.V.S.S. as estimates of microbial populations, but, as has been detailed in the earlier chapters, this work was without success, at least for enumerating populations of an autotrophic sludge growing on thiocyanate.

When it became evident that some other means of enumerating bacterial populations was necessary, and in fact vital for the continuance of the work, the possibility of using A.T.P. was re-examined. The fears expressed above were overcome when E.I. Du Pont (U.K.) Ltd. offered the loan of an A.T.P. analyser for the purpose of testing its efficiency for samples containing the constituents of coke-oven liquors. A research program was then drawn up to determine.

(a) A standard method for A.T.P. assay.
(b) The quenching effect of the luminescent response by the constituents of a coke-oven liquor.
(c) The optimum assay conditions for the boiling TRIS method.
(d) The optimum A.T.P. extraction method for coke-oven effluents.

(e) The relevance of A.T.P. measurement.

(f) The effect of homogenisation of the mixed liquor prior to sampling.

5.4 A Standard Method for ATP Assay

Equipment

1 x Du Pont Luminescence Biometer
1 x Hamilton syringe - 50 µl.
1 x Shandon Repro-Jector
Reaction cuvettes (Du Pont)
1 x B-D Automatic pipettor - 0.1 ml
Pipettor tips (Du Pont)

Reagents

Luciferin - luciferase reaction powder (vials ex Du Pont)
Buffer salt tablets (Du Pont)
Stabilised water (see Appendix 9)

Method

To prepare a reaction mixture (for 25 assays), one buffer salt tablet is totally dissolved in 3.0 ml. of stabilised water. Then, the luciferin-luciferase reaction powder is slowly added to the buffered solution and mixed by gentle shaking. It is important that no air bubbles are produced by the mixing process, as these will interfere with accuracy when aliquots of the reaction mixture are pipetted. 0.1 ml volumes of this reaction mixture are then dispensed into the reaction cuvettes using the B-D automatic pipettor. The cuvettes containing the reaction mixture are allowed to stand for 20 minutes at room temperature in order to dissipate inherent light.

After extraction of A.T.P. from a sample (see Sections 5.6 and 5.7) aliquots of the extract are injected into the reaction mixture. A 50 microlitre Hamilton syringe, to which is attached a Shandon Repro-Jector to expel the syringe contents at a reproducible velocity, is used for this purpose. In order to prevent sample carryover, the syringe is flushed several times with the solution to be assayed. The syringe is set to deliver a 10 µl

aliquot, and a sample of the extract is then carefully drawn into the syringe. Immediately before assay, this syringe is checked visibly to ensure that no air bubbles or foreign matter have been inadvertently drawn up.

When an acceptable aliquot of the extract has been obtained, the syringe is pushed through a light-tight septum in the biometer, into a reaction cuvette containing the reaction mixture. The 10 µl sample is then injected into the cuvette, and the light resulting from the luminescent reaction is amplified and converted to a digital readout proportional to the A.T.P. content of the sample.

5.5 The Quenching Effect on the Luminescent Response due to the Constituents of a Coke-Oven Liquor

Experimental

A series of four tests was undertaken in this investigation. The aims, method and conclusions of each test are given below. The results of the four tests are given in Table 5.1. A.T.P. was extracted in every case by the boiling-TRIS method.

5.5.1 Test A1 - observation of the quenching effect of a coke-oven effluent containing small concentrations of thiocyanate or phenol.

Solutions

A - 1.0 ml. stock A.T.P. solution* diluted to 1.0 litres with stabilised water. i.e. 1.0×10^9 fg/ml A.T.P. or 1.0 $gm.m^3$ A.T.P.

B - 1.0 ml. stock A.T.P. solution diluted to 1.0 litres with sterilised, treated coke-oven effluent* (COE) to which potassium thiocyanate (10 $gm.m^{-3}$ as CNS) had been added.

C - 1.0 ml. stock A.T.P. solution diluted to 1.0 litres with sterilised, treated COE to which phenol (10 $gm.m^{-3}$ as C_6H_5OH) had been added.

Samples from each solution were assayed for A.T.P. content. The results indicate that whilst the actual A.T.P. content is the same in all three solutions, the indicated A.T.P. is 5-10% less in the samples from solutions B and C.

*Details for the production of stock solutions, and the procedure for glassware cleaning, are given in Appendix 9.

5.5.2 Test A2 - (i) the quenching effect due to unknown constituents of a COE.

(ii) the quenching effect of a COE containing high concentrations of thiocyanate or phenol.

Solutions

A - as in Test A1.

D - 1.0 ml. stock A.T.P. solution diluted to 1.0 litres with sterilised COE.

E - 1.0 ml. stock A.T.P. solution diluted to 1.0 litres with sterilised COE to which 500 $gm.m^{-3}$ CNS had been added.

F - 1.0 ml. stock A.T.P. solution diluted to 1.0 litres with sterilised COE to which 500 $gm.m^{-3}$ C_6H_5OH had been added.

The results of this test show a decrease in indicated A.T.P. of approximately 5% in all solutions made up with COE. There is no significant difference between the COE solutions with, or without thiocyanate or phenol.

5.5.3 Test A3 - the quenching effect of different thiocyanate concentrations.

Solutions

A - as in Test A1.

G - K - 1.0 ml stock A.T.P. solution diluted to 1.0 litres with stabilised water to which thiocyanate in concentrations varying from 60-600 $gm.m^{-3}$ had been added.

These results indicate that thiocyanate levels upto 600 $gm.m^{-3}$ have no quenching effect on the luminescent reaction.

5.5.4 Test A4 - The quenching effect of different phenol concentrations.

Solutions

A - as in test A1.

L and M - 1.0 ml. stock A.T.P. solution diluted to 1.0 litres with stabilised water to which phenol in concentrations of 50 and 500 $gm.m^{-3}$ had been added.

These results indicate that phenol levels up to 500 $gm.m^{-3}$ have no quenching effect on the luminescent reaction.

5.5.5 Results

TABLE 5.1 TESTS ON THE QUENCHING EFFECT OF VARIOUS COMPOUNDS ON THE LUMINESCENCE REACTION

Test	Solution	Constituent and Diluent	Instrument A.T.P. Readings x 10^{8} fg.ml^{-1}	Mean A.T.P. Reading x 10^{8} fg.ml^{-1}	Standard Deviation
	A	stabilised water	9.23, 9.16, 9.03, 8.88, 8.98	9.06	0.14
A1	B	COE + 10 gm.m^{-3} CNS	8.67, 8.44, 8.01, 8.07, 8.40, 8.28	8.31	0.25
	C	COE + 10 gm.m^{-3} Phenol	8.59, 8.68, 8.49, 8.53	8.57	0.08
	A	stabilised water	9.37, 9.30, 9.32	9.33	0.04
A2	D	COE	9.15, 8.75, 8.55, 8.89, 8.82	8.83	0.22
	E	COE + 500 gm.m^{-3} CNS	8.58, 8.88, 9.06, 8.56, 8.80	8.78	0.21
	F	COE + 500 gm.m^{-3} Phenol	8.90, 8.90, 8.50, 8.97, 9.19, 8.77	8.87	0.23
	A	stabilised water	9.1, 9.3	9.2	0.10
	G	H_2O + 60 gm.m^{-3} CNS	9.3, 9.2	9.3	0.10
A3	H	H_2O + 120 gm.m^{-3} CNS	8.3, 9.4, 9.2	9.0	0.60
	I	H_2O + 210 gm.m^{-3} CNS	9.3, 9.3	9.3	0.00
	J	H_2O + 300 gm.m^{-3} CNS	9.5, 9.5	9.5	0.00
	K	H_2O + 500 gm.m^{-3} CNS	8.8, 9.5, 9.2	9.2	0.40
	A	stabilised water	9.2, 9.4	9.3	0.10
A4	L	H_2O + 50 gm.m^{-3} Phenol	9.5, 9.0, 8.9	9.1	0.30
	M	H_2O + 500 gm.m^{-3} Phenol	9.3, 9.2	9.3	0.10

5.5.6 Conclusions

The above tests indicate that neither phenol or thiocyanate at concentrations likely to be found in an activated-sludge mixed liquor significantly affects the luminescent response of the firefly reaction.

However, it appears that other constituents of the coke oven liquor do have a small effect on the instrument's output, decreasing the actual A.T.P. by approximately 5%. Since it is not an absolute value of A.T.P. in which we are interested, but rather a response given by conditions of cellular growth or death, this slight depression of the true A.T.P. content is of little significance in this work.

5.6 Optimum Assay Conditions - Boiling TRIS* Method

A very brief investigation was made into the optimum conditions for extraction of A.T.P. by the boiling TRIS method, and later assay with the biometer. Observations were made upon the effects of

(1) duration of boiling
(2) increased cooling
(3) increased aliquot volume.

It was not possible to undertake a full investigation into the assay method as supplies of necessary chemicals were severely limited. The results of the few investigations made are given below:

(1) TABLE 5.2 OPTIMUM A.T.P. ASSAY CONDITIONS - THE EFFECT OF DIFFERENT BOILING TIMES

Sample	Substrate	Boiling Time min	ATP Readings x 10^6 fg.ml	Mean ATP Reading x 10^6
Autotrophic	CNS	1	7.98, 7.73	7.86
Autotrophic	CNS	5	1.32, 1.41	1.37
Heterotrophic	Phenol	1	9.06, 8.98	9.02
Heterotrophic	Phenol	5	5.36, 6.78	6.07
Mixed	CNS and Phenol	1	0.64, 0.68	0.66
Mixed		5	0.68, 0.65	0.67

* TRIS buffer:- 2-amino-(hydroxymethyl) propane - 1,3 - idol

(2) Effect of Increased Cooling

Six samples were simultaneously taken from a reactor containing an autotrophic sludge degrading thiocyanate. A.T.P. was extracted from each of these samples by the TRIS method. Immediately after extracting A.T.P. by boiling in a Kjeldahl flask, three of the flasks were transferred to an ice bath. The remaining three flasks were immersed in a solution of salt and ice at -12 ^{0}C.

TABLE 5.3 OPTIMUM A.T.P. ASSAY CONDITIONS - THE EFFECT OF INCREASED COOLING

Sample	Coolant Temperature ^{0}C	A.T.P. Readings x 10^{7} fg.ml^{-1}				Mean ATP Reading x 10^{7} fg.ml^{-1}	Standard Deviation
A	0	1.52	1.69	1.65		1.62	.09
B	0	1.68	1.43	1.42		1.51	.15
C	0	1.58	1.53	1.60		1.57	.04
D	-12	1.73	1.77	1.76	1.74	1.75	.02
E	-12	1.53	1.53	1.54		1.53	.01
F	-12	1.76	1.74	1.59	1.69	1.70	.08

(3) Increased Aliquot Volume

Several samples of different sludges were extracted by the TRIS method. Two aliquots of each extract, one of 0.01 ml. volume, the other of 0.02 mls, were assayed for A.T.P.

TABLE 5.4 OPTIMUM A.T.P. ASSAY CONDITIONS - ACCURACY OF REPROJECTOR

Sample	Substrate	A.T.P. Readings x 10^{7} fg.ml^{-1}			Mean ATP Reading x 10^{7} fg.ml^{-1}	Aliquot Volume(mls)
A	CNS	5.46	5.43	5.52	5.47	0.01
A	CNS	10.9	10.82	10.92	10.88	0.02
B	Phenol	85.9	86.8	85.3	86.0	0.01
B	Phenol	162.0	156.0	166.0	161.0	0.02
C	CNS	0.87	0.79	0.86	0.84	0.01
C	and Phenol	1.63	1.68	1.69	1.67	0.02

Conclusions

From the very small number of investigations made, the following points arise.

Table 5.2 indicates that a boiling time of 5 minutes results in decomposition of A.T.P. in both the autotrophic and heterotrophic sludges. No decomposition is found in the third sample. It is likely that the reason for this is the low A.T.P. content of the sample. It would be of benefit to test a much wider range of boiling times, but as explained limitations of chemical supplies prevented this.

Table 5.3 indicates very little difference between the two coolant temperatures. However, it does appear that the lower temperature coolant gives a slightly higher A.T.P. content, and reduced error.

Table 5.4 shows that the Reproject injector is reasonably accurate - i.e. doubling the injected aliquot results in a doubling of indicated A.T.P. It therefore makes no difference whether 0.01 mls or 0.02 mls of extract are assayed.

To conclude therefore, preferred conditions for the boiling TRIS extraction method are;

(1) a boiling time of 1 minute
(2) use of salt-ice mixture for reduced coolant temperature
(3) Reproject injector scale is sufficiently accurate.

5.7 Optimum A.T.P. Extraction Method for Coke-Oven Effluents

Several schemes have been reported in the literature for extraction of A.T.P. from a sample. Possibly the most common method used for activated-sludge samples is the addition of Boiling TRIS Buffer to the sample. This kills the activated-sludge immediately, and the cellular A.T.P. is extracted quantitatively. After cooling and diluting, an aliquot is centrifuged, and if not analysed immediately, it is frozen until required. Amongst the workers that have successfully used this method are Levin et al [123,133], Patterson et al [129], Varnham and Grainger [131] and Statham

and Langton[134]. Other extractant liquids that have been used are butane-1-ol[139], perchloric acid[138], dimethyl-sulfoxide[136] and chloroform[135].

Quantitative extraction of the A.T.P. is the prerequisite condition of accuracy, and all the variations in methods described in the literature are attempts to meet this end. No single method of extraction is universally applicable, and each must be considered on its own merits for each specific material under investigation. Discussions with Dr. Varnham[137] suggested that boiling TRIS buffer was the initial method to try.

This method was in fact used for the first series of tests, the results of which are given in Chapter 6 and 7. However, it was found somewhat cumbersome, entailing much glassware cleaning - a time-consuming job as the glassware has to be chemically and biologically clean - and liable to errors caused by the heating and rapid cooling processes.

Once the possibility of using A.T.P. as a measure of microbial activity had been established in the first series of tests, it was decided to investigate other extraction methods. Three extractant liquids were compared - boiling TRIS, butane-1-ol, and dimethyl sulfoxide (D.M.S.O). The Perchloric acid method was not investigated as a simple and safe method was sought. Perchloric acid is a very strong oxidising agent and needs to be handled with care. Similarly, it was thought unwise to attempt to use chloroform considering the large numbers of samples that need to be quickly analysed. Details of the three extraction methods investigated are given in Appendix 10.

5.7.1 Experimental

The activated-sludge used in these tests was taken from one of the 5.5 l batch reactors (see Fig. 4.6). The contents of the reactor had been homogenised in a domestic cream-maker prior to testing (see Section 5.9). The mixed liquor comprised a sludge actively growing on phenol in a coke-oven effluent environment.

Firstly, two samples of the mixed liquor were simultan-

eously extracted by the butan-1-ol method. The extracts were then assayed for A.T.P. content and the results noted.

Two more samples were then taken, one of which was extracted by TRIS, the other by D.M.S.O. The results of the A.T.P. assays were again noted. This test was repeated 20 minutes later.

Finally, a full batch test was investigated. Thiocyanate was added to a starved culture, and the activity of the culture was monitored by measuring the A.T.P. at regular intervals. The A.T.P. was extracted both by the boiling TRIS and DMSO methods, each method being used alternatively. Residual thiocyanate was also monitored.

5.7.2 Results

TABLE 5.5 COMPARISON OF SIMULTANEOUS A.T.P. SAMPLES EXTRACTED BY BUTAN-1-OL

	A,T,P. Readings x 10^9 $fg.ml^{-1}$				Mean A.T.P.	Standard Deviation
Sample A	3.98;	3.72;	4.81;	4.61	4.28	0.51
Sample B	4.86;	5.26;	4.37;	4.53	4.76	0.39

TABLE 5.6 COMPARISON OF SIMULTANEOUS A.T.P. SAMPLES EXTRACTED BY TRIS AND DMSO

Time mins.	Sample	Extraction Method	A.T.P. Readings x 10^9 $fg.ml^{-1}$				Mean ATP	Standard Deviation
0	A	TRIS	3.26;	3.07;	3.26		3.20	0.11
0	B	DMSO	2.96;	3.25;	3.30;	3.32	3.21	0.17
20	A	TRIS	3.37;	3.47;	3.36		3.40	0.06
20	B	DMSO	3.47;	3.44;	3.41;	3.29	3.40	0.08

TABLE 5.7 THIOCYANATE DECAY-BATCH TEST. COMPARISON OF DMSO AND TRIS EXTRACTION METHODS

Time(hours)	Extraction Method	A.T.P. (mean) $fg.ml^{-1} \times 10^8$	Residual CNS $gm.m^{-3}$	M.L.S.S. $gm.m^{-3}$
0	DMSO	8.34	3.6	1410
0.25	TRIS	7.35	3.6	1432
0.50	DMSO	7.77	3.6	1385
0.75	TRIS	8.54	3.3	1390
1.00	DMSO	15.48	448.0	1475
1.25	TRIS	21.98	422.0	1405
1.50	DMSO	21.24	383.6	1443
1.75	TRIS	24.08	-	-
2.00	DMSO	22.44	287.7	1475
2.25	TRIS	24.99	-	-
2.50	DMSO	24.48	163.0	1480
2.75	TRIS	21.70	-	-
3.00	DMSO	24.72	22.0	1498
3.25	TRIS	7.84	3.8	1458
3.50	DMSO	7.44	3.8	1488
3.75	TRIS	9.87	3.6	1503
4.00	DMSO	9.66	3.8	1510
4.25	TRIS	10.01	-	1568
4.50	DMSO	8.08	-	1515

Discussion

The results indicate that there is very little to choose between the TRIS and DMSO extraction methods for coke-oven works mixed liquors. They both give repeatable results (Table 5.6) and extract similar amounts of A.T.P.

The batch test illustrates this very well. The results of Table 5.7 are plotted in Figure 7.4. Both sets of results show a good fit to the same curve.

Table 5.5 however shows that the butan-1-ol method gives different results for two similar samples. This is probably due to the difficulty encountered in pipetting the extract, as there is only 1 ml. extract available at the bottom of the test-tube. No doubt this method could be improved to make the butan-1-ol

extraction competitive, but in view of the simplicity and reproducibility of the TRIS and DMSO methods, and because of the toxicity of the butan-1-ol fumes, it was decided to forgo any further work on butan-1-ol/octanol extraction.

The choice then lay between TRIS and DMSO. The batch test decided between the two, as samples were taken and analysed every fifteen minutes, for 4½ hours. It was found much easier and quicker to extract with DMSO than with boiling TRIS. The TRIS method suffers on the following counts:

(a) boiling, followed by rapid cooling, involves a temperature change of over 110 °C within 3 minutes. During preceding work, glass flasks had often broken because of this temperature change, thus losing the sample.

(b) a slight variation in boiling time can have a very great effect on the amount of A.T.P. extracted. Too little boiling and the A.T.P. may not be fully extracted; too much, and it will decompose.

(c) the method is cumbersome, requiring vast amounts of ice.

The DMSO method on the other hand was found in practice to be an extremely simple one, and it was thus decided that this was the most suitable method for extracting A.T.P. from the mixed liquor of a coke oven effluent activated-sludge plant.

5.8 The Relevance of A.T.P. Measurement

After the first series of A.T.P. tests, when it became apparent that measurement of A.T.P. was linked in some way to the active biomass content of the sludge, it was felt necessary to gain further knowledge as to the actual relevance of the A.T.P. measure.

As a consequence, Mr. R. Bolus - an M.Sc. project student on the M.Sc. course "Biology of Water Management" at the University of Aston - offered to analyse typical populations of the three cultures grown in the laboratory using microbiological plating techniques.

To recap, the three cultures investigated were:-

(i) A phenol-oxidising culture, nominated the HETEROTROPHIC culture

(ii) A thiocyanate-oxidising culture, nominated the AUTOTROPHIC culture

(iii) A mixed culture from the continuous plant, able to oxidise both phenols and thiocyanate.

The results of this investigation are summarised in Table 5.8 below.

TABLE 5.8 TYPICAL POPULATIONS AND COMPOSITIONS OF LABORATORY CULTURES

		Populations x 10^7 per ml.		
Group	Assay Medium	"Autotrophic" Culture	"Heterotrophic" Culture	Mixed Culture
autotrophs	SCN medium	1.1	0.75	1.0
heterotrophs	Nutrient agar	1.4	4.5	0.7
fluorescent pseud.	Kings B	0.09	1.3	0.4
bacteria	Phenol and	0.42	2.0	1.0
fungi	SCN medium	0.01	0.032	0.067

Several points arise from Table 5.8. Firstly, the assumed labels of heterotrophic and autotrophic cultures previously used are misnomers, as both cultures contain appreciable quantities of both heterotrophs and autotrophs. However, it can be seen that the "heterotrophic" culture is mainly comprised of heterotrophs, with only a relatively small proportion of autotrophs present. The "autotrophic" culture on the other hand contains both heterotrophs and autotrophs in approximately equal proportions. It is not known whether the heterotrophs present in this culture have the ability to oxidise thiocyanate, or whether they exist due to the residual polyhydric phenols present in the base liquor. The reason is not of great importance in this project, as we are investigating the kinetics of a culture which oxidises thiocyanate, which culture we have labelled the "autotrophic" culture to distinguish it from the phenol-oxidising culture.

What is important is that two separate cultures are shown to be present, one predominantly heterotrophic, and the other with a much larger proportion (almost 50%) of autotrophs, and that these correspond to the labels given.

The lack of fungi in all three cultures is indicative of a healthy state of affairs. Fungi are not normally present in activated-sludge plants treating coke-oven effluents unless the plant is in a poor state of health. Excessive residence time and sludge-age tends to promote fungal growth.

The most important observations from Table 5.8, at least in relation to A.T.P. measurement, are the orders of magnitude of the bacterial populations. In each of the cultures, there are something like 1 x 10^7 cells per ml. The range of A.T.P. values previously encountered from similar cultures has been 0.1 to 10 x 10^7 fg. per ml.

Du Pont advise a standard conversion factor of 0.5 fg. A.T.P. per cell[140]. Thus the cell populations of the three cultures have ranged from 0.2 to 20 x 10^7 cells/ml as measured by the luminescence biometer. This is the same order of magnitude as the populations determined by microbiological plating techniques.

What then is A.T.P. and why can it be used to enumerate bacterial populations?

A.T.P. is found in all living cells and is the prime energy donor in cellular life processes. A.T.P. has aptly been termed the "currency" of the energy metabolism in living cells. A schematic of the A.T.P. molecule is shown below in Figure 5.1.

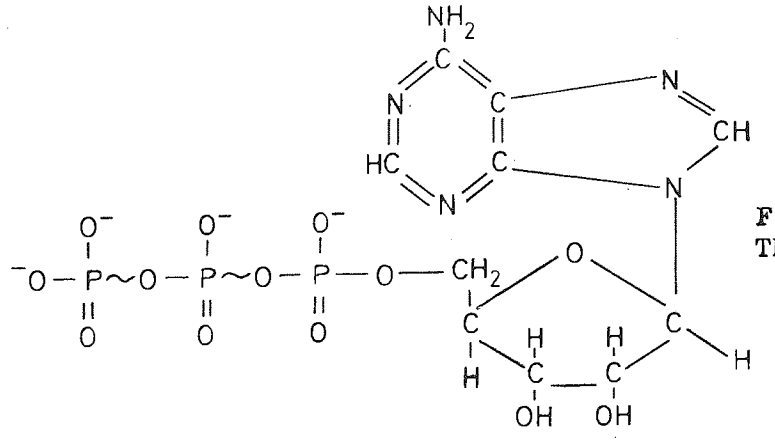
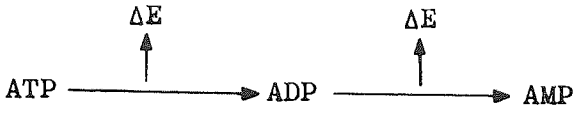


FIG. 5.1
THE ATP MOLECULE

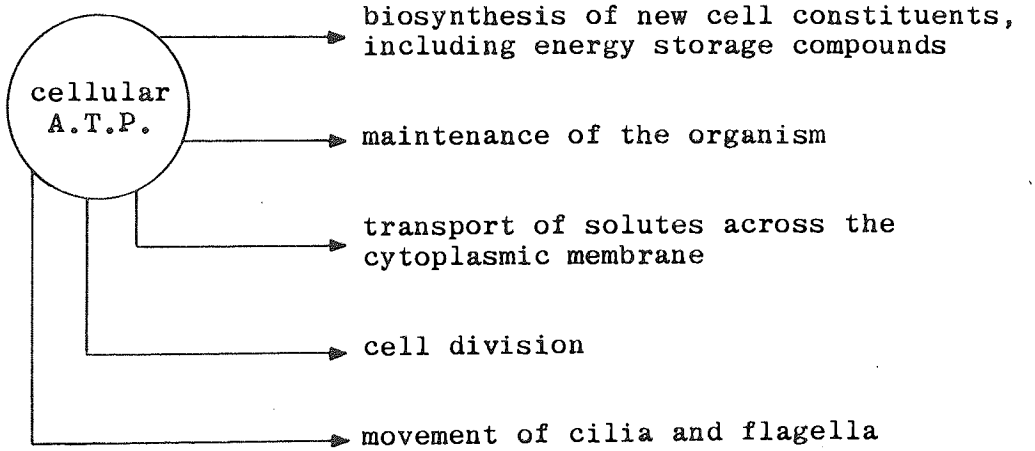
The biochemically utilisable energy in the molecule is stored in the two terminal energy-rich phosphate bonds (designated by the symbol ~). It is thus that when A.T.P. is utilised as an energy source, the reaction products are normally Adenosine di-phosphate (ADP) or adenosine monophosphate (AMP).

Thus



Energy from Cellular ATP is used to initiate or sustain many of the necessary metabolic processes of active micro-organisms. Figure 5.2 illustrates some of the processes in which A.T.P. is known to act as an energy donor.

Figure 5.2 A.T.P. Expenditure Processes



Synthesis of A.T.P. from ADP occurs during the catabolism of dietary materials by living organisms. Thus the three adenine nucleotides (ATP, ADP and AMP) act as metabolic energy mediators and are ideally placed to regulate the whole metabolic economy of the organism[141].

Because of its fundamental role in cellular processes, ATP seems an attractive parameter to study the response of cells to their environment (e.g. the response of activated-sludge organisms to inhibitors, substrate concentration, temperature etc.).

ATP is also believed to be indicative of the concentration of active biomass. D'Eustachio and Johnson[142] and Allen[143] found that the amount of ATP per cell remains fairly constant across all phases of the growth cycle. A study of thirteen representative species of bacteria showed a range from 2.2 to 10.3 x 10^{-10} µgATP per cell, and indicated a mean of 5 x 10^{-10} µgATP/cell could be used as an ATP-to-bacteria conversion factor[142]. Levin et al[133] grew cultures of E.coli , Z.ramigera, and Bacillus sp. and analysed their ATP content at several time intervals using a luminescence biometer. They too found that during the growth cycle, the ATP content in µgATP per cell remained reasonably constant, although a wider range of ATP content per cell was found for the three species - 0.74 to 13 x 10^{-10} µgATP per cell.

If then it is accepted that the amount of ATP per cell remains reasonably constant across all phases of the growth cycle, it follows that an increase in active biomass concentration due to growth should result in a proportionate increase in total ATP content. It does not matter that the conversion factor for ATP-to-bacteria may not be accurately known, as we are not interested in the absolute enumeration of bacteria; rather we are interested in changes in concentration due to growth and to nutrient deficiency.

In conclusion, it appears that measurement of the ATP content of a culture will be proportionate to the total active biomass concentration. The choice of conversion factor (ATP-to-bacteria) will affect the absolute figures obtained, but if a particular conversion factor is exclusively used, absolute enumeration of bacteria is unimportant. By observing the changes in ATP content of a batch culture oxidising either thiocyanate or phenol, it should be possible to determine the kinetic coefficients of the separate cultures based on changes in active mass.

5.9 The Effect of Homogenisation of the Mixed Liquor Prior to Sampling

When enumerating bacterial populations by direct means - such as microbiological plating techniques or ATP, TTC or DNA estimation, it is vital that a representative sample is obtained. An activated-sludge mixed liquor comprises particulate matter of

varying size and shape. The particles consist of clumped bacterial cells and solid matter agglomerated or flocculated by bacteria, (Pipes [144]). Bacteria are also present as isolated cells or filaments. Thus, to enable accurate enumeration of the micro-organisms, some means of homogenising the mixed liquor is required.

Pike et al[145] found that sonication of a sample of activated-sludge mixed liquor reduced the viable count by less than 10% but that homogenisation generally gave much higher counts. They recommended sonication of the sample in an ultra-sonic bath for one minute because of the reduced errors in bacterial enumeration, and because of the simplicity of the method. They reported a reduction in the coefficient of variation of similar samples from 69% piror to homogenisation, to 12% after homogenisation.

Gayford and Richards[146] combined the use of a laboratory homogenisor with a deflocculating agent - a mixture of sodium pyrophosphate and Lubrol W -and found a threefold increase in viable counts. Williams et al[147] using a novel ultra-sonic instrument of their own design, found a twenty-fold increase in total heterotrophs and thiobacillus-type organisms. They reported that sonication appears to release cells from their flocs without affecting their viability.

Although each of these workers has found homogenisation of the sample to be beneficial in respect of reducing errors due to sampling, there seems to be some difference of opinion as to the effect of homogenisation (or sonication) on the viable count.

There was unfortunately insufficient time to carry out a full investigation into the effects of homogenisation and soni-cation of samples of the coke-oven effluent activated-sludge mixed liquor either prior to, or following sample extraction. However, a brief test was made on the effects of substrate degradation by homogenisation of the mixed liquor prior to sampling.

Three reactors, as illustrated in Figure 4.6, were used in this test. A sludge, acclimatised to thiocyanate, was completely

mixed, and split into three equal volumes. One such volume was homogenised with a domestic cream-maker prior to testing; a second volume was given 3 minutes of sonication at 50 watts, using an ULTRASONICS RAPIDIS 50 disintegrator, fitted with a 9 mm tip[148]. This volume was divided into 150 ml samples for sonication, and then remixed. The third volume was not homogenised in any way.

Fresh, treated-coke oven effluent and necessary nutrients (see Section 4.3.1) was added to each of the sludges, the volume in each reactor being made up to 4.0 litres. Thiocyanate, in equal concentrations, was then added to each reactor, and aeration begun. The temperature of each reactor was held constant at 20 $^{0}C \pm 0.5\ ^{0}C$, and the pH maintained at pH 7.0 ± 0.2.

Table 5.9 gives the results of this test.

TABLE 5.9 EFFECT OF HOMOGENISATION ON SUBSTRATE REMOVAL EFFICIENCY

	Reactor 1	Reactor 2	Reactor 3
Observations	No Homogenisation	Sludge Homogenised with Domestic Cream Maker	3 Minutes Sonication of Sludge
Time (hours)	No Foaming	Excessive Foaming	Foaming
	Residual CNS $gm.m^{-3}$	Residual CNS $gm.m^{-3}$	Residual CNS $gm.m^{-3}$
0	404.1	406.9	417.8
½	364.4	401.4	401.4
1	297.3	374.0	364.4
1½	220.6	326.1	306.9
2	137.0	297.3	238.4
2½	41.6	267.2	179.5
3	2.7	231.5	110.0
3½	-	200.0	42.5
4	-	139.2	3.6
4½	-	46.8	-

The results given in Table 5.9 suggest that homogenisation of the activated-sludge causes foaming in the reactor, and has a deleterious effect on the oxidative efficiency of the sludge. It is reasonable to hypothesise that there was disruption of the cells particularly when homogenised with the domestic cream-maker, and that the overall viability of the sludge was thus reduced.

However, it would be unwise to place too much reliance on these results, as only this one brief test was undertaken, and no measurement of the bacterial mass in each reactor was undertaken.

As will be seen in the next two chapters, the sludges under investigation were homogenised in the later tests. It was found that homogenisation of the mixed-liquor enabled extraction of a much improved representative sample.

CHAPTER 6. A MODEL FOR THE BATCH BIODEGRADATION OF PHENOLS IN COKE-OVEN EFFLUENTS

Chapter 4 has shown that the kinetics of a heterotrophic sludge may be approximated using MLSS or MLVSS as a measure of biomass. However it was found impossible to similarly determine the kinetics of an autotrophic system. A more accurate and representative measure of active cellular concentration is needed to determine the concentration of living cells in an autotrophic system. The results of the initial tests of Chapter 5 suggest that ATP is such a measure.

Batch tests were carried out on both the heterotrophic and autotrophic systems in an attempt to determine their separate kinetic coefficients. ATP was used as a measure of biomass in both cases, and the responses to batch biodegradation of substrate were determined. It was found that in general the response of each system was very different, and that the standard Monod model needed to be altered to fit the observed data.

The results obtained, models developed and numerical values found for the kinetic coefficients of the two sludges are given in Chapters 6 and 7.

6.1 Experimental Procedure

The 5.5 litre batch reactors as illustrated by Fig. 4.6 were used in all of the tests. The pH and temperature of the system were controlled as outlined in Section 4.7. The Dissolved Oxygen (D.O) concentration was occasionally measured to ensure that sufficient available oxygen was present. Due to the efficiency of the diffuser stones used, the D.O. was normally about 10 $gm.m^{-3}$.

The diluent for the Mixed Liquor was, as before, completely treated coke-oven effluent to which growth-inducing nutrients had been added. The composition of this diluent is given in Section 4.3.1. Heterotrophic sludge as cultured in Section 4.3.2 was added to this diluent and the resultant mixture comprised the Mixed Liquor. Between batch tests, phenol was added to the

aerated, completely-mixed liquor to prevent starvation of the micro-organisms. A wastage rate of between 10% and 20% of the Mixed Liquor was maintained daily to ensure both minimising the effect of inhibitors and maintenance of an actively-growing culture.

The contents of the reactor were allowed to settle periodically, normally once or twice each week, and the supernatent was discarded. Fresh culture media was then added. This process prevented the accumulation of toxic materials produced during the oxidation of the substrate.

The actual conditions caused for each of the batch tests differed according to the type of information sought. Normally however, the culture was left in the absence of substrate for several hours to ensure that the micro-organisms were in a starved state. Phenol would then be added to the mixed liquor. Sampling would begin just prior to addition of substrate, and continue until steady-state conditions had been re-attained. Each sample was analysed for ATP content and residual phenol concentration. In the first few tests, the samples were also analysed for MLSS and MLVSS. This was discontinued later because of the much increased work load.

Because of the rapidity of the ATP analysis, it was found possible to monitor the cell concentration whilst the test was proceeding. In this way, the end-point of each test, i.e. that stage at which the ATP or cell concentration attained a steady value, could be observed. Approximate analyses were also made for the phenol content of the sample using a Lovibond Comparator. In this way it was found possible to determine the point at which substrate became limiting. Sampling frequency would then be increased so that the effect of substrate limiting conditions on cellular populations could be observed more accurately.

6.1.1 Sampling Technique

Two separate samples were taken, one for ATP content and another for determination of residual phenol, MLSS and MLVSS. A 50 ml beaker was used to extract a representative sample from the reactor. Whilst vigourously agitating the contents of this

beaker, 40 ml were measured into a pre-weighed 50 ml nominal capacity centrifuge tube containing one drop of saturated mercuric chloride. The mercuric chloride has the effect of "killing" the micro-organisms in the sample, thus freezing residual phenol concentration and suspended solids concentration at the moment of sampling. The contents of the centrifuge tube were immediately stirred, and then left whilst another sample of the mixed liquor was taken for ATP assay.

Approximately 20 ml of the mixed liquor was removed from the reactor in a 50 ml beaker. Whilst vigourously agitating the contents of this beaker, a 1.0 ml sample was quickly withdrawn using a disposable, sterile, microbiological pipette. The A.T.P. content of this sample was immediately extracted using either the boiling TRIS or neutral DMSO extraction method as given in Appendix 10. On completion of the extraction stage the ATP count for the sample was determined from the biometer using the technique given in Section 5.4. After noting this count, the "frozen" sample in the centrifuge tube was prepared for analysis as outlined in Section 4.5.1.

Figure 6.1 illustrates the technique and time periods involved in the sampling and subsequent analysis of each sample. Extraction Method in this case is DMSO.

Time minutes/secs	0/00	0/10	0/20	3/00	4/00	24/00
Action	Withdraw 40 ml sample to centrifuge tube. "Freeze" with $HgCl_2$	Withdraw 20 ml sample; pipette 1.0 ml to extractant liquor	Extract ATP (DMSO method)	Assay for ATP Note count	Centrifuge 40 ml sample	Analyse supernatent for phenol; sludge for MLSS and MLVSS

FIG. 6.1 SAMPLING TECHNIQUE AND TIMING

In the later tests, those Runs designated ATP-P7 to ATP-P12, the mixed-liquor was homogenised with either a domestic cream maker or an Ultrasonics disintegrator in order to improve upon representative sampling.

6.1.2 Analytical Methods

The analytical methods for MLSS and mono-phenols are given in Section 4.5.1; that for MLVSS in Section 4.10.1.

Approximate phenol assays were made using a Lovibond comparator. The method is given in Appendix 6.

Two ATP extraction methods were used, the boiling TRIS and the neutral DMSO methods. Details of these are given in Appendix 10. The procedure for ATP assay is given in Section 5.4

6.2 Results

The results of all the batch tests made on the heterotrophic sludge are given in Tables 6.1 to 6.12. Above each set of results, the aim of that particular test is shown. These results are interpreted graphically in Figures 6.2 to 6.12.

6.3 Discussion

Several points arise from the results. Firstly, and most importantly, a decline in ATP simultaneous with the decrease in substrate is shown in every case. Such results were wholly unexpected as, according to conventional kinetics, as substrate is utilised, bacteria multiply and active mass as measured by suspended solids content will rise. This was observed in practice in the earlier experiments detailed in Chapter 4. Given the assumption that the ATP per cell ratio is constant (Section 5.8), the data observed in Tables 6.1 to 6.12 cannot be explained by conventional Monod kinetics, and development of the Monod model must therefore be undertaken.

A second point arising from the results is the degree of fit of the ATP data to curves drawn through the points. Particularly in the first two runs, a great deal of scatter is shown in the data (Figures 6.2 and 6.3). In the later runs (Figures 6.7 to 6.12) the scatter is very much reduced. The boiling TRIS extraction method was used in Runs ATP-P1 to ATP-P3, and the indicated ATP count for each sample was usually taken as the mean of three assays. In all of the other tests, the extraction method was DMSO, and as sufficient confidence in the repeatability of results using this method had been obtainted from Run ATP-P7 onwards, only one ATP assay per sample was normally made.

"continued P.152"

AIM Both tests to observe curve shapes.

TABLE 6.1 RUN ATP-P1

Time h	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	ATP $gm.m^{-3}$
0	1560	2752	4.65
	1550	2737	3.60
	1540	2785	3.50
½	1480	2832	4.55
1	1390	2882	4.00
2	1245	3087	1.9
3	1065	2912	4.8
4	830	3080	4.7
5	670	3177	4.1
6	521	3242	3.1
7	355	3382	3.8
8	129	3460	3.2
8	62	3492	1.15
8½	31	3458	3.2
8	9	3508	1.75
9	9.3	3460	0.57

TABLE 6.2 RUN ATP-P2

Time h	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	MLVSS $gm.m^{-3}$	ATP $gm.m^{-3}$
0	1380	7230	6963	8.65
½	1260	7270	6919	20.0
1	1165	7660	7237	22.25
1½	1035	7508	7242	28.5
2	925	6538	6258	17.75
3	695	7225	6917	19.5
4	500	7450	7136	14.4
4½	390	7263	6969	9.15
5	266	7308	6996	11.9
5½	180	7508	7188	11.9
6	80	8285	7620	16.2
6	55	8175	7456	8.75
6	33	8263	8003	15.3
6½	17	8538	8127	16.7
6	10.7	8523	8031	15.8
7	10.3	8505	8076	14.6

Comments

Boiling TRIS Extraction method in both tests.

ATP results normally the mean of 3 individual assays.

Ratio of MLVSS: MLSS varies between 94-97%; i.e. remains reasonably constant.

Observed variation between three ATP assays = ± 10% of mean value.

AIM To observe curve shape

TABLE 6.3 RUN ATP-P3

Time h	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	MLVSS $gm.m^{-3}$	ATP $gm.m^{-3}$
0	1185	1862	1257	1.63
½	1150	3052	2036	1.87
1	1140	3122	2094	2.24
1½	1115	2887	1904	1.79
2½	1060	2792	1824	-
3½	995	2710	1774	1.67

Comments

(1) Test discontinued because phenol decay too slow.

(2) MLVSS: MLSS ratio between 65-68%.

(3) ATP results normally mean of 3.

(4) Extraction method TRIS.

AIM To observe ATP pool during starvation conditions, and to observe effect on pool of sudden loading

TABLE 6.4 RUN ATP-P4

Time minutes	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	4.5	9.5
15	4.1	10.0
30	4.1	10.0
45	4.4	10.0
105	4.5	11.2
107	940	11.4
110	885	12.8
115	890	11.6
120	895	12.9
130	860	13.9
175	750	13.4

Comments

(1) Extraction method DMSO.

(2) Maximum observed error ±3% of mean for 3 ATP assays of 1 sample.

AIM Using DMSO extraction method, to gain impression of curve shape.

TABLE 6.5 RUN ATP-P5

Time h	Phenol $gm.m^{-3}$	ATP Instrument Reading			ATP Mean $\times 10^{8}$ $fg.ml^{-1}$	ATP Concentration $gm.m^{-3}$
0	986	1.69	1.68		1.69	10.1
½	903	2.11	2.14		2.13	12.78
1	797	1.83	1.94	1.88	1.88	11.28
2	609	2.06	2.11		2.09	12.54
3	416	1.65	1.65		1.65	9.90
4	106	1.24	1.40	1.43	1.42	8.52
5	4.5	1.80	1.83		1.82	10.92
6	3.0	1.54	1.56		1.55	9.30
24	3.1	1.40	1.39		1.40	8.40

Comments

(1) As shown, repeated assays are very close. Three assays are made only when first two differ significantly.

(2) The "mean" ATP reading is the mean of the 2 "best" results.

(3) ATP concentration is determined from the following formula:

$$\text{ATP in } gm.m^{-3} = \frac{\text{mean indicated ATP} \times 10^{7} \times \text{dilution factor of extraction method}}{10^{9}}$$

$$= \text{mean } \frac{\text{ATP} \times 10^{7} \times 60}{10^{9}} \quad \text{for DMSO method}$$

AIM To determine Endogenous Decay Rate

TABLE 6.6 RUN ATP-P6

Time hours	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$	$\log_e$ATP
0	3.4	9.84	2.29
½	3.6	10.56	2.36
1	-	10.62	2.36
2	-	9.78	2.28
3	3.6	9.00	2.20
4	-	8.82	2.18
5	3.1	9.12	2.21
6	-	8.76	2.17
24	3.1	6.48	1.87

Theory

If a culture is left in the absence of nutrient, the population of cells will decline. This is expressed in terms of Monod kinetics by -

$$-\frac{dx}{dt} = k_e X$$

From which

$$\log_e X = -k_e t + C$$

If the logarithm of the cell population is plotted against time, the gradient of the resultant linear curve will equal k_e, the endogenous decay coefficient.

Comments

(1) Extraction Method DMSO.

(2) ATP results normally the mean of two. As before, if two readings are dissimilar, a third is taken and the mean of the two "best" is noted.

(3) The above points do not show very good linearity (see Figure 6.6). However, by drawing a line through the data k_e may be approximated as:

$$k_e \simeq 0.02 \text{ hours}^{-1}$$

AIM To observe curve shape

TABLE 6.7 RUN ATP-P7

Time h	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	2.7	9.42
¼	324	11.40
½	285	10.44
	220	9.24
1	168	7.92
1½	70	5.64
2	3.4	8.52
2¼	3.6	10.02
2½	3.2	11.04
2	-	11.64
3	-	11.82
3½	-	11.22
3	-	10.98
4	-	9.90
4¼	-	10.68
4½	-	10.44

TABLE 6.8 RUN ATP-P8

Time minutes	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	8.2	10.02
15	8.5	10.44
30	8.4	10.82
40	312	13.98
50	280	14.40
60	280	14.64
75	219	14.28
90	125	12.84
120	69	11.76
150	19	12.78
180	8.7	13.68
210	8.4	13.50
240	-	13.56
255	-	13.14
270	-	13.08
285	-	13.56
285		13.56
300		13.68

Comments

(1) Mixed Liquor homogenised in each test.

(2) Extraction method DMSO.

(3) Normally, only one ATP assay was made per sample. This was because of the high cost of necessary chemicals, and increased confidence in the results obtained. As the ATP results were plotted whilst the run was in progress, any obviously deviant result was repeated.

AIM Both runs to observe curve shapes. Run ATP-P9 to observe effects of phosphate limiting conditions.

TABLE 6.9 RUN ATP-P9

Time minutes	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	8.3	13.68
20	4.5	11.82
40	4.3	12.12
60	4.3	13.50
65	PHENOL SUBSTRATE ADDED	
73	346	11.94
80	326	7.38
90	291	10.26
105	231	8.58
120	152	8.10
150	12	7.26
180	6.8	11.16
200	4.3	11.04
220	4.1	11.16
240	-	10.74
253	PO_4 (10 $gm.m^{-3}$) ADDED	
255	-	10.74
270	-	12.72
285	-	13.74
300	-	13.20
350	-	14.46
375	-	14.22
390	-	14.64

TABLE 6.10 RUN ATP-P10

Time minutes	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	8.2	11.88
20	4.3	12.54
40	3.9	13.86
60	4.1	12.36
65	PHENOL SUBSTRATE ADDED	
70	791	13.86
80	738	12.54
90	745	12.96
105	718	11.70
120	686	11.76
150	587	10.62
180	523	10.38
205	383	9.90
235	294	9.54
265	158	9.18
295	26	8.46
325	4	14.04
355	4	14.58
370	3.8	15.90
385	3.9	15.42

Comments

Only 1 ATP assay per sample for both runs. Extraction method DMSO. No phosphate was added when Run ATP-P9 commenced. Mixed liquor of Run ATP-P9 homogenised, that of Run ATP-P10 dispersed ultrasonically.

AIM To observe curve shape. Also to ensure that curve shapes previously found were not due to phosphate - limiting conditions

TABLE 6.11 RUN ATP-P11

Time minutes	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	3.6	17.04
20	3.9	14.4
50	3.4	14.28
65 PHENOL SUBSTRATE ADDED		
70	565	13.26
85	510	13.14
100	455	10.92
120	375	9.06
165	175	6.84
210	3.6	10.44
230	4.3	12.54
250	4.7	12.9
270	6.3	13.14
300	7.5	13.92
330	5.0	14.1

Method

Previous tests have shows that the ATP declines as the phenolic substrate is metabolised. It was thought that this decline might be deficiency of phosphate.

When phosphate (as sodium hexametaphosphate) is added to a coke-oven liquor, a precipitate is always observed. This precipitate is caused by the very high concentrations of calcium in the effluent - the addition of phosphate precipitating the calcium as calcium phosphate.

For Run ATP-P11, most of the calcium in the base coke-oven effluent was removed raising by the pH of the effluent to 12.0 with concentrated NaOH, discarding the precipitate, and neutralising the supernatent with concentrated HCl. When phosphate was added to this liquor, it immediately went into solution, and no signs of precipitation were observed.

Observations

A similar pattern of ATP decline during substrate metabolism is shown, suggesting that the previous tests were not made under phosphate-limiting conditions.

Comments

Extraction method DMSO. One ATP assay per sample. Phosphate concentration in de-calcified liquor approximately 20 $gm.m^{-3}$. Mixed liquor homogenised.

AIM To observe curve-shape

TABLE 6.12 RUN ATP-P12

Time hours	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	4.7	10.40
¼	4.4	10.27
½	366	11.92
	297	10.43
1	232	9.01
1½	96	7.57
1	28	7.18
2	4.3	8.92
2½	-	11.26
3	-	11.37
3½	-	10.73
4	-	10.97

Comments

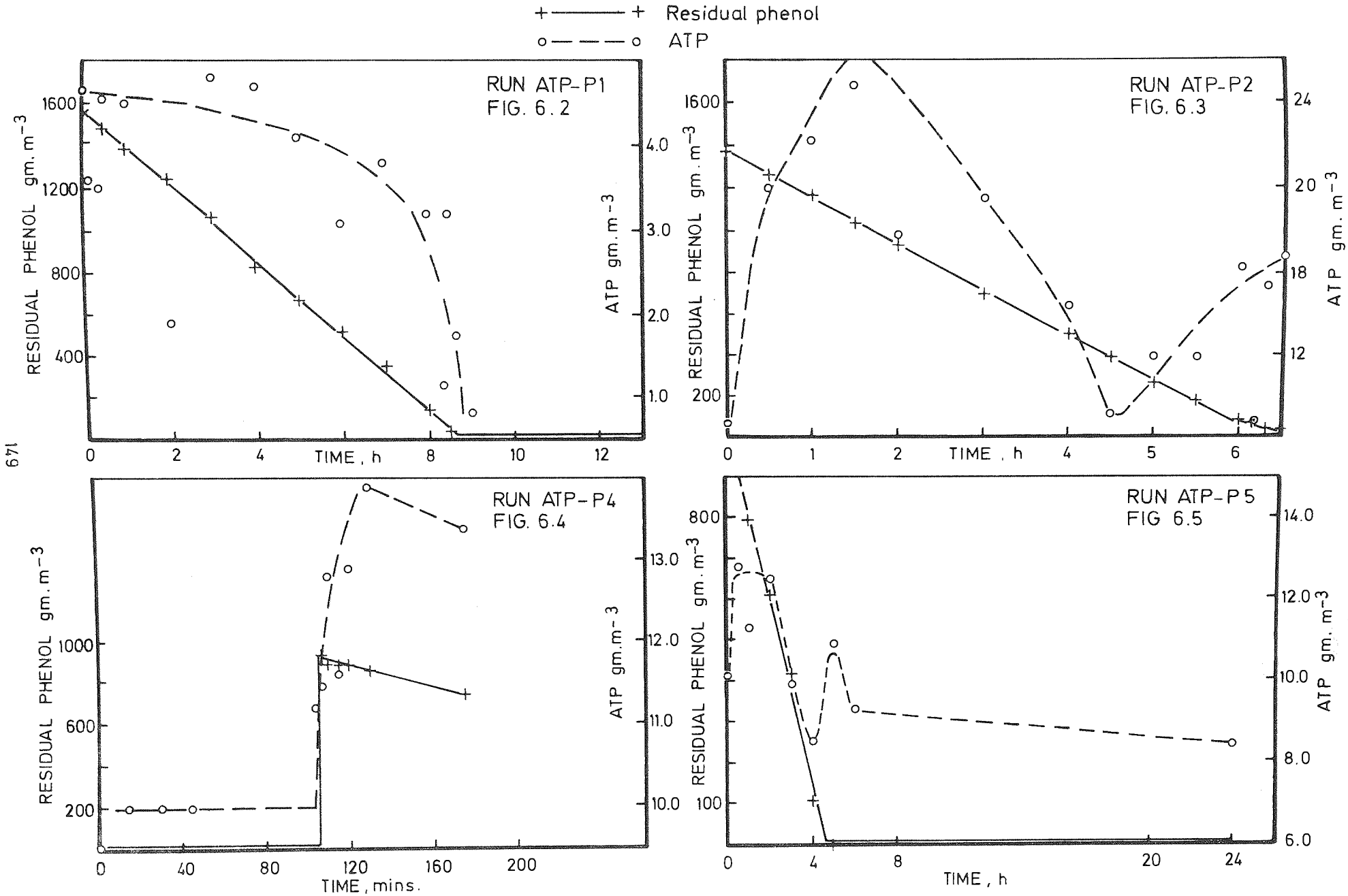
(1) Calcium in coke-oven effluent removed as in Run ATP-P11.

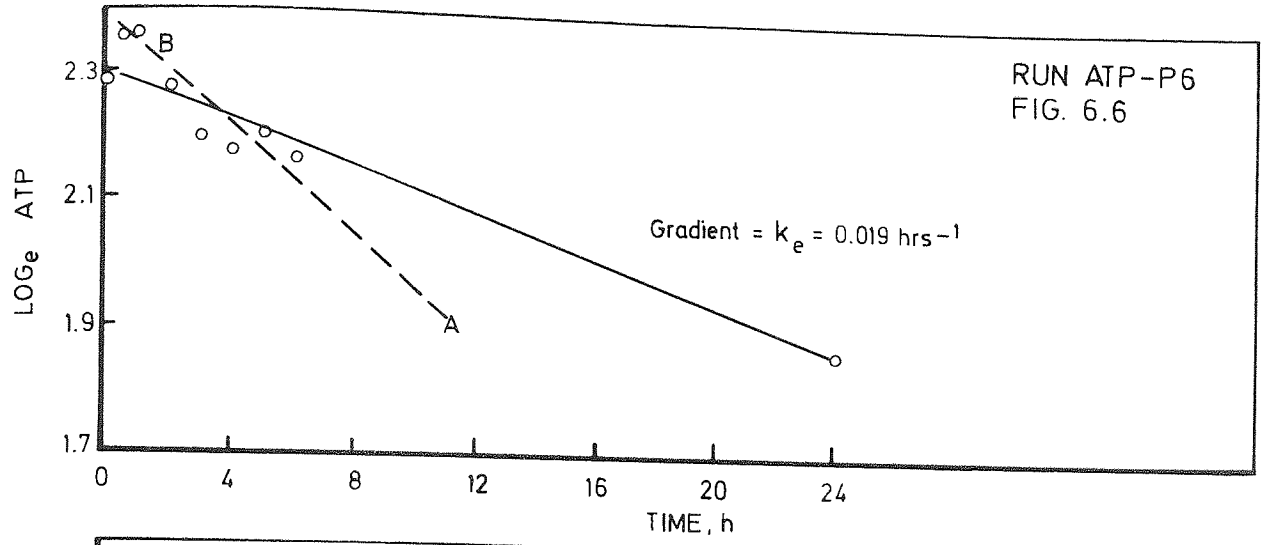
(2) 10 $gm.m^{-3}$ phosphate added to mixed liquor prior to substrate addition.

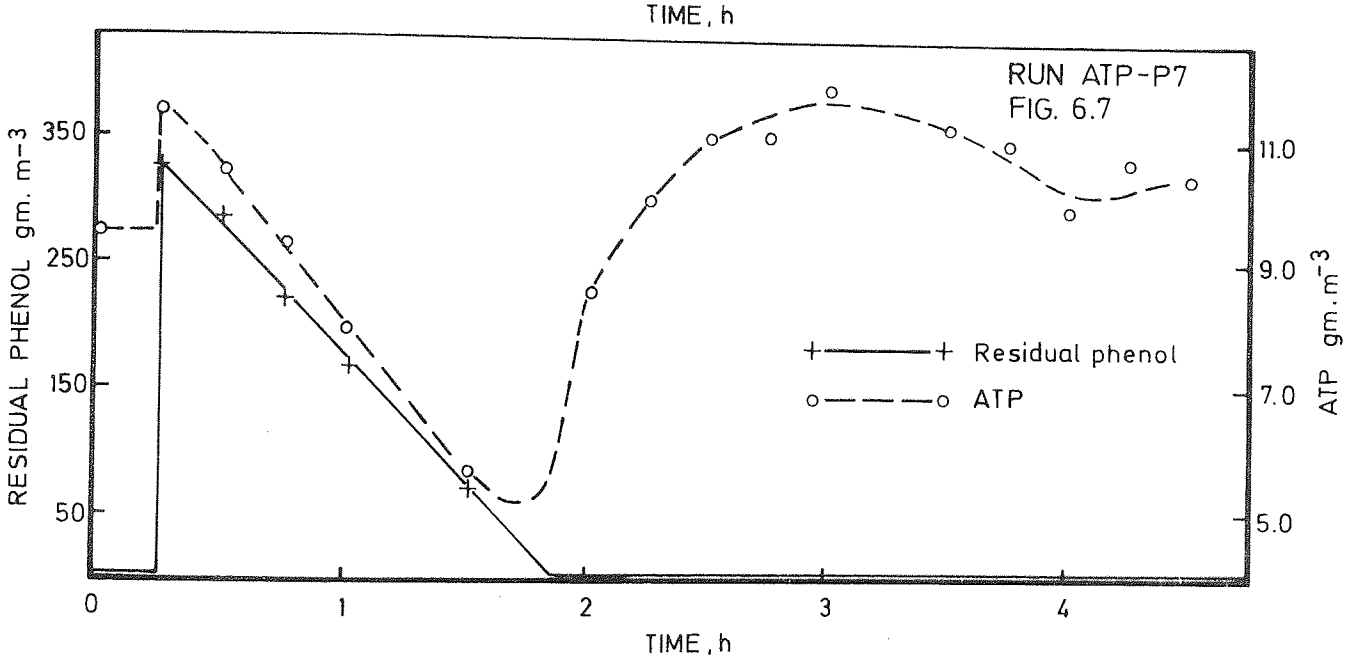
(3) Extraction method DMSO.

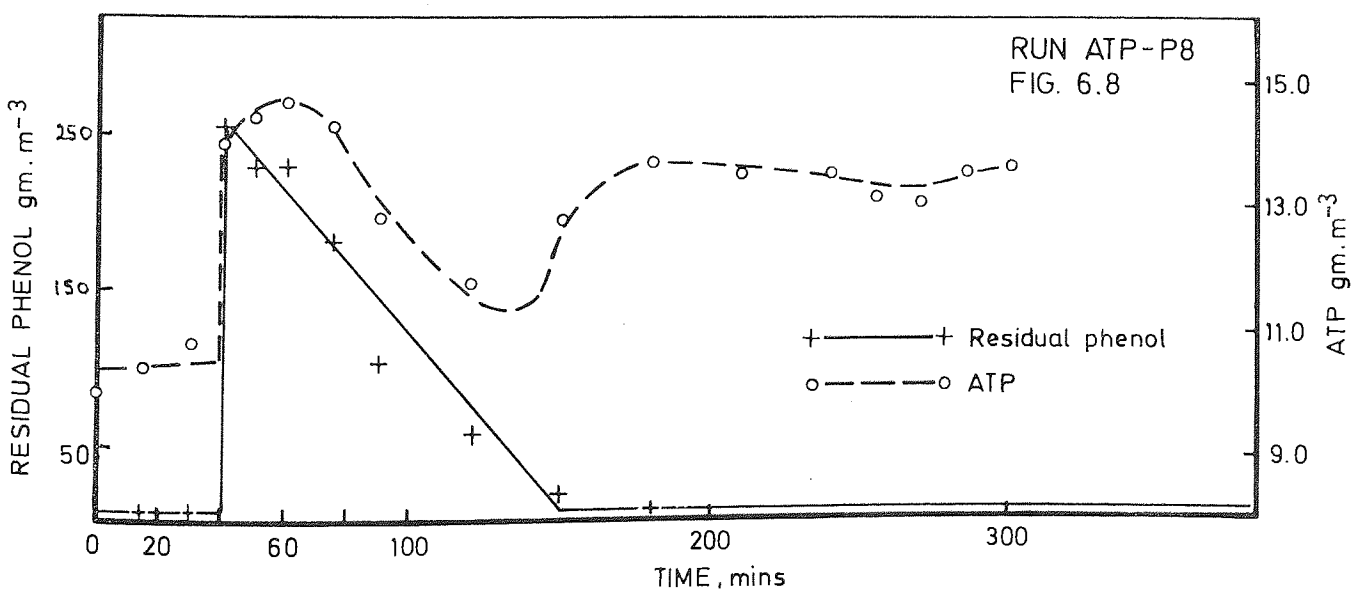
(4) One ATP assay per sample.

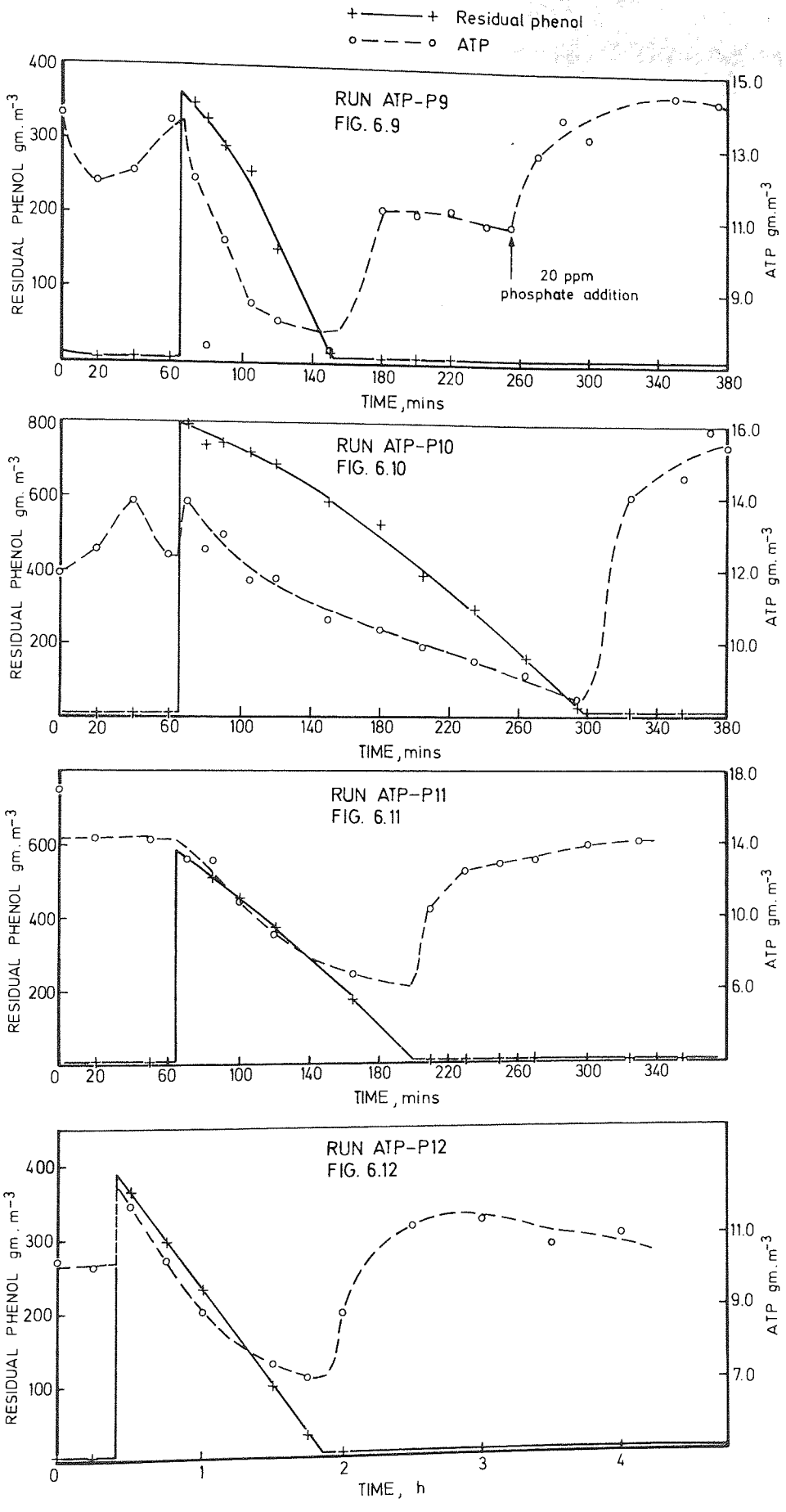
(5) Mixed Liquor homogenised.


+——— + Residual phenol
o— — — o ATP
RUN ATP-P1
FIG. 6.2
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, h
RUN ATP-P2
FIG. 6.3
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, h
RUN ATP-P4
FIG. 6.4
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, mins.
RUN ATP-P5
FIG 6.5
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, h


RUN ATP-P6
FIG. 6.6
B
A
Gradient = k_e = 0.019 hrs^{-1}
LOG$_e$ ATP
2.3
2.1
1.9
1.7
0
4
8
12
16
20
24
TIME, h


RUN ATP-P7
FIG. 6.7
RESIDUAL PHENOL gm. m^{-3}
ATP gm.m^{-3}
350
250
150
50
11.0
9.0
7.0
5.0
Residual phenol
ATP
0
1
2
3
4
TIME, h


RUN ATP-P8
FIG. 6.8
RESIDUAL PHENOL gm. m^{-3}
ATP gm.m^{-3}
250
150
50
15.0
13.0
11.0
9.0
Residual phenol
ATP
0
20
60
100
200
300
TIME, mins


+——+ Residual phenol
o– – –o ATP
RUN ATP-P9
FIG. 6.9
20 ppm
phosphate addition
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, mins
RUN ATP-P10
FIG. 6.10
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, mins
RUN ATP-P11
FIG. 6.11
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, mins
RUN ATP-P12
FIG. 6.12
RESIDUAL PHENOL gm.m-3
ATP gm.m-3
TIME, h

Run ATP-P6 was an attempt to determine a value of the endogenous decay coefficient (k_e) by measuring the decay of active biomass against time in the absence of substrate. The data does not show very good linearity. It may be that at this stage the ATP analytical method had not been sufficiently well developed. Alternatively, the last point on the curve - the one at 24 hours, might be a rogue point. If this was the case, a better fit might have been obtained by the dotted-line A-B. This test should be repeated with more samples taken between 6 hours and 24 hours. It was not possible to repeat the test at the time due to the shortage of necessary chemicals.

Run ATP-P9 shows the necessity of phosphate availability. In the absence of phosphate, cellular multiplication was severely curtailed, although it appears that in this particular test metabolism of the substrate did proceed at an apparently normal rate. What probably occurred in this run was a partial metabolism, in which phenol was converted to some intermediate product, which was not further metabolised until phosphate was made available.

Lastly, the rate of substrate removal seems to be reasonably linear in all the tests except for Run ATP-P10. In this run, the initial substrate concentration was appreciably higher than in most of the other tests. The rate of removal of substrate is slower at higher phenol concentrations, indicating possible metabolic toxicity by high phenol concentrations. However, it should be pointed out that in Runs ATP-P1, P2, and P5, initial substrate concentrations are even higher, yet no toxic effects are shown.

To summarise, the batch tests on the biodegradation of phenol using ATP as a measure of viable biomass have shown that ATP declines during the stage of substrate removal, and does not begin to increase until substrate becomes limiting. At this point, the ATP increases very rapidly, until it attains a steady-state value which is normally slightly above the value observed prior to substrate injection.

The reason for the ATP decline is not known, but

a hypothesis was made that the phenol is not immediately metabolised, but that it is instead adsorbed on to the flocs. Such a process would explain the lack of increase of ATP, but does not satisfactorily explain the observed decrease.

However, tests were then instigated to prove, or disprove this hypothesis, and the results of these tests are given in the next section.

6.4 Experiments into the Nature of Substrate Removal

The phenol/ATP tests have invariably shown a decline in ATP concurrent with decrease of residual phenol. This suggests that there is little or no growth whilst the phenol is being removed, and that growth occurs only when the substrate becomes limiting. However, this hypothesis seems to be denied by the earlier work with MLSS and MLVSS, when the suspended solids content increased during substrate decline.

As a check to determine whether the decline in ATP was due to zero (or negative) growth, or due to some other metabolic intermediary stage, tests were undertaken to determine:

(i) is the phenol adsorbed on to the floc during the initial stage of the batch test?

(ii) at what stage of the test does substrate metabolism proceed?

6.4.1 Adsorbtive Capability of the Heterotrohpic Sludge

Method

A heterotrophic mixed liquor was inoculated with phenol as substrate. Samples of the mixed liquor were removed periodically, each sample being centrifuged for 20 minutes at 3000 rpm. The supernatent liquor from each sample was analysed for phenol content.

Another sample, taken simultaneously, was acidified to pH 2 with concentrated HCl, in order to break up the cells and release any adsorbed phenol. After centrifuging, the supernatent was neutralised with NaOH and analysed for phenolic content.

The two phenol concentrations thus determined were then compared. An accumulation of phenol in the second sample (from

the supernatent liquor and the sludge) would indicate an adsorptive process.

A second test was undertaken in which the total phenolic content was determined by making the sludge alkaline. The pH of the sample was increased to pH 12 with concentrated NaOH. This process should ionise any phenols adsorbed onto the sludge, ensuring they go into solution in a form that may then be analysed.

Results

TABLE 6.13 ACIDIFICATION OF THE MIXED LIQUOR pH 2.0

Time minutes	Phenol Concentration in Supernatent $gm.m^{-3}$	Total Phenol Concentration (Supernatent + Sludge) $gm.m^{-3}$
0	4.7	4.3
5	Phenol Innoculation	
10	112	117
30	36	39
60	5.7	4.8
90	4.4	4.0

TABLE 6.14 ALKALINATION OF THE MIXED LIQUOR pH 12.0

Time minutes	Phenol Concentration in Supernatent $gm.m^{-3}$	Total Phenol Concentration (Supernatent + Sludge) $gm.m^{-3}$
0	219	210
15	125	117
45	69	74
75	19	8.7

Conclusions

The results indicate that there is no significant degree of adsorption and storage of phenol on to the sludge flocs during batch biodegradation of phenol.

6.4.2 Oxygen - Uptake Tests to Determine the Metabolic Stage

Method

Using one of the continuous reactors illustrated in Figure 6.13, a test was undertaken to determine at what stage during batch biodegradation of phenol does the metabolic process proceed. The influent and effluent flows to the reactor were isolated, so that it acted as a batch reactor. Phenol was added as substrate to a mixed liquor comprising of a treated coke-oven effluent base with nutrient addition, and a heterotrophic sludge. The Dissolved Oxygen concentration in the mixed liquor was continuously monitored. Samples of the mixed liquor were periodically removed for later assay of phenolic content.

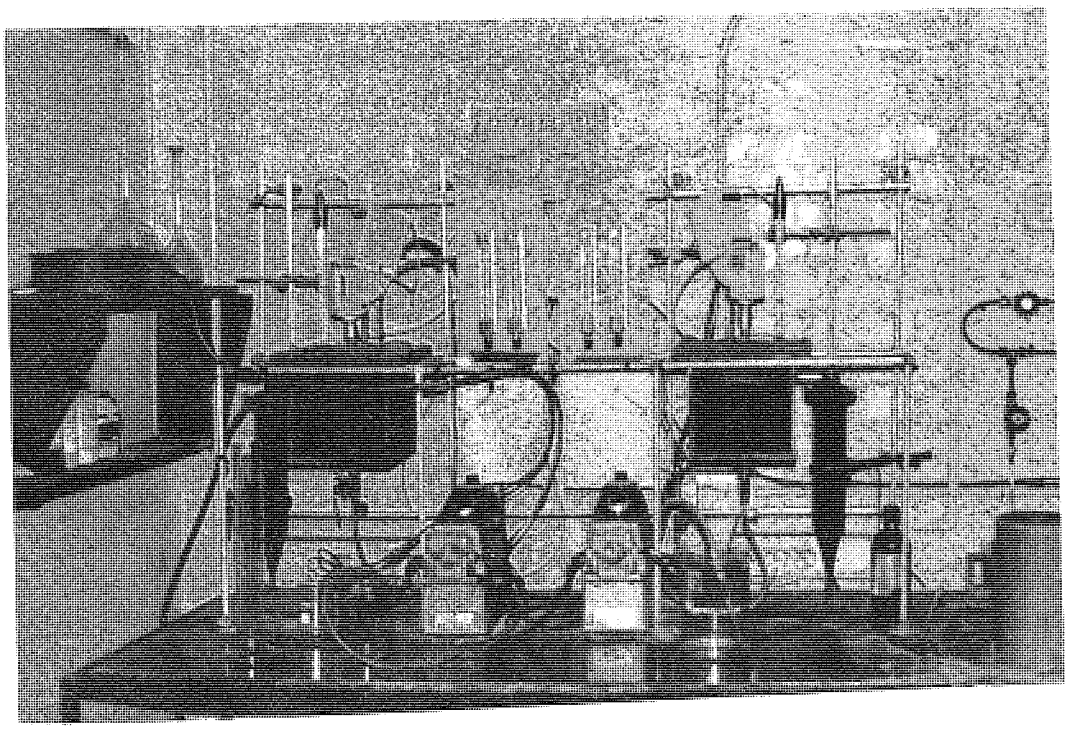


FIG.6.13

Results

The results of the test are illustrated in Figure 6.14.

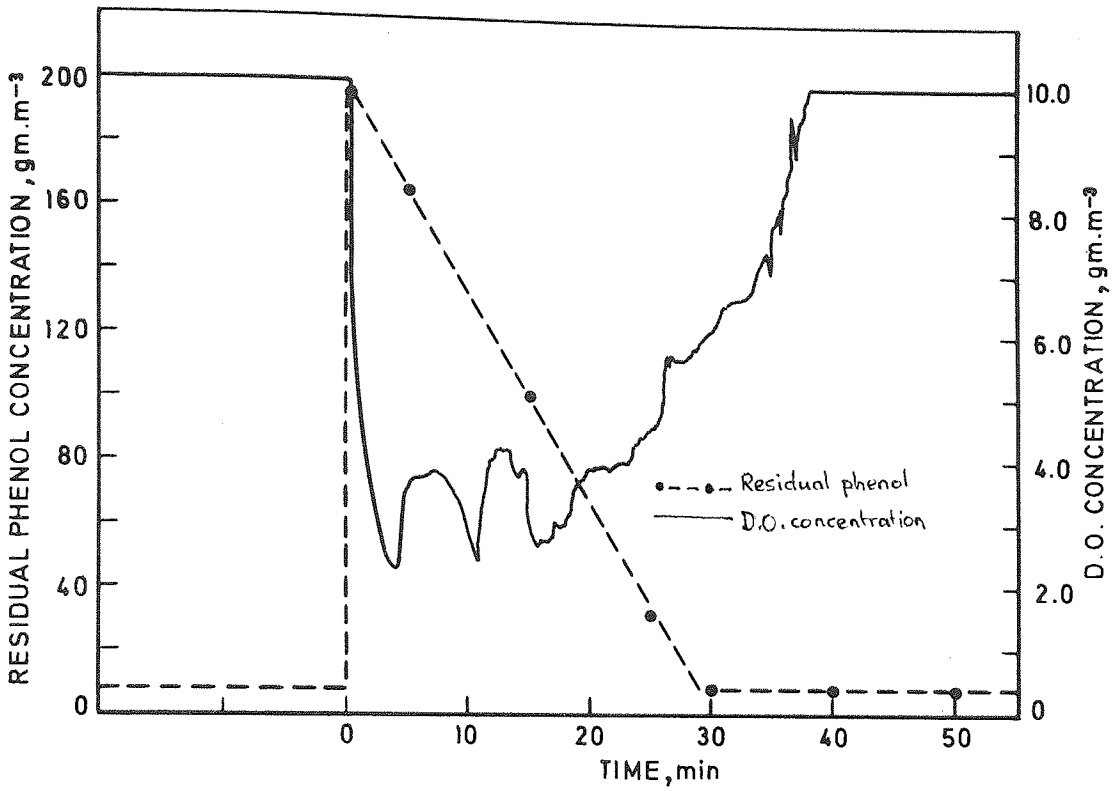


FIG.6.14 OXYGEN-UPTAKE TEST

Conclusions

The graph indicates that oxygen-uptake proceeds simultaneously with phenolic removal, but that there still remains an oxygen requirement after the phenol has disappeared from the system. The suggestion is that although metabolism proceeds from the instance of the substrate innoculation, metabolism of some intermediate compounds continues after the phenol has disappeared.

6.5 A storage Model for Batch Cultures Utilising Phenol as Substrate

The previous sections of this chapter have indicated the following:-

(i) ATP declines whilst substrate is lost from a batch system.

(ii) An adsorptive process in which substrate is initially lost from the system by adsorption onto cellular flocs does not appear to be shown.

(iii) Metabolism of substrate occurs from the instance of

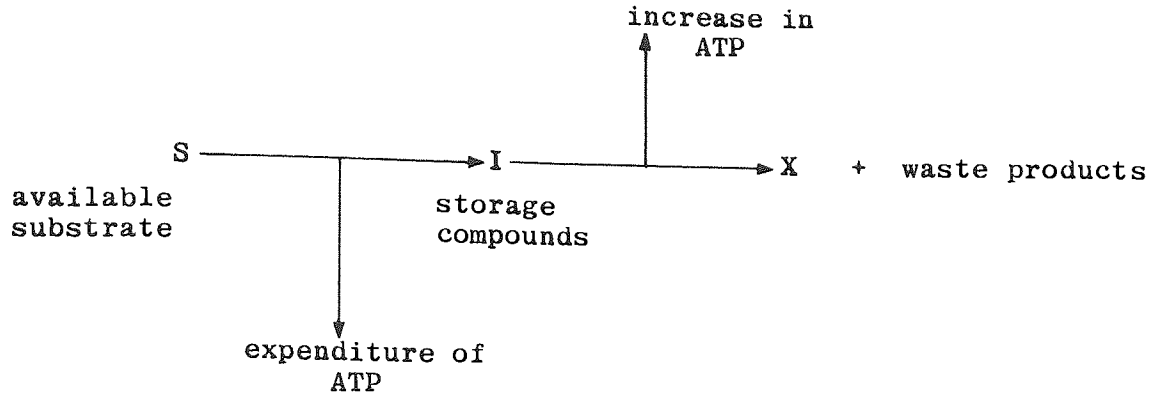
phenol injection, and continues after the phenol has disappeared from the system.

If ATP is regarded only as a measure of active biomass, then the observed results indicate a reduction in the number of cells whilst substrate is being metabolised. Such a process is inexplicable as metabolism is normally expected to increase cellular populations, and the earlier work reported in Chapter 4 had shown an increase in biomass as measured by the suspended solids content of the culture.

However, ATP is not only a measure of viable biomass, but is also a measure of available energy for cellular processes. If one hypothesises that prior to biomass increase due to growth, there is some intermediate stage where the substrate is converted to a STORAGE COMPOUND within the infra-structure of the cell, the energy-requirement for this conversion might be responsible for the observed decline in ATP. Porges et al[149] in tests on the biodegradation of dairy wastes, found that the rate of uptake of substrate was twelve times the rate of oxidation. They found that the carbohydrate waste was firstly stored in the cells as an insoluble glycogen-like storage product, and then subsequently utilised. Wilkinson[150] found that storage compounds often tend to be formed when organisms are grown under conditions of nutrient sufficiency, and utilised when the organism is placed in a nutritionally less favourable environment. Rose[72] discussed energy expenditure within the cell, and stated that active transport of solutes across the cytoplasmic membrane and conversion to storage compounds requires an appreciable amount of energy in the form of ATP.

Such a process would fit the observed results; i.e. an increase in MLSS caused by an overall increase in total dried solids; but a decrease in ATP due to expenditure of energy in the conversion of phenol to storage compounds prior to metabolism.

A two-stage system is thus envisaged:



6.5.1 Formulation of Mathematical Expressions for a Storage Model

Rate of Removal of Substrate; $\frac{dS}{dt}$

It is assumed that substrate is lost from the system solely by being converted to stored products within the active biomass. The rate of loss of substrate will then depend upon the concentration of active cells present in the system at any given time, i.e.:

$$-\frac{dS}{dt} = C_1 X \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Eq. 6.1}$$

However, as has been explained above, during periods of nutrient sufficiency, substrate is being stored, and anabolism is low. The concentration of active biomass will therefore remain reasonably constant over this period, any slight increase due to metabolism of stored products being offset by loss due to basal metabolism.

The initial, steady-state, A.T.P. content of the system prior to substrate addition is representative of the concentration of active biomass over this period, and equation 6.1 thus becomes:

$$-\frac{dS}{dt} = C_1 X_i \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Eq. 6.2}$$

Rate of Change of Stored Products; $\frac{dI}{dt}$

The increase in stored products is connected to the removal of substrate by the relationship:

$$\frac{\text{Weight of stored products formed}}{\text{weight of substrate lost}} =$$

$$-\frac{dI}{dS} = Y_I = \text{yield coefficient} \quad \ldots\ldots\ldots \text{Eq. 6.3}$$

Thus, over a small period of time, dt:

$$\left(\frac{dI}{dt}\right)_{\text{increase}} = -Y_I \frac{dS}{dt} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. 6.4}$$

Since we have no information either upon the structure of the storage compounds, or upon the nature of their conversion from substrates, it will be impossible to determine a value for Y_I. However, the concentration of (I) is only used as a mathematical tool, and any value may therefore be assumed for Y_I. Taking the simplest assumption that 1 gm of stored products is formed by a loss of 1 gm of substrate:

$$\text{then } Y_I = 1.0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 6.5}$$

Storage compounds are also being constantly lost from the system by metabolism and respiration. If one assumes Monod kinetics to describe the growth rate of new cells, then:

$$\mu = \mu_{max} \frac{I}{K_I + I} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 6.6}$$

The change in (I) due to metabolism is related to the growth rate (μ), to the concentration of A.T.P. available for growth (X), and to the yield of A.T.P. per unit mass of stored products utilised (Y_{ATP}), by the expression:

$$\left(\frac{dI}{dt}\right)_{loss} = \frac{\mu X}{Y_{ATP}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 6.7}$$

The nett rate of change of storage compounds is thus described by equations 6.2 and 6.4 to 6.7.

i.e. $$\frac{dI}{dt} = C_1 X_i - \frac{\mu X}{Y_{ATP}} \quad \text{..... Eq. 6.8}$$

Rate of Change of A.T.P.; $\frac{dX}{dt}$

The nett change in concentration of active biomass is given by the difference in the increase due to growth, and the decrease due to basal metabolism, thus:

$$\frac{dX}{dt} = \mu X - k_e X \quad \text{..... Eq. 6.9}$$

where k_e is termed the basal metabolism rate, or the endogenous respiration rate.

However, a further expression must be included in this equation to represent loss in A.T.P. due to conversion to storage products. As storage is a linear process (equation 6.4), one can reasonably assume that energy expenditure due to the storage process may also be described by a linear expression:

Thus $$\left(\frac{dX}{dt}\right)_{\text{storage}} = C_2 \quad \text{..... Eq. 6.10}$$

Note that equation 6.10 is only valid whilst substrate is being converted to storage compounds. As soon as substrate becomes limiting, there is no A.T.P. expenditure due to storage, and C_2 becomes zero. Examination of experimental results indicates that this occurs at substrate levels of the order of 5.0 gm/m^3.

Thus, the overall rate of change of A.T.P. is represented by:

$$\frac{dX}{dt} = (\mu - k_e) X - C_2 \quad \text{..... Eq. 6.11}$$

where $C_2 = 0$ for $S < 5.0$ ..... Eq. 6.12

The storage model thus comprises equations 6.2, 6.6, 6.8, 6.11 and 6.12.

6.5.2 Process Modelling Technique

The behaviour of the system is simulated by solving the basic mathematical relationships given above. Three methods were used to fit the equations over the whole of the growth cycle, thereby determining the values of the kinetic coefficients.

Firstly, "guessed-values" of the coefficients were inputted, and the equations solved over the time period of the test. The calculated values were then compared with the experimental data, and a value was obtained for the Least Squares Fit of the differences. By altering each coefficient in turn, a minimum Least Squares Fit (L.S.F.) was obtained.

Secondly, each coefficient was altered in turn by a very small amount, and the new value of the L.S.F. determined. The gradient due to the original value of Least Squares minus the new value and divided by the difference was then calculated. By altering the coefficient with the maximum gradient, an improved Fit was progressively obtained.

However, both of these methods proved to be too insensitive to obtain a reasonable fit to the experimental data. By either method, a minimum L.S.F. could be obtained, but it was found that this was not the absolute minimum for the system, but rather a local minimum of a particular "valley".

The method adopted then was to simulate the experimental results paying most attention to curve shapes that alteration of each coefficient produced. Then, guided by the Least Squares gradient of that particular coefficient, the curve shape was progressively altered to produce a reasonable fit of the experimental data.

6.5.3 Simulation Hardware

Computer time and availability being limited, programmes

were written on a small programmable calculator to obtain an idea of the values of the coefficients. A programme was then written in PRIME FORTRAN to output the L.S.F., the gradients due to alteration of coefficients, and the experimental and computed values of the data. By calculated use of an inter-active terminal, reasonable fits to the experimental data could then be obtained quickly.

A copy of the program used, together with sample data and output, is given in Appendix 11.

6.5.4 Results

Figure 6.15 shows the result of fitting the model to the experimental data of one of the batch tests. It was found impossible to change the shape of the left-hand side of the X-curve from a sigmoidal to an exponential decay, as is illustrated by the experimental data, no matter how the coefficients were altered. It was therefore assumed that the model was invalid, and required further development.

A survey of the literature on phenol biodegradation had shown many references relating to the toxicity of phenol substrate at high concentrations. Hill and Robinson[151] explained this phenomenon in terms of competitive inhibition by the substrate itself. At high substrate concentrations, ineffective Enzyme-Substrate complexes would be formed which would not react to form a product.

Haldane[152] modelled this process, and developed an expression for the growth rate that incorporated a term representing toxicity at elevated substrate concentrations. Various other kinetic models have been advanced to describe the metabolic rates of inhibitory substrates. Pawlowsky and Howell[153] tested five such functions on data from batch experiments on a phenolic substrate with acclimatised sludge, and found no significant difference between them on the basis of fit. They preferred the Haldane model because of its simplicity. Edwards[154] also tested various inhibition models, and he too preferred the Haldane function.

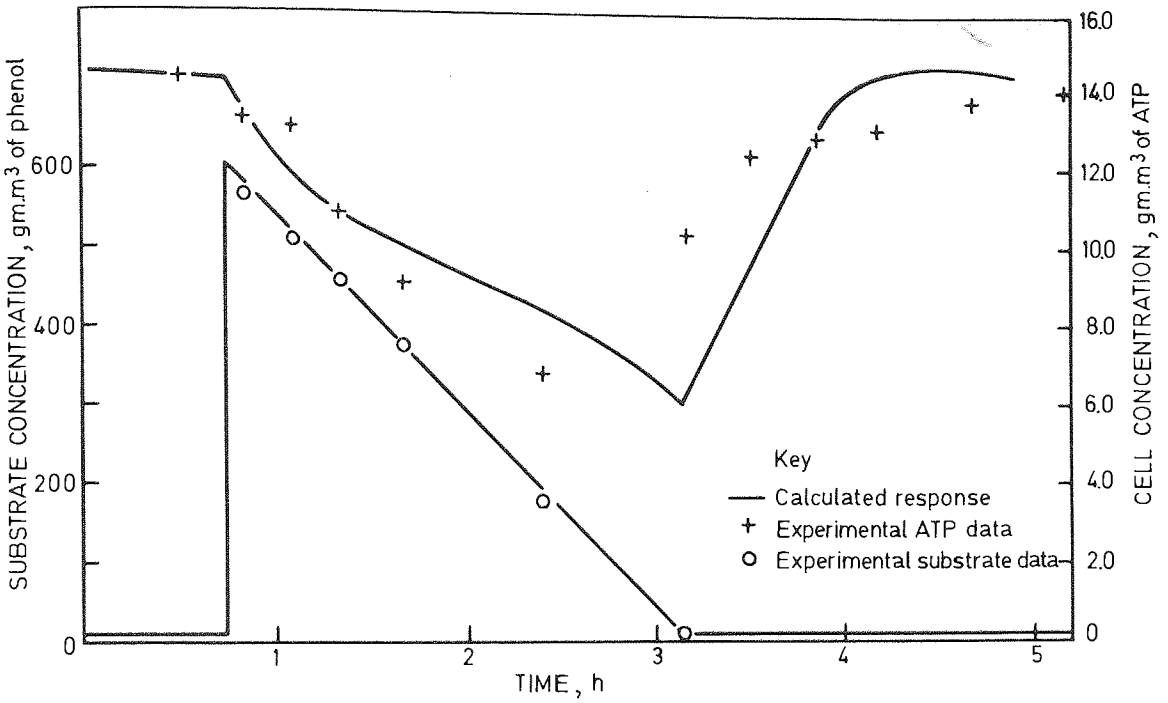


FIG.6.15 EXAMPLE OF EXPERIMENTAL AND COMPUTED CURVES FOR STORAGE MODEL – NO ALLOWANCE FOR TOXICITY, RUN ATP-P11

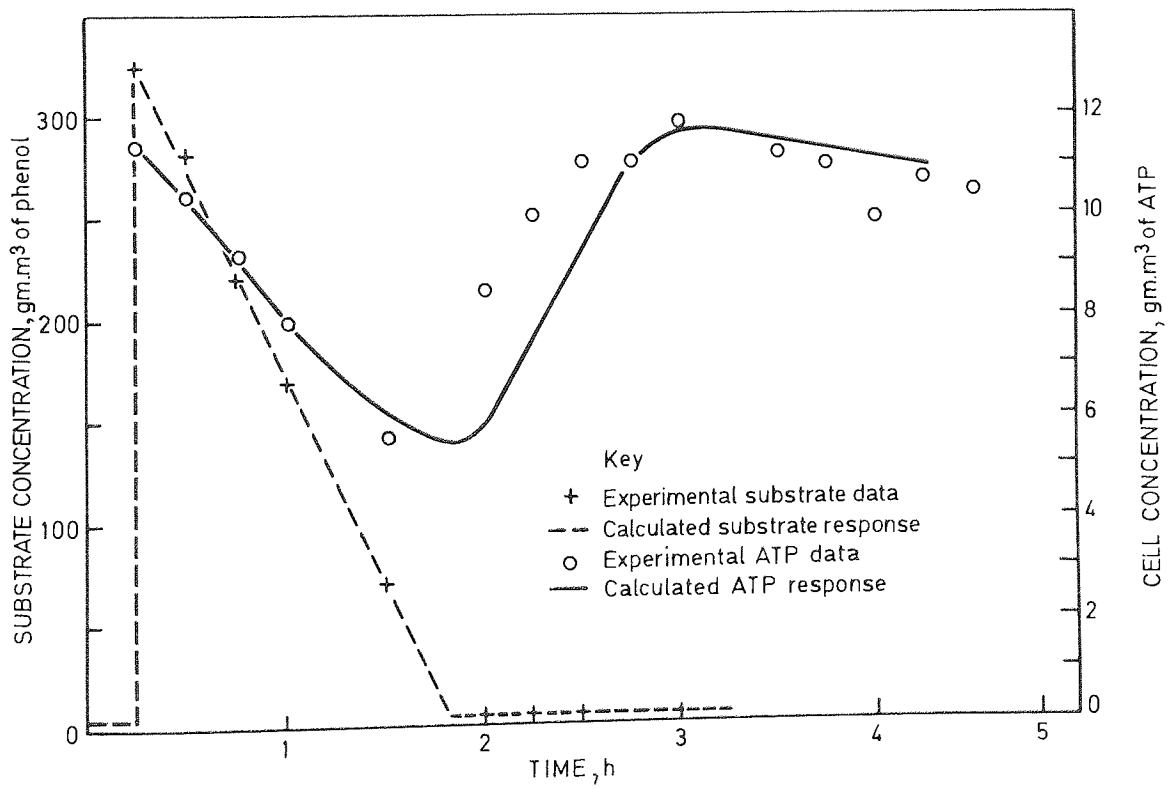


FIG.6.16 EXPERIMENTAL AND COMPUTED CURVES FOR STORAGE MODEL WITH ALLOWANCE FOR TOXICITY, RUN ATP-P7

6.6 Modification of the Storage Model to Incorporate an Expression for Toxicity

Haldane[152] modified the Monod model for the growth rate of single-substrate cultures by incorporating an expression which considerably reduces the growth rate at very high concentrations of substrate, this effect being much less significant at lower levels of ambient substrate:

$$\mu = \mu_{max} \frac{S}{K_s + S + S^2/K_t} \quad \text{..... Eq. 6.13}$$

where K_t is a constant of toxicity.

In the storage model previously advanced, the growth rate does not depend on ambient substrate concentration (S), but rather upon the concentration of storage compounds (I). However it is still the ambient substrate concentration that will have a toxic effect on the growth rate, as it is this that will form ineffective Enzyme - Substrate complexes that will reduce the conversion of substrate to storage compounds.

Thus, it is proposed that the Haldane model be modified to allow for growth due to storage compounds, yet have a toxicity effect due to substrate concentration.

$$\mu = \mu_{max} \frac{I}{K_I + I + S^2/K_t} \quad \text{..... Eq. 6.14}$$

The storage model, with allowance for toxicity at high substrate concentrations, now comprises equations 6.2, 6.8, 6.11, 6.12 and 6.14.

6.6.1 Results

The above model was solved using the simulation techniques outlined previously. Values of coefficients were obtained which gave Best Fit results for three batch experiments. Table 6.15 shows calculated values for each of the tests, and Figures 6.16 to 6.18 illustrate the fit obtained in each case.

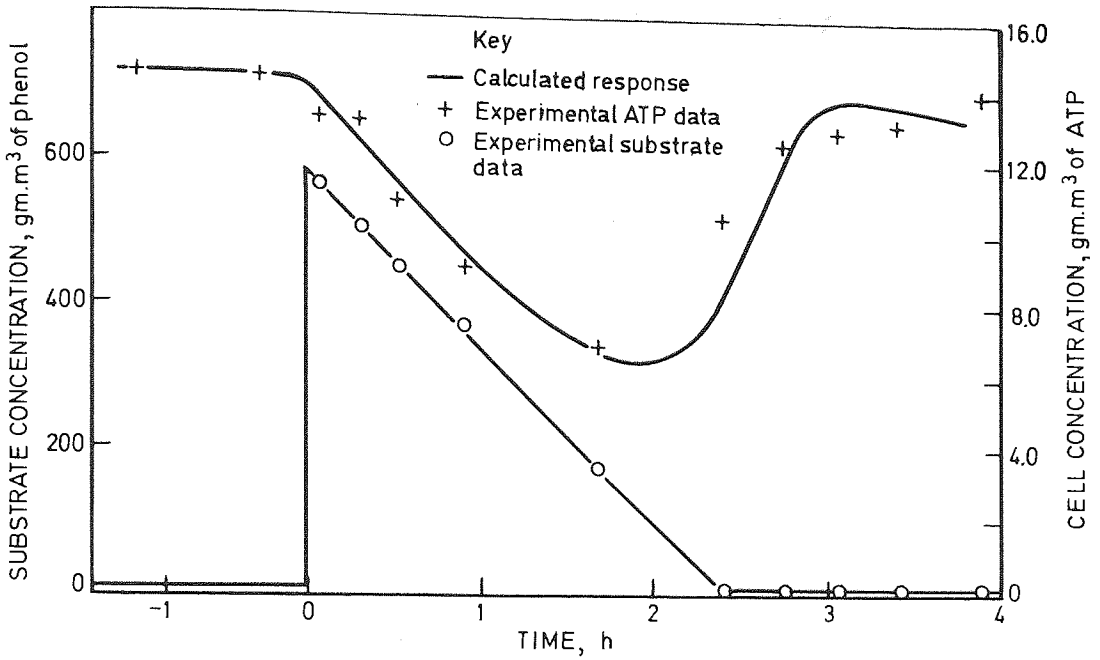


FIG.6.17 COMPUTED vs EXPERIMENTAL CURVES, RUN ATP-P11

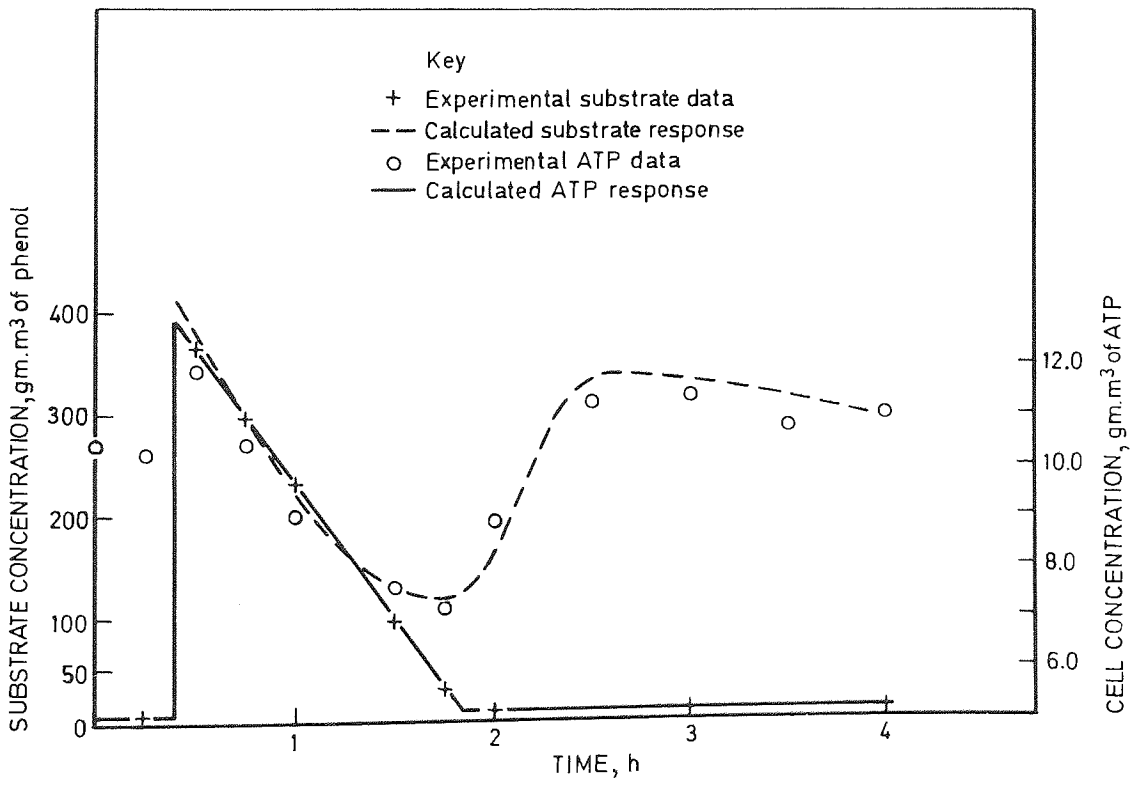


FIG.6.18 COMPUTED vs EXPERIMENTAL CURVES, RUN ATP-P12

TABLE 6.15 CALCULATED KINETIC COEFFICIENTS FOR A PHENOL-OXIDISING CULTURE

EXPT.	S_o $gm.m^{-3}$	X_i $gm.m^{-3}$	C_1 h^{-1}	C_2 $gm.m^{-3}$ h^{-1}	μ_m h^{-1}	k_e h^{-1}	K_I $gm.m^{-3}$	K_t $gm.m^{-3}$	Y_{ATP}
ATP-P7	325	11.4	18.86	5.8	1.5	0.06	60.0	40.0	0.032
ATP-P11	600	14.2	17.60	5.0	1.6	0.05	60.0	40.0	0.022
ATP-P12	390	13.2	18.94	6.0	1.5	0.062	60.0	40.0	0.024
MEAN			18.47	5.6	1.53	0.057	60.0	40.0	0.026

6.7 Discussion

A plausible mathematical model has been described to represent the kinetics of growth and substrate utilisation for phenolic bacteria. There are some weaknesses in the model which will require strengthening, the principal one being the assumption that substrate falls at a linear rate, independent of changes in either substrate or of biomass concentration. This can be partially explained by assuming that as concentration of substrate falls, the "activeness" of the biomass increases,off-setting the lower probability of contact with substrate molecules. This is supported by the evidence that MLSS increases from the time when substrate is added.

However, it should be emphasised that this is only one of several possible models that could be developed to fit the observed data. As an example of another model which could have been developed to fit the experimental results, consider the possibility that the ATP decline during conditions of substrate availability is caused by enzymatic activity. During the catabolic process, there may be a nett decrease in ATP as it is being used to activate the regulatory enzymes necessary for the degradation of phenol. In such a case the ATP would be converted to other adenine nucleotides such as Adenosine Di - or Mono-phosphate (ADP or AMP). Such a system could explain the very rapid increase in ATP level once substrate becomes limiting. At this time the enzymatic demand on the ATP would cease, and there would be a sudden increase in ATP as AMP and ADP are converted. In such a case, the nett increase in cell population caused by the addition of substrate would be reflected by the difference in steady-state

ATP pool level before substrate injection, and after substrate utilisation.

Knowles [141] and Simpson et al[135] reported on the use of the Adenylate energy charge (EC) as a preferred measure of microbial viability, where

$$EC = \frac{ATP + \frac{1}{2} ADP}{ATP + ADP + AMP}$$

It would have been helpful to monitor the energy charge in order to show the individual and total energy states throughout the experiment. Unfortunately, the facilities for measurement of AMP and ADP were not available when the experimental work was being undertaken. It is planned to continue investigative work in this field, and it is hoped in future to monitor the three adenine nucleotides in order to enable calculations of the energy charge.

In the three experiments reported in which curve-fitting techniques were used to determine values of the kinetic coefficients, a range of values for k_e was found between 0.05 and 0.062 hours^{-1}. This does not compare very favourably with the value of approximately 0.02 hours^{-1} found in experiment ATP-P6. However, as pointed out earlier, if the 24 hour ATP data-point in this Run is regarded as a rogue point, the line AB may be drawn through the remaining data (see Figure 6.6). The gradient of this line then gives a value of $k_e = 0.044^{-1}$ which is rather more compatible with the results found by curve-fitting techniques.

In presenting the information given in this chapter, the intention has been to explain the possible benefits of ATP measurement, to show how modelling may be used to support, or deny, a hypothesis, and to illustrate the power of inter-active computers in simulation exercise. Some advice is given on the strategy to use in developing a model, and in fitting parameters to it. Automatic optimising procedures are not likely to be beneficial during model development because of the difficulty in deriving a benefit function that includes a description of shape. Simulation, preferably with inter-active graphic terminals, is

recommended for use at the development stage, although supplementary output on degree of fit and parameter sensitivity may be useful.

The model requires further validation before extending the consequences of the hypothesis into continuous process activated-sludge plants.

CHAPTER 7. A MODEL FOR THE BATCH BIODEGRADATION OF THIOCYANATE IN COKE-OVEN EFFLUENTS

Coincident with the testing of the heterotrophic cultures, as reported in Chapter 6, experiments were undertaken on the response of a batch autotrophic culture to substrate injection. ATP was again used as a measure of active biomass.

It was found that the ATP response of an autotrophic system is very different to that response observed in the heterotrophic system.

This chapter details the results obtained and model developed for the batch biodegradation of thiocyanate in coke-oven effluents.

7.1 Experimental Procedure

The experimental procedure for the autotrophic tests was exactly as outlined for the heterotrophic tests, given in Section 6.1, except for the following;

(1) An autotrophic sludge, rather than a heterotrophic one, was added to the diluent to make up the Mixed Liquors. Details of the production of this sludge are given in Section 4.7.

(2) Between batch tests, thiocyanate (as potassium thiocyanate) was added to the aerated, completely-mixed liquor to prevent starvation of the micro-organisms.

(3) The daily wastage rate of the autotrophic Mixed Liquor was much reduced owing to the very slow growth rates observed. Dependant upon the amount of available sludge, 0% to 5% was wasted daily.

(4) Settlement of the contents of the reactor was much more frequent, normally once daily, to prevent accumulation of ammonium ions (see Section 4.3.1).

(5) As with the heterotrophic tests, a Lovibond Comparator was used to approximate the residual thiocyanate content of the Mixed Liquor in each Run.

7.1.1 Sampling Technique

Again, this was exactly the same as the technique used in the heterotrophic tests and reported in Section 6.1.2.

7.1.2 Analytical Methods

The analytical methods are given in the following sections;

MLSS	- Section 4.5.1
MLVSS	- Section 4.10.1
Thiocyanate	- Section 4.10.1
TRIS Extraction method	- Appendix 10
DMSO Extraction method	- Appendix 10
ATP assay	- Section 5.4

Approximate thiocyanate assays were made using a Lovibond Comparator. The method is given in Appendix 6.

7.2 Inhibitive Effect of Phenol on Thiocyanate Biodegradation

Several tests were also made to observe what effect the presence of phenol has upon the biodegradation of thiocyanate by an autotrophic sludge.

In each test, two reactors were sampled over a given time period. Each reactor contained a mixed liquor comprised of

(a) a similar quantity of sludge - obtained by mixing the sludges from both reactors, and dividing the mixed sludge between the two reactors by volume,

(b) the same volume of base liquor (see Section 4.3.1),

(c) the same concentration of thiocyanate ions - obtained by pipetting potassium thiocyanate from a stock solution.

To one of the reactors, phenol was added at a known concentration. Addition of the phenol was either at the beginning of the test, or part-way through it.

7.3 Results

The results of all of the thiocyanate batch tests are

given in Tables 7.1 to 7.10, and are illustrated graphically in Figures 7.1 to 7.10.

The results of the four inhibition tests are given in Tables 7.7 to 7.10.

7.4 Discussion

The results obtained for the batch tests on the autotrophic cultures are inconclusive. The first two tests should be viewed with considerable caution, as they were in essence "proving" tests to develop the ATP extraction method and sampling procedure. In spite of this, both of these tests indicate a substantial increase in ATP as substrate disappears from the system, followed by a very sudden fall in ATP when substrate becomes limiting.

In the third test, Run ATP-T3, it may be seen that there is much less scatter through the ATP data. This effect is to be expected as, by continuously improving both sampling and extraction techniques, the maximum observed variation between assays of the same sample has been reduced from 11% of the mean in the first two tests, to 5%. The results of this test indicate a sudden rise in ATP as substrate is made available, and a rapid fall in ATP when substrate becomes limiting. As residual substrate disappears from the system, the ATP curve shows a slightly negative gradient (see Figure 7.3). However, this section of the curve should be viewed with care because if one of the ATP data-points in the mid-section is a rogue point, the shape of the curve could be flat, or even positive.

More data is obviously called for, and this was provided in the next test, ATP-T4. At the same time, it was decided to compare two extraction methods, boiling TRIS buffer and DMSO. The comparison of these two methods has been previously discussed in Section 5.7. Several other points arise from this test however. Firstly, there appears to be an immediate and substantial increase in ATP at the moment of substrate injection. This is then followed by a steady increase as substrate is being removed from the system, and there is again a sudden decrease in ATP as substrate becomes limiting. Finally, there appears to be a nett increase in

 "continued P.180"

AIM Both tests to observe the correlation between ATP results and thiocyanate decay

TABLE 7.1 RUN ATP-T1

Time hours	CNS $gm.m^{-3}$	MLSS $gm.m^{-3}$	MLVSS $gm.m^{-3}$	% VSS/SS	ATP $gm.m^{-3}$
0	1108	2288	1507	65.9	0.47
⅙	1074	2460	1616	65.7	0.57
⅓	1032	2262	1825	80.7	0.61
½	989	2500	1995	79.8	0.45
¾	949	2355	1926	81.8	0.60
1	912	2525	2116	83.8	0.63
1½	781	2347	1962	83.6	0.90
2	601	2448	2078	84.9	0.53
2½	423	2317	1993	86.0	0.61
3	199	2462	2119	86.1	0.47
3½	4.9	2205	1900	86.2	0.88
3⅔	3.8	2552	2202	86.3	0.81
3¾	4.9	2635	2282	86.6	0.69
3⅘	3.6	2495	2170	87.0	0.73
3¹¹⁄₁₂	4.7	2195	1895	86.3	0.65
4	2.7	2562	2220	86.7	0.51
4½	2.5	2520	2160	85.7	0.58

TABLE 7.2 RUN ATP-T2

Time hours	CNS $gm.m^{-3}$	MLSS $gm.m^{-3}$	ATP $gm.m^{-3}$
0	1062	1987	0.500
⅙	1047	2152	0.515
⅓	1040	2502	0.665
½	1037	2377	0.685
1	1025	2302	0.585
2	985	2285	0.75
3	893	2390	0.95
4	816	2192	0.875
5	696	2292	0.7
6	521	2585	0.635
7	375	2485	0.76
8	149	*3360	0.835
8⅓	66	*4675	0.6
8½	31	*4512	0.785
8⅔	4.7	*3532	0.3
9	3.3	*4192	

Comments (1) Extraction method TRIS in both cases.

(2) ATP results normally the mean of 3 individual assays. Observed variation between 3 assays ± 11% of mean value.

(3) Although the ratio of MLVSS:MLSS in Run ATP-P1 gradually increases throughout the test, a curve plotted through the MLVSS data still shows no correlation to the thiocyanate decay. In the interest of brevity, this curve has not been included.

AIM Run ATP-T3:- to observe curve shape.

Run ATP-T4:- to compare TRIS and DMSO extraction methods.

TABLE 7.3 RUN ATP-T3

Time hours	CNS $gm.m^{-3}$	MLSS $gm.m^{-3}$	MLVSS $gm.m^{-3}$	% VSS/SS	ATP $gm.m^{-3}$
0	766	4105	2948	72	0.49
½	741	4170	2839	68	breakage
1	721	4197	2851	68	1,51
1½	660	3902	2664	68	1.43
2½	463	3295	2438	74	1.34
3½	162	4502	3013	67	breakage
4	1.4	2857	2253	79	1.09
4½	-	3500	2407	69	0.75
5	-	3150	2318	74	0.43
5½	-	3980	2800	70	0.48
6	-	4217	2952	70	0.47

Comments: ATP-T3

(1) Relatively stable MLVSS:MLSS ratio.

(2) Extraction method TRIS.

(3) ATP results normally the mean of 3 individual assays. Maximum observed variation = ± 5%.

TABLE 7.4 RUN ATP-T4

Time hours	CNS $gm.m^{-3}$	MLSS $gm.m^{-3}$	Extraction Method	ATP $gm.m^{-3}$
0	3.6	1410	DMSO	0.83
¼	3.6	1433	TRIS	0.74
½	3.6	1385	DMSO	0.78
¾	3.3	1390	TRIS	0.85
450 $gm.m^{-3}$ CNS addition at T = 0.98 hrs				
1	448	1475	DMSO	1.55
1¼	422	1405	TRIS	2.20
1½	384	1443	DMSO	2.12
1¾	-	-	TRIS	2.41
2	288	1475	DMSO	2.24
2¼	-	-	TRIS	2.50
2½	163	1480	DMSO	2.45
2¾	-	-	TRIS	2.17
3	22.0	1498	DMSO	2.47
3¼	3.8	1458	TRIS	0.78
3½	3.8	1488	DMSO	0.74
3¾	3.6	1503	TRIS	0.99
4	3.8	1510	DMSO	0.96
4¼	-	1568	TRIS	1.01
4½	-	1515	DMSO	0.90

Comments: ATP-T4

(1) Two extraction methods compared - TRIS and DMSO.

(2) In each case, ATP result normally mean of 2 assays.

(3) Mixed Liquor homogenised (domestic cream maker) prior to testing.

AIM Run ATP-T5:- to observe curve shapes.
Run ATP-T6:- to determine decay rate, k_e.

TABLE 7.5 RUN ATP-T5

Time minutes	CNS $gm.m^{-3}$	ATP $gm.m^{-3}$
0	11.8	1.24
5	6.0	1.20
10	3.6	1.19
20	3.3	1.34
30	3.3	1.27
CNS addition at T = 31 mins		
32	129	1.10
37	125	1.25
41	121	1.17
50	110	1.33
80	72	1.19
95	54	1.23
110	35	1.55
115	28	1.60
120	23	1.41
125	15	1.49
130	8	2.12
150	3.8	1.40
180	4.1	1.53
210	-	1.44
240	-	1.13
300	-	0.96

TABLE 7.6 RUN ATP-T6

Time in hours	CNS $gm.m^{-3}$	ATP $gm.m^{-3}$	logn ATP
0	-	1.03	0.030
½	4.4	1.19	0.174
1	-	1.37	0.315
2	-	1.16	0.148
3	-	1.31	0.270
4	-	1.27	0.239
5	3.3	1.00	0
6	-	0.87	-0.139
24	-	0.53	-0.635

Comments ATP-T5 Extraction Method DMSO. ATP results normally mean of 2. Maximum variation found between three assays = ± 4%. Mixed liquor homogenised.

ATP-T6 Extraction Method DMSO. ATP results normally mean of 3. Maximum variation observed = ± 2%.

A curve plotted of ℓ_nATP against time does not show very good linearity - see Figure 7.6. However, by drawing a line through the data, k_e may be approximated as:

$$k_e \triangleq 0.037 \text{ hours}^{-1}.$$

AIM To observe the ATP response of an autotrophic culture to phenol injection. Initial Phenol concentration = 70 $gm.m^{-3}$

TABLE 7.7 RUN ATP-I1

(a) No Phenol Addition						
CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	MLVSS $gm.m^{-3}$	% VSS/SS	ATP $gm.m^{-3}$	Time hours
334	2.1	2872	2016	70	1.23	0
315	-	3147	2208	70	1.27	¼
278	-	2967	2198	74	1.27	½
256	-	3455	2430	70	1.33	¾
221	-	3355	2319	69	1.01	1
185	-	3142	2300	73	1.39	1¼
158	-	3280	2313	71	1.30	1½
126	-	3455	2494	72	1.58	1¾
90	-	3547	2433	69	1.24	2
59	-	3975	2531	64	1.65	2¼
23	-	3272	2302	70	1.88	2½
-	-	-	-	-	-	2¾
5.5	-	3315	2420	73	1.28	3
6.0	-	3512	2463	70	1.02	3¼
-	-	-	-	-	-	3½
-	-	-	-	-	-	3¾

(b) Phenol Addition at T = 0 hours					
CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	MLVSS $gm.m^{-3}$	% VSS/SS	ATP $gm.m^{-3}$
337	70	3475	2376	68	1.44
307	68	3102	2228	72	1.47
284	64	2977	2181	73	-
268	66	3042	2201	72	1.57
240	68	3297	2278	69	1.83
210	70	3157	2222	70	1.45
187	66	2850	2064	72	1.50
162	67	2952	2107	71	1.50
143	68	3115	2204	71	1.23
-	-	-	-	-	-
88	68	3272	2302	70	1.80
-	-	-	-	-	-
47	64	2927	2149	73	1.19
-	-	-	-	-	-
16	63	3587	2526	70	1.46
16	68	2717	1954	72	2.09

Comments Extraction method TRIS; ATP assays normally mean of 3; Maximum observed variation between 3 assays = ± 12%.

AIM To observe the ATP response of an autotrophic culture to phenol injection. Initial phenol concentration = 50 $gm.m^{-3}$

TABLE 7.8 RUN ATP-I2

(a) No Phenol Addition					(b) Phenol Addition at T=0 hours			
CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	ATP $gm.m^{-3}$	Time hours	CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	MLSS $gm.m^{-3}$	ATP $gm.m^{-3}$
426	3.0	2700	0.52	0	419	58	2820	1.11
411	2.7	4220	0.80	¼	411	50	2525	1.01
401	-	3520	-	½	399	52	2485	0.82
389	-	2870	0.84	¾	389	52	2682	0.96
351	-	2710	0.45	1¼	349	51	2650	0.59
326	3.1	2522	-	1¾	307	53	2660	0.52
270	-	2872	0.66	2¼	255	54	2990	0.71
199	-	2837	0.46	3	173	54	2810	0.41
96	-	5345	0.47	4	52	50	3680	0.57
-	-	-	-	4¼	17	50	2937	0.58
31.5	2.4	2672	0.03	4½	6.6	50	2740	0.42
11.0	-	2645	0.43	4¾	6.6	48	2887	0.45
5.5	-	2785	0.54	5	-	-	-	-

Comments Extraction method TRIS. ATP assays normally mean of 3. Maximum observed variation between 3 assays = ± 11%.

AIM To observe the ATP response of an autotrophic culture to phenol injection. Phenol addition part-way through test.

TABLE 7.9 RUN ATP-I3

(a) No Phenol Addition			(b) Phenol Addition at T = 120 mins			
Time minutes	CNS $gm.m^{-3}$	ATP $gm.m^{-3}$	Time minutes	CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
10	4.7	0.46	0	5.5	4.9	0.69
30	241	0.47	25	243	4.4	0.68
50	241	0.56	45	223	-	0.83
70	241	0.53	65	192	4.7	0.86
120	227	0.56	115	138	-	0.95
185	197	0.52				
240	175	0.56	130	115	106	0.80
275	135	0.65	150	81	105	0.91
305	127	0.66	180	49	-	0.99
335	-	0.55	210	8.2	110	0.56
			240	5.7	-	0.63
			270	3.8	59	0.57
			300	-	67	0.54
			330	-	60	0.54

Comments Extraction method DMSO. ATP assays normally mean of 2. Maximum observed variation between 2 assays = ± 4%.

Both mixed-liquors homogenised (cream-maker) prior to testing.

Probability of faulty pH controller in Run ATP-I3(a).

AIM To observe the ATP response of an autotrophic culture to phenol injection. Phenol addition part-way through test.

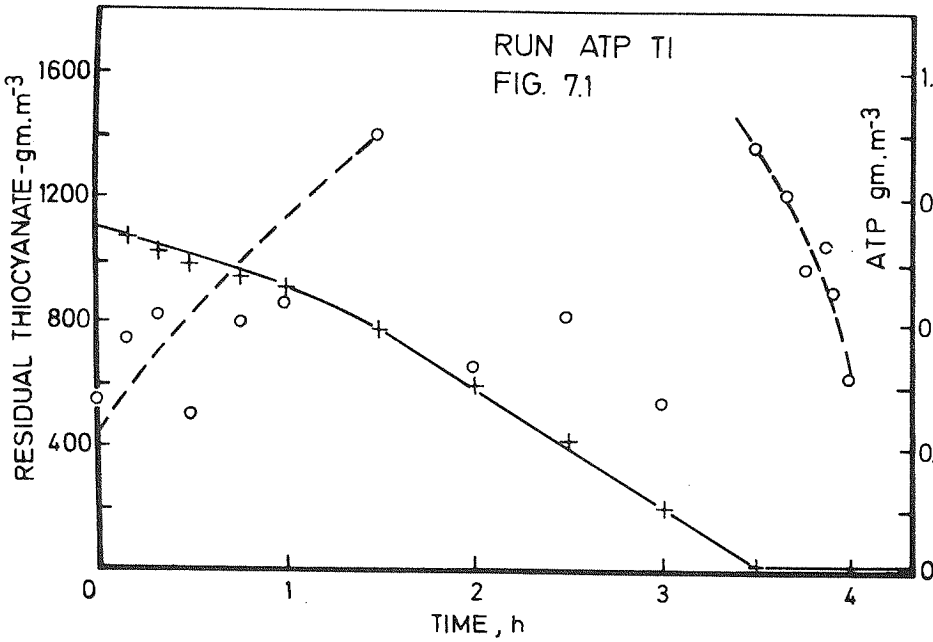
TABLE 7.10 RUN ATP-I4

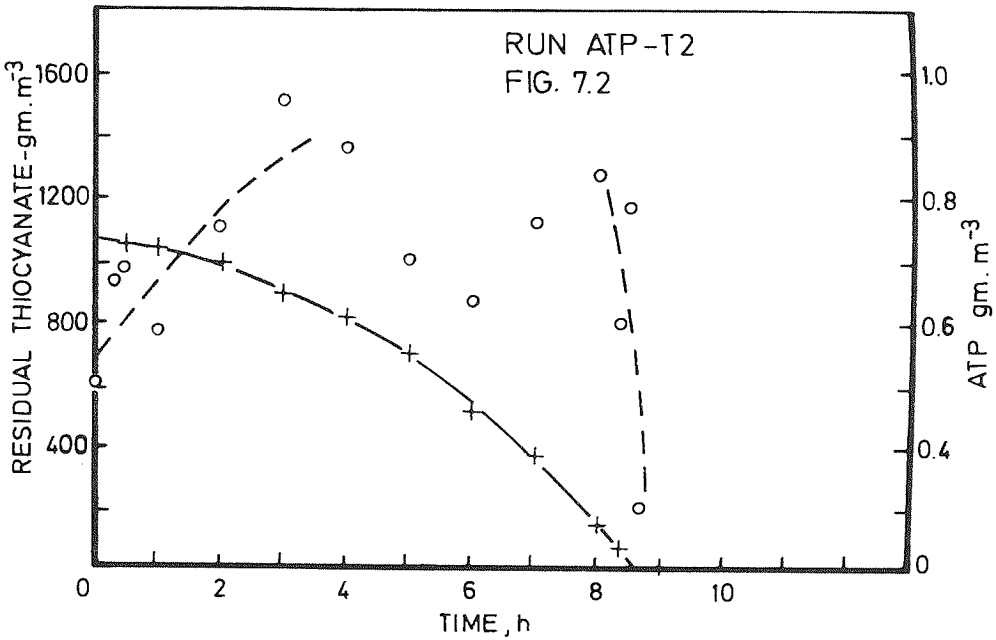
(a) No Phenol Addition			
Time minutes	CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
5	2.7	4.3	1.83
25	2.7	3.8	1.37
55	2.5	3.6	1.24
75	3.3	-	1.12
110	3.3	-	1.15
CNS Addition at T = 120 mins			
130	324	-	1.68
150	310	3.9	1.78
170	288	-	2.03
180	283	-	1.94
190	277	3.3	2.16
215	249	-	2.37
255	189	-	2.44
345	40	3.7	2.38
360	16	-	2.34
380	1.9	-	1.09

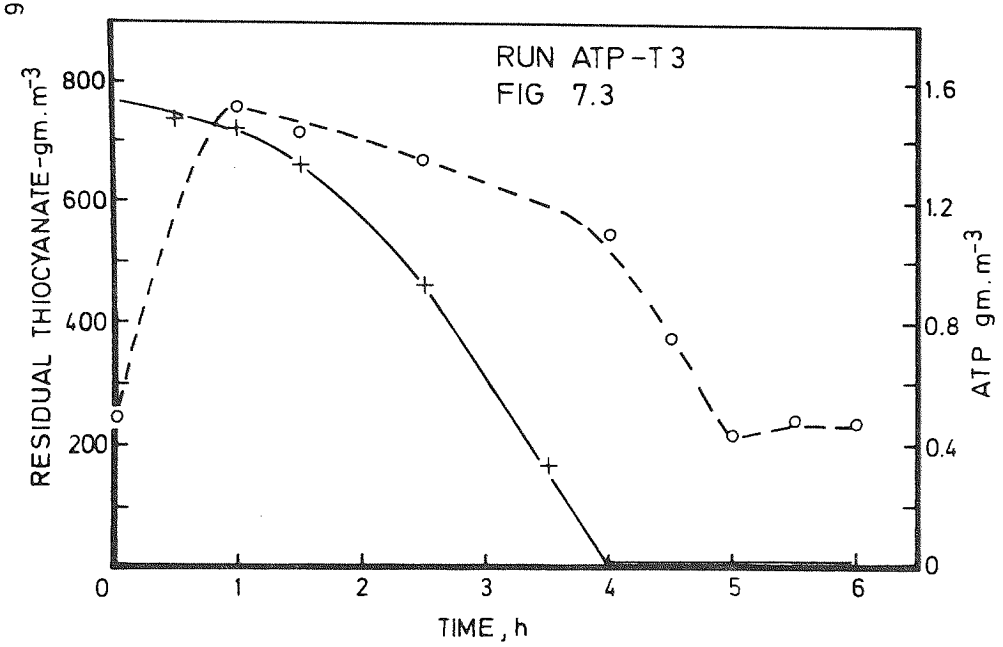
(b) Phenol Addition at T = 175 mins			
Time minutes	CNS $gm.m^{-3}$	Phenol $gm.m^{-3}$	ATP $gm.m^{-3}$
0	3.0	4.1	2.05
20	2.7	4.8	1.57
50	-	-	1.36
70	2.5	-	1.14
105	2.5	4.4	1.22
CNS Addition at T = 120 mins			
125	322	-	1.64
145	312	4.1	1.97
165	296	-	2.00
Phenol Addition at T = 175 mins			
177	275	95	2.25
185	270	93	2.31
200	256	92	2.32
220	227	95	2.26
260	171	95	2.55
340	23	89	2.46
355	1.9	89	1.61
370	1.6	89	0.97

Comments Extraction method DMSO. Normally only one ATP assay taken.

Both mixed liquors homogenised (cream-maker) prior to testing.

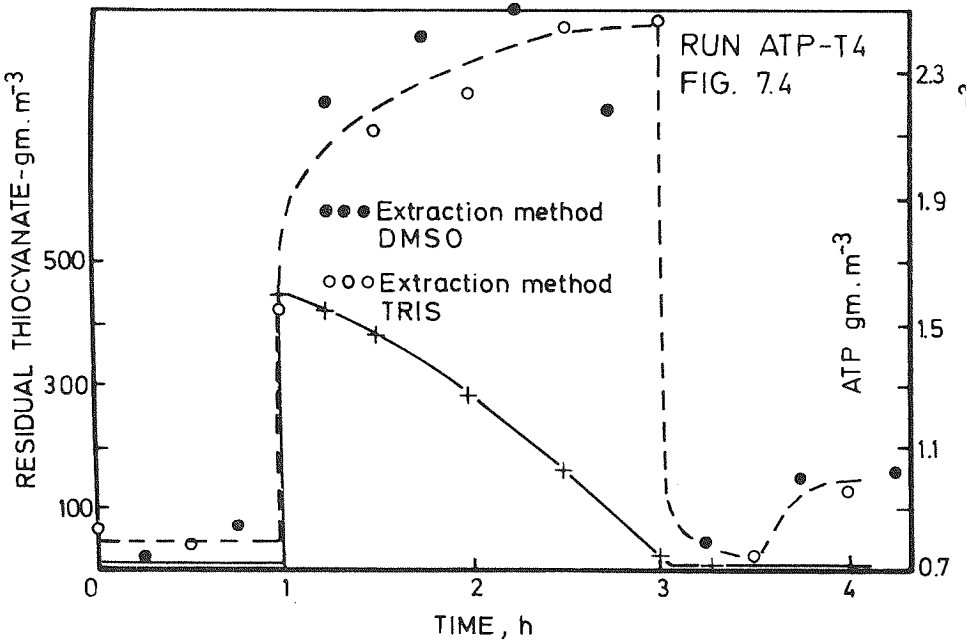


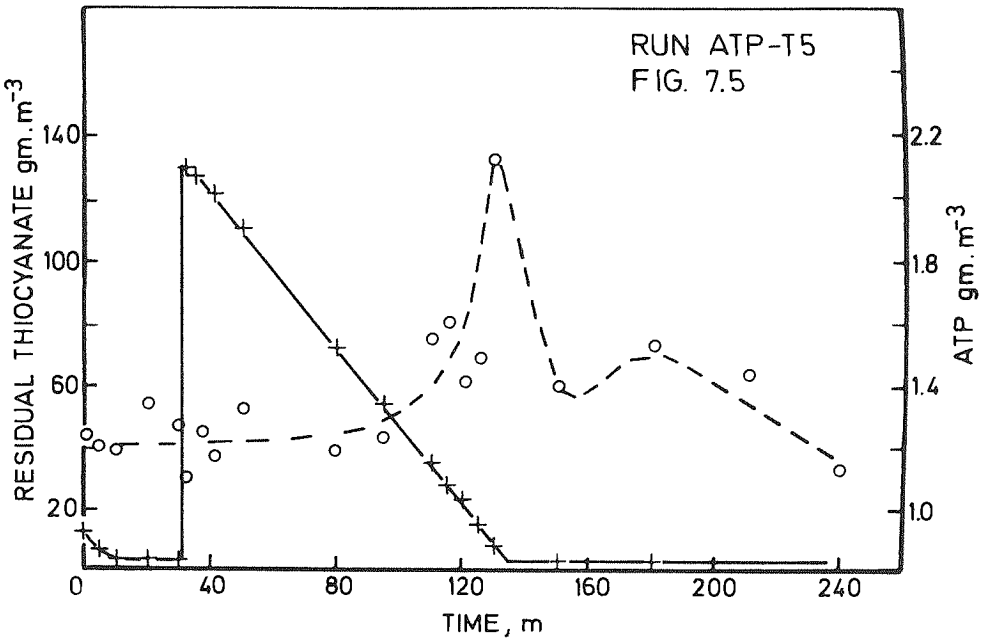


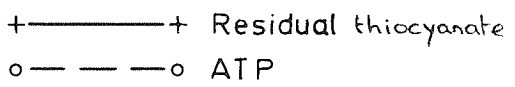


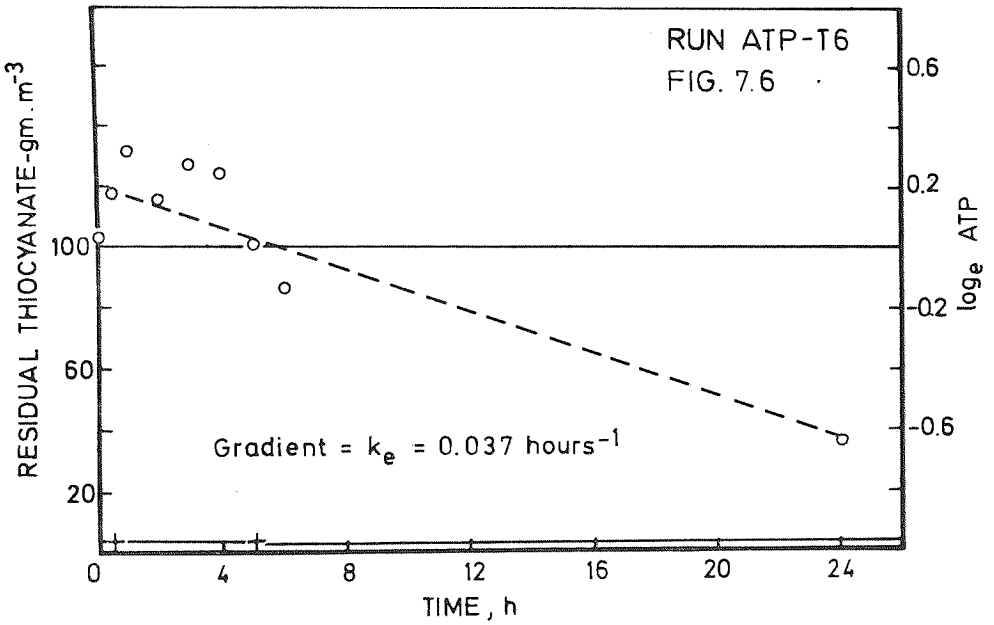
+——+ Residual thiocyanate

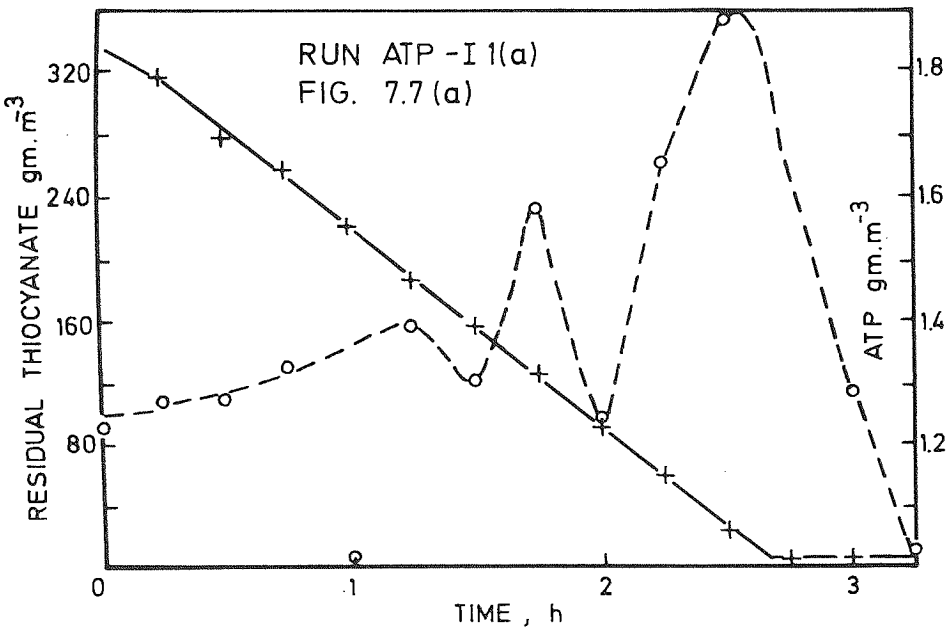
o— — —o ATP

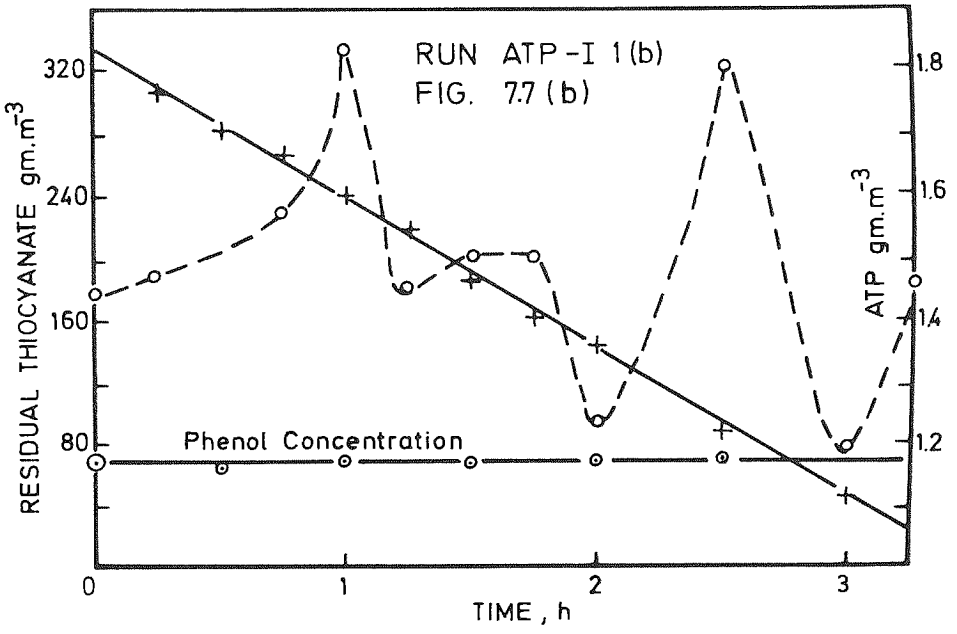

RUN ATP-T4
FIG. 7.4
●●● Extraction method DMSO
○○○ Extraction method TRIS
RESIDUAL THIOCYANATE-gm.m⁻³
ATP gm.m⁻³
TIME, h
0
1
2
3
4
100
300
500
0.7
1.1
1.5
1.9
2.3

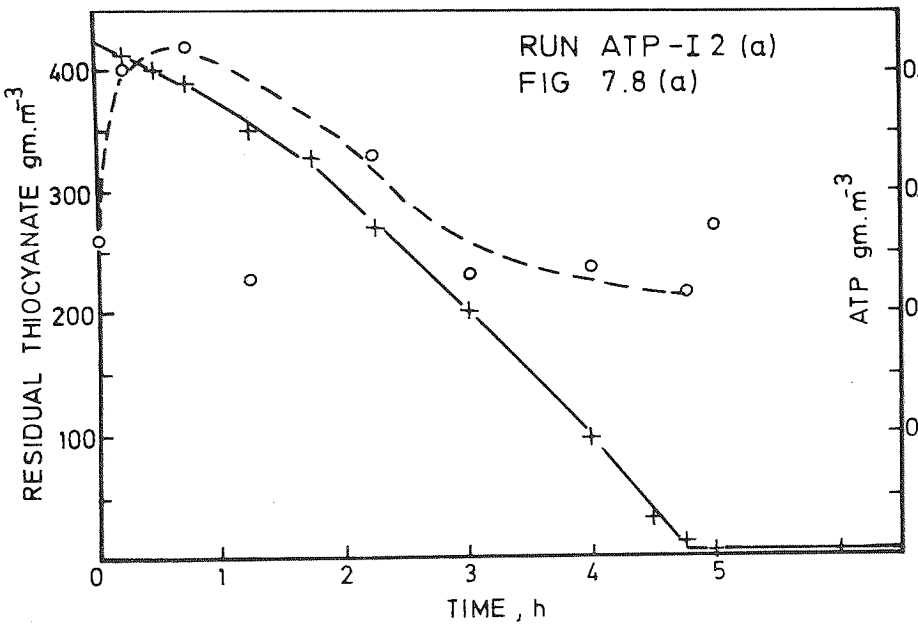

RUN ATP-T5
FIG. 7.5
RESIDUAL THIOCYANATE gm.m⁻³
ATP gm.m⁻³
TIME, m
0
40
80
120
160
200
240
20
60
100
140
1.0
1.4
1.8
2.2

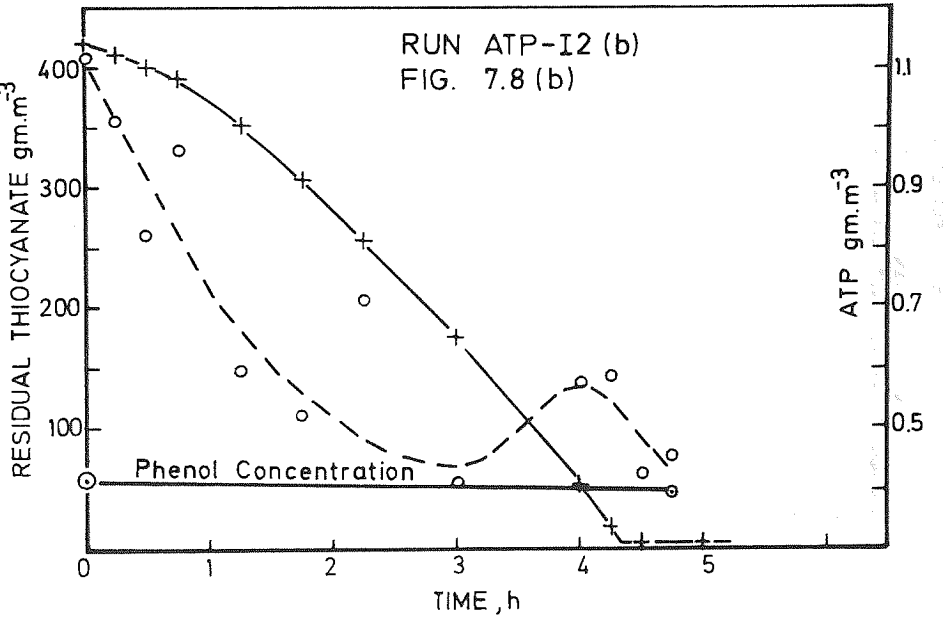

Residual thiocyanate
ATP

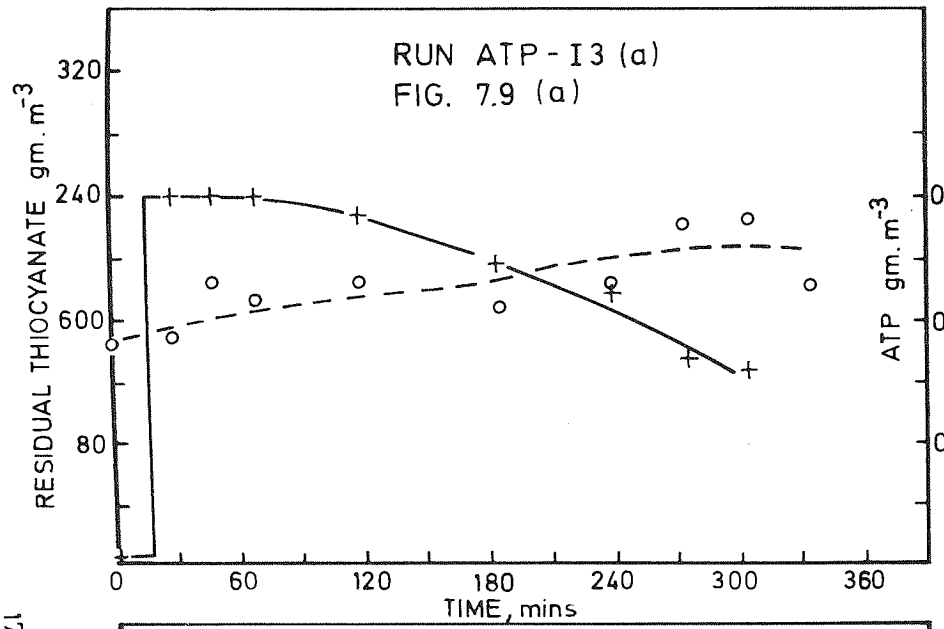

RUN ATP-T6
FIG. 7.6
RESIDUAL THIOCYANATE-gm.m⁻³
log_e ATP
Gradient = k_e = 0.037 hours⁻¹
TIME, h
0
4
8
12
16
20
24
20
60
100
-0.6
-0.2
0.2
0.6

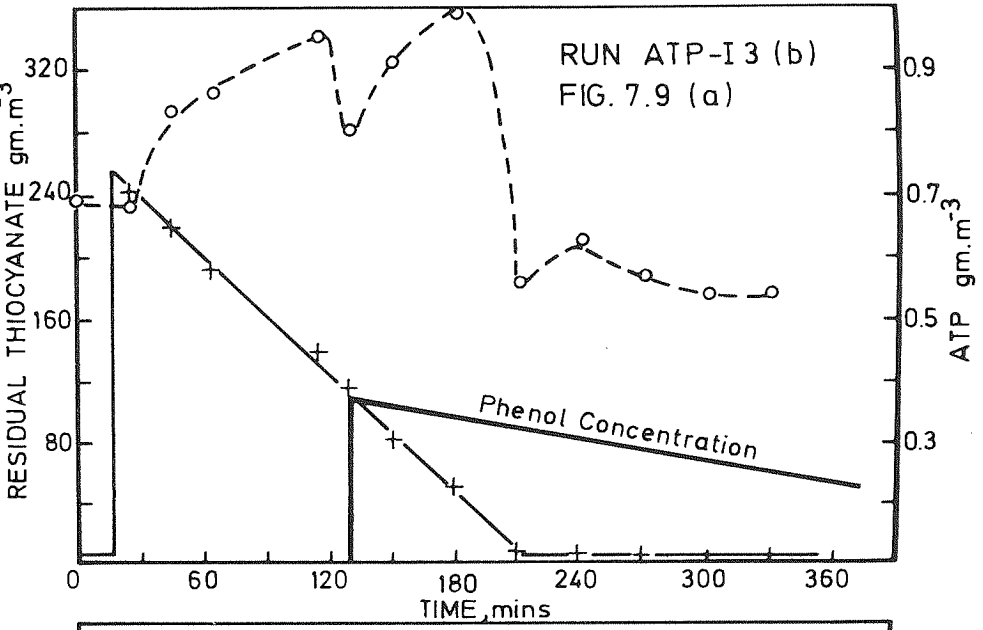

RUN ATP -I 1(a)
FIG. 7.7 (a)
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
TIME , h

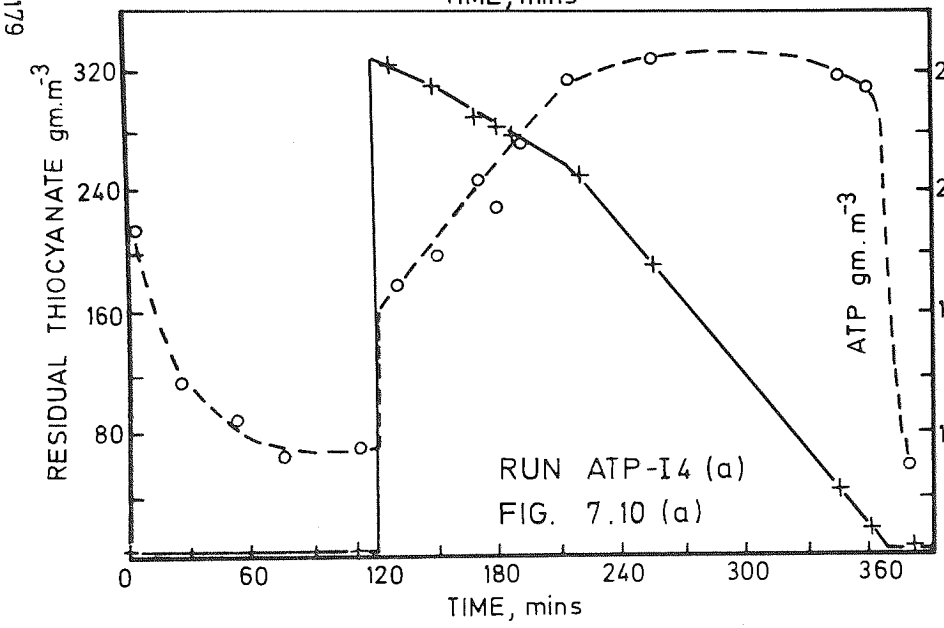

RUN ATP -I 1 (b)
FIG. 7.7 (b)
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
Phenol Concentration
TIME , h

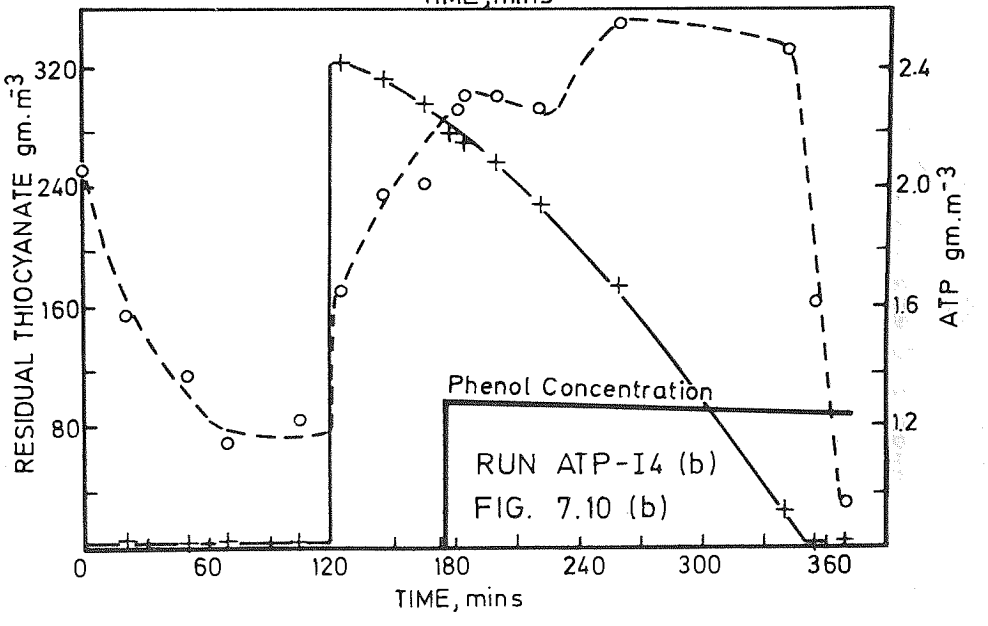

RUN ATP-I 2 (a)
FIG 7.8 (a)
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
TIME , h


RUN ATP-I2 (b)
FIG. 7.8 (b)
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
Phenol Concentration
TIME , h


RUN ATP-I3 (a)
FIG. 7.9 (a)
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
TIME, mins


RUN ATP-I3 (b)
FIG. 7.9 (a)
Phenol Concentration
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
TIME, mins


RUN ATP-I4 (a)
FIG. 7.10 (a)
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
TIME, mins


RUN ATP-I4 (b)
FIG. 7.10 (b)
Phenol Concentration
RESIDUAL THIOCYANATE gm.m-3
ATP gm.m-3
TIME, mins

endogenous ATP pool level once all of the substrate has been degraded.

At this stage, it was thought that a pattern of ATP response to thiocyanate degradation is an autotrophic system was emerging. It was believed that the initial increase in ATP was too sudden for it to be due to growth, and that instead there was an instantaneous change in energy state as ADP or AMP was converted to ATP. Similarly for the ATP response observed as substrate becomes limiting - the decay in ATP is far too fast, and much too sudden to be solely due to endogenous decay, and it was thus hypothesised that there was again a change in energy state, though this time in the converse direction, i.e. ATP being reduced to ADP or AMP.

Unfortunately the next test did not fit these hypotheses. As can be seen from Figure 7.5, there was a hyperbolic rise in ATP reaching a peak at substrate-limiting conditions. A sudden, and fast rate of decay was then observed as before. Similar curves were observed in other tests - see Figure 7.7, and no known explanation is given for these completely different responses. It would be all too easy to suggest that these results were rogue ones, due possibly to a poorly acclimatised sludge, or due perhaps to an undetected fault in either the pH or temperature controllers. However, the rate of loss of thiocyanate in this test (78 $gm.m^{-3}.hr^{-1}$) is similar to that found in Run ATP-I3b (75 $gm.m^{-3}.hr^{-1}$) and in Run ATP-I4a (79 $gm.m^{-3}.hr^{-1}$), both of which show a similar response to that observed in Run ATP-T4. It is not known why different responses are obtained under similar conditions. Further work is required in this area to determine exactly what processes are occurring.

A test was made in an attempt to determine the rate of endogenous decay of the autotrophic culture, with the assumption that the decay may be explained mathematically by

$$-\frac{dX}{dt} = k_e X$$

Figure 7.6 shows the result of a test in which the culture was left in the absence of substrate for 24 hours. By plotting $\log_e$ ATP against time, the above equation should give a

straight line. A straight line has in fact been drawn through the data, giving a value of k_e = 0.037 hours^{-1}. However, a great deal of scatter may be observed around this line, and one should not place too much reliance on this value.

As with the similar test for the heterotrophic sludge - Run ATP-P6, this test needs to be repeated with more samples taken between the 6 hour and 24 hour time periods. It was not possible to repeat the test at the time due to the shortage of necessary chemicals.

Four investigations were made into the inhibitory effect of phenol on autotrophic degradation of thiocyanate (see Runs ATP-I1 to ATP-I4, Figures 7.7 to 7.10). Again, these were very inconclusive.

In each of these tests, a portion of the acclimated autotrophic sludge was divided into two equal volumes, each of which being made up to 4.0 litres in one of the batch reactors with treated coke-oven effluent culture media (Section 4.3.1). An equal volume of stock thiocyanate solution (as potassium thiocyanate) was pipetted into each reactor. To one of the reactors, a known volume of phenol stock solution was also added. The ATP response to thiocyanate removal in both reactors was then compared.

In Run ATP-I1, a residual phenol content of approximately 70 gm.m^{-3} seems to have depressed the rate of removal of thiocyanate from 130 gm.m^{-3}.hr^{-1} to 95 gm.m^{-3}.hr^{-1}. The ATP response of the culture containing phenol is also relatively erratic. The thiocyanate removal rates remain fairly constant however in Runs ATP-I2 and ATP-I4, although in Run ATP-I2, a completely different ATP response is observed.

The only test in which an inhibitive effect due to phenol is really noticeable is the ATP response in Run ATP-I3. Figure 7.9b shows that as the culture is innoculated with phenol, there is an immediate depression in the ATP curve. The curve quickly recovers, attaining a peak just prior to substrate limiting conditions as before. This effect is also shown in Run ATP-I4

(Figure 7.10.b) although the depression in the ATP curve in this case is not as sudden or as severe as before.

It is interesting to note that in the last two tests, i.e. Runs ATP-I3 and ATP-I4, the ATP response fits the general shape of a sudden increase in ATP when substrate is injected, followed by a gradual increase as substrate is being lost from the system, and ending with a sudden fall in ATP level under substrate limiting conditions. This format is not shown in Run ATP-I3a however; for some reason there was a very slow rate of removal of substrate coincident with a flat ATP response. It was later noticed that the pH controller to this reactor occasionally gave erroneous readings. Replacement of the pH probe cured this fault.

There were two differences between these two tests and the two previous inhibition tests. Firstly, the DMSO extraction method was used instead of the TRIS method. Because of its simplicity, it is believed that the DMSO method is much less susceptible to errors (see Section 5.7). Secondly, the mixed liquor in each of the latter Runs was homogenised prior to testing. As explained in Section 5.9 it is believed that homogenisation of the mixed liquor considerably reduces errors due to sampling and representative extraction of ATP. More weight is therefore given to these later results than to those obtained previously.

To conclude then, whilst there is some doubt as to the actual ATP response obtained when an autotrophic culture is subjected to growth-inducing conditions by the addition of thiocyanate as substrate, it is thought that the following pattern may be representative of the general effect.

As substrate is added, there is an immediate rise in ATP concentration due to a change in energy state, rather than to growth. There then follows a period in which substrate is being lost from the system, and a gradual increase in ATP due to growth is associated with this. Once substrate becomes limiting, there is an immediate drop in ATP concentration, again caused by a change in energy state, rather than being due solely to endogenous decay.

With such a hypothesis, and by selection of results thought to be representative of such an effect, an attempt may now be made to model this response, so that the kinetic coefficients of an autotrophic sludge may be evaluated.

7.5 Evaluation of the Kinetic Coefficients of an autotrophic sludge, using ATP as a measure of biomass

7.5.1 Theory

The general shape assumed for the ATP response to substrate injection and utilisation for an autotrophic culture is illustrated below in Figure 7.11.

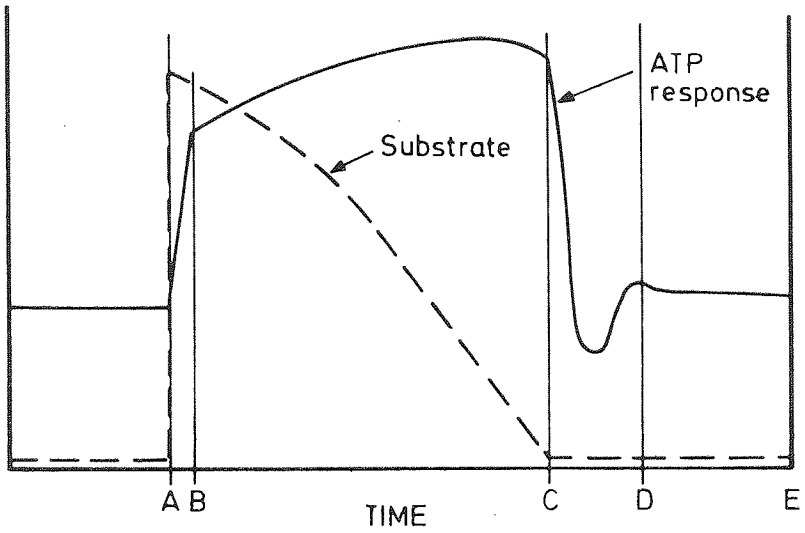


FIG. 7.11 ATP RESPONSE IN AN AUTOTROPHIC CULTURE

There are two very sharp jumps in ATP content in this curve. The first, shown in region A → B is a sudden increase in ATP when substrate is added. The second, shown in region C → D is a sudden decrease in ATP, occurring during substrate-limiting conditions. Neither of these jumps can be explained by the normal theory of growth and death, as the rates are virtually instantaneous.

It is therefore hypothesised that the sudden increase in ATP caused by addition of substrate is the immediate increase in metabolic activity caused by the addition of an energy source. Such activity may be the conversion of AMP or ADP to ATP, or some other similar process. It is not within the scope of this project to determine the actual cause.

In a similar way, it is hypothesised that the sudden decrease in ATP when substrate becomes limiting is caused by the immediate cessation of metabolic activity due to the lack of an energy source. A plausible example of what may be occurring is

the conversion of exogenous ATP to intracellular ADP.

To determine the kinetics of such a system, neither of these regions are of importance. It is regions B → C and D → E that require observation. During the time period B → C, it is hypothesised that increase in ATP is caused by growth. If one assumes Monod kinetics to be valid over this region, then the kinetic coefficients may be determined by fitting the observed results from this particular part of the graph to the Monod equations, that is;

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad \ldots\ldots\ldots \text{Eq. 3.1}$$

$$\frac{dX}{dt} = \mu X - k_e X \quad \ldots\ldots\ldots \text{Eq. 4.4}$$

$$-\frac{dS}{dt} = \frac{\mu X}{Y} \quad \ldots\ldots\ldots \text{Eq. 3.7}$$

Assistance in this operation may be forthcoming by observance of the region D → E. During this period, there are no exogenous energy sources available for bacterial multiplication and this period thus comprises the "decay period" during which bacteria are dying and, in so doing, are releasing endogenous nutrients for living cells.

Thus, this period is represented in the above equations by the term;

$$-\frac{dX}{dt} = k_e X \quad \ldots\ldots\ldots \text{Eq. 3.5}$$

where k_e = endogenous decay rate.

Integration of this equation yields

$$-\log_e X = k_e t + C \quad \ldots\ldots\ldots \text{Eq. 7.1}$$

By plotting values of $\log_e X$ against time for a batch system operating in conditions of endogenous decay, the endogenous decay rate may be estimated from the gradient of the curve.

Figure 7.6 shows such a plot, the value of k_e determined from the curve being:

$$k_e = 0.037 \text{ hours}^{-1}$$

7.5.2 Development of the batch model for an autotrophic culture

Regarding Figures 7.1 to 7.10, it can be seen that the general shape of the substrate curve is characterised by a flat curve at the moment of substrate innoculation, whose gradient then increases negatively until it attains a constant value, which it then maintains until substrate concentration is reduced to base-level.

Such a curve is illustrated in Figure 7.12.

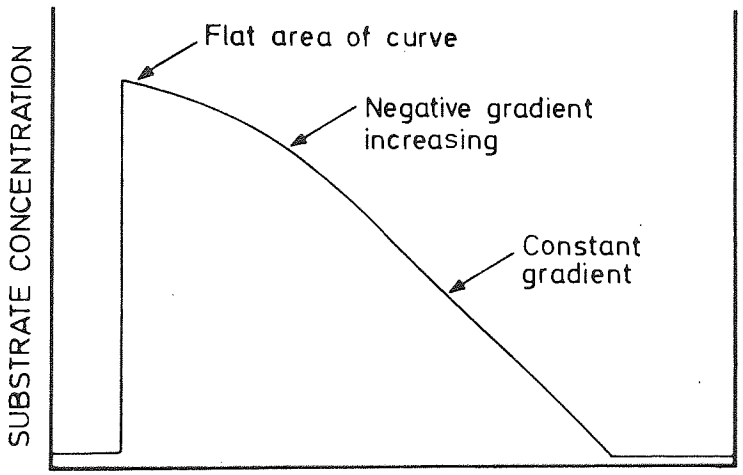


FIG. 7.12 CHARACTERISTICS OF A TYPICAL SUBSTRATE CURVE - AUTOTROPHIC SYSTEM

This type of substrate curve differs from the standard Monod curve in that the gradient remains constant until base-level substrate concentration is obtained. The Monod growth model thus requires modification to incorporate a term which will allow for this effect. What is required is a term which will produce self-inhibition at higher substrate levels, and which reduces to a negligible value at low substrate levels.

The Haldane model, as described in Section 6.6 fits the above criteria, and the basic Monod model is thus developed to include the Haldane term for substrate self-toxicity. To recap,

the Haldane model is given by equation 6.13,

$$\text{i.e. } \mu = \frac{\mu_{max}\ S}{K_s + S + S^2/K_t} \qquad \ldots\ldots\ldots \text{ Eq. 6.13}$$

where K_t is a constant of toxicity.

7.5.3 Process Modelling Technique

The behaviour of the system is simulated by solving the basic mathematical relationships given above. The values of five kinetic coefficients require enumerating, and "guesstimates" of each of these coefficients are input to the model, and the calculated curve compared with the experimentally derived curve.

It would be helpful to determine the value of at least one of the coefficients by experimental means. In Section 7.5.1 it has been shown that the value of k_e might be so obtained. However, the curve obtained in Run ATP-T6, when attempting to determine k_e in this fashion, showed much scatter in the data, and it would be unwise to place too much reliance on the straight line drawn through these points. The value of k_e obtained in this test is therefore suspect.

The method adopted then was to simulate the experimental results paying most attention to curve shapes that alteration of each coefficient produced. Then, guided by the Least Squares gradient (see Section 6.5.2) of that particular coefficient, the curve shape was progressively altered to produce a reasonable fit of the experimental data.

7.5.4 Simulation Hardware

- As in Section 6.5.3.

7.5.5 Results

Figure 7.13 illustrates a fit of the above model to the data of experiment ATP-T4. The values of the coefficients that are responsible for this fit are also shown.

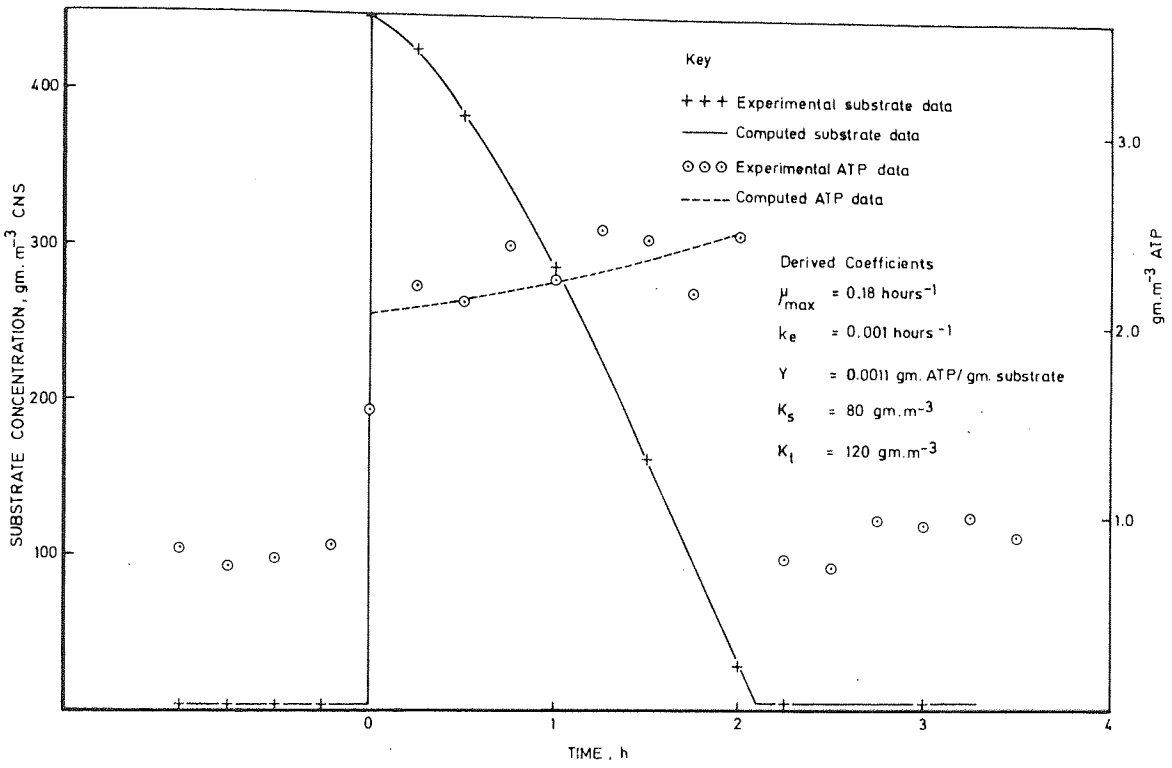


FIG.7.13 COMPUTED AND EXPERIMENTAL CURVES FOR RUN ATP - T4

7.6 Discussion

Only one of the experimental runs has been fitted to the model. This is because the model advanced for thiocyanate degradation in an autotrophic culture, using ATP as a measure of biomass, is only submitted as an example of the modelling technique.

It has earlier been stated that the ATP response to substrate loading and utilisation in an autotrophic system is not yet fully understood. Out of a total of fourteen batch experiments, only four give the type of response illustrated by Figure 7.11 and simulated in Section 7.5. Without being sure that this is the "correct" response, it would be unwise to devote too much attention to this particular hypothesis.

Furthermore, it is impossible at the present state of knowledge to determine at what ATP level growth begins. Regarding Figure 7.13, a computed ATP curve has been drawn through the data. However, this curve could have a shape totally different to the one drawn. Its starting point might well be at an ATP value lower, or higher than the one indicated. Before a curve can be reasonably fitted to such data, quantitative information regarding the sudden "jump" in ATP levels at the moment of substrate innoculation is required. If this jump is due to a change in energy state due to synthesis of ATP from ADP, then the ADP, and AMP concentrations before, and after the "jump" must be monitored in order to better understand the process, and arrive at a starting-point for the growth curve.

In the discussion on the results of the fitting of the phenol storage model, Section 6.12, it was stated that monitoring of the energy charge, which is a function of the concentrations of all three adenine nucleotides, would have been helpful to better understand the changing energy states within the system. The same applies in this case, and it is recommended that future work should ensure that the concentrations of all three adenine nucleotides are monitored, and that the use of the energy charge as a measure of microbial activity be tested.

ATP is a measure of the activity of the whole of the biomass. However, in a given "autotrophic" culture, less than 50% of the cells are in fact autotrophs (see Table 5.8). The effects of growth, or death, of the other types of cells present on the total ATP count have not been considered in this case. For the "heterotrophic" cultures tested in Chapter 6, non-heterotrophic cells are in a much lower proportion, and are much less likely therefore to affect the results.

The coefficient values determined from the fit of Run ATP-T4 are given as examples only. Without knowing the true shape of this sector of the curve, nor even whether this region is due to gorwth and decay alone, it would be foolhardy indeed to attempt to use these values as control parameters in a continuous process activated-sludge plant.

CHAPTER 8 VALIDATION OF THE MODEL

8.1 Introduction

To date, a model has been developed, based on Monod Kinetics, which attempts to represent the kinetics of a completely-mixed activated sludge plant treating a coke oven effluent. The model assumes that there are two main constituents of the effluent which are to be metabolised - organic compounds such as phenol, and a group of inorganics, namely thiocyanate, thiosulphate and cyanide. The model further assumes that each of these two groups of compounds are metabolised by a particular group of micro-organisms, each having a very different kinetic response. These two groups have been loosely labelled the heterotrophs - for organic metabolism, and the autotrophs - for inorganic metabolism. Finally, it is assumed that the presence of high concentrations of phenol in the mixed liquor in some way interferes with autotrophic metabolism.

Tests upon the kinetic response of each of these microbial groups have indicated that accurate quantification of the kinetic coefficients is of prime importance (see Section 4.12). Difficulties were found however in the measurement of bacterial populations. The use of MLSS or MLVSS as an indirect measure of biomass was found to be too insensitive, at least for the auto-trophic system. A more direct method of enumerating bacterial populations was then investigated - the analysis of the ATP content of the sludge. The results obtained in batch tests using such a measure of viability indicated that the standard Monod model does not sufficiently describe the kinetic response of either system. A model was then developed for the heterotrophic system which appeared to fit the experimental results. A model was also developed for the autotrophic system, but lack of consistency of results, together with a shortage of available time, means that this model must be viewed with scepticism.

However, estimates of the kinetic coefficients have been obtained for each of the microbial systems. No matter how unsure one may be of these values, they should be tested against real data if only to prove their erroneousness.

A search was therefore instigated to obtain plant data

with which to test the model. As mentioned in Section 1.5 there is an acute lack of historical data concerning the activated sludge treatment of coke-oven effluents, due in the main to the relative unimportance with which this subject was viewed in the past. Two paths of investigation were however pursued. At the moment of writing, the BSC has underway an ECSC sponsored project to gain data on all aspects of coke-oven effluent treatment in an integrated Iron- and Steelworks[155]. It was hoped that this project might provide data for validation of the model of the continuous process, completely-mixed activated sludge plant. However, examination of the data generated to date has shown wide gaps due mainly to problems in the commissioning of instrumentation. There are several constraints upon the information required. Some of these are outlined below

(a) Daily Phenol and thiocyanate influent and effluent concentrations must be available over the time period observed.
(b) Daily flow rates must be monitored.
(c) A time-period must be chosen in which the treatment both collapses and returns to design specification.
(d) The cause of the plant collapse must be one that is possible to model, e.g. sudden increase in volumetric or organic loading.
(e) All other constraints, such as environmental factors, must remain reasonably constant over the period.

No time period was observed in the data generated to date from the above project in which all of these constraints were met.

A second avenue of investigation was the Activated-Sludge Treatment plant at the Appleby-Frodingham steelworks, Scunthorpe. This plant was designed in the latter part of 1972 and commissioned in June 1974. Since that date, comprehensive records have been kept on its performance. These records were therefore searched in an attempt to find a time-period which met the above criteria. One such period was in fact found, although as is normally the case in practical problems, there were some items of data required which had not been recorded. However, sufficient data is available with which, together with some minor assumptions, the

model may be validated. In order to explain the necessity of these assumptions, a brief explanation of the Scunthorpe Biological Treatment Plant is required.

8.2 The Scunthorpe Works Biological Treatment Plant

Liquors from five Works in the Scunthorpe area are pumped through a series of pipelines to the treatment plant where they are mixed in a large reservoir before being fed into the aeration cells. A total of nine aeration cells, each fitted with a surface aerator, is provided. For the purpose of solids removal and sludge recirculation, the nine cells are operated as three streams, each comprising three cells. A schematic of the plant is given in Figure 8.1.

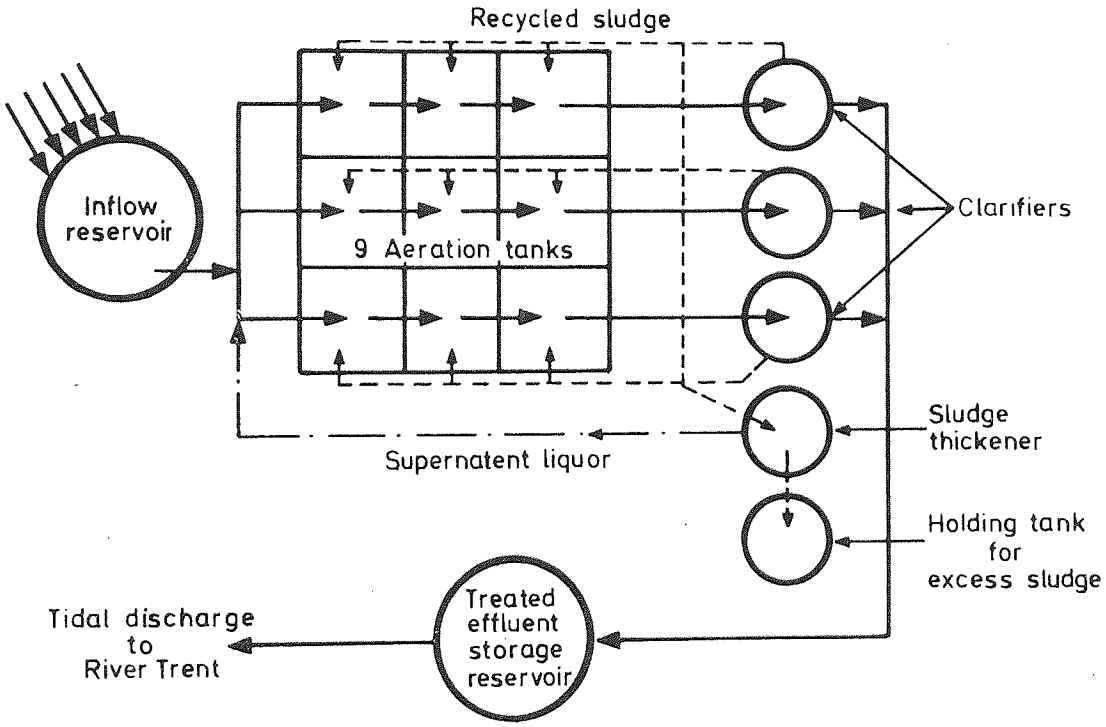


FIG. 8.1 Scunthorpe Works Biological Treatment System

The inflow reservoir is provided to smooth out fluctuations in effluent flows and qualities as well as to provide a reserve in the case of temporary failure of collecting pipelines or of the treatment plant itself.

The treated liquor from each of the three streams flows to a separate clarifier and under normal operation biological

sludge is returned only to those aeration cells serving the particular clarifier.

The supernatent liquor from the clarifiers flows by gravity to a "tidal storage" reservoir and is then discharged by gravity to the River Trent. Discharge takes place only during a period of about four hours around each high tide to ensure maximum dispersion of the treated liquors in the tidal reaches of the river.

Excess sludge withdrawn from the treatment process is thickened to approximately 3% in a picket fence thickener, and is then pumped into a sludge holding tank prior to sludge disposal.

8.2.1 Acquisition of Suitable Works Data for Model Validation

Amongst the parameters continuously monitored at the Scunthorpe Works Biological Treatment Plant are

(a) Influent Mono-phenol } daily samples at the inlet
(b) Influent thiocyanate } reservoir.
(c) Effluent Mono-phenol } daily samples for each of the
(d) Effluent thiocyanate } three streams at the clarifier.
(e) Total daily flow rate through all 9 streams.
(f) Influent pH, sampled at the inlet reservoir.
(g) Effluent pH, sampled at the tidal reservoir.

The above list indicates some of the problems encountered when attempting to utilise the Works data. Firstly, whilst influent and effluent compositions are available on a daily basis for each of the three streams, they cannot be accurately correlated to the flow rate, as this is only measured as a total daily flow throughout the whole of the plant. Secondly, because of the retention time of the system (normally 22 hours through the cells), and due to the fact that samples are only taken daily, an upset caused by increased loading might not be noticed in the effluent until two days later. Furthermore, although this has now been corrected, no information was available over the time period observed on the pH of the individual streams. Finally, neither the return-sludge flow rate nor the sludge wastage rate is metered.

It is possible to overcome these objections to a "perfect state of affairs" by

(i) Treating the nine cells as one whole. A mean effluent composition may then be determined from the individual compositions of each stream. These mean values can then be correlated to the average daily flow-rate.

(ii) Choosing a sufficiently large time-period such that a time-lag of up to two days will not be significant.

(iii) Assuming the effluent pH in the tidal reservoir to be representative of the order of pH in each stream.

(iv) Assuming the return-sludge flow rate to be constant. Under normal conditions, one sludge recycle pump is pumping full bore for each stream. The rating of the pumps is such that a recycled-sludge flow-rate of 227 $m^3.h^{-1}$ is obtained.

(v) Assuming a value of 5% for the sludge wastage ratio. This is the value given by the Scunthorpe Group Water Engineer as typical.

A search was therefore made through the plant records in an attempt to find one time-period in which plant-collapse had occurred due to an unexpected increase in hydraulic or organic loading. Several such upsets were in fact discovered, but most of these lasted only a day or two. It would be impractical to attempt to model such a short time period, as the time-lag due to retention and sampling-times would become extremely significant.

However, a period was found between January and March 1975 in which the plant had collapsed. Shortly after a sudden doubling of the flow rate, the concentrations of phenol and thiocyanate in the discharge began to rise. Both constituents eventually attained their influent concentrations, although the phenol quickly recovered. The thiocyanate however remained at its influent concentration for a period of about 25 days. Such an effect is similar to the simulation given in Figure 3.2, which was caused by the Inhibition effect incorporated into the model (see Section 3.9).

The raw data for this time period is given in Appendix 12, and the reduced data, i.e. the mean compositions of influent and effluent phenol and thiocyanate are plotted, together with the fluctuations in daily flow-rate, in Figure 8.2.

Figure 8.2 illustrates the plant response to a sudden (1 day) doubling of organic loading. Other factors such as pH and temperature may be incorporated into this effect, but it is assumed that their contribution is negligible. An analysis of the available pH records yields the following values.

For the Inlet Reservoir; Mean pH = 7.84,
Standard Deviation = 0.60.

For the Treated Effluent Storage; Mean pH = 7.38,
Standard Deviation = 0.20.

Thus, although there is some fluctuation in pH before treatment, it would appear that the treatment process itself tends to reduce the fluctuations and bring down the pH to a value which lies within the normal operating range for thiocyanate metabolism of pH 6.8 to 7.6[86].

Temperature measurements over this period were not continuously recorded. However, the only variations in temperature that would normally occur in a plant of this type would be of a seasonal nature. As this period lies within a single season, it is to be expected that temperature fluctuations would have been minimal. Some mixed-liquor temperatures were taken however over this period and their average of 28 ^{0}C lies close to the optimal temperature of 30 ^{0}C reported for the autotrophic biodegradation of thiocyanate[86].

There are two particular points of interest recognisable from Figure 8.2. Firstly, after its sudden increase, the flow-rate remains reasonably constant for a period of about 30 days, and then falls quickly to a value of about 3400 $m^3.day^{-1}$, at which value it then remains. Secondly, the thiocyanate concentration in the discharge is shown to be higher than its influent concentration on certain occasions. This is almost certainly due

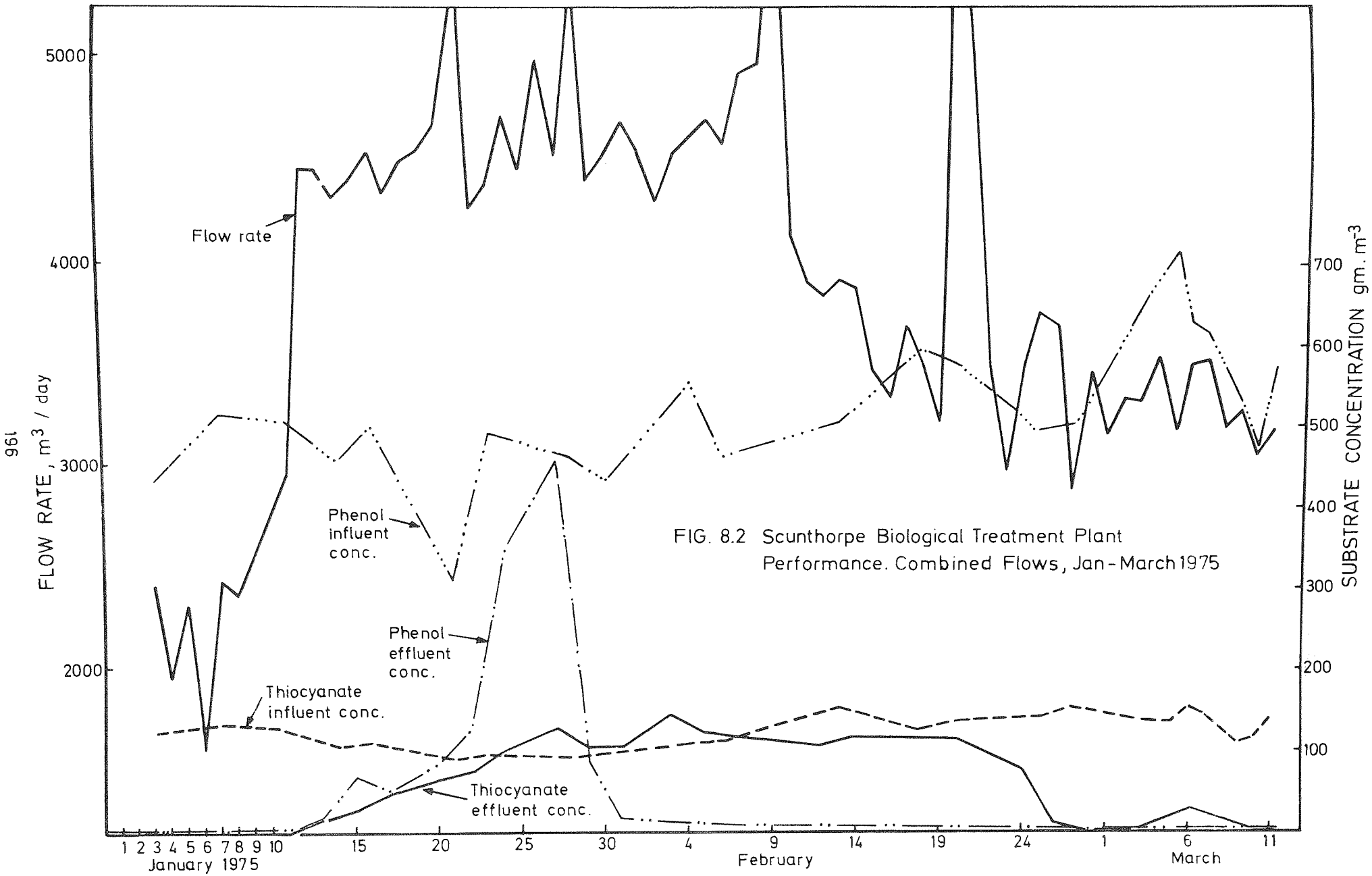


FIG. 8.2 Scunthorpe Biological Treatment Plant Performance. Combined Flows, Jan-March 1975

both to the time-lag through the plant, and to the fact that sampling intervals are often as long as three days.

Figure 8.3 shows the effect of "smoothing" the data, and illustrates more clearly the response of the plant to organic shock. It is this "smoothed" plot that is to be used to test the model.

8.3 Simulation of the Scunthorpe Biological Treatment Plant

The model developed previously may now be used to effect a simulation of the Scunthorpe Works. Actual plant data is to be input to the model, and the organic load varied as shown in Figure 8.3.

The model for ATP response to phenol utilisation will not at this stage incorporate a term for storage. As stated in Section 6.12, this model requires further validation before extending its consequences into continuous process activated-sludge plants. However, the Haldane model for both heterotrophic and autotrophic growth will be incorporated.

8.3.1 Assumptions

The nine aeration units of the Scunthorpe plant are to be considered as one reactor for the purpose of modelling.

The sludge-recycle flow-rate is assumed to be constant at 227 $m^3.h^{-1}$, i.e. the sludge-return pumps are assumed to be working continuously at full-bore.

It is assumed that no biological solids are lost in the plant discharge effluent.

It is assumed that pH and temperature effects over the period of observation are negligible, and that the plant upset observed is solely due to the organic shock.

In the absence of better information, an inhibition factor due to residual phenol content as given in Section 3.9 is to be incorporated into the model.

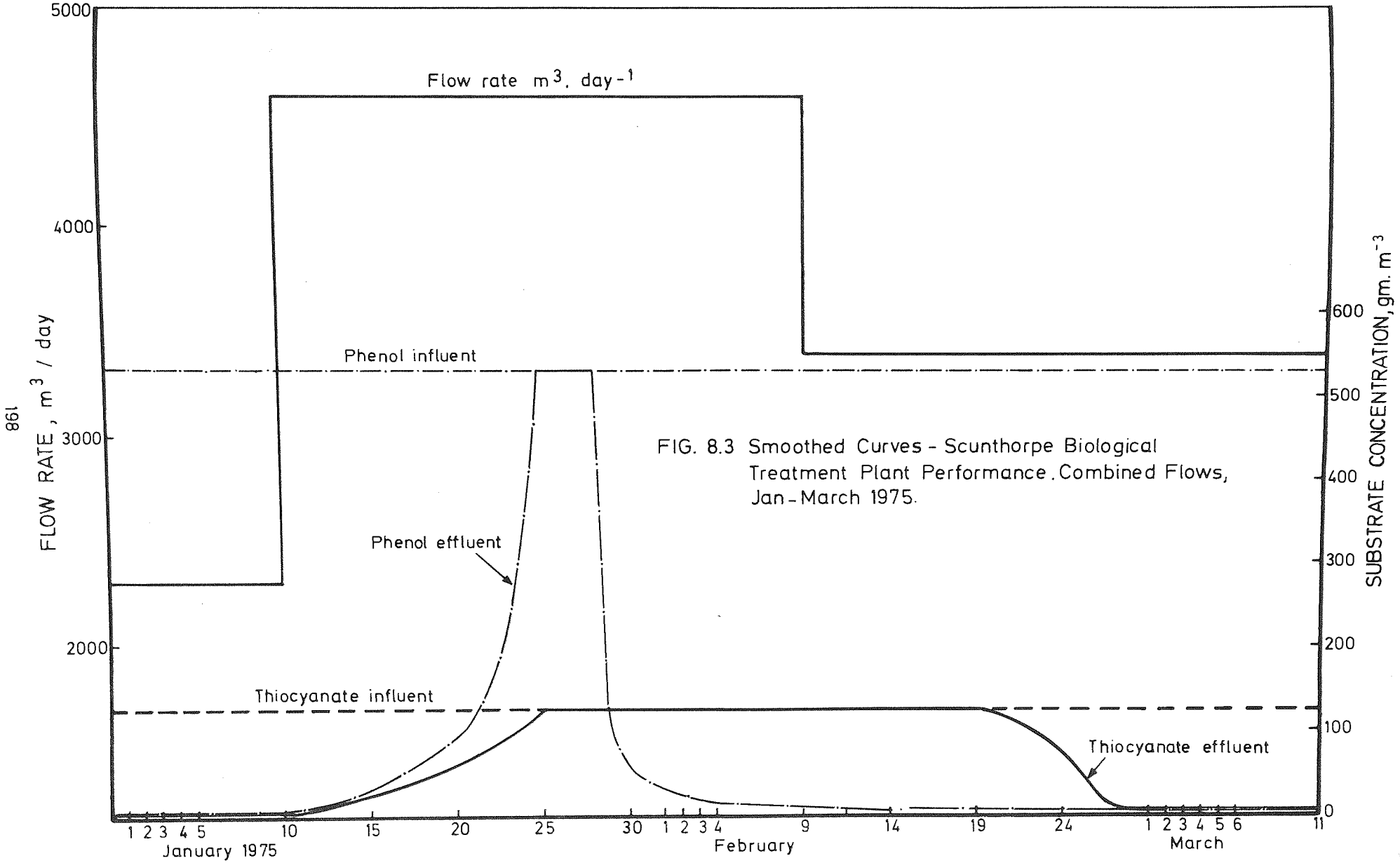


FIG. 8.3 Smoothed Curves - Scunthorpe Biological Treatment Plant Performance. Combined Flows, Jan - March 1975.

8.3.2 Plant Data

This is taken from plant design specifications, and from the assumptions given above.

V = Reactor Volume (summation of 9 aeration units)
= 5130 m^3

w = wastage ratio = 0.05

aq = Recycle-sludge flow-rate = 5448 $m^3.day^{-1}$

a = Recycle-sludge ratio = 5448/q

8.3.3 Design Data

The kinetic coefficients that have been previously evaluated for the two sludges (see Chapters 6 and 7) are to be tested in this simulation. Their values are given below.

	μ_{max} $days^{-1}$	k_e $days^{-1}$	K_s $gm.m^{-3}$	Y $\frac{gm.ATP}{gm.Substrate}$	K_t $gm.m^{-3}$
Autotrophic sludge	4.32	0.024	80.0	0.0011	120.0
Heterotrophic sludge	36.72	1.37	60.0	0.026	40.0

The inhibitive effect of residual phenol on autotrophic metabolism is given by the linear equation:

$I.F. = S_p/50.0$ ..... see Section 3.9

8.3.4 Initial Conditions

The initial substrate concentrations and flow-rate are taken from Figure 8.3.

S_{op} = initial phenol influent concentration
= 530 $gm.m^{-3}$

S_{oT} = initial thiocyanate influent concentration
= 125 $gm.m^{-3}$

qo = initial flow rate
= 2300 $m^3.day^{-1}$

The initial substrate concentrations in the discharge effluent, and the initial ATP concentrations in the mixed-liquor may then be determined from the steady-state equations of the

model, as illustrated earlier in Section 3.10.1.

Their values are thus determined:

S_P = phenol concentration in discharge effluent
= 2.45 $gm.m^{-3}$

S_T = thiocyanate concentration in discharge effluent
= 1.89 $gm.m^{-3}$

X_P = initial ATP concentration due to heterotrophic sludge
= 4.28 $gm.m^{-3}$

X_T = initial ATP concentration due to autotrophic sludge
= 0.648 $gm.m^{-3}$

NB These values have been determined using the kinetic coefficients given in Section 8.3.3. The actual measured substrate discharge concentrations are approximately 2.5 $gm.m^{-3}$ for thiocyanate, and 3.5 $gm.m^{-3}$ for phenol, which are very close to the computed values given above. The ATP concentration of the mixed-liquor was not measured and it is therefore impossible to know whether or not the values computed above are typical. However, it is interesting to note that there is a ten-fold difference calculated between the ATP concentration due to the heterotrophic sludge, and that due to the autotrophs, this factor of ten was also shown in the experimental work of Chapters 6 and 7.

8.3.5 Effect of organic-shock

The organic shock shown in Figure 8.3 is input to the model using the SLAM function SSTEP. This function is similar to the SRAMP function, details of which are given in Section 3.10.3, the only difference being that the variable is immediately increased, rather than allowed to increase linearly at a defined rate.

The flow-rate varies twice in Figure 8.3 and these effects are simulated thus:

```
Q = SSTEP (TIME, 10.0, 2300.0, 4600.0)      ..... Eq. 8.1
Q = SSTEP (TIME, 40.0, 4600.0, 3400.0)      ..... Eq. 8.2
```

Equation 8.1 states that the flow rate remains constant at

2300 m^3.day for the first ten days. It then immediately rises to a value of 4600 $m^3.day^{-1}$, at which value it then remains. Equation 8.2 states that the flow rate reduces from 4600 $m^3.day^{-1}$ to 3400 $m^3.day^{-1}$ on the fortieth day, and remains at this value thereafter.

8.3.6 Simulation Software

The model is programmed using SLAM (see Section 3.6.1). The dynamic equations are integrated one hundred times per day. The integration method used is SIMPSONS RULE. Copies of the listings produced, and outputs obtained are attached in Appendix 13.

The program is held under file-name SCUN.

The program name is BACTIN.

The initialisation and data-values are held in data-file SCUN-DATA.

8.4 Results

The results given in Appendix 13 show that the performance of the Scunthorpe Biological Treatment Plant is not satisfactorily simulated by the model proposed. When the hydraulic load is doubled on the tenth day of the simulation, both phenol and thiocyanate concentrations in the discharge rise only slightly. The growth of heterotrophs is shown to account for the doubling of load within one day, whilst although the autotrophs are shown to grow much more slowly, the increase in thiocyanate concentration in the discharge is not significant. These responses are very different to those actually observed, see Figure 8.2. It is impossible at this stage to say whether the difference is due to a fault in basic theory, to faulty coefficients, or to a combination of both these factors.

However, it should be stated that it was not expected that this simulation would be representative of the Scunthorpe Works response over the period tested. There are several factors which contributed to this statement. Firstly, as stated earlier in Section 7.6 no real credence was given to the kinetic coefficients obtained for the autotrophic sludge. Secondly, as detailed in Section 6.7, there are several models which could

have been developed to fit the results of the heterotrophic batch tests, each of which would have given different values of the kinetic coefficients of this sludge. Furthermore, no allowance was made in this simulation for the effect of storage compounds, if indeed such compounds are present and have effects on the ATP response and/or inhibitive effects on autotrophic growth. Finally, doubts have been expressed[156] about the composition of the activated-sludge at the Scunthorpe Works, and also about the degree of aeration. It was suggested that DO levels in this plant might be insufficient under periods of high organic loading. It was further suggested that thiocyanate oxidation at this Works is accomplished by heterotrophic organisms, such as those reported by Hiam[84], rather than by strictly autotrophic bacteria. If this is the case, the model developed to date will be inapplicable to such a system. Investigations are at present underway to prove, or disprove these two possibilities.

The main intention in presenting this simulation has been to make the program available to other workers. Improved results, rather more equitable with the observed response, could have been obtained by alteration of the values of the coefficients. However, these results would have had no real basis for inclusion, and the danger would then have been present of the possibility of their being taken out of context.

Finally, the results of this simulation show that there is yet much work to be done in order to satisfactorily model the activated-sludge process for the treatment of coke-oven waste-liquors. The negative results obtained are at least useful in showing this.

CHAPTER 9 DISCUSSION

In Section 1.5 the original aims of the project are outlined. It was stated that after preliminary investigations of the effluents arising from a modern, integrated iron- and steel-works, a decision was made to concentrate on quantitative modelling of the activated-sludge system for the treatment of coke-oven liquors.

The reasons for such a choice may be most clearly seen from Figure 8.2. This shows the actual performance of the Scunthorpe Biological Effluent Treatment Plant between January and March 1975. A doubling in organic load is shown to have caused plant failure, such that the discharge phenol concentration was above design specification for about 25 days, whilst the discharge thiocyanate concentration grossly exceeded design specification for almost 50 days.

Such occurrences are all too frequent, and can no longer be tolerated. However, lack of knowledge of the interactions involved in the biological treatment of coke-oven effluents make the solution of such problems exceedingly difficult, if not impossible. Control strategies are required in order to correct malfunctions such as these. Prior to the development of such control strategies however, the physical, chemical and biological interactions involved must be investigated and determined. An attempt has been made in this project to further knowledge in this area.

Firstly, and possibly most importantly, the measure of biological active mass used for modelling has not been treated as a homogenous, single species. Instead, the biomass has been split into two parts, nominated the "autotrophic" and the "heterotrophic" cultures, and two groups of substrate, inorganics such as thiocyanate,thiosulphate and cyanide, and organics such as phenols, have been associated with them. The work undertaken in the project has shown the usefulness, and the necessity of such an approach.

The Literature Survey of Chapter 2 indicated the need for this approach. In Section 2.5.4 it was reported that many workers have found that the presence of phenolics, or of actively-growing

masses of phenol-degrading bacteria, are inhibitory to biodegradation of thiocyanate. Without separating the two cultures for modelling purposes, a description of the inhibition process cannot be included in the model.

When tests were instigated in the laboratory in an attempt to determine the kinetic coefficients of each of the cultures, significant differences between the two cultures were observed. The heterotrophs were found easy to culture, with large masses of light, flocculent and easily-settleable material growing daily. The autotrophic culture on the other hand was of a gritty, particulate nature, and was very slow to grow.

The investigation carried out by Mr. R. Bolus into the composition of each of the cultures (see Section 5.8) showed the "heterotrophic" culture to be comprised predominantly of heterotrophic bacteria, whilst the "autotrophic" culture in fact contained only about 50% autotrophs. However, the composition of the two cultures was shown to be very different.

Although too much reliance should not be placed on the values obtained for the kinetic coefficients of each of the cultures, the growth rate obtained for the heterotrophic sludge (1.5 $hours^{-1}$) is much greater than that obtained for the autotrophs (0.18 $hours^{-1}$). Furthermore, the heterotrophic yield (0.026 g.ATP per g.phenol utilised) is much greater than the autotrophic yield (0.0011 g.ATP per g.thiocyanate utilised). Both of these results are in agreement with the observed behaviour of the cultures.

Furthermore, the observed response of the Scunthorpe Plant to shock-loading indicates some process of autotrophic inhibition due to the presence of phenol, or to the presence of actively-growing heterotrophs (see Figures 8.2 and 8.3).

The second stage of the project centred about the determination of the kinetic coefficients of each of the cultures. It was found that in an autotrophic culture, the use of the MLSS concentration to represent bacterial populations was too insensitive a measure. In this case, the total suspended solids contain

only a small proportion of active cells, and any increase in biomass due to growth is too small to be readily determined by this measure. Later experiments showed that the use of the Volatile Suspended Solids concentration (MLVSS) was just as inadequate for the autotrophic culture. The proportion of volatile solids to total solids was shown to remain reasonably constant over the whole of the growth cycle of the micro-organisms.

For the heterotrophic culture however, the MLSS concentration was shown to be a fairly representative measure of active biomass. The reason for this is the relatively high growth rate and high yield of the heterotrophs when growing on phenol. The proportion of active cells in the biomass would thus be much higher than in the autotrophic culture, and changes in "activeness" due to growth, or to death, would thus be more easily observed.

Unfortunately, in order to satisfactorily model the processes involved, a single measure of active biomass is required which is applicable to each of the cultures, so that interactions between them, such as inhibition effects, may be investigated.

Other measures of microbial activity were therefore investigated, and the use of Adenosine Triphosphate was decided upon. When measuring the ATP content of either an autotrophic or a heterotrophic batch culture, relatively smooth curves were obtained from which hypotheses were made about the ATP response to substrate metabolism.

One particularly interesting point which emerged from the ATP batch tests concerned the inhibition effect. In Section 2.5.4 three different inhibition paths are mentioned. They are as follows:

(a) Phenol itself is inhibitory.
(b) Actively growing cultures of phenol-degrading bacteria create inhibitory conditions.
(c) Oxidation intermediates produced by partial metabolism of phenol are inhibitory.

The last two of these paths are very similar. They both

suggest that inhibition of the thiocyanate organism occurs simultaneously with phenol metabolism, rather than because of the presence of a certain concentration of phenol alone.

Comparing these hypotheses with the results of the inhibition tests reported in Chapter 7 (Runs ATP-I1 to ATP-I4), some evidence is given that the inhibitive effect is not due to the addition of phenol itself. In Runs ATP-I3 and ATP-I4, shown in Figures 7.9 and 7.10, the inoculation of 100 $gm.m^{-3}$ of phenol does not affect the rate of removal of thiocyanate, in spite of the fact that such a phenolic concentration has been reported in the past to completely inhibit thiocyanate metabolism (see Section 2.5.4)

However, some effect is shown in these two experiments on the ATP response. The ATP is depressed temporarily as the phenol is added. The reason for this depression, and subsequent recovery is not known, but it probably represents the cellular response to a sudden change in environmental conditions.

In the other two inhibition tests, shown in Figures 7.7 and 7.8, there appears to be no common relationship between the ATP response and substrate removal. It should be noted however that in neither of these tests was the mixed liquor homogenised prior to testing, and the ATP data are probably just the result of a high degree of scatter. Homogenisation of the mixed liquor prior to sampling appears to be a necessary requirement for accurate determination of the ATP response.

The results of Chapter 6 suggest that complete metabolism of phenol might be a fairly lengthy process, in which two or more steps are required prior to the complete breakdown of the phenol. A storage process has been hypothesised in which the phenol is firstly metabolised to some intermediate storage compound, which is then later utilised when conditions allow (e.g. substrate-limiting conditions).

If, as Jones and Carrington [91] suggested, it is the presence of actively-growing masses of phenol-degrading bacteria that are responsible for the inhibition of the autotrophs, an explanation

may be forthcoming for the performance of the Scunthorpe Plant as shown in Figure 8.2. Between the 1st and 20th February, the difference between the utilisation rate and the production rate of the storage compounds was such that there was present a sufficiently high concentration of active, phenol-degrading bacteria to completely inhibit thiocyanate metabolism. After this time, their concentration diminished sufficiently to enable increasing metabolism of thiocyanate until, by the end of February, both residual storage product and thiocyanate concentrations in the discharge effluent were at base level.

Such a hypothesis would also fit the physical characteristics of the system. Virtually all of the sludge is recycled back to the aeration unit after settling, and only a volume equal to 5% of the influent flow-rate is wasted. It would thus take 20 days to wash-out the sludge from the system. From the date at which discharge phenol concentration has achieved base level, the 4th February, and thus presumably the date from which the storage products are being utilised at a significant rate, it takes a further 15 days before the thiocyanate begins to disappear. During this time, utilisation of storage compounds will be progressing at the same time as their concentration is diminishing due to sludge wastage. Furthermore, the absence of actively-growing thiocyanate-degrading organisms since the 25th January will have reduced their concentration, adding to the time required to re-establish a thiocyanate-degrading culture.

All of this is of course only one possible explanation of the observations recorded. Other hypotheses could have been proposed for the results of the batch tests reported in Chapter 6, and then developed to fit the observed response of the Scunthorpe Plant. What is most apparent from this work is that it is incomplete. If ATP is to be used as a measure of microbial activity, further information is required in order to satisfactorily explain what exactly is represented by a sudden change in ATP. It seems evident from the results of the batch tests on both the heterotrophic and the autotrophic cultures that ATP is not a good measure of total cell count under rapidly-changing conditions. If ATP is proportional to the total cell count, a reduction in ATP implies fewer cells. In this event, have the cells died? Instances are

shown in several batch tests of virtually instantaneous doubling, or halving, of the ATP concentration (see for example Figures 7.10.a and 7.10.b where the ATP concentration has dropped from 2.4 to 1.0 $gm.m^{-3}$ in less than 30 minutes). A reduction of this magnitude cannot be accounted for by decay alone. However, if as has been suggested, ATP is regarded as an energy substance which does not have a constant quantity per cell, then the observed changes in ATP can be explained without having extremely fast growth, and decay rates. It has been hypothesised in Section 7.4 that these sudden changes in ATP content are due to a change in energy state, when AMP and ADP are converted to ATP, or vice versa. It is this process that makes it impossible to model the ATP response of the cultures with any degree of certainty. More information is required in order to fully explain these sudden increases and decreases.

As mentioned in Section 6.7, it is planned to monitor the three adenine nucleotides in order to enable calculations of the Energy Charge. Other workers[135,141] have found this parameter to be a better measure of microbial activity than ATP alone. If it is found that the Energy Charge is a satisfactory measure of bacterial populations over the whole of the growth cycle, it will then be much easier to determine kinetic coefficients for each of the sludges, and to simulate the characteristics of a full-scale works.

On the positive side however, ATP has been shown to be a good measure of active biomass under steady-state conditions. Its analysis is relatively simple and quick, it appears to be accurate, and repeated assays give similar results. It follows therefore that ATP monitoring might prove useful in assessing the "health" of a plant. For example, a steady decline in ATP in the mixed-liquor might indicate a drop in performance long before this is shown by increases in discharge pollutant levels. Plant management could then take appropriate steps before treatment collapsed. Levin et al.[133] recommended the use of the food-to-micro-organisms ratio as a control strategy for maximum efficiency of the activated-sludge process, using ATP as the measure of viable micro-organisms. They found correlation between ATP and BOD levels in the mixed-liquor, whilst the volatile suspended solids concen-

tration was too insensitive to show any change.

Although an attempt was made in Chapter 8 to simulate the response of a completely-mixed, continuous process activated-sludge plant to a sudden doubling of organic load, it was fully realised that the results obtained would be very different to the actual response. The kinetic coefficients used in this model were those values determined by experiment, and were based on ATP. However, no allowance was made in the model for changes in ATP due to conversion to, or from, AMP or ADP, for the simple reason that there is no quantitative information available on this process. Furthermore, the inhibitory model used has no satisfactory experimental or theoretical basis, but is included to show the necessity of such a factor. The simulation is given both to show that much further work is required, and to ensure that the data is available with which to validate future models.

The basic approach of the model developed in this project is believed to be the correct one. Because of the interactions between the phenol-degrading and the thiocyanate-degrading micro-organisms, they must be modelled separately, and an inhibitory effect incorporated. However, more work of a quantative nature needs to be undertaken in order to more fully understand the process of inhibition, and to enable its modelling. The process of phenol metabolism has been shown to be much more complex than was previously thought. It is insufficient only to relate disappearance of substrate to increase in biomass; the products of metabolism and the change in energy levels need also to be taken into account.

Very high substrate levels have been shown to affect the metabolism of both autotrophic and heterotrophic cultures, and the Haldane model, which incorporates a constant of toxicity, has been used in preference to the Monod model for growth to better simulate the experimental results.

CHAPTER 10 GENERAL OBSERVATIONS AND CONCLUSIONS

1) Any attempt at modelling the activated-sludge treatment of coke-oven effluents using a fundamental, rather than a "black-box" approach, requires that the sludge be considered as being comprised of at least two general types of bacteria, those responsible for the biodegradation of phenol, and those responsible for the biodegradation of thiocyanate.

2) A factor should be incorporated into the model to allow for the inhibition of autotrophic metabolism caused by the presence of high concentrations of phenol, or more likely by the presence of actively-growing masses of phenol-degrading bacteria.

3) A factor should be incorporated into the models of both autotrophic and heterotrophic growth to allow for self-toxicity at high substrate concentrations. The Haldane model for self-toxicity appears to be a suitable expression of this effect.

4) The numerical values of the kinetic coefficients used in the model are of prime importance, and their determination should take place where possible under similar operating conditions as the plant under investigation. All environmental conditions (temperature, pH, etc.) must be held constant at normal operating conditions.

5) The measurement of MLSS and MLVSS does not satisfactorily represent the concentration of active autotrophic bacteria.

6) Under starved conditions, measurement of ATP concentration appears to represent total cell count.

7) Measurement of ATP is slightly affected by the constituents of a coke-oven liquor, but concentrations of phenol and thiocyanate such as are normally present in such a liquor do not significantly affect measurement.

8) Determination of ATP concentration is reliable and reasonably accurate. It is also a very rapid analysis.

9) Under rapidly-changing conditions (e.g. during periods of high growth or sudden substrate insufficiency), observed changes in ATP cannot be accounted for by growth or by decay alone. Therefore the concentration of ATP per cell does not remain constant under such conditions.

10) Further investigations into the use of ATP as a measure of active biomass are required. These should take into account both the products of metabolism, and the changes in energy state as measured by ADP and AMP. The use of the Energy Charge as a measure of active biomass should also be investigated.

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APPENDICES

APPENDIX 1 NOTATION AND ABBREVIATIONS

1.1 Definitions

AUTOTROPHS - a thiocyanate oxidising sludge comprised mainly of autotrophic bacteria.

HETEROTROPHS - a phenol oxidising sludge comprised mainly of heterotrophic bacteria.

1.2 Abbreviations

A.M.P.	-	Adenosine Mono-Phosphate
A.D.P.	-	Adenosine Di-Phosphate
A.T.P.	-	Adenosine Tri-Phosphate
B.C.R.A.	-	British Carbonisation Research Association
B.O.S.	-	Basic Oxygen Steelworks
B.O.D.	-	Biochemical Oxygen Demand
B.S.C.	-	British Steel Corporation
C.E.L.	-	Corporate Engineering Laboratories
CNS	-	Thiocyanate
COE	-	Coke Oven Effluent
DMSO	-	Dimethyl Sulfoxide
DNA	-	Deoxyribonucleic Acid
D.O.	-	Dissolved Oxygen
E.C.	-	Energy Charge
E.C.S.C.	-	European Coal and Steel Community
fg.	-	femtogram = 10^{-15} gram
I.H.D.	-	Interdisciplinary Higher Degree
I.S.I.	-	Iron and Steel Institute
L.S.F.	-	Least Squares Fit
M.L.S.S.	-	Mixed Liquor Suspended Solids
M.L.V.S.S.	-	Mixed Liquor Volatile Suspended Solids
N.W.C.	-	National Water Council
P.V.	-	Permanganate Value
R.W.A.	-	Regional Water Authority
S/S	-	Steady State
SLAM	-	Simulation Language for Analogue Modelling
Stab.Water	-	Stabilised Water - see Appendix 9
TRIS buffer	-	2-amino-2-(hydroxymethyl) propane-1,3 idol
TSS	-	Total Suspended Solids
TTC	-	Tetrazolium Chloride
TOC	-	Total Organic Carbon
VSS		Volatile Suspended Solids

1.3 Notation

a - Sludge recycle ratio

b - sludge concentration factor $= \left(\frac{1+a}{a+w}\right)$

c - integration constant

C_1 - constant ..... time^{-1}

C_2 - energy rate constant ..... concentration/time

D - dilution ratio ..... time^{-1}

I - concentration of storage compounds .. mass/volume

IF - Inhibition Factor

k_e - endogenous respiration rate or basal energy requirement ..... time^{-1}

K_s - saturation constant, related to substrate ..... mass/volume

K_I - saturation constant, related to storage compounds ..... mass/volume

K_t - constant of toxicity ..... mass/volume

q - flow rate ..... volume/time

S - Substrate concentration ..... mass/volume

t - time

V - reactor volume ..... volume

W - wastage ratio

X - concentration of biomass (as MLSS or ATP) ..... mass/volume

Y - Yield Coefficient - gms. of biomass produced per gm. substrate utilised.

μ - specific growth rate ..... time^{-1}

μ_{max} - maximum specific growth rate ..... time^{-1}

μ_x - growth rate determined from equation 4.8 ..... time^{-1}

μ_s - growth rate determined from equation 4.10 ..... time^{-1}

θ - steady-state detention time = $^1/\mu - k_e$ ..... time

Subscripts

o - relates to influent values
i - relates to values at time = 0
p - represents heterotrophs
T - represents autotrophs
av - average
I - relates to storage compounds

CONFIDENTIAL

TECHNICAL NOTE

No. CEL/CE/TN/34/75
Date JULY 1975

BATTERSEA LABORATORY
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ORIGIN

CONSERVATION ENGINEERING DEPARTMENT

TITLE

SLAM - SIMULATION LANGUAGE FOR ANALOGUE MODELLING

AUTHOR(S)

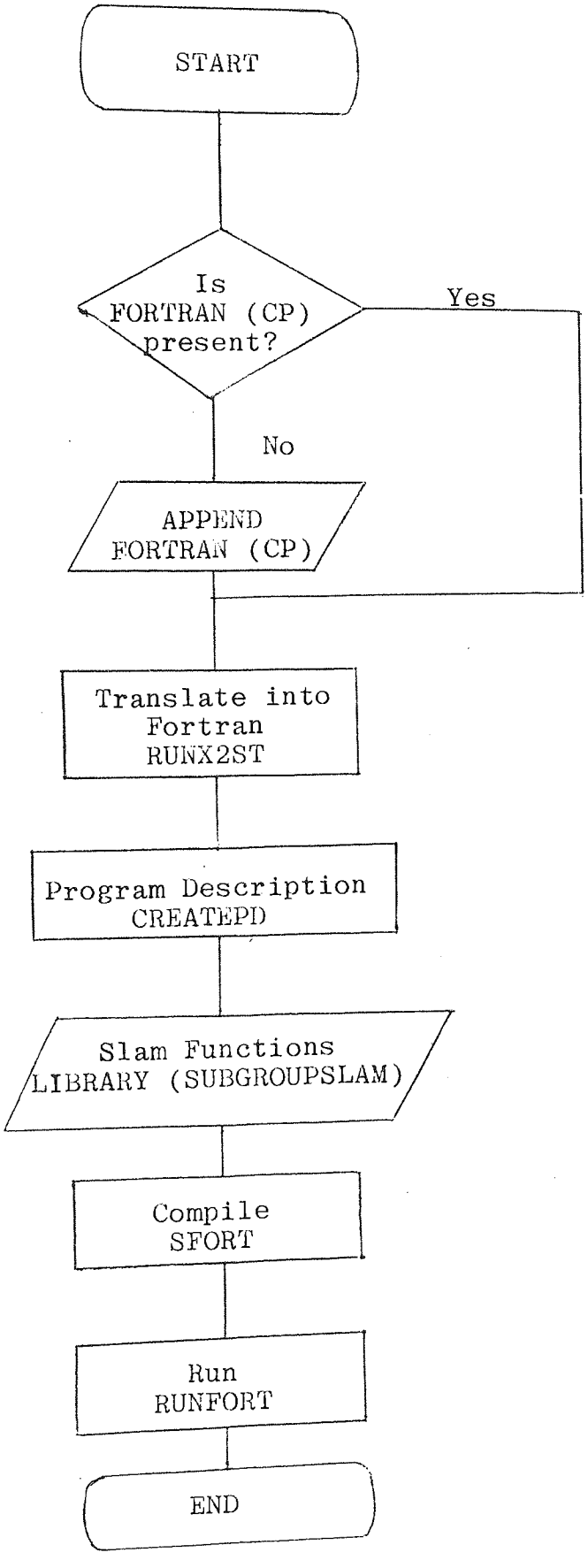
G. F. TOMLINS

DISTRIBUTION LIST

APPENDIX 2

APPENDIX 2.1

SCHEMATIC REPRESENTATION OF USER MACRO - SLAM



APPENDIX 2.2

USER MACRO SLAM

```
DOCUMENT     SLAM

WE CONERR,GO 3CL1
IF STR(%C)=(),GO 3CL0
MF "       FORTRAN(CP)"
CY %A,!(APPE)
CY !,%A
ER !
3CL0 RUNX2ST *CR%A,*CP%A-SOURCE,ER 3CL1
CREATEPD %A,FORT
MF "       LIBRARY(SUBGROUPSLAM)"
CY %A-SOURCE,!(APPE)
CY !,%A-SOURCE
ER !
SFORT %A,*LPL,LIBSLAM,ER 3CL1
RUNFORT %A,*TR%A,*ER 3CL1,*LP
DP 0, SLAM RUN OK
EX
3CL1 DP 0, SLAM RUN FAILED
IF COR,DL
```

APPENDIX 2.3

USER MACRO SLMG

```
DOCUMENT     SLMG

WE CONERR,GO 3CL1
IF STR(%C)=(),GO 3CL0
MF "       FORTRAN(CP)"
CY %A,!(APPE)
CY !,%A
ER !
3CL0 RUNX2ST *CR%A,*CP%A-SOURCE,ER 3CL1
CREATEPD %A,FORT
MF "       LIBRARY(SUBGROUPSLAM)"
CY %A-SOURCE,!(APPE)
CY !,%A-SOURCE
ER !
MF "       LIBRARY(A.SUBROUTINES)"
CY %A-SOURCE,!(APPE)
CY !,%A-SOURCE
ER !
SFORT %A,*LPL,LIBSLAM,LIBPLOT,ER 3CL1
RUNFORT %A,*TR%A,*ER 3CL1,*LP,*GP
DP 0, SLAM RUN OK
GO 3CL2
3CL1 DP 0, SLAM RUN FAILED
IF COR,DL
3CL2 IF NOT MOF,EJ
```

S L A M T R A N S L A T I O N B Y #X2ST MARK 1.4

SOURCE INPUT LISTING

```
     1              FORTRAN(CP)
     2              MASTER DYNAS1
     3                 TITLE 'DYNAMIC, MIXED, ACTIVATED SLUDGE, TRIAL 1.
     4             1           EQUATIONS BY DOWNING, PAINTER, CURDS ET AL. 1, 4L.
     5             2      '       B              D           THETA          SO            XO
     6             3              S1      '
     7              REAL Q,V,A,B,MU,KE,Y,SO,XO,STEP,HOURS,MUMAX,KS
     8       C
     9       C   INITIALISATION
    10       C
    11              Q=227.0
    12              V=5000.0
    13              A=0.35
    14              W=0.05
    15              MUMAX=0.8
    16              KS=350.0
    17              KE=0.007
    18              Y=0.39
    19              B=(1.0+A)/(A+W)
    20              D=Q/V
    21              THETA=1.0/D
    22                 INPUT SO,XO,S1,STEP,HOURS,IM
    23                 OUTPUT B,D,THETA,SO,XO,S1,3L
    24                 OUTHD '     TIME      ',       X       ',       S       '
    25       C  DYNAMIC EQUATIONS
    26              MU=MUMAX*S/(KS+S)
    27              DX=D*A*B*X+MU*X-D*X*(1.0+A)-KE*X
    28              X=INTGRL(DX,XO)
    29              DS=D*(SO-S)-MU*X/Y
    30              S = INTGRL (DS,S1)
    31              INTINF
    32              ALG;IM=SIMP,RKFS,RKVS
    33              CI;STEP
    34              END
    35              OUTEQ TIME,X,S
    36              TERMINATE (TIME.GE.HOURS)
    37              REPEAT (SO .GT. 0.0)
    38              END
    39              FINISH

          **** ~ END OF INPUT
```

EQUATIONS BY DOWNING, PAINTER, CURDS ET AL.

DYNAMIC, MIXED, ACTIVATED SLUDGE, TRI - 1.

B	D	THETA	SO	XO	S1
3.3750	4.54000E-02	22.026	2000.0	100.00	1500.0

TIME	X	S
0.00000E 00	100.00	1500.0
1.0000	186.97	1298.6
2.0000	339.16	957.98
3.0000	563.23	406.52
4.0000	722.40	55.775
5.0000	759.36	22.629
6.0000	783.48	20.903
7.0000	807.32	20.235
8.0000	830.61	19.631
9.0000	853.55	19.071
10.000	876.15	18.550
11.000	898.42	18.064
12.000	920.36	17.610
13.000	941.98	17.185
14.000	963.28	16.785
15.000	984.27	16.409
16.000	1004.9	16.055
17.000	1025.2	15.721
18.000	1045.4	15.405
19.000	1065.2	15.106
20.000	1084.7	14.823

DYNAMIC, MIXED, ACTIVATED SLUDGE. TRIAL 2.

EQUATIONS BY DOWNING, PAINTER, CURDS ET AL.

B	D	THETA	S0	X0	S1
3.3750	4.54000E-02	22.026	2000.0	1000.0	1000.0

TIME	X	S
0.00000E 00	1000.0	1000.0
0.10000	1058.2	859.79
0.20000	1116.5	703.66
0.30000	1173.7	558.98
0.40000	1227.9	422.43
0.50000	1276.7	300.00
0.60000	1317.6	198.23
0.70000	1348.6	121.95
0.80000	1369.7	71.478
0.90000	1382.6	41.937
1.0000	1390.2	26.255
1.1000	1394.7	18.662
1.2000	1397.6	16.686
1.3000	1399.8	12.907
1.4000	1401.6	12.072
1.5000	1403.2	11.677
1.6000	1404.8	11.688
1.7000	1406.2	11.396
1.8000	1407.7	11.346
1.9000	1409.2	11.315
2.0000	1410.7	11.295
2.1000	1412.1	11.279
2.2000	1413.6	11.265
2.3000	1415.0	11.253
2.4000	1416.5	11.240
2.5000	1417.9	11.228
2.6000	1419.4	11.217
2.7000	1420.8	11.205
2.8000	1422.3	11.193
2.9000	1423.7	11.181
3.0000	1425.1	11.170
3.1000	1426.6	11.158
3.2000	1428.0	11.147
3.3000	1429.4	11.135
3.4000	1430.9	11.124
3.5000	1432.3	11.112
3.6000	1433.7	11.101
3.7000	1435.1	11.090
3.8000	1436.6	11.078
3.9000	1438.0	11.067
4.0000	1439.4	11.056
4.1000	1440.8	11.045
4.2000	1442.2	11.033
4.3000	1443.6	11.022
4.4000	1445.0	11.011
4.5000	1446.4	11.000
4.6000	1447.8	10.989
4.7000	1449.3	10.978
4.8000	1450.7	10.967
4.9000	1452.0	10.956
5.0000	1453.4	10.946
5.1000	1454.8	10.935
5.2000	1456.2	10.926
5.3000	1457.6	10.913
5.4000	1459.0	10.903
5.5000	1460.4	10.892
5.6000	1461.8	10.881
5.7000	1463.2	10.871
5.8000	1464.5	10.860
5.9000	1465.9	10.850
6.0000	1467.3	10.839
6.1000	1468.7	10.829
6.2000	1470.0	10.818
6.3000	1471.4	10.808
6.4000	1472.8	10.798
6.5000	1474.1	10.787
6.6000	1475.5	10.777
6.7000	1476.9	10.767
6.8000	1478.2	10.757
6.9000	1479.6	10.747
7.0000	1480.9	10.736
7.1000	1482.3	10.726
7.2000	1483.6	10.716
7.3000	1485.0	10.706
7.4000	1486.3	10.696
7.5000	1487.7	10.686
7.6000	1489.0	10.676
7.7000	1490.4	10.666
7.8000	1491.7	10.657
7.9000	1493.1	10.647
8.0000	1494.4	10.637
8.1000	1495.7	10.627
8.2000	1497.1	10.618
8.3000	1498.4	10.608
8.4000	1499.7	10.598
8.5000	1501.0	10.589
8.6000	1502.4	10.579
8.7000	1503.7	10.569
8.8000	1505.0	10.560
8.9000	1506.3	10.550
9.0000	1507.6	10.541
9.1000	1509.0	10.531
9.2000	1510.3	10.522
9.3000	1511.6	10.513
9.4000	1512.9	10.503
9.5000	1514.2	10.494
9.6000	1515.5	10.485
9.7000	1516.8	10.475
9.8000	1518.1	10.466
9.9000	1519.4	10.457
10.000	1520.7	10.448

```
      MASTERDYNAS1
      DIMENSION$A1(2),$A2(2),$W1(2),$W2(2),$W3(2),$W4(2),$W5(2),$W6(2),$
     $W7(2),$W8(2),$W9(2),$W10(2),$W11(2),$W12(2),$W13(2)
      REAL*12 I
      REAL Q,V,A,B,MU,KE,Y,SO,XO,STEP,HOURS,MUMAX,KS
      LOGICAL*1 I005,I013
      COMMON/SLAMC1/KEEP,I001,I002,I003,I004
      EXTERNAL$ERROR
50004 FORMAT(8G0.0)
C     ******************************************************************
C     SEGMENT INITIALISATION
C     ******************************************************************
      CALL$TRAP($ERROR)
50000 CONTINUE
      KEEP=1
      I001=1
      I002=2
      I003=0
      I005=.TRUE.
      I006=1
      MINS=10
      MAXS=100
      I=ON=0
      TIME=0.0
      RLER=.01
      I020=1
      I021=1
      I022=1
C     ******************************************************************
C     PRE-INITIAL REGION
C     ******************************************************************
51002 FORMAT(1H1,'DYNAMIC, MIXED, ACTIVATED SLUDGE, TRIAL 1.
     $              EQUATIONS BY DOWNING, PAINTER, CURDS ET AL. !////1X,'
     $      B               D           THETA         SO            XO
     $      S1      ')
      WRITE(I002,51002)
C     ******************************************************************
C     INITIAL REGION
C     ******************************************************************
      Q=227.0
      V=5000.0
      A=0.35
      B=0.05
      MUMAX=0.8
      KS=350.0
```

```
      RE=.007
      V=0.39
      B=(1.0+A)/(A+W)
      D=Q/V
      THETA=1.0/B
      READ(1001,50004,END=50003)SO,XO,S1,STEP,HOURS,IM
51003 FORMAT(1X,1PG13.5,1PG13.5,1PG13.5,1PG13.5,1PG13.5,1PG13.5,1PG13.5///1X)
      WRITE(1002,51003)B,D,THETA,SO,XO,S1
51004 FORMAT(1X,'    TIME      ','       X       ','       S       ')
      WRITE(1002,51004)
C     ****************************************************************
C     DYNAMIC REGION
C     ****************************************************************
50001 CONTINUE
C     ****************************************************************
C     DERIVATIVE SECTION 1
C     ****************************************************************
      IF(I005)GOTO51001
51000 CONTINUE
      GOTO(51006,51007,51008),IM
51006 CALLSIMP (2,1006,1020,MINS  ,MAXS  ,IMON  ,STEP  ,TIME  ,RLER  ,0
     E,SA1,SA2,SW1,SW2,SW3,SW4)
      GOTO51001
51007 CALLRKFS (2,1006,1021,MINS  ,MAXS  ,IMON  ,STEP  ,TIME  ,RLER  ,0
     E,SA1,SA2,SW5,SW6,SW7,SW8,SW9)
      GOTO51001
51008 CALLRKVS (2,1006,1022,MINS  ,MAXS  ,IMON  ,STEP  ,TIME  ,RLER  ,0
     E,SA1,SA2,SW10,SW11,SW12,SW13)
51001 CONTINUE
      IF(I005)SA2(1)=XO
      X=SA2(1)
      IF(I005)SA2(2)=S1
      S=SA2(2)
      MU=MUMAX*S/(KS+S)
      DX=D*A*B*X*MU*X+D*X*(1.0+A)*RE*X
      SA1(1)=DX
      DS=D*(SO-S)-MU*X/Y
      SA1(2)=DS
      IF(I006.NE.1)GOTO51000
51005 FORMAT(1X,1PG13.5,1PG13.5,1PG13.5)
      WRITE(1002,51005)TIME,X,S
      I013=(TIME.GE.HOURS)
      IF(I013)GOTO50002
      I005=.FALSE.
      GOTO50001
50002 CONTINUE
C     ****************************************************************
C     TERMINAL REGION
C     ****************************************************************
      I013=(SO.GT.1.0.0)
      IF(I013)GOTO50000
50003 STOPOK
      END

END OF SEGMENT, LENGTH   420, NAME  DYNAMI
```

S L A M T R A N S L A T I O N H Y #X2ST MARK 2.1

SOURCE INPUT LISTING

```
    1            OPTIONS ELIST,DEBUG1,EXPLICIT
    2            FORTRAN(CP)
    3            MASTER BACTIN
    4             DIMENSION TAXS(2),SAXS(8)
    5             DATA TAXS(1)/14HTIME IN HOURS./,SAXS(1)/61HST AND SP IN MG PER L
    6           1.    DASH=LINE=THIO,  SOLID=LINE=PHENOLS./
    7             REAL I,MUP,MUT,MUMAXP,MUMAXT,KSP,KST,HOURS,KEP,KET
    8             REAL LASTSP,LASTST,LASTXP,LASTXT,LASTT,LATSOP
    9             INITIAL
   10                 NOSORT
   11                 COMMON ISET
   12                 IF (ISET.EQ.123456) GO TO 10
   13                 CALL HGPSET(9)
   14                 ISET=123456
   15          10     CONTINUE
   16                 CALL HGPLOT (-1,0,-1,0,0,4)
   17                 CALL HGPAXIS (0.0,0.0,TAXS,-14,20.0,0.0,0.0,0.0,2.5)
   18                 CALL HGPAXIS (0.0,0.0,SAXS,60,11.0,90.0,0.0,0.0,150.0)
   19                 END
   20            TITLE 'SINGLE-STAGE, MIXED ACTIVATED-SLUDGE UNIT ALLOWANCE
   21           1 MADE FOR DIFFERENT BACTERIA AND INHIBITION BY PHENOL.',4L,
   22           2 '       D              XP0            SP0            XT0          ST0
   23           3 ',       SOT       '
   24      C
   25      C
   26      C          FILESTORE SOP7
   27      C         INITIALISATION
   28      C
   29                 Q=227.0
   30                 V=5000.0
   31                 A=0.35
   32                 W=0.05
   33                 KET=0.0023
   34                 MUMAXT=0.05
   35                 KST=10.0
   36                 KEP=0.09
   37                 KSP=10.0
   38                 MUMAXP=0.134
   39                 YP=1.8
   40                 YT=0.4
   41                 B=(1.0+A)/(A+W)
   42                 D=Q/V
   43                 THETA=1.0/D
   44                 INPUT      SOT,SP0,ST0,XP0,XT0,STEP,HOURS,IM
   45                 INPUT IRUN
   46                 LASTT=0.0
   47                 LASTSP=SP0/150.0
   48                 LASTST=ST0/150.0
   49                 LATSOP=1000.0/150.0
   50                OUTPUT D,XP0,SP0,XT0,ST0,SOT
   51                OUTPUT 2L
```

S L A M T R A N S L A T I O N B Y #X2ST MARK 2.1

```
                OUTPUT '      TIME     ',      XP       ',       SP       ',       XT
     1          ',       ST       ',      SOP      '
                END
C
                DYNAMIC
                 DERIVATIVE
                  SOP=SRAMP(TIME,10.0,20.0,1000.0,1400.0)
                  MUP=MUMAXP*SP/(KSP + SP)
                  MUT=(MUMAXT*ST/(KST +ST))*(1.0-I)
                  I=SP/50.0
                  DXT=D*A*B*XT +MUT*XT-KET*XT-D*XT*(1.0+A)
                  DXP=D*A*B*XP +MUP*XP-KEP*XP-D*XP*(1.0+A)
                  DSP=D*(SOP-A*SP)-MUP*XP/YP-D*SP*(1.0+A)
                  DST=D*(SOT-A*ST)-MUT*XT/YT-D*ST*(1.0+A)
C
                  XT=INTGRL(DXT,XT0)
                  XP=INTGRL(DXP,XP0)
                  SP=INTGRL(DSP,SP0)
                  ST=INTGRL(DST,ST0)
C
                   INTINF
                   ALG:IM=SIMP,RKFS,RKVS
                   CI:STEP
                   END
                 END
C
                  PARALLEL
                NOSORT
                 IF (ST .GE. SOT) ST=SOT
                 IF (SP .GE. 50.0) I=1.0
                END
                   OUTPUT TIME,XP,SP,XT,ST,SOP
                   TIMEG=TIME/2.5
                   SPG=SP/150.0
                   STG=ST/150.0
                  SOPG=SOP/150.0
                   HOURSG=HOURS*10.0
                     NOSORT
                      CALL HGPLOT (LASTT,LASTSP,3,0)
                      CALL HGPLOT (TIMEG,SPG,2,0)
                      CALL HGPLOT (LASTT,LASTST,3,0)
                      CALL HGPLOT (TIMEG,STG,3,7)
                      CALL HGPLOT (TIMEG,STG,2,7)
                     CALL HGPLOT (LASTT,LATSOP,3,0)
                      CALL HGPLOT (TIMEG,SOPG,2,0)
                      LASTT=TIMEG
                      LASTSP=SPG
                      LASTST=STG
                     LATSOP=SOPG
                     END
                   TERMINATE (TIME .GE. HOURS)
                  END
                END
```

SINGLE-STAGE, MIXED ACTIVATED-SLUDGE UNIT.ALLOWANCE MADE FOR DIFFERENT BACTERIA AND INHIBITION BY PHENOL.

D	XP0	SP0	XT0	ST0	SUT
4,54000E-02	816.30	26.874	880.00	9.1000	500.00

TIME	XP	SP	XT	ST	SOP
0.00000E 00	816.30	26.874	880.00	9.1000	1000.0
0.50000	816.21	26.452	880.37	8.2258	1000.0
1.0000	815.97	26.132	880.61	7.7130	1000.0
1.5000	815.61	25.896	880.77	7.4109	1000.0
2.0000	815.17	25.726	880.88	7.2278	1000.0
2.5000	814.67	25.608	880.97	7.1137	1000.0
3.0000	814.12	25.531	881.04	7.0416	1000.0
3.5000	813.55	25.485	881.10	6.9967	1000.0
4.0000	812.97	25.465	881.16	6.9704	1000.0
4.5000	812.38	25.463	881.20	6.9574	1000.0
5.0000	811.79	25.475	881.25	6.9540	1000.0
5.5000	811.22	25.498	881.29	6.9577	1000.0
6.0000	810.65	25.528	881.33	6.9668	1000.0
6.5000	810.10	25.564	881.37	6.9797	1000.0
7.0000	809.57	25.605	881.40	6.9955	1000.0
7.5000	809.05	25.647	881.44	7.0133	1000.0
8.0000	808.55	25.691	881.47	7.0325	1000.0
8.5000	808.07	25.736	881.51	7.0527	1000.0
9.0000	807.62	25.782	881.54	7.0734	1000.0
9.5000	807.18	25.827	881.57	7.0944	1000.0
10.000	806.76	25.871	881.60	7.1154	1000.0
10.500	806.39	26.122	881.62	7.1623	1020.0
11.000	806.10	26.719	881.60	7.3189	1040.0
11.500	806.29	27.581	881.51	7.6182	1060.0
12.000	806.76	28.646	881.36	8.0702	1080.0
12.500	807.65	29.872	881.13	8.6882	1100.0
13.000	808.97	31.226	880.82	9.4967	1120.0
13.500	810.75	32.688	880.41	10.534	1140.0
14.000	812.93	34.238	879.86	11.857	1160.0
14.500	815.67	35.864	879.15	13.539	1180.0
15.000	818.79	37.556	878.23	15.678	1200.0
15.500	822.35	39.306	877.06	18.392	1220.0
16.000	826.32	41.106	875.55	21.818	1240.0
16.500	830.70	42.949	873.65	26.110	1260.0
17.000	835.48	44.831	871.28	31.419	1280.0
17.500	840.64	46.746	868.36	37.889	1300.0
18.000	846.17	48.689	864.84	45.642	1320.0
18.500	852.07	50.655	860.66	54.766	1340.0
19.000	858.31	52.640	855.78	65.320	1360.0
19.500	864.89	54.639	850.17	77.326	1380.0
20.000	871.81	56.648	843.82	90.776	1400.0
20.500	879.03	58.444	836.74	105.57	1400.0
21.000	886.52	59.832	829.08	121.32	1400.0
21.500	894.22	60.851	821.00	137.60	1400.0
22.000	902.09	61.532	812.65	154.04	1400.0
22.500	910.09	61.904	804.15	170.31	1400.0
23.000	918.19	61.994	795.63	186.11	1400.0
23.500	926.36	61.828	787.20	201.21	1400.0
24.000	934.56	61.429	778.95	215.40	1400.0
24.500	942.78	60.821	770.96	228.51	1400.0
25.000	950.97	60.028	763.30	240.41	1400.0
25.500	959.13	59.071	756.02	251.00	1400.0
26.000	967.21	57.972	749.19	260.20	1400.0
26.500	975.20	56.753	742.82	267.98	1400.0
27.000	983.06	55.436	736.96	274.31	1400.0
27.500	990.78	54.042	731.62	279.17	1400.0

28.000	998.35	52.561	726.82	282.60	1400.0
28.500	1005.7	51.103	722.56	284.62	1400.0
29.000	1012.8	49.598	718.85	285.28	1400.0
29.500	1019.7	48.094	715.68	284.63	1400.0
30.000	1026.3	46.607	713.03	282.74	1400.0
30.500	1032.7	45.154	710.91	279.69	1400.0
31.000	1038.8	43.747	709.27	275.57	1400.0
31.500	1044.5	42.398	708.11	270.45	1400.0
32.000	1050.0	41.117	707.40	264.45	1400.0
32.500	1055.2	39.911	707.11	257.64	1400.0
33.000	1060.0	38.784	707.22	250.12	1400.0
33.500	1064.6	37.740	707.68	242.00	1400.0
34.000	1068.8	36.780	708.49	233.35	1400.0
34.500	1072.8	35.901	709.60	224.26	1400.0
35.000	1076.5	35.102	710.99	214.82	1400.0
35.500	1080.0	34.379	712.63	205.10	1400.0
36.000	1083.2	33.727	714.50	195.18	1400.0
36.500	1086.1	33.140	716.56	185.11	1400.0
37.000	1088.9	32.614	718.81	174.95	1400.0
37.500	1091.5	32.142	721.22	164.77	1400.0
38.000	1093.8	31.719	723.77	154.60	1400.0
38.500	1096.1	31.340	726.43	144.48	1400.0
39.000	1098.2	31.000	729.20	134.46	1400.0
39.500	1100.1	30.694	732.06	124.58	1400.0
40.000	1101.9	30.418	734.99	114.85	1400.0
40.500	1103.6	30.170	737.97	105.32	1400.0
41.000	1105.2	29.944	741.00	96.020	1400.0
41.500	1106.7	29.739	744.04	86.974	1400.0
42.000	1108.1	29.553	747.10	78.218	1400.0
42.500	1109.4	29.382	750.14	69.788	1400.0
43.000	1110.7	29.225	753.15	61.726	1400.0
43.500	1111.8	29.082	756.09	54.079	1400.0
44.000	1113.0	28.949	758.95	46.902	1400.0
44.500	1114.0	28.826	761.69	40.259	1400.0
45.000	1115.0	28.712	764.27	34.222	1400.0
45.500	1115.9	28.606	766.65	28.867	1400.0
46.000	1116.8	28.508	768.80	24.262	1400.0
46.500	1117.6	28.416	770.69	20.453	1400.0
47.000	1118.4	28.330	772.30	17.445	1400.0
47.500	1119.2	28.250	773.64	15.186	1400.0
48.000	1119.9	28.175	774.75	13.568	1400.0
48.500	1120.6	28.104	775.67	12.456	1400.0
49.000	1121.2	28.038	776.46	11.712	1400.0
49.500	1121.8	27.975	777.15	11.221	1400.0
50.000	1122.4	27.917	777.78	10.895	1400.0
50.500	1122.9	27.861	778.37	10.674	1400.0

APPENDIX 6 LOVIBOND COMPARATOR TESTS FOR THIOCYANATE AND PHENOL

The Lovibond Comparator is a simple and inexpensive instrument for the approximate quantification of selected chemical concentrations in a sample. The compound under investigation is reacted with certain chemicals as detailed in the Lovibond test to create a colour change that is proportional to its concentration. This colour is then compared with a set of standard colours to determine the concentration. In this project, the standard Lovibond tests were further simplified to enable very quick approximations of phenol and thiocyanate concentration. The reagents and procedures used are outlined below.

Simplified Lovibond Test for Thiocyanate

Reagent: Dissolve 100 gm. of hydrated ferric chloride ($FeCl_3$ $6H_2O$) in water containing 50 ml concentrated HCl. Make up to 500 mls.

Blank: Dilute 10 mls. of the ferric chloride reagent to 110 mls. with distilled water.

Technique: To 100 mls. of sample, add 10 mls. of ferric chloride solution. Test for colour against blank.

Simplified Lovibond Test for Phenol

Reagents: (A) concentrated HCl

(B) Ammonia Solution

1 vol. 0.88 NH_4OH in 8 vols. H_2O

(C) Antipyrene Solution

2% 4-amino-antipyrene in water.

(D) Ferricyanide Solution

8% Potassium ferricyanide in water

(E) Buffer Solution pH 10

Dissolve 12.37 gm. boric acid and 14.91 gm. potassium chloride in 1 litre distilled water. Take 250 mls. of this solution, add 44 mls. of N.NaOH and make up to 1 litre.

Technique: Take a suitable volume of the sample (normally 1-10 mls.) in a 100 ml. graduated flask. Add approximately 60 mls. distilled water, add 10 mls. buffer solution and make up to about 90 mls. with water. Shake. Check that the pH of the contents of the flask is in the range 9.6 to 10.0 and adjust if necessary with HCl or the Ammonia solution. Make up to 100 mls. Shake.

Divide this solution into two equal volumes. Add 1.0 ml Antipyrene solution to each. One of these solutions constitutes the blank. To the other add 1.0 ml. of Ferricyanide solution. Shake, and leave for 10 minutes for the colour to develop.

Test for colour against blank.

APPENDIX 7 HEWLITT-PACKARD (HP 65) MONOD PROGRAM

This program solves the basic Monod equations for S and X in a batch system. Values of the coefficients, initial S and X, and the integration time differences are required as inputs. The equations solved are:

$$\frac{dS}{dt} = \frac{\mu X}{Y} \quad \text{..... Equation 3.7}$$

$$\frac{dX}{dt} = \mu X - k_e X \quad \text{..... Equation 4.4}$$

$$\mu = \mu_{max} \frac{S}{K_s + S} \quad \text{..... Equation 3.1}$$

THE PROGRAM

KEY	CODE	KEY	CODE	KEY	CODE	KEY	CODE	KEY	CODE
LBL	23	6	06	RCL 8	34 08	-	51	9	09
A	11	R/S	84	÷	81	CHS	42	+	61
1	01	STO 6	33 06	g	35	R/S	84	2	02
R/S	84	7	07	4	04	STO 5	33 05	÷	81
STO 1	33 01	R/S	84	RCL 1	34 01	CLX	44	STO	33
2	02	STO 7	33 07	X	71	RCL 4	34 04	9	09
R/S	84	RCL 5	34 05	ENTER	41	-	51	RCL 5	34 05
STO 2	33 02	STO 8	33 08	ENTER	41	RCL	34	ENTER	41
3	03	RCL 6	34 06	ENTER	41	9	09	RCL 8	34 08
R/S	84	STO	33	RCL	34	X	71	+	61
STO 3	33 03	9	09	9	09	RCL 7	34 07	2	02
4	04	LBL	23	X	71	X	71	÷	81
R/S	84	9	09	RCL 3	34 03	RCL 6	34 06	STO 8	33 08
STO 4	33 04	RCL 8	34 08	÷	81	+	61	GTO	22
5	05	ENTER	41	RCL 7	34 07	STO 6	33 06	9	09
R/S	84	RCL 2	34 02	X	71	R/S	84	RTN	24
STO 5	33 05	+	61	RCL 5	34 05	RCL	34		

PROGRAM EXECUTION

Step	Procedure	Enter	Press	Display
1	Initiate Program	-	A	1.00
2	Enter μ_{max}	Value of μ_{max}	R/S	2.00
3	Enter K_s	Value of K_s	R/S	3.00
4	Enter Y	Value of Y	R/S	4.00
5	Enter k_e	Value of k_e	R/S	5.00
6	Enter S_1	Value of S_1	R/S	6.00
7	Enter X_1	Value of X_1	R/S	7.00
8	Enter ΔT	Value of ΔT	R/S	S_2
9	Calculate X_2	-	R/S	X_2
10	Calculate S_3	-	R/S	S_3
11	Calculate X_3	-	R/S	X_3
⋮	⋮	-	⋮	⋮
	Continue			

APPENDIX 8 METHODS FOR THE ENUMERATION OF BACTERIAL POPULATIONS

The population of a sample of microbial mass may be estimated on the basis of:

8.1 The Mixed Liquor Suspended Solids - M.L.S.S.

8.2 The Mixed Liquor Volatile Suspended Solids - M.L.V.S.S.

8.3 Determination of sludge activity by oxygen utilisation.

8.4 Determination of sludge activity by carbon dioxide generation.

8.5 Determination of sludge activity by micro-organism counts.

Other indices such as Sludge Age and Sludge Density Index are merely variations on the M.L.S.S. concentration.

8.1 M.L.S.S.

8.1.1 Measurement: A known volume of sample is filtered, the filtrate then being dried to a constant weight at 105 °C. The M.L.S.S. concentration is then expressed in terms of mg. per litre. The M.L.S.S. is usually then incorporated in some expression such as the sludge age:

$$\text{Sludge Age} = \frac{V \times S_m}{Q \times S_s} \text{ days}$$

V = aeration tank volume

S_m = suspended solids in mixed liquor

Q = waste liquor flow rate

S_s = suspended solids in plant effluent

For a given liquor, and for a given plant, the optimum sludge age may easily be determined by empirical methods.

8.1.2 Advantages and Disadvantages: The determination of M.L.S.S. is a very simple and quick process. However, it does not give detailed information about the activity of the sludge. When used to determine the Sludge Density Index (S.D.I.) it gives an idea of the settleability of the sludge, but tells nothing about the activity of the organisms, or concentration of mineralised material.

8.2 M.L.V.S.S.

8.2.1 Measurement: In the same way as before, the M.L.S.S. is determined. The dried solids are then ignited in an electric muffle (temperature of the order of 500 ^{0}C). The difference in weight between the dried and ignited solids is the volatile solids.

8.2.2 Advantages and Disadvantages: Determination of M.L.V.S.S. gives more information as to the activity of the sludge. It can be used along with S.V.I., or Sludge Age to control the plant. However, it is not an accurate measure of the volatility of the sludge, because, at the temperatures needed to volatise organic matter, mineral matter will also be dehydrated. Once again, it does not give an accurate picture of the ecology of the sludge, but it is simple, and relatively cheap, and is an improvement upon determination of M.L.S.S. only.

8.3 Sludge Activity by Oxygen Utilisation

Sludge activity - not to be confused with sludge volatility - indicates the oxidising ability of a sludge, and is thus a direct indication of living bacterial population. It can be determined by measuring the rate of oxygen utilisation of the waste-liquor.

8.3,1 Measurement: The usual method of determining the sludge activity, via the oxygen utilisation of the sludge, is manometrically in a micro-respirometer, such as the Warburg Respirometer. However, this method is somewhat clumsy and time-consuming, and again requires trained laboratory personnel.

8.4

A better method of determining the sludge activity is by measuring the amount of carbon-dioxide evolved. Carbon dioxide-free air is bubbled under pressure or vacuum through a mixture of the activated sludge and waste liquor. The spent air is then passed through standard baryta solution. The amount of CO_2 is determined by titrating the baryta with standard oxalic acid using

phenolphthalein as indicator. The apparatus is simple, and the method is easy and rapid but again, trained laboratory personnel are required.

8.5 Determination of Sludge Activity by Micro-Organism Counts

8.5.1 Direct Plate Counts: The first such method to be discussed is the microbiological plating technique. This is a simple and cheap method, in which the sample is diluted many times, and then poured onto a plate containing an agar which comprises the necessary nutrients for the bacterial types under study. The plate is then incubated for a definite period (this may be in excess of one day), and the number of colonies present is then counted.

Obvious disadvantages of such a method are the time it needs, and the fact that a trained microbiologist is required.

8.5.2 Measurement of A.T.P. without culturing: Adenosine triphosphate (A.T.P. - found in all living organisms) is extracted by chemical means from a sample of the sludge, and is injected into a reaction mixture of luciferase and luciferin. This results in a light-flash, the intensity of which is directly related to the amount of A.T.P. liberated, and hence to the number of living organisms present in the sludge sample. The intensity of the light-flash can be measured, and related to the bacterial population.

Comments: This method enables reasonably accurate and quick microbial population counts. The actual analysis may only take a matter of seconds using an A.T.P. analyser. No especially trained personnel are needed, as the analysis practically consists of inoculating the sample into the reaction mixture (contained within the equipment), and reading off the number of counts from the digital display. However, one major drawback - capital costs of such equipment are high, of the order of £4,000.

APPENDIX 9 PREPARATION OF REAGENTS FOR ATP ANALYSIS AND PROCEDURE FOR GLASSWARE CLEANING

9.1 Stabilised Water

Stabilised water must be used in all analytical manipulations and sample preparation. It should also be used for final washing of all glassware.

Freshly prepared distilled water is acidified with a drop of concentrated hydrochloric acid, and then boiled for 10 minutes. After cooling to ambient temperature, the pH is brought back to 7.0 using sodium hydroxide solution. The water is then filtered through a standard Millipore filter (0.45μ).

It is recommended that fresh stabilised water is prepared daily.

9.2 Stock ATP Solution

0.1193 gm. adenosine 5' triphosphate disodium salt trihydrate is dissolved in 100 mls. stabilised water. This gives a stock solution of 1000 $gm.m^{-3}$ ATP.

It is recommended that this solution is prepared weekly. Dilutions of this reagent should be discarded immediately after use, as ATP rapidly deteriorates when present in low concentration.

9.3 Sterilised Coke Oven Effluent

Treated coke-oven effluent from the laboratory-scale, continuous plant (see section 3.9) is acidified with a drop of concentrated hydrochloric acid and filtered. It is then boiled for 10 minutes. After cooling to ambient temperature, the pH is brought back to neutral using sodium hydroxide solution. The Coke-oven effluent is then filtered through a standard Millipore filter (0.45μ).

9.4 Mops Buffer (0.01m)

Dissolve 2.09 gm. of morpholinopropane sulfonic acid (MOPS) in 1 litre of stabilised water. Adjust to pH 7.4 with sodium hydroxide solution. Sterilise by filtration through a

Millipore filter (0.45).

The buffer should be stored in stoppered flasks and prepared daily.

9.5 Procedure for Glassware Cleaning

All vessels used during ATP extraction and measurement must be both chemically and biologically clean. Where an autoclave is not available, the following procedure may be followed:

(a) Wash glassware five times in tap water.

(b) Rinse with 0.2N hydrochloric acid.

(c) Rinse three times with ordinary distilled water.

(d) Rinse three times with MOPS buffer to neutralise remaining acid.

(e) Rinse with several washes of stabilised water.

APPENDIX 10 THREE METHODS FOR THE EXTRACTION OF ATP

10.1 Boiling TRIS Extraction

To a 70 ml. Kjedahl flask containing approximately 40 mls. of boiling TRIS buffer (0.025 M, pH 7.75), add 1.0 ml. of sample. It is recommended that sterile, disposable microbiological pipettes are used for transferring the sample.

Boil for 1 minute exactly. Transfer the flask to a bucket containing a salt/ice mixture, and cool as quickly as possible until the contents are frozen. Put on ice.

When it is required to assay the extract, thaw the contents of the flask, and make up the volume to 70.0 ml. with TRIS buffer. Shake, and allow to settle for 5-10 minutes before assay.

N.B. Many workers recommend filtration of the extract before assay, but this has not been found necessary when small samples of mixed-liquor are taken.

The ATP concentration is then given by:

ATP in femtograms per ml. = Instrument reading x 70.

10.2 Butan-1-ol Extraction

A sample of the mixed liquor is diluted 50x with sterile treated coke oven effluent. A 1.0 ml. sample of this dilution is transferred to a test tube, and 1.0 ml of butan-1-ol plus 0.1 ml. of 0.1 M MOPS buffer are added. Shake vigorously for ten seconds. Add 8.0 ml. of octinol. Shake vigorously for 10 seconds. Centrifuge for 3 minutes at 3000 rpm.

The top, organic layer is removed by pipetting, and an aliquot of the aqueous layer is then assayed for ATP content. The concentration of ATP iin the original sample is then given by:

ATP in $fg.ml^{-1}$ = biometer reading x 50.

10.3 DMSO Extraction

This is the method found most favourable for coke-oven effluent mixed-liquors.

Reagent Preparation: 90% DMSO; Certified grade Dimethylsulfoxide is recommended. Prepare the extractant solution daily by diluting 9 parts DMSO with 1 part 0.01 M MOPS buffer.

To a 70 ml. Kjeldahl flask containing 9.0 ml. of 90% DMSO, add 1.0 ml. sample. Shake vigorously for 20 seconds. Allow to stand for 2 minutes at room temperature. Add 50.0 ml. of 0.01 M MOPS buffer and shake for 15 seconds. Assay immediately or put on ice.

The ATP concentration of the mixed liquor is then given by:

$$\text{ATP in } fg.ml^{-1} = \text{biometer reading} \times 60$$

```
.NULL.
      REAL HX,MU,TIME
C     DETERMINATION OF OPT. VALUES OF COEFFS.-(CON)S- FOR ADSORPTION
C     CURVE-X-CURVE.              G.F.T.     25.2.77
C
C      INCLUDES HALDANE MODEL FOR SELF TOXICITY OF PHENOL
      DIMENSION HX(20),B(20),CON(7),C(7),P(8),G(8),BB(20)
C
      DIMENSION TIME(20)
      READ (7,10) SQ,AO,XO,T
 10   FORMAT (4F5.0)
      READ (7,20)(HX(I),I=1,10)
      READ (7,20)(HX(I),I=11,20)
 20   FORMAT (20F5.0)
      K9=0
      L=7
 25   READ (L,30) CON
      L=1
      DO 35 I=1,7
 35   C(I)=CON(I)
 30   FORMAT (7F7.0)
      TIMEX = 0.0
      K9=K9+1
      DO 350 J=1,8
      S1=SO
      A1=AO
      X1=XO
      S2=S1
      A2=A1
      X2=X1
      DO 180 K=1,20
      DO 170 K7=1,20
      N=1
      S1=S2
      A1=A2
      X1=X2
 50   S=(S1+S2)/2.0
      A=(A1+A2)/2.0
      X=(X1+X2)/2.0
      S3=S2
      A3=A2
      X3=X2
      N=N+1
      MU=C(2)*A/(C(3)+A+S*S/C(7))
      IF(S2-5.0) 60,61,62
 60   S2=5.0
 61   C(1)=0.0
      C(5)=0.0
      GO TO 63
 62   S2=S1-C(1)*T
 63   A2=A1+C(1)*T-MU*X*T/C(6)
      X2=X1+MU*X*T-C(4)*X*T-C(5)*T
      IF (ABS((S3-S2)/S2).GE.0.01) GOTO 50
      IF (A2 .EQ. 0.0) GOTO 65
      IF (ABS((A3-A2)/A2).GE. 0.01) GOTO 50
 65   IF (ABS((X3-X2)/X2).GE. 0.01) GOTO 50
 170  CONTINUE
      E(K)=X2
      IF (J .GT. 1) GOTO 180
      BB(K)=X2
      TIME(K) = TIMEX + 0.2
      TIMEX = TIME(K)
 180  CONTINUE
      S7=0.0
      DO 200 K=1,20
 200  S7=S7+(E(K)-HX(K+1))*(E(K)-HX(K+1))
      P(J)=S7
      IF (J.EQ.1) GOTO 300
      CON(J-1)=CON(J-1)-D1
      G(J-1)=(P(J)-P(1))/D1
      IF (J.EQ.8) GOTO 350
 300  D1=CON(J)*.001
      CON(J)=CON(J)+D1
 350  CONTINUE
      GOTO 398
      WRITE(1,399)
 399  FORMAT(44H SUM SQUARES OF DIFFERENCES WITH INITIAL DATA)
 398  WRITE (1,400) P(1)
 400  FORMAT (F20.9)
      WRITE(1,449)
 449  FORMAT(20H        GRADIENTS      ,19H        CONSTANTS      )
      DO 450 J=1,7
 450  WRITE (1,460) G(J),CON(J)
 460  FORMAT (6X,F9.7,10X,F9.7)
      WRITE (1,499)
 499  FORMAT(23H  HISTORICAL X-VALUES  ,19HCALCULATED X-VALUES,11H
     1 TIME)
 501  DO 500 K=1,19
 500  WRITE (1,510) HX(K+1),BB(K),TIME(K+1)
 510  FORMAT (6X,F9.7,10X,F9.7,15X,F4.2)
 520  IF (K9 .LE. 100) GOTO 25
      CALL EXIT
      END
BOTTOM
```

APPENDIX 11
EXPERIMENT 4.7 BEST FIT
Sheet 1

APPENDIX 11

DATA AND OUTPUT FOR EXPERIMENT 4.7

DAT 4

```
.NULL.
590.0  6.6  14.2  .01
14.2  13.1  12.6  10.8  9.7  8.7  8.0  7.4  6.9  6.6
 6.5  6.5  10.0  11.4  12.3  12.8  13.2  13.4  13.6  13.8
250.0  1.6  60.0  0.05  5.0  0.022  40.0
BOTTOM
```

```
S.S.Q
 178.3593750000
GRADIENTS                CONSTANTS
$976.7021                $249.9999
$152609.7                1.5999997
$4069.592                $59.99999
$48835ll.                0.0500000
$48835.11                4.9999990
$11098888                0.0220000
$6104.389                $39.99999
HISTORICAL X-VALUES   CALCULATED X-VALUES   TIME
$13.09999                $13.07724          0.40
$12.00000                $11.99573          0.60
$10.79999                $10.96067          0.80
9.6999989                9.9792652          1.00
8.6999989                9.0609341          1.20
8.0000000                8.2207203          1.40
7.3999996                7.4838524          1.60
6.8999996                6.8907642          1.80
6.5999994                6.5029564          2.00
6.5000000                6.4230738          2.20
6.5000000                6.7829781          2.40
$10.00000                7.9584780          2.60
$11.39999                $10.15846          2.80
$12.29999                $12.45601          3.00
$12.79999                $13.74911          3.20
$13.19999                $13.80010          3.40
$13.39999                $13.67060          3.60
$13.59999                $13.53485          3.80
$13.79999                $13.40016          4.00
```

APPENDIX 12

January 1975 to March 1975 Inclusive

SCUNTHORPE BIOLOGICAL EFFLUENT TREATMENT PLANT
EFFLUENT FLOW RATES

Date	Flow $m^3.day^{-1}$	Date	Flow $m^3.day^{-1}$	Date	Flow $m^3.day^{-1}$
Jan 3	2392	Jan 26	4971	Feb 18	3501
4	1942	27	4522	19	3231
5	2299	28	4321	20	6126
6	1610	29	4401	21	4700
7	2422	30	4518	22	3485
8	2361	31	4677	23	2980
9	-	Feb 1	4535	24	3495
10	-	2	4304	25	3763
11	2966	3	4528	26	3703
12	4458	4	4609	27	2889
13	4453	5	4693	28	3468
14	4315	6	4571	Mar 1	3162
15	4398	7	4913	2	3342
16	4542	8	4949	3	3330
17	4339	9	5787	4	3546
18	4490	10	4152	5	3182
19	4544	11	3923	6	3501
20	4650	12	3849	7	3525
21	5285	13	3922	8	3196
22	4266	14	3890	9	3272
23	4830	15	3472	10	3059
24	4713	16	3350	11	3186
25	4463	17	3692	12	-

APPENDIX 12

December, 1974 to March, 1975 Inclusive

Biological Effluent Treatment Plant

INLET RESERVOIR

Date	pH	SS	PV	CNS	S_2O_3	Mono Phenol	Total Phenol	NH_3	PO_4	NVM	Free CN	BOD	COD	Diss. Metals
3/12/74	8.5	81	1600	149	157	636	745	1400	-	3	12.8	1550	2800	0.57
5/12/74	8.3	75	1730	139	-	660	795	-	-	-	-	-	-	-
10/12/74	8.1	52	1320	123	128	494	575	988	-	10	12.7	-	-	-
12/12/74	8.1	55	1480	113	-	556	635	-	-	-	-	-	-	-
17/12/74	5.6	51	970	66	74	354	420	740	-	8	13.7	1250	2800	0.40
19/12/74	-	91	1225	113	-	426	555	-	-	-	-	-	-	-
24/12/74	7.5	61	1315	108	106	460	560	1282	-	36	11.7	-	-	-
31/12/74	7.8	65	1220	122	125	416	460	1025	-	17	8.1	1100	2270	-
2/ 1/75	6.5	43	1220	122	-	430	515	-	-	-	-	-	-	-
7/ 1/75	7.8	84	1440	132	198	514	580	958	-	13	10.5	-	-	-
11/ 1/75	7.2	62	1395	125	-	506	575	-	-	-	-	-	-	-
14/ 1/75	8.1	56	1325	105	126	456	495	676	-	18	9.4	1075	2650	0.33
16/ 1/75	8.2	46	1380	110	-	496	595	-	-	-	-	-	-	-
21/ 1/75	7.4	61	1185	89	121	310	410	596	-	28	12.9	-	-	-
23/ 1/75	7.2	39	1330	95	-	490	565	-	-	-	-	-	-	-
28/ 1/75	6.3	40	1250	91	102	460	565	1061	-	9	13.8	1000	2314	0.72
30/ 1/75	8.2	92	1240	96	-	432	510	-	-	-	-	-	-	-
4/ 2/75	8.2	46	1540	110	139	554	640	890	-	7	11.3	-	-	-
6/ 2/75	8.0	55	1315	110	-	462	545	-	-	-	-	-	-	-
13/ 2/75	8.1	60	1415	152	-	506	600	-	-	-	-	-	-	-
18/ 2/75	7.8	50	1530	125	158	594	685	1078	-	14	11.6	1550	-	0.22
20/ 2/75	8.1	82	1540	134	-	580	625	-	-	-	-	-	-	-
25/ 2/75	8.2	66	1330	140	157	494	585	1271	-	21	16.4	-	-	-
27/ 2/75	8.3	78	1500	152	-	506	650	-	-	-	-	-	-	-
3/ 3/75	8.2	-	1755	138	-	684	840	-	-	-	-	-	-	-
4/ 3/75	8.1	103	1765	139	152	712	855	1243	-	20	7.9	1400	3474	0.29
5/ 3/75	8.5	-	1585	137	-	630	770	-	-	-	-	-	-	-
6/ 3/75	8.4	212	1840	152	-	616	860	-	-	-	-	-	-	-
7/ 3/75	-	-	1840	144	-	686	885	-	-	-	-	-	-	-
9/ 3/75	8.7	-	1470	108	-	536	728	-	-	-	-	-	-	-
10/ 3/75	8.6	-	1325	113	-	474	605	-	-	-	-	-	-	-
11/ 3/75	8.5	37	1535	138	121	570	710	1011	-	29	18.4	-	-	-
12/ 3/75	8.5	-	1410	136	-	494	615	-	-	-	-	-	-	-
13/ 3/75	8.2	42	1500	150	-	478	680	-	-	-	-	-	-	-
14/ 3/75	8.4	-	1575	150	-	566	750	-	-	-	-	-	-	-
16/ 3/75	8.3	-	1540	155	-	566	700	-	-	-	-	-	-	-
17/ 3/75	8.4	-	1600	164	-	606	730	-	-	-	-	-	-	-
18/ 3/75	8.6	-	1610	159	177	588	700	1120	-	12	11.5	1300	3058	0.31
19/ 3/75	8.6	-	1555	156	-	570	665	-	-	-	-	-	-	-
20/ 3/75	8.6	31	1675	163	-	620	710	-	-	-	-	-	-	-
21/ 3/75	8.6	-	1470	142	-	524	635	-	-	-	-	-	-	-
23/ 3/75	8.7	-	1700	151	-	630	750	-	-	-	-	-	-	-
24/ 3/75	8.7	-	1625	158	-	580	685	-	-	-	-	-	-	-
25/ 4/75	8.7	90	1570	144	190	548	695	1100	-	31	14.4	-	-	-
26/ 3/75	8.7	-	1345	129	-	530	610	-	-	-	-	-	-	-
27/ 3/75	8.4	53	1385	136	-	488	620	-	-	-	-	-	-	-
31/ 3/75	8.5	-	1520	157	-	550	620	-	-	-	-	-	-	-

CLARIFIER NO.1

Date	pH	S.S.	3 min. P.V.	CNS	PO_4	Mono Phenol	Total Phenol	NVM
2/1/75	-	102	-	7.0	-	3.3	-	-
3/1/75	-	82	-	9.8	-	4.3	-	-
6/1/75	-	62	-	5.4	-	3.6	-	-
7/1/75	-	47	-	4.2	-	4.0	-	-
8/1/75	-	42	-	3.0	-	4.1	-	-
9/1/75	-	77	-	2.7	-	4.3	-	-
10/1/75	-	87	-	2.8	-	4.3	-	-
13/1/75	-	112	-	5.6	-	49.6	-	-
14/1/75	-	113	-	34.0	-	136.0	-	-
15/1/75	-	128	-	59.6	-	190.0	-	-
16/1/75	-	156	-	80.0	-	260.0	-	-
17/1/75	-	176	-	102.0	-	136.0	-	-
20/1/75	-	143	-	122.0	-	246.0	-	-
21/1/75	-	128	-	110.0	-	416.0	-	-
22/1/75	-	180	-	105.0	-	320.0	-	-
23/1/75	-	197	-	104.0	-	454.0	-	-
24/1/75	-	206	-	110.0	-	474.0	-	-
27/1/75	-	79	-	127.0	-	400.0	-	-
28/1/75	-	71	-	124.0	-	166.0	-	-
29/1/75	-	92	-	113.0	0.4	59.0	-	-
30/1/75	-	70	-	106.0	-	58.6	-	-
31/1/75	-	46	-	106.0	3.0	19.6	-	-
3/2/75	-	48	-	154.0	2.5	11.6	-	-
4/2/75	-	46	-	127.0	-	10.4	-	-
5/2/75	-	93	-	125.0	-	10.4	-	-
6/2/75	-	116	-	119.0	-	8.5	-	-
7/2/75	-	177	-	121.0	1.8	8.4	-	-
11/2/75	-	246	-	110.0	-	7.2	-	-
12/2/75	-	171	-	105.0	1.8	6.8	-	-
13/2/75	-	100	-	120.0	-	6.6	-	-
14/2/75	-	130	-	122.0	2.1	7.0	-	-
17/2/75	-	375	-	119.0	1.6	6.8	-	-
18/2/75	-	358	-	117.0	-	7.2	-	-
19/2/75	-	136	-	121.0	1.2	9.2	-	-
20/2/75	-	301	-	125.0	-	6.8	-	-
24/2/75	-	204	-	129.0	1.8	6.1	-	-
25/2/75	-	444	-	105.0	-	5.1	-	-
26/2/75	-	499	-	29.0	1.8	4.7	-	-
27/2/75	-	285	-	2.3	-	4.1	-	-
28/2/75	-	182	-	3.2	3.2	5.2	-	-
3/3/75	-	193	-	17.0	4.1	6.4	-	-
4/3/75	-	161	-	4.2	-	6.3	-	-
5/3/75	-	177	-	3.5	4.1	5.2	-	-
6/3/75	-	129	-	4.1	-	5.5	-	-
7/3/75	-	132	-	6.9	3.1	7.4	-	-
8/3/75	-	-	-	3.5	-	6.2	-	-
9/3/75	-	-	-	1.5	-	5.2	-	-
10/3/75	-	76	-	3.1	-	5.4	-	-
11/3/75	-	74	-	2.6	4.0	5.3	-	-

CLARIFIER NO.2

Date	pH	S.S.	3 min. P.V.	CNS	PO_4	Mono Phenol	Total Phenol	NVM
2/1/75	-	99	-	1.8	-	3.2	-	-
3/1/75	-	99	-	2.1	-	4.0	-	-
6/1/75	-	84	-	1.8	-	3.7	-	-
7/1/75	-	77	-	2.7	-	4.3	-	-
8/1/75	-	156	-	2.5	-	4.2	-	-
9/1/75	-	95	-	2.3	-	4.4	-	-
10/1/75	-	115	-	2.7	-	4.6	-	-
13/1/75	-	145	-	43.0	-	5.5	-	-
14/1/75	-	116	-	36.0	-	6.6	-	-
15/1/75	-	92	-	24.4	-	6.3	-	-
16/1/75	-	95	-	32.0	-	4.8	-	-
17/1/75	-	110	-	36.8	-	6.4	-	-
20/1/75	-	97	-	62.0	-	4.8	-	-
21/1/75	-	78	-	62.5	-	10.8	-	-
22/1/75	-	83	-	65.0	-	15.6	-	-
23/1/75	-	168	-	79.0	-	84.8	-	-
24/1/75	-	204	-	97.5	-	280.0	-	-
27/1/75	-	205	-	125.0	-	480.0	-	-
28/1/75	-	138	-	119.0	-	476.0	-	-
29/1/75	-	76	-	101.0	1.5	195.0	-	-
30/1/75	-	59	-	98.0	-	20.6	-	-
31/1/75	-	61	-	104.0	1.4	18.6	-	-
3/2/75	-	66	-	136.0	3.1	12.8	-	-
4/2/75	-	61	-	124.0	-	11.6	-	-
5/2/75	-	90	-	123.0	3.0	12.0	-	-
6/2/75	-	109	-	118.0	-	10.4	-	-
7/2/75	-	148	-	119.0	2.6	9.2	-	-
11/2/75	-	122	-	110.0	-	8.6	-	-
12/2/75	-	148	-	107.0	2.3	10.8	-	-
13/2/75	-	114	-	125.0	-	8.6	-	-
14/2/75	-	123	-	124.0	2.6	9.0	-	-
17/2/75	-	603	-	109.0	1.2	5.4	-	-
18/2/75	-	416	-	104.0	-	5.1	-	-
19/2/75	-	222	-	103.0	1.4	7.6	-	-
20/2/75	-	375	-	98.0	-	5.3	-	-
24/2/75	-	250	-	6.0	1.8	4.9	-	-
25/2/75	-	250	-	3.3	-	5.0	-	-
26/2/75	-	172	-	2.5	1.9	3.9	-	-
27/2/75	-	141	-	3.0	-	3.9	-	-
28/2/75	-	115	-	2.9	2.6	4.7	-	-
3/3/75	-	169	-	3.8	4.2	5.5	-	-
4/3/75	-	201	-	6.2	-	5.3	-	-
5/3/75	-	141	-	4.0	4.3	5.7	-	-
6/3/75	-	132	-	5.9	-	5.4	-	-
7/3/75	-	168	-	27.8	3.5	7.4	-	-
8/3/75	-	-	-	33.5	-	6.2	-	-
9/3/75	-	-	-	16.8	-	5.0	-	-
10/3/75	-	84	-	3.6	-	5.3	-	-
11/3/75	-	99	-	4.0	4.8	5.4	-	-

APPENDIX 12 cont.

CLARIFIER NO.3

Date	pH	S.S.	3 min. P.V.	CNS	PO_4	Mono Phenol	Total Phenol	NVM
2/1/75	-	124	-	1.7	-	2.3	-	-
3/1/75	-	97	-	1.8	-	3.0	-	-
6/1/75	-	102	-	1.6	-	3.0	-	-
7/1/75	-	124	-	1.9	-	3.3	-	-
8/1/75	-	126	-	1.9	-	3.3	-	-
9/1/75	-	110	-	1.5	-	3.2	-	-
10/1/75	-	142	-	1.9	-	3.8	-	-
13/1/75	-	83	-	2.0	-	4.3	-	-
14/1/75	-	110	-	1.9	-	3.8	-	-
15/1/75	-	115	-	1.5	-	3.8	-	-
16/1/75	-	127	-	1.4	-	3.5	-	-
17/1/75	-	144	-	4.0	-	3.9	-	-
20/1/75	-	86	-	8.6	-	2.7	-	-
21/1/75	-	78	-	18.2	-	3.0	-	-
22/1/75	-	78	-	49.0	-	43.4	-	-
23/1/75	-	138	-	74.0	-	69.6	-	-
24/1/75	-	178	-	94.0	-	290.0	-	-
27/1/75	-	194	-	130.0	-	484.0	-	-
28/1/75	-	80	-	110.0	-	300.0	-	-
29/1/75	-	92	-	93.0	2.1	18.0	-	-
30/1/75	-	63	-	97.0	-	16.2	-	-
31/1/75	-	80	-	104.0	3.2	12.8	-	-
3/2/75	-	83	-	135.0	4.1	10.6	-	-
4/2/75	-	60	-	124.0	-	10.0	-	-
5/2/75	-	73	-	120.0	3.9	9.5	-	-
6/2/75	-	89	-	120.0	-	9.0	-	-
7/2/75	-	128	-	110.0	2.5	7.6	-	-
11/2/75	-	246	-	110.0	-	7.0	-	-
12/2/75	-	205	-	106.0	1.6	7.0	-	-
13/2/75	-	148	-	113.0	-	6.8	-	-
14/2/75	-	227	-	106.0	2.6	6.6	-	-
17/2/75	-	494	-	117.0	1.5	6.4	-	-
18/2/75	-	540	-	111.0	-	5.7	-	-
19/2/75	-	212	-	117.0	2.9	9.6	-	-
20/2/75	-	302	-	121.0	-	6.5	-	-
24/2/75	-	321	-	97.0	2.3	5.5	-	-
25/2/75	-	261	-	44.0	-	5.2	-	-
26/2/75	-	276	-	2.6	1.9	4.1	-	-
27/2/75	-	239	-	2.6	-	4.2	-	-
28/2/75	-	205	-	2.3	4.0	4.7	-	-
3/3/75	-	184	-	3.5	4.5	5.8	-	-
4/3/75	-	171	-	15.6	-	6.0	-	-
5/3/75	-	155	-	3.4	4.4	5.9	-	-
6/3/75	-	199	-	12.6	-	5.4	-	-
7/3/75	-	172	-	61.0	3.9	9.0	-	-
8/3/75	-	-	-	53.5	-	6.2	-	-
9/3/75	-	-	-	1.0	-	4.8	-	-
10/3/75	-	155	-	2.6	-	5.1	-	-
11/3/75	-	108	-	1.8	4.3	5.6	-	-

APPENDIX 12

January to March, 1975

Biological Effluent Treatment Plant

TIDAL RESERVOIR

Date	pH	SS	PV	CNS	S_2O_3	Mono Phenol	Total Phenol	NH_3	PO_4	NVM	Free CN	BOD	COD	Diss. Metals
27/12/74	7.6	113	111	4.2	-	3.6	14.4	-	3.2	-	-	-	-	-
30/12/74	7.3	156	117	2.6	nil	3.4	13.4	1243	2.8	23	0.37	-	-	-
3/ 1/75	7.4	128	134	4.4	-	3.8	16.0	-	2.7	-	-	-	-	-
6/ 1/75	7.2	95	118	2.6	nil	3.5	12.4	1016	5.6	6	0.37	-	-	-
8/ 1/75	7.0	127	132	2.8	-	3.7	14.2	-	1.0	-	-	-	-	-
10/ 1/75	7.4	116	143	2.6	-	4.1	14.7	-	3.1	-	-	-	-	-
13/ 1/75	7.2	107	163	17.2	nil	14.8	27.6	848	0.8	7	0.40	-	-	-
15/ 1/75	7.4	103	236	28.6	-	60.0	81.0	-	1.0	-	-	160	692	0.27
17/ 1/75	7.4	149	380	53.0	-	140.0	163	-	1.0	-	-	-	-	-
20/ 1/75	7.7	98	526	66.0	18.0	132.0	175	692	1.4	4	0.79	-	-	-
22/ 1/75	6.8	96	270	55.0	-	64.0	73.0	-	0.7	-	-	-	-	-
24/ 1/75	7.6	153	802	100.0	-	296.0	320	-	4.4	-	-	-	-	-
27/ 1/75	7.9	160	1210	130.0	36.0	444.0	520	694	3.3	16	1.5	-	-	-
29/ 1/75	7.1	102	442	98.0	-	88.0	132	-	3.0	-	-	340	1096	0.33
31/ 1/75	7.4	85	296	104.0	-	15.6	40.0	-	6.8	-	-	-	-	-
3/ 2/75	7.4	83	309	137.0	nil	10.8	43.5	722	-	7	1.1	-	-	-
5/ 2/75	7.3	81	266	122.0	-	10.5	37.6	-	-	-	-	-	-	-
7/ 2/75	7.5	143	241	120.0	-	9.2	32.4	-	1.6	-	-	-	-	-
10/ 2/75	7.3	194	226	110.0	trace	7.6	33.0	1170	-	3	0.34	-	-	-
12/ 2/75	7.3	162	224	107.0	-	7.2	30.2	-	2.2	-	-	-	-	-
14/ 2/75	7.6	145	231	114.0	-	7.0	25.9	-	2.1	-	-	-	-	-
17/ 2/75	7.4	504	304	117.0	4	6.3	26.0	972	1.7	89	1.2	-	-	0.21
19/ 2/75	7.3	186	259	111.0	-	9.0	37.8	-	2.6	-	-	310	1218	-
21/ 2/75	7.3	331	263	107.0	-	5.9	26.8	-	2.0	-	-	-	-	-
24/ 2/75	7.4	261	221	83.0	5.3	6.0	27.0	1154	2.3	22	0.7	-	-	-
26/ 2/75	7.4	298	145	8.0	-	3.4	19.4	-	1.8	-	-	-	-	-
28/ 2/75	7.5	-	145	2.3	-	4.4	20.2	-	3.6	-	-	-	-	-
3/ 3/75	7.4	168	155	6.6	4.0	5.1	38.2	1310	4.3	10	0.27	-	-	-
5/ 3/75	7.4	153	151	4.2	-	5.7	33.8	-	4.0	-	-	102	646	0.25
7/ 3/75	7.4	192	174	35.6	-	7.2	34.8	-	3.5	-	-	-	-	-
10/ 3/75	7.5	104	131	2.4	trace	7.6	30.6	-	-	-	0.33	-	-	-
12/ 3/75	7.5	92	132	2.4	-	5.3	30.5	-	3.1	-	-	-	-	-
14/ 3/75	7.6	117	143	3.1	-	5.1	31.8	-	1.7	-	-	-	-	-
17/ 3/75	7.6	105	141	12.6	nil	4.8	21.2	1030	1.6	8	0.35	-	-	-
19/ 3/75	7.7	89	136	3.7	-	5.6	16.0	-	1.3	-	-	63	564	0.25
21/ 3/75	7.6	80	141	3.9	-	4.9	20.2	-	1.9	-	-	-	-	-
24/ 3/75	7.7	109	141	5.7	2.7	5.1	26.1	966	4.4	11	0.31	-	-	-
26/ 3/75	7.7	100	141	4.0	-	6.4	26.0	-	4.6	-	-	-	-	-

APPENDIX 13 - LISTING AND OUTPUT OF THE SCUNTHORPE SIMULATION - SHEET 1

S L A M T R A N S L A T I O N B Y # X 2 S T M A R K 2 . 1

FILENAME, SCUN, DATA-FILE SCUNDATA

SOURCE INPUT LISTING

```
      1              OPTIONS FLIST,DEBUG1,EXPLICIT
      2              FORTRAN(CP)
      3              MASTER BACTIN
      4              REAL I,MUP,MUT,MUMAXP,MUMAXT,KSP,KST,KEP,KET,KIP
      5              REAL KIT
      6              INITIAL
      7              TITLE 'SINGLE-STAGE, MIXED ACTIVATED-SLUDGE UNIT,ALLOWANCE
      8             1 MADE FOR DIFFERENT BACTERIA AND INHIBITION BY PHENOL.',4L,
      9             2 '      XPO            SPO           XTO           STO          SOP
     10             3 ',' SOT '
     11       C
     12       C
     13       C           FILESTORE SCUN
     14       C
     15       C   ALL TIME PERIODS IN DAYS
     16       C   100 STEPS BETWEEN COMMN. INTERVALS.
     17       C   INTEGRATION PERIOD 1 DAY
     18       C
     19       C
     20       C          INITIALISATION
     21       C
     22                  V=5130.0
     23                  W=0.05
     24                   Z=0.0
     25                  INPUT KET,MUMAXT,KST,YT,KEP,MUMAXP,KSP,YP
     26                  INPUT SOP,SOT,SPO,STO,XPO,XTO,KIP,IRUN
     27                  INPUT IM,DAYS,KIT
     28                  OUTPUT XPO,SPO,XTO,STO,SOP,SOT
     29                  OUTPUT 2L
     30                  OUTPUT '      TIME     ',' XP       ',' SP      ',' XT
     31             1       ',' ST      ',' Q       '
     32              END
     33       C
     34              DYNAMIC
     35                  DERIVATIVE
     36                      NOSORT (Q,B,A,D,THETA=TIME,V,W)
     37                       IF (TIME .GE.40.0) GOTO 15
     38                       Q=SSTEP(TIME,10.0,2300.0,4600.0)
     39                       GOTO 16
     40            15         Q=SSTEP(TIME,40.0,4600.0,3400.0)
     41            16         D=Q/V
     42                       A=5448.0/Q
     43                       B=(1+A)/(A+W)
     44                       THETA=1.0/D
     45                      END
     46                      MUP=MUMAXP*SP/(KSP + SP*SP*SP/KIP)
     47                      MUT=(MUMAXT*ST/(KST +ST+ST*ST/KIT))*(1.0-I)
     48                      I=SP/50.0
     49                      DXT=D*A*B*XT +MUT*XT-KET*XT-D*XT*(1.0+A)
     50                      DXP=D*A*B*XP +MUP*XP-KEP*XP-D*XP*(1.0+A)
     51                      DSP=D*(SOP+A*SP)-MUP*XP/YP-D*SP*(1.0+A)
```

S L A M T R A N S L A T I O N B Y #X2ST M A R K 2.1

```
52                    DST=D*(SOT+A*ST)-MUT*XT/YT-D*ST*(1.0+A)
53      C
54                    XT=INTGRL(DXT,XT0)
55                    XP=INTGRL(DXP,XP0)
56                    SP=INTGRL(DSP,SP0)
57                    ST=INTGRL(DST,ST0)
58      C
59                       INTINF
60                     ALG;IM=SIMP,RKFS,RKVS
61                     CI;CI=.1
62                      END
63                  END
64      C
65                  PARALLEL
66                   NOSORT
67                    IF (TIME .LT. (Z-0.01)) GOTO 28
68                    IF (TIME .GT. (Z+0.01)) GOTO 28
69                    OUTPUT TIME,XP,SP,XT,ST,Q
70                    Z=Z+1.0
71        28          IF (ST .GE. SOT) ST=SOT
72                    IF (SP .GE. 50.0) I=1.0
73                   END
74
75                    TERMINATE (TIME .GE. DAYS)
76                  END
77              END
78      C
79                  TERMINAL
80                    REPEAT (IRUN .GT. 1)
81                    NOSORT
82                    IF (IRUN .GT. 1) GO TO 20
83           20       CONTINUE
84                    END
85                  END
86              END
87              FINISH

            **** - END OF INPUT
```

APPENDIX 13 - SHEET 3

SINGLE-STAGE, MIXED ACTIVATED-SLUDGE UNIT, ALLOWANCE

MADE FOR DIFFERENT BACTERIA AND INHIBITION BY PHENOL

XPO	SPO	XTO	STO	SOP	SOT
4,2800	2,4500	0,64800	1,8900	530,00	125,00

TIME	XP	SP	XT	ST	Q
0,00000E 00	4,2800	2,4500	0,64800	1,8900	2300,0
1,0000	4,3675	2,3937	0,67238	1,8000	2300,0
2,0000	4,3887	2,3864	0,69545	1,7391	2300,0
3,0000	4,3939	2,3835	0,71730	1,6854	2300,0
4,0000	4,3952	2,3827	0,73799	1,6377	2300,0
5,0000	4,3955	2,3826	0,75759	1,5948	2300,0
6,0000	4,3956	2,3825	0,77615	1,5563	2300,0
7,0000	4,3956	2,3825	0,79372	1,5215	2300,0
8,0000	4,3956	2,3825	0,81037	1,4900	2300,0
9,0000	4,3956	2,3825	0,82613	1,4613	2300,0
10,000	4,3952	2,7768	0,84099	1,5275	4600,0
11,000	7,5317	2,8031	0,87160	2,8138	4600,0
12,000	8,2716	2,5301	0,90069	2,7054	4600,0
13,000	8,4446	2,4845	0,92699	2,6242	4600,0
14,000	8,4851	2,4720	0,95076	2,5569	4600,0
15,000	8,4946	2,4691	0,97224	2,4994	4600,0
16,000	8,4968	2,4684	0,99166	2,4498	4600,0
17,000	8,4974	2,4683	1,0092	2,4066	4600,0
18,000	8,4975	2,4682	1,0251	2,3688	4600,0
19,000	8,4975	2,4682	1,0394	2,3357	4600,0
20,000	8,4975	2,4682	1,0523	2,3066	4600,0
21,000	8,4975	2,4682	1,0640	2,2809	4600,0
22,000	8,4975	2,4682	1,0746	2,2582	4600,0
23,000	8,4975	2,4682	1,0841	2,2381	4600,0
24,000	8,4975	2,4682	1,0928	2,2202	4600,0
25,000	8,4975	2,4682	1,1006	2,2043	4600,0
26,000	8,4975	2,4682	1,1076	2,1901	4600,0
27,000	8,4975	2,4682	1,1140	2,1775	4600,0
28,000	8,4975	2,4682	1,1197	2,1662	4600,0
29,000	8,4975	2,4682	1,1249	2,1560	4600,0
30,000	8,4975	2,4682	1,1296	2,1470	4600,0
31,000	8,4975	2,4682	1,1338	2,1389	4600,0
32,000	8,4975	2,4682	1,1376	2,1316	4600,0
33,000	8,4975	2,4682	1,1411	2,1251	4600,0
34,000	8,4975	2,4682	1,1442	2,1192	4600,0
35,000	8,4975	2,4682	1,1470	2,1139	4600,0
36,000	8,4975	2,4682	1,1496	2,1092	4600,0
37,000	8,4975	2,4682	1,1519	2,1049	4600,0
38,000	8,4975	2,4682	1,1540	2,1011	4600,0
39,000	8,4975	2,4682	1,1558	2,0976	4600,0
40,000	8,4975	2,4682	1,1575	2,0945	4600,0
41,000	6,9110	2,2332	1,1599	1,5334	3400,0
42,000	6,5237	2,3722	1,1614	1,5359	3400,0
43,000	6,4308	2,4082	1,1629	1,5352	3400,0
44,000	6,4085	2,4170	1,1643	1,5337	3400,0
45,000	6,4031	2,4192	1,1655	1,5321	3400,0
46,000	6,4018	2,4197	1,1667	1,5306	3400,0
47,000	6,4015	2,4198	1,1678	1,5291	3400,0
48,000	6,4014	2,4198	1,1688	1,5278	3400,0
49,000	6,4014	2,4198	1,1697	1,5266	5400,0
50,000	6,4014	2,4198	1,1706	1,5254	3400,0
51,000	6,4014	2,4198	1,1714	1,5244	3400,0
52,000	6,4014	2,4198	1,1721	1,5234	3400,0
53,000	6,4014	2,4198	1,1728	1,5225	3400,0
54,000	6,4014	2,4198	1,1735	1,5217	5400,0
55,000	6,4014	2,4198	1,1740	1,5209	3400,0
56,000	6,4014	2,4198	1,1746	1,5202	5400,0
57,000	6,4014	2,4198	1,1751	1,5195	3400,0
58,000	6,4014	2,4198	1,1756	1,5189	5400,0
59,000	6,4014	2,4198	1,1760	1,5183	3400,0
60,000	6,4014	2,4198	1,1764	1,5178	3400,0
61,000	6,4014	2,4198	1,1768	1,5173	3400,0
62,000	6,4014	2,4198	1,1772	1,5168	3400,0
63,000	6,4014	2,4198	1,1775	1,5164	3400,0
64,000	6,4014	2,4198	1,1778	1,5160	3400,0
65,000	6,4014	2,4198	1,1781	1,5157	3400,0
66,000	6,4014	2,4198	1,1783	1,5153	3400,0
67,000	6,4014	2,4198	1,1786	1,5150	3400,0
68,000	6,4014	2,4198	1,1788	1,5147	3400,0
69,000	6,4014	2,4198	1,1790	1,5145	3400,0
70,000	6,4014		1,1792	1,5142	