FERMENTATION OF MILK WASTES

by

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Fermentation of Milk Wastes .

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There is a world shortage of high quality protein whilst valuable raw materials from food processing which could be used for protein production are wasted. Dairy effluents in particular fit this situation,but in addition provide a major source of water pollution.

This study utilised a continuous tower fermentation system to treat dairy effluents with a specially selected lactose-utilising filamentous fungus to provide a single cell protein animal feed while achieving a high degree of effluent treatment.

Sixty-one species of lactose-utilising fungi were isolated from a conventional milk effluent treatment system and synthetic milk effluent treated soil. Ten of the most rapidly growing species were cultivated in batch submerged culture in order to select those most suitable for the fermentation system from which P.javanicum van Beyma was ultimately chosen.

Experiments performed with semi-defined media established the optimum temperature,dilution rate and pH value for maximum lactose utilisation and biomass production.

The results were confirmed with whey substrate on which high levels of treatment efficiency were attained although foaming resulted in some restriction of dilution rate. Biomass yield from whey was slightly lower than that reported for yeasts,but harvesting costs would be lower due to the pelleted morphology adopted by P.javanicum in the fermenter. Attempts to ferment whole factory effluent were unsuccessful,probably because of the variability of the commercial conditions.

The industrial usefulness of P.javanicum was demonstrated in the semi-defined media experiments because of its ability to grow over a wide range of temperatures and pH values and to tolerate plant failure.

P.javanicum biomass produced from whey was of high protein quality and quantity and compared well with other single cell proteins or soya protein when considered as an animal feed.

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1.1 The Food Problem

The world population was approximately four billion in 1975, will be around six-seven billion at the turn of the century, and is unlikely to stabilise before ten-twelve billion, unless other phenomena intervene (Handler, 1975). Continued population growth at these rates constitutes the principal obstacle to true development of most countries, but more particularly in second and third world countries. The planet's resources must, therefore, be stretched in order to fulfil the requirements of these people for food, shelter, clothing and energy. These constitute the minimum required for existence, and take no account of the basic human needs we in the developed countries assume by right, for example, education, medicine, sewage treatment. On almost all counts only one third of the present world population is adequately provided for. Even to remain at that proportion it will be necessary to perform the miracle of producing, within twenty-five years, much more food, water, energy, forest and mineral products than previously in all of man's history (Borgstrom, 1975). If this is achieved, continued population growth will still ensure that the high proportion of starving, underfed and malnourished will remain.

There are great imbalances in the distribution of the world's resources. The developed countries have taken a far greater share of these than they would be entitled to on a population basis. The chief recipients of the world's flow of food and animal feeds have not been the developing countries, which have by definition the lowest standards of living, but

rather Europe and Japan. Europe each year buys on the world market more plant protein than the whole Indian subcontinent is eating (Borgstrom, 1975). The world's increasingly serious nutritional problem is magnified by the uneven distribution of available food amongst countries, and between socio-economic strata within individual countries. In 1972, the Protein-Calorie Advisory Group (PAG) of the United Nations stated that the results of surveys of total food produced per person (on a global basis) demonstrated there was no world-wide shortage of food in terms of both quantity (calories) and quality (protein) (P.A.G., 1976). However, in the developing countries there is overwhelming clinical evidence of undernutrition (too few calories) and malnutrition (particularly lack of protein). Clearly, millions of people are not receiving adequate supplies of food. It is noteworthy that the character of malnutrition has changed in the last 40-50 years. Apart from occasional acute famines, "classic malnutrition" was the consequence of some dietary imbalance giving rise to specific deficiency diseases such as beriberi (thiamine deficiency), scurvy (ascorbic acid deficiency) and pellagra (nicotinic acid deficiency). The current major forms of malnutrition are diseases resulting from protein deficiency (kwashiorkor) in individuals also deprived of an adequate calorific intake, that is, semistarvation, for example marasmus (Handler, 1975).

In the 1970's a number of factors precipitated what was generally termed a "food crisis". Natural calamities and weather conditions contributed to a fall in world food output.

Substantial increases in prices for fuel, fertilizers, and transport after 1973 added substantially to the costs affecting the prices of foods in world markets. The food shortages were particularly pernicious for third world countries, almost all of whom are substantial food importers (Teubal, 1980). Much research has been devoted to increasing the world food supply, mainly in three areas: firstly the redistribution of supplies to overcome regional deficiencies; secondly increasing "conventional" agricultural production; and thirdly developing new or novel food sources. No one source or scheme, conventional or unconventional will achieve this: a combination of methods will be necessary (Delaney and Donelly, 1973).

Redistributing the food supply may help to alleviate temporary shortfalls in specific areas, for example in the event of crop failure in a third world country, but ultimately will be of little benefit to the overall world shortage.

Proposals for increasing food production by conventional agricultural practices include land reclamation, irrigation, pest control, increased use of fertilizers, and better crop yields through both improved breeding programmes and farm management. Such improvements have thus far helped the food supply keep pace with population growth in most parts of the world, but a limit as to how much more technology can achieve is being reached. To aggravate matters, the supply of additional arable land is dwindling (Flannery, 1975); the most readily available and fertile lands are already under cultivation.

Large areas do remain for development, particularly in South America and Africa, but these will be very expensive to develop, both in economic and environmental terms. This has been demonstrated in the Amazon Basin, where uncontrolled felling and burning has caused severe environmental damage.

The water supply may also limit world agricultural production. Although only four percentage (%) of the world's river flow is presently diverted for irrigation, virtually all readily available opportunities have been utilised. To gain access to major additional water supplies, by river diversion or desalination of brackish waters, would require enormous engineering projects and a large increase in annual energy expenditure, thereby increasing the cost of food production (Handler, 1975).

Some new foods produced by "conventional" agricultural means are being developed, for example, cottonseed protein, peanut flour, triticale, and alfalfa protein. Advances are being made in fish farming through applying the techniques learned with red-meat and poultry production to optimising feed compositions and environmental conditions for maximum fish production (Flannery, 1975). Nevertheless, these developments are still tied to agriculture and its climate dependence, and land and water requirements.

All these "conventional" agricultural techniques are also dependent upon abundant energy supplies, usually fossil fuels. Petroleum and Natural Gas will be effectively exhausted in the

next century, and coal will be available for only one or two centuries thereafter (Flannery, 1975). The development of renewable energy sources which can be utilized as fossil fuel reserves diminish must be given priority. One such energy source is ethanol which can be produced by fermentation from carbohydrate crops, and which thus ultimately derives from solar energy via photosynthesis. Brazil is investing heavily in the production of ethanol from sugar-industry-wastes to reduce the dependence upon imported oil (Blake, 1980). New Zealand is currently planning a similar programme (Hopkinson, 1979 [a, b]; Fielding Herald, 1980), as are Australia and the United States of America. There is an analogous situation developing with regard to the fertilizers used in vast quantities to support present levels of agricultural production. Guano deposits from islands such as Nauru provide the main reservoirs for phosphate fertilizer production, and these are being extracted and exhausted at an alarming rate.

In primitive agriculture, the "farm" constituted a closed or nearly closed ecosystem. The farmer used self-generated wastes (of human, animal or vegetable origin) as fuel or fertilizer. There was, therefore, no, or only slight losses, of essential soil nutrients, for example, potassium, calcium, magnesium, zinc etc, which are required for good plant growth and crop yields. Hence when an area becomes an exporter of agricultural produce, whether in the form of plant or animal material, there is a net loss to the ecosystem

of these metal ions, with a commensurate requirement for the application of imported fertilizers if yields are not to diminish. This can become very expensive, especially to a country such as New Zealand, which imports vast amounts of phosphate fertilizers every year. Moreover, there will always be some losses to the ecosystem through run-off into waterways and to the sea, ensuring that there will always be a minimum requirement for fertilizer to make good the balance. As with fossil fuels, the raw materials required for fertilizer manufacture are finite resources and cannot be regarded as inexhaustible. Recycling of the elements in organic and inorganic substances occurs in nature on a global scale, although ultimately the oceans constitute a "sink". Until we develop the technology necessary for the economic extraction of minerals from the sea, they will thus remain unavailable to us.

Pirt (1978) has defined waste reclamation as "recycling the waste material in a useful form", and states that one of the aims of biotechnology should be to discover the mechanisms whereby the natural cycling of elements occurs and to apply these processes where they are needed. In principle, all those elements which make up biomass (that is, matter derived from living cells, whether microbial, plant or animal) can be recycled by means of microbial action. If this were not so, the planet's surface would rapidly become buried in a layer of undecomposed detritus derived from dead animals, leaves, paper, excreta and all the other residues of plant and animal

life. These decomposition cycles function continuously, independent of any actions of man. Problems arise when the microorganisms responsible for them are overloaded by the production of large volumes of organic waste in a limited space, for example in feed-lot farming of cattle. This problem has been intensified since the Industrial Revolution by the production of vast quantities of synthetic materials which may be resistant to microbial degradation, for example plastics.

Overloading the natural "recycle pathways" results in pollution of the environment and it has, therefore, become necessary to assist the microbial recycling of biomass by "pretreating" these wastes before they are released. In most developed countries it is now standard practice for domestic and industrial effluents to undergo some type of sewage treatment before release. Moreover the natural cycling of materials between organic and inorganic states can take very long periods of time, but effluent treatment allows these cycles to be shortcircuited and permits the "re-use" of certain materials without requiring their extraction from the environment. For example, processes using microbes to extract metals from sewage are being developed (Smilie, 1977). These metals may be reprocessed directly, economising on the mining of metal ores. Similarly, effluent treatment permits the salvaging of complex organic materials such as proteins, forestalling their breakdown into constituent molecules and elements. Since all life is ultimately dependent upon the fixation of nitrogen by

microorganisms, any process which economises on the breakdown of organic nitrogenous compounds should be exploited.

Recycling of materials by effluent treatment will, in addition to reducing environmental pollution, allow the more efficient use of available resources, but is unlikely to have much effect upon the total quantities of food produced. It is in the area of novel or new food sources that the best prospects for increasing the world's food supply lies. Microbial products in various forms have attracted particular attention because of their amenability to controlled intensive cultivation by fermentation, and lower dependence on the vagaries of climate, weather and soil characteristics (Moraine, Shelef, Meydan and Levi, 1979). Single cell protein (SCP) has been widely accepted as a generic term for crude or refined sources of protein whose origin is unicellular or simple multicellular organisms, that is bacteria, yeasts, fungi, algae and perhaps protozoa and even bacteriophage (PAG, 1970).

There are many industries which make use of microorganisms to perform the biological transformation of low-grade materials into more valuable products. For example, the production of lactic acid in cheese and casein plants, alcohol in breweries, citric acid from molasses, antiobiotics and vinegar from alcohol (Chapman, 1966). Microbial products have consitituted important portions of the diets of certain peoples for centuries, for example, mushrooms, truffles and cheeses are well-accepted foods in Europe and similarly miso and tempeh are important foods in the Orient (Laskin, 1977).

There is adequate evidence to indicate that some species of microorganisms can be safe and useful sources of proteins, vitamins and minerals for animal and human feeding. The safety of such materials will depend on the organism selected, the quality of the substrates utilised and the conditions of growth. PAG has set out guidelines for determining both safety and nutritional characteristics of a microbial food and these have been discussed by Andrews (1980). At the present time, SCP products grown in purified petroleum hydrocarbon fractions are safely used in animal feeding at a practical level. No carcinogenic, mutagenic, or embryotoxic effects have been observed from the use of such materials (PAG, 1976). The American Food and Drug Administration has approved the use of a Saccharomyces fragilis derived SCP for both animal and human foods (Anon, 1975).

SCP's can be produced by fermentation from substrates which would otherwise be dumped as wastes. This could be critical to the continued development of SCP for food and animal feed. A process which is only marginally economic (compared with, for example, the production of soya or fishmeal concentrates) could be made profitable if it is adapted to the use of a substrate which is supplied at zero, or near zero, cost. For example, the production of yeast protein from wood process waste (the Finnish Pekilo process) and potato process waste (the Swedish Symba process) (Pirt, 1978). The economics of SCP production is an extremely complex subject, depending on many complicated and volatile factors, including political issues, as in, for example, the halt in Toprina production by British Petroleum in Sardinia (Done, 1978), in

addition to process details such as choice of organism, choice of feedstock, details of construction, location, operation etc., Consideration must also be given to the costs of competing products, for example, soya (Laskin, 1977). Present trends in prices for animal and vegetable proteins indicate that SCP production will become increasingly profitable, leading to greater investment by industry and resulting in an expansion in the quantity of information available. This will benefit the third world countries who may lack the necessary finance to initiate research into SCP production, but who will be able to purchase the technology from the developed nations. These third world countries frequently generate large volumes of wastes which are particularly suited to SCP production by fermentation, for example, sugar, coffee, oil seed and cassava processing residues. Many of these countries have what is virtually a one-crop economy, the wastes from which may contribute to the pollution of the environment. Single-cell-protein would provide an additional source of revenue for the nation, as well as preventing pollution. An example of this is provided by Malaysia which imports almost all the concentrates required for animal feeding, thus SCP produced from substrates such as palm-oil wastes, which at present cause severe river pollution, would find a ready market (Davis, 1978).

There are available for fermention a great variety of wastes: Ratledge (1977) and Moo-Young (1977) have presented comprehensive reviews of the literature describing these fermentation substrates. The most important potential substrates are waste carbohydrates and a partial listing of the annual world or United States output

of them is given in table 1.

Table 1.

Partial listing of annual world (or US) output of carbohydrates (potentially) suitable for SCP production.

source		quantity in million tons
sugars	: molasses	9.3
	whey	(1.5 US)
fruit wastes	: waste sulphite liquor	(12 US)
	starch	
	potato wastes	
	cassava	
	manure	200
cellulose	: bagasse	104.2
	wheat bran	57.3
	wheat straw	850
	rice straw	590
	soybean vines	162
	corn cob	30.1
	corn stover	120
	manure	(200 US)
	urban refuse	(150 US)
	wood remnants	(60 US)

After Moo-Young (1977).

Some wastes may be of especial interest as fermentation substrates in specific locales. Sulphite waste liquor and dairy effluents are largely composed of water, rendering their transport to alternative sites for processing uneconomic. The most efficient use of these types of effluent would be achieved by a fermentation plant adjacent to the production point.

New Zealand is one of the world's largest producers of dairy products, with the corollary of also being one of the world's largest producers of dairy wastes. At the present time the treatment of these effluents in New Zealand is extremely limited. Only one dairy factory in the country possesses an effluent treatment plant, the remaining factories either spray irrigating their wastes (on to pastures) or discharging them directly to natural waterways (Galpin, 1979). This represents the loss of extremely valuable substances, both nutritionally and economically and provides one of the greatest single contributions to the pollution of inland waterways.

The development of a fermentation process producing single-cell protein from dairy effluents and concurrently reducing the pollution caused by those effluents would, therefore, be of especial value to New Zealand and was selected as the aim of this project.

1.2 Dairy Effluents

Fluid wastes from dairy plants consist primarily of water-borne milk solids of various origins but may also include domestic wastes, lubricants, sodium chloride, boiler treatment compounds, detergents and sanitizing agents (usually alkali solutions). About 90% of the materials which contribute to the Biological Oxygen Demand (BOD) of the effluent are milk solids (Harper, 1974). Dairy effluents can be separated into two groups on the basis of origin of the milk solids: dairy waste waters and process byproducts.

1.2.1 Dairy Waste Waters

The majority of milk solids in dairy waste waters arise from

1. Spillages and leakages on to floors.
2. Milk and milk products adhering to or left in churns, piping, tankers and plant before washing out.
3. Processing losses such as those from entrainment in evaporators, butter washings, discharges from bottle washers, vat washings and pressroom washings in cheese making (Royal,1976).

Table 2 illustrates the characteristics that could be expected of an effluent from a New Zealand Dairy Factory.

Table 2 Expected Characteristics of New Zealand Dairy Factory Effluent

Volume	m^3/day	30-2500
Waste water volume coefficient	m^3 water/m^3 whole milk	0.69-9.8
Milk fat	mg/l	0-2100
Total nitrogen	mg/l	8-1300
Lactose	mg/l	120-2300 excluding whey
Nitrate	mg/l	0.3-70
Phosphorous	mg/l	4-150
Temperature	^oC	11-72
pH		3-13.2

Source: Galpin (1979)

1.2.2 The Treatment of Dairy Waste Waters

Milk has a high BOD of the order of 120,000 mgl^{-1} (Royal, 1976) and even when diluted in waste waters, conventional effluent treatment is expensive. A manufacturing creamery with an intake of 500,000 kg of milk per day of which 1% in terms of whole milk is lost would produce an effluent with a BOD load of 600 kg per day. This represents approximately 11,000 people in terms of population equivalents (the daily BOD load of domestic sewage per person is 55g) (Royal, 1976). Coton (1976) discussed the costs of effluent treatment and illustrated the problem by the example that a large modern creamery with a throughput of 100,000 gallons per day and costing around £5 million will probably include an effluent plant costing approximately £0.4 million.

These costs have increased sharply since 1976. Sample data for dairy wastages in terms of both quantities and BOD loads have been presented by Harper (1974) and Royal (1976).

The conventional treatment of dairy effluent depends largely on the use of various types of percolating filter and activated sludge processes. Such plants may, of course, be highly efficient and able to treat a wide variety of effluents to acceptable standards. The byproducts of these systems are the treated water, which may or may not be suitable for recycling, and quantities of sludge which requires disposal by incineration or spraying onto land (Pannell and Greenshields, 1976). The latter may not be practicable if insufficient land is available.

The most efficient method of dealing with dairy waste waters is to avoid their occurrence - these wastes constitute valuable product losses. Improved management techniques can reduce wastage arising from drainage and washing of tankers and equipment as well as from spillages (Harper, Blaisdell and Grosskopf, 1971). Attempts have been made to segregate first plant rinses from other waste streams and to recover the material from this directly back into product. One dairy in Sweden has installed this type of system (Coton, 1976). Unfortunately, these methods often involve concentration and evaporation stages and thus may be relatively expensive to operate. Baltjes (1976) has described waste water control in the Dutch dairy industry and Harper (1974) has covered a similar topic with reference to the British industry.

Hayashi and Ishioka (1976), in their discussion of the re-use of dairy waste waters in Japan, have suggested three courses of action: firstly - preventing the milk component from flowing into the water, secondly - recovering solid materials from the water, and thirdly - re-utilising the waste water directly. They cite a Japanese example of the latter in the use of waste waters for fish culture. In rural areas where there is sufficient land available, fish such as eels, gibels and carp are being cultivated in dairy-waste waters.

Even with improved techniques as outlined above there will always be some unavoiadable losses of milk solids to waste waters, necessitating some type of effluent treatment prior to disposal. Efficient use could be made of these waste waters as substrates for microbial growth, with the proviso that the fermentation system applied must be suitable for the use of dilute substrates (see section 1.3).

1.2.3 Dairy Process Byproducts

These derive from the manufacture of dairy products such as butter, cheese, casein and acidified milk products, for example, yoghurt. In terms of total quantity produced, cheese whey is by far the most important, although at periods of peak production casein whey can yield high seasonal volumes (Smith, 1979).

Composition of Whey

Whey is not a substance of defined composition, it varies considerably according to the product from which it derives and

the particular manufacturing process involved. There are many varieties of cheese, which all yield a different whey. Water soluble substances such as sodium or calcium chloride added during processing may also be found in whey. Whey usually contains approximately half the initial total solids content of the influent milk and averages about 6% solids. These solids typically contain around 75% lactose, 14% protein, 9% minerals, 2% fat (Smith, 1979; Anon, 1975). Lactose is a disaccharide comprising one molecule of glucose and one molecule of galactose joined by a 1-4β-galactosidic linkage. Whey lactose occurs in two forms, α and β, normally in the proportions 40α to 60β. When whey is concentrated sufficiently then cooled the α lactose tends to crystallise, resulting in the mutarotation of β-lactose to the α form, to maintain the equilibrium. This property of lactose is important in the drying of milk and milk byproducts.

The most valuable nutritional component of whey is the milk protein fraction, approximately 20% of the original protein content of whole milk. Whey protein is a complex mixture of a number of protein fractions, the principle members being α-lactalbumin and β-lactoglobulin which between them comprise approximately 90% of the total. There are also various globulin, serum albumin and proteose-peptone fractions present. Whey proteins are nutritionally superior to most other proteins in human and animal nutrition, principally due to their favourable amino acid composition and in particular to the high lysine content (Smith, 1979).

The major mineral constituents of whey are potassium, calcium, sodium, magnesium, chloride and phosphate. The calcium and phosphate are to a certain degree retained in cheese, but the remainder of

the other minerals are present in virtually the same quantities as in whole milk.

The acid content of whey varies according to the manufacturing process and the period and conditions of storage of the whey. The pH value of cheese whey is typically in the region of 6.0, whereas that of casein whey is in the region 4.3 - 4.6 (Smith, 1979). The lactic acid content of cheese whey derives from the partial fermentation of lactose during cheese manufacture (Oborn, 1968).

Many analyses of the composition of whey are available, for example, the reports of Atkin, Witter and Ordal (1967), Oborn, (1968), and Pace and Goldstein (1975). Typical figures for cheddar whey are presented in Table 3.

Table 3

Composition of Liquid Cheddar Whey

Item	Liquid Whey (amount/100g)
Water	91.3%
Crude protein (N x 6.25)	0.9%
True protein (N x 6.25)	0.7%
Fat	0.3%
Lactose	5.1%
Ash	0.6%
Calcium	51 mg
Phosphorous	53 mg
Iron	0.1 mg
Sodium	-
Potassium	-
Vitamine A	10 IU
Thiamin	0.03 mg
Riboflavin	0.14 mg
Niacin	0.1 mg
Ascorbic acid	-
Food energy	26 kcal

After United States Dairy Association (1963)

1.2.4 Treatment of Dairy Process Byproducts

Dairy processing plants tend, because of the economy of scale, to be relatively large, resulting in large volumes of byproducts such as whey. Current figures for whey production throughout the world are given in tables 4 and 5. The treatment of dairy process byproducts will be discussed with particular reference to cheese wheys, although the process systems are also applicable to other byproducts. The quantities of whey available at any time during the year are closely related to the seasonal production of milk. In Australia this is dependent on the availability of pasture (Oborn, 1968). The New Zealand dairy industry is also based entirely on pasture feeding, few concentrates are used and cows are not housed in winter. Virtually all cows calve in July and August, reach peak milk production in November, and are dried off in May (Marshall, 1976).

When discussing the "treatment" of whey it is necessary to distinguish between "disposal" and "utilization". Disposal implies the discard of whey; any treatment applied will be in order to prevent pollution, without regard for recycling any part of the whey. In contrast utilization is applied to prevent wastage of potentially valuable substances in addition to preventing pollution. Wix and Woodbine (1958 [a,b]) have made a comprehensive review of the methods available in 1958 for the disposal and utilization of whey.

A Disposal of Whey

The dairy industry has traditionally regarded whey as a waste product to be disposed of by suitable dumping. Bissett and Riddle (1976) conducted a survey of dairy effluent disposal practices in Canada and reached the following conclusions:

firstly - 72% of the plants surveyed discharged effluents to municipal sewers;

secondly - approximately 19% of all diary plants utilised land disposal such as holding ponds, irrigation, and septic tanks;

thirdly - less than 10% of all plants discharged directly to natural watercourses, with half of these plants having some form of biological treatment.

Oborn (1968 reported that most of the whey produced in Australia is discarded.

There has recently been a large increase in the quantity of whey produced in New Zealand, and particularly in the quantity available at a single location. Ruiz, Gurnsey and Short (1978) report that 1.2×10^6 tonnes of acid whey are produced annually in that country. Only one dairy factory in New Zealand includes a biological effluent treatment plant. Initially this was a trickling filter system, but the company has since installed an aerated lagoon as the operation of the trickling filter was unsatisfactory. The remaining dairy factories either spray irrigate their wastes or discharge them directly to natural waterways (Galpin, 1979). Marshall (1976) has reported statistics of daily whole milk intake waste water volume BOD_5 and suspended solids concentrations in the waste water for six New Zealand diary factories. The average chemical composition, temperature and pH of the waste water from these factories is presented in Table 6.

Table 4

Estimated World Whey Production

(estimated at 8kg and 5kg whey per kg of cheese and cottage cheese respectively)

'000 tons

	1966	1971	1972	1973 (Preliminary)
USA	8616	10883	11804	11836
Canada	774	998	1019	935
Belgium-Luxembourg	282	290	282	230
Denmark	1000	960	1048	1024
France	4878	5603	5960	6136
Germany, Federal Republic	2512	3285	3482	3568
Ireland	136	264	364	328
Italy	3918	3840	3880	3960
Netherlands	1864	2424	2504	2616
United Kingdom	872	1296	1469	1447
Total Western Europe	20105	23045	24189	24541
Australasia	1352	1440	1502	1512
Other developed countries	496	768	800	832
Total developed countries	31345	37134	39314	39656
USSR	3456	3624	3808	4016
Eastern Europe	4968	6088	6090	6300
Total developing countries	17344	19344	19736	20723
Total World	57113	66190	68948	70695

After Coton, (1976)

Table 5

Estimated World Whey Production

Country	Original data	in 10^6 tonnes	Source
New Zealand	1.2×10^6 tonnes (acid whey)	1.2 (acid whey)	Ruiz, Gurnsey, Short, 1978
Australia	350×10^6 gallons	1.59	Lane, 1977
	1.6×10^6 tonnes	1.6	Holder & Sewards, 1976
UK	113.1×10^3 tonnes (whey solids)	1.89*	Smith, 1979
Ireland	7.3×10^6 gallons	0.03	Delaney et al, 1973
Canada	8.0 billion kg	8.0	Bisset & Riddle, 1976
USA	2.5×10^9 lb	11.35	Volesky & Emond, 1979
EEC	1060.6×10^3 tonnes (whey solids)	17.68*	Smith, 1979
World	100 billion lb	45.4	Vananuvat and Kinsella, 1975[a]

* assumes whey contains 6% solids (Smith, 1979).

Table 6

Average Chemical Composition, Temperature, and pH of Waste water from six New Zealand Dairy Factories

Factory*		1	2	3	4	5	6
Temperature °C		19-46	20-65	17-42	19-62	19-54	12-32
pH		2.1-12.3	3.3-10.5	4.5-10.5	5.9-11.3	7.6-11.0	4.2-12.2
Nitrogen	mean	50	26	305	180	114	200
mg/l	range	40-80	8-50	209-408	160-280	30-200	47-580
Fat	mean	300	33	470	1800	840	800
mg/l	range	200-1320	0-100	310-634	400-2100	400-1400	300-1500
Carbohydrate	mean			3.95		2.1	4.3
g/l	range			2.1-6.0		1.0-4.1	1.3-14.8
Phosphorous	mean	25	8		62	34	99
mg/l	range	12-56	6-10		29-79	17-59	45-280
Ash	mean			1.6	2.0	1.6	2.04
g/l	range			1.0-2.2	1.4-2.4	0.9-2.2	0.7-5.6

* 1. Butter and buttermilk

2. Lactic casein

3. Multiproduct

4. Cheddar cheese

5. Cheddar cheese

6. Cheddar and Colby cheese

After Marshall (1976)

B Utilisation of Whey

In finding a satisfactory method for utilising large quantities of whey, there are two major difficulties. The greatest problem results from the low solids content - whey is 94% water (Perzow, 1974; Chapman, 1966). This renders transportation and storage extremely expensive unless part or all of this water is removed, processing which in itself is expensive. Moreover, whey is subject to spoilage, causing further problems during transportation and storage (Reddy, Henderson, and Erdman, 1976). Refrigeration or the addition of preservatives such as formalin may be required, but the former is expensive and the latter will affect any later processing. A significant element in the calculation of the economic feasibility of processes for the utilisation of whey will centre on (i) surcharges for disposal to municipal sewage systems; and (ii) capital investment required for installation of plant.

Feeding to Animals

Liquid whey has traditionally been used as a raw material for stock feeding. The procedure was developed when milk was separated on the farm but the advent of bulk tanker collection and centralised processing has contributed to its decline. In the United Kingdom 40% of the whey produced is still used for animal feeding (Coton, 1976), but higher transport costs, seasonality of supply and poor storage properties make it comparatively unattractive to the farmer (Oborn, 1968). Moreover, the high lactose content of whey may cause digestive problems in the animals (Alkin et al, 1967).

Spray Irrigation

Spray irrigation is the most common method of effluent treatment and disposal used by the New Zealand manufacturing dairy industry and is generally associated with productive farming enterprises. Approximately 20% of the total effluent from all of New Zealand's butter, cheese and milk powder factories and 50% of the effluent from all casein factories is disposed of in this manner. Factories are normally situated in rural areas and therefore disposal of effluent to domestic sewage treatment plants is rare (Parkin and Marshall, 1976). Spray irrigation of whey is also practised in Australia (Hodder, et al, 1976). The use of milk effluents in this manner fulfils two important agricultural functions - irrigation and the addition of nutrients to the soil. McDowell and Thomas (1961) have reported that each cubic metre of whey contains the equivalent of 1.3kg K_2SO_4, 8kg $(NH_4)_2\ SO_4$, 2.7kg superphosphate, and 1.5kg $CaCO_3$. Parkin and Marshall (1976) have reported that milk and milk-fat production have been increased by up to 50% by the application of whey to pasture. Sharrat (1959) claimed that spray irrigation with whey could cause temporary excessive acidity in soil and plant damage, but this has not been observed in New Zealand provided correct pasture management procedures are observed (Parkin et al, 1976).

Drying of Whey

Dried whey can be produced by a variety of methods (Amundson, 1966; Weber, 1967; Oborn, 1968) and can be incorporated into a

large number of human foods, for example ice-cream, confectionery, bread, soups and meat and cheese products (Chapman, 1966; Coton, 1976) and into concentrate mixtures for animal feeds (Schingoethe, Ludens, Tucker and Dash, 1976). Recent figures for whey powder production are presented in table 7.

Table 7 Whey Powder Production

	'000 tons					whey drying as %
	1966	1970	1971	1972	1973	total whey supply 1973
USA total	214	282	308	346	338	40
of which for food	110	133	145	171	178	-
Canada	19	20	24	25	24	36
EEC	93	210	274	325	389	30
of which France	26	80	115	148	170	39
Netherlands	28	51	65	71	99	53
Germany, F.R	15	43	48	56	66	26
UK	11	13	14	15	15	15
Belgium	6	7	10	9	9	55
Others	7	16	22	26	30	6
Austria	1	7	8	9	11	35
Finland	6	12	14	17	17	65
Total 13 countries	333	531	628	722	779	34

After Coton (1976)

Demineralization

Demineralized whey, produced by electrodialysis or, less commonly, ion exchange, followed by drying to a powder finds a major outlet in the babyfood industry (Tomarelli, 1962; Barness, Omans, Rose, and György, 1963; Coton, 1976; Smith, 1979) in the manufacture of "humanized" babyfoods.

Lactose and Whey Chemicals

Descriptions of the manufacture of lactose, lactic acid, albumin and other chemicals from whey are well covered by several authors (Whittier and Webb, 1950; Wix, 1958 [a,b]; Webb and Johnson, 1965) who provide details of specific processes and their many variations.

Whey Proteins

Originally whey proteins were extracted by heat coagulation (Wendorff, Amundson, and Olsen, 1970) but recently much research has been devoted to their extraction in undenatured form. True protein amounts to about 0.6 - 0.7% in whey and has an excellent amino acid spectrum (Table 8). Whey proteins are used in the flour and sugar confectionery industries, in soft-drink manufacture and in babyfoods (Winston, Saperstein and Lutwod, 1970; Horton, Goldsmith and Zall, 1972; De Vilbiss, Holsinger, Posati and Pollansch, 1974; Coton, 1976; Lane, 1977).

Table 8 Amino Acid Composition of Whey Proteins Obtained by Ultrafiltration

Amino Acid	mg/gN	Amino Acid	mg/gN
Lysine	176	Cystine	60
Histidine	52	Valine	117
Arginine	50	Methionine	44
Aspartic acid	219	Isoleucine	114
Threonine	129	Leucine	221
Serine	92	Tyrosine	62
Glutamic acid	364	Phenylalanine	65
Proline	118	Tryptophan	36
Glycine	38		
Alanine	100		

Source: Coton, 1976

Whey proteins are extracted in undenatured form by some type of membrane process and these have been reviewed by Lacey (1972); Zall (1971, 1976); Delaney et al (1973); Donnelly, O'Sullivan and Delaney (1974); Coton (1976); Ruiz et al (1978); Short and Hughes (1978) and Smith (1979). The two membrane processes most commonly operated are Ultrafiltration and Reverse Osmosis. The main handicap to the expansion of protein extraction techniques is that the majority of the original BOD load from the whey remains in the permeate from the filtration process, thus generating a secondary effluent problem (Vananuvat et al, 1975[a]). The permeate contains most of the whey lactose (approximately 45 grams per litre) and minerals (Weetall, Havetwalla, Pitcher, Detar, Vann and Yarerbaum, 1974; Ruiz et al, 1978) and is usually termed "lactose permeate".

Lactose Permeate

Donelly, O'Sullivan and Delaney (1974) suggested that lactose permeate could be used in three types of process - lactose crystalisation; enzyme processing; fermentation substrate.

It is quite easy to extract lactose from the permeate (see section 1.2.3) but the market for lactose is strictly limited, being confined almost entirely to the pharmaceutical industries and, to a lesser degree, for animal feeding. The uses of lactose in normal food and confectionery are minimal due to its low solubility, lack of sweetness, and laxative effects (Smith, 1979; Volesky et al, 1979).

Lactose can be upgraded in value by enzymatic hydrolysis to glucose and galactose (Pomeranz, 1964). Addition of a further small quantity of glucose and glucose isomerase results in a product with the sweetening value of sucrose on an equivalent weight basis (Weetall et al, 1974). The Dairy Industry is interested in the production of lactase enzyme (β-galactosidase) for use in lowering the concentration of lactose in milk and whey and for preventing precipitation in ice-cream and frozen cream desserts (Kosikowski and Wierbicki, 1973; Volesky et al, 1979). The majority of research into the utilisation of lactose permeate has been devoted to its potential as a substrate for fermentation.

Fermentation

Historically, whey was first used for the production of penicillin, (Perzow, 1974) although this was discontinued after problems with foaming and the purification of the product developed. Whey and lactose permeate can be fermented both aerobically and anaerobically, the latter process usually resulting in the production of methane which may be used as an energy source.

Coton (1976) has reported that lactose permeate is at a temperature of about 50°C as it leaves the ultrafiltration plant, requiring no further energy input to operate an anaerobic process at about 30°C at which the residence time and, therefore, capital involved in plant is minimal. The particular advantages of the anaerobic fermentation of lactose are;

firstly - no aeration costs,

secondly - no additional nutrients required,

thirdly - a valuable substance (methane) is produced, and fourthly - little sludge results.

In contrast to the anaerobic fermentation of lactose permeate, one of the principal disadvantages of the anaerobic digestion of whey is that the fermentation may require heating to achieve economic rates of reaction (Holder et al, 1976). If the whey is extremely dilute (for example if process wash waters have been combined with it) heating may be too expensive and therefore aerobic fermentation would be more appropriate.

Aerobic fermentation of whey and whey-processing byproducts has generally been in order to produce single-cell-protein. The majority of this research has been devoted to the fermentation of whey by yeasts, and in particular Saccharomyces fragilis, (Wasserman, Hopkins and Porges, 1959; Wasserman, 1960[a,b,c], 1961, 1962; Wasserman, Hampson, Alvare and Alvare, 1961 ; Amundson, 1967; Muller, 1969; Marth, 1970; Bechtle and Clayton, 1971; Knight, Smith, and Mickle, 1972; Perzow, 1974; Vananuvat et al, 1975; Coton, 1976; Terra, 1976; Laskin, 1977).

Pace and Goldstein (1975) have described a proposed cheese whey ultrafiltration/fermentation plant in which the lactose permeate is supplemented with the necessary nutrients and continuously metered into a conventional, aerated, stirred tank reactor. The exit stream from the fermenter is centrifuged to yield a yeast cream of 15 - 20% solids which is then drum dried. The two best known processes for the fermentation of whey by yeast are the "Wheast Process" operated by Knudsen Dairy Products of the USA, and the SAV process developed in France by the

Societe des Alcools du Vexin. The former process is a batch fermentation of cottage cheese whey by S. fragilis (Oborn, 1968; Perzow, 1974), whilst the latter involves two fermentation stages followed by evaporation and spraydrying (Oborn, 1968). According to the SAV process description, the lactose is completely consumed in one fermentation stage and the majority of the lactic acid in the second stage. The feed material may be either cheese or casein whey and there is no liquid effluent from the process as all the matter is recovered from the spray drier. A novel fermentation system - dialysis culture - for production of food yeast from lactose permeate, has been described by Lane (1977) (Fig. 1)

In this technique a semi-permeate membrane is used to separate fluid containing dialysable nutrients, in this case whey or lactose permeate, from a suspension of microorganisms, which thus depend for their growth upon the dialysis of nutrients across the membrane. Membrane fouling is minimised

by maintaining high circulation rates on both sides of the membrane. The operating parameters (flow rates, concentrations of solutes and biomass) can be adjusted so that the growth of organisms remains substrate limited; the concentration gradient across the membrane, and hence the rate of dialysis, thus remains at a maximum. The dialysis step can be regarded as affecting concentration of the microbial nutrients, and high cell densities can be obtained because the reservoir volume can be large relative to the fermenter volume. Dialysis culture of food yeast on whey ultrafiltrate (lactose permeate) might reduce capital costs by reducing the capacity of both the fermenter and the harvesting equipment required to process a given volume of ultrafiltrate.

Ruiz et al (1978), have described the reduction in lactic acid, nonprotein nitrogen and ash in lactic acid whey by fermentation with Candida ingens. Lactose was not utilised and could be used as the carbon-substrate for a further fermentation stage with a different organism.

Amber Laboratories, a division of Milbrew Inc., Wisconsin, USA, have developed a whey fermentation process featuring a closed loop system with essentially no waste effluent. A prototype full-size commercial facility rated to produce 5000 tons per year of yeast product from three times as much whey solids has been installed. The yeast selected is a strain of S. fragilis and the system can operate batchwise, semicontinuously, and continuously, although the process is more efficient when operated in the continuous mode. Conversion of lactose to yeast

is virtually complete and results in 0.45 - 0.55 lb of yeast per lb of lactose. By operating at low pH, large innoculum concentrations and high cell contents, the fermentation sidesteps contamination problems and thus the need for sterile or aseptic equipment and techniques. Partial evaporation followed by spraydrying of the fermentation effluent stream yields a "yeast-fermentation-solubles product" approved by the United States Food and Drug Administration (USFDA) as an animal feed. An FDA approved human food grade yeast product can also be produced, although at the expense of a slight drop in fermentation yield. Ethanol is recovered as a byproduct of the fermentation and increased yields of this valuable energy source can be achieved by altering the fermentation conditions (Anon, 1975; Bernstein, 1976; Laskin, 1977). Ethanol, as a whey fermentation product, has been suggested to be worthy of serious investigation (Coton, 1976). Rising oil prices will increase the profitability of such a process.

Burgess (1977) believes the economics of SCP production from whey by conventional stirred tank reactor (STR) technology to be marginal, and that a cheaper fermentation system is required for a more economically viable process. He suggests the use of the tower fermenter, and reports that yeast (S. fragilis) yields of 67% of the theoretical maximum have been achieved in the laboratory tower fermenter. This is lower than yields achieved in STR's, but the process should be economically viable because of the reduction in fermentation costs possible with the simpler fermentation system. These costs account for about 50% of the capital investment and approximately 60% of the operating costs in conventional systems.

In comparison with yeasts, few mould or bacterial fermentations of dairy effluents have been performed. Apart from their early use as substrates for penicillin production, two other mould fermentation products are mentioned specifically in the literature: riboflavin (Wix and Woodbine, 1958 [b]) and fat (Robinson, 1952; Wix and Woodbine, 1958 [a,b]). Bacterial fermentations of whey have been reported by Meade, Pollard and Rodgers (1947) with Clostridium acetobutylicum, and by Wix and Woodbine (1958 [b]) using either Streptococcus lactis or Lactobacillus bulgaricus.

In summary, a flow diagram of the alternative processes available for the utilisation of whey is presented in Fig 2.

Fig 2. Alternative Processes for the Utilisation of Whey

It is obvious that the technology for the fermentation of dairy effluents by yeasts to produce SCP has been well developed. The main disadvantage of the production of yeasts (and also bacteria) is that harvesting can be rather expensive, generally requiring the use of some type of centrifugation process. These procedures have large power requirements and thus are expensive to operate, as well as requiring large initial capital investment.

In contrast, filamentous fungi can be harvested much more readily and may, therefore, provide a more economic alternative for SCP production. Filamentous fungi are widely used in the traditional food technologies of the East to modify various dietary staples for example, those based on oilseeds or cereals, producing fermented foods with improved taste, texture and digestibility (Hesseltine and Wang, 1967). A further advantage of filamentous fungi over yeasts results from their structure which may facilitate texturing of the product (Smith, Palmer and Reade, 1975; Solomons, 1980). Moreover, Spicer (1971) has claimed that the sulphur-amino acid content of filamentous fungi is higher than that of yeasts, suggesting that the former possess a superior essential amino acid profile and biological value. Filamentous fungi may exhibit lower growth rates than yeasts, but they also have a relatively low RNA content, which suggests greater applicability to human foods (Laskin, 1977).

Consequently, it was decided to use a species of the filamentous fungi for the production of SCP from dairy effluents. The fermentation system producing this SCP should:

(i) be applicable to all types of dairy effluents;

(ii) reduce or eliminate lactose from the effluent;

(iii) allow for easy harvesting of the product, preferably in one operation.

(iv) be flexible, for example, tolerate the substitution of one type of effluent for another without requiring further investment or serious modification of operating techniques.

1.3 Fermentation Systems

Hatch (1975) has defined a fermenter as "a vessel which provides the nutritional and physiological environment required for single-cell growth". The earliest large-scale fermenters, as described by de Becze and Liebmann (1944), were used in Central Europe for the aerobic production of compressed yeast. At first, the fermenter consisted of a large cylindrical tank with air introduced at the base. This design was soon modified by the use of impellers to increase the rate of mixing and to disperse the gas phase. Mechanical agitation was found to provide a reduction in the compressed air requirement, better mixing, and higher oxygen-transfer-rates, than could be obtained in the gas-sparged tank. As a result, the mechanically-agitated fermenter became standard in the early years of the fermentation industry (Hatch, 1975).

Before a basic fermenter design can be chosen, criteria for comparison of the possible designs must be selected. Commercial criteria for performance must be used and it is in this area that little comparative data is available, even on the more "conventional" fermenters (Emery, 1976). Commercial viability is based not only on mass-energy and income/expenditure balances, but also on reliability, stability, ease of control and flexibility. One method for evaluating the economics of various fermenter designs is to compare the performance ratios, which are the mass of oxygen transferred per unit power requirement at the same oxygen-transfer-rate; that is, power requirements for a given degree of efficiency of oxygen transfer (Hatch, 1975).

Efficiency of oxygen transfer is usually considered one of the most important factors when designing a fermentation system (Moo-Young, 1975). The medium component with the lowest solubility in the liquid phase is typically oxygen and therefore for aerobic cell growth the fermenter is usually designed to maximise the oxygen-transfer-rates while minimising the power requirements. Katinger (1977), in his discussion of new fermenter configurations, has considered the parameters which must be considered when designing a fermenter. He states that fermenters are required to perform a great number of functions which may sometimes impose contradictory or conflicting requirements on the design of the equipment. These requirements are summarised in table 9.

Table 9. Desired Properties for Fermenters

A Functional Requirements

1. High gas/liquid mass transfer
2. Creation of gas/liquid interfaces without causing foaming problems
3. Sufficient hold up of dispersed phases (gaseous, liquid or solid)
4. Reasonable heat transfer
5. Avoidance of "dead" zones in large fermenters and in highly viscous fermentation broths
6. Prevent aggregation, but with no damage to the mircroorganisms.

B Economic Requirements

1. Cheap, robust and simple mechanical design
2. Easy to operate
3. Well understood scale-up characteristics
4. Reasonable flexibility with respect to various process requirements
5. Stable operation under fluctuating process conditions
6. Low specific power consumption.

After Katinger, 1977

The choice of fermenter design will, of course, depend on a large number of variables, including

1. The final product(s)
2. The nature of the substrate and its availability

   for example: wheys tend to be produced on a seasonal basis and it may, therefore, be more economic to invest in a system which can use an alternative substrate when the primary substrate is not available
3. The land area and "on-line services" available

   for example: if the fermentation system is to be incorporated into an existing plant, the design choice may be limited by the necessity to use already available equipment, services, etc., There may be physical constraints placed on the design by a shortage of land on which to site the plant.
4. Technical services available.

   Some fermenters may require a large technical "back-up" from trained personnel; others may be more simple in operation. The availability of such trained personnel must be considered when choosing between these two types of design. This is of especial significance to the "third world countries".

A fermentation system can be operated in three possible modes: batch, semi-continuous and continuous. Which mode is selected will depend on the parameters outlined above, that is, substrate, desired product(s) etc., Continuous operation can offer considerable advantages over batch or semi-continuous operation. It is ideally suited to automation; being self-regulating and selfperpetuating it can be fitted into a

production line of continuous medium make-up, sterilization (if required), production, extraction and processing of products. Unproductive phases of batch cultivation, namely the lag periods after inoculation, harvesting, cleaning, refilling, sterilizing and reinoculation are avoided (Evans, 1965; Langlykke, 1970). Thus for a given output of product a smaller plant, than that required for batch cultivation, is necessary; alternatively the same working volume gives a bigger throughput. At the same time there should be a more consistent uniformity of product (Hough and Wase, 1966).

A further important advantage of continuous culture is that the growth rate is controlled by the supply of one freely chosen nutrient. The result of this choice of limiting nutrient can have dramatic effects upon the physiology of the organisms and hence on the character of the fermentation. As the availability of the limiting nutrient is controlled by the medium flow rate (dilution rate), any rate of growth less than a well-defined maximum value can be selected at will. The concentration of available limiting substrate will, therefore, be very low at all growth rates except those approaching the maximum; this allows the use of potentially toxic substrates, for example, phenol (Evans, 1965).

When using continuous cultivation techniques a stable condition, termed "steady state" will be achieved in the fermenter for a given set of parameteres, for example, pH, temperature, aeration rate (for aerobic fermentations) and substrate supply rate. If one of these parameters is changed, a new steady state will be established (Hough and Wase, 1966). The steady state will correspond to a transient stage found in the comparable batch fermentation. This means that microorganisms can be held in a selected physiological condition and enables greater product control than is possible with batch cultivation.

Trilli (1977) predicting costs in continuous fermentation demonstrated mathematically that for each particular continuous fermentation there is a critical dilution rate at which an abrupt change in the relationship between unit cost of product and fermentation time takes place. For values on one side of the critical value, the tendency of the unit cost is the opposite of that existing for values lying on the other side of the critical value. This means that the continuous fermentation has an economic advantage over the batch fermentation only at dilution rates lying on one side of the critical dilution rate. Which side this is depends on the nature of the particular fermentation process, that is on the relationship between specific production rate and dilution rate, while the magnitude of the critical dilution rate depends upon the economic, physical and biological parameters of the process.

Traditionally, most fermentations have utilised the batch mode of operation; the continuous mode has only been developed, on an industrial scale, more recently. Commercial fermentation systems available tend to be of the former type, requiring modification for continuous use and as capital investment in plant makes up a large proportion of the costs of a fermentation system, established plants are unlikely to be so modified unless three criteria are fulfilled:

firstly - process modifications will have to produce significant increases in product yield,

secondly - such modifications must involve a minimum of investment in new plant, and

thirdly - such modifications must be compatible with extisting production strategies.

When completely new plant is being selected, the greater yields for a given capital investment possible with continuous cultivation will probably dictate the choice of this option.

Continuous cultivation is particularly useful when large volumes of dilute substrate, for example cheese wheys, are available. The batch fermentation of these types of effluents would require large fermenter volumes and thus a much greater investment in equipment than is necessary for their continuous fermentation. Consequently a continuous cultivation system was selected for this project as being more appropriate for the substrates to be fermented.

The most common apparatus used in fermentations today is the mechanically agitated, fully-baffled fermenter provided with an open-blade turbine mixer - the conventional stirred tank reactor (Prokop and Votruba, 1976). The classic example of an STR is the chemostat, see Fig 3.

This basic design may be modified somewhat as, for example, in the Waldhof fermenter by the addition of a draught tube (Chapman, 1966). This type of STR is often used in fodder yeast production in Europe (Prokop and Sobotka, 1975). Various aspects of the design variables of the STR have been reviewed in the literature: Miller (1964); Sideman, Hortassu, and Fulton (1966); Schaftlein and Russell (1968); Solomons (1968); Reith (1970); Rowley and Bull (1973).

The addition of impellers to an aerated tank has two beneficial effects:

firstly - radial mixing is stimulated which tends to even out the void fraction distribution across the column cross section. Lowering the void fraction tends to reduce coalescence and tends to maintain a higher interfacial area for mass transfer (Radovich and Moissis, 1962),

secondly - the viscous sheer imported by the impellers breaks up large bubbles and reduces the bubble size distribution (Hatch, 1975).

These two effects lead to a greater rate of mass transfer of oxygen. As previously stated, the oxygen-transfer rate can be a critical design parameter for fermentation vessels. High oxygen-transfer rates are frequently required for the economical production of SCP (Serieys, Goma and Durand, 1978). British Petroleum have reported a required oxygen-transfer rate of 15g/litre hour (Orazem and Erickson, 1979). Study of oxygen-transfer requirements has led to modifications of internal reactor geometry. Thus many new reactors have recently become available, each of novel design but tailored for particular processes. Examples of these include the high oxygen-transfer rate reactors marketed by Vogelbusch (Schreier, 1974); the "Effigas" reactors distributed by Chemap (Pickett, Topiwala and Bazin, 1979), the ICI Pressure Cycle Fermenter (Gow, Littlehailes, Smith and Walter, 1975); The Miniloop Culture Vessel (Pirt, Panikov and Lee, (1979); The Multistage Vibrating Disc column with cocurrent gas-liquid flow (Tojo, Miyanami and Yano,(1974).

Schügerl, Lücke and Oels (1977) have compared data for oxygen-transfer in bubble columns (single-stage aerated tower fermenters) with published data for other fermentation systems. Prokop and Votruba (1976) have reviewed different fermenter configurations classifying them according to the mixing time of the vessel contents and the ratio of axial and radial flows within the vessel. There have been published many reports which compare the efficiency of different aeration schemes in stirred tank reactors, but these reports are difficult to interpret. Efficiency of aeration in a STR is affected by many different factors, such as the shape and relative proportions of the vessel, the aeration rate, impeller speed and air bubble size. The multiplicity of variables make it difficult to compare results obtained by different workers (Chapman, 1966).

In recent years there has been a realisation that stirred fermenters have disadvantages. Under viscous conditions, for example in mycelial fermentations, it is extremely difficult to achieve adequate mixing such that the broth is not depleted of oxygen before it is recirculated back to the impeller for reaeration. Furthermore, under these conditions (of viscosity) much of the energy applied to the broth (via the impeller) is dissipated as heat, thus resulting in lower oxygen transfer efficiencies (Cooney and Wang, 1971).

The need for cooling places severe economic penalties on a commercial plant, especially in tropical countries where the ambient temperature may be higher than the temperature maximum

for the process organism (Greenshields, 1978a). Heat removal in external cooling loops requires high pumping rates with consequent large energy demands, particularly if the process organism imposes a temperature shock constraint. These problems of STR's tend to increase with size (Gow, Littlehailes, Smith and Walter, 1975). Large STR's also require expensive seals and moving parts (Cocker and Greenshields, 1977). All these factors have led Hatch (1975) to conclude that when the nature of the fermentation is such that the design criteria require a minimum power input, then alternative designs to the STR must be sought. The most favoured of these involve air-lift principles in which air is introduced at or near the base of the fermentation vessel, providing the impetus for mixing as it rises. Thus the two operations of agitation and aeration are performed concurrently (Prokop et al, 1976). The design may include a draught tube (Hatch, 1975) or some arrangement of "downcomers and uprisers" for example the ICI pressure cycle fermenter (Gow et al, 1975). The original patent for an airlift fermenter was granted to Lefrancois, Mariller and Mejane (1955). Orazem et al (1979) have described a type of airlift fermenter where the vessel is a bubble column divided into two sections, gas being sparged into one section causing the dispersion density in that section to be lower than in the unsparged section. The resulting pressure difference causes liquid circulation. An upward liquid velocity is observed in the sparged section and a downward liquid velocity in the unsparged section. The downward liquid flow entrains some gas and therefore mass transfer can take place in all parts of

the fermenter. Further variants of the airlift fermenter are the split cylinder airlift tower (Belfield, 1976) which consists of a cylinder divided into two sections by a baffle, and the basin fermenter (Cooper and Silver, 1975; Laskin, 1977).

The simplest type of airlift fermenter is the bubble column. Bubble columns are popular in the chemical industry because of their versatility and economical advantages, that is, low investment costs due to their simple construction, and low variable costs of production due to low energy requirements of their operation which is maintained by fluid dynamical mixing and dispersion of the phases (Schügerl et al, 1977). All these advantages are also valid for the application of bubble columns in biotechnology.

The airlift fermenter is claimed to have advantages for bacterial fermentations of methanol and at the laboratory scale it has proved useful in such widely differing fields as fungal SCP production, itaconic acid production by Aspergillus terreus and even microbial polysaccharide production (Emery 1976).

The continuous tubular reactor (Continuous Tower Fermenter, CTF) and its various modifications described by Greenshields and Smith (1971, 1974) and Greenshields, Morris, Daunter, Alagaratnam and Imrie (1971) is a simple bubble column and has the advantages of low capital investment requirements and operating costs of this type of airlift fermenter. It has proved suitable for the cultivation of bacteria, yeasts and moulds (Cocker and Greenshields, 1975) and for metabolite production, for example acetic acid (Nerantzis, 1979). The CTF is

particularly suited to the fermentation of large volumes of dilute substrate (Pannell and Greenshields, 1976) and therefore was considered to be well suited to the fermentation of dairy effluents.

In summary, the aim of this project was the development of a continuous tower fermentation system for the production of a filamentous fungal SCP from a variety of dairy effluents.

2. Selection of Microorganism

2.1 Introduction

The microorganism selected for SCP production must be nonpathogenic and nontoxogenic and the products of its metabolism must be innocuous (Laskin, 1977). Certain species of microorganism have been consumed by Man for many years with no ill effects, for example Saccharomyces cerevisiae which is used for brewing and baking. This may have contributed to the early dominance of yeasts as SCP process organisms, especially when dairy wastes constitute the substrate. Many authors have described yeast fermentations of dairy effluents (Tewes and Gavel, 1953; Wasserman, Hopkins and Porges, 1958, 1958; Wasserman 1960 [a,b,c], 1961, 1962; Wasserman and Hampson, 1960; Wasserman et al, 1961 ; Tomisek et al, 1961; Amundson, 1966; Chapman, 1966; Atkin et al, 1967; Perzow, 1974; Anon, 1975; Delaney, Kennedy and Walley, 1975; Pace et al, 1975; Vananuvat et al, 1975 [a,b]; Terra, 1976; Burgess, 1977; Lane, 1977). Food yeasts are also produced in various parts of the world from raw materials other than dairy effluents, for example Candida yeast has been produced from such diverse substrates as Hydrocarbons (Sherwood, 1974; Paca and Gregr, 1977; Goma and Ribot, 1978) and cassava (Musenge, 1980). Other examples of substrates for food yeast production are citric acid wastes (Braun, Meyrath, Stuparek and Zerlauth, 1979), molasses and sugar-containing waste liquors from the wood pulp industry (Chapman, 1966).

Bacteria have been proposed as process organisms for SCP production (PAG, 1970) because they possess a number of advantages

over yeasts, for example higher growth rates and much higher protein contents. However, they also present the disadvantages of susceptibility to bacteriophage infection and higher nucleic acid content. Reddy et al (1976) have described the fermentation of cheese whey by Lactobacillus bulgaricus and reported that this process has been developed to pilot-plant-scale. This bacterium had previously been reported to ferment "waste lactose" from butter manufacture in association with "a mycoderm" (Olive, 1936). Bacterial SCP has been produced from other substrates, for example Pseudomanas fluorescens from poultry wastes (Shuler, Roberts, Mitchell, Kargi, Austic, Henry, Vashion and Seeley, 1979).

Gray, Och and El Seoud (1964) screened 175 isolates of the Fungi Imperfecti for protein production from crude glucose. They selected this group of filamentous fungi because of the inherent ability of many of them to grow very rapidly. In addition, this group of microorganisms can utilise many different types of substrate. They have been investigated for SCP production by Church, Nash and Brosz (1972) who report that these organisms are efficient in converting dissolved and suspended organic matter into a macroscopic, high protein,mycelium which can easily be harvested. These authors claim that the high protein quality of the Fungi Imperfecti may enable part or all of the costs of the waste treatment to be recovered from the fungal feed value.

Although many different species of filamentous fungi have been studied to determine their suitability for SCP production

the majority of work has been concentrated on members of four genera - Geotrichum (Fabel, 1949; Atkin et al, 1967); Hang, Splittstoesser and Landschoot, 1975; Kier, Allerman, Floto, Olsen and Sortkjaer, 1976; Olsen, Allerman and Kier, 1977; Kier, Olsen and Floto, 1978; Quinn and Marchant, 1978; Schneider, Anderson and Allerman, 1978); Aspergillus (Weaver, Heisler, Porges, McClennan, Treadway, Howerton and Cordon, 1953; Chastukin, Goncharova and Golubchina, 1957; North Star Research and Development Institute, 1970; Church et al, 1972; Smith et al, 1975; Soderquist, 1975; Worgan, 1976; Pannell, 1976; Spensley, 1977; Hang, 1978; Davies, 1978; Stockbridge, 1979); Fusarium Chastukin et al, 1957; Delaney et al, 1973; Smith et al, 1975; Worgan, 1976; Macris and Kokke, 1978); and Trichoderma (Church et al, 1972; Soderquist, 1975). Studies of SCP production by filamentous fungi in the CTF have been confined almost entirely to Aspergillus niger (Pannell, 1976; Cocker et al, 1977; Spensley, 1977; Stockbridge, 1979), although Sporotrichum thermophile (Anzoulatos, 1980) and a species of Penicillium (Greenshields, 1978[b]) have also been investigated.

The only mould fermentation of a dairy effluent in a CTF was performed by Pannell and Greenshields (1976) who cultivated A. niger on an effluent from Cadbury Schweppes Ltd., Chocolate Crumb Factory, Leominster, which contained both lactose and sucrose. The authors observed that while A. niger removed a large portion of the sucrose, it did not utilise the lactose (see section describing milk effluent isolations). The first priority of this project was, therefore, to select a species of filamentous fungus able to utilise lactose while growing in a CTF. This was achieved by a series of experiments; a large

number of filamentous fungi able to grow in surface culture with lactose as their sole carbon source were isolated. Ten of these species were selected for an experiment to compare their ability to grow in batch submerged culture with lactose as the sole carbon source, and finally four species were cultivated in a continuous Tower Fermenter in order to select the filamentous fungus most suited to SCP production from dairy effluents.

2.2 Isolation Experiments

Lactose utilizing fungi were isolated from two sources - (1) a milk effluent and (2) soil to which a synthetic milk effluent was added.

2.2.1 Milk Effluent Isolations

Materials

Samples were collected from various stages of the sewage treatment system at Cadbury's Chocolate Crumb Factory (Leominster). This plant produces a sweetened evaporated milk and thus the effluents generated during processing contain considerable amounts of both lactose and sucrose. Samples were stored at $4^{o}C$ for approximately 18 hours prior to analysis.

Sample points:
(i) raw effluent
(ii) activated sludge
(iii) intermediate humus sludge (effluent from the roughing trickle filter)

(iv) final humus sludge (effluent from the final trickle filter

(v) roughing trickle filter stones

(vi) final trickle filter stones

All dilutions were made in sterile distrilled water.

Methods

pH values of the liquid effluents were measured using a Pye Model 78 pH meter. Serial dilutions up to 10^{-5} of each dilution were innoculated on modified buffered yeast agar (MBYA) plates (appendix 1) and these were incubated at 25°C for seven days. Samples of the fungal "mat" on the trickle filter stones were innoculated on to MBYA plates and incubated as above. After incubation, representative isolates were subcultured, if necessary, and identified.

Results

For table of results see appendix 2.

The isolates appeared to fall into three main genera - Aspergillus, Trichoderma, Geotrichum - and there appeared to be a trend in genus distribution within the treatment system. For example, Trichoderma spp. were found throughout almost all the system but were not isolated from the raw effluent, whilst Aspergillus species were found in the raw effluent and intermediate humus sludge, but not at other stages of the system.

Few fungi were isolated from the filter effluents, (none from the final trickle filter effluent) probably because they remained attached to the fungal "mat" formed on the stones.

Becker and Shaw (1955) reported that if the habitat in sewage, more especially in trickle filters, predisposes fungi to vegetative growth with little or no production of "diseminules" then plating of liquid effluent samples may not be representative of the fungal species present and the relative species abundance at the various treatment stages. Bacteria and actinomycetes were isolated from all stages of the treatment system and may have affected the species of fungi growing on the plates. This may be via competition for nutrients, or the production of antagonistic substances.

The distribution of fungal genera may reflect the varying growth conditions prevalent throughout the system. For example: (i) temperature. The activated sludge is at 26 - 30^{o}C, with the raw effluent flowing in slightly warmer and there is progressive cooling as the effluent flows through the system (Pengilly, 1977).

(ii) pH. The pH of the effluent rises from approximately 5.0 initially to 8.0 at the end of the system. These two parameters probably influence fungal species distribution. Further experiments would be required to clarify the situation and since the aim of this experiment was to isolate lactose utilising fungi, such experiments would be beyond the scope of this project. Ten lactose-utilising moulds were isolated during this experiment. Some of these isolates grew slowly in surface culture and were, therefore, not considered further experimentation. Additionally, Aspergillus fumigatus, although one of the most frequently isolated species, is not acceptable for SCP production as it is known to be a causative agent of disease (Farmer's Lung,

Aspergillosis). Geotrichum spp. were eliminated from consideration because they have been observed to grow in submerged culture in short hyphal fragments (Olsen et al, 1977), and this morphology is not suited to the continuous Tower Fermenter (Stockbridge, 1978).

Thus, the following species isolated from a milk effluent treatment system were selected for further comparative studies (section 2.3).

Aspergillus terreus

Trichoderma hamatum

Trichoderma viride

Trichoderma harzianum

2.2.2 Soil isolations

Materials

Modified Buffered Yeast Agar (appendix 1)

Two 1 litre solutions of pasteurized milk (Unigate) in distilled water were prepared as 20% and 40% solutions yielding final milk solids concentrations of 2% and 4% respectively.

Methods

On Day 0, the milk solutions were each sprayed on to a 40 cm^2 area of exposed garden soil. A third plot was reserved for a control. On the following days after spraying, 0, 3, 7, 14, soil samples (selected randomly within each plot), were collected into sterile containers and were treated as follows:-

Five replicate soil crumb plates were prepared for each sample (average crumb size 0.002g) using three crumbs per plate.

Five replicate pour plates of a 10^{-2} dilution (in sterile distilled water) of each sample were prepared. Preliminary experiments revealed that this dilution produced a suitable amount of fungal growth. All plates were incubated at 25°C for seven days and stored at 4°C until examined.

All filamentous fungi were identified, the majority to species level and those isolated infrequently to generic level. The species were scored for presence or absence and the percentage frequency of isolation calculated; that is, presence on one replicate plate scored 20%; presence on all five plates scored 100%.

The experiment was performed twice, in December 1977, and February 1978. The ground was frozen and under snow for most of the latter month.

Results and Discussion.

For tables of results see appendix 2.

The graphs of various selected genera (appendix 3) show no regular pattern. No statistically significant changes in isolation frequencies as a result of the experimental treatment can be observed. Several factors may account for this:

(i) Insufficient effluent was added to elicit a detectable response.

(ii) Sampling times were unsuitable, for example, effects might have been apparent only between days 0 and 3, when no samples were collected.

(iii) Low soil temperatures could influence the growth of soil organisms and their isolation frequency.

(iv) Incubations were carried out at 25°C; organisms degrading the effluent in the soil at lower temperatures were probably unsuited to the culture conditions provided.

(v) The effluent utilisation may result from bacterial or other microorganism activities rather than from those of the filamentous fungi. No microorganisms other than filamentous fungi were identified.

It was possible to make a subjective selection from the identified fungal species of those which appeared to increase in isolation frequency after addition of a synthetic milk effluent and which appeared best able to utilise lactose as their sole carbon source. These species were:

Mucor hiemalis

Penicillium javanicum

Fusarium tabacinum

Fusarium nivale

Gliocladium roseum

Trichoderma viride

Trichoderma hamatum

Fusarium semitectum *

* This species did not appear to increase in isolation frequency, but was observed to grow rapidly in surface culture with lactose as the sole carbon source.

2.3 Batch Fermentations

Materials

Ten fungal species isolated as described in sections 2.2.1 and 2.2.2.

Microorganisms:

Penicillium javanicum

Gliocladium roseum

Mucor hiemalis

Fusarium nivale

Fusarium semitectum

Fusarium tabacinum

Trichoderma harzianum

Trichoderma hamatum

Trichoderma viride

Aspergillus terreus

Lactose concentration was determined with a Lactose/Galactose Assay Kit (Boehringher-Mannheim catalogue number 176303) using a Pye-Unicorn SP800 spectrophotometer.

Methods:

Modified Buffered Yeast broth (appendix 1) was dispensed in 20 cm^3 aliquots into 100 cm^3 flasks and sterilised.

Five replicate flasks per species were innoculated with $2x10^5$ spores and incubated in an orbital incubator (Gallenkamp Ltd.) at 27°C and 100 revolutions per minute for five days. The mycelium was harvested by filtration onto tared Whatman No. 1 (qualitative) filters and overnight drying; biomass production

was determined by weighing. Lactose concentration in the filtrate was measured. Means and standard deviations were calculated for biomass production and lactose utilisation.

Results and Discussion

A table of results is given in Appendix 4.

This experiment was designed to compare growth rates (as measured by biomass production) and lactose utilisation in submerged culture of the ten selected species. This would allow a more informed decision as to the filamentous fungus most applicable for SCP production in the CTF.

No one mould gave the highest figures for both parameters measured and therefore a compromise decision had to be made.

(i) F. tabacinum and F. semitectum used the greatest amounts of lactose but produced minimal amounts of mycelium and thus could be utilised in the treatment of dairy wastes when biomass production is not required. However, since this project is designed for the production of SCP from dairy wastes, these two species proved unsuitable.

(ii) F. nivale ranked second for biomass production, but only fifth for lactose utilisation and was, therefore, discarded.

(iii) The Trichoderma species tested performed well in both biomass production and lactose utilisation (T. hamatum third and sixth; T. harzianum fourth and tenth; T. viride sixth and eighth).

Unfortunately, some Trichoderma spp. have been reported to produce toxins (Turner, 1971) making them undesirable organisms for SCP production. Additionally, Trichoderma spp. spore easily and one was observed in a CTF to grow poorly, in a morphology which was unsuited to the turbulent conditions within the fermenter (Stockbridge, 1979). Trichoderma spp. were, therefore, rejected from consideration for testing in the CTF.

(iv) Gliocladium roseum performed eighth best for biomass production and seventh for lactose utilisation, producing a very small amount of biomass and as with the species above, was not studied further.

(v) The moulds selected for testing in the CTF as a result of this experiment were:

Penicillium javanicum - this isolate used the third largest amount of lactose and produced the greatest amount of biomass.

Aspergillus terreus - ranked fourth for lactose utilisation and fifth for biomass production.

Mucor hiemalis - ranked seventh for biomass production and ninth for lactose utilisation. Although this last isolate performed more poorly than did some of the other rejected species, it was included because it was the only Phycomycete species in the study. The differing structure (for example aseptate hyphae) and physiology of a Phycomycete species might be expected to cause some differences in behaviour in a continuous tower fermentation from that which has been observed for deuteromycete species and therefore could prove of interest for future studies in the CTF.

2.4 Preliminary Tower Fermentations

Materials

Microorganisms:

Penicillin javanicum

Mucor hiemalis

Aspergillus terreus

Aspergillus niger strain 38 (Source: University of Aston in Birmingham Fermentation Laboratory Culture Collection.)

The first three species were selected as described in section 2.3. A. niger (38) was included because a considerable amount of information about its behaviour in the CTF was available (Cocker, 1975; Pannell, 1976; Spensley, 1977; Stockbridge, 1979) including its behaviour when cultivated on an effluent containing lactose (Pannell et al, 1976).

Fermenters:

Two fermenters, one of one litre volume, the other five litres volume, were used for these fermentations.

The One Litre Fermenter (Fermenter A)

The one litre fermenter was constructed in glass by the University glassblowers (fig 4). It was a one-piece construction, with two integral water jackets for attemperation. Ports for the admission of air and medium were provided at the base. A port for fermenter contents sampling was positioned between the two water jackets. No provisions were made for thermometer, pH, or dissolved oxygen probes. Stockbridge (1979) has reported that a fermenter of this size will not produce reliable steady-state results, due to wall effects and that the intrusion of probes into

such a small space will seriously affect the operation of the fermenter.

No special air distribution system was employed. The outlet consisted of an inverted glass cone situated at the top of the fermenter, connected to which was a 5.0 mm internal diameter "swan-neck". The internal diameter of the fermenter was 5.0 cm and the height 60 cm, which resulted in a working volume of approximately 1.0 litre. Attemperation of the fermenter contents was achieved using a Churchill thermocirculator (Churchill Instruments Ltd., Perivale, Middlesex), which maintained the temperature to within $\pm 0.5^{o}C$ of the set temperature.

Medium was supplied to the fermenter by a Baron Yemm BYO 800 Peristaltic pump (Norris Industries, Rushton Ltd., Rushton, Northants) which operated on 2.0 mm internal diameter silicone rubber tubing.

Compressed air was fed from the laboratory air system via a pressure reducer and air/oil filter unit (Air Power Minett Ltd., Birmingham) to a second pressure reducer and oil filter (Spirax Monnier, Cheltenham, models MR1 and MF1 respectively), mounted on the fermenter framework. The air supply rate was monitored by a 2-25 L/min. flow meter (Gapmeter Ltd.,) which fed air to a Whatman Gamma 12 filter sterilizer, fitted with a 0.3μm filter (ex Gallenkamp Ltd.,) from which it was led by a plastic tube, to the roof of the laboratory and back down to the fermenter air inlet, thus protecting the air supply system from fermenter contents during any air failures.

Fig.4 Fermenter A The One Litre Fermenter

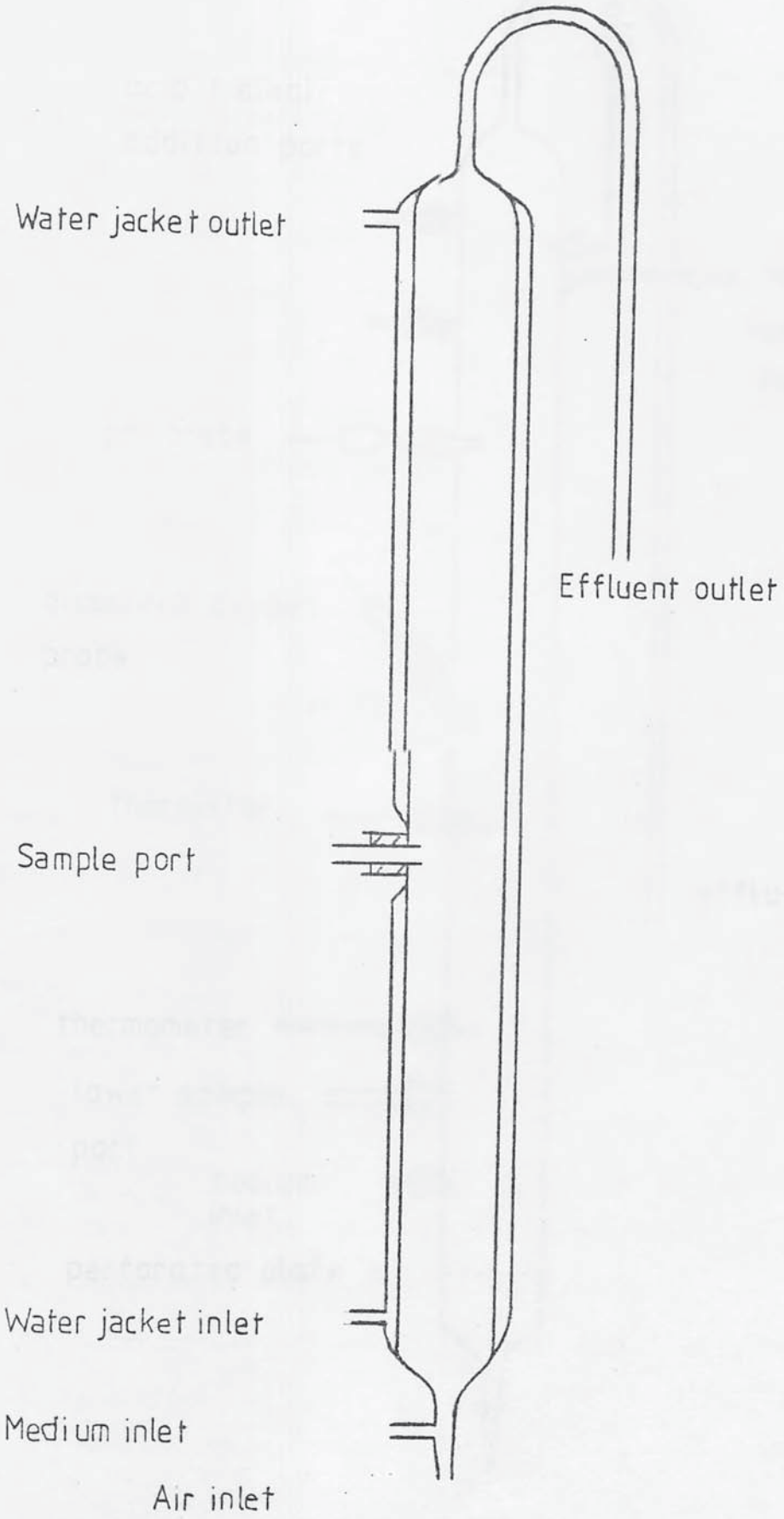
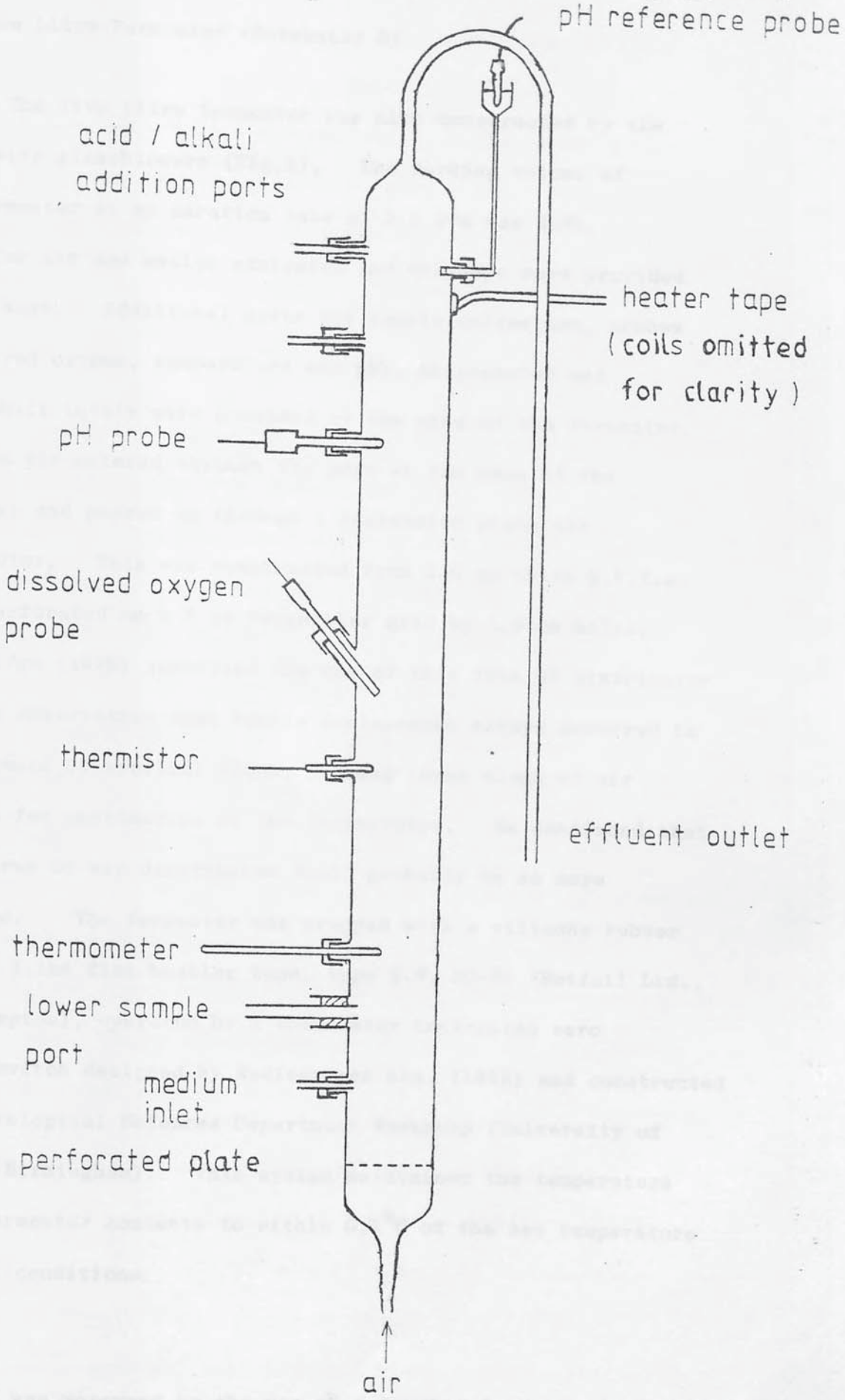


Fig 5 Fementer B The Five Litre Fermenter



The Five Litre Fermenter (Fermenter B)

The five litre fermenter was also constructed by the University glassblowers (Fig.5). The working volume of the fermenter at an aeration rate of 2.5 vvm was 3.8L. Ports for air and medium admission and drainage were provided at the base. Additional ports for sample collection, probes (dissolved oxygen, temperature and pH), thermometer and acid/alkali inlets were provided at the side of the fermenter. Filtered air entered through the port at the base of the fermenter and passed up through a perforated plate air distributor. This was constructed from 2.0 mm thick p.t.f.e. plate perforated on a 2 cm triangular grid by 1.5 mm holes. Stockbridge (1979) justified the use of this type of distributor with the observation that bubble coalescence always occurred in the presence of mycelial flocs, forming large slugs of air within a few centimetres of the distributor. He concluded that other forms of air distributor would probably be no more effective. The fermenter was wrapped with a silicone rubber sheathed 1.1kw flat heating tape, type G.W. 50-70 (Hotfoil Ltd., Wolverhampton), operated by a thermister controlled zero voltage switch designed by Radiospares Ltd. (1975) and constructed in the Biological Sciences Department Workshop (University of Aston in Birmingham). This system maintained the temperature of the fermenter contents to within 0.1^{o}C of the set temperature under all conditions.

pH was measured by the use of a toughened, steam sterilisable probe in conjunction with a remotely positioned reference

electrode (both electrodes ex E.I.L., Richmond, Surrey) and a pH meter controller (E.I.L. model 918). pH control solutions were pumped to the fermenter by Delta peristaltic pumps (Watson-Marlow Ltd., Falmouth, Cornwall). Control was maintained to within $\pm$0.1 pH units of the set value. Media were made up with tap water and are summarised in Appendix 1.

Methods

Lactose was determined by the phenol-sulphuric acid method of Dubois, Gilles, Hamilton, Rebers and Smith (1956). Burgess (1977) measured lactose concentration by this method in his investigation of the fermentation of a milk effluent in a Tower Fermenter by S. fragilis. Liquid samples were volumetrically diluted to give lactose concentrations of between 20.0 and 100 $\mu g cm^{-3}$. 1.0 cm^3 aliquots of the diluted samples were added to 1.0 cm^3 of 5% analar phenol in a pyrex test tube followed by the addition of 5.0 cm^3 of concentrated analar sulphuric acid. The solution was mixed and left standing for a minimum of 20 minutes to allow the colour to develop. Colour was then measured in an EEL spectra (Evans Electroselenium Ltd., Halstead, Essex) spectrophotometer set at 488 nm, against a lactose (analar) standard.

Ammonium nitrogen was determined by a distillation procedure. 5.0 cm^3 of sample were run into a Markham still, followed by 5.0 cm^3 of 400gl^{-1} sodium hydroxide. The first 20 cm^3 of distillate were collected into 10.0 cm^3 of a saturated boric acid solution and this mixture was backtitrated against 0.01M HCl, using Tashiro's indicator

($2.0g.l^{-1}$ methyl red, plus $1.0g,l^{-1}$ methylene blue in absolute ethanol) to determine the endpoint. The nitrogen concentration was calculated from the following equation:

$$N = 0.028V$$

where N = Nitrogen concentration in $g.l^{-1}$

V = Volume of titre in cm^3

Fermentation "start-up", unless otherwise specified, was by the following procedure. The fermenter was sterilised by steaming for 24 hours at atmospheric pressure. A small amount of steam was allowed to escape from all ports in order to avoid the formation of air pockets which might have prevented complete sterilisation. The medium inlet tubing and air filters were autoclaved at 103.5 kilopascals for 15 minutes then aseptically connected to the fermenter. Sterile air was passed into the fermenter, together with steam which was then slowly turned off. The fermenter was allowed to cool before filling with sterile media, which was then brought up to the operating temperature. A spore suspension was aseptically introduced into the fermenter and the mould allowed to grow batchwise (that is, with no medium feed) for approximately 24 hours, or until it had grown to the required concentration, after which the medium feed was started and the aeration rate increased to the desired level.

Samples of the fermenter contents were taken in the following manner:

One litre samples of fermenter effluent were collected and filtered through tared Whatman No. 1 filter paper. The resultant solids were dried at $105^{o}C$ to constant weight, thus enabling the effluent biomass concentration (X_E) to be

determined. The time taken to collect the sample was used to calculate the dilution rate (D).

After a 20 cm^3 discard, 100 cm^3 samples were taken from the fermenter contents sampling port. Stockbridge (1979) has reported that the faster such a sample is taken, the further into the fermenter the effective sampling area extends and therefore samples were collected as rapidly as possible. These were filtered, dried and weighed as described above to determine the fermenter biomass concentration (X_F). A 30 cm^3 aliquot of the filtrate was collected into a sterile container and used for lactose and nitrogen analysis. Several investigators (Pannell, 1976; Spensley, 1977); Stockbridge, 1979) have reported that the liquid portions of the fermenter contents and the effluent were identical under all conditions used. Thus only the fermenter contents liquid (fermenter broth) was analysed.

Morphology samples comprising approximately 25 cm^3 of the fermenter contents mixed with 5 cm^3 of formalin were stored in glass universal containers until examination.

Medium samples were collected from the medium reservoir into sterile universal containers. All liquid samples were analysed immediately or stored at 4°C for a maximum of 24 hours before analysis.

Formulae for the calculation of fermentation parameters used to describe fermentations presented in Chapters 2, 3 and 4 are summarised below:

Dilution Rate (D, h^{-1}) $= \frac{F}{W}$

Growth Rate (μ, h^{-1}) $= \frac{X_E . D}{X_F}$

where F = flow rate in $l.h^{-1}$.

W = working volume of the fermenter in l

X_E = effluent biomass concnetration in $g.l^{-1}$

X_F = fermenter biomass concentration in $g.l^{-1}$

Substrate Utilisation ($\triangle A_{1-7}$, $g.l^{-1}$) = $B_2 - B$,

where B_2 = medium concentration in $g.l^{-1}$

B_1 = fermenter broth concentration in $g.l^{-1}$

$\triangle A_1 = \triangle S$ = lactose utilisation

$\triangle A_2 = \triangle N$ = nitrogen utilisation

$\triangle A_3 = \triangle P$ = phosphate utilisation

$\triangle A_4 = \triangle Na$ = sodium ion utilisation

$\triangle A_5 = \triangle Mg$ = magnesium ion utilisation

$\triangle A_6 = \triangle Ca$ = calcium ion utilisation

$\triangle A_7 = \triangle K$ = potassium ion utilisation

Substrate Utilisation Rate (dA_{1-7}, $g.h^{-1}$) = $\triangle A_{1-7}.F$

where $\triangle A_{1-7}$ = substrate utilisation in $g.l^{-1}$

F = flow rate in $l.h^{-1}$

Biomass Production Rate ($d X_E$, $g.h^{-1}$) = $X_E.F$

where X_E = effluent biomass concentration in $g.l^{-1}$

F = flow rate in $l.h^{-1}$

Productivity (Qs, $g.g^{-1}h^{-1}$) = $\dfrac{X_E D}{\triangle S}$

where X_E = effluent biomass concentration in $g.l^{-1}$

D = dilution rate in h^{-1}

$\triangle S$ = lactose utilisation in $g.l^{-1}$

Results

A. Mucor hiemalis

Experiment 1.

Fermenter A

Medium : 1

Temperature : 30°C

D : 0.09 h^{-1}

Air : 1.5 v.v.m

The mould grew slowly in the form of loose flocs, but within two days of start-up had started "washing-out". That is, the growth rate was insufficient to compensate for the flocs leaving the fermenter in the overflow. By day four (after start-up), the mould had completely washed-out of the fermenter.

B. Aspergillus terreus

Experiment 2.

Fermenter A

Medium : initially 3, thereafter see experimental details.

Temperature : initially 30°C, thereafter see experimental details.

D : 0.1 h^{-1}

Air : 1.5 v.v.m

On day four, the medium was altered by the addition of extra salts (medium 4) because the mould was growing poorly - the tower contained few flocs and these exhibited an inhibited morphology. Cocker (1975) has classified flocs on the basis of morphology, and reported that growth in the form of hard smooth pellets is symptomatic of an inhibited fermentation. Within 24 hours the fermentation improved, the mould concentration was observed to have

increased, although the floc morphology was still very inhibited - the flocs were large with a diameter of approximately 0.7 cm. On day seven, the temperature was lowered to 25°C, and the medium concentrations of ammonium sulphate and magnesium sulphate raised (medium 5). By day nine, the fermentation appeared to be much improved, although the flocs were small and still somewhat inhibited. The medium pH was 5.35, the effluent pH 2.65, the mould was using 5.0 $g.l^{-1}$ of lactose (ΔS) and producing 2.1 $g.l^{-1}$ of biomass (X_E).

At this point, the fermentation was disrupted by compresser failure, requiring four days to recover, however, by day fourteen, X_E was 2.23 $g.l^{-1}$ and ΔS was 4.2 $g.l^{-1}$.

On day fifteen the medium was changed to a synthetic milk effluent (medium 6) whose lactose content was determined to be 8.2 $g.l^{-1}$. Fermentation parameters on day sixteen were as follows: medium pH = 6.6; effluent pH = 4.2; X_E = 11.25 $g.l^{-1}$; ΔS = 7.6 $g.l^{-1}$. This X_E figure was high and probably reflected the presence of filtrable milk solids trapped within the fungal mycelium. From this point bacteria and yeasts were observed to be present in the fermentation broth and on day twenty-two the mould began washing out.

Aspergillus terreus

Experiment 3

Fermenter A

Medium : initially 5, thereafter see experimental details

Temperature : 25°C

D : 0.1 h^{-1}, then 0.5 h^{-1}

Air : 1.5 v.v.m, then 2.0 v.v.m

The mould grew in the same morphology as in the initial stages of Experiment 2 (that is, in an inhibited morphological form) and therefore the yeast extract concentration in the medium was increased (medium 7). This did not result in an improvement, so the aeration rate was increased to 2 v.v.m and the dilution rate to 0.5 h^{-1}.

On day eighteen, fermentation parameters were as follows: medium pH = 4.7; effluent pH = 3.4; $X_E = 0.54\ g.l^{-1}$; $\Delta S = 2.5\ g.l^{-1}$ From day twenty dried milk was used as the lactose source for the medium (medium 8) but this proved unsuitable for the growth of A. terreus - overnight the mould washed out.

Aspergillus terreus

Experiment 4

Fermenter B

Medium : initially 12, thereafter see experimental details

Temperature : 30°C during start-up, 25°C after germination.

D : 0.2 h^{-1}

Air : 1.5 v.v.m

The mould grew well and by day thirteen the fermentation parameters were as follows: $X_E = 1.24\ g.l^{-1}$; $X_F = 5.82\ g.l^{-1}$; $\Delta S = 2.46\ g.l^{-1}$; $\Delta N = 0.076\ g.l^{-1}$; $\mu = 0.041\ h^{-1}$.

Over the following nine days, the medium was altered by the gradual substitution of pasteurised whole milk for lactose. The concentration of added lactose was reduced by 1.0 $g.l^{-1}$ per day and a volume of milk (Unigate) containing the equivalent amount of lactose was added. On day twenty-three, the medium carbohydrate was provided wholely by milk, with no addition of lactose. At this

stage, the mould appeared to be growing less well than earlier in the experiment, that is, before the medium substitution with milk. Fermentation parameters were as follows:

X_E = 2.02 g.l^{-1}; X_F = 17.29 g.l^{-1}; ΔS = 3.31 g.l^{-1};

effluent pH <3.0.

This fermenter mould concentration (X_F) was artificially high and as in Experiment 2 reflected the high concentration of milk solids within the fermenter where the pH was sufficiently acid to cause coagulation of most of the milk proteins.

Over the next three days, the fermentation became increasingly inhibited, the fermenter contained a high concentration of yeasts and few mould flocs and consequently the experiment was halted.

C. Aspergillus niger

Experiment 5 A. niger strain 38

Fermenter B

Medium : initially 9, thereafter see experimental details

Temperature : 30^{o}C

D : initially 0.2h^{-1}, thereafter see experimental details

Air : 2.0 v.v.m

The fermentation was started with a sucrose medium because the innoculum consisted of biomass from a fermenter being operated with a sucrose medium by P J Stockbridge. The mould continued to grow well, exhibiting a loose "cotton-wool" type of morphology and on day three, when it was changed to a lactose medium, (medium 10), X_E was 3.13 g.l^{-1}. Concurrently, D was lowered to 0.1h^{-1}. By day five, the mould morphology had changed to a "stringy form" and

little biomass was present in the effluent. The dilution rate was increased to $0.2h^{-1}$, and the medium lactose concentration was reduced (medium 11, sterilized). On day seven fermentation parameters were as follows:

X_E = 0.08 $g.l^{-1}$; ΔS = 1.40 $g.l^{-1}$; medium pH = 5.5;

effluent pH = 4.0.

On successive days, the mould grew less well and fermentation parameters for days eight, nine and ten are given below:

Day	X_E, $g.l^{-1}$	X_F, $g.l^{-1}$	ΔS, $g.l^{-1}$	ΔN, $g.l^{-1}$	μ, h^{-1}
8	0.36	6.52	1.20	0.010	0.360
9	0.18	8.91	1.88	-	0.003
10	0.14	10.98	3.99	0.056	0.002

The growth rate diminished steadily and on day eleven biomass production had effectively ceased. The extremely low figures for μ as compared with previous findings for this strain of A. niger (Stockbridge, 1979), reflect the great difference between X_E and X_F. The mould appeared to be retained in the tower, possibly because of two factors:

(i) the mould grew in a dense, heavy form

(ii) fermenter B has a longer than usual "settling zone" at the top (see Fig 5).

Aspergillus niger strain 38L

Experiment 6

Organism : Aspergillus niger strain 38 was subcultured for several generations on MBYA medium (appendix 1) in order to select a strain better able to utilize lactose. The resulting strain was designated A niger strain 38L.

Fermenter B

Medium : 11

Temperature : 30°C

D : 0.20h^{-1}

Air : 1.5 v.v.m

During the first three dyas, the mould grew in large (almost 1.0 cm in diameter) flocs which had tight central zones but loose hairy outer zones. Fragments broken off from these flocs grew in a more loose "cotton-wool" morphology (that exhibited by A. niger grown on sucrose). By day five this loose form was the only morphological type present in the tower. Results of analyses performed on the tower contents and effluents on days seven, eight and nine are summarised below:

Day	X_E,g.l-1	X_F,g.l^{-1}	ΔS,g.l^{-1}	ΔN,g.l^{-1}	μ,h^{-1}
7	0.25	2.92	0.775	0.007	0.020
8	0.46	3.26	0.520	0.007	0.030
9	0.20	5.20	1.110	0.027	0.007

By day nine, the morphology was changing to the "stringy" form described in experiment 5 and X_E was very small and the fermentation was stopped.

Aspergillus niger strain 38L

Experiment 7

Fermenter B

Medium : 12

Temperature : 30°C

D : 0.4h^{-1}

Air : 1.5 v.v.m

For the first seven days, the fermentation proceeded well, although ΔS was only $1.0 g.l^{-1}$ and X_E only $0.1 g.l^{-1}$, the mould was growing in a filamentous morphology. On day eight, the morphology started changing into the more inhibited (stringy) form observed in Experiments 5 and 6. Subsequently, the fermenter became infected with Trichoderma viride and the experiment was stopped.

D. Penicillium javanicum

Experiment 8 P. javanicum

Fermenter A

Medium : initially 1, thereafter see experimental details

Temperature : $30^{o}C$

D : initially $0.09 h^{-1}$, thereafter see experimental details

Air : 1.5 v.v.m

Initially the mould grew in "tight" flocs, indicating that the fermentation was inhibited by some factor(s). On day three D was increased to $0.3\ h^{-1}$ and hence forth the flocs appeared to be less "tight" and the fermenter mould concentration to be higher. On day five fermentation parameters were as follows:

$X_E = 1.16\ g.l^{-1}$; medium pH = 5.9; effluent pH = 2.75.

The mould continued to grow well in an uninhibited morphology and on day nine the experiment was halted.

Penicillium javanicum

Experiment 9

Fermenter B

Medium : 12

Temperature : $30^{o}C$

D : $0.4\ h^{-1}$

Air : 1.5 v.v.m

The mould grew well, generating the data presented in Table 10 and after fifteen days, the experiment was halted.

Table 10

Data from Chapter 2 Experiment 9

Day	D,h^{-1}	X_E,$g.l^{-1}$	X_F,$g.l^{-1}$	ΔS,$g.l^{-1}$	ΔN,$g.l^{-1}$	μ,h^{-1}
7	0.406	0.996	3.872	1.930	0.067	0.104
8	0.417	1.036	4.645	2.625	0.138	0.093
9	0.465	1.551	4.738	3.560	0.043	0.152
10	0.400	1.017	4.638	2.000	0.077	0.087
11	0.472	0.943	4.443	2.025	0.069	0.100
12	0.397	0.574	7.967	2.280	0.098	0.029
13	0.397	1.009	4.120	1.850	0.062	0.097
14	0.398	0.337	3.466	1.600	0.049	0.039
15	0.304	0.775	3.064	1.500	0.352	0.077

The mould was dispatched to the Commonwealth Mycological Institute (Kew, London) who confirmed its identification as a strain of Penicullium javanicum van Beyma.

Discussion

Four mould species were cultivated in the CTF to determine which was the most suitable for SCP production from dairy effluents. The first fungus tested was Mucor hiemalis which is a common soil isolate (Gilman, 1959) but which proved completely unsuited to the growth conditions provided within the fermenter, washing out within four days of start-up. This wash-out was unlikely to have occurred as a result of a nutrient deficiency since the medium provided was similar to that which supported the growth of M. hiemalis in both surface and batch-submerged culture (sections 2.2 and 2.3), merely differing in the substitution of the sodium salt for potassium dihydrogen orthophosphate, and a slightly reduced yeast extract concentration. Similarly it seems unlikely that a difference of $3^{o}C$ in temperature between the batch fermentation (section 2.3) and the CTF would have such a drastic effect on the metabolism of M. hiemalis as to prevent its growth in the fermenter.

The second mould species tested Aspergillus terreus - proved much more amenable to growth in the CTF, as might have been expected since it has previously been used for itaconic acid production in submerged culture (Emery, 1976). Once a suitable medium composition and temperature had been determined, the mould grew well in the fermenter, utilising more than $2.0 g l^{-1}$ lactose, and producing more than $1.0 g.l^{-1}$ biomass. However, A. terreus would not grow on either dried milk/salts or whole milk/salts media containing the same levels of lactose or minerals; the mould appeared to be inhibited by the addition of whole milk. There

are two possible reasons for this:

(i) the lack of an essential nutrient. This seems improbable, as the "milk medium" contained all the constituents of the "lactose medium", plus additional nutrients from the milk,

(ii) the presence of some inhibitory factor(s) in the milk. These substances might have been normal milk constituents, or may have been formed in the medium as a result of the growth of contaminating microorganisms. Fresh medium was prepared every 24 hours, but during the last 5 to 8 hours of each period a noticeable "souring" occurred and there was microscopic evidence of yeast growth within the medium. Moreover, the pH value of the "milk medium" was somewhat higher than that of the "lactose medium", resulting in a higher value for the fermenter pH. This may have encouraged yeast infection of the fermenter during the later stages of the fermentation. Several authors have reported that the acid conditions within the tower fermenter restrict the growth of other organisms (Pannell, 1976; Stockbridge, 1979). These yeasts may have interfered with the growth of A. terreus either directly by competing for nutrients, or indirectly by the elaboration of inhibitory substances. Although competition for nutrients may have taken place, it was not likely to have seriously affected the growth of A. terreus since relatively large amounts of the carbon and nitrogen sources remained in the effluent, but the production of inhibitory substances remains a possible explanation. Further experiments with A. terreus were not advisable because this mould is known to produce a toxin, patulin (Moss, 1977).

Aspergillus niger, as previously stated, was included in this set of experiments because a great deal of information about its

behaviour in the CTF was available, including its use to ferment a dairy effluent (Pannell et al, 1976). In the latter experiments it was observed that while A. niger removed a large amount of the BOD of the effluent (sweetened condensed milk effluent), it appeared to be preferentially utilising the sucrose, and not lactose (Pannell, 1977). This was not unexpected since the lac - operon is repressed in the presence of more easily metabolised sugars such as sucrose (Kobayashi and Suzuki, 1972).

In these experiments, three attempts were made to cultivate A. niger in the CTF with lactose as the sole carbon source (Experiments 5, 6, and 7). It was not possible to continue the fermentation for more than ten days because by this stage the mould exhibited an inhibited morphology (described as "stringy"). Once A. niger was in this state, any infecting organism able to grow even moderately well in the prevailing conditions could take over the fermentation, as happened with T. viride in Experiment 7. A niger was, therefore, not suited to the fermentation of dairy effluents, where the major objective is the utilisation of lactose.

The isolate which performed best of the four moulds tested in the CTF was Penicillum javanicum. This is clearly demonstrated in Table 11.

Table 11

Comparison of Performance in the CTF of four moulds

	$dS\ g.h^{-1}$	$dX_E\ g.h^{-1}$	Data from
P. javanicum	5.138	1.748	Exp. 9
A. terreus	1.873	0.942	Exp. 4
A. niger	1.064	0.517	Exp. 5
A. terreus	1.025	0.270	Exp. 3
A. niger	0.589	0.190	Exp. 6
M. hiemalis	0.0	0.0	Exp. 1

The data in table 10 have been presented in the form of "rates" (i.e. grams per hour) in order to compensate for the different dilution rates used in the experiments. P. javanicum utilised more than twice the amount of lactose and produced almost twice the biomass than did its nearest rival, A. terreus and was therefore, better suited to the conditions provided within the CTF. Thus P. javanicum was the best filamentous fungus for cultivation in the CTF to produce SCP from dairy effluents with the proviso that it is shown to be a non-producer of antibiotics or microbial toxins. The Penicillia as a group are important industrially for the production of antibiotics, but these substances are not desirable components of products destined for animal or human food. Until recently antibiotics were routinely incorporated into commercial pig and poultry diets in the belief that this would lead to improved yields. The practice was discontinued however, after it was discovered that the incidence of antibiotic-resistant bacteria in both the animals and farmworkers handling them had increased.

(Report of the Joint Committee, 1969). It was necessary, therefore, to ascertain whether P. javanicum produces any antimicrobial substances when cultivated in the CTF (see section 2.5).

2.5 Antibiotic Testing

Materials

Bacillus subtilis spores suspended in alcohol at a concentration of 2×10^7 spores per cm^3 (supplied by Mr J D Brown, Microbiology Department, Birmingham General Hospital). This strain is sensitive to all antibiotics tested against it and is used as an indicator bacterium by the Hospital laboratories.

Nutrient Agar (Oxoid Hd) prepared as in the manufacturer's instructions, autoclaved at 103.5 kilopascals for 15 minutes, then held at $44^{\circ}C$ until required.

Effluent from Exp. 3.4.1.1. filtered through Whatman No. 1 filter paper.

Liquid medium from Exp. 3.4.1.1.

Methods

A suspension containing 1×10^5 spores per cm^3 of B. subtilis in nutrient agar was dispensed in 15 cm^3 amounts in sterile petri plates. Three wells were cut in the agar of each plate using an alcohol flamed No. 3 cork borer.

0.1 cm^3 amounts of filtered effluent were dispensed into each well on 5 plates (test plates) while a further 5 plates

received the same quantity of medium (control plates). After the liquid had been absorbed by the agar, the plates were incubated for three days at 37°C.

After incubation, each plate was examined to determine whether any inhibition of the growth of B. subtilis (as demonstrated by a clear zone in the bacterial lawn around each well) had occurred.

Results

All test and control plates showed no inhibition of growth of B. subtilis. The bacterial lawn reached the edge of each well.

Discussion

Within the limits of the experimental technique, this experiment demonstrated that P. javanicum does not produce any antibiotic substances when grown in submerged culture in a CTF. The control plates containing the fermentation medium were included to determine whether any antibacterial action resulted from a medium constituent or from the growth of P. javanicum. Since no inhibition occurred, there was no necessity for the control plates.

Although no antibiotic production was detected in this experiment, this does not prove that P. javanicum cannot produce such substances. The experiment was, therefore, repeated at regular intervals throughout the duration of this work and at all times there was no indication of a positive result. Thus P. javanicum when cultivated in the CTF did not produce any detectable antibiotic substances.

Experiments to determine whether this strain of P. javanicum produces any mycotoxins were not performed because the necessary facilities were not available, but there is no evidence in the literature to suggest that P. javanicum is a producer of toxins. Consequently, this organism was selected for SCP production from dairy effluents in a CTF.

2.6 Penicillium javanicum

Description

Lockwood, Ward, May, Herrick and O'Niell (1934) presented the following description of P. javanicum van Beijma;

> "Colonies on cornmeal agar colourless, mycelium sparse, covered with brownish yellow perithecia after three days. Conidia very sparse. On wort agar mycelium abundant, at first white, later mouse grey. Perithecia and conidia sparse. Mycelium spectate, hyaline, 2 - 5µm in diameter. Conidiophores 10 - 250µm, usually consisting only of a single verticil of 3 - 5 sterigmata, occasionally with a side branch or only a single sterigma. Sterigmata linear, tapering evenly to long acuminate points, 2.3-3. 5 x 7-12µm(Fig 6A). Conidia smooth, hyaline or straw colour in mass, subspherical to elliptical, 1.5-2 x 2-4µm (Fig 6B) Perithecia spherical or nearly so, whitish to brownish straw colour, 100-200µm in diameter (Fig 6F). Asci subspherical, oval or occasionally flask-shaped, 7-12 x 10µm; 4-8, usually 6-8 spores (Fig 6C,D). Ascospores elliptical to spherical, minutely echinulate, hyaline, without furrow or frills, 2.4-4 x 2.514µm (Fig 6E)".

Fig 6

Penicillium javanicum van Beijma

Legend

A penicilli x 1800

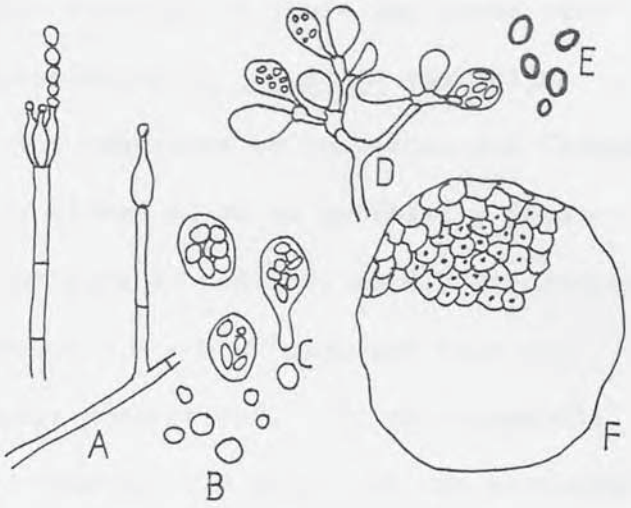
B conidia x 1800

C asci x 1800

D asci x 1800

E aerospores x 1800

F perithecium x 400



A more recent description of the mould has been reported by Pitt (1979), who stated that Eupenicillium javanicum (of which P. javanicum is the type isolate) is "probably the most abundant of all Eupenicillium species, and has a worldwide distribution as a soil fungus". It has previously been isolated from soil in Japan, Nigeria, Brazil, India, Nicaragua and Australia.

Penicillium javanicum was investigated in the 1930's for the purposes of fat production. Ward, Lockwood, May and Herrick (1935) found that the mycelium of P. javanicum van Beijma could contain as much as 41.5% fat, depending on culture conditions. This fat was composed of palmitic, stearic tetracosanic oleic and α and β - linoleic acids, in addition to glycerol and a small quantity of unsaponifiable matter (Ward and Jamieson, 1934).

Little work, as judged by the literature, appears to have been performed with this mould since that period. The University

of Aston in Birmingham Library undertook a "Medline" computer search of the literature since 1960, using the key words "Penicillium javanicum, toxicity, pathogenicity and antibiotic production" but no references relating to these key words were discovered. One report associating P. javanicum van Beijma with antibiotic production was published by Mukherjee and Chaudhuri (1975), who investigated the effect of pH on antibiotic production. However, in their report they give no results, merely concluding that medium pH within the range 4.5 - 9.0 "does not have any significant role on antibiotic production". It is impossible to determine from this paper whether the mould was (a) producing no antimicrobial substances, or (b) the mould was producing some antimicrobial substances but that production was not affected by medium pH.