THE CONTINUOUS FERMENTATION OF VINEGAR IN TOWER FERMENTER

by

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B.Sc.

A thesis submitted to the University of Aston in Birmingham in partial fulfilment for the award of the Degree of Doctor of Philosophy.

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A continuous Tower fermentation system has been operated for the production of vinegar. Five different raw materials were investigated. These were: cocoa 'sweatings', a substrate derived from the cocoa fermentation 'sweat' boxes and is a highly polluting waste in the country of origin; beet molasses, a by-product of sugar industry; malt charging wort; pure ethanol, and wine.

Batch and continuous modes of fermentations were studied. The substrates were tested under different conditions of temperature and aeration rate. The influence of the medium total acidity on the productivity and specific productivity, was measured.

The equation $K = a\mu + b$ was found to be valid for all substrates tested (K is the specific product formation rate, μ is the specific growth rate and a, b experimental parameters).

The Tower fermenter proved to be an efficient reactor for the production of vinegar. Pilot and production scale fermentation systems were developed for the production of cider vinegar and are now commercially viable. The future of the Tower fermenter as a reactor for the production of other metabolites was also considered.

Key words: Continuous, Fermentation, Vinegar, Tower fermenter.

To

My Parents

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OBJECTIVES

One of the major problems of the World is that of starvation or poor nutrition. Its cause is not only the lack of food, but also the lack of knowledge of food preservation and storage.

An answer to the problem, apart from that of finding new sources of food could be given by the improvement of food storage and preservation technology.

Statistics show that vinegar already plays an important role in food storage and preservation, in industrialized countries. Greenshields (1978), in his world-wide survey, indicates that the world usage of vinegar is approximately 8,000 million litres per annum. He has pointed out that the use of vinegar world-wide will increase, at least by a factor of two not including the natural increase due to population.

One of the objectives of this study was to examine different raw materials for the production of vinegar, and to determine the kinetic parameters which enable us to evaluate the production of acetic acid (vinegar).

The use of the Tower fermenter was chosen because of its comparatively low technology as a fermentation system and its easy maintenance. The use of the continuous Tower fermenter for the production of vinegar in Third World countries could prove a viable proposition. The use of old systems with their low productivity or the expensive construction and maintenance of more sophisticated generators is not the ideal for village-level technology. The knowledge of vinegar production for food preservation and food flavour can help the solution of poor nutrition especially with countries who do not either make vinegar or even know how to use it. Another objective of this study was a theoretical consideration of the continuous Tower fermentation for product formation kinetics problems for future bacterial fermentations.

SECTION 1

Section 1. INTRODUCTION

1.1. DEFINITION AND REGULATIONS

According to the joint U.N. Food and Agriculture Organisation and the World Health Organisation Food standards programme (1974), vinegar is a liquid produced from a suitable raw material containing starch or sugar or starch and sugar by the process of double fermentation, alcoholic and acetous, and which contains a specified amount of acetic acid.

The alcoholic fermentation is carried out by using various strains of Saccharomyces spp. such as: S. cerevisiae, S. carlsbergensis, S. diastaticus and S. ellipsoideus.

The acetous fermentation is the oxidation of alcohol to acetic acid under aerobic conditions by acetic acid bacteria such as: Species of the genus Acetobacter Beijerinck and Acetobacter Asai (Bergey's Manual of Determinative Bacteriology, 8th edition.

1.1.1. Categories of vinegar

From the technological point of view, there are three categories of vinegar: (FAO/WHO, 1974):

1. Natural fermented vinegar
2. Acetified distilled vinegar
3. Artificial vinegar

The first type contains fermentation by-products from alcoholic and acetous fermentations. The second type contains only those from the distillation and acetous fermentation and artificial vinegar contains none of them.

In the first two categories belong the following types of vinegar. According to the origin of the initial mash these are named:

1. Malt vinegar from barley malt or cereals whose starch has been hydrolysed by amylase
2. Sugar vinegar from sugar syrup, molasses or refined syrup.
3. Cider vinegar or apple vinegar from apple juice.
4. Wine vinegar or grape vinegar from grape juice.
5. Glucose vinegar from a solution of glucose.
6. Spirit vinegar or distilled vinegar from dilute distilled alcohol.

Other sources of vinegar are: honey, whey, palm juice, coconut juice, cocoa sweatings, grape juice, rice, pineapple, etc.

1.1.2. Restrictive regulations for vinegar in production

The following regulations are valid for one or more countries (FAO/WHO, 1974):

1. Vinegar must not be produced from raw materials which by regulation are unfit for that purpose.
2. Vinegar must not be prepared with mineral acids.
3. Vinegar must not be manufactured or stored in metallic containers which are affected by acetic acid.
4. Flavouring substances to be added must be destined exclusively for this purpose.
5. Organic acids other than acetic acid should be derived from the raw material in the appropriate proportions.
6. Addition of antiseptics is prohibited.

7. Addition of mineral substances other than salt is not allowed.

8. Vinegar may not contain "mother of vinegar" sediments or vinegar eels.

9. Other regulations are related to the composition of vinegars to be defined as a certain variety.

1.2. HISTORY OF VINEGAR

Vinegar is a word derived from the french 'vinaigre' which means sour wine. It was known by the alchemists and it was indicated by the symbol ☥, ☓, ☥ while for the distilled vinegar the characters ☩, ※, ☩ were used. (Greenshields, 1978).

It is one of the most known food commodities of the ancient and modern world. People used vinegar as food, cosmetics and for therapeutical reasons.

USES OF VINEGAR

1.2.1. As Food

The use of vinegar as food starts from prehistoric times. "Shekkar", fermented apple juice, was known to the Phoenicians and to the Aryan race from the beginning of the recorded history. The souring of apple cider to yield vinegar was an ancient art (Alwood, 1903).

In historical times, first the Babylonians started producing vinegar from the sap of palm trees. They used to collect this sweet sap in vessels by piercing the palm trees. Because that sweet juice or sap had a low concentration of sugar, the produced wine was not stable, and after three or four days converted into vinegar (Huber, 1927a).

Another type of vinegar, which was stronger than that of the palm sap, was that from dates. A thick syrup, known as honey, was extracted from ripe dates. The date honey was fermented in vessels and yielded a wine which was stronger than the palm sap wine so the produced vinegar was stronger as well.

An ancient cultural tradition of the Babylonians as early as 4000 B.C. was vinegar making from bread. This bread was made from germinated cereals, which were ground, and processed with water. After baking on both sides, the bread was stable for many months. The bread was cut or crushed and moistened with water and beer. The product from the fermentation of the sugars in the bread was ethanol, which, in turn converted to acetic acid. (Huber 1927b). In general, vinegar was used by the Babylonians in cooking and in preserving meat, fish, fruits and vegetables.

Since vinegar played an important role in the Babylonian household and economy, about 3000 B.C., the commercial vinegar production from dates started. In about 234-149 B.C., Cato was the first to write about the Roman contribution to vinegar making (Cato and Varro transl. by Hooper and Ash, 1934; Cato trans. by Brehault, 1933). 'Posca' was a common drink to Romans. Actually, this was a mixture of vinegar with water and eggs. Greeks and Romans made use of vinegar both in cooking and at their meals.

Information from Columella who lived from about 4 B.C. to 65 A.D. makes it known that a way of preparing vinegar was used at that period. Carbon sources were: dryed figs and date honey,

inoculated with heavy concentrations of vinegar to avoid contamination. (Columella, trans. Forester and Heffner, 1955).

For the period 23-76 A.D., there is information from Pliny (Pliny, transl. by Rockham, 1945) about, 'the peculiarity of wine among liquids to go mouldy or else to turn into vinegar'. From the Renaissance till today, vinegar is continuously being used both as food and as food preservative, in pickling processes.

1.2.2. As a cosmetic

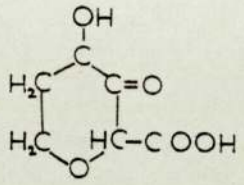
Vinegar has been used as a cosmetic today for many purposes. Three parts of distilled white vinegar mixed with one part of citrus fruit juice serves as a shave lubricant (Gibbs, 1974). Also, vinegar is used with lemon juice in various shampoos.

Kromer (1972) records the use of vinegar as a beautifier. Fowler ahd Schwartz (1973) also record that for some time women have been known to finish a shampoo with a vinegar rinse to give low pH.

1.2.3. As a biologically active substance

Simultaneously, with the occurrence of vinegar as a food commodity, it appears that the early man understood the antibiotic value of vinegar. Since antibiosis was one of the first principles of medicine that found application very early, vinegar was one of the first to serve this purpose. Greeks and Babylonians were using vinegar for antibiotic purposes (Hippocrates, transl. by Adams, 1949, Huber, 1927 a,b, Columella, transl. by Forester and Heffner, 1955). Also the Assyrians had a treatment for chronic middle ear diseases applying vinegar to the ear (Ochs, 1950a). Jarvis (1959)

describes various applications of vinegar for the treatment of many skin diseases such as: ringworm, impetigo, shingles. Antoniani et al. (1958) and Yamamoto et al. (1959) reported the presence of a biologically active substance in vinegar that markedly inhibits the growth of human tumor cells cultivated in vitro. The substance was isolated in pure state as a white powder with a melting point of 184°C. Its probable structural formula is:



Another substance found by Steel and Walker, (1957a,b) in cultures of different strains of Acetobacter, to have antibiotic activities. Two mutants were A.acetigenus NClB 8132, 5346 and the strain A.xylinum var africanum (NClB 7029). That substance reacted inhibitorily to the growth of Proteus vulgaris. Also, the substance which was produced by the parent strain inhibited the growth of all the mutants (Steel and Walker, 1957a and b). Another activity of the bacteria of acetic acid was the lethal action of an Acetobacter sp, a close relative of A.mesoxydans, to yeasts. Gilliland and Lacey, (1963) reported that the above Acetobacter sp. prevented the growth of yeasts and caused them to die when both organisms were present in the ratio of 1:1. The lethal action was found in beer. Also, the lethal action of the Acetobacter was found on strains of the genera Pichia, Schizosaccharomyces, Torula, Candida, Zygosaccharomyces Saccharomyces, (Asai, 1968).

1.2.4. Miscellaneous uses

Löw (1901) refers that vinegar was used to release olives from their pits. It is also said that when Hannibal marched over the Alps to Rome his soldiers used vinegar to crack rocks which were blocking his way (Titus Livius, transl. by Spillman and Edmonds, 1895). But this was proved to be a fiction, invented by the Romans, to account for their defeat by Hannibal (Pliny, 1945).

SECTION 2

2. METHODS OF PRODUCTION

2.1. BATCH PROCESSES

2.1.1. Old Methods

One of the oldest methods for the production of vinegar is the 'let alone process'. This method was used for the production of wine vinegar (Cruess, 1948). The wine was converted to vinegar in containers by the action of bacteria which were occurring in the air or on the grapes. Mitchell (1926) refers to the 'fielding process' in which the casks of the wine were left open in the fields, adding amounts of vinegar to start the process.

2.1.2. More recent methods.

2.1.2.1. The Orleans process.

The Orleans process is the refinement of the fielding process. Its name derived from the district of France where the process was used (Mitchell, 1926).

The process consisted of making vinegar in barrels of fifty gallons capacity (approximately 220 l). The barrels were laid on their sides with two air holes in the side. Three quarters of the casks were filled with mash prepared by mixing about five gallons of 5% vinegar by volume with thirty gallons of alcoholic liquid containing 5 to 6% ethanol by weight. For inoculation, a small amount of vinegar "mother of vinegar" was added to the barrels. "Mother of vinegar" is a mass of vinegar bacteria, often a pellicle (Jones, 1970). Instead of being laid to the fields, the barrels were stored in heated buildings or cellars. When the acidity of vinegar reached about 5% by weight, the vinegar was drawn for the next batch. The process required a period of three to four

months to complete the acetification of ethanol.

Since the acetic acid bacteria are oxidizing ethanol to acetic acid in an aerobic way only on the surface of the liquid, there is always an unfavourable ratio of the free surface of the liquid to the total volume of the liquid, which causes low acetification rates. The long time of fermentation involves great losses of ethanol and of acetic acid by volatilization, so that the values of the yield may sink to as low as 50% of the theoretical output (Enenkel, et al. 1953). Wüstenfeld (1930) and Joslyn (1970) present values of conversion efficiency from 77 to 84%.

2.1.2.2. The Quick Process

In the early part of the 18th century, Boerhaave (1732) a Dutch technologist, found that the rate of acid production was directly proportional to the amount of surface exposed to air. He constructed the first generator using packing material and applied the trickling principle for vinegar production.

Wüstenfeld (1930) reports that Kastner conducted experiments in 1823 to improve the old Boerhaave process. At about the same time, Schutzenbach, a German chemist, introduced the use of a vat instead of a cask, and provided mechanical means for the repeated distribution of the acidic liquor over the packing (Mitchell, 1926). He also drilled holes in the Boerhaave reactors for ventilation near the bottom of the vat. The method, because of the large contribution of Schutzenbach, was called the "German process" (Wüstenfeld, 1930).

In 1824, a British patent granted to Ham for the production of vinegar. The patent describes a system which recirculates the liquid with a pump located at the top of the vat and distributes the liquid over the packing material with the aid of a sprinkling device composed of two perforated pipes.

It seems that Ham's acetator was the forerunner of Frings circulating generator (paragraph 2.1.2.3.).

Since the innovations of the four above mentioned workers, many patents appeared which advanced the state of the art. Description of these have been made by Bitting (1928, 1929).

The modern "Quick" vinegar process vats are constructed from a variety of different woods including redwood, cypress, oak and various types of pine (New Zealand Kauri pine or Columbian pine). Other materials have also been introduced for the construction of the vat such as: stainless steel, glass-lined steel, plastic and fibreglass (Devey and Dakin, 1971).

The packing of the vats is made from various materials and its choice is a matter of availability and economics. Packing materials which have been used include ceramics, pumace, charcoal, various types of wood (beechwood, cypress, redwood, willow and certain types of pine) in the form of shavings, twigs and segments. In France, the most common packing material for wine vinegar acetifiers is grapevine prunings and in the U.S.A. corn cob husks.

The preparation of the packing material follows a certain procedure related to its nature. In the case of using birch twigs, the twigs are boiled for long periods to drive

off essential oils which may contaminate the product. The preparation of the twigs is a costly and time consuming procedure. Devey and Daking (1971) referred to an example of packing preparation for a 130000 litre capacity vat. This vat is loaded with two tons of treated birch twigs and these are steamed continuously for two days prior to charging to vat.

After about twenty times of successive batches of vinegar have been made in the same acetifier, the birch twigs are discarded and the process is started again with fresh twigs. The reason for changing the packing material is that certain Acetobacter species, such as A.xylinum produce slime (cellulosic material) which gradually coat the packing materials.

The "Quick" process has been established in many countries for many years, because there appeared to be advantages over the older Orleans method. This system increased the production rate and reduced the acetification rate from weeks to days. It is also less labour intensive. Although it has advantages over the Orleans method the "Quick" process it also has certain disadvantages over the submerged processes.

a. Low conversion of ethanol to acetic acid (75-80% often falling to 60% or less)(Greenshields, 1978).
b. Time consuming, costly and laborious use of packing materials
c. Occupies large space
d. Each batch fermentation consumes the necessary time for the development of the bacterial lag phase, which reduces the output rate of vinegar.

Greenshields (1978) reports that rolling generators have also been used commercially, but these require long acetification times and consume much power. He also mentioned that some manufacturers run two acetators in tandem, in order to gain time and efficiency by shortening the lag phase of bacterial growth. Although this system succeeds conversion efficiencies of 95% or greater, their volumetric efficiency is lower in comparison with the submerged processes.

2.1.2.3. Frings Circulating generator

An improvement of the "Quick" vinegar process was made when the circulating generator was introduced by Frings (1932, 1937). The circulating generator was similar to the "Quick" vinegar process but had many advantages over the latter. For example, vinegars of higher acidity were produced, requiring less tankage and an increased production rate was achieved. The innovation of the Frings circulating generator consists of the use of meters and regulators to monitor the following parameters in the operation:

1. the circulation rate of the mash
2. the flow of cooling water to the mash cooler or heat exchanger, and
3. the quantity of air delivered to the generator.

Thermometers are also installed at several different heights on the tank to indicate the temperature of the mash as it trickles through the generator.

As in the older "Quick" process reactors, the circulating generator provides the main characteristics, i.e. the vat, which is usually wood, and a false bottom to support the beechwood twigs above the collection chamber.

Air is supplied by a single fan-type blower and is distributed to the generator by a number of equally spaced inlets located in the sides of the generator just beneath the fals bottom. A pump for circulating the feed-vinegar mixture from the holding section beneath the false bottom, through a heat exchanger to the top of the generator. A spray mechanism distributes the liquid over the surface of the packing. The spray mechanism consists of a sparger-wheel which operates in such a manner that it permits an even distribution of the infusion liquid over the packing area. The cooled mash passes through the pipe (1), (Figure 2.1) into the sparger wheel which rotates in a known manner by the reaction caused by liquid flowing therefrom. When the flow of liquid is relatively weak, the discharge of liquid takes place from a single arm (2). Only when the flow rate exceeds a certain limit does a siphon (3) commence to supply a second arm (4). This arrangement ensures an even distribution of the acidic liquid at different rages. The number of sparger arms may be increased according to each particular process (Frings, 1932).

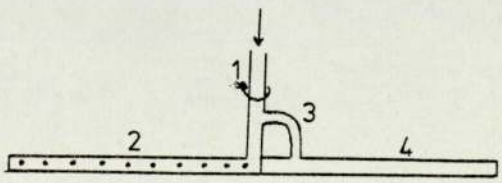


Figure 2.1. Sparger wheel with siphon

(From Frings, 1932)

Generally the Frings generator does not require close

attention. Three parameters are important for its operation:

a) The circulation rate of the mash

b) The flow rate of the water into the heat exchanger

c) The air flow rate

Investigations on Frings type plants in Hungary, by Pandi (1974), show that losses of ethanol due to over-oxidation and evaporation can occur in appreciable amounts. At five plants, the losses of ethanol due to overoxidation were 4.16% by volume and losses due to evaporation were 4.33% by volume.

According to Pandi (1974) the high overoxidation losses are mostly due to Acetobacter xylimum growing in the lower layers of the fermenter, where the ethanol content is low. The conditions in this part of the fermenter are favourable to the development of mucous layer by the acetic acid bacteria.

2.1.3. Vinegar production by submerged cultures

The study of the submerged culture for the production of vinegar started by Tait and Ford (1876) and Fowler and Subramaniam (1923). They started to investigate some of the parameters which affect the submerged acetification. Despite these studies, no practical applications were derived from them. The appreciation of the submerged culture technique came only after the success of the production of antibiotics and bakers' yeast. The submerged culture for the production of vinegar was studied by Hromatka and co-workers (Hromatka and Ebner, 1949, 1950, 1951, 1955; Hromatka et al, 1951; Hromatka and Kastner, 1951). In 1949, Haeseler also studied

the submerged acetification. The results of the investigations of these workers showed that the acetic acid bacteria can grow in submerged conditions only when there was a continuous supply of oxygen. An interruption of the aeration could prove fatal to a high percentage of the Acetobacter population depending on the duration of the interruption and on the total acidity of the medium. This fundamental knowledge made the industrial realization of submerged vinegar fermentation possible. In 1953, Enenkel et al. published a work concerning aerated towers for the production of carbonilic acids. Although this patent was not restricted to the production of vinegar, it led to the development of the Frings Acetator.

2.1.3.1. Frings Acetator

The Acetator consists of a wooden, aluminium or stainless steel tank fitted with internal colling coils and a bottom-entering, high-speed agitator, Figure 22. Automatic devices control the aeration rate and the flow of the cooling water. The agitator is designed to pull air from the room to the bottom of the tank, where it is finely dispersed by the agitator and distributed throughout the liquid in the tank.

The Acetator is operated semicontinuously but may be operated under certain conditions continuously. To start fermentation, the Acetator is continuously filled with a mixture of vinegar, mash and nutrients and run batchwise. When the ethanol content falls to 0.3 or 0.2% by volume, about 35-40% of the contents of the tank are removed as finished product. Fresh feed is pumped in to restore the original level and the cycle starts again. The agitator and air supply operate

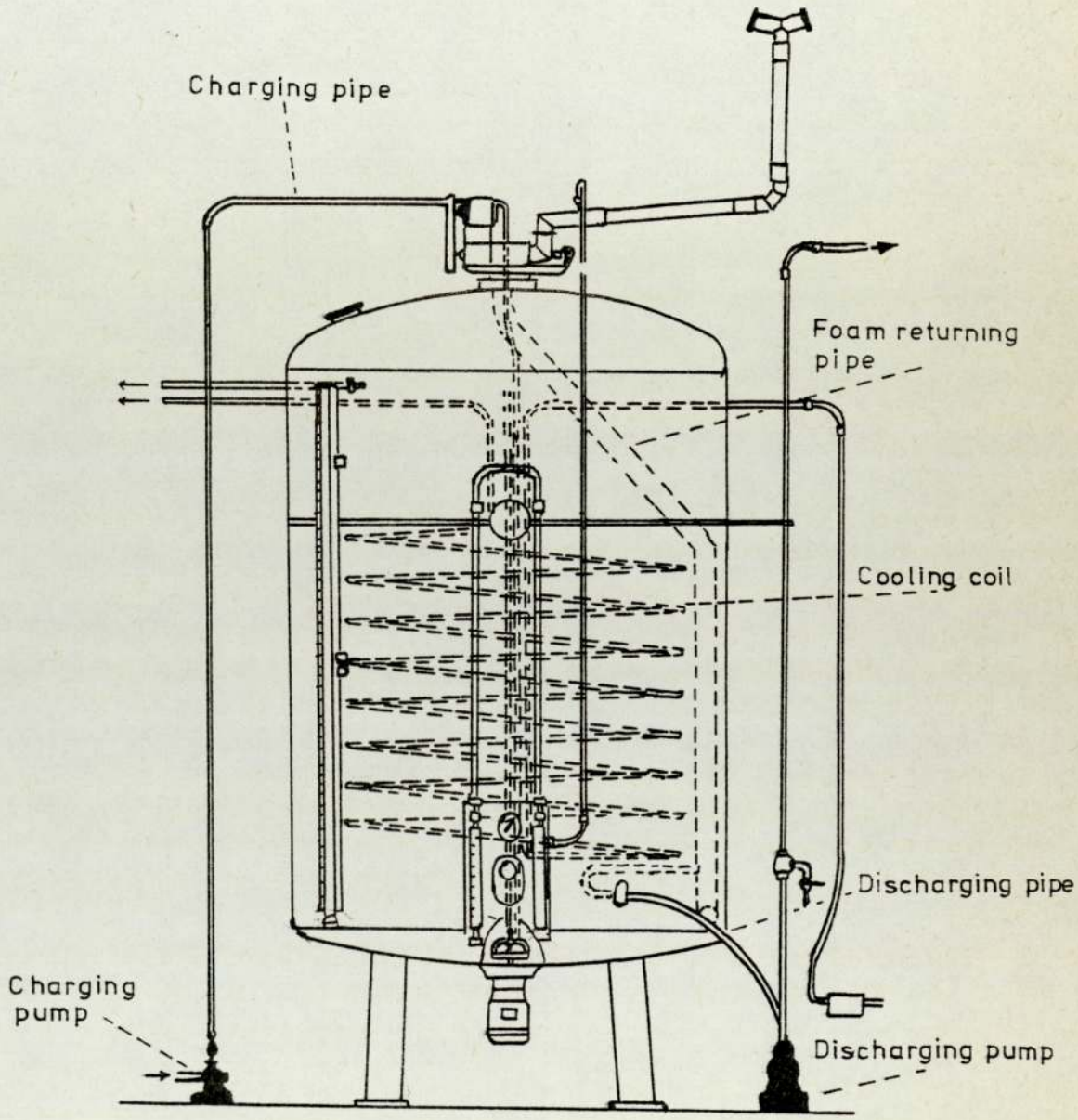


Figure 2.2. Diagram of the Frings Acetator

continuously during the change.

Advantages of the Acetator over the trickling type of generator, are:

1. Better performance
2. Smaller size (higher volumetric efficiency)
3. It can run continuously with few modifications
4. No problems caused by the packing process
5. They are capable of producting at 12% by weight of acetic acid at 98% conversion efficiency

Disadvantages of the Acetator are:

1. Susceptibility to complete production stoppage (possibly lack of air for 30 seconds at high acidity could cause total loss of bacterial viability.).
   Some manufacturers install stand-by generators which supply instantaneous power in the event of a failure.
2. Higher cost of equipment (relatively to that of the Tower fermenter which is made from polypropylene).
3. High rate of power consumption (0.30 to 0.35 Kw./per litre of absolute alcohol converted) (Hromatka and Ebner, 1955).
4. Production of cloudy vinegar which requires some type of fining treatment before it can be filtered.

2.1.3.2. Various types of bioreactors operating on a continuous mode for the production of vinegar.

In 1959, Cohee and Steffen described another type of acetifier which was operating on the continuous principle, the Cavicator. This reactor was developed initially for the biological oxidation of sewage. Basically the Cavicator is

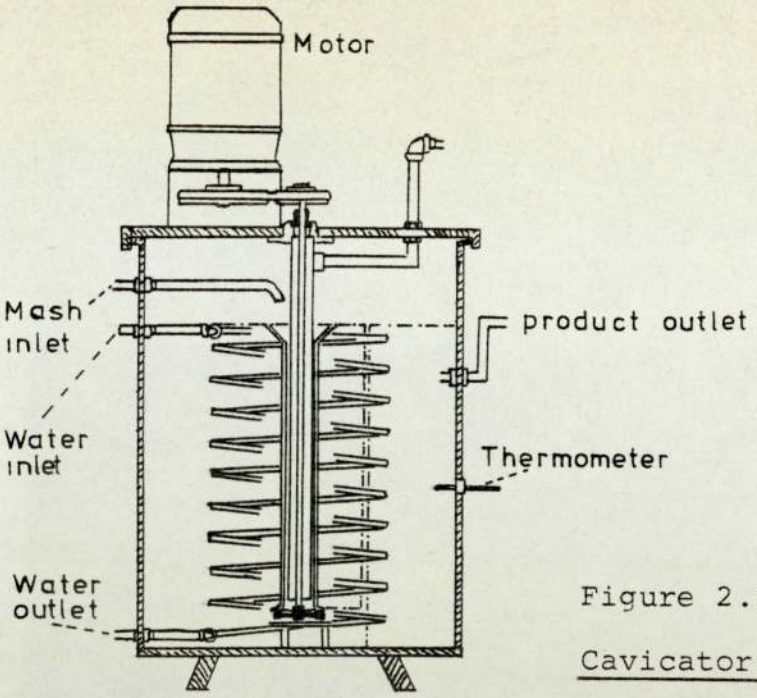
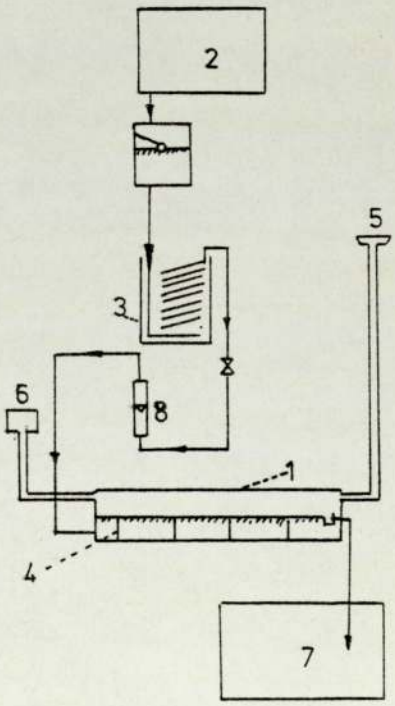


Figure 2.3. Diagram of the Cavicator (Cohee and Steffen 1959)



1 Fermenter
2 Medium tank
3 Preheating tank
4 Flow regulating means
5 Air outlet
6 Air inlet
7 Collection tank
8 Flow meter

Figure 2.4. Surface fermentation system for the prodcution of vinegar (Kaisha, 1971)

a modification of the Frings Acetator. The main difference of the Cavicator from the Acetator is its operation which is manual, in contrast to the Acetator which operates automatically. The Cavitator has a conversion efficiency of over 95%. There are no more details about its performance and energy consumption so far published. Despite attempts to use the Cavicator for vinegar production commercially, technical difficulties have forced it to be abandoned.

Another acetifier was described by White (1961). Figure 2.5. This acetifier was operating on the principle of jet-aeration and recirculation in submerged culture. The system was easily installed in existing wooden vats and was adapted to continuous production. White (1966) states that his acetifier is believed to be the first continuous submerged culture carried out for the manufacture of vinegar in Great Britain. White's system has been operated continuously on a commercial basis, but full details of its operation have not been published, other than that 98% conversion efficiencies are achieved and some reports suggest that this system has now been withdrawn (Greenshields, 1977).

A well-equipped acetifier has been recently described by Chemap (Chemap, CH-8708 Mannedorf, Alte Landstrasse 415, Zurich), the Vinegator (Müller, 1978), Figure 2.6. The Vinegator consists of a stainless steel tank and is equipped with an automatic oxygen control system. This system regulates the amount of oxygen by the use of aerators which incorporate atmospheric air or pure oxygen into the reactor.

The oxygen transfer and the circulation of the mash are

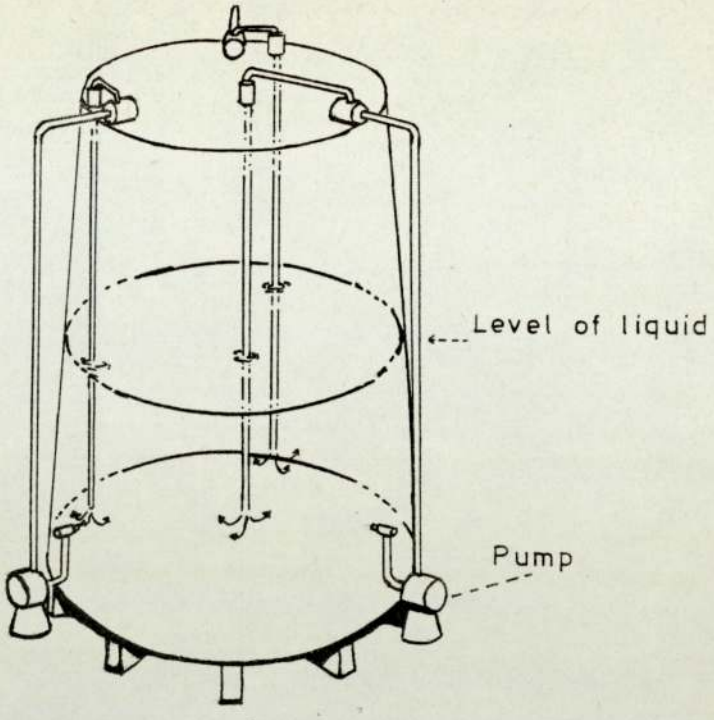


Figure 2.5. Diagram of White's acetifier (White, 1961)

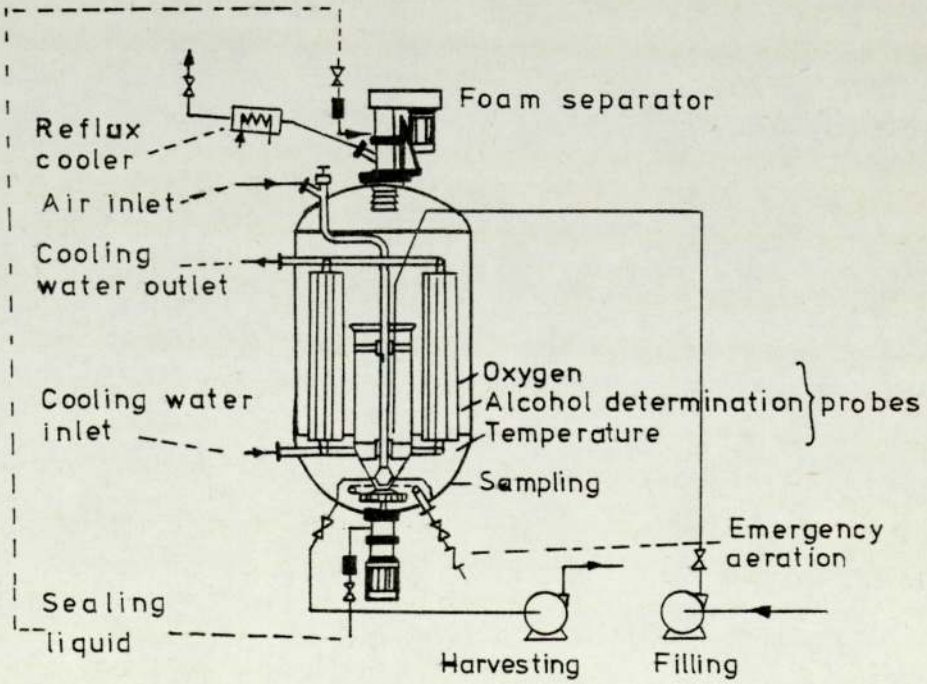


Figure 2.6. Diagram of the Chemap Vinegator (Müller, 1978)

achieved by means of an agitator-turbine system. There is also an emergency aeration system incorporated to the bottom of the tank. The stirrer, cooling attemporators and mechanical foambreaking system are controlled automatically. Müller (1978) states that Vinegator is capable of producing 300 Kg acetic acid/day, but he does not describe the other parameters of the reactor, such as the volumetric capacity, for comparison studies.

In Japan, Kaisha (1971) has developed a method of producing vinegar, based on the surface fermentation technique. Kaisha's system consists of one or many vessels inert to the acetic acid, Figure 2.4. The vessels may be semicylindrical, square or ellipsoidal in shape. The alcoholic medium is introduced to the first vessel which has a flow regulator to control the feed rate. The liquid flows from the one vessel to the other and forms a layer on the top of the surface. The acetic acid bacteria utilize the oxygen which is available on the surface of the liquid film, and oxidizes the ethanol to acetic acid. Kaisha claims that the systems can reach mean acidification rate of 3.5% per day or 1.45 g $l^{-1}.h^{-1}$ with conversion efficiency of 95.5%. The operating temperature of the vessels is between 28-29°C and the temperature of the liquid is elevating to 35°C.

The Kaisha's system presents certain advantages over the submerged fermentation systems:

1. It does not involve high cost for construction and operation
2. It is not dependent on continuous power supply for aeration and feeding purposes.

Disadvantages:

1. Uncontrollable temperature of the liquid leads to ethanol and acetic acid losses due to evaporation.
2. Bacterial film forming facilitates the spoilage of finished product fromthe death and lysis of cells, and consequently the production of not good quality vinegar. The cleaning of the vessel surfaces from the bacterial film is time consuming and the sequential lag phase of the next fermentation reduces the overall production rate of the system.

Schügerl et al. (1977) published a paper which compares the different types of bioractors. The study is based on measurements of the specific surface areas (A) which are achieved in different bioractor types under comparable conditions (sulfite oxidation). These are compared as a function of the specific power input ($\frac{E}{V}$), where E is the energy in Kw and V is the volumetric capacity of the reactors (Table 2.1.1.).

Fermenter type	Aerator type	A m^{-1}	E/V kw/m^3	Aeration S.G.V. cm/sec
STR	-	600	3.0	
TF	PE	650	0.6	4.5
FA		1000	1.2	
AL	EN	1300	0.9	3
TF	PO	2000	0.9	4
AL	EN	2500	7.2	8
TF	IN	6000	1.5	3
TF	EN	8000	2.2	7

Table 2.1.1. Comparison of specific surface areas in various bioractors (Schügerl et al 1977)

STR Stirred tank ractor

TF Tower fermenter

FA Frings Fermenter

AL Air lift fermenter with draught tube

PE Perforated plate aerator

PO Porous plate aerator

EN Ejector nozzle aerator

IN Injector nozzle aerator

As can be seen from the table, the stirred tank reactors STR, yield the smallest specific areas with the highest power input, while Frings Acetator, FA, with its self-aspirating aerator is the best of this type. Frings Acetator is better than air-lift bioreactors with draught tube, AL. The highest specific surface areas are obtained by Tower fermenters with ejector and/or injector nozzle aerators. The comparison (in Table 2.1.1) shows also that the Tower fermenters with porous plate aerators are the most economical reactors, since they yield medium to high specific areas with relatively low specific power input. The size of the specific gas/liquid surface area controls the oxygen transfer rate in a liquid and consequently the oxidation rate in aerobic fermentation.

2.2.4. Tower fermentation system

2.2.4.1. Definition

In 1971, Greenshields et al. described the tower or tubular fermenter as an elongated non-mechanically stirred fermenter with an aspect ratio (height to diameter ratio) of at least 6:1 on the tubular section or 10:1 overall, through which there is an unidirectional flow of medium or gases.

2.2.4.2. Classification of Tower fermentation systems

A previous classification of Tower fermentation systems has been described by Pannell (1976).

According to this classification, the Tower fermentation system can be divided into two types.

1: Single and multistage plug-flow Tower fermenters, and

2: Single-stage well mixed Tower fermenters.

Although the above classification includes satisfactorily the various types of Tower fermentation systems, the position of the multistage Tower fermenters remains unclear. The multistage Tower fermenters have been desiqned for fermentations where high aeration is needed cannot be placed in the plug-flow systems neither in the single-stage well mixed Tower fermenters.

A classification based on the aeration intensity used for the individual fermentation system could be proved more appropriate. According to the last classification scheme the Tower fermentation systems can be placed into two categories:

1: Anaerobic (Microaerophilic) and

2: Aerobic (single and multistage).

1: Anaerobic (microaerophilic) Tower fermentation systems

In this type of fermentations belong the processes which

need no aeration or need low intensity of aeration as in the case of alcoholic fermentation which is microaerophilic.

In microaerophilic processes the generation of gases and the introduction of small amounts of air per time unit creates a low mixing of the medium in the fermenter. Uses of the microaerophilic Tower fermentation systems have been reviewed by Hough and Button (1972) and by Coote, (1975). Greenshields (1968) used this type of fermentation for the production of malt vinegar charging wort. The type of yeasts which are used for this type of fermentation belong to group three, according to Greenshields et al., (1971) classification.

The group three includes the Flocculent-fermentation limited yeasts. These yeasts attain concentrations of 25 to 40% (w/w) and are retained in the fermenter at high dilution rates, at wide range medium specific gravities.

The same process has been used by the author for the production of molasses vinegar charging wort (see section 4.2.)

A development of the Tower fermentation system for laboratory, pilot- and production scale is described by Royston (1966) Klopper et al (1965) Shore and Royston (1968)

In Figure 2.7 is shown the APV Co. Ltd., Tower fermentation system, for the production of beer.

2: Aerobic (Single and multistage) Tower fermentation systems

The aerobic Tower fermenters have been used for the production of both microbial biomass and metabolites. (Smith and Greenshields, 1974 and Greenshields and Smith, 1974). Rosen (1968) and Simek et al, (1973) have worked on the production of yeasts. In our laboratories also previous works on the production of biomass have been studied by Morris (1973)

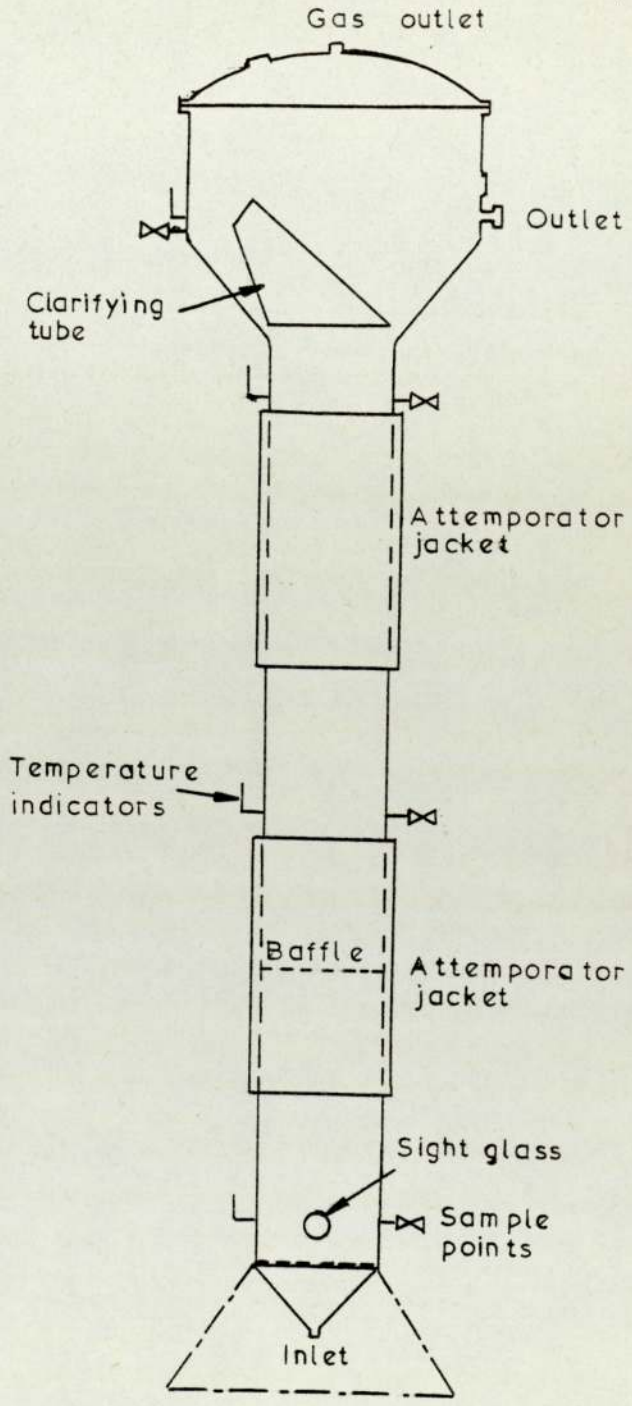


Figure 2.7. A.P.V. Tower fermenter.

and Daunter, (1972). They both worked on studies of fungi in Tower Fermenters.

Also, the growth of thermophilic fungi on cellulose has been described by Barnes (1974). In 1975 Cocker studied the morphology of Aspergillus niger M1 in continous tower systems. In 1976 Pannell studied the growth kinetics of A. niger M1 in Tower fermenters at low and high dilution rates.

Also, the growth of A. niger M1 on starchy materials has been studied by Spensley (1977).

The continuous malt vinegar production in Tower fermenters studied by Greenshields (1968), Jones (1970) and Humphreys (1976).

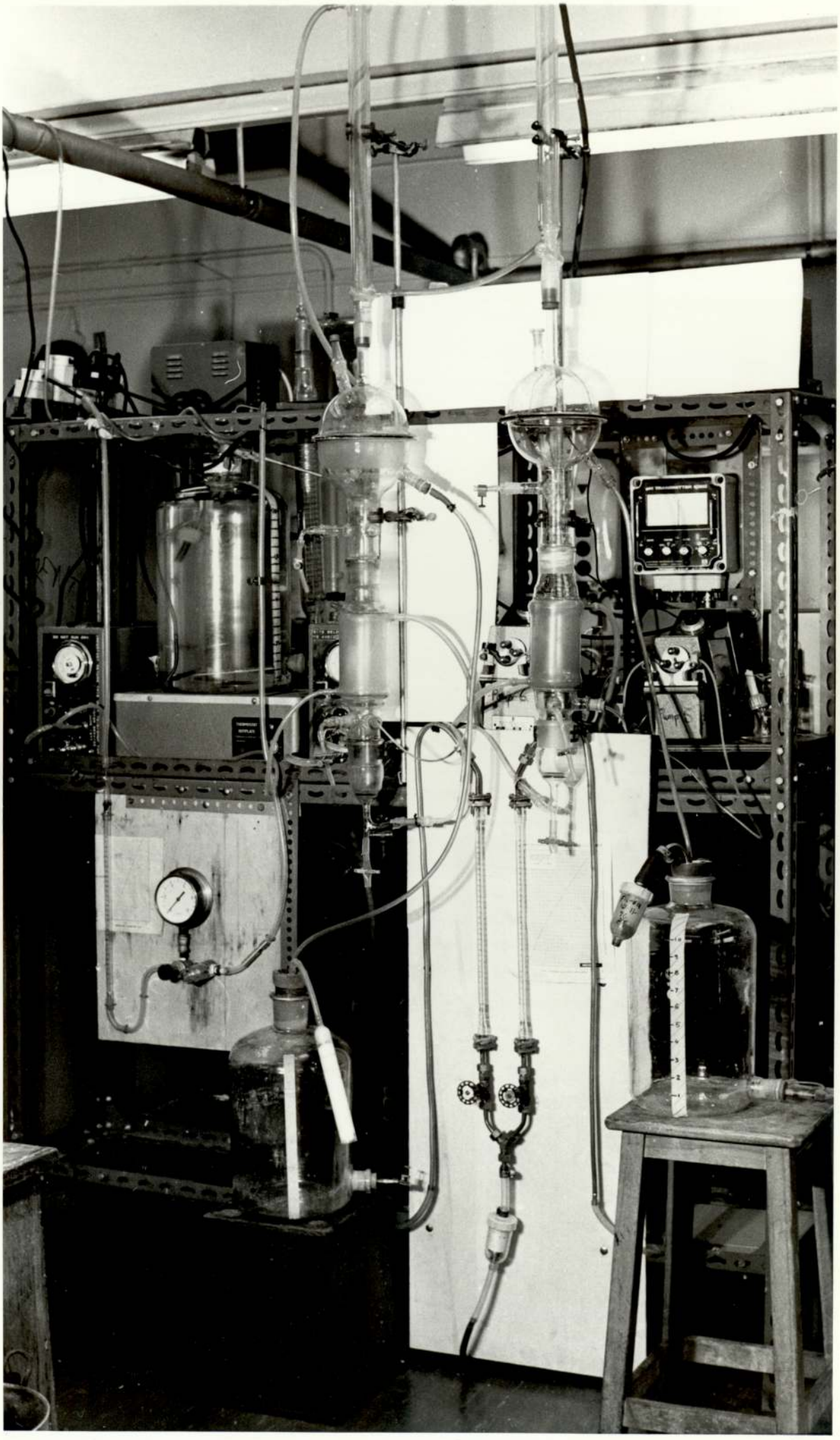
The multistage tower fermenters can be described as tubular reactors in which the air distributors are more than one (Erickson et al., (1972).). These fermenters have been studied also by Hsu et al., (1975).

Hsu et al., (1975) described a new type of air distributor named the Koch mixer. They found that the Koch mixer was better than the sieve tray air distributor in terms of oxygen transfer phenomena.

Generally, the air flow tower fermentation systems may be characterized by three flow patterns according to the magnitude of the superficial gas velocity (S.G.V.) (Morris et al., (1973)).

1. "Bubbly flow". (S.G.V. 1cm/sec) Air bubbles travelling up the column in nearly straight lines and producing little back mixing.
2. "Turbulent flow". (S.G.V. 3 cm/sec) Production of considerable backmixing.
3. "Slug flow". (S.G.V. > 5 cm/sec) Air bubble size is

Plate 2.1. Tower fermentation system



varying more than it does in the previous two flow patterns.

In flow patterns 1 and 2, the oxygen transfer is better than that in flow pattern 3, because of the bubble sizes (In situation 3 the bubble size is larger than in 1 and 2).

A hybrid stirred single-stage Tower fermenter has been described by Feustel and Humfeld (1946) for the production of yeasts and by Yates (1971) for the production of vinegar.

2.2.4.3. Description of the Tower fermentation system for the production of vinegar.

The Tower fermentation system used consisted of the following:

1. The Tower fermenter (Figure 2.8. plate 2.1)

Two fermenters of different volumetric capacity were used.

1. The fermenters consisted of two main parts. The lower part which was tubular and the upper part which was an expanded spherical shape continuation of the tubular part.

The material was standard Q.V.F. glass pipeline (J.A. Jobling Ltd., Stone, Staffs) modified by the addition of ports from glass parts where necessary, by the University glassblowers.

a) 3.1 l fermenter.

The lower tubular part was 750 mm long with 70 mm internal diameter pipe sections which had six ports fitted.

Near the bottom of the tubular part a sintered glass filter (porosity 2) was fitted which acted as an air distributor.

The tubular part also was covered by water jacket. The upper part was a spherical shaped expansion. Two ports were fitted, one on the top for air exit purposes and one on the side for medium exit. The expansion consisted of two separated hemi-spherical parts with 165 mm (i.d.).

When the tower fermenter was used for anaerobic fermentations, a yeast separator was used.
The air exit was connected with a double system of condensers.
The liquid exit was connected through a silicon rubber tubing with a 20 litre aspirator bottle.
The ports had 12 mm (i.d.) and were extended with silicon rubber tubing 70 mm long, closed with metallic clips.

b) 1 l fermenter.
Tubular part 443 mm long, 45 mm i.d.
Expansion 73 mm i.d.

2. The air supply

The air was supplied via a Norgren Ultraine filter and pressure regulator (Air Power Minett, 41, Great Lister Street, Birmingham 7.)

A rotameter flow measuring device was used to monitor the aeration rate (Rotameter Manufacturing Co. Ltd., Croydon) Also a pressure regulator (type MR1, Spirax-Monnier, Cheltenham) was connected to maintain the air pressure constant. The saturation of air by water vapour was achieved by passing it through a 20 l aspirator filled with sterile water (after leaving the rotameter).

3. Temperature

The temperature of the fermenter was regulated with the use of a system of water jacket and thermocirculator (Churchill Instruments Co., Perivale, Middlesex) through which water was circulated. A thermometer (-10, 110^{o}C) was also used to enable the temperature of the fermenter contents to be checked.

3. The medium supply

The medium was prepared in a 20 l glass aspirator equipped with "Gamma 12" inlet air filters. From the aspirator through a 1 mm silicon rubber tubing the medium was pumped to the fermenter with the use of a peristaltic pump (Watson-Marlow Ltd., Marlow, Bucks). The part of rubber tubing which was in touch with the peristaltic pump had to be changed every three days because after that period of time deformation of the tube occured, which resulted in alteration on the internal diameter of the tube and consequently the feed rate was not remaining constant.

For low dilution rates it was necessary to use an electric timer device so that the electric power was only supplied to the pump for 15 secs per minute thus the feed rate was reduced to a more suitable range.

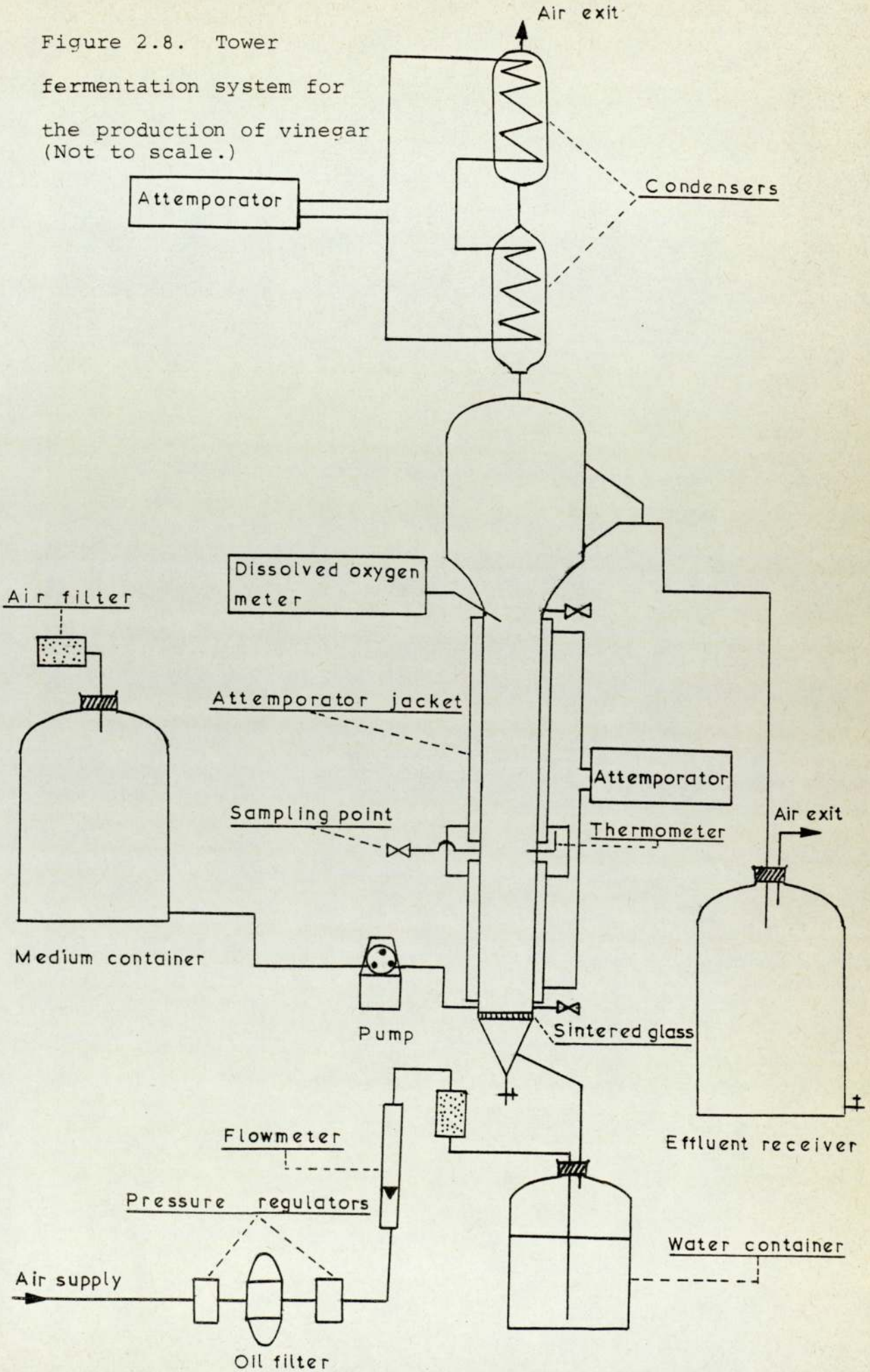
4. Oxygen analysis equipment

Dissolved oxygen was monitored by a New Brunswick DO-50 analyser (V.A. Howe and Co. Ltd., 88 Peterborough Road, London, S.W.6.). and a New Brunswick meter-recorder and probe.

Entry of the probe was through a port mounted at 45^{o} to the fermenter wall, at the top of the tubular part.

The concentration of oxygen in the input and exit air lines could be monitored by a Servomex (OA 101) oxygen analyser (Servomex Ltd., Crowborough, Sussex) but in practice the difference was not high and the measurement was of little value.

Figure 2.8. Tower fermentation system for the production of vinegar (Not to scale.)



SECTION 3

3.1. NUTRITIONAL REQUIREMENTS

3.1.1. Nitrogen and Carbon

Rao and Stokes (1953a) observed that most acetic acid bacteria can grow in media containing NH_4-nitrogen as a sole source of nitrogen. Many strains of A.suboxydans and A. melanogenus were found to have this ability. Also, Shimwell (1957) found that A.xylinum did grow well in a modified Hoyer's glucose medium (containing 2% glucose instead of ethanol). Only three species of those which grew in the Frateur (1950) modified Hoyer's ethanol medium A. peroxydans NCIB 861B, A.aceti NCIB 8621 and A.lovaniense NCIB 8620 did not grow in the Hoyer's glucose medium, and even these could grow in the latter in the presence of yeast extract. These observations were of great importance because they established that the utilization of a particular nitrogen source by the acetic acid bacteria was not limited by the kind of nitrogen source itself, but on the combination of the nitrogen source and the carbon source and, in some species, on the presence of animo acid, vitamin, purine bases, etc., as growth factors.

The Hoyer's (1899) medium consists of ammonium phosphate 0.1g, primary potassium phosphate 0.1g, magnesium phosphate 0.1g, sodium acetate 0.1g, ethanol 3 cc and distilled water 100 cc

Frateur modified Hoyer's solution as follows:

$(NH_4)_2 SO_4$	1g
$KH_2 PO_4$	0.9g
K_2HPO_4	0.1g
$MgSO_4.7H_2O$	0.25g
95% ethanol	30 cc
1% w/v $FeCl_3.6H_2O$ (aqueous solution)	0.5 cc

In distilled water to make 1 litre

Gray and Tatum (1944) reported that a strain of A. melanogenus utilized ammonium nitrogen in the presence of thiamine, pantothenic acid, nicotinic acid and p-aminobenzoic acid.

Cultures of A.schutzenbachii, A.curvum and A.aceti which were found in the quick vinegar process, showed a much poorer growth with glucose as a carbon source than with ethanol, acetate or lactate. That was found to be true, even with the addition of various nitrogen sources or growth factors (Loitsyanskaya, 1955).

The acetic acid bacteria which are capable of growing in ethanol as a source of carbon and energy, can be shown to oxidize ethanol, but the inverse is not always true. Also, the same workers found that strains which assimilated ethanol were found to belong to the genus Acetobacter and only one strain, A.capsulatus, utilized glucose as a sole carbon source. The strains which assimilated glucose were found to belong to the genus Gluconobacter. In the experiments of Asai et al. (1962), there were no strains of Gluconobacter able to utilize ethanol as a sole carbon course.

Although the selective assimilation of ethanol or glucose are properties of the genera Acetobacter and Gluconobacter, this property should not be considered as the sole criterion for distinguishing the two genera, but as evidence, since there are Acetobacter strains which do not assimilate ethanol, and Gluconobacter strains which do not assimilate glucose. Many strains of A.suboxydans and A.melanogenus cannot utilize ethanol (Rao and Stokes, 1953a).

3.2. GROWTH FACTOR REQUIREMENTS

The knowledge of the nutrient requirements of Acetobacter genus is part of an overall consideration of these, which influence the efficiency of the conversion of ethyl alcohol to acetic acid in the production of vinegar.

3.2.1. Vitamins

In 1942, Lampen et al, in 1943, Underkofler et al, started to study the nutrient requirements of the acetic acid bacteria.

They reported that A. syboxydans 621 required p-aminobenzoic acid, nicotinic and pantothenic acids for growth. Most strains of A.suboxydans as well as other species tested conform to this pattern of growth factor requirement.

Foda and Vaughn (1953) found that A.rancens required thiamine. Rao and Stokes (1953a) also found that A.melanogenus required thiamine as growth factor.

Biotin is required for growth by A.ascendens, A. pasteurianus and A.acidum-mucosum (Hall et al. (1953)

Pantothenic acid is the most extensively studied vitamin as a growth factor of the acetic acid bacteria. Jlli et al. (1965) reported that four strains of Gluconobacter required pantothenic acid when glucose was the carbon source, and two strains required nicotinic acid in addition. Only one of ten strains of Acetobacter spp tested required p-aminobenzoic acid when glucose was the carbon source.

Pantothenate is also required for the growth of A. ascendens, A. capsulatus, A.pasteurianus, A.turbidans (Hall et al., 1956).

Goldman et al, (1958) found an unknown substance or

substances in yeast autolysate essential for the growth of A. gluconicus. This compound was stable in acid or alkali, more soluble in ethanol or acetone than in chloroform. It was extracted with ethyl ether at pH 2.

3.2.2. Amino acids

The amino-acid requirements of A.suboxydans were determined by Stokes and Larven (1945). Valine, isoleucine, alanine and histidine were sufficient to support the growth of the organism; but growth was improved upon the addition of cystine or methionine.

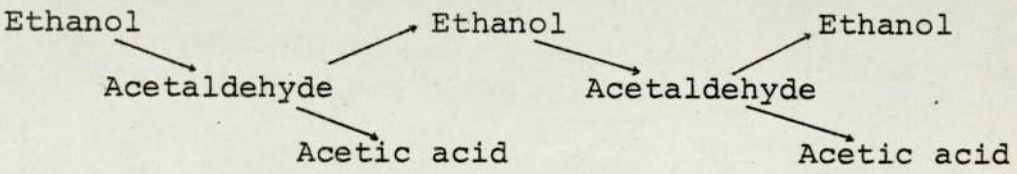
Ammonium sulphate alone did not support the growth of A.suboxydans, but showed a stimulatory effect when added to suboptimal concentrations of the required amino acids. Jlli et al. (1965) reported that two strains of Acetobacter required alanine and one strain of Gluconobacter required cystine when glucose was the carbon source. When ethanol was the carbon source, four of seven Acetobacter strains required alanine. The amino acid requirement of Gluconobacter was studied by Yamada et al. (1965). They found that when carbon source was glucose, or fructose, or sorbitol, or ethanol, the Gluconobacter strains required glutamic acid, essentially or accesorily.

3.3. BIOLOGICAL OXIDATION OF ETHANOL TO ACETIC ACID

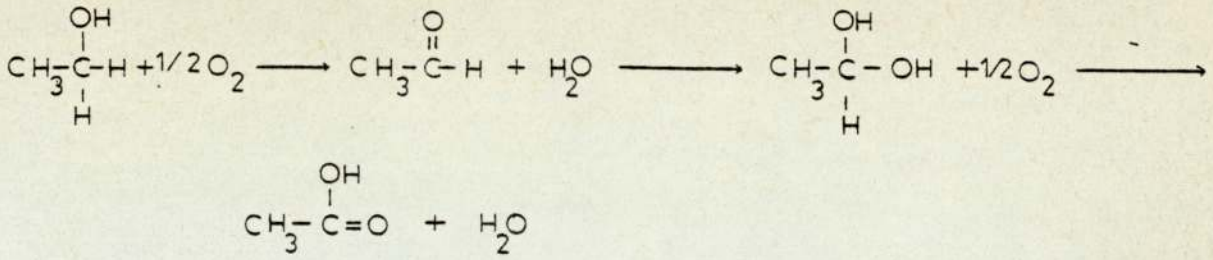
3.3.1. Mechanism of the Biological oxidation of Ethanol to Acetic Acid

The conversion of ethanol to acetic acid by bacteria was first noticed by Pasteur (1868) when he found that the reaction required the presence of oxygen. In 1897, Henneberg found that the oxidation of ethanol to acetic acid was carried out in two steps: the first step was the oxidation of ethanol to acetaldehyde, and the second step was the oxidation of acetaldehyde to acetic acid. (Neuberg and Windisch, 1925). The oxidation of acetaldehyde is a dismutation reaction in which one mole of acetaldehyde is reduced to ethanol while another mole is oxidized to acetic acid (Neuberg, 1928).

Previously, Neuberg and Morinari (1926) showed that only 75% of the acetaldehyde is converted to acetic acid and 25% to ethanol. Therefore, the process of the conversion of ethanol to acetic acid consists of an oxidation and a dismutation which are produced side by side.

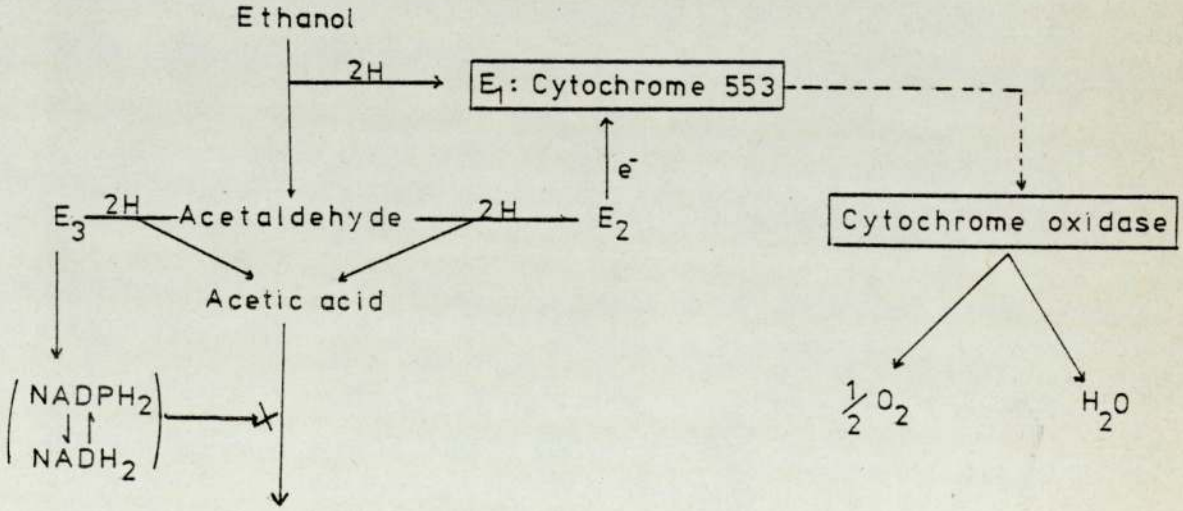


In 1928, Wieland and Bertho, proposed another scheme for the oxidation of ethanol to acetic acid, because they observed an extremely low activity of dismutation in A. orleansis, A. ascendens and A.pasteurianus.



Jones and Greenshields (1970a,b) suggested that a sequential oxidation reaction mechanism took place, since they detected only low concentrations of acetaldehyde (less than 84 mg per 100 cc) during the acetification process.

Nakayama (1961a,b)by combining all the available knowledge with his own results, concluded the following scheme for the oxidation of ethanol by acetic acid bacteria. (Figure 3.1)



E_1 = alcohol-cytochrome-553 reductase

E_2 = coenzyme-independent aldehyde dehydrogenase

E_3 = NADP-dependent aldehyde dehydrogenase

Figure 3.1. Oxidation of ethanol by Acetic acid Bacteria (Acetobacter) (After Nakayama, 1961a,b).

With the help of E_1 which is an alcohol-cytochrome-553 reductase, ethanol is oxidized to acetaldehyde. The electrons which are produced from the oxidation are delivered by the heme iron of the cytochrome-553 which is the E_1 itself. There are two ways for the resulted acetaldehyde to be oxidized further, by E_2 or by E_3. The liberated electrons are transferred to the heme bound to E_1, cytochrome-553 or via E_3 they reduce NADP. The resulting electrons are transferred to a cytochrome oxidase by the reduction of the cytochrome-553. The cytochrome system operates in the ethanol and acetaldehyde oxidation by E_1 and E_2 whereas the $NADPH_2$ produced by E_3 inhibits further oxidation of acetic acid through the tricarboxylic acid cycle by upsetting the equilibrium $NADPH_2 \rightleftarrows NADH_2$ (Nakayama, 1959).

Also, Nakayama, 1959, suggested that the large quantities of acetic acid in the medium as a metabolic intermediate of ethanol can be explained with two hypotheses:

1. "Bound coenzymes" in the cells of Acetobacter were all used for the oxidation of ethanol to acetate, thus making the enzymes of acetate oxidation nonfunctionable, and
2. The odixation of ethanol to acetate and the further oxidation of acetate known as peroxidation (overoxidation) are regulated in an unknown way by particle-bound enzymes, coenzymes and the cytochrome system.

3.3.2. Factors affecting the oxidation of ethanol to acetate

3.3.2.1. Optimum pH of E_1 and E_2 (Figure 3.1.)

The pH which is in favour for the accumulation of acetic acid must also favour the functioning of E_1 and E_2 systems. Nakayama (1959) found this to be acidic. Also,

Dupuy and Margenet (1962) found in A.rancens fermentation that the cells grown at pH 3.8 and 6.0 were able to oxidise ethanol.

3.3.2.2. Temperature

In experiments of Alian et al (1963a, b) it was shown that the optimal temperature for submerged cultures of Acetobacter aceti depend on the acidity of the medium. At the temperature of 22°C, growth and acid production rates were slowed up. Also, the total concentration of acid in the medium was dependent on the temperature of the bacterial culture.

They found also, that the maximum specific growth rate was only apparent within the range of 3-4.5% w/v concentration of acetic acid in the medium. In concentrations higher than 8% w/v, the death rate of the bacteria increased and less than 10% of the cells survived.

3.3.2.3. Aeration

Hromalka and Ebner (1950) noticed that acetic acid bacteria have a considerable requirement for oxygen in submerged cultures. They noticed that, because the bacteria utilize constantly the dissolved oxygen of the medium, continuous aeration is necessary. The demand for oxygen has been shown by the same workers in experiments in which they found that interruption of aeration for more than 30-60 secs. leads to deterioration of the acetic acid bacteria and to their death.

In the work of Gigineishrilli (1960) on the effect of

aeration conditions on the intensity of the oxidation process in vinegar manufacture, it has been shown that when the natural aeration was replaced by a forced aeration, the mean duration of the production cycles could be decreased from 119-158 to 88-106 hours.

Since acetic acid is a growth-associated product, many workers appeared to study the aeration conditions on the vitality of the acetic acid bacteria. (Cohee and Steffen 1959, Allgeier and Hildebrandt, 1960).

In these works it was noted the qualitative dependance of the vitality of acetic acid bacteria on the aeration, and they observed the high demand of these bacteria for oxygen.

Alian et al (1963b) studying the submerged cultivation of acetic acid bacteria in different aeration rates, found that when the cultivation was carried out in flasks or in a fermenter with air access and a stirrer, the specific growth rate was directly proportional to the aeration rate. When the aeration was increased in the range of 0.5 to 8.8 g O_2/ litre/h, the specific growth rate was increasing from 0.038 to 0.08(h^{-1}). The organism was the A.aceti and the total acidity was 5% w/v.

3.3.2.4. Total acidity

(Inhibition effects)

Total acidity is the amount of acid (in terms of acetic acid) which exists in the medium plus the amount of ethanol converted into acetic acid.

The total acidity has been found to act inhibitorily against

the growth of the bacteria of acetic acid. Both ethanol and acetic acid are growth inhibitors of bacteria in the concentrations they are used for vinegar production (Divies, 1973).

Divies (1973) found that fluctuations in population and total acidity varied according to the dilution rates in continuous stirred tank reactors (C.S.T.R.) in fermentation with A. mesoxydans.

In 1963a, Alian et al also had found that the process of alcohol oxidation by A.aceti depends on the concentration of acid. Bacteria in a medium with low acidity propagate vigorously and oxidize alcohol. With an increase in acidity to 4-5% w/v, the growth rate and the alcohol oxidation rate become low, as they were inhibited by the high acidity.

In the literature there are indications of the ability of the acetic acid bacteria to adapt in high concentrations. These bacteria become acclimatized to quantities of acetic acid, which increase as the alcohol is oxidized, with the result that the adapted bacteria tolerate concentrations of it which if added at the beginning of the culture would preclude the possibility of growth of the bacteria (Janke, 1921; Manteifel, 1939; Shaposhnikov, 1948 Greenshields, 1977).

3.3.3. Peroxydation or Overoxydation

Peroxydation (or overoxydation) is the phenomenon. according to which, a further oxidation of acetic acid to CO_2 and H_2O by acetic acid bacteria occurs. In the manufacture of vinegar the peroxidation of alcohol is extremely undesirable since it lowers the output of vinegar manufactures.

The problem of the conditions under which the acetic acid

bacteria complete the process of the oxidation of ethanol to CO_2 and H_2O is complex. According to Dupuy (1958); Nakayama (1959) & Shimwell and Carr (1959), when any amount of ethanol exists in the medium the bacteria cannot metabolize acetic acid. However, there are other data indicating that acetic acid can be utilized in the presence of ethanol (Antoniani et. al., 1950; Loitsyanskaya, 1955). By cultivating acetic acid bacteria in a defined medium containing alcohol, glucose, glycerine, peptone and yeast extract at pH 3.5, Nakayama (1959) found that the bacteria could oxidize acetic acid at a lower rate than when the bacteria were cultivated in a medium with a supplementation of 2% w/v of acetic acid.

The phenomenon could be explained by adaptation of the bacteria to acetic acid or by natural selection and reproduction of cells better adapted to the acidic conditions. This first explanation has been generalized for all fermentations by Herbert (1977). He mentioned that in order to achieve the production of a particular metabolite the conditions for the fermentation have to conduct from the beginning of the continuous process. The cells carry the parental properties in the whole course of the consequent continuous culture.

Loitsyanskaya and Mamkaeva (1962) found that acetic acid is used by A. aceti not only in energy catabolism but in anabolism as well. In order to avoid peroxidation occurring in vinegar manufacture Loitsyaskaya and Mamkaeva (1962) suggested the ..... p.t.o.

lowering of total acid (as has been defined in paragraph 3.3.2.4), and the addition of carbohydrates. The addition of carbohydrates helps to keep constant the equilibrium of the bacterial mass and the process of oxidation of alcohol to acetic acid involving diauxic phenomena. (Pirt, 1975). The Acetobacter xylinum which is used in industry for the production of vinegar is prototrophic with ethanol and auxotrophic with glucose. (Shimwell, 1957). Also, lactate appeared to be the best source of carbon for the growth of A. suboxydants in comparison with sugars and sugar alcohols and acetic acid. (Rainbow and Mitson 1953). When ethanol is present the assimilation of glucose was inhibited in the experiments of Hall et al. (1956). This confirmed the diauxic growth of certain Acetobacter-strains used in industry. In later experiments of Asai et al. (1962) it was found that the strains which assimilated the ethanol in the presence of glucose were belonging to the genus Acetobacter.

3.3.4. Composition of vinegar

The finished product of the acetification process, the vinegar, apart of the acetic acid which comprises the major constituent, there are several other compounds in smaller concentrations which play important role in the quality of vinegar.

Since the process of vinegar production consists of a double fermentation where the higher alcohols obtained in the initial alcoholic fermetnation are subjected to acetification, it would be expected that a number of alcohols and their esters would be found in the final product.

Also, some of the products which result in the aroma of vinegar come to the finished product from the wort and some of them are formed during storage. Asai and Shoda (1958) and Asai et al. (1964) found in spirit vinegar, ethyl acetate as the major volatile constituent of the aroma producing compounds. Most of the ethyl acetate was found to form during storage, very small proportions of it were formed during the actual acetification process.

The same workers had also found in wine vinegar a number of compounds of which the majority had developed during storage. The compounds namely were: acetoin, iso-amyl alcohol, amylalcohol, iso-butyl alcohol and amylacetate.

Kahn et al. (1966) working with cider vinegar and distilled vinegar detected 12 compounds in the first and 5 compounds in the second respectively. The compounds detected were methanol, ethyl alcohol, sec butyl alcohol and C_5 alcohol, methyl and ethylacetate, ethyl lactate, 3-hydroxy-2-butanone, acetic, propionic, iso-butyric and C_5 acids.

Aurand et al. (1966) studying the volatile compounds of cider, wine, tarragon and distilled vinegars identified 19 compounds in cider vinegar, 17 compounds in wine, 20 in tarragon vinegar and 11 in distilled vinegar. They also found that four compounds were present in all the vinegars. These included acetaldehyde, acetone ethyl acetate and ethyl alcohol.

Aurand et al. (1966) concluded that the most important carbonyl compounds contributing to vinegar flavour were the esters. Since esters are known for their aromatic flavour it was not surprising that they were the largest group of

volatile flavour components. Also, different vinegars could be identified by the relative concentration of the alcohols and esters.

Jones and Greenshields (1969) studied the volatile constituents of malt vinegar using gas liquid chromatography. The subject of the work was the construction of a survey of commercial types of vinegar. The compounds detected were acetic propionic and isobutyric acids, ethyl, n-propyl, sec-butyl, isobutyl acids, ethyl, n-propyl, sec-butyl, isobutyl, iso and n-amyl alcohols, isobutyl, sec-butyl, ethyl and n-amyl acetates; ethyl formate, acetaldehyde and acetoin.

The same workers (1970a) made a comparative study of commercial types of vinegar against a vinegar produced in the laboratory in different stages; immediately after production, after filtration and after 6 months' storage.

A comparison between compounds in the wort and compounds in the vinegar resulted in two compounds, propionic and isobutyric acids, which were present in vinegar but not present in the wort. All the other compounds were present in both.

In a second paper, Jones and Greenshields (1970b) concluded that the process of acetification produces a pattern and the production of volatiles remains the same as far as their number and their concentration is concerned, during storage.

In 1971, Jones and Greenshields made a comparison of malt vinegars producted in a Tower fermenter, in Frings generator and by the Quick process. They found little difference in the composition of the resultant vinegars

in the different methods.

In 1974, Yamazida et al. determined the amino acids of raw materials for the production of vinegar from rice, sake', malt, grapes and apples. They found that these mashes contained alanine, proline, aspartic acid and glutamic acid with smaller quantities of arginine methionine and histidine. The concentration of these amino acids decreased during the acetic acid fermentation, because of bacterial assimilation.

SECTION 4

Section 4. SUBSTRATES USED

4.1. COCOA SWEATINGS

4.1.1. Origin

Cocoa beans are the fruits of the tree Theobroma cocao Linn. The origin of Theobroma cacao L., is believed to be the Amazon and Orinoco. The genus Theobroma belongs to the family Sterculiaceae. This family of 50 genera is distributed in the tropics and subtropics of both hemispheres; estimates as to the number of its species vary from 750 to 1250 (De Witt, 1965). From the members of the family Sterculiacae, the most economically important is the genus T. cacao L. or cocao tree. The cacao tree attains its full height when about ten years old. According to variety and local conditions, a fully grown tree is between 3.5 and 7.5 meters high. The main stem of a young cacao plant may grow to one or two meters in height before branching. The young cacao leaves vary in colour, according to type, from pale green or pink to dark red. Mature leaves are evergreen and sometimes attain a length of 35 cm and a breadth of 7 cm.

4.1.2. Kinds of Cocoa and description of cacao fruits

Numerous varieties of cocoa are cultivated. The most important of these are the 'Criollo' (which means 'native') and the 'Ferastero' (which means 'foreign').

According to the origin of the cacao tree, the cocoa beans have a distinguishing shape which characterizes them.

In general, the cocoa beans when mature, are composed of a fairly thick husk, containing the seeds which are invested by a mucilaginous pulp. The number of seeds varies from twenty to forty and occasionally as many as fifty.

The pulp is developed from the outer integument of the ovule.

Detailed descriptions of different types of fruits are given by Zipperer, (1913); Hart, (1911); van Hall, (1914) and Kaden, (1936). A general idea of the cocoa bean is given by the photo (4.1).

4.1.3. Composition of the Cocoa Beans

a The pulp:

This is the white, or sometimes pale pink, mucilaginous mass in which the beans are embedded. Nicholls (1913) reported the following composition for pulps examined in St. Lucia (Table 4.1)

Table 4.1 Composition of the pulp — per cent

After Nicholls, (1913)

Moisture	79.73-88.5
Albuminoids	00.56-00.72
Dextrose	8.34-13.12
Sucrose	0.40-00.95
Starch	traces
Non volatile acid, calculated as tartaric acid ..	0.25- 0.42
Ion oxide	0.03
Salts (potassium, sodium, calcium, magnesium	0.40- 0.45
Volatile acids & alcohol ..	absent

Hardy (1943) established that the non volatile acid was citric acid and not tartaric acid. The initial pH was 4.0.

b. The Sweatings as a result of the fermentation

After harvesting, the cocoa beans are put in the fermentation boxes, and are covered with banana leaves. The fermentation of the cocoa beans is achieved naturally by yeasts which can grow easily in a medium similar to the one described in Table 4.1.

With the action of pectic enzymes, the cells of the pulp are broken down and a turbid yellow liquid trickles away. These liquids are the 'sweatings'.

The composition of sweatings has been investigated by Knapp (1937) in Trinidad. The samples were after 18, 19 and 40-42 hours' fermentation. The analysis by Knapp gave the following figures (Table 4.2):

Table 4.2. Sweatings composition by Knapp

	18 h	19 h	40-42 h
Specific gravity	1.066	1.050	1.039
Total solids	15.2%w/v	10.9%w/v	7.9%w/v
Vol. acid calculated as acetic acid	1.4%w/v	1.2%w/v	2.0%w/v
Theobromine	-	trace	trace
Caffeine	-	trace	trace

The action of the acetic acid bacteria obviously had started before the forty-second hour.

The type of yeasts which are responsible for the alcoholic fermentation varies in different places.

Knapp's (1937) literature survey includes the following list of yeasts, found in fermenting cacao:

Saccharomyces cerevisiae: reported on Trinidad cacao by Chittenden in 1899

Saccharomyces Theobromae Preyer, discovered by Preyer in 1901

on Ceylon cacao and has been renamed Eutorulopsis theobromae.

Saccharomyces ellipsoideus, and apiculatus: were observed on cacao from Puerto Rico by Loew in 1907.

Saccharomyces anomalus: found by Bainbridge and Davies on Jamaican cacao in 1912.

S.ellipsoideus, S. bussei, S.ellipsoideus var domingensis and Torulopsis lilienfeld-toallii: In fermenting cacao of San Domingo by Giferri (1931).

After the zenith of yeast activity has been passed, a large proportion of the pulp has already trickled away in the form of sweatings. The composition of the remainder is favourable to the growth of bacteria. Most of the bacteria belong to the Acetobacter genus.

Plate 4.1. Cocoa beans before the fermentation process takes place. (Photo by R.N. Greenshields.)

4.2. BEET MOLASSES

Beet molasses is a by-product of the sugar refining industries. The raw material is the roots of the plant sugar beet (Beta vulgaris, Family Chenopodiaceae). The average sugar content of the beet roots is about 15 to 20%. About one-third of the world's sugar production is from beets and two-thirds from cane. The principal beet-sugar growing areas are the U.S.S.R. (European and Asiatic), the U.S.A., the U.K., France, Poland, the Federal Republic of Germany and Czechoslovakia. (Encyclopaedia Britannica).

4.2.1. Molasses composition and quality

The molasses were obtained during 1976 from the British Sugar Corporation refinery at Kidderminster.

Basic analyses gave the following results:

Total reducing substances (after hydrolysis)	54.53 w/w%	(wet weight)
Total nitrogen	1.47 %	(wet weight)
Total solids	61.5%	

During 1966-1972 a detailed research on the quality of molasseswas made by the Warsaw and Krakow yeast and alcohol Research Institute. The results show that a consistent fall in the lower limits of yields, for the production of yeasts and alcohol, which could indicate a deterioration in the quality of raw material. It was suggested that increased content of weedkillers and chemical fertilizers in sugar beet and consequently in the molasses, inhibits the growth of yeasts. It was concluded, that technical progress in the cultivation of sugar beet appears to result in a deterioration of the quality of molasses (Hrobani and Koladziejezak, 1973).

4.2.2. Continuous tower fermentation of molasses

4.2.2.1. Medium preparation

Four different media were prepared for the continuous alcoholic fermentation. The molasses concentration was increasing from A medium to D (see Table 4.2.1.)

The nitrogen source was ammonium sulphate (B.D.H.) and the phosphate was added as sodium dihydrogen phosphate (B.D.H.). The amounts of sulphate and phosphate salts were added according to Coote, (1975).

Media	A	B	C	D
Molasses g/l	169	182	224	265
Ammonium sulphate, g/l	3.57	3.57	3.57	3.57
Sodium dihydrogen phosphate, g/l	1	1	1	1

Table 4.2.1. Media composition for continuous alcoholic tower fermentation (Coote, 1975)
Total solids in molasses 61.5%

The medium pH was adjusted with con. H_2SO_4 to 4.5 before autoclaving at 1.05 Kg/cm^2 for 30 minutes.

The medium was prepared in 20 l aspirators.

4.2.2.2. Size, preparation, and operation of the tower fermenter for the alcoholic fermentation

The fermenter used was a 3 l tower fermenter similar to the one described in paragraph 2.2.4.3. with no air distributor. Before the fermentation was commenced the fermenter was cleaned and afterwards steam sterilized for 24 hours.

A part of the medium (2 l) was pumped into the fermenter

with the help of a peristaltic pump (Watson-Marlow)

The inoculum used was a strain of Saccharomyces diastaticus (CFCC 54) p.107. It was shown by previous workers in the author's laboratories, that mashes containing up to 20 g% w/v sugar could be successfully fermented using the above mentioned strain S.diastaticus (Coote, 1975). The temperature conducted was 28°C and the aeration rate was 0.4 v/v/m. The first stage was aerobic for production of yeast biomass.

The fermentation continued until most of the sugar was utilized. This stage lasted 72 hours. The second stage was anaerobic (Microaerophilic). The aeration rate was decreased to 0.01 v/v/m and the temperature to 25°C. Medium started to enter the fermenter with flow rate of 0.1 l/h in the beginning, increasing to 0.20 l/h after 10 days.

Media of different molasses concentrations were fermented and collected in aspirators of 20 l for the subsequent acetous fermentation. The aspirators with the charging wort were kept in a refrigerator at 4°C.

4.3. MALT

The raw material for the production of malt vinegar was malted barley. Barley is one of the most important cereals and belongs to the genus Hordeum L. of the family Graminae.

4.3.1. Production of charging wort

The production of charging wort was made by the Beechams (Food and Drinks) Ltd., Worcester. There are two ways for the production of charging wort: the batch and the continuous and semi-continuous. An interesting review on the different methods and processes used in brewing has been made by

Hough and Button (1972).

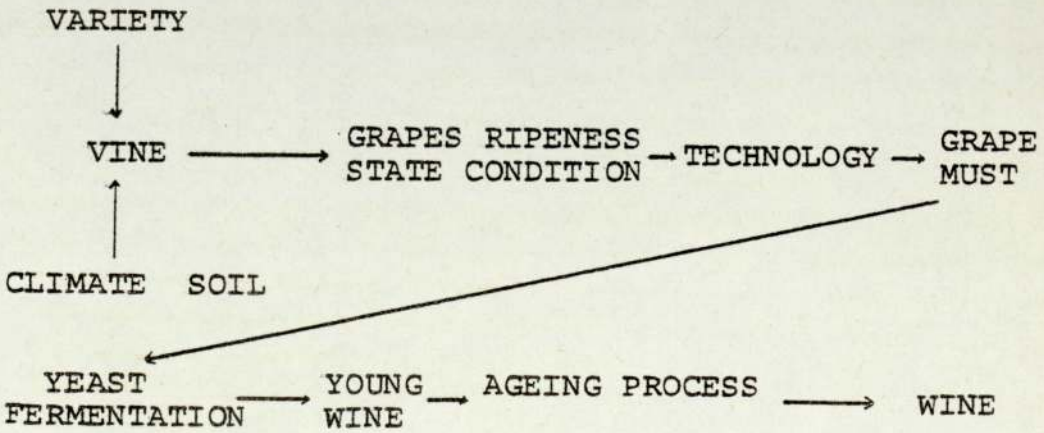
4.4. ALCOHOL FOR SPIRIT VINEGAR

Spirit vinegar is made from pure alcohol. The raw materials used for the production of ethanol were initally molasses. The alcohol was obtained from Hammond Vinegar Company Ltd.

Other sources for the production of ethanol include ethylene, a by-product of oil (Greenshields, 1978). The alcohol from ethylene started to be used as a carbon source for the production of spirit vinegar in the early 1950's. But the idea of producing spirit vinegar from synthetic ethanol has been abandoned by many countries because of the carcinogenic substances that exist in it. (Greenshields, 1978).

4.5. WINE

Wine is the alcoholic liquor which is produced from the fementation of grape juice. The cycle for the production of wine is shown schematically below:



The wine used for the production of vinegar was provided by John H. Swift Wines Ltd. Company (Nelson Lane Warwick).

SECTION 5

Section 5. MATERIALS AND METHODS

The following materials and methods were used in the routine chemical analyses and experiments during this study.

5.1. Alcohol determination

The concentration of ethanol in the fermenter was determined by two methods:

a) The specific gravity which was employed in continuous processes

b) The Widmark method which was used in batch processes for reasons of substrate economy.

a) The specific gravity method

100 cc of the medium was neutralised by 5N NaOH and distilled slowly until approximately 95 cc of distillate had been collected in a 100 cc volumetric flask. The distillate was made up to 100 ml with distilled water and the specific gravity of the distillate measured at 15°C with a Westphal type specific gravity balance (Stanton Type Instruments Ltd.) The concentration of ethanol in the distillate was determined by reference to Thorpe's alcoholometric tables (Thorpe, 1915).

b) Widmark method (Widmark, 1922)

In order to perform this method, special Widmark flasks were constructed. These were 100 cc Erlenmeyer flasks of borosilicate glass with No.B19 standard taper ground glass stoppers. One end of a glass rod (diameter 4 mm) with a small cup at the other end was attached to the bottom of each stopper.

The cup was about 1 cm above the bottom of the flask. Flask and stopper were furnished with glass hooks to permit the

stopper to be held on with rubber bands. The reagents used, prepared according to Lundquist (1959).

The concentration of alcohol in mg per cc of sample was given by the formula (B-A) 11.3 (Lundquist 1959), where B was the mean value of the blank titrations, A is the titration figure for the analysis (in cc of thiosulphate) The original sample was diluted 10 times with distilled water.

5.2. Acetic acid determination

6 cc of sample was titrated against 1N NaOH with phenolphthalein as indicator. The amount of NaOH used for the neutralization of the acid represents the amount of the acid in g per 100 cc of sample (expressed in terms of acetic acid). (Humphreys, 1976). When the substrate was dark coloured, the titration was made with the use of a pH meter and pH-probe.

5.3. Estimation of biomass dry weight concentration (DeMoss and Bard, 1957)

30 cc of sample was placed in preweighed tubes and was centrifuged for 10 min at 10,000 r.p.m. in a M.S.E. bench centrifuge. The supernatant was decanted and the remaining sediment was resuspended in 50 cc of distilled water. After recentrifuging, the new supernatant was decanted and the tube dried to constant weight at 85°C and weighed. The formula (b-a) 33.34 gave the concentration of biomass in g per litre, where a is the weight of tube without bacteria and b is the weight of tube with bacteria.

5.4. Fermented medium total nitrogen determination (Jacobs, 1965)

Three samples of 1 cc each were incinerated in 3 micro-Kjeldahl digestion flasks with added catalyst (potasium sulphate: copper sulphate; selenium; in proportion of 32:5:1 parts by weight and 1 cc concentrated sulphuric acid). The samples were taken from the original medium which had been diluted to a suitable dilution, with distilled water.

Steam distillation of the ammonia in a Markham still was effected by the addition of 4 cc 10N Sodium hydroxyde.

The distillate (approximately 15 cc) was collected in 10 cc saturated boric acid solution. Titration was with 0.01 N HCl, using BDH '4-5' indicator. The result was the titration mean value of the three samples.

5.4.1. Determination of the nitrogen taken up from the medium

A suitably sized sample from the feeding medium was distilled and titrated as above (5.4). The procedure was executed in triplicates. The mean value of the fermented medium total nitrogen was subtracted from the feeding medium total nitrogen mean value. The result was the medium utilized total nitrogen. This was found to have little value because of the high nitrogen concentration in the feeding medium.

5.5. pH measurements

The pH was measured with a Pye Model 98 pH-meter.

5.6. Total reducing substances determination

Total reducing substances in the samples were measured by the ferricyanide method (Somogyi, 1945) after acid hydrolysis, which was achieved by adding 1 cc of concentrated

hydrochloric acid for every 10 cc of sample and heating in a water bath at 95°C for 10 minutes.

5.7. Dissolved oxygen measurement

The dissolved oxygen was monitored by a New Brunswick DO-50 analyser with a galvanic probe (see paragraph 2.2.4.3.)

The calibration of the dissolved oxygen analyser was made as follows:
The zero was set after immersing the probe in a 0.01-M borax solution (pH 9.2) containing 100 mg of sodium sulphate per 5 cc of solution. The point 100 was that where the indicator was stabilized after intensive aeration of the medium. The consequential indications were expressed as per cent of the saturated value of the dissolved oxygen in the medium.

5.8. Medium flow rate or feed rate (f)

The medium flow rate, f, is given by the ratio $\frac{V}{t}$ $(l.h^{-1})$ where V in litres is the volume of the inflowing medium at a time interval, t (in hours).

The flow of medium in the fermenter was maintained constant by a peristaltic pump (MHRE-72) Watson-Marlow Ltd.) fitted with silicon rubber tubing (Jenkins Scientific Ltd., Mark Road, Hemel Hempstead, Herts).

The specifications of the silicon rubber tubing were: 1 mm i.d., 3 mm o.d. During the continuous operation it was found that the tubing had to be changed every 3 days, because if it was left longer, its elasticity deteriorated at the point of contact with the pump. This had a direct effect on the medium flow rate and consequently on the dilution rate

(see next paragraph).

5.9. Medium dilution rate (D)

The medium dilution rate, D, is given by the ratio $\frac{f}{V}$ where f is the feed rate in $l.h^{-1}$ and V is the active volume of the fermenter in litres. The active volume of the fermenter V was measured before the fermentation commenced. For this reason the fermenter was filled with known volume of water and at each aeration rate the remaining water volume measured.

5.10. Specific substrate utilization rate, q_s

The substrate utilization rage, q_s, was calculated by the formula $q_s = D.\Delta S.\frac{1}{X_E}$ where D is the dilution rate in h^{-1} and ΔS is the difference $S_o - S$ where So is the initial substrate concentration in $g.l^{-1}$ and S is the final substrate concentration in $g.l^{-1}$; X_E is the bacterial biomass dry weight concentration in the effluent line, measured in $g.l^{-1}$ (Pirt 1975)

5.11. Specific bacterial growth rate, μ

The specific bacterial growth rate, μ, was calculated by the formula $\mu X_F = D.X_E$ or $\mu = \frac{D.X_E}{X_F}$ (in h^{-1}) where D is the medium dilution rate (in h^{-1} X_F and X_E is the bacterial dry weight concentrations in the fermenter and in the effluent line, respectively (Pannell, 1976).

5.12. Air flow rate measurements

The air flow rate in the fermenter was monitors by a calibrated Rotameter and maintained constant by a subsidiary pressure regulator (for specifications see paragraph 2.3.4.3.)

5.13. Storage of Acetobacter cultures

Liquid cultures directly from the fermenter were kept in conical flasks with wide flat-bottomed surface. The cultures were active up to three months. A long lag phase was noticed when the inoculum originated from the flasks rather than if it were taken directly from acetifiers.

5.14 Storage of the yeast cultures

For the storage of the yeast culture, agar plates and agar tubes were prepared as given below:

Malt extract (oxoid)	3 g
Yeast extract (oxoid)	3 g
Glucose	10 g
Mycological peptone (oxoid)	5 g
Agar	2 g
Distilled water	1 l

5.15. Foam control

The control of the culture foam was affected partly by the configuration of the fermenter. The upper part of the fermenter which consisted by an expansion of the tubular lower part was found to affect the production of foam considerably. The reason was that the superficial gas velocity, at the expansion was smaller than that at the tubular part (see paragraphs 6.1.11 and 6.2.10).

In cases where the foam did not cease with physical methods, the addition to the fermenter of a silicon-based antifoam agent (Sillcolapse437 I.C.I. Ltd., Piccadilly, Manchester) was necessary.

The supplied antifoam before use was diluted with an equal

volume of distilled water. After autoclaving, the oil-based carrier compound was removed and the remaining solution re-autoclaved. The antifoam was injected into the fermenter as required. The use of antifoam was kept to a minimum. (In the range of 0.02 to 0.04 $g.l^{-1}$.)

SECTION 6

COCOA SWEATINGS VINEGAR

RESULTS AND DISCUSSION

6.1. PRODUCTION OF COCOA "SWEATINGS" VINEGAR IN CONTINUOUS TOWER FERMENTER

6.1.1. Medium examination and preparation; Fermenter design

In Section (4.1), the origin and the derivation of the cocoa "sweatings" have been described. The fermented juice before the acetification experiments had commenced, was analysed for the determination of ethanol, reducing sugars, acid, (expressed in terms of acetic acid), suspended solids and NH_4 nitrogen. The results of the analysis are shown in table (6.1.1).

Compounds	Concentration ($g.l^{-1}$)
Ethanol	38.5
Acetic acid	18.5
NH_4-Nitrogen	0.1
Total reducing sugars	4.0
Suspended solids	72.0

Table (6.1.1). Composition of the Cocoa "sweatings" effluent as was delivered from Malaysia. The pH was 4.3.

Also, a microbiological examination of samples from cocoa "sweatings" gave a variety of microorganisms with predominant species the Acetobacter. There were also many yeasts (section 4.1.4). The conclusion of the chemical and microbiological analyses was that the given substrate was fermented to completion as far as the alcoholic fermentation was concerned because the level of the concentration of the reducing sugars was low in comparison with the high concentration of ethanol.

The presence of the acetic acid bacteria, the amount of acetic acid (1.85% v/v) and the low pH, were the indicators that the acetic acid fermentation had started but slowed down, presumably because of the inadequate supply of oxygen in the fermentation 'boxes' (section 4.1).

The high concentration of ethanol in comparison with that of acetic acid, also the amount of ammonia nitrogen proved the cocoa "sweatings" effluent was suitable as an acetic acid fermentation medium.

In order to stabilize the substrate, the medium was centrifuged to remove the suspended solids and the existing microbial population.

The fermentation system used has been described in section (2.3.4.3). Because of the small amount of medium available, the volumetric capacity of the fermenter used was 1 litre.

6.1.2. Preliminary fermentation (cocoa "sweatings")

A preliminary fermentation for the preparation of active inoculum was made and found useful for the following total programme of the cocoa "sweatings" fermentation design.

According to the preliminary fermentation results, it was found that the limit of the aeration rate was 0.7 v/v/m (s.g.v. 0.73 $cm.sec^{-1}$) and beyond that point, vigorous foaming occurred. A change of the medium colour was seen during the preliminary fermentation (from light yellow to brown) thus, it was necessary to determine biomass concentrations with methods other than the optimal density method.

6.1.3. EFFECTS OF TEMPERATURE ON THE PRODUCTION OF COCOA SWEATINGS VINEGAR IN TOWER FERMENTERS

In growth associated products, the effects of a variable on the growth rate of the bacteria, has an indirect effect on the formation rate of the product. In the case of temperature, the effects on the growth rate of the bacteria occur in two ways:

1. With an increase of temperature, the growth rate increases and subsequently the metabolic activity for the production of metabolites increases.
2. Yet again, the increase of temperature reduces the oxygen solubility of the medium and if the aeration of the medium does not substitute the consumed oxygen, the growth rate of the bacteria slows down.

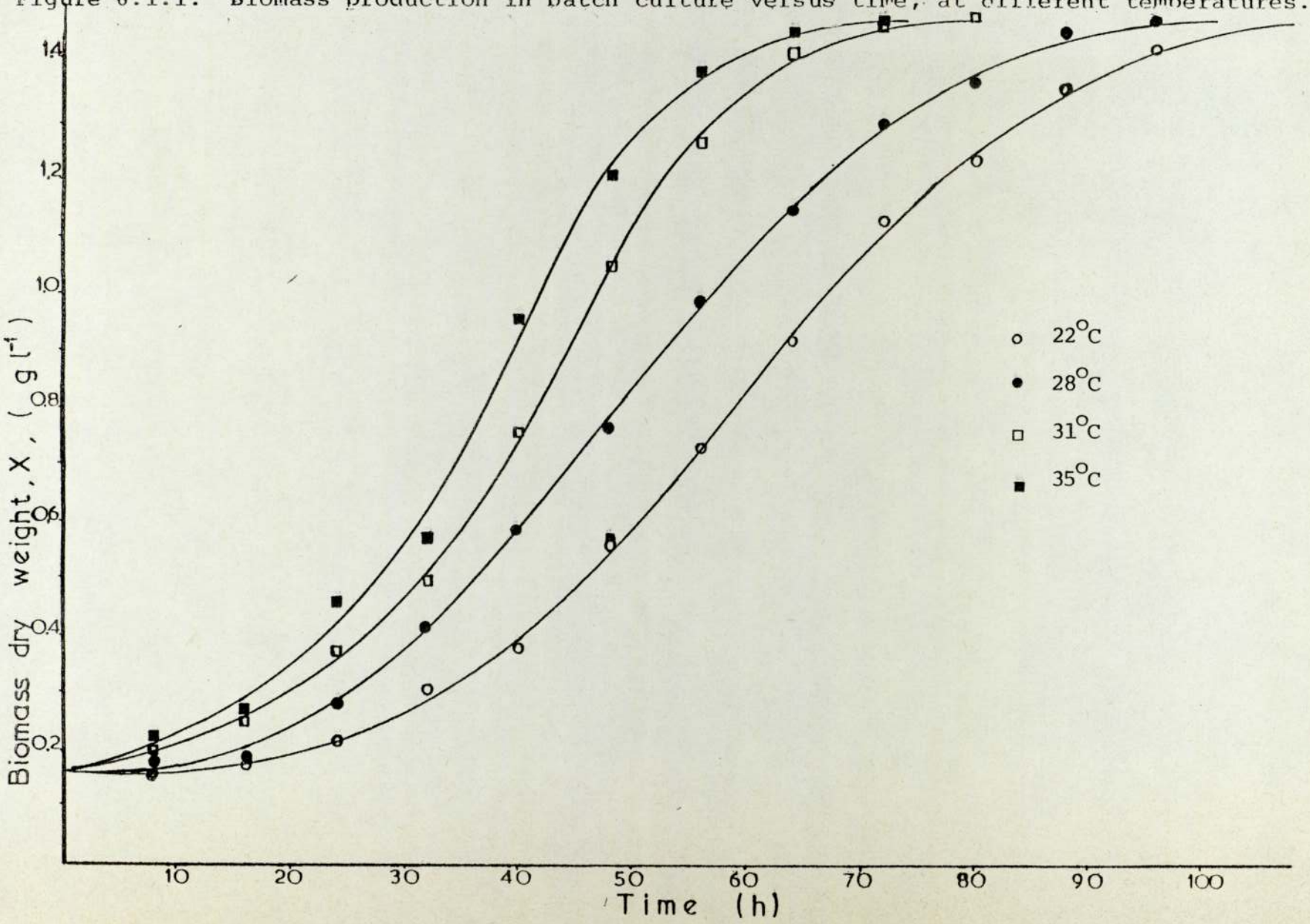
Four different temperatures were tested, under constant conditions of aeration and substrate concentration.

When the aeration rate was selected, the important criterion was to avoid excessive culture foaming. Accordingly, an aeration rate of 0.2 v/v/m (s.g.v. = 0.21 cm. sec^{-1}) was chosen, so the use of antifoam for the temperature experiment was kept to the minimum. (0.02 g/l)

The temperatures tested were 22, 28, 31 and 35°C, to include the optimum temperature (30°C for the genus Acetobacter (Bergey's Manual of Determinative Bacteriology, 8th Edition, 1974). (De Ley 1960, 1961, 1962).

Figures (6.1.1)-(6.1.14): Tables (6.1.1)-(6.1.8).

Figure 6.1.1. Biomass production in batch culture versus time, at different temperatures.



6.1.3.1. Batch cultures

It has been recognized, by Bull and Bushell (1975), that the closed culture systems (paragraph 2.1.) are most unsuitable to determine the effects of temperature of microbial growth because changes in temperature produce simultaneous changes in other batch culture variables (see paragraph 6.1.2). There are also changes in the growth yield and maintenance energy. This point has been discussed further in paragraph (6.1.3.2.5).

The use of the batch culture data, discussed below, are for qualitative elucidation of temperature effects on the cocoa vinegar production. Although the quantitative results were extracted from the continuous culture, some of the batch data results played an important role for comparison studies and were also used as a tool for the design of the continuous culture experiments.

6.1.3.1.1. Effect of temperature on the product formation rate and bacterial growth rate.

Effects on lag phase duration (cocoa "sweatings")

In figure 6.1.1, results are shown for the biomass concentration when the 1 l fermentor was operating in a batch mode. The development of the biomass was considerable after the first 25 hours. This is a characteristic property of the acetic acid bacteria. Long lag phases is a problem which exists in the vinegar industry (Greenshields, 1977).

The duration of the lag phase increases as the temperature is reduced (Figure 6.1.1). The results for the experiment of the effect of temperature in the duration of lag phase are shown in table 6.1.2.

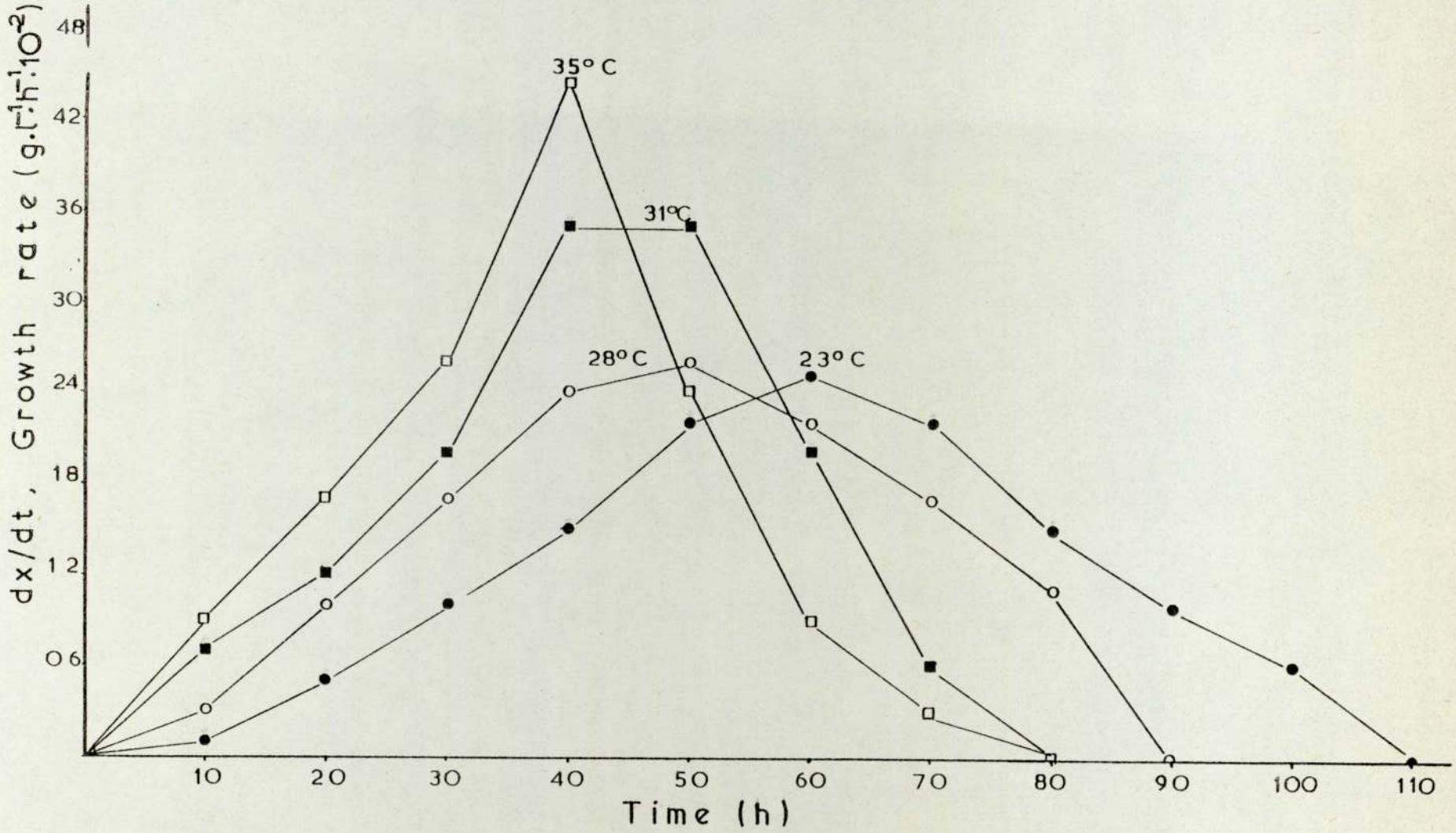


Figure 6.1.2. Bacterial growth rates during batch culture cycles. Cocoa "sweatings"
Aeration rate 0.2 v/v/m

Temperature ^{o}C	Duration of lag phase (h)
23	26
28	16
31	8
35	6

Table 6.1.2. Effects of the temperature on the duration of the Lag phase in cocoa 'sweatings' vinegar batch fermentation Aeration rate 0.2 v/v/m (S.G.V. 0.21 cm sec^{-1})

The temperature also appears to have a strong influence on the growth rate. At the temperature of 35°C, the maximum growth rate appears at the 40th hour and at the temperature of 23°C at the 60th hour (Figure 6.1.2)

This means that, as the temperature increases from 23°C to 35°C, the duration of the batch culture (keeping constant all the other parameters) is reduced from approximately 110 to 80 hours.

Similar results were found in Staphylococcus in peptone medium. The increase of temperature resulted in a decrease in the duration of the lag phase (Cooper, 1963). An explanation of these phenomena has been given by Hunter and Rose (1972) who found changes of the cell composition with changes in temperature. The changes occurred mostly in protein lipid and RNA content of the cell. They also noticed changes in the structure of certain cell components. There is probably a continuous change of the membrane lipid structure with

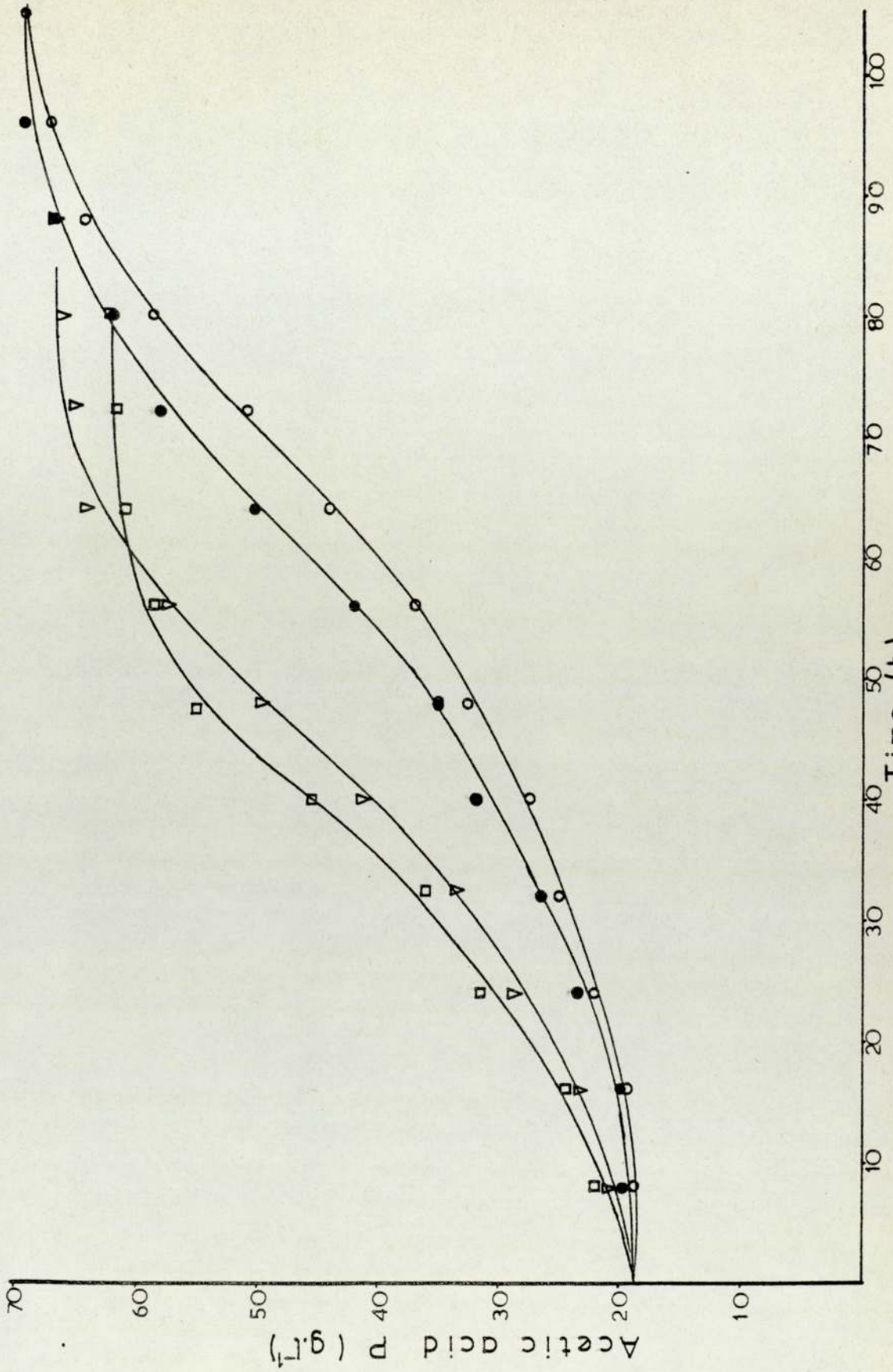


Figure 6.1.3. Production of acetic acid in batch culture at different temperatures.

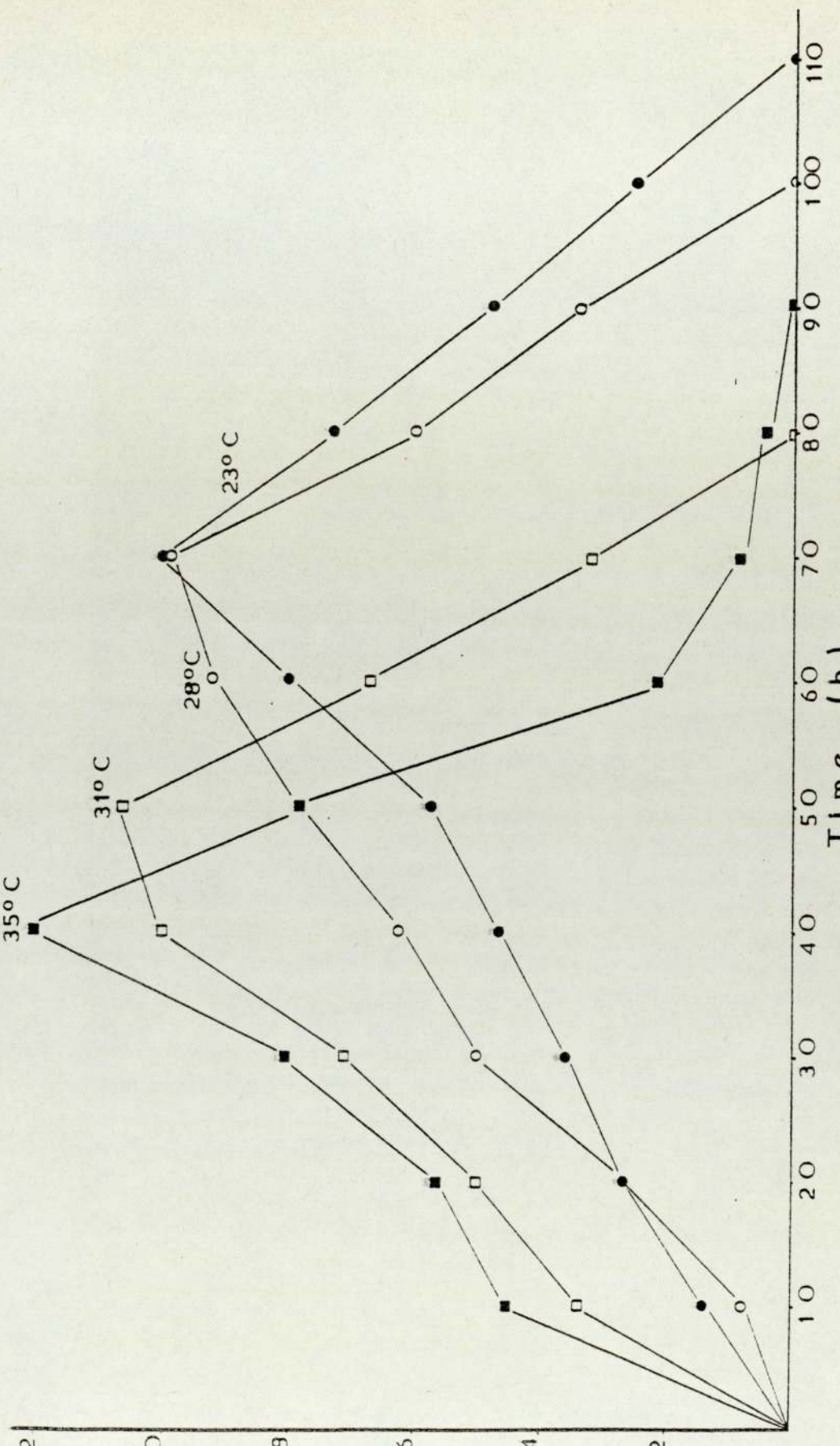


Figure 6.1.4. Effects of temperature on the product formation rate in batch culture.

Cocoa "sweatings"

Aeration rate 0.2 v/v/m

change of temperature to maintain the lipid function. Pirt (1974) suggests that sometimes breakdown of membrane structure by the temperature changes might cause loss of metabolites and stimulate their over production.

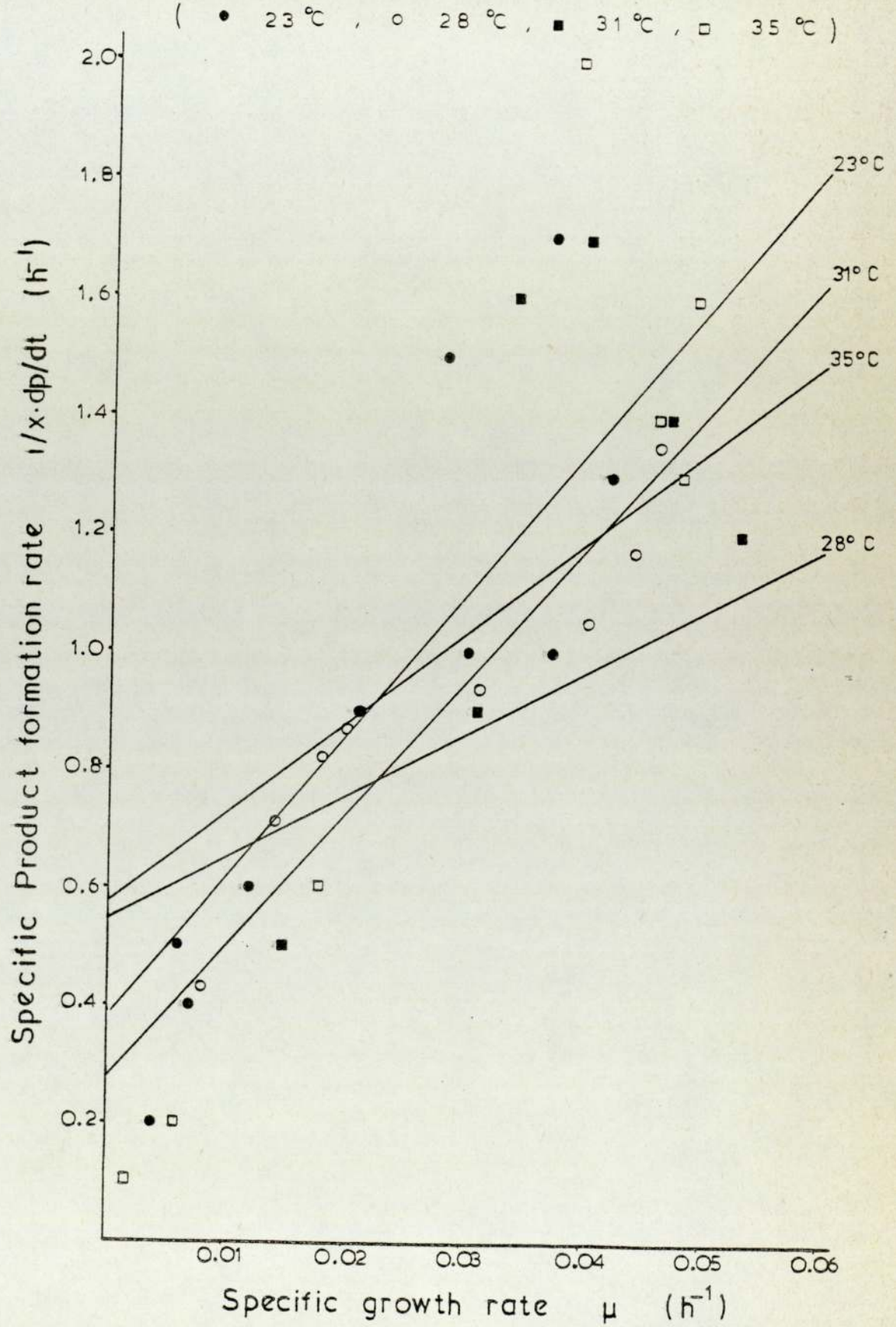
Because the acetic acid is a growth associated product (Divies 1973; Aiba et al 1973), its development follows the same pattern as that of the biomass. The development of the acetic acid in cocoa vinegar fermentation is shown in figure 6.1.3. The growth curves for the different temperatures are parallel to the product concentration curves, for the same temperatures respectively (Figures 6.1.1, 6.1.3).

The product formation rate, $\frac{dP}{dt}$, (where P is the product concentration in the fermenter) is higher for higher temperatures (Figure 6.1.4). The rate of product formation occurs at the same physiological phase where the growth rate ($\frac{dx}{df}$) has its maximum value, Table (6.1.3). The differences at the temperatures 23°C and 28°C are not substantial if the whole of the period of the fermentation is considered.

Temperature °C	Time in which maximum growth rate appears (h)	Time in which maximum product formation rate dp/dt appears (h)
23	70	60
28	70	50
31	50	50
35	40	40

Table (6.1.3) Effect of temperature on the time that the maxima of growth rate and product formation occur. Cocoa sweatings. Aeration rate 0.2 v/v/m, mean s.g.v. 0.21 cm sec^{-1}.

Figure 6.1.5. Specific product formation rate versus specific bacterial growth rate at different temperatures.



6.1.3.1.2. Relationship between specific product formation rate (K) and bacterial specific growth rate (μ) at different temperatures (cocoa "sweatings")

The dependence of product formation on biomass growth, for lactic acid formation has been discussed in detail by Lu̇deking and Piret (1959 a,b).

Regression analysis gave the regression equations which are represented by the straight lines in figure (6.1.5). The dependence of product formation rate from both the instantaneous growth rate and from the biomass concentration found by Divies (1973), has been confirmed. Divies (1973) found that the relationship $\frac{1}{x}\frac{dP}{dt} = a\frac{1}{x}\frac{dx}{dt} + b$ is valid for acetic acid fermentations, working in defined medium.

In Table (6.1.4), are the values of the constants a and b for the different temperatures.

Temperature ^{o}C	a	b (h^{-1})	Degrees of freedom n	Correlation coefficient r	Probability p
23	24.5	0.35	9	0.840	0.01
28	10.5	0.55	8	0.674	0.05
31	24.5	0.29	6	0.792	0.02
35	14.7	0.59	6	0.448	less than 0.1

Table (6.1.4). Regression analysis values for the constants a, b in equation $\frac{1}{x}\frac{dP}{dt} = a\frac{1}{x}\frac{dx}{dt} + b$ for different temperatures.

Substrate: Cocoa sweatings.

Aeration rate: 0.2 v/v/m (mean s.g.v. 0.21 cm sec^{-1})

For the temperatures of 23, 28 and 31°C, the regression analysis resulted in a strong positive correlation between

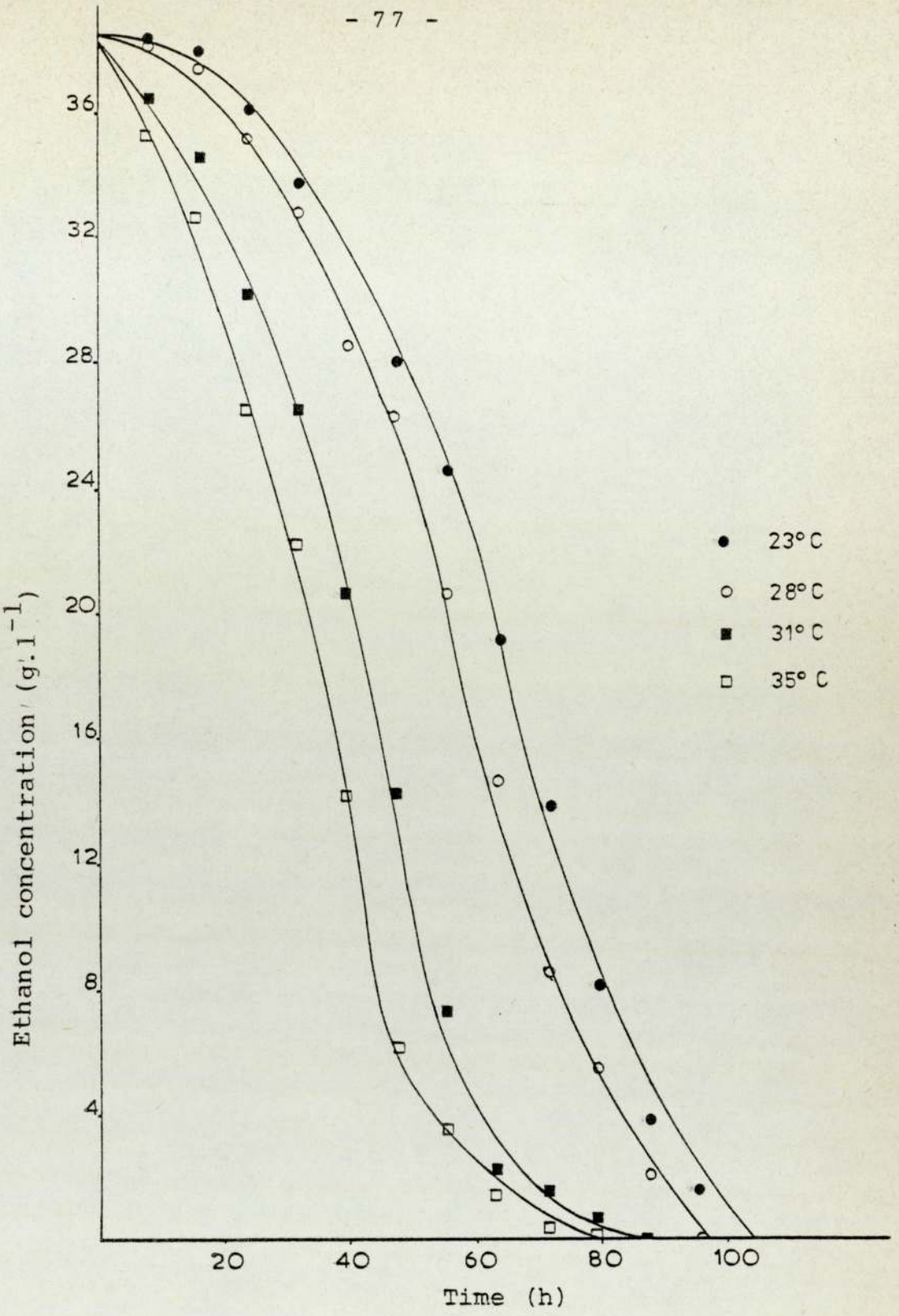


Figure 6.1.6. Substrate concentration during batch culture, at different temperatures.
Substrate: ethanol (cocoa 'sweatings')
Aeration rate 0.2 v/v/m.

specific product formation rate (k) and specific bacterial growth rate (μ). At the temperature of 35°C, the probability of correlation of the K and μ found to be less than 0.1. This can be explained by substrate loss due to evaporation which at the temperature of 35°C is 0.6 $g.l^{-1}.h^{-1}$ (given with the use of condensers). More details are given later (paragraph 6.1.5).

At the temperature of 35°C, 50% w/v of the ethanol was utilized at the 35th hour, and at the 23°C the utilization of 50% of ethanol was at the 64th hour (Figure 6.1.6).

This conclusion resulted from the observation that the substrate was utilized faster at higher temperatures than at lower temperatures. Without condensers, both ethanol and acetic acid were lost due to evaporation during the fementation with all the temperatures which have been tried.

The evaporation of ethanol was so great that it was not possible to monitor the batch fermentation in tower fermenters without the use of double system of condensers.

With the use of condensers and the introduction of water saturated air (paragraph 2.3.4.3), the evaporation of both substances ceased at the temperatures of 23 and 28°C. At the temperatures of 31°C and 35°C, it reached the level of 0.014 $g.l^{-1}.h^{-1}$ and 0.06 $g.l^{-1}.h^{-1}$ respectively.

6.1.3.2. CONTINUOUS CULTURE

6.1.3.2.1. Effect of dilution rate on ethanol conversion to acetic acid and on the biomass concentration in the fermenter at different temperatures (Cocoa "sweatings")

After each batch culture for each temperature, the

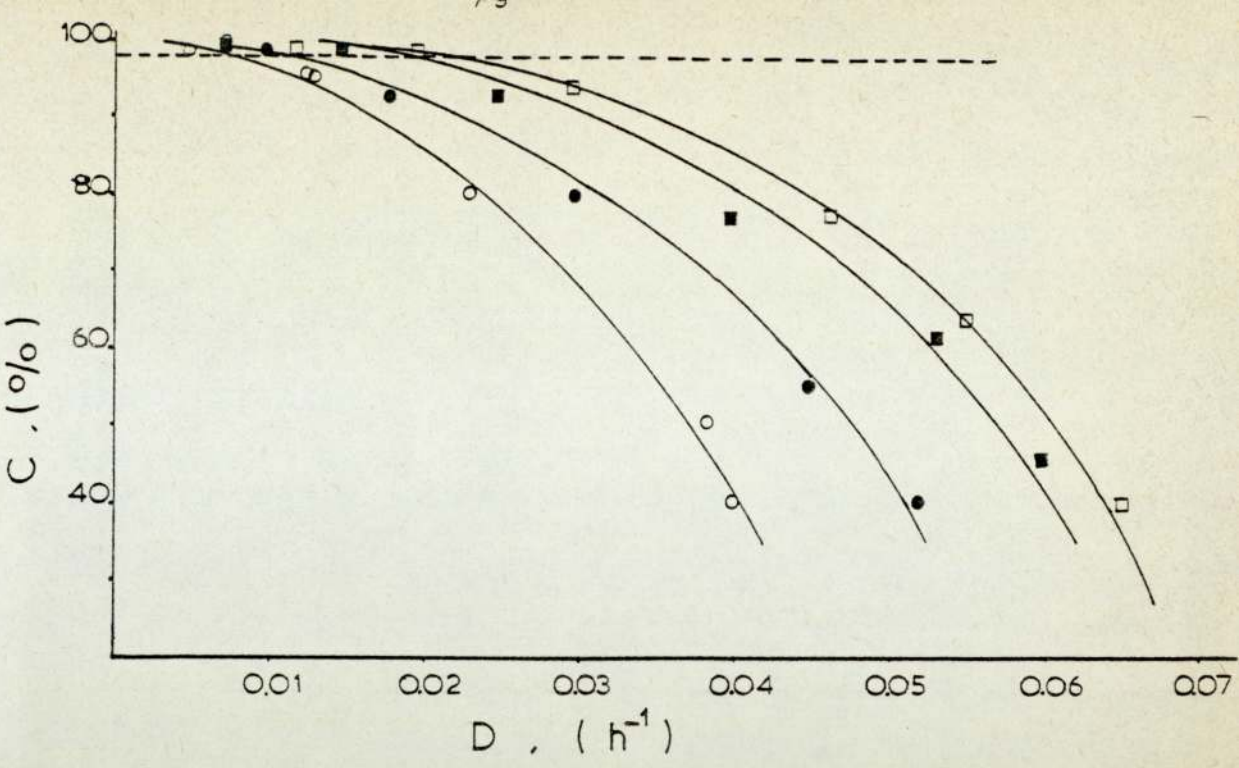


Figure 6.1.7. Effect of temperature on the conversion of ethanol to acetic acid during continuous vinegar fermentation of cocoa 'sweatings'

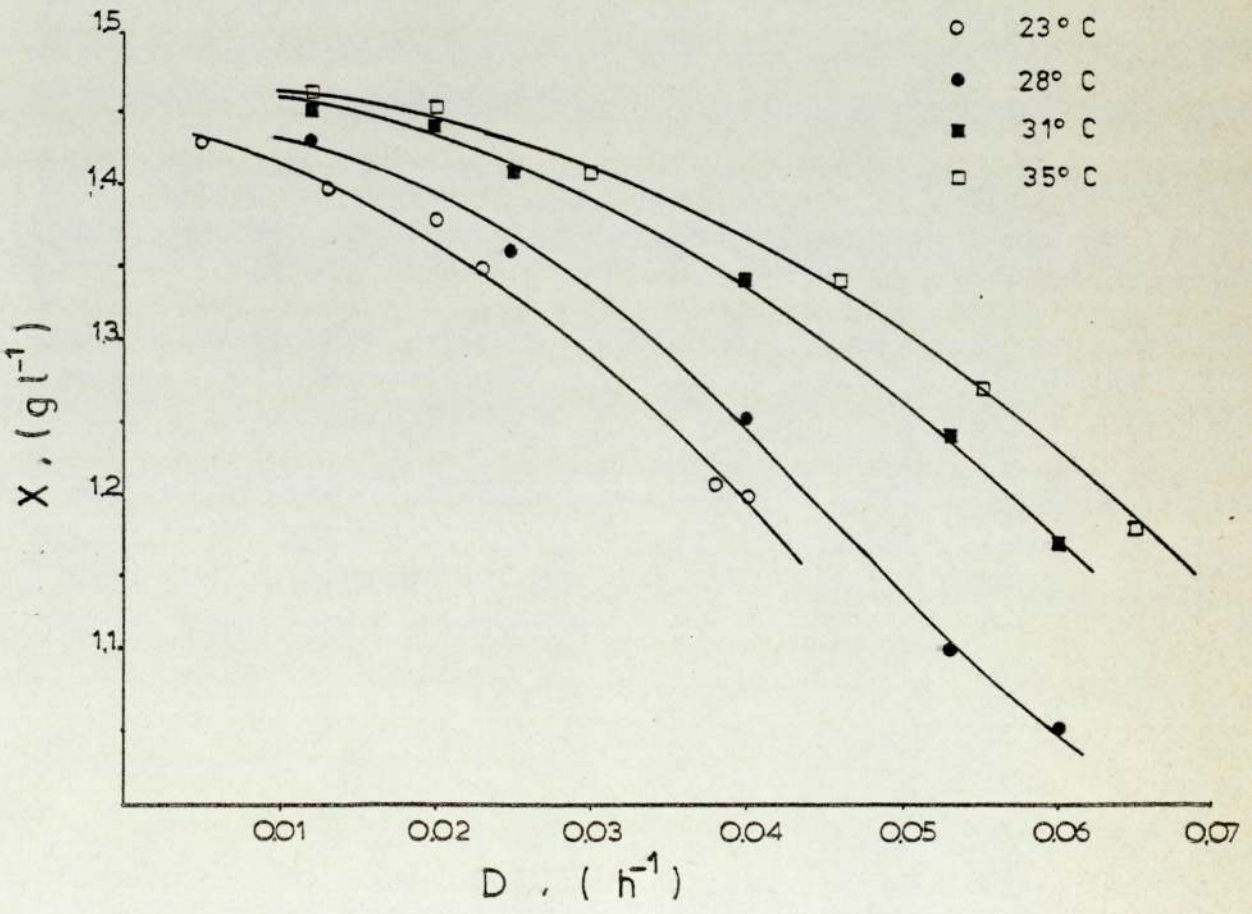


Figure 6.1.8. Effect of temperature on the biomass concentration in the fermenter during continuous vinegar fermentation of cocoa 'sweatings'

experiment was followed by continuous fermentation experiments (CTF). The observations from the conversion of ethanol to acetic acid in the CTF agreed with those of the conversion in batch culture. The higher the temperature the easier the fermentation proceeded.

In figure (6.1.7), 'washout curves' which represent loss of conversion ability of ethanol are given for the temperatures of 23, 28, 31 and 35°C.

Temperature °C	Medium dilution rate (h^{-1})	Medium residence time (h)
23	0.008	125.0
28	0.011	90.9
31	0.019	52.6
35	0.022	45.5

Table (6.1.5). Medium dilution rates and residence times for 97.5% conversion of ethanol to acetic acid, for different temperatures.
Substrate: Cocoa sweatings
Aeration rate: 0.2 v/v/m
Mean s.g.v.: 0.21 cm sec^{-1}

The conversion of ethanol in the CTF follows smooth biomass wash-out patterns as the dilution rate increases. This probably occurs because acetic acid is a growth associated product (figure 6.1.5). However, the acetic acid is both partially growth associated and partially biomass concentration associated, the wash-out of the biomass affects the specific product formation rate, $\frac{1}{x_E} \cdot \frac{dP}{df}$, and as a consequence, the ratio of instantaneous acid concentration over

the total acid, i.e. ethanol conversion to acetic acid. The biomass concentration in the tower fermenter follows a different pattern from that of a chemostat (Figure 6.1.8).

In the continuous tower fermenters, the weight and thus the internal recycle of the biomass result in different kinetics from that in continuous stirred reactors, where there is a perfect homogeneity.

Since the bacterial weight is not substantial, the curves of bacterial concentration in the fermenter for the different dilution rates in CTF do not decline from the theoretical chemostat curves as much as those at fungal biomass (Greenshields and Pannell, 1974).

Again, since acetic acid is an inhibitor (as is also ethanol), Divies, (1973), the biomass concentration versus dilution rate follows the theoretical chemostat curves for competitive inhibition (Figure 6.1.9).

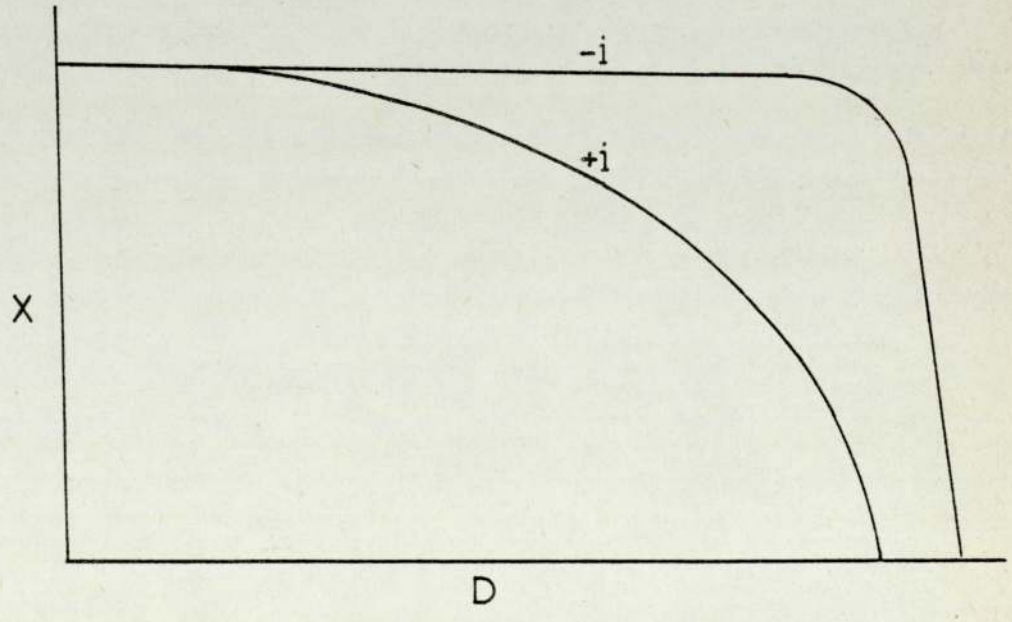


Figure 6.1.9. Effect of competetive inhibitor added to culture medium of chemostat culture (+i with inhibitor, -i without inhibitor.) (The product yield is considered constant.) (Pirt, 1975)

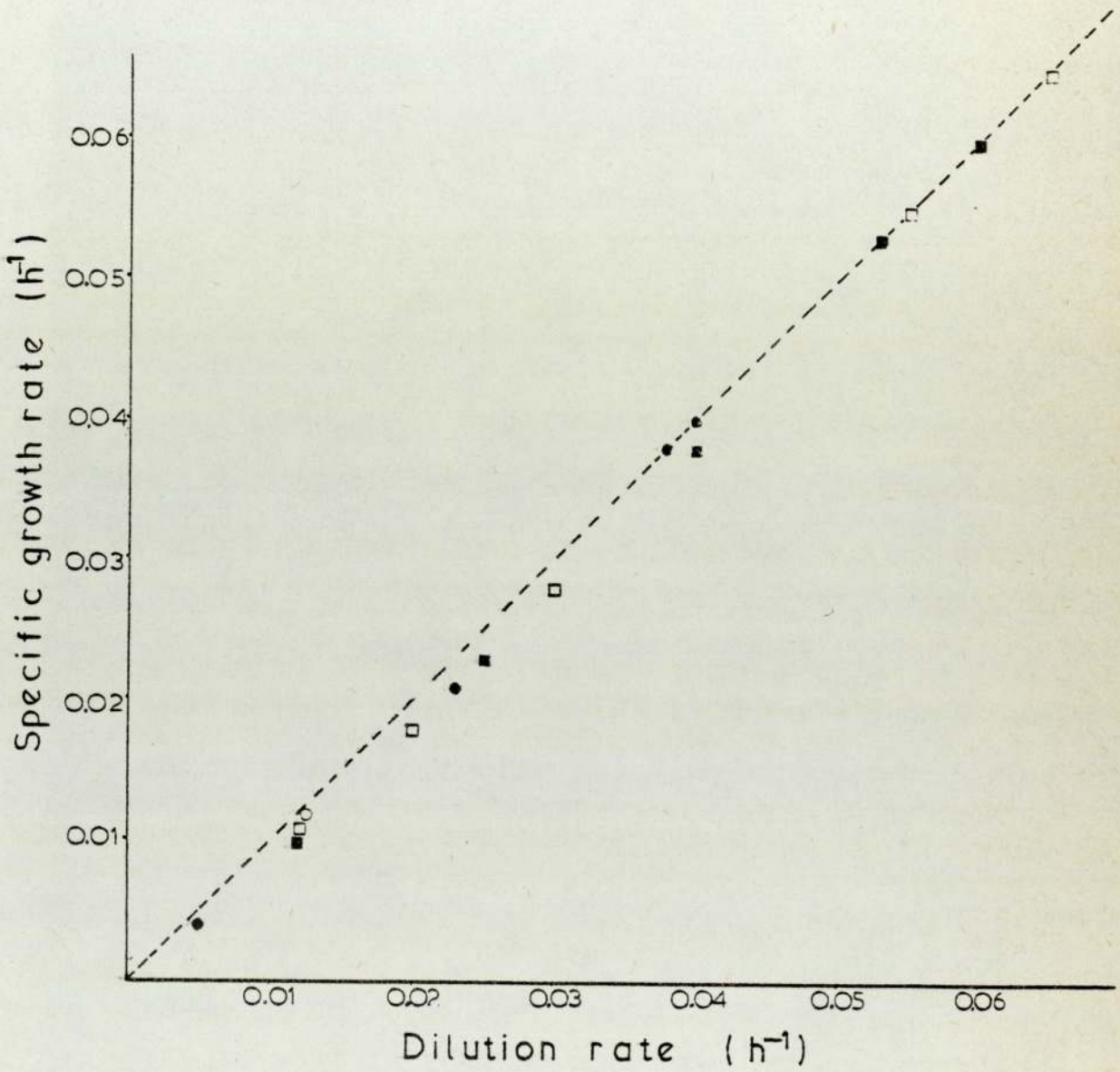


Figure 6.1.10. Relationship between specific growth rate, μ, and dilution rate at four different temperatures.

● 23°C, o 28°C, ■ 31°C, □ 35°C

The broken line represents the conditions where:

$$X_{ferm.} = X_{eff.} \quad \text{or} \quad D = \mu$$

6.1.3.2.2. Effect of dilution rate on the bacterial specific growth rate

The tower fermenter, because of its geometry, exhibits a different relationship between specific growth rate of bacteria and medium dilution rate. In a conventional C.S.T.R. at steady state, there is the relationship $\mu = D$. In CTF, because of the sedimentation effect the equation $\mu = D$ is not entirely valid. Instead, the equation $\mu . X_F = D . X_E$ exists, where X_F is the biomass concentration in the fermenter and X_E the biomass concentration in the effluent stream (Pannell, 1976).

From the last equation, $\mu = \frac{X_E}{X_F} D$

If the biomass in the fermenter is heavy, or flocculent, then the ratio $\frac{X_E}{X_F}$ becomes small because the denominator X_F becomes large, i.e. biomass is accumulating in the fermenter and only part of it is washed out.

If the biomass in the fermenter is light, since the bacterial biomass is, the ratio $\frac{X_E}{X_F}$ becomes equal to one and as a consequence $D = \mu$.

Figure 6.1.10 shows that in some of the steady state conditions the kinetics of the fermentation follows the relationship $\mu = D$. Most of the points correspond at high dilution rates. In the lower dilution rates μ is always smaller than D. In fungal fermentation where the weight of the microbial pellets is high, the relationship $X_E < X_F$ always applies except in cases where the morphology of the microorganism is bound up with the viscosity of the medium (Spensley, 1977).

In figure 6.1.10 it is shown that for the temperature

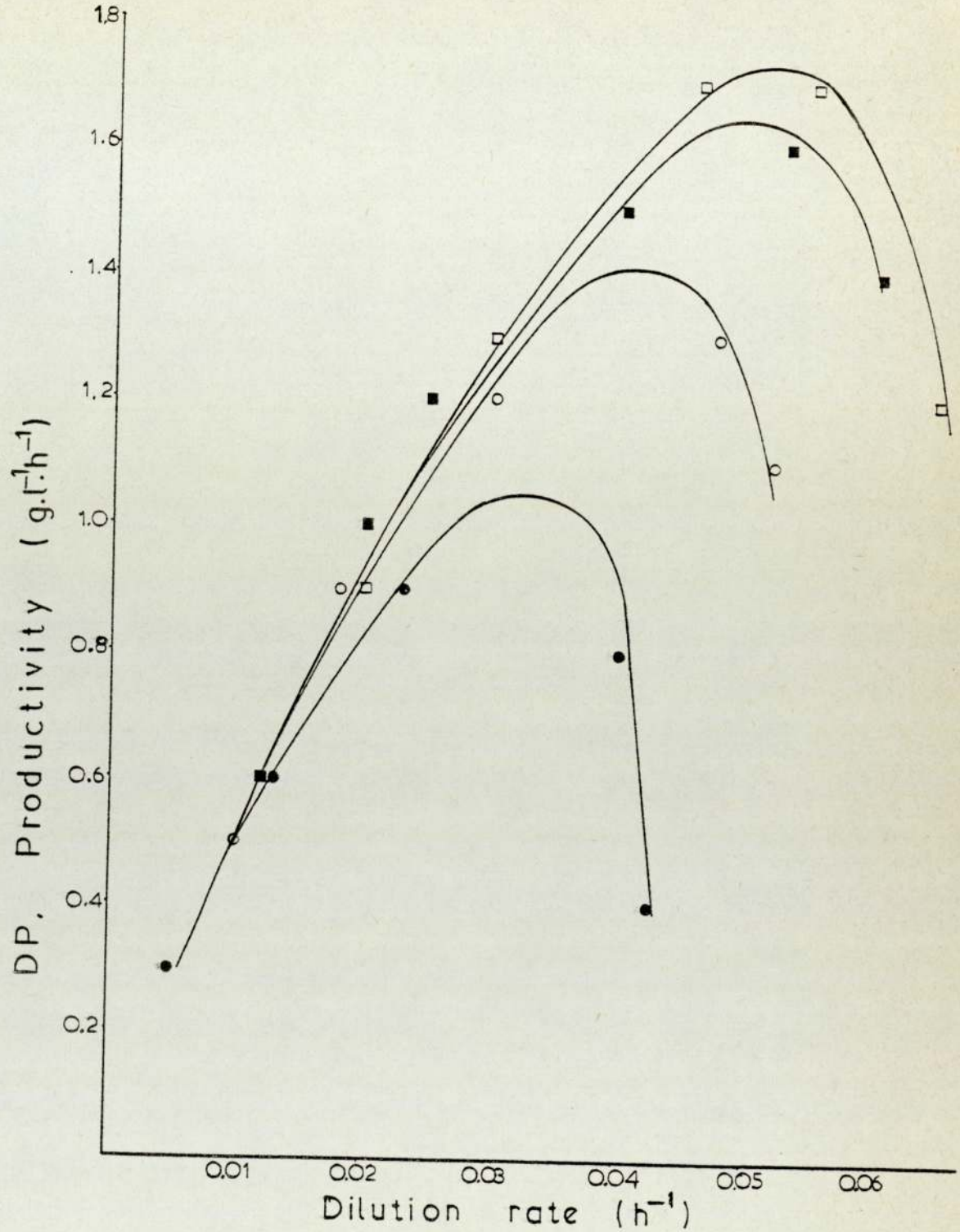


Figure 6.1.11. Relationship between acetic acid productivity, D.P., and medium dilution rate, D, at four different conditions of temperature.

• 23°C, ○ 28°C, ■ 31°C, □ 35°C

Substrate: Cocoa "Sweatings"

of 23°C, $\mu = D$ occurs in two cases ($0.038 h^{-1}$ and $0.04 h^{-1}$).

At the temperature of 28°C only when $\mu = 0.06 h^{-1}$ was $\mu = D$. (Details are given in tables A1.4. to A1.5. A.1.6.)

At the temperatures of 31°C and 35°C $\mu = D$ occurred at dilution rates 0.055, $0.06 h^{-1}$ and 0.053, $0.06 h^{-1}$ respectively.

As was referred to previously, a common point in the above observation is that the relationship $\mu = D$ starts to apply at high dilution rates, i.e. the homogeneity of the continuous tower fermenter starts to become a property of the fermenter only at high dilution rates (i.e. for bacterial cultures).

6.1.3.2.3. Acetic acid productivity in continuous tower fermenter at different temperatures (Cocoa "sweatings")

Acetic acid productivity is the product D.P. where D is the dilution rate and P is the acid concentration (in terms of acetic acid) in the fermenter.

At the temperature of 35°C it was noticed that the maximum productivity was at the dilution rate of $0.015 h^{-1}$ but at this temperature the evaporation rate of ethanol was $0.06 g.l^{-1}.h^{-1}$ (see paragraph 6.1.3.1.1, figure 6.1.11) and the output was reduced to the value of 94% of theoretical.

The loss of ethanol due to evaporation is an important parameter which affects the economics of the vinegar fermentation. In large industries the use of high capacity condensers reduces the evaporation of ethanol and acetic acid but not at considerable rates. In laboratory scale, the use of condensers brought a substantial reduction of the losses,

but not when high temperatures were used. The high temperatures in combination with high aeration rates contribute to the problem of the evaporation of volatile substances and is a more difficult problem to solve by conventional methods.

Temperature (^{o}C)	Maximum observed productivities ($g.l^{-1}.h^{-1}$)	Dilution rate (h^{-1})
23	1.05	0.032
28	1.33	0.044
31	1.61	0.049
35	1.73	0.050

Table 6.1.6. Maximum productivities observed at different temperatures.

Substrate: Cocoa Sweatings

Aeration rate: 0.2 v/v/m

Mean s.g.v.: 0.21 $cm.sec^{-1}$

Temperature (^{o}C)	Productivity ($g.l^{-1}.h^{-1}$)	Volumetric efficiency (h^{-1})
23	0.42	0.19
28	0.55	0.26
31	0.97	0.44
35	1.07	0.51

Table 6.1.7. Productivites at volumetric efficiency of the tower fermenter in which the conversion C=97.5% (of the total acid).

Substrate: Cocoa Sweatings

Aeration rate 0.2 v/v/m, (mean s.g.v 0.21 $cm\ sec^{-1}$)

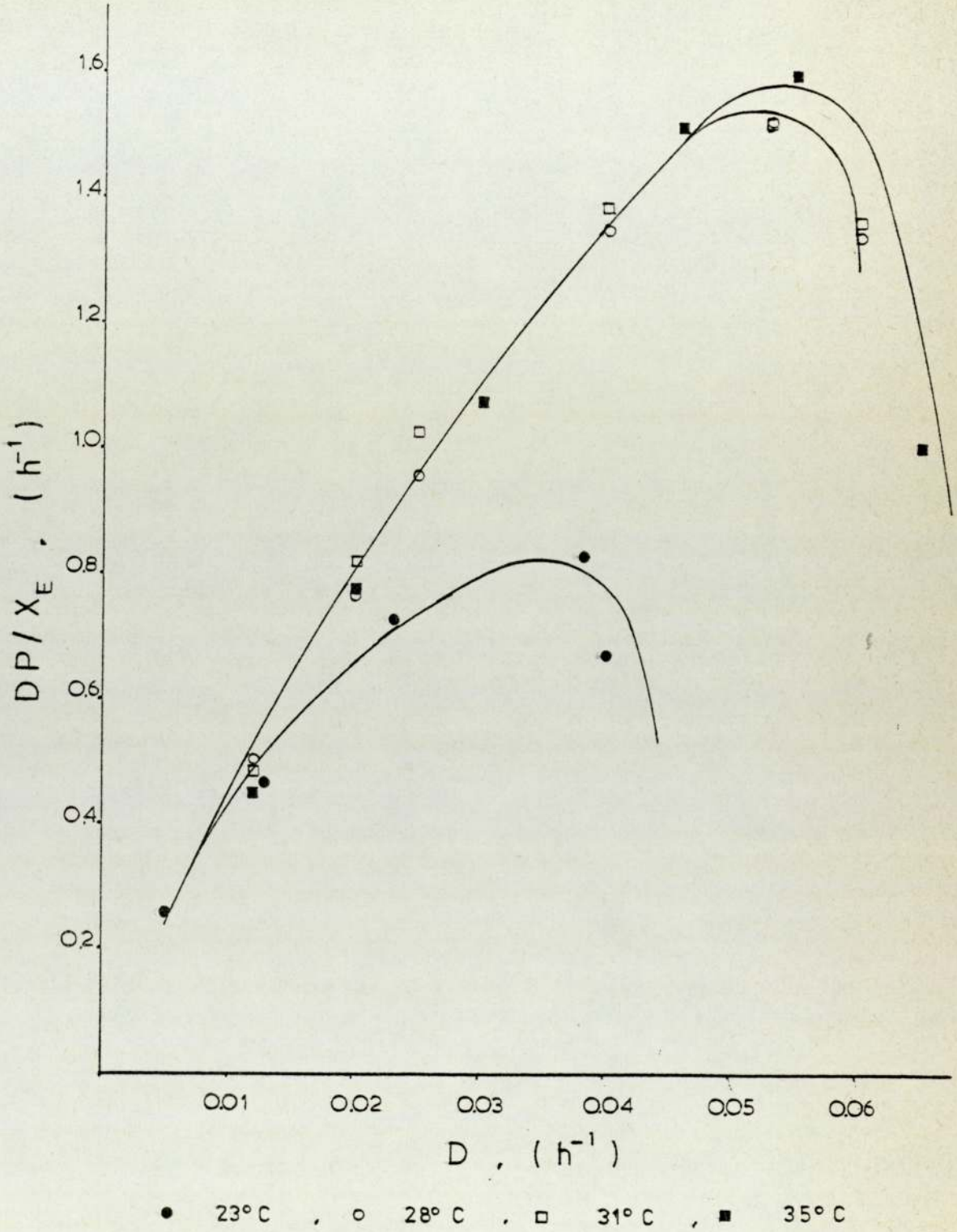


Figure 6.1.12. Relationship between specific productivity DP/X_E and medium dilution rate, D, at four different conditions of temperature.

Substrate: Cocoa "sweatings"

6.1.3.2.4. Acetic acid specific productivity in continuous tower fermenter, at different temperatures

(Cocoa "sweatings")

In 1963a,b, Alian et al studied the effects of temperature on the acetic acid bacteria, and noticed that the higher the temperature, the more active were the cells. This agrees with the results which are represented in figure (6.1.12).

For low temperatures, the specific productivity of the fermenter was low and at higher temperatures it was high. Specifically, at the temperature of 22°C the maximum specific productivity (K) was 0.82 h^{-1}, at the temperatures of 28°C and 31°C the K values were about the same and the maximum specific productivity (K max) reached the value of 1.55 h^{-1}. At the temperature of 35°C, the K max was 1.59 h^{-1}.

At temperatures higher than 28°C the specific productivity of the fermenter changes little in comparison with the value of that at 23°C. From the above results, it can be concluded that at a specific temperature, the fermentation producing almost the same amount of acid per hour per biomass unit for the same concentration of total acid. Moreover, the fall in the specific productivity begins at a temperature of 35°C and a dilution rate of 0.055 h^{-1} for 28, and 31°C and at a dilution rate of 0.053 h^{-1} for the temperature of 23°C at a dilution rate of 0.035 h^{-1}.

6.1.3.2.5. Determination of the true growth yield, Y_G, and maintenance energy coefficient, m, at different temperatures (Cocoa "sweatings")

Yield studies on microorganisms have the purpose of finding the relationship between substrate utilization and the formation of new cell material. Monod (1942) demonstrated that the amount of growth was directly proportional to the amount of energy source added.

Generally, the following scheme of energy balance exists in microorganisms (Pirt, 1975).

total rate of consumption = rate of consumption for growth = rate of consumption for maintenance

$$\text{or: } \frac{ds}{dt} = \frac{\mu X}{Y_G} + mX \qquad \text{hence: } \frac{1}{X}\frac{ds}{dt} = \frac{\mu}{Y_G} + m \qquad (1)$$

where X is the dry weight concentration of the bacteria

μ is the bacterial specific growth rate

Y_G is the true growth yield

m is the maintenance energy coefficient

S is the substrate concentration

The maintenance energy coefficient gives the energy which is used independently of growth. This amount of energy is needed for many purposes including the turnover of cell material, the preservation of the right ionic composition and intracellular pH and for maintenance of a large intracellular pool of metabolites against a concentration gradient.

In the case of acetic acid fermentations, the energy source is ethanol, but this also served as a carbon source.

In order to determine the true growth yield (Y_G) and the maintenance energy coefficient m, the plot of $\frac{1}{x}\cdot\frac{ds}{dt}$ against μ

is constructed from the equation (1). The slope of the straight line (e) gives the reciprocal of Y_G and the intercept on the ordinate gives the value of m (Figure 6.1.13).

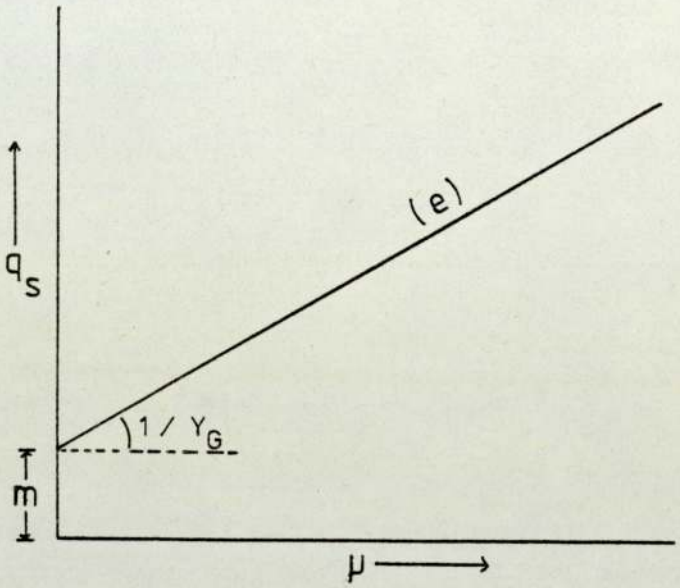


Figure 6.1.13 Graphical method for the determination of the true growth yield, Y_G, and the maintenance energy, m.

Figure 6.1.14 represents the relationship between $\frac{1}{X_E} \cdot \frac{ds}{dt}$ and μ. X_E has been used for the calculation of Y_G and m because the tower fermenter geometry effect on the biomass concentration. The maintenance energy in reality is the energy which is consumed when the fermenter is producing zero biomass, i.e. the specific growth rate is equal to zero.

In C.S.T.R., $X_E = X_F$, hence there is no difference for the calculation of Y_G and m if X_F was used instead of X_E (Pannell, 1976).

The maintenance energy coefficient, (m), increases as the temperature increases. The true growth yield, (Y_G) also increases as the temperature increases. Table 6.1.8. In Figure 6.1.14, the straight lines represent the equations

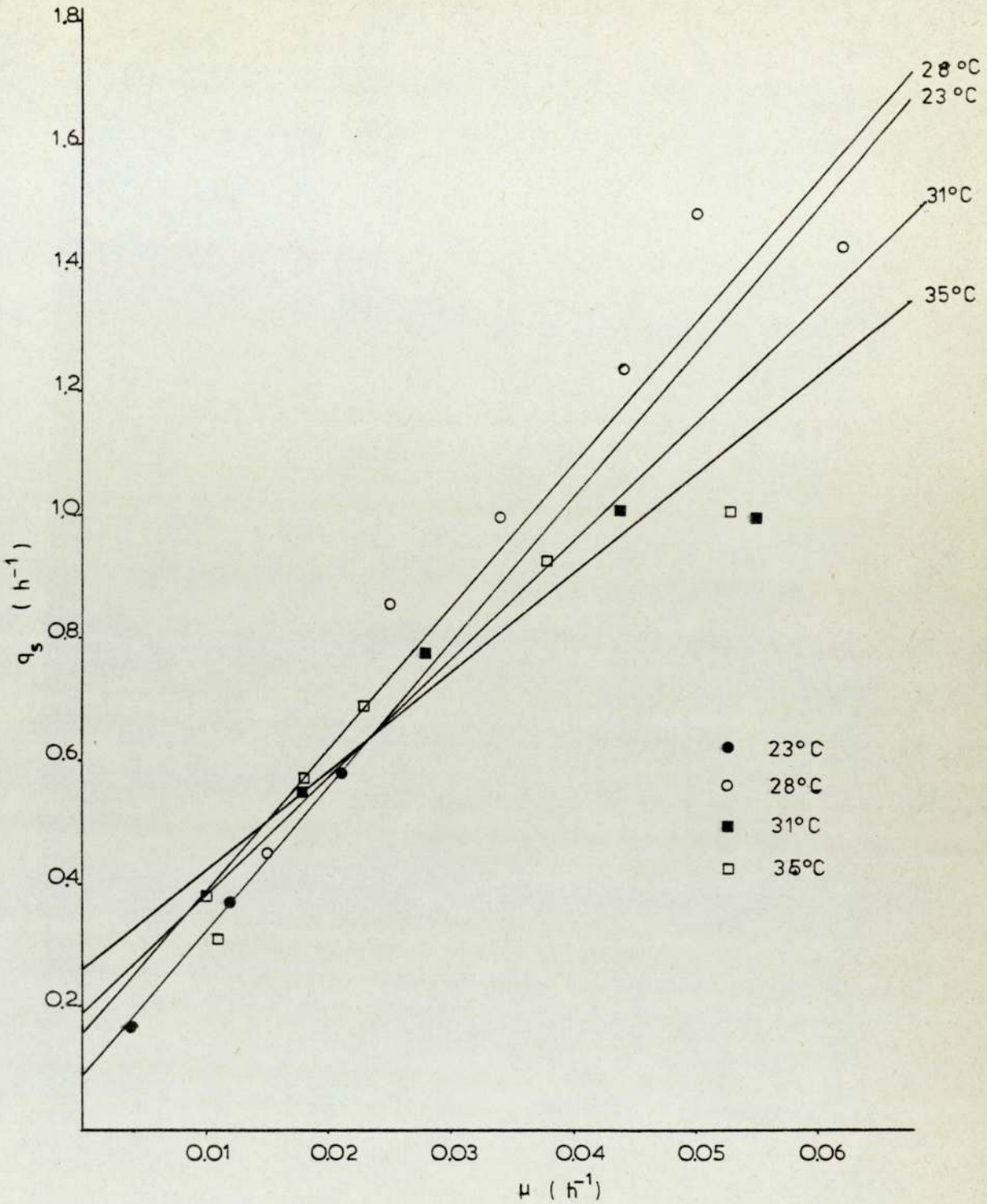


Figure 6.1.14. Yield studies. Determination of Y_G and m. Substrate cocoa sweatings.

of q_s on μ.

Temperature ^oC	Y_G	m, g. of ethanol/g biomass dry weight per h
23	0.041	0.09
28	0.043	0.16
31	0.052	0.19
35	0.062	0.26

Table 6.1.8. Values of true growth yield and maintenance energy coefficient at different conditions of temperature.
Cocoa sweatings.
Aeration rate 0.2 v/v/m.

The increase of the maintenance energy with the temperature increasing can be explained as a result of the increase of metabolic activity of the cells. The cells demand more energy to maintain their metabolic activities in higher temperatures (Stouthamer, 1976). The true growth yield also increases for similar reasons.

6.1.4. EFFECTS OF AERATION RATE ON THE PRODUCTION OF COCOA "SWEATINGS" VINEGAR IN CONTINUOUS TOWER FERMENTATION

The importance of oxygen and the necessity of the continuous aeration of the culture, have already been discussed in paragraph 3.3.2.3.

A series of batch and continuous processes were conducted in order to elucidate the effects of aeration on the acetic acid fermentation for the production of cocoa vinegar.

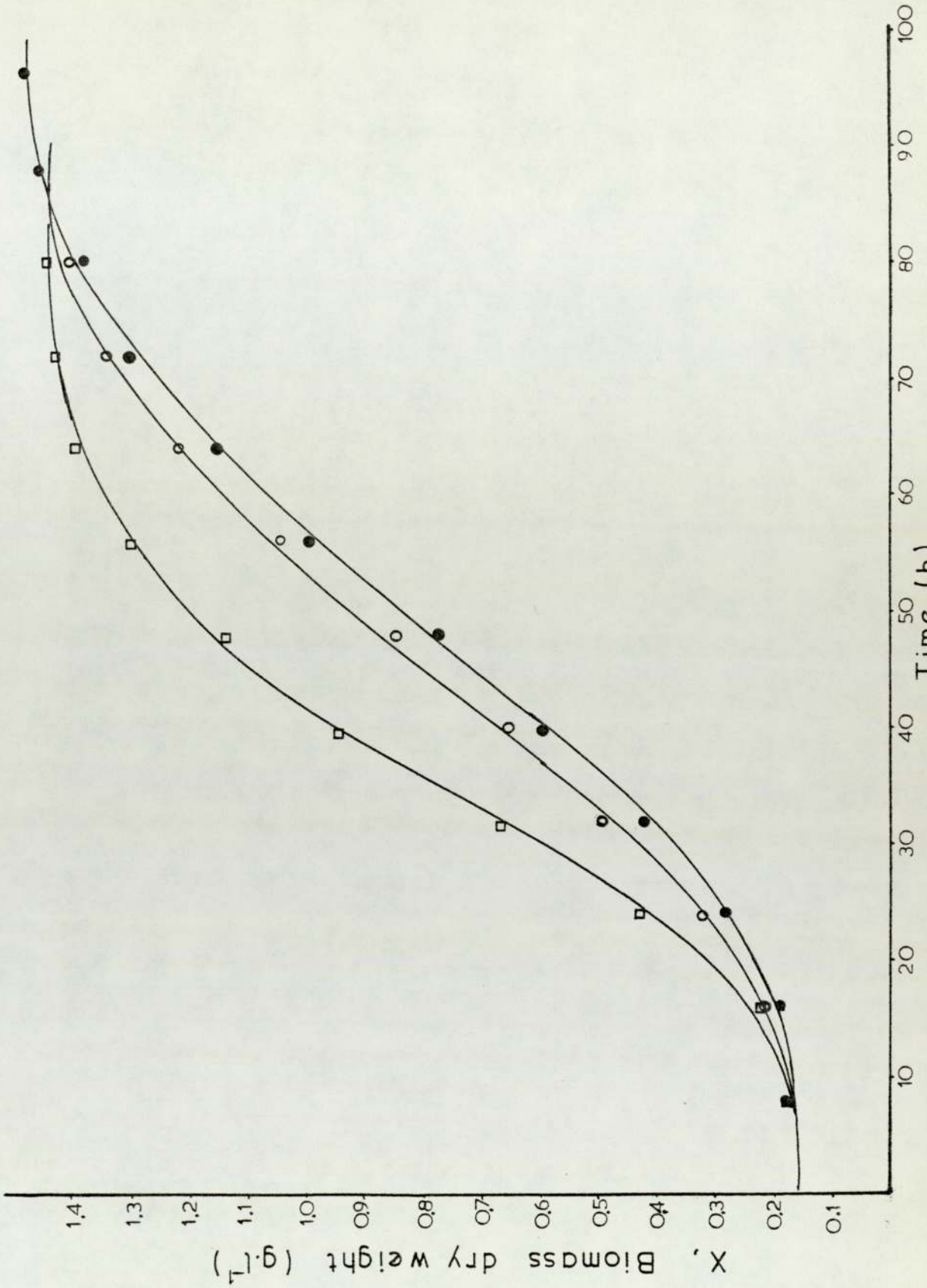
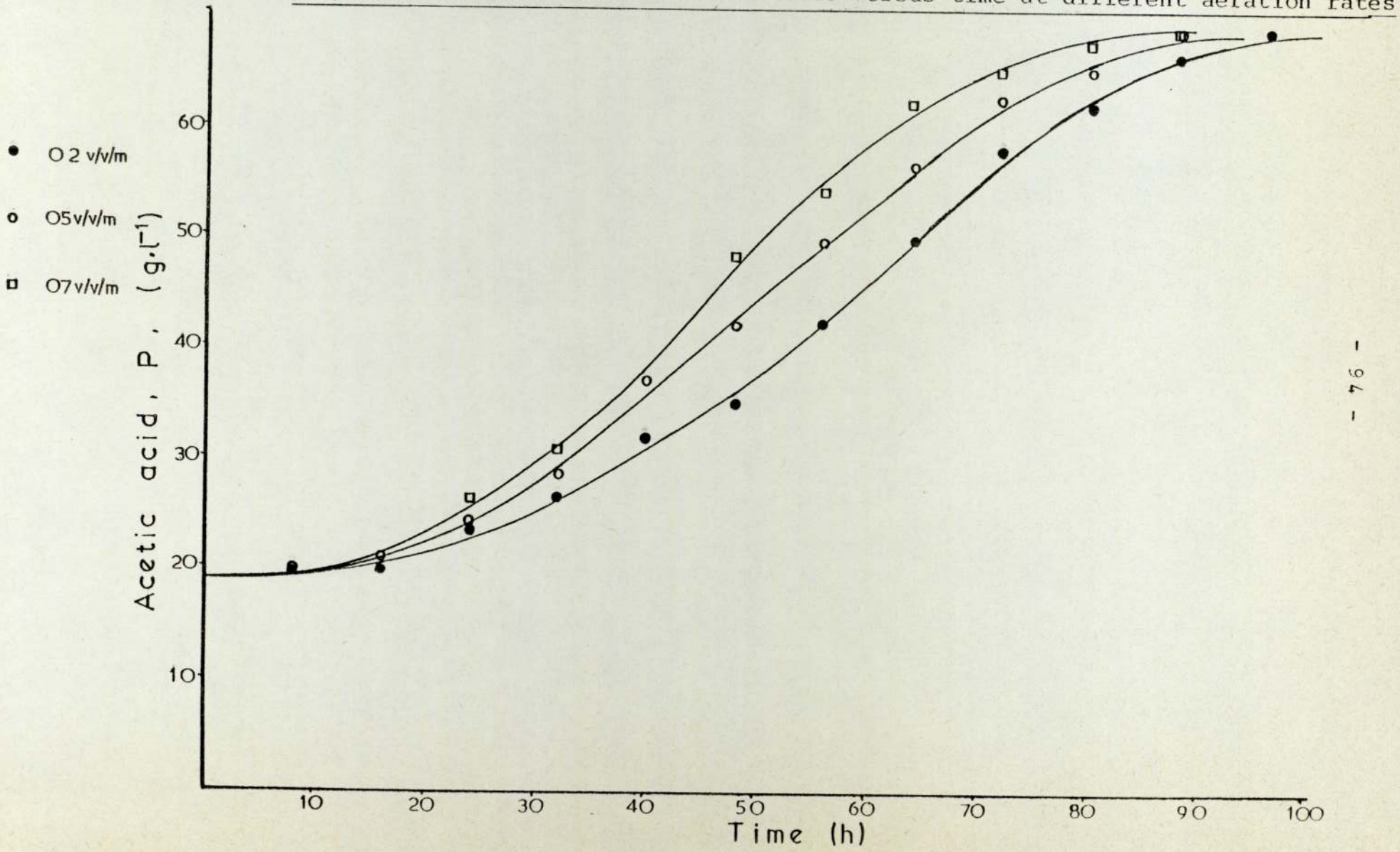


Figure 6.1.15. Biomass production in batch culture versus time at different aeration rates.

Figure 6.1.16. Acetic acid production in batch culture versus time at different aeration rates



6.1.4.1. Batch cultures

Three aeration rates have been chosen for testing the behaviour of the cocoa 'sweatings' medium for the production of vinegar.

The selection of the aeration rates was made on the basis of the preliminary fermentation observations (paragraph 6.1.2).

The selected aeration rates were: 0.2 v/v/m, 0.5 v/v/v and 0.7 v/v/m.

Figure 6.1.15 shows the relationship of biomass concentration at the three aeration rates. At the aeration rate of 0.2 v/v/m, the culture reached the stationary phase in 95 hours, whilst at the 0.7 v/v/m aeration rate it reached the stationary phase in 80 hours. Also, at 0.7 v/v/m aeration rate the maximum growth rate of the bacteria shows a considerable difference from that under 0.2 and 0.5 v/v/m maximum growth rates (Figure 1.1.17).

The maximum product formation rates ($\frac{dp}{dt}$) at 0.2 and 0.5 v/v/m differ only slightly, whilst under 0.7 v/v/m the difference is larger than either of the other cases.

6.1.4.1.1. Availability and utilization of oxygen (Cocoa "sweatings")

The amount of dissolved oxygen given by the lowest aeration rate of 0.2 v/v/m to the medium for the complete oxidation of the available ethanol to acetic acid is 0.2 1.1^{-1}. min^{-1} x 5400 min = 1080 $\ell.\ell^{-1}$. Theoretically, the amount of oxygen required to oxidize the amount of ethanol was 38.5 $g.1^{-1}$ i.e. 38.5 $g.1^{-1}$ x $\frac{32}{46}$ = 26.78 g of oxygen, which is equivalent to 26.78/32 = 0.84 moles of oxygen. This is equivalent to

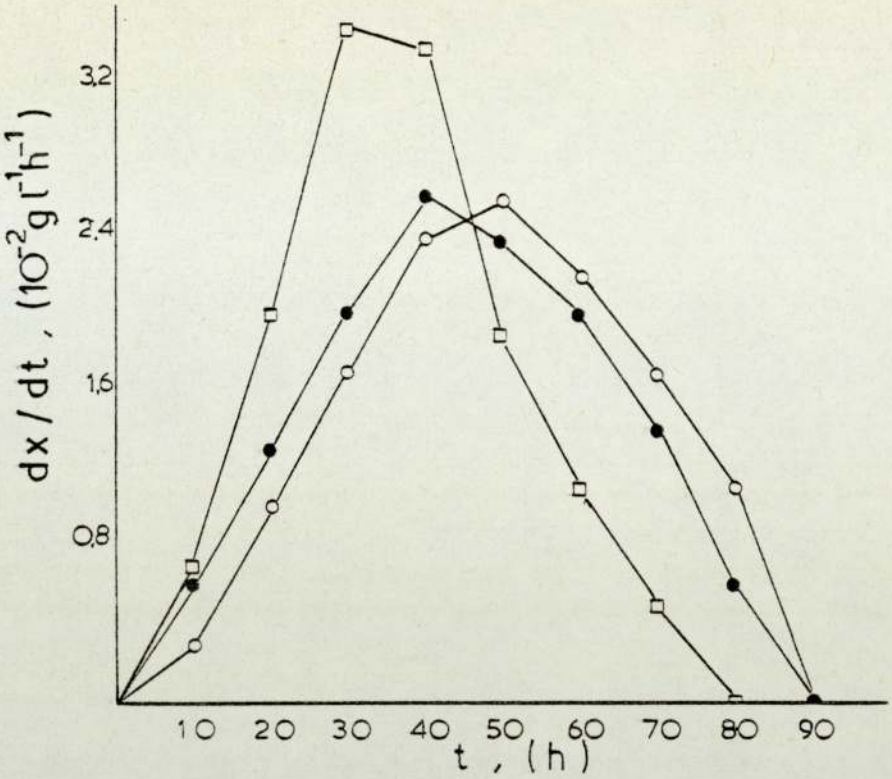


Figure 6.1.17. Bacterial growth rates at different aeration rates in batch cultures

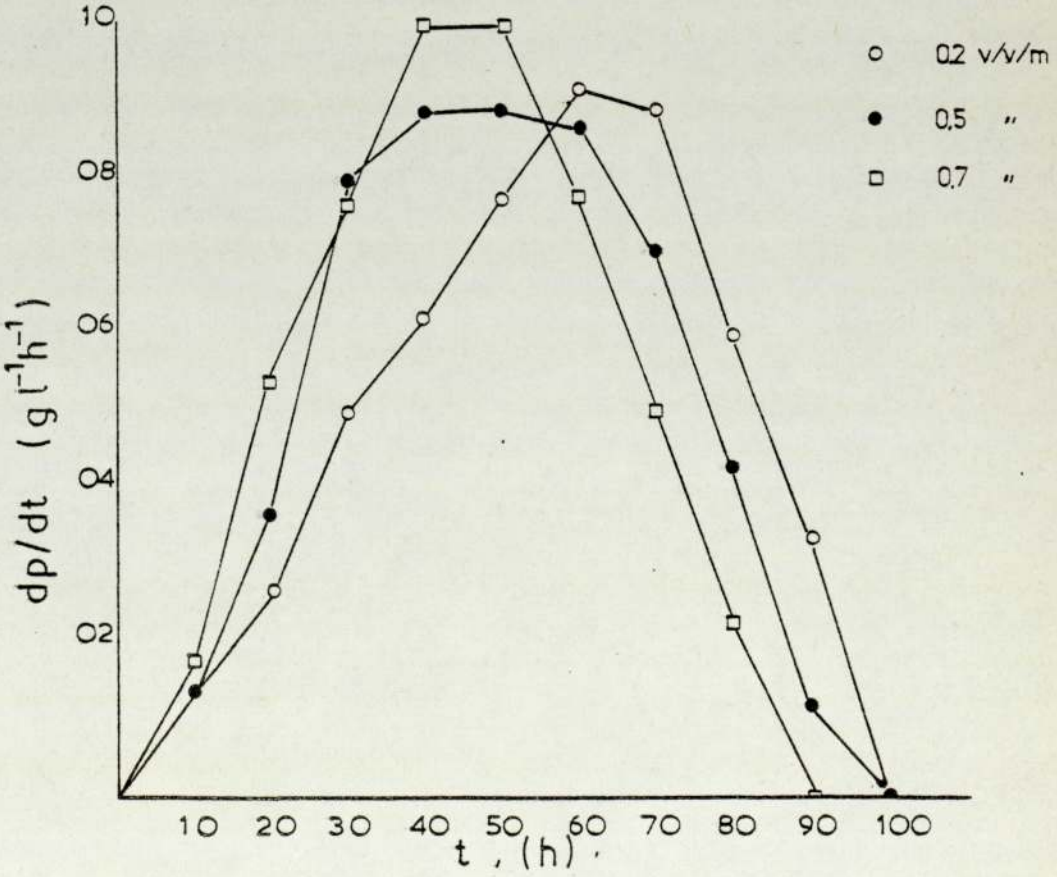


Figure 6.1.18. Product formation rates at different aeration rates, in batch cultures

0.84 x 22.4 = 18.82 l of oxygen. Since air is approximately one-fifth oxygen (Vaughn, 1954), 18.82 x 5 = 94.1 l of air are required to oxidize the ethanol in the 1 ℓ of stock. The amount of air which has been passing through the medium during the whole fermentation course until completion was 1080 $\ell.\ell^{-1}$. From the difference of 1080 - 94.1 = 985 $\ell\cdot\ell^{-1}$ of air is not utilized, for the oxidation of ethanol to acetic acid. A part of the excess oxygen was utilized by the bacteria for growth and other metabolic needs. The rest of air was necessary for medium agitation purposes. During the fermentation the level of oxygen solubility was 80% of saturation level.

From the above theoretical considerations, the observed difference in the maximum growth rate and maximum product formation rate between 0.7 v/v/m and 0.5 and 0.2 v/v/m aeration rates, Figures (6.1.17) and (6.1.18) could be explained partly as inability of the tower fermenter to hold enough air at low aeration rates and partly by the amount of fat and lipid constituents of the medium which lower the transfer rate of oxygen from the air phase to the liquid phase. Of course, another cause could be the use of antifoam which although used in small amounts ($0.02g.l^{-1}$) might result in the decrease of the oxygen transfer rate from the air to liquid phase. The relationship between transfer rate coefficient ($K_L.a$) within the medium and the aeration rate for oxygen transfer phenomena, need further investigation.

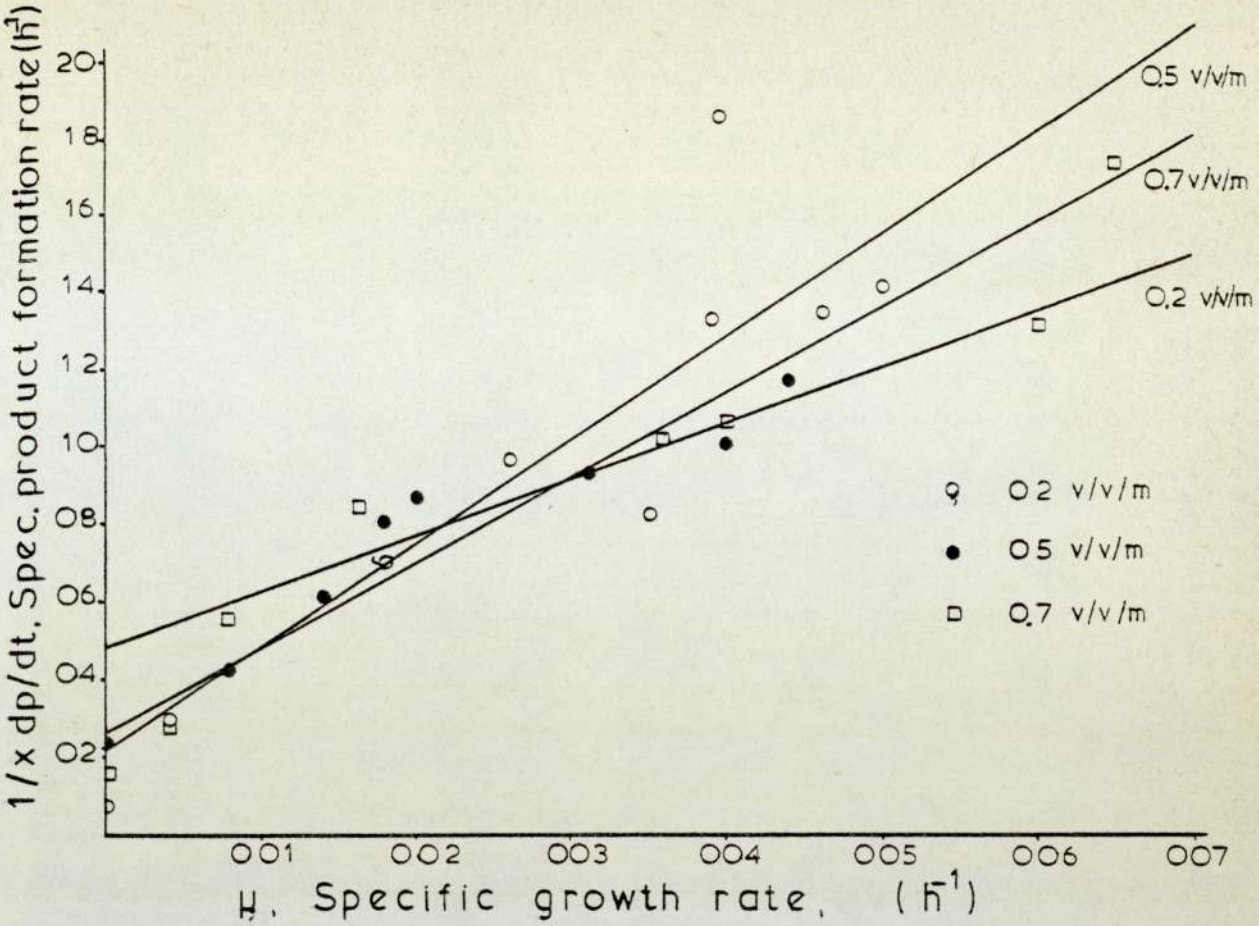


Figure 6.1.19. Relationship between specific product formation rate, K, and specific bacterial growth rate, μ, in batch culture. Cocoa 'sweatings'.
Temperature 28°C.

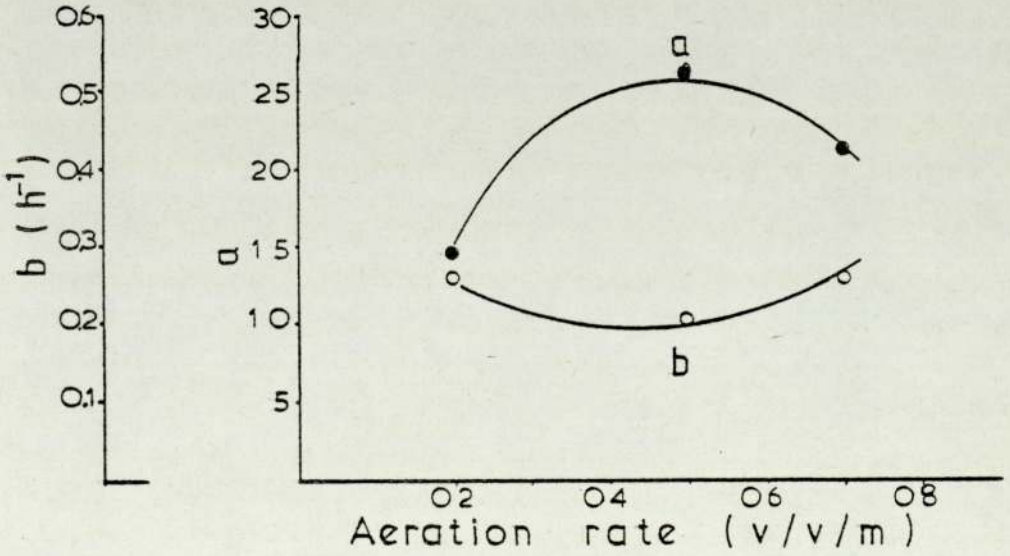


Figure 6.1.20. Values of a and b of the equation K = aμ+b, at different aeration rates.
Cocoa 'Sweatings'
Temperature 28°C

6.1.4.1.2. Relationship between specific product formation rate, K, and bacterial specific growth rate at different conditions of aeration. (Cocoa "sweatings")

The specific product formation rate $\frac{1}{x}\cdot\frac{dp}{dt}$ or K was plotted against the specific growth rate for the three batch cultures (Figure 6.1.19). The results of the plot are shown in table 6.1.9.

The equation $K = \frac{1}{x}\cdot\frac{dP}{dt} = a\mu + b$ in all three cases. A strong positive correlation between K and μ was found. The values of the experimental parameters a and b follow an inverse relationship. Hence, when the values of (a) show maximum the values of (b) show minimum. (Figure 6.1.20).

For the explanation of the phenomenon, the original equation $\frac{dP}{dt} = a\frac{dx}{dt} + bx$ would show that the (a) and (b) being the experimental parameters of the growth rate and the biomass concentration are developing to give a product formation rate which increases at a constant rate. This is a property of growth associated and partially-growth associated product, as in lactic acid fermentation (Luedeking & Piret 1959b) and acetic acid fermentation (Divies, 1973)

6.1.4.2. Continuous culture.

6.1.4.2.1. Effects of dilution rate on the conversion ability of ethanol to acetic acid. Biomass concentration at different dilution rates and aeration rates. (Cocoa "sweatings")

The conversion of ethanol to acetic acid in the continuous tower fermenter is shown by the wash-out curve in Figure 6.1.21. The higher the aeration rate the faster and more complete is the conversion. In table (6.1.10) have been

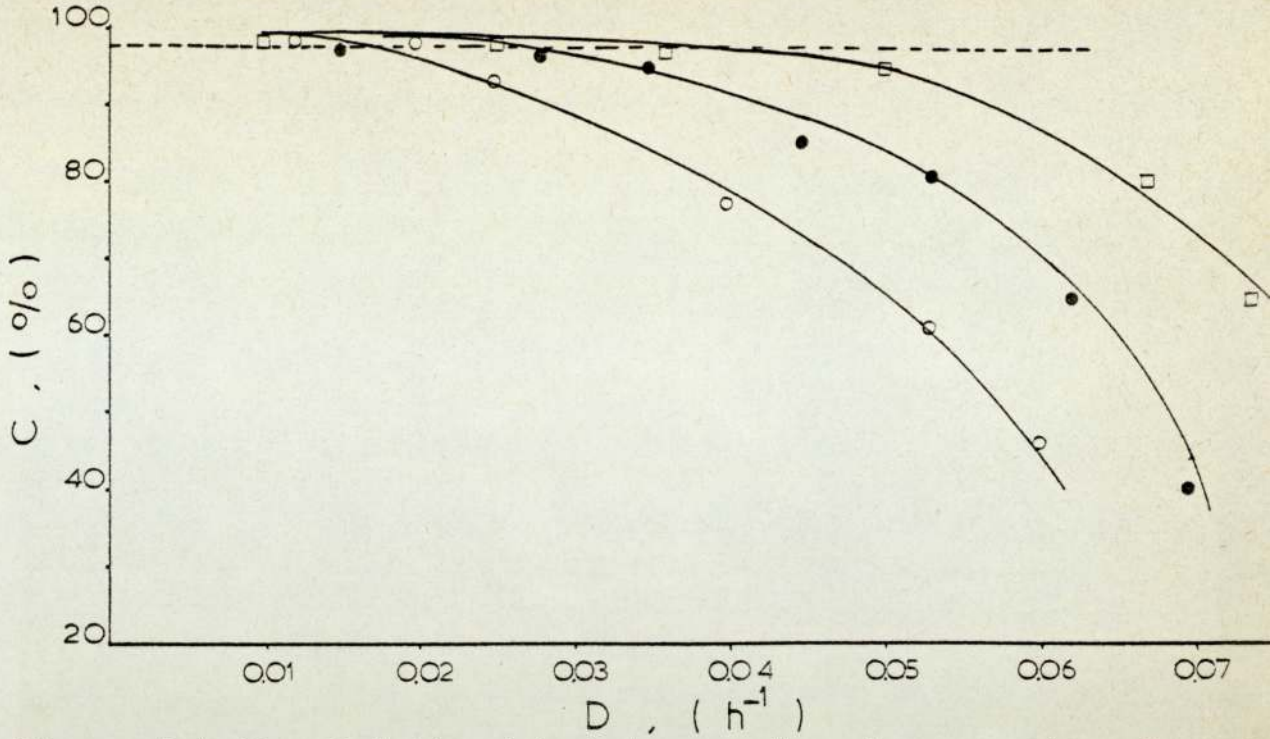


Figure 6.1.21. Relationship between ethanol conversion, C, and dilution rate, D, at different conditions of culture aeration.
Cocoa "sweatings"
Temperature 28°C

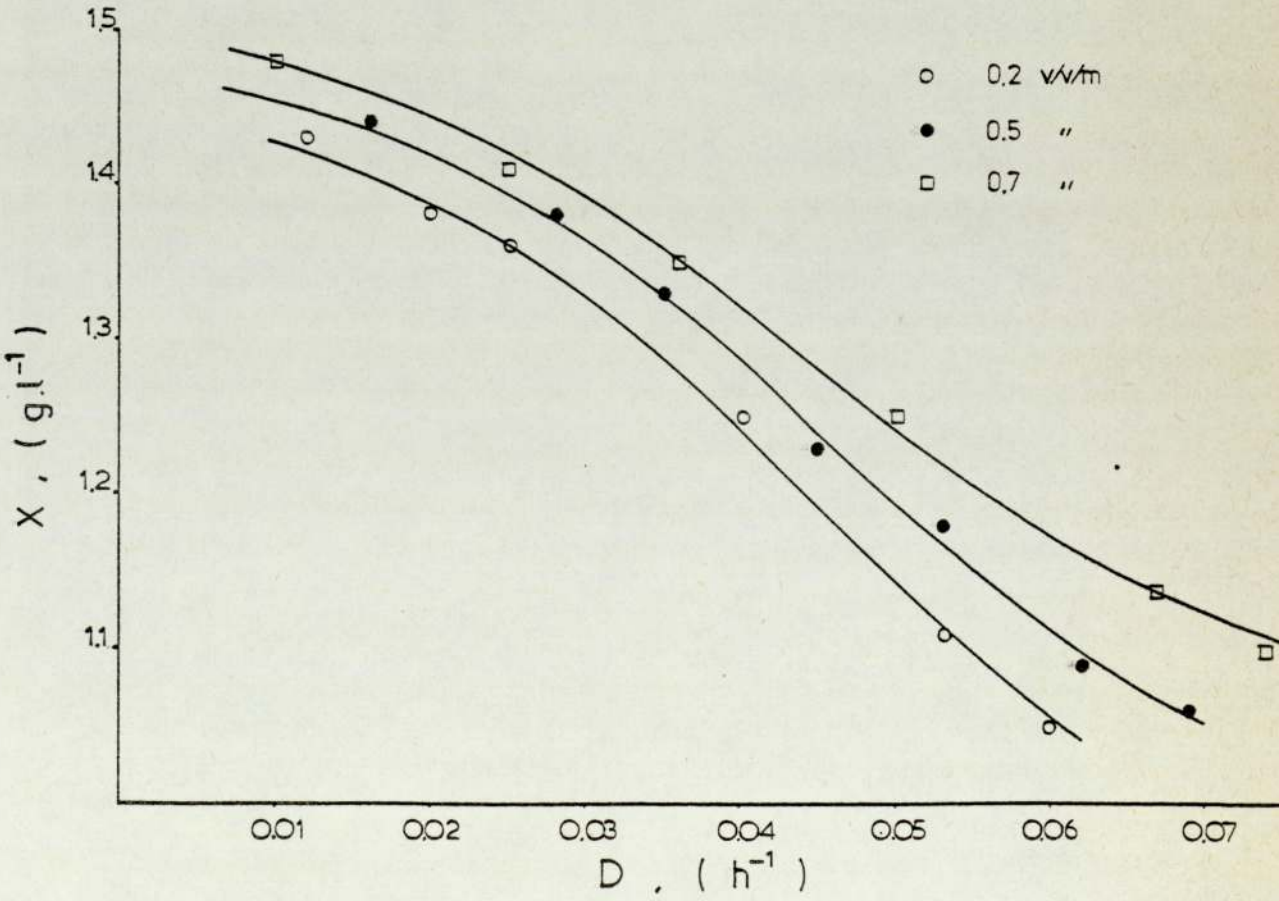


Figure 6.1.22. Bacterial concentration in the fermenter versus dilution rate at different aeration rates.
Cocoa "sweatings"
Temperature 28°C

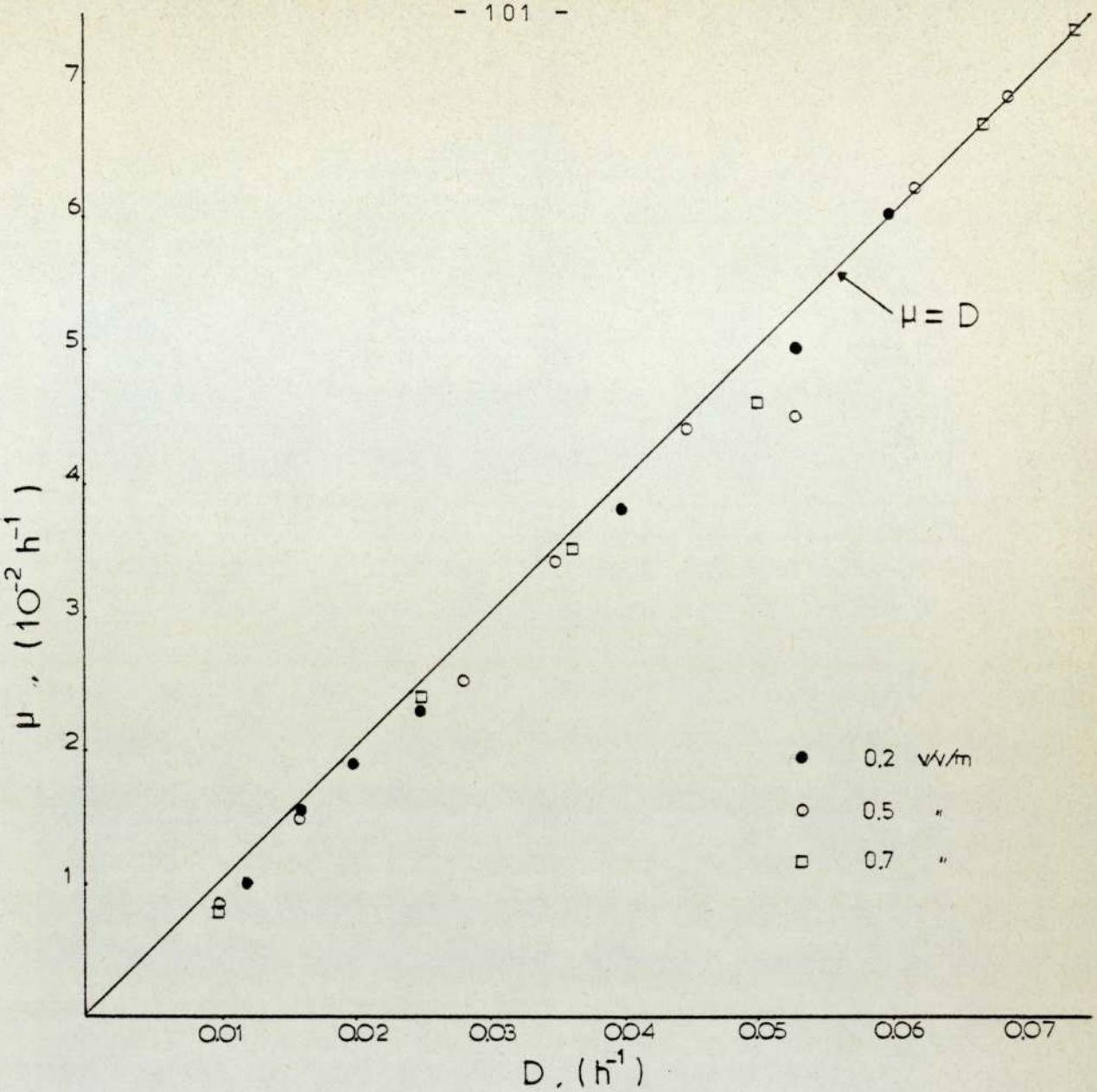


Figure 6.1.23. Specific growth rate versus dilution rate at different conditions of culture aeration.

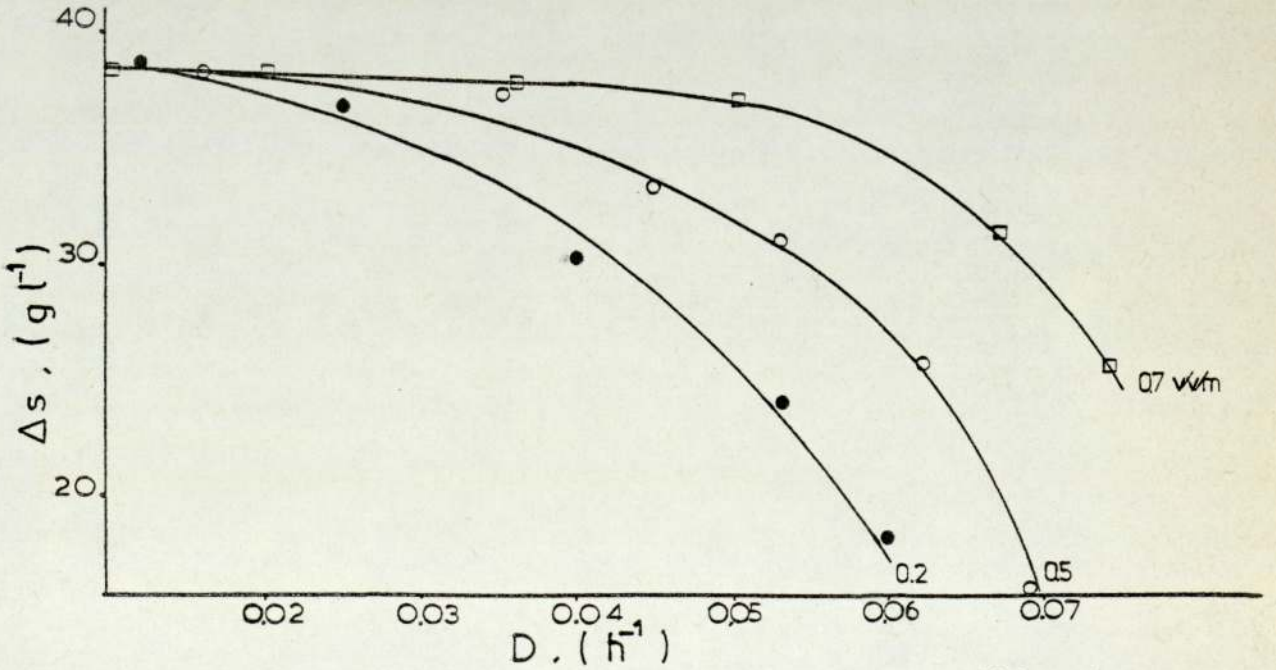


Figure 6.1.24. Relationship between substrate utilization, Δs, and dilution rate, D.

included values of dilution rates and residence times when 97.5% (of the total acid) conversions was achieved.

Aeration rates v/v/m	Max. SGV cm sec^{-1}	Dilution rate (h^{-1})	Residence time (h)
0.2	0.21	0.017	59
0.5	0.52	0.026	39
0.7	0.73	0.041	24

Table (6.1.10) Values of dilution rate and residence time of the medium when the fermentation was under different aeration rates. See also paragraph 6.1.4.2.3. Cocoa sweatings.
Temperature 28°C

At the aeration rate of 0.2 v/v/m the conversion was much slower than that at 0.7. The reasons have already been explained (paragraph 6.1.4.1.2). The wash-out of the biomass and of the acid followed the same pattern as that in the temperature experiment. The continuous tower being a fermenter which has a behaviour between that of a plug flow pipe and a continuous stirred reactor gave washing out patterns as in Figures (6.1.22, 6.1.23 and 6.1.24). Some explanations are given in paragraph 6.1.3.2. It has also been observed that froth flotation occurs.
This phenomenon becomes more important at higher superficial gas velocities. As a result there is a higher concentration of biomass at the top of the fermenter and sometimes there is a higher biomass dry weight in the effluent stream than in the fermenter. In many cases and especially at high

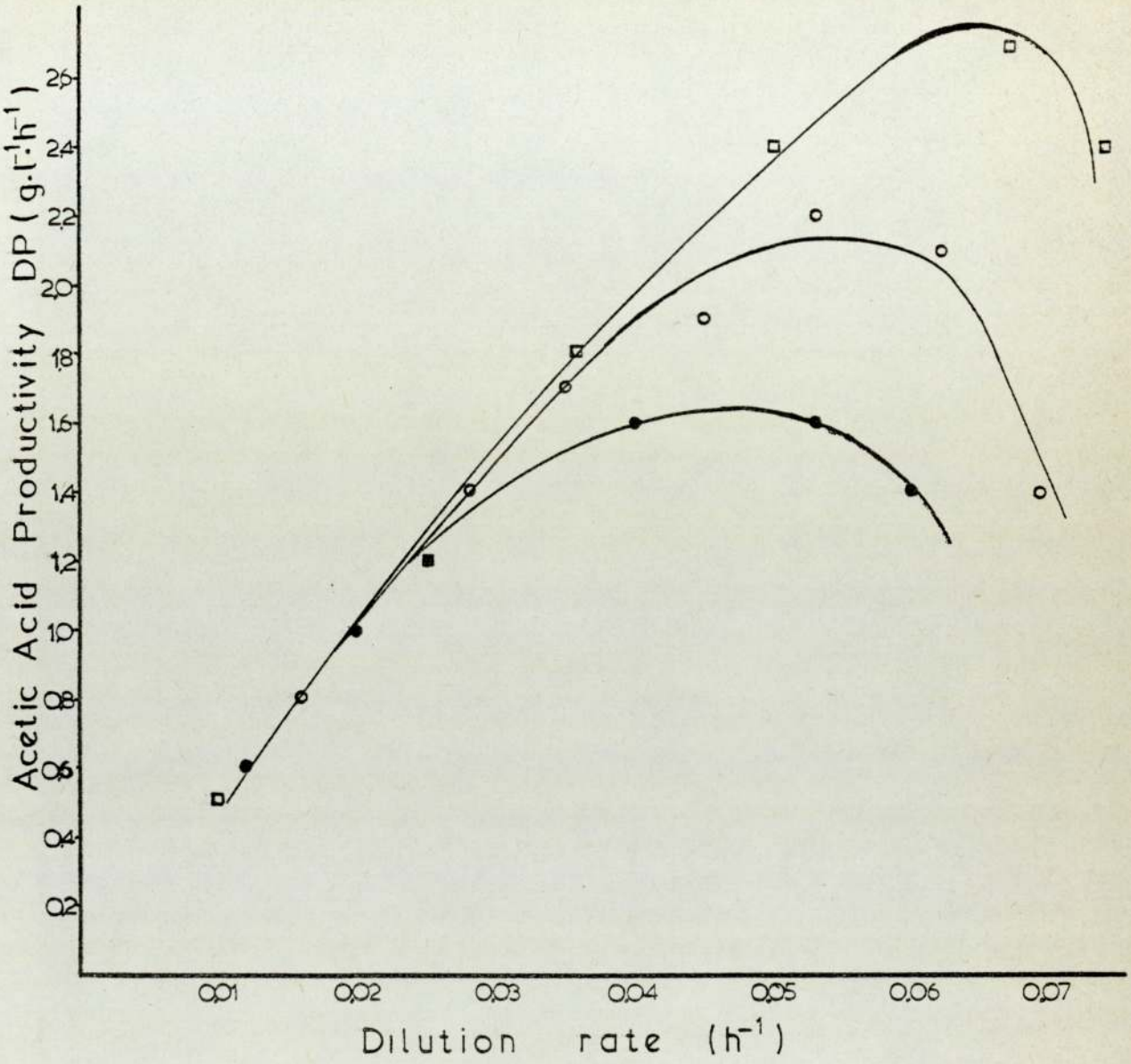


Figure 6.1.25. Relationship between acetic acid productivity and dilution rate at different aeration rates.

● 0.2 v/v/m ○ 0.5 v/v/m □ 0.7 v/v/m

Substrate: Cocoa "sweatings"

Temperature 28°C

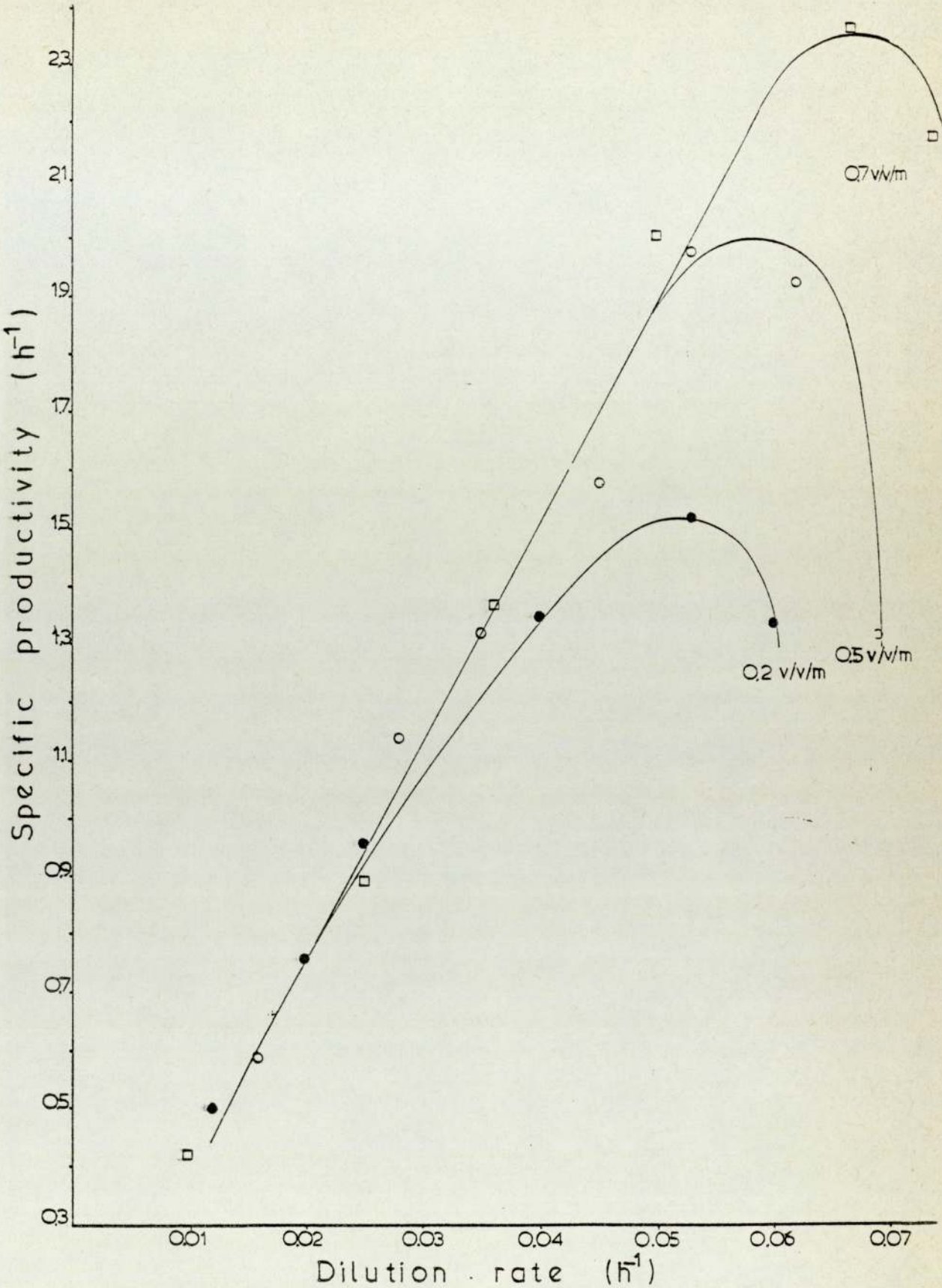


Figure 6.1.26. Relationship between specific productivity DP/X_E and dilution rate, D, under different aeration rates.
Cocoa 'sweatings'
Temperature 28°C

dilution rates, the biomass dry weight concentration in the fermenter was equal to the dry weight concentration in the effluent stream (see figure 6.1.23). The points which are on the $\mu = D$ line represent these cases.

6.1.4.2.2. Effect of dilution rate on the acetic acid productivity and specific productivity at different aeration rates (Cocoa "sweatings")

The productivity at high aeration rates was greater than that under lower aeration rates. The tower fermentation showed that at an aeration rate of 0.7 v/v/m it had a maximum productivity of 2.58 $g.l^{-1}.h^{-1}$ and at aeration rates of 0.2 v/v/m it had a maximum productivity of 1.62 $g.l^{-1}.h^{-1}$. (Figure 6.1.25). This could be explained by the higher specific productivities which were observed at aeration 0.7 v/v/m.

(Figure 6.1.26). The productivity of the fermenter per biomass unit showed that this was improved when the aeration rate was higher. The higher volume of oxygen which was coming into contact with the medium because of an increased interfacial area resulting from higher aeration rates could be a possible reason for the explanation of this phenomenon. Each cell was receiving more oxygen which was the limiting factor for oxidation of ethanol to acetic acid (Table 6.1.11).

Aeration rate v/v/m	Max S.G.V.* cm sec^{-1}	Maximum Productivity ($g.l.^{-1}h^{-1}$)	Maximum specific Productivity (h^{-1})
0.2	0.19	1.64	1.52
0.5	0.47	2.13	2.00
0.7	0.66	2.76	2.36

Table 6.1.11. Maximum productivity and maximum specific productivity which was observed during continuous tower fermentation for the production of cocoa sweatings at different aeration rates.
Temperature 28°C.

* See also paragraph 6.1.4.2.3, table 6.1.12

6.1.4.2.3. Superficial gas velocities

Calculation of the superficial gas velocities in both expansion and tubular parts of the fermenter gave the following results:

Aeration rate (v/v/m)	Tubular part s.g.v. $cm.sec^{-1}$	Expansion minimum s.g.s. cm sec^{-1}
0.2	0.21	0.08
0.5	0.52	0.20
0.7	0.73	0.28

Table 6.1.12. Values of superficial gas velocity in the tubular and expansion part of the fermenter for different medium aeration rates.

The values of s.g.v. in the expansion have been calculated at the maximum section and consequently represent the minimum possible values.

MOLASSES VINEGAR

6.2. PRODUCTION OF MOLASSES VINEGAR IN TOWER FERMENTER

6.2.1. Medium examination and preparation; Fermenter design

The media for acetification, derived from the alcoholic fermentation as described in paragraph (4.2), were subjected to chemical and microbiological examination. The results from the chemical examination are shown in table (6.2.1.)

Medium	A'	B'	C'	D'
Ethanol	41.8	44.8	52.5	65.1
Acid (as acetic)	0.43	0.52	0.62	0.70
Total reducing sugars	4.25	4.72	6.27	7.30
pH	4.5	4.55	4.5	4.45

Table 6.2.1. Composition of molasses based media. The figures represent grams per litre.

The microbiological examination showed that no serious bacterial contamination had appeared. Predominant micro-organisms were yeasts of Saccharomyces from the alcoholic fermentation. The molasses media A, B, C, D (Table 4.2.1.) had been inoculated with S. distaticus (G.606) ex Guinness and Sons Ltd. Each fermented medium (A', B', C', D') (Table 6.2.1) was centrifuged (15 min at 15000 r.p.m.) in order to remove the existing yeasts and suspended solids. Part of the centrifuged medium was used for the experiment of concentration, the rest was kept at 4° in a refrigerator.

The fermenter which was chosen was a 3.1 l volumetric capacity. The general outline of the fermenter was similar

to that described in paragraph 2.2.4.

6.2.2. Preliminary fermentation

A preliminary fermentation of molasses mash was conducted for three reasons:

a) Preparation of a large active inoculum, for the subsequent experimental fermentations,

b) Determination of the maximum aeration rate which would cause no foam formation, and

c) Determination of the method for acid titration and biomass estimation.

The results show that the maximum aeration rate with little foam formation was 0.4 v/v/m. (s.g.v. 0.55 cm. sec^{-1}). The molasses was titrated using a pH meter, because the dark coloured substrate did not permit the pH measurements based on the colour changes. The estimation of the bacterial biomass concentration had to be measured by means other than optical density because the molasses tends to change colour when the pH drops from 4.5 to 3.8.

6.2.3. Effect of total acid concentration on the production of molasses vinegar in tower fermenter

Since the acetic acid and the ethanol in concentrations which are used for the production of vinegar, act as inhibitors of the bacterial growth (see paragraph 6.1.3.2.1) and subsequently of the product formation rate, continuous culture experiments were tried with different total acidities (54.3, 58.2, 68.3, 84.6 $g.l^{-1}$)

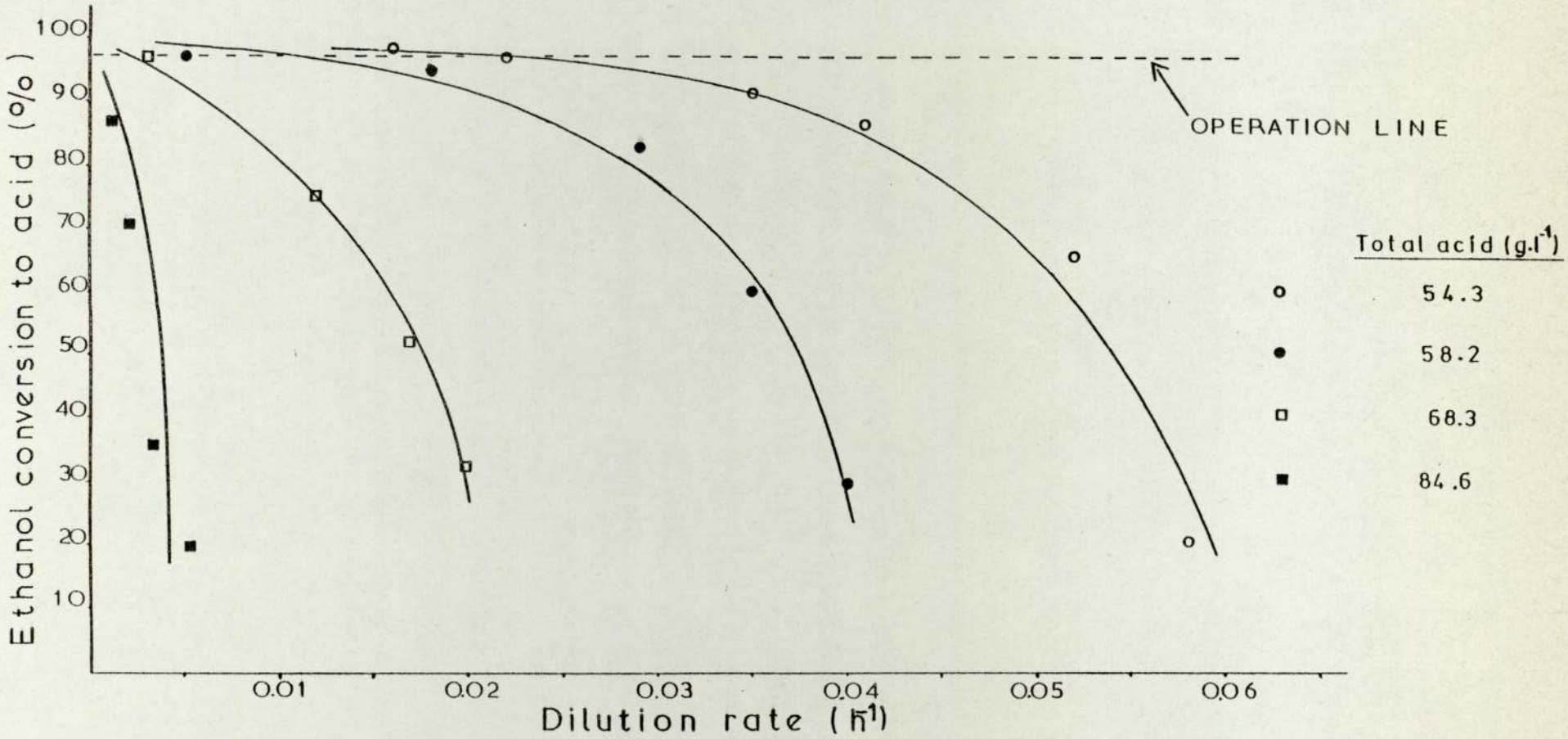


Figure 6.2.1. Effect of total acid concentration on the conversion ability of ethanol to acetic acid in continuous fermentation of molasses based medium
Temperature 28°C
Aeration rate 0.4 v/v/m

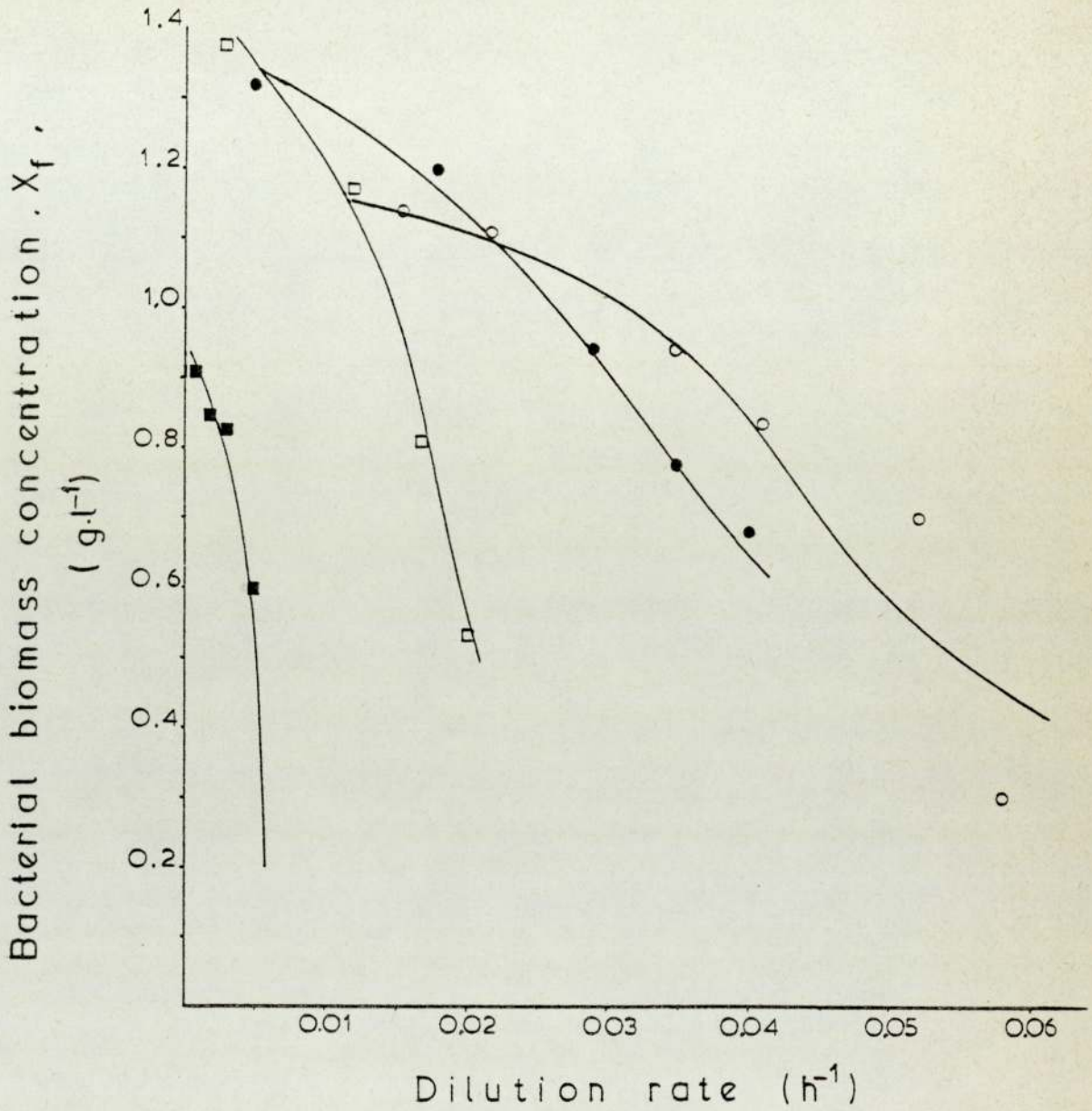
6.2.3.1. Effect of total acid concentration on the ability of conversion of ethanol to acetic acid

It was outlined in paragraph 6.1.3.2.1. that the product formation in the acetic acid fermentation was inhibited by the concentration of acetic acid and by the concentration of ethanol which exists in the medium. Subsequently, the conversion of ethanol to acetic acid was influenced for the same reasons and exhibited the pattern of the curves in Figure (6.2.1.) In actual fact, the dilution rates for 97.5% (of total acid) conversion was .024h^{-1} for 54.3 $g.l^{-1}$ total acid, and for 84.6 $g.l^{-1}$ total acid was 0.001 h^{-1} which shows the strong effect of total acid to the conversion of ethanol to acetic acid. (Table 6.2.2.)

Total acid concentration ($g.l.^{-1}$)	Dilution rate at 97.5% conversion ($h.^{-1}$)	Residence time at 97.5% conversion (h)
54.3	0.024	41.7
58.2	0.012	83.4
68.3	0.003	333.4
84.6	0.001	1.000

Table 6.2.2. Dilution rates and residence times of the medium for ethanol conversion abilities of 97.5% (of the total acid) at different concentrations of total acid.
Molasses Media
Aeration rate 0.4 v/v/m (s.g.v. 0.55 cm sec^{-1})
Temperature 28°C

Figure 6.2.2. Relationship between bacterial biomass dry weight and dilution rate at different total acid concentration



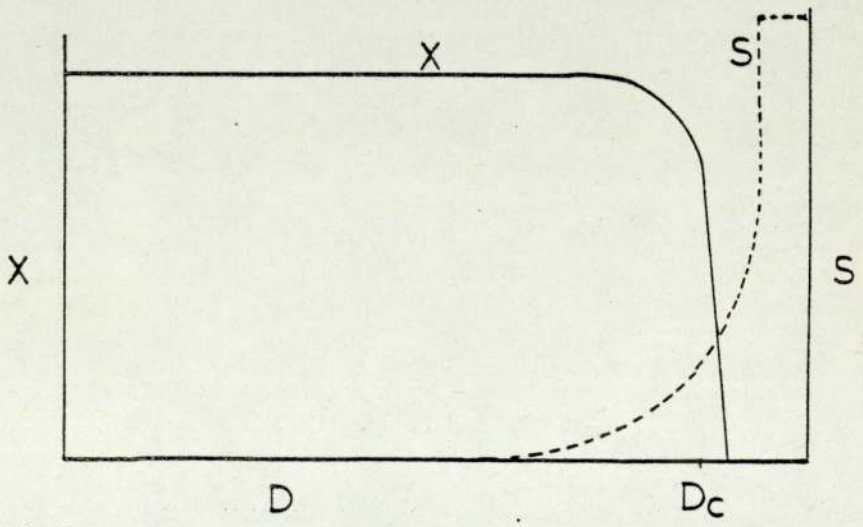


Figure 6.2.3. Bacterial dry weight, X, and substrate concentration, S, in a continuous stirred reactor (S.C.T.R)

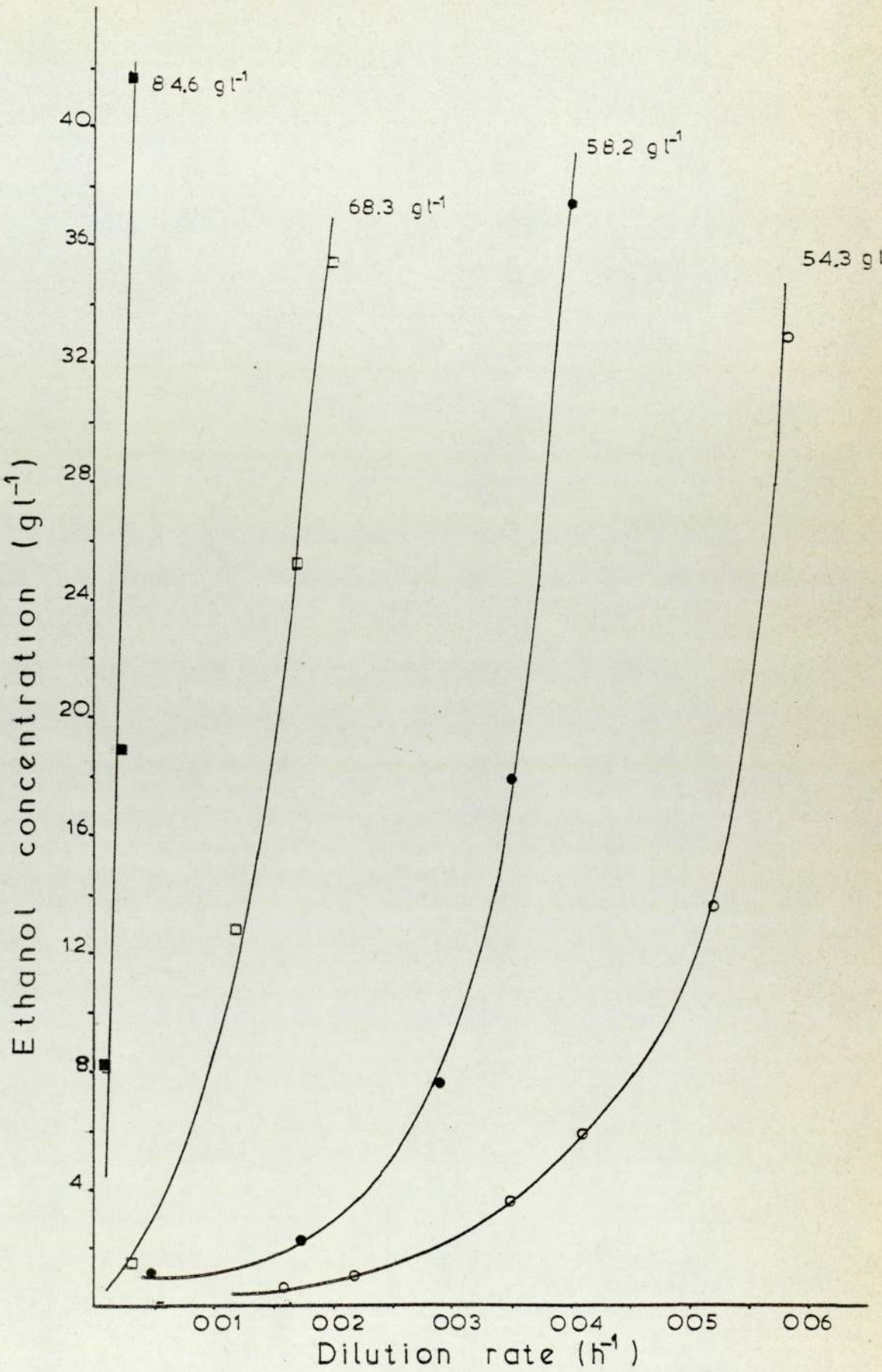


Figure 6.2.4. Substrate concentration versus dilution rate for different total acid concentrations. Substrate Molasses.

6.2.3.2. Effect of total acidity on the concentration of biomass (X_F) and substrate utilization in the fermenter

In figures (6.2.2. and 6.2.4.) are shown graphs of bacterial biomass washout and substrate utilization. The biomass concentration in the fermenter for low acidities and increasing dilution rates exhibits a smooth pattern of curves which approach the continuous stirred reactor behaviour. As the total acid concentration increases the washout of the bacteria is dependent on the changes of dilution rate. With total acidities, 84.6 $g.l.^{-1}$ and 68.3 $g.l^{-1}$ small changes in dilution rate result in a gross change in the bacterial concentration in the fermenter. With total acid concentration of 54.3 $g.l^{-1}$ (Figure 6.2.2.), the change in dilution rate does not result in marked change in the biomass concentration in the fermenter. Consequently, the dilution rate acts as the limiting factor in high acidities rather than in low acidities, in continuous tower fermentations.

The concentration of substrate in C.S.T.R. has a dramatic change at the critical dilution rate from low level concentrations to high concentrations. (Figure 6.2.3.).

In continuous tower fermentations the substrate concentration in the fermenter approaches the C.S.T.R. idealized pattern only at high total acidities. In Figure (6.2.4), at the acidity of 54.3 $g.l^{-1}$, the relationship between substrate concentration and dilution rate exhibits a pattern similar to that of C.S.T.R.

In high total acidities, as in the case of 68.3 $g.l^{-1}$ and 84.6 $g.l^{-1}$, the change of the ethanol concentration in the fermenter is depending more sensitive to changes in dilution rate.

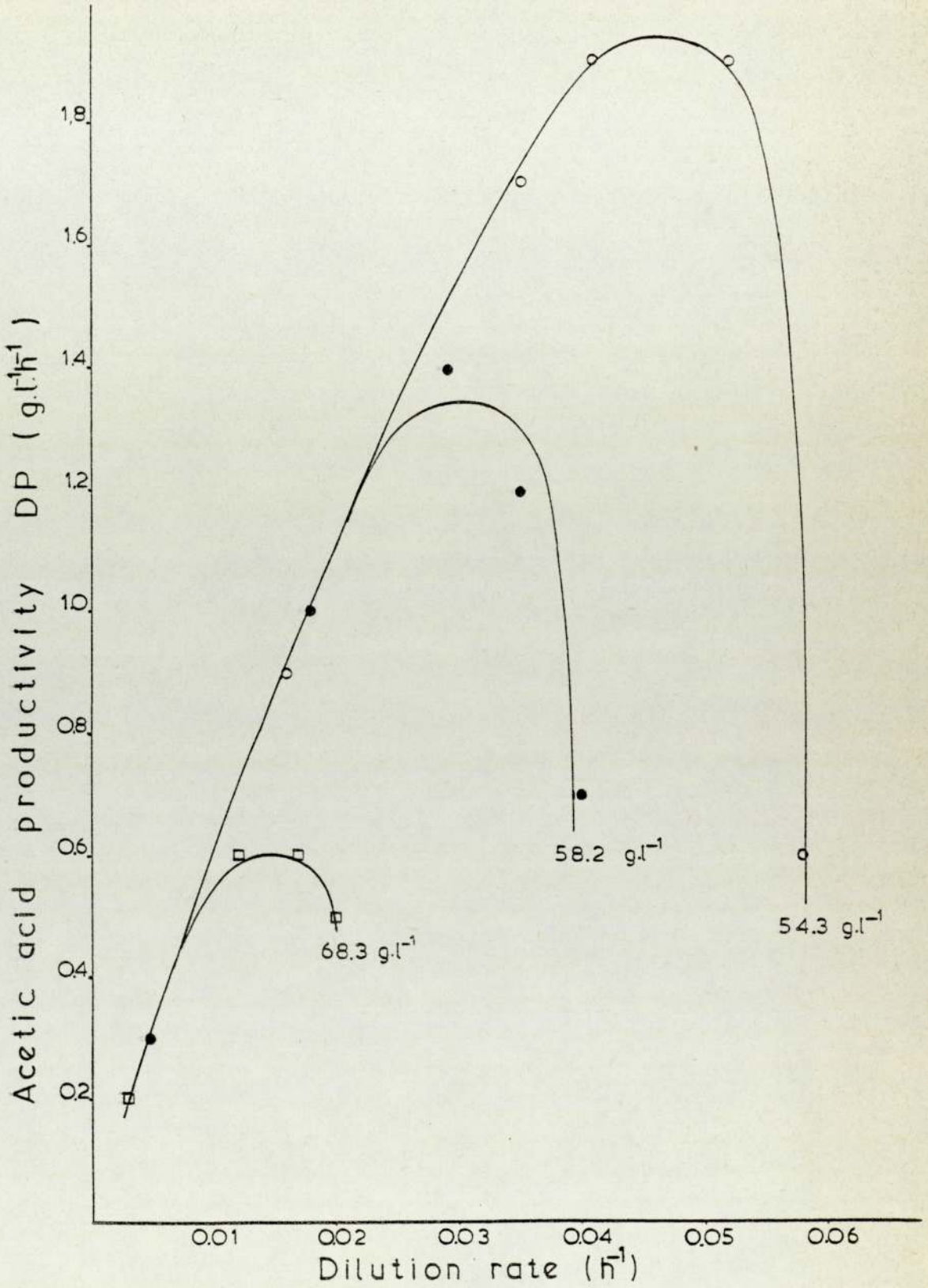
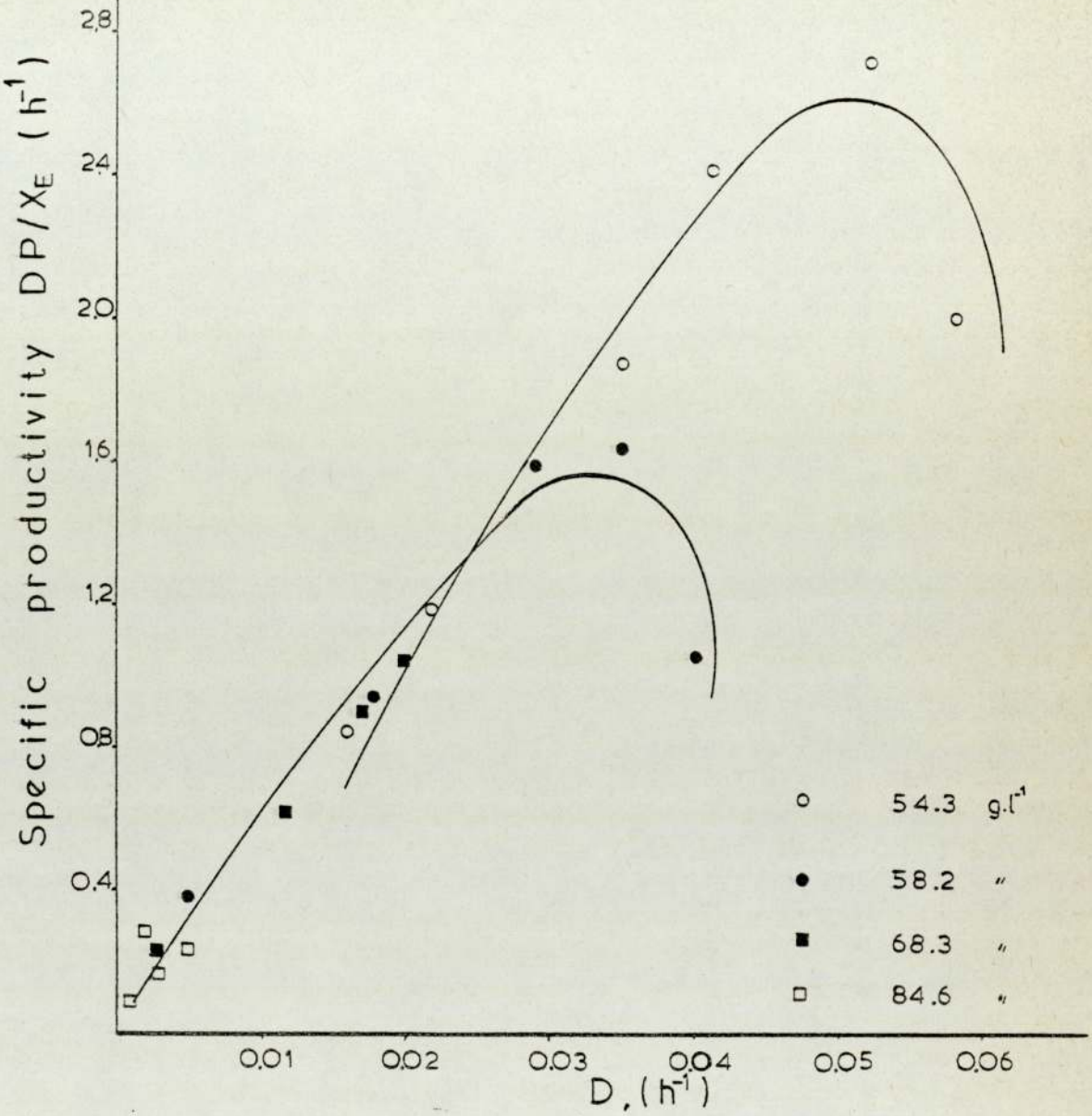


Figure 6.2.5. Acetic acid productivity versus dilution rate for different total acid concentrations. Substrate Molasses.

Figure 6.2.7. Specific productivity versus dilution rate for different total acid concentrations, in molasses medium.



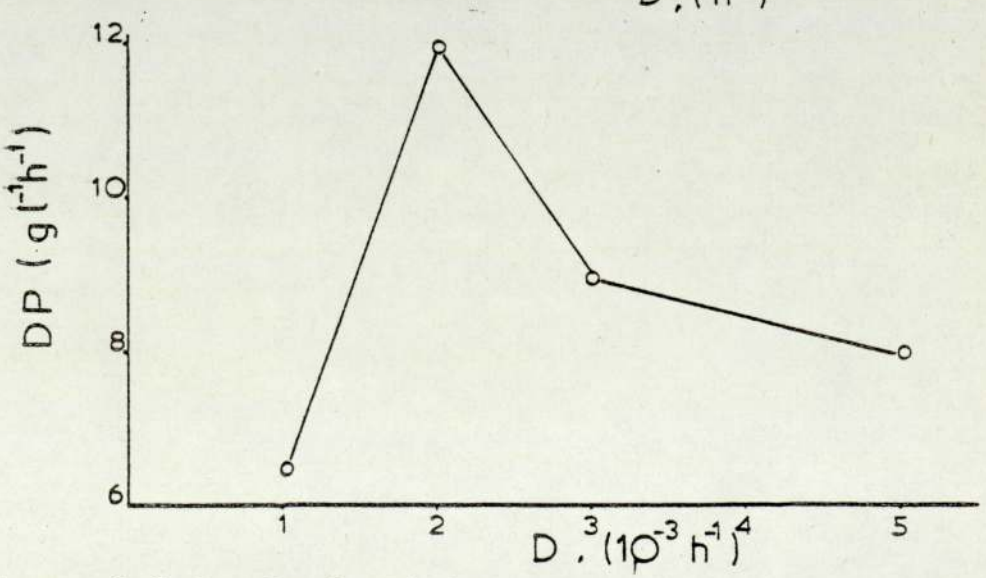


Figure 6.2.6. Productivity versus dilution rate for high total acid concentration (84.6g.l^{-1})

6.2.3.3. Effect of total acidity on the acetic acid productivity and on the acetic acid specific productivity

In figures (6.2.5) and (6.2.6), is shown the relationship between total acidity and acetic acid productivity (D.P).

In low acidities the maximum productivity is higher than the maximum productivity in high acidities.

A similar phenomenon has been observed between specific acetic acid productivity DP/X_E and total acidity.

At low acidities as in the case of 54.3 $g.l^{-1}$, the maximum specific productivity is higher than that in high acidities. Figure (6.2.7). (Table 6.2.3.)

At low dilution rates, the relationship between specific productivity and dilution rate is linear. In higher dilution rates the linearity disappears. The linear relationship between DP/X_E (specific productivity) and D has been found by Divies (1973) who was working with defined media. In Table 6.2.3. are the results of the maximum productivity and maximum specific productivity at different acidities.

Total acidity $g.l^{-1}$	Maximum productivity $g.l.^{-1}.h.^{-1}$	Maximum specific productivity (h^{-1})
54.3	1.95	2.5
58.2	1.35	1.6
68.3	0.6	-
84.6	0.16	-

Table 6.2.3. Maximum productivities and maximum specific productivities observed at different total acidities.
Molasses vinegar
Aeration rate 0.4 v/v/m (s.g.v. 0.55 cm $sec)^{-1}$
Temperature 28°C

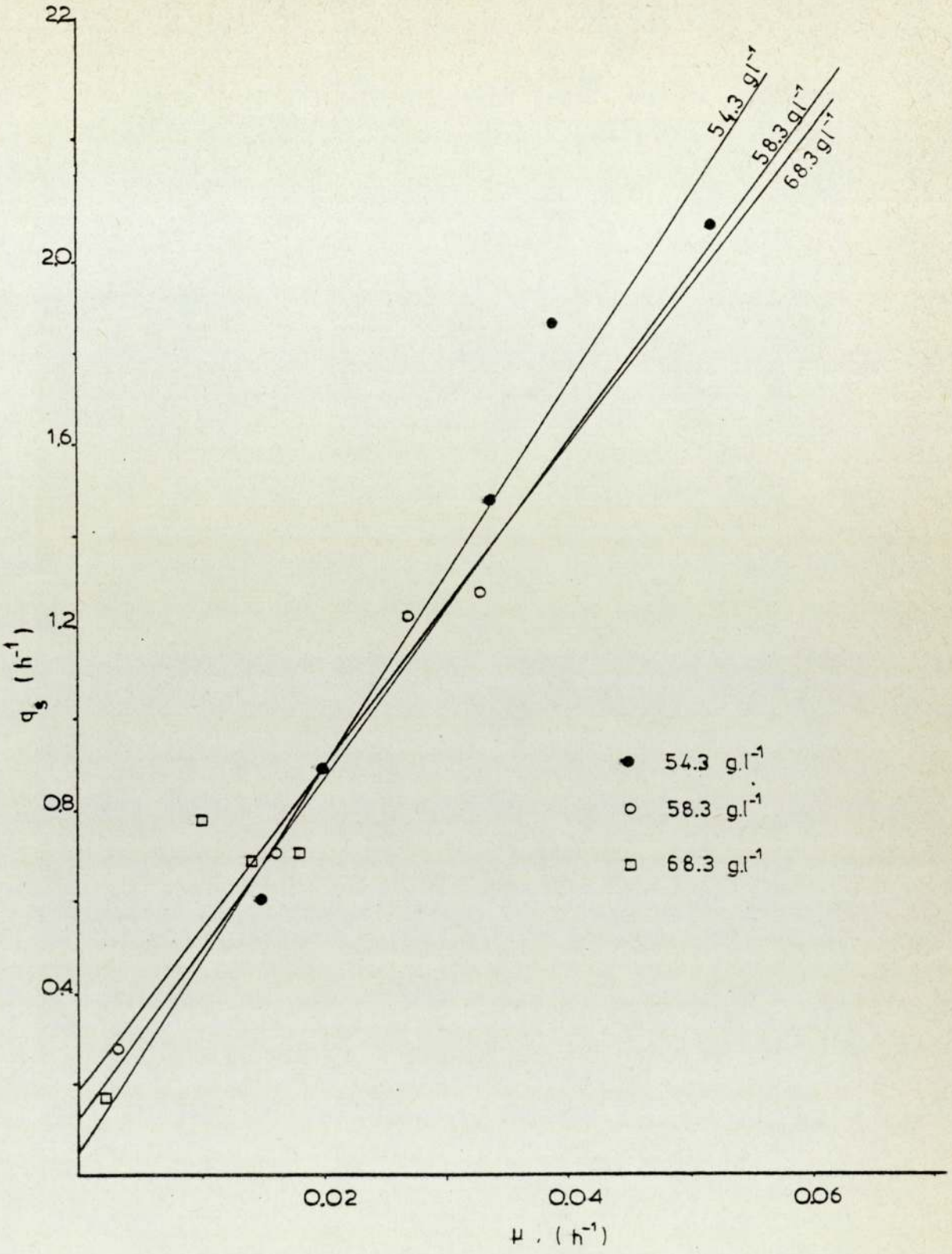


Table 6.2.8. Yield Studies. Determination of Y_G and m Substrate molasses.

6.2.3.4. Yield studies: Determination of the true growth yield and maintenance energy coefficient

The results from the continuous tower fermentation for the production of molasses vinegar were plotted (Figure 6.2.8). The values of q_s versus the values of μ for the different total acid concentration gave the straight lines of figure 6.2.8. According to the equation $q_s = \frac{\mu}{Y_G} + m$ (Pirt, 1975), the values of Y_G and m were obtained. For total acidity 54.3, 58.2 and 68.3 $g.l^{-1}$ the Y_G found to be 0.022, 0.027, 0.029 respectively and the m found to be 0.04, 0.14, and 0.20 g ethanol per g of dry weight bacterial biomass per hour respectively.

Both parameters Y_G and m were increased with the total acidity increasing. The increase of the ionic concentration of the medium increasesthe demand for higher maintenance energy of the cells. (Stouthamer, 1976). The true growth yield also is influenced by both the medium composition and the maintenance energy coefficient. The values of Y_G and m found for different total acid concentrations of the medium are compared with the values of Y_G and m for different temperatures later on in the text (paragraph 6.2.4.2.4).

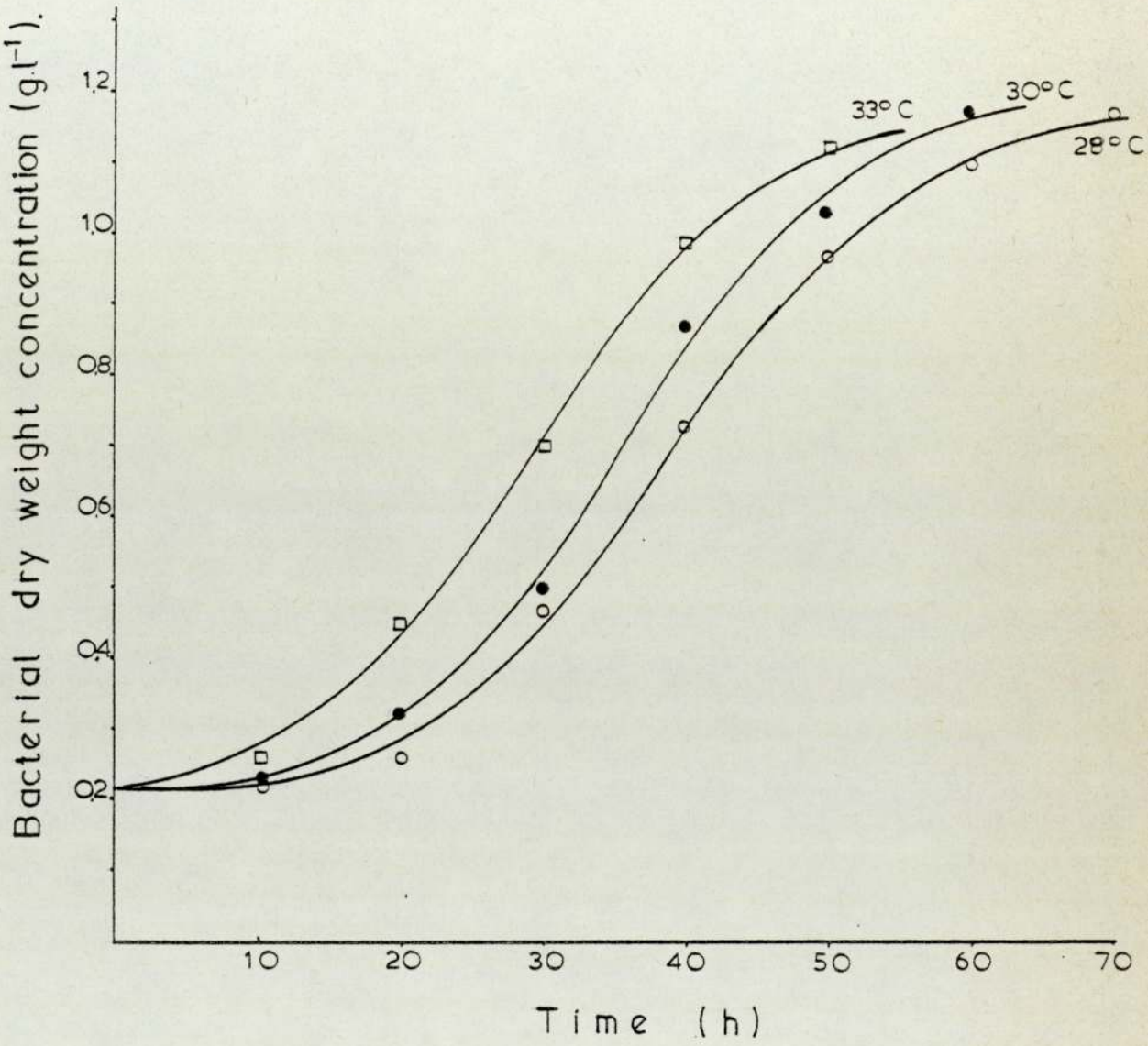


Figure 6.2.9. Bacterial dry weight concentration versus time at different conditions of temperature
Substrate molasses
Batch fermentation

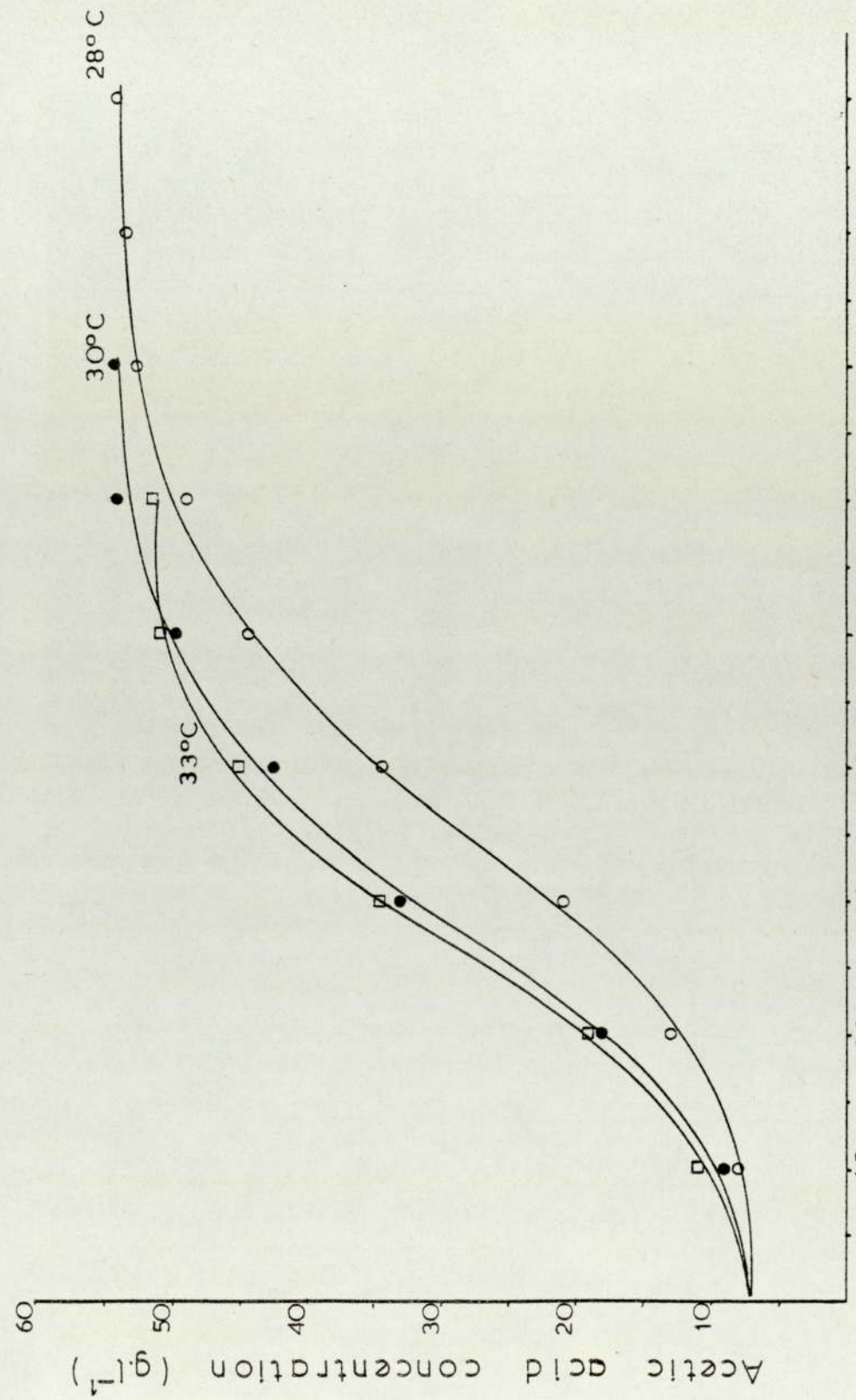


Figure 6.2.10. Product formation against time in batch fermentation at different temperatures.

Substrate Molasses

6.2.4. EFFECTS OF TEMPERATURE ON THE PRODUCTION OF MOLASSES VINEGAR IN TOWER FERMENTERS

6.2.4.1. Batch cultures

Three fermentation systems were prepared in order to perform the experiments for the determination of the optimum temperature, in batch, first, and continuous later. The medium chosen was the A (Table 4.2.1). The aeration rate was chosen 0.4 v/v/m (s.g.v. 0.55 cm.sec^{-1}), because of the comparatively low foam formation at this aeration rate. The temperatures chosen were 28°C, 30°C and 33°C. At temperatures of 28°C and 30°C, there was no loss of ethanol due to evaporation.

6.2.4.1.1. Effect of temperature on the product formation rate and bacterial growth rate

The development of the bacterial growth is shown in Figure (6.2.9.). As indicated on the table, the duration of the batch culture at 28°C was longer than at 33°C. At the temperature of 30°C, also, the duration of the growth is longer than that at 30°C and shorter than that at 28°C. This indicates that the temperature, when all other parameters remain constant, affects the duration of completion of the acetic acid fermentations for molasses vinegar production. The product formation follows the pattern of the bacterial growth, (Figure 6.2.10). The reasons are that the acetic acid is a partially growth associated and partially biomass concentration (non growth) associated. The maximum growth rate at tmperature 28°C appeared at the 40th hour and at 33°C at the 30th hour from the beginning of the culture, (Figure 6.2.11). This is an indication that the growth rate was affected by the increase in temperature. Also, the product formation rate had shown a similar behaviour towards the

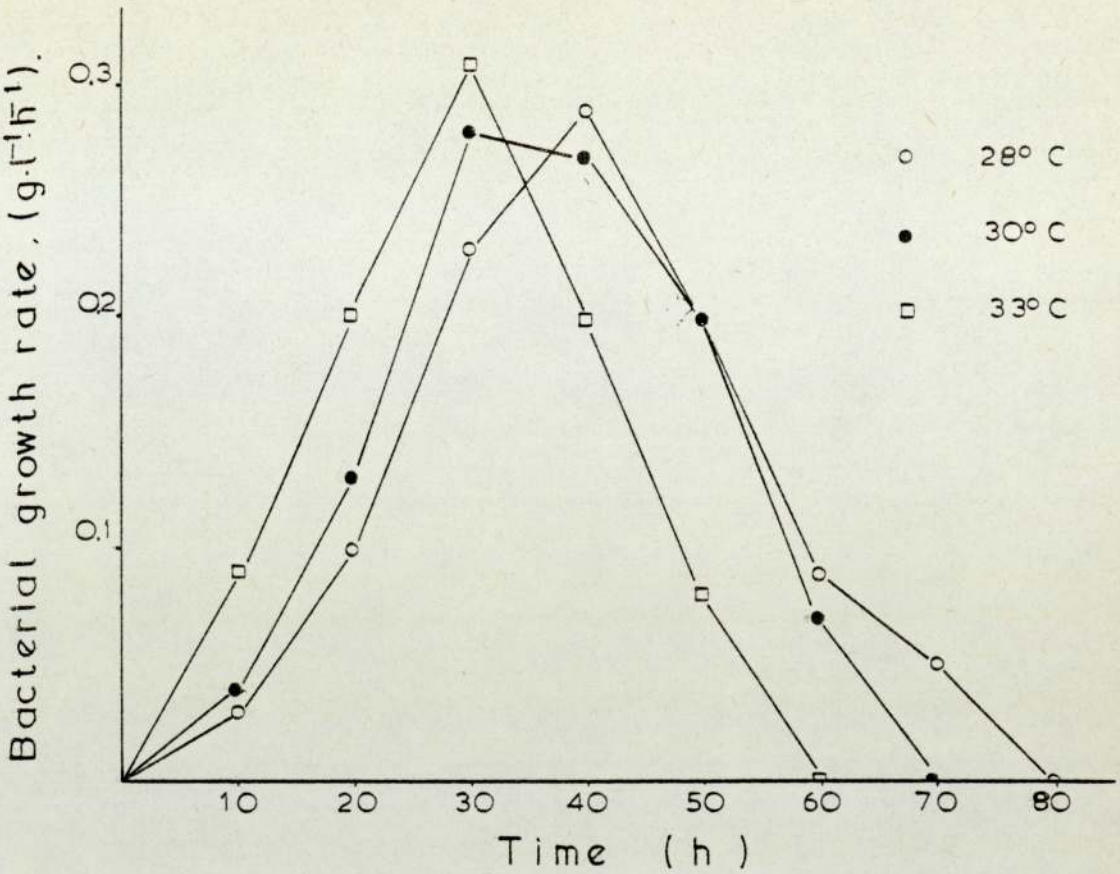


Figure 6.2.11. Bacterial growth rate versus time for different conditions of temperature.
Substrate molasses.

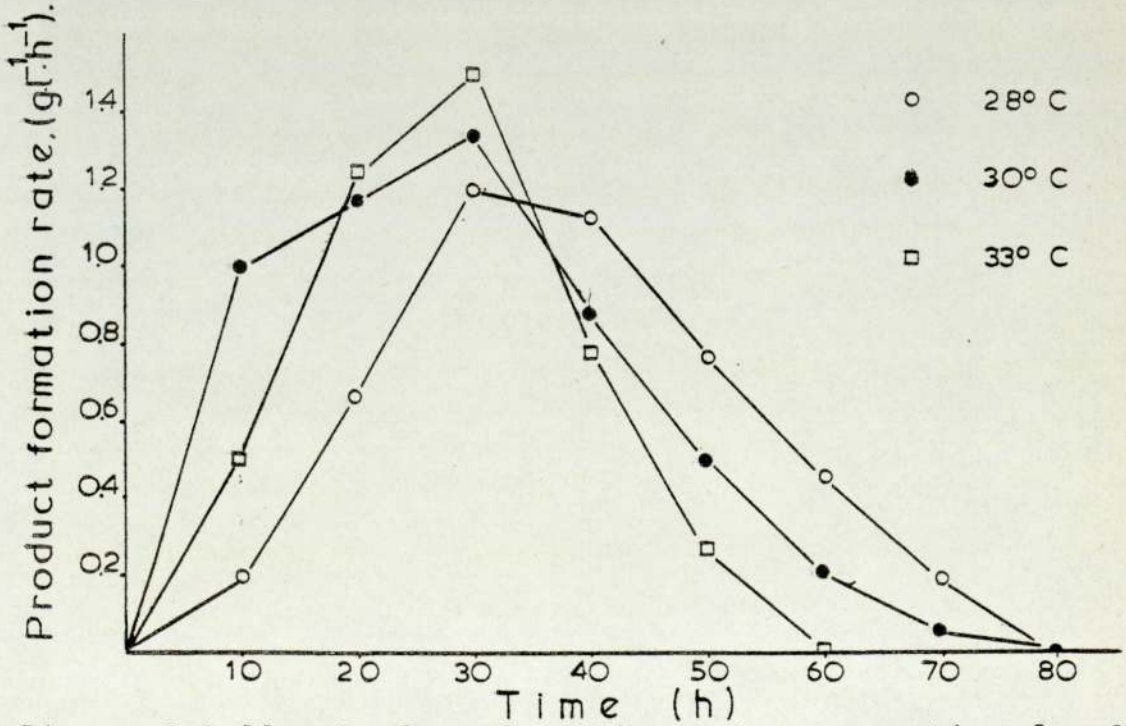


Figure 6.2.12. Product formation rate versus time for different conditions of temperature
Substrate molasses.

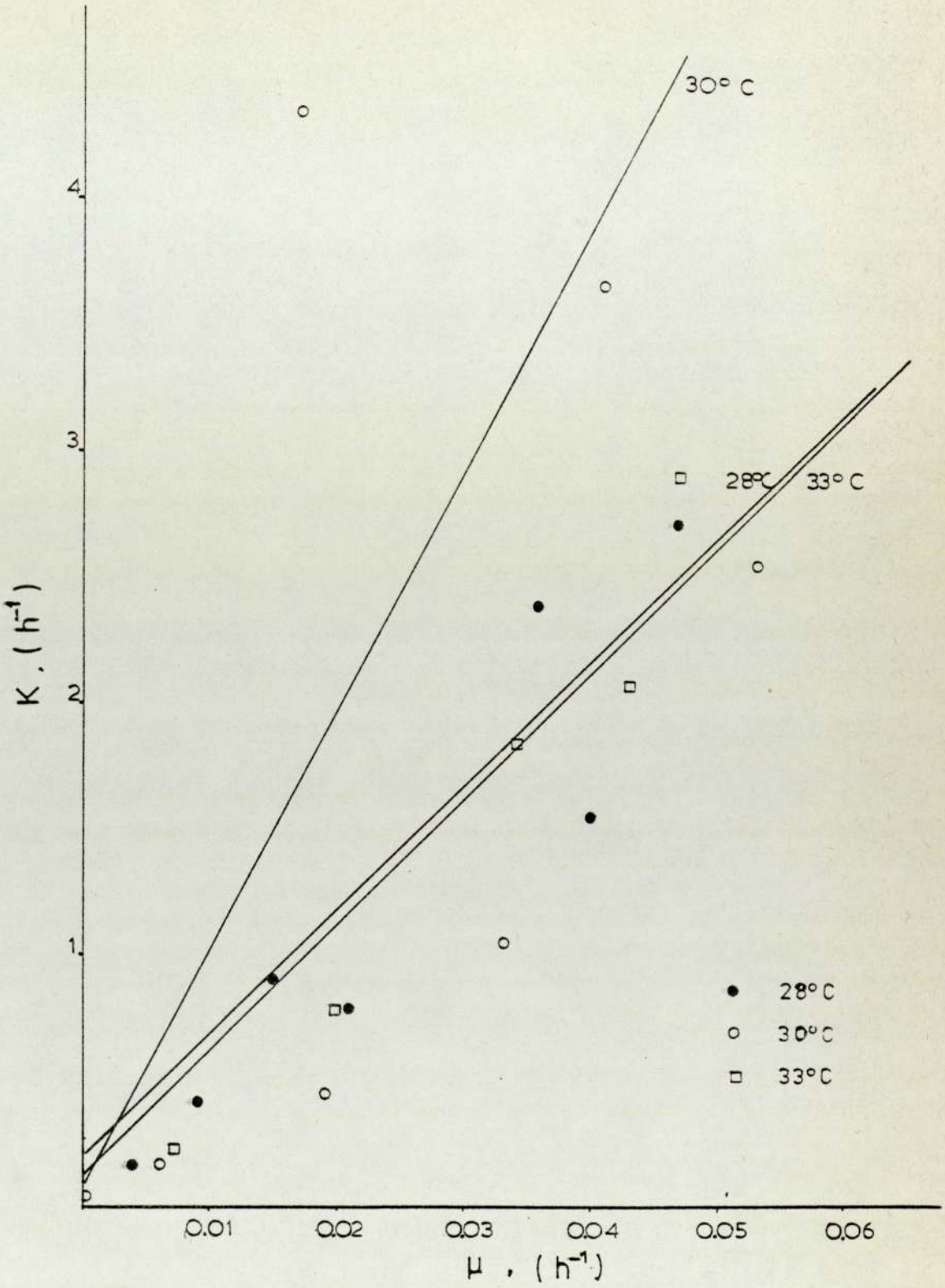


Figure 6.2.13. Relationship between specific product formation rate, K, and specific growth rate, μ, at different conditions of temperature.
Substrate molasses

temperature changes (Figure 6.2.1.2.).

In table (6.2.4.) results are included from the temperature experiment concerning the effects of temperature on the product formation rate, growth rate and lag phase duration.

Temperature ^{o}C	Time period at which max. growth rate observed. (h)	Time period at which max. product formation rate observed. (h)	Lag phase duration. (h)
28	40	40	15
30	30	30	10
33	30	30	6

Table 6.2.4. Time periods at which maximum growth rate and product formation rate occurred. Also the duration of lag phase at different temperature conditions
Molasses vinegar
Aeration rate 0.4 v/v/m (s.g.v. 0.55 $cm.sec^{-1}$)
Total acidity 54.3 $g.l^{-1}$

The lag phase appeared to be shorter at the temperature of $33^{o}C$ and longer at the temperature of $28^{o}C$. A reason for this is given in Section (3).

6.2.4.1.2. Relationship between specific product formation rate (K) and bacterial specific growth rate (μ) at different temperatures. Substrate: molasses

Results of the three batch cultures of the specific product formation rate ($\frac{1}{X} \cdot \frac{dP}{dt} = K$) have been plotted against the values of specific growth rate of bacteria ($\frac{1}{X} \cdot \frac{dx}{dt} = \mu$) Figure (6.4.13). Regression analysis proves that the relationship between K and μ is linear, of the type $K = a\mu + b$.

Table (6.2.5.) includes the results of the regression analysis of k on μ and gives the values of the experimental parameters a and b for the different temperatures.

Temperature ^{o}C	a	b (h^{-1})	r	P
28	51.6	0.2	0.986	0.001
30	70	0.07	0.680	0.05
33	45.67	0.12	0.882	0.01

Table 6.2.5. Values of the experimental parameters a, b for different temperatures in batch culture.
r = correlation coefficient
p = probability
Molasses vinegar
Aeration rate 0.4 v/v/m (s.g.v. 0.0-5 cm sec^{-1})
Total acidity 54.3 $g.l^{-1}$

6.2.4.2. Continuous culture

Continuous operation of the tower fermenter was applied for three different temperatures keeping constant all other parameters, i.e. aeration rate o.4 v/v/m (s.g.v. 0.55 cm sec^{-1}), medium total acidity 54.3 $g.l^{-1}$ and volumetric capacity of the tower fermenter 3.1 l.

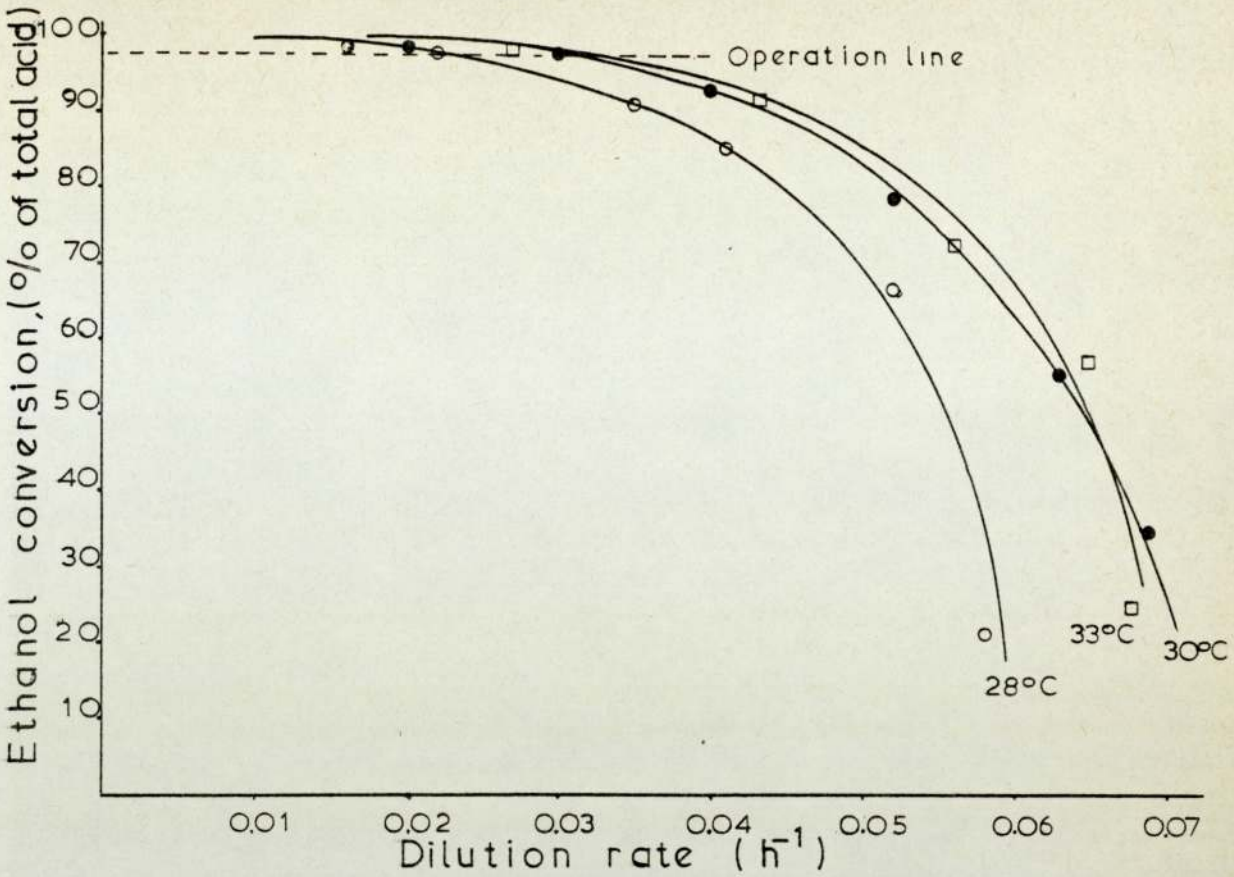


Figure 6.2.14. Effect of temperature on the conversion in continuous molasses vinegar fermentations

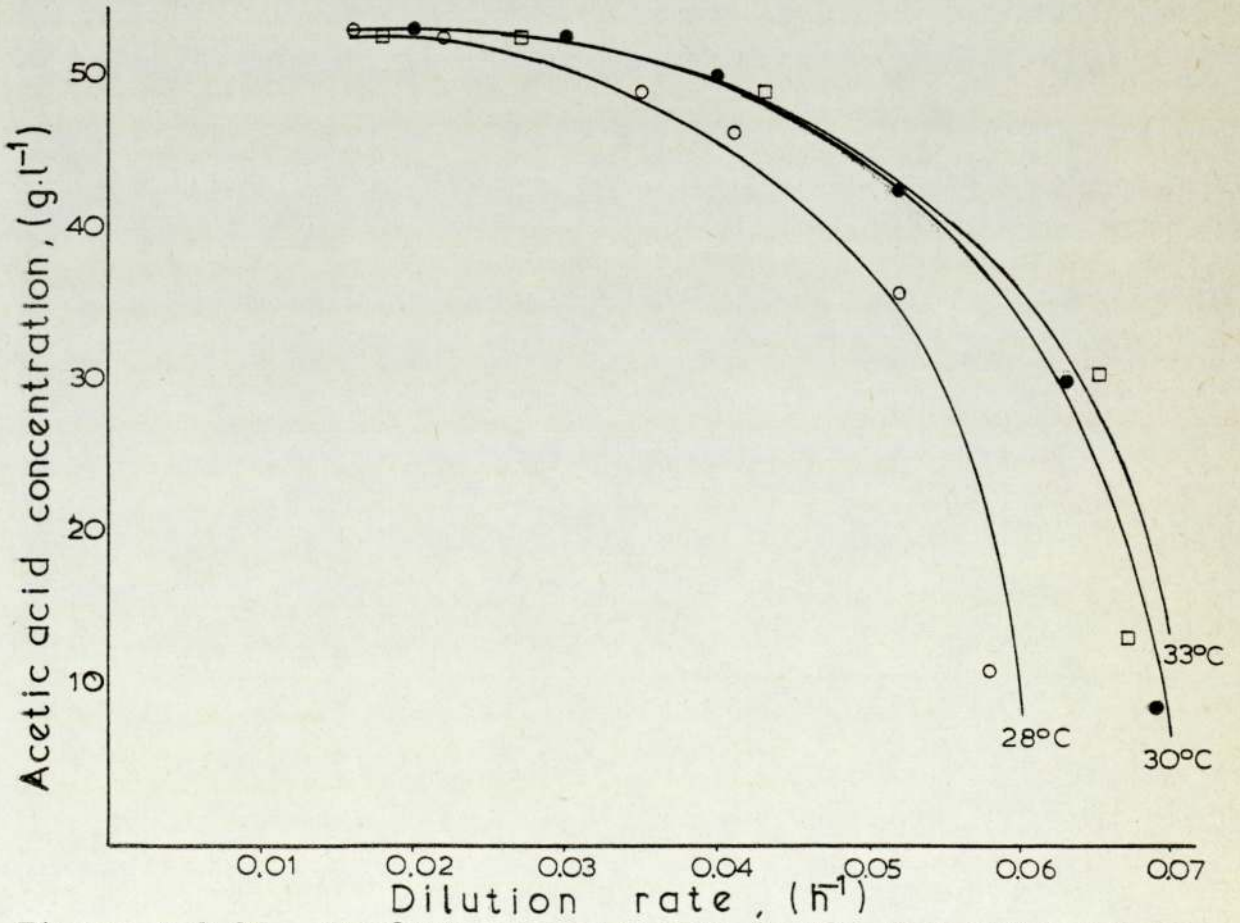


Figure 6.2.16. Product concentration in the fermenter versus dilution rate for different temperatures.

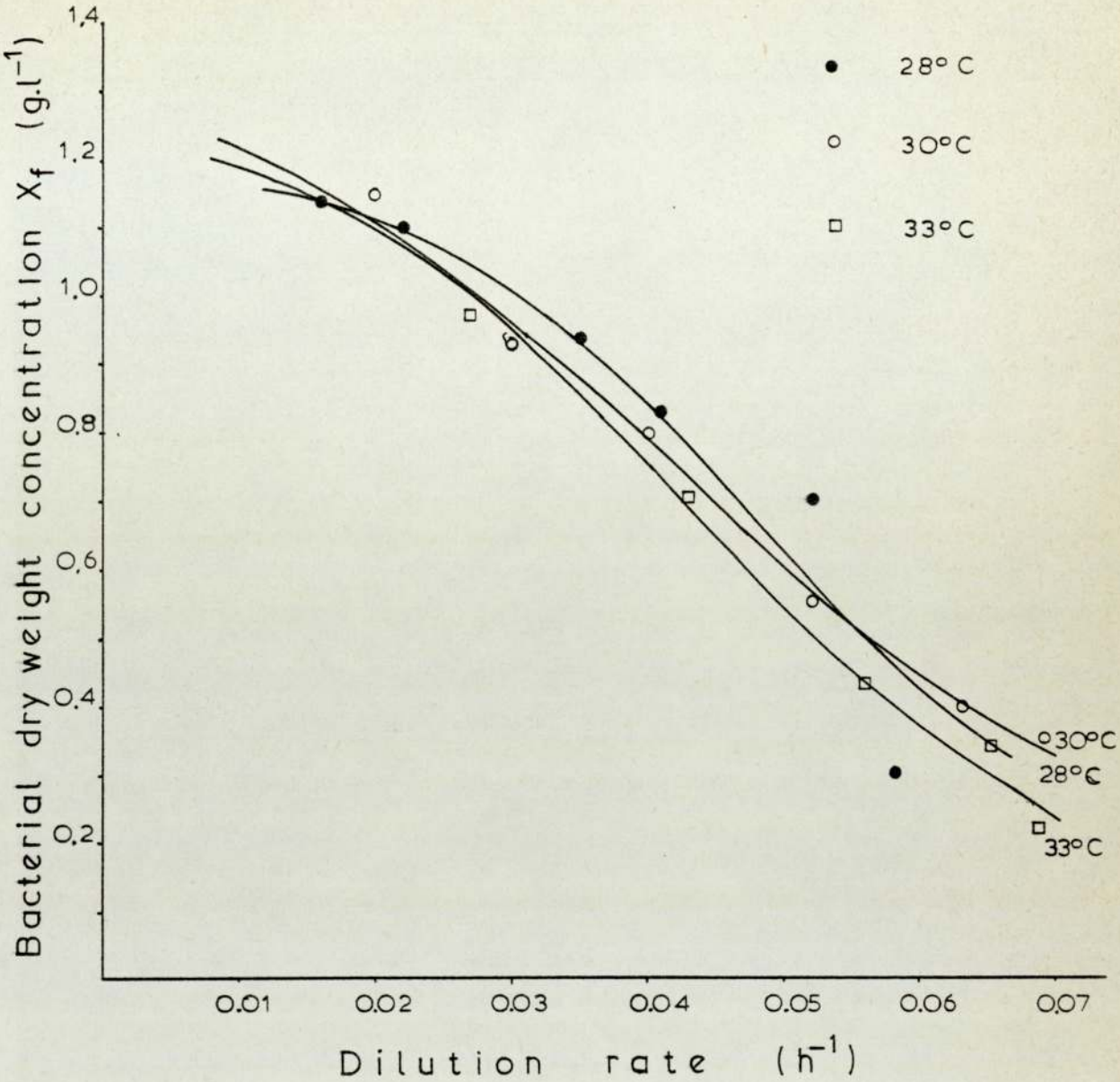


Figure 6.2.15. Effect of temperature on the dry weight concentration, X_F, in the fermenter in continuous molasses vinegar fermentation

6.2.4.2.1. Effect of temperature on ethanol conversion to acetic acid in continuous tower fermentation for the production of molasses vinegar

The temperatures which were tested were 28°C, 30°C and 33°C. At temperatures of 28°C and 30°C the ethanol and acetic acid evaporation was not appreciable. At the temperature of 33°C, the substrate loss due to evaporation was 0.073 $g.l^{-1}$ h^{-1}.

The conversion of ethanol at the temperature of 28°C was slower than that at temperatures of 30°C and 33°C. See Figure (6.2.14), Table (6.2.6).

At the temperatures of 33°C and 30°C, there was no substantial difference. Generally at the three temperatures mentioned above, the kinetics of the substrate conversion follow the typical tower fermentation kinetics which have been previously observed in cocoa sweatings vinegar fermentation.

Temperature (°C)	Dilution rates at 97.5% substrate conversion (h^{-1})	Medium residence times at 97.5% substrate conversion (h)
28	0.023	43.5
30	0.032	31.3
33	0.033	30.3

Table (6.2.6) Dilution rates and residence times of the medium at the level of 97.5% ethanol conversion, Figure (6.4.4.)

Molasses vinegar

Aeration rate 0.4 v/v/m

Total acidity 54.3 $g.l^{-1}$

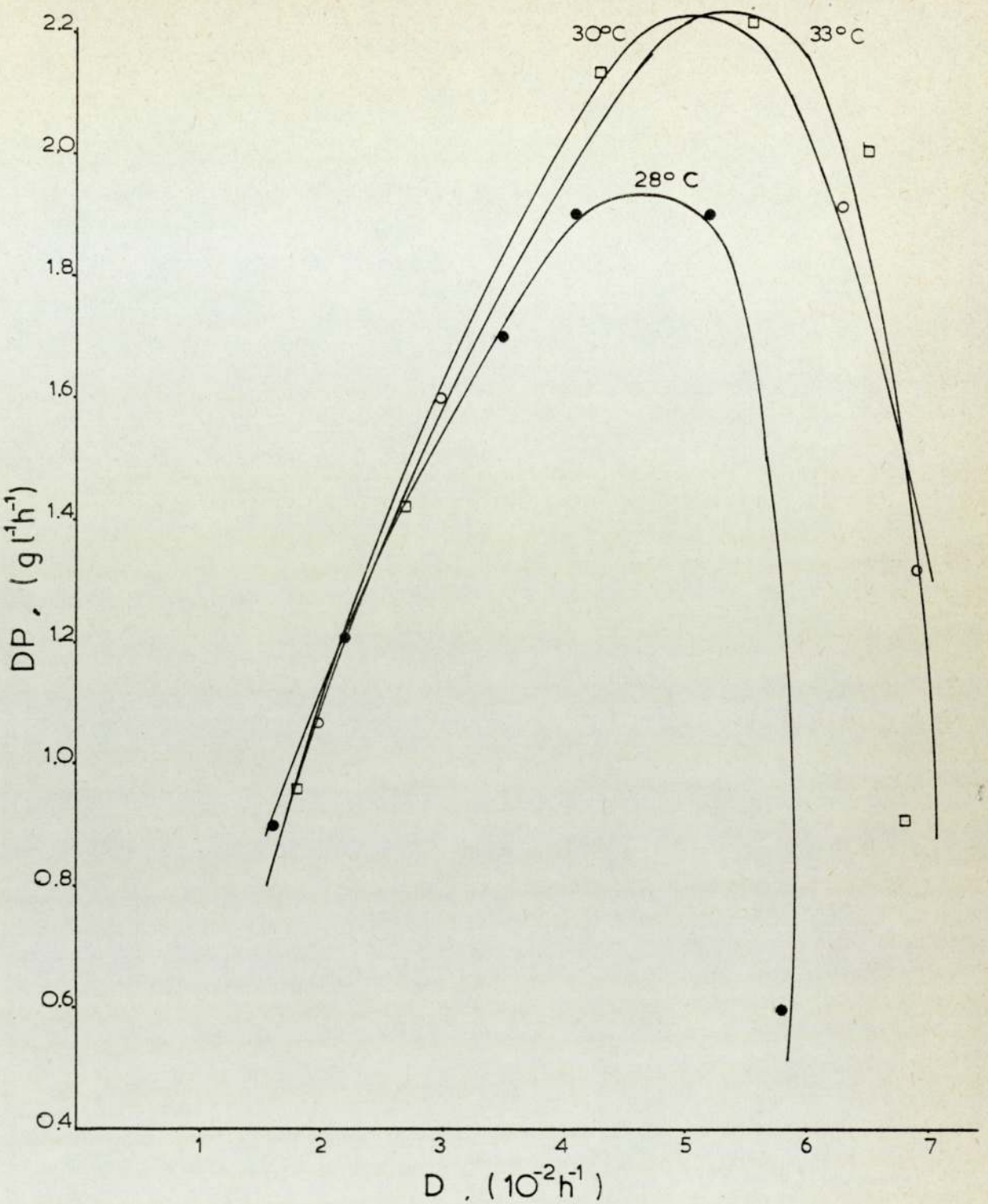


Figure 6.2.17. Acetic acid productivity, DP, versus dilution rate, D, for different conditions of temperature. Substrate molasses.

6.2.4.2.2. Effect of temperature on the bacterial dry weight concentration and product concentration in the fermenter

In Figures (6.2.15) and (6.2.16), the relationship between bacterial dry weight in the fermenter and the product concentration at increasing dilution rates is shown. The pattern of the curves for the product concentration in the fermenter does not decline from that of a C.S.T.R. In contrast the washout of the bacterial biomass from the fermenter exhibits the characteristic tower fermenter effect which influences the whole kinetics of the fermentation (Figure 6.2.16).

6.2.4.2.3. Effect of temperature on the productivity and specific productivity

At temperatures lower than 30°C, the productivity (D.P.) of the tower fermentation system showed a decrease equal to 0.29 g. of acetic acid per ℓ of medium per h. Figure (6.2.17).

At temperatures of 30°C and 33°C, the productivities did not exhibit appreciable difference, table (6.2.7). The specific productivities $(\frac{DP}{X_E})$, was increased with increasing temperature Fig. 6.2.18. A cause for the increase of the productivity and the specific productivity with increase in temperature could be that the product formation rate is increasing when the temperature increases (Paragraph 6.2.4.1.1.) At the temperatures of 30°C and 33°C, the maximum productivity is almost similar, but the specific productivities differ substantially. This means that the productivity per bacterial biomass unit increases when the temperature increases. (Tables A.2.5-A.2.9)

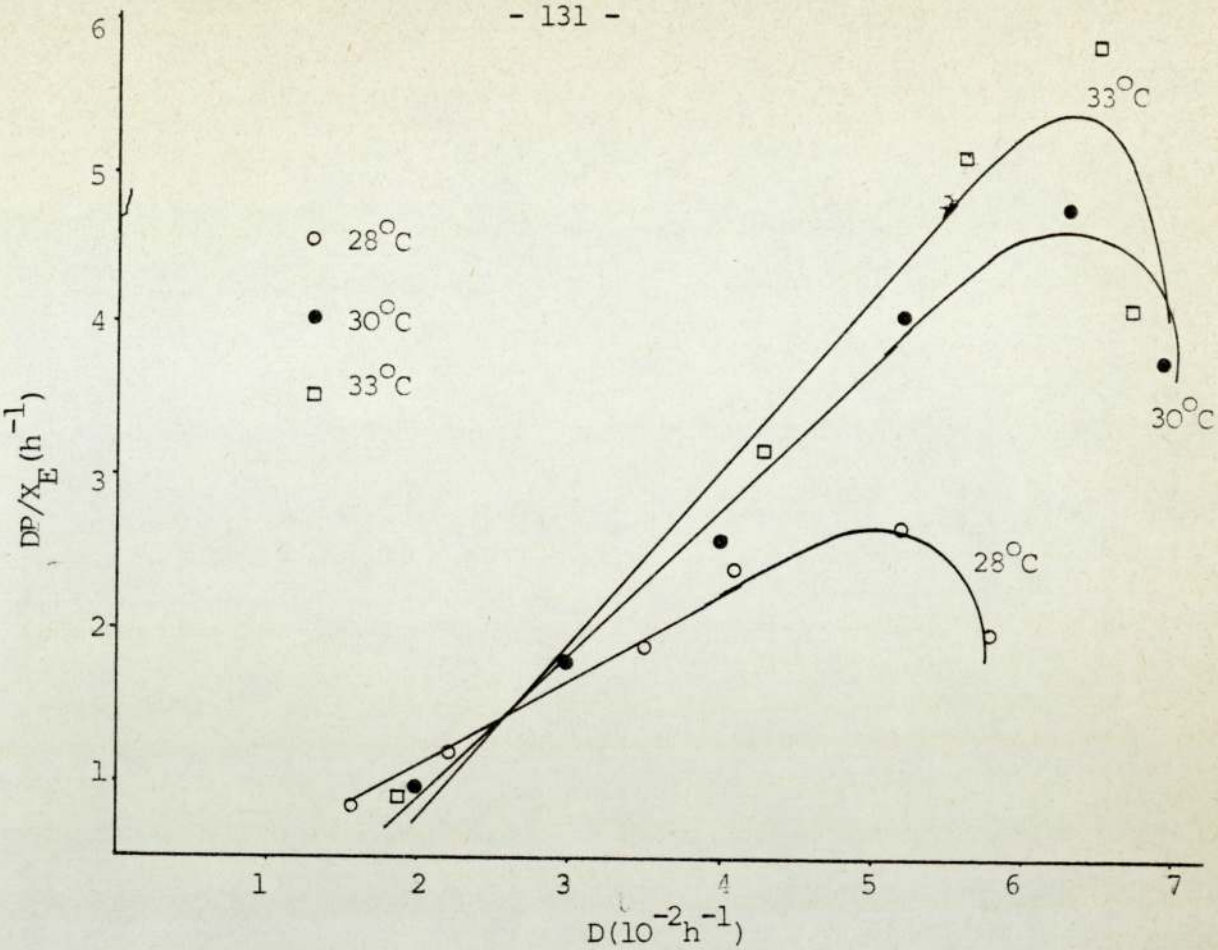


Figure 6.2.18. Specific productivity DO/X_E versus dilution rate, D for different conditions of temperature.

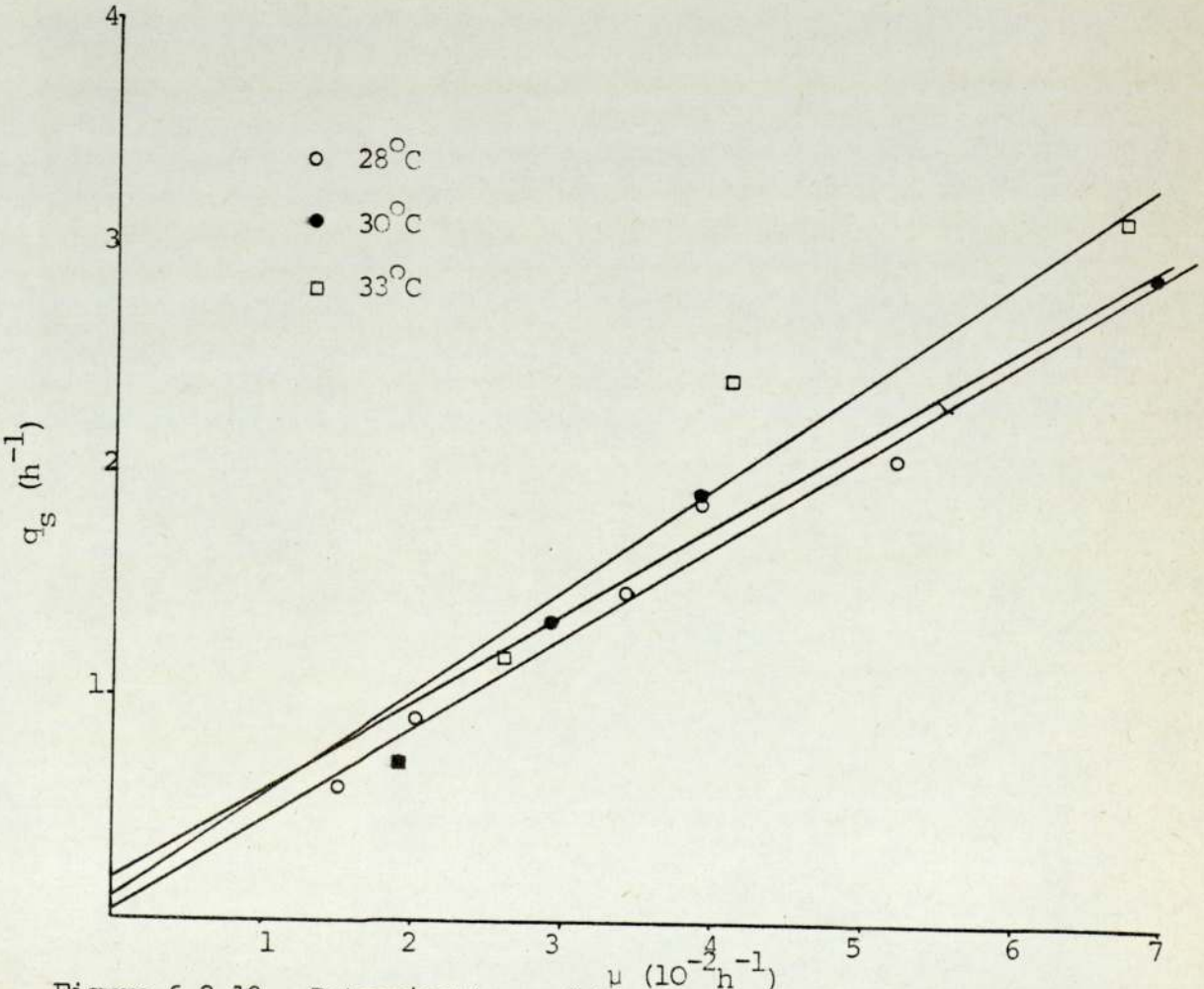


Figure 6.2.19. Determinations of the true growth yield, Y_G, and maintenance coefficient, m, for different conditions of temperature

Temperature (^{o}C)	Maximum productivity ($g.l^{-1}.h^{-1}$)	Maximum specific productivity (h^{-1})
28	1.94	2.70
30	2.23	4.65
33	2.24	5.45

Table (6.2.7) Values of maximum productivity and maximum specific productivity at different temperatures
Molasses vinegar
Aeration rate 0.4 v/v/m (s.g.v. 0.55 cm sec^{-1})
Total acidity 54.3 $g.l^{-1}$

6.2.4.2.4. Determination of the true growth yield, Y_G, and maintenance energy coefficient, m, for different conditions of temperature

In Figure (6.4.19), values of q_s are plotted against μ, in order to obtain the true growth yield, Y_G, and the maintenance energy coefficient, m. The results are shown in Table 6.2.8.

Temperature ^{o}C	Y_G $g.g^{-1}$	m $g.g^{-1}.h^{-1}$	Total acid $g.l^{-1}$
28	0.022	0.04	54.3
30	0.025	0.18	54.3
33	0.021	0.10	54.3

Table (6.2.8.) Values of Y_G and m at different temperatures. Molasses vinegar. Aeration rate 0.4 v/v/m

When the temperature increases from 28° to 30°C the maintenance energy coefficient increases for the organisms studied. The low values of m and Y_G at 33°C could be explained

from the evaporation of ethanol at this temperature.

Comparison of these values with those obtained in paragraph 6.2.3.4. show that both the total acidity and the temperature play an important role on the maintenance energy coefficient. Small change in the ionic composition of the medium causes gross change in the values of m for the same temperature. A small change of $2^{o}C$ from 28^{o} to $30^{o}C$ for the same total acidity, 54.3 $g.l^{-1}$ the maintenance energy changed from 0.14 to 0.18 $g.g^{-1}.h^{-1}$. The difference is more obvious when the total acid changed from 54.3 to 68.3 $g.l^{-1}$. The corresponding values of m were changed in the range of 0.16 $g.g^{-1}.h^{-1}$. Although the changes of Y_G were not high, in order to permit conclusive remarks, it has been noticed that the same pattern occurred for changes of temperature and total acid but in smaller scale. At temperatures lower than $30^{o}C$, the maintenance energy coefficient m is decreasing and normally the requirements of maintenance energy are higher for higher temperatures. The lower value of m at $33^{o}C$ could be explained from the evaporation of ethanol at this temperature.

6.2.4.2.5. <u>Relationship between specific growth rate (μ) and dilution rate (D) at different conditions of temperature for the production of molasses vinegar</u>

In Figure (6.4.20), values of μ have been plotted against D. Most of the values of μ are lower than the corresponding values of D. There are some values of μ for dilution rates higher than 0.052 h^{-1} in which the equation $\mu = D$ applies. It could be concluded that for dilution rates higher than 0.052 h^{-1} the continuous tower fermenter behaves as an ideal continuous stirred reactor

for the fermentation of acetic acid in the molasses vinegar production.

6.2.5. EFFECTS OF AERATION RATE ON THE PRODUCTION OF MOLASSES VINEGAR IN CONTINUOUS TOWER FERMENTATION

The aeration rates for testing were chosen to be 0.2, 0.3 and 0.4 v/v/m (or s.g.v. 0.27, 0.41 0.55 $cm.sec^{-1}$) respectively.

The reasons for testing this range of aeration are that in the preliminary fermentation (paragraph 6.2.2.), aeration rates higher than 0.4 v/v/m caused vigorous foam formation and consequently it was necessary to use large amounts of antifoam. The use of antifoam prevents the foam formation and consequently the loss of bacteria which are contained in the foam because of froth flotation phenomena. On the other side, the effect of antifoam on the oxygen solubility is negative. Very little amounts of antifoam ($0.02-0.03 g.l^{-1}$) can cause a dramatic fall in the oxygen transfer rate from the air phase to the liquid phase. The reduction of solubility of oxygen in the medium has a direct negative effect on the acetic acid bacteria. (Paragraph 3.3.2.3).

Therefore, it was 0.4 v/v/m at which the maximum aeration rate was tested.

The temperature 30°C was chosen because the productivity was almost equal to that of 33°C with the advantage that no loss of substrate occurred during that temperature. (See paragraph 6.2.4.2.3.).

The medium A was chosen because at this total acidity was observed the highest productivity (see paragraph 6.2.3.3.)

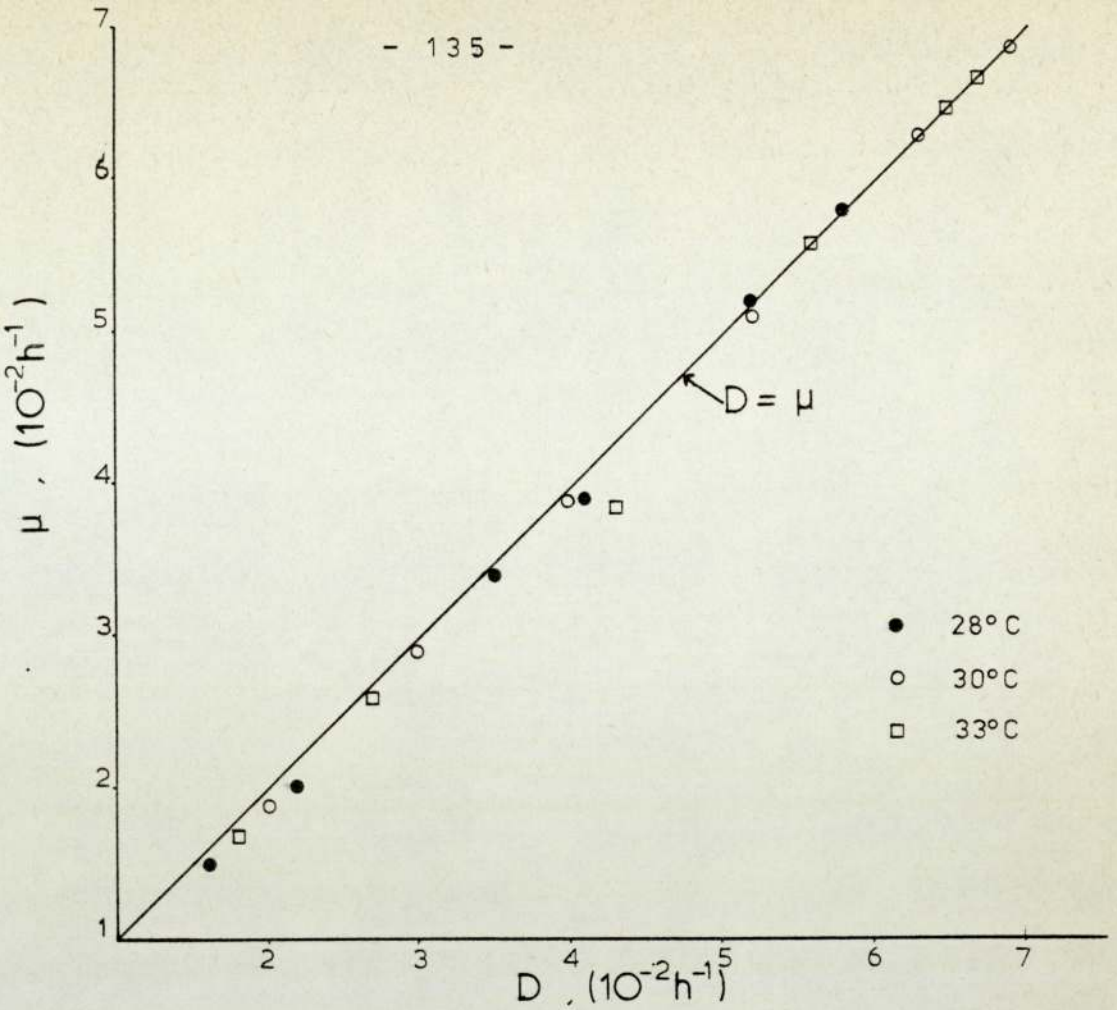


Figure 6.2.20. Relationship between specific growth rate, μ, and dilution rate, D, at different conditions of temperature

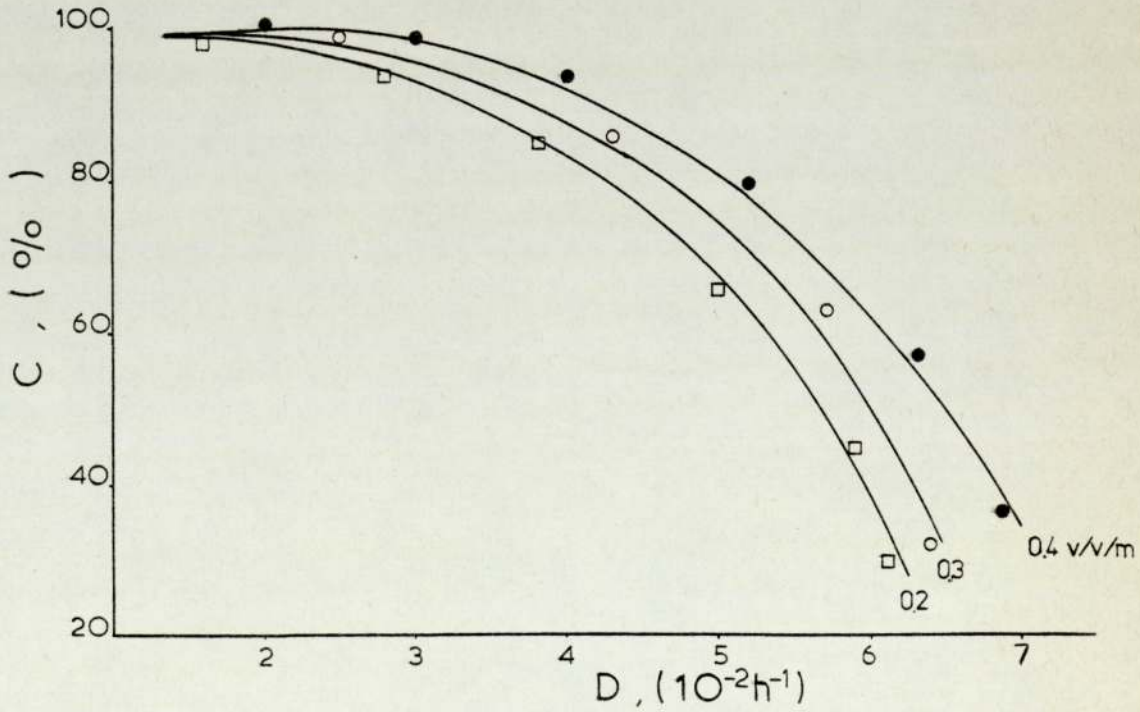


Figure 6.2.21. Effect of aeration on the conversion in continuous molasses vinegar fermentation

6.2.5.1. Effect of aeration rate on the conversion ability of ethanol to acetic acid for the production of molasses vinegar

The ability to convert ethanol to acetic acid was plotted as percentage of the total acid against the dilution rate increase.

With aeration rate 0.4 v/v/m (s.g.v. 0.55 cm sec^{-1}), the conversion was faster than that with 0.3 and 0.2 v/v/m (s.g.v. 040, 0.27 $cm.sec^{-1}$). (Figure 6.4.21.)

As in paragraph 6.1.4.2.2. the phenomenon could be explained in a similar way. The excess of air which is not utilized by the bacteria for the oxidation of ethanol to acetic acid, is used for agitation purposes. Also for increasing the interfacial area. In table (6.2.9), included are values of dilution rates at which the conversion is 97.5% (of total acid).

Aeration rates	Max* S.G.V. $cm.sec^{-1}$	Dilution rate at 97.5% conversion level (h^{-1})	Residence times at 97.5% conversion level (h)
0.2	0.27	0.021	47.6
0.3	0.40	0.026	38.5
0.4	0.55	0.031	32.3

Table 6.2.9. Dilution rates and Residence times values at which 97.5% conversion at ethanol was achieved.

Molasses vinegar

Temperature 30°C

Total acid 54.3 $g.l^{-1}$

* See also table 6.2.11

6.2.5.2. Effect of dilution rate on the biomass, product and substrate concentration at different aeration rates

The bacterial biomass is washed out when the dilution rate is increased. In ideal C.S.T.R., the washout of biomass happens abruptly and the dilution rate at which the wash out phenomenon occurs is called critical dilution rate. In the continuous tower fermenter, the bacteria biomass starts to wash out from small dilution rates and continues the washing out phenomenon in high dilution rates depending on the weight of the microorganisms.

In the case of molasses fermentation, the bacterial dry weight concentration in the fermenter (X_F) at dilution rates of 0.07 (h^{-1}) was high in the tower.
However, the remaining amount of the bacterial dry weight concentration in the fermenter of 0.3 $g.l^{-1}$ was unable to convert the substrate satisfactorily (Figure 6.4.23.)

In dilution rates higher than 0.03h^{-1} the substrate concentration in the fermenter was unacceptable from the economical point of view.

The change in aeration rate from 0.4 v/v/m to 0.2 v/v/m did not affect the wash out pattern of the bacterial biomass. Figure (6.4.22)

On the contrary, in the case of the substrate utilization and product washout the aeration rate of 0.4 v/v/m(s.g.v. 0.55 cm. sec^{-1}) proves more efficient from that of 0.2 or 0.3 v/v/m (s.g.v. 0.27 cm. sec^{-1} and 0.40 cm.sec^{-1} respectively).

The concentration of substrate in the fermenter with constant dilution rate was higher in aeration rates of 0.2 v/v/m and 0.3 v/v/m than that at the aeration rate of 0.4 v/v/m.

The acetic acid concentration in the fermenter with

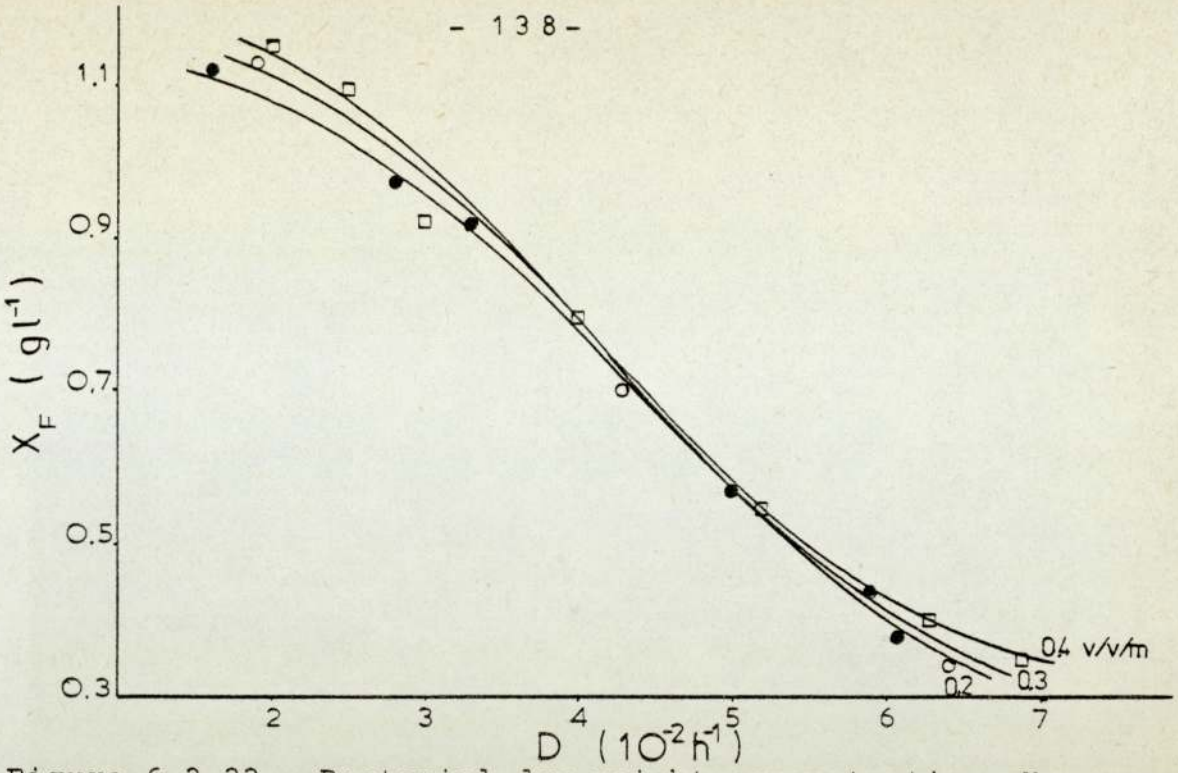


Figure 6.2.22. Bacterial dry weight concentration, X_F, versus dilution rate at different aeration rates.
Substrate molasses.

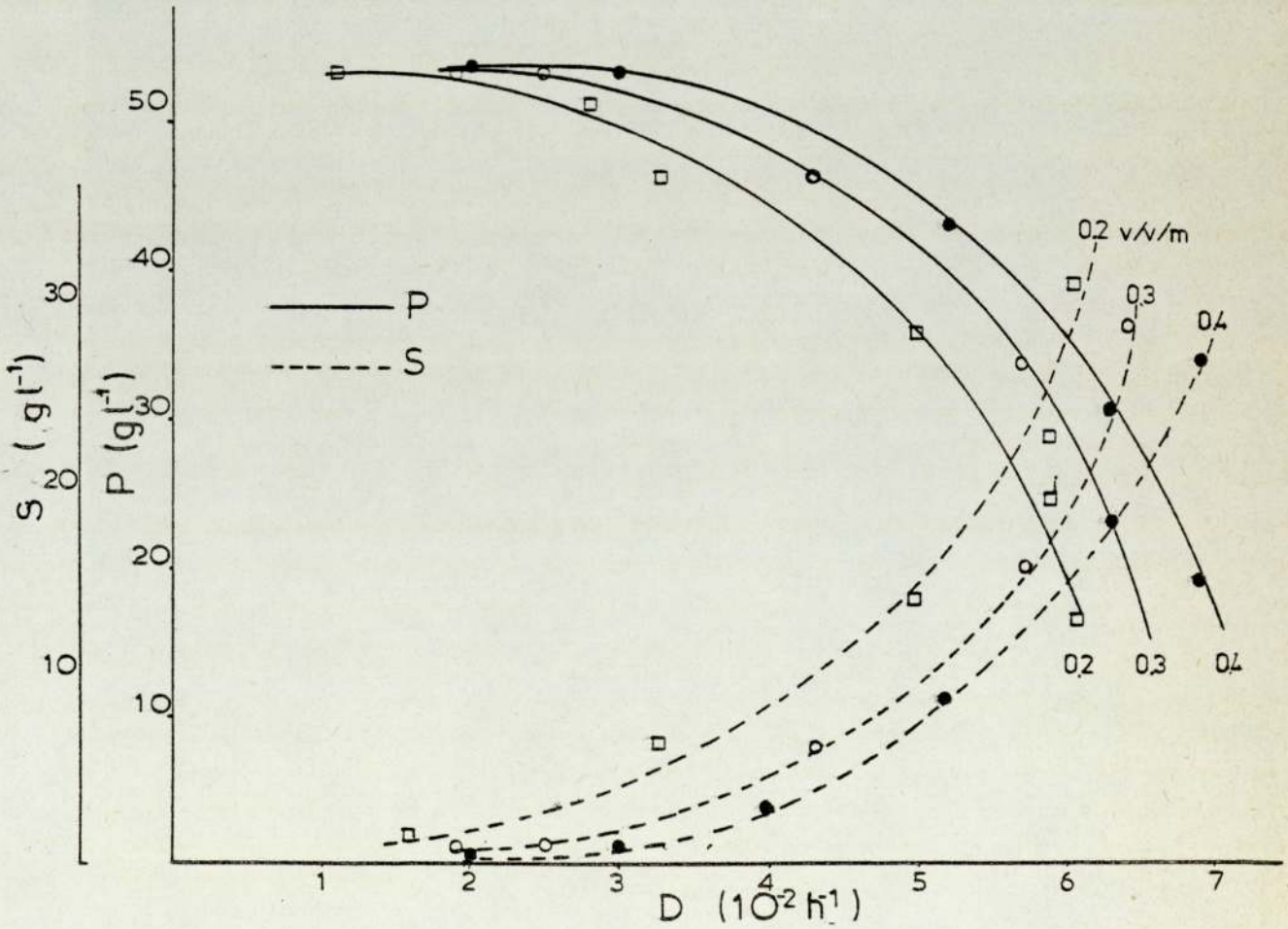


Figure 6.2.23. Product concentration, P, and substrate concentrations versus dilution rate at different conditions of aeration.
Substrate molasses.

constant dilution rate was higher at the aeration rate of 0.4 v/v/m than that at 0.2 and 0.3 v/v/m, Figure (6.2.23).

As was previously mentioned (paragraph 6.1). the aeration rate of 0.2 v/v/m provided sufficient amounts of oxygen for the oxidation purposes, however the excess amount of air was used for other mentioned purposes. (Paragraph 6.2.5.1)

6.2.5.3. Effect of dilution rate on the acetic acid productivity and specific productivity at different aeration rates

The acetic acid productivity of the tower fermenter for the production of molasses vinegar occurred at the maximum aeration rate of 0.4 v/v/m (s.g.v. 0.55 cm sec^{-1}). At aeration rates of 0.2 and 0.3 v/v/m, the maximum productivities were 1.67 $g.l^{-1}.h^{-1}$ and 2.13 $g.l^{-1}.h^{-1}$ respectively (Figure 6.4.24), Table (6.2.10).

Also, the maximum specific acetic acid productivities, i.e. the maximum acetic acid productivity per biomass unit presented a similar pattern. At the aeration rate of 0.4 v/v/m occurred the higher maximum specific productivity.

Aeration rate (v/v/m)	$s.g.v._{max}^{*}$ ($cm.sec^{-1}$)	Max acid productivity ($g.l^{-1}.h^{-1}$)	Max. acid sp productivity (h^{-1})
0.2	0.27	1.67	3.45
0.3	0.4	2.13	4.10
0.4	0.55	2.24	4.70

Table 6.2.10. Maxima of acid productivities (DP) and specific acid productivities ($\frac{DP}{X_E}$) at different aeration rates.

Molasses vinegar

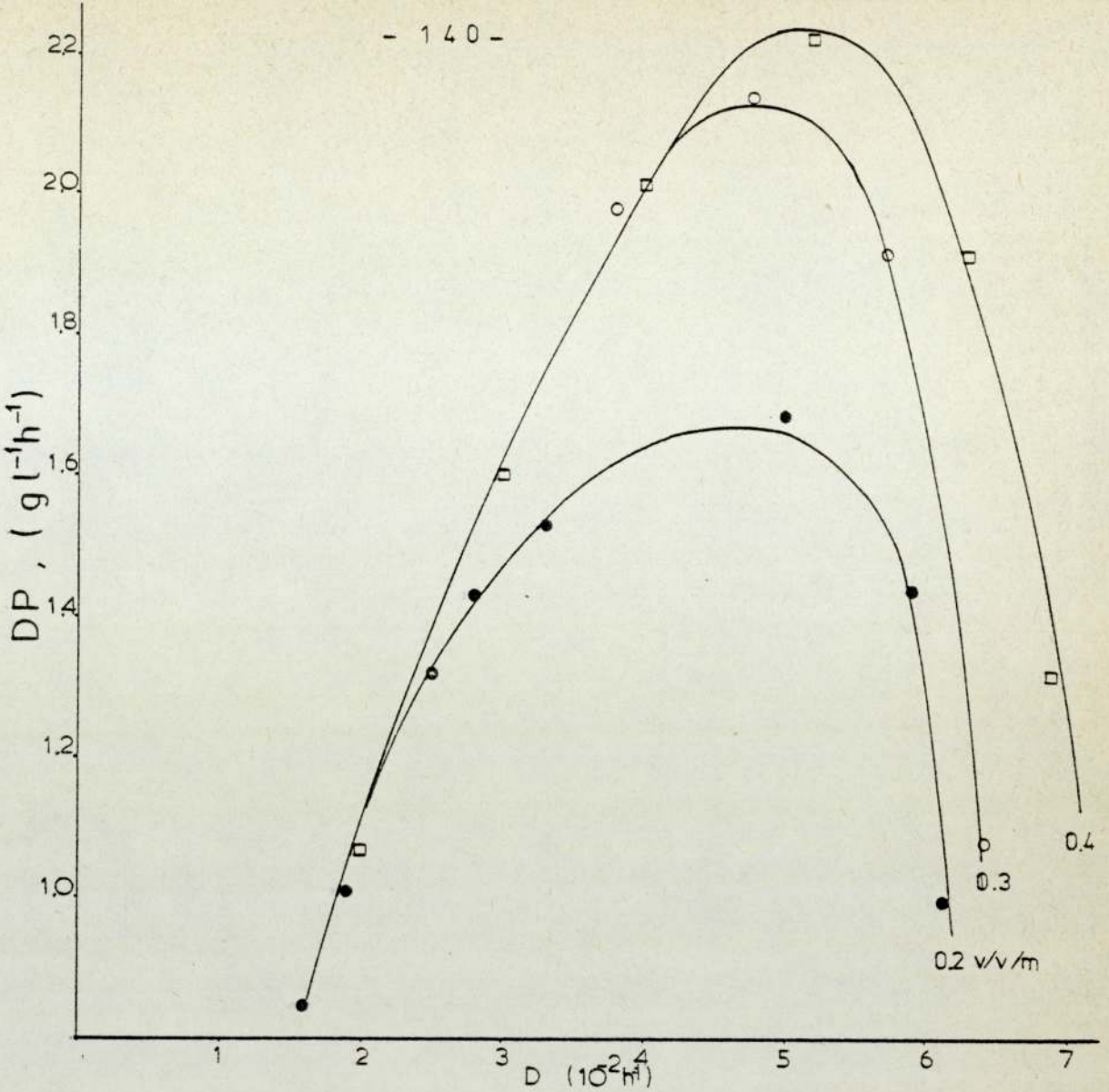


Figure 6.2.24. Productivity, DP, versus dilution rate, D, in molasses vinegar fermentation at different aeration rates.

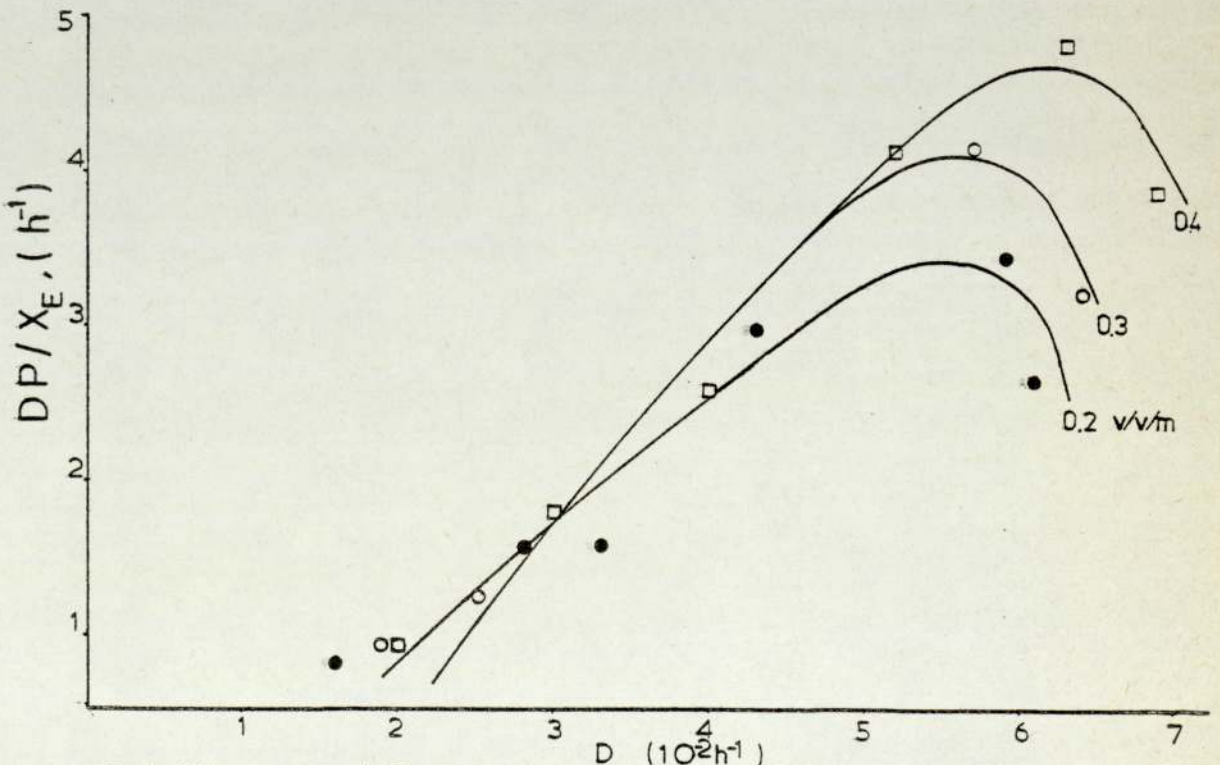


Figure 6.2.25. Specific productivity DP/X_E, versus dilution rate at different conditions of aeration.
Substrate molasses.

Aeration rate (v/v/m)	Tubular part s.g.v. $cm\ sec^{-1}$	Expansion minimum s.g.v. $cm\ sec^{-1}$
02	0.27	0.05
03	0.40	0.07
04	0.55	0.12

Table 6.2.11. Values of superficial gas velocity in the tubular and expansion parts of the fermenter for different medium aeration rates.

The values of s.g.v. in the expansion have been calculated at the maximum section and consequently represent the minimum possible values.

MALT VINEGAR

6.3. PRODUCTION OF MALT VINEGAR IN CONTINUOUS TOWER FERMENTER

6.3.1. Medium, size of fermenter and inonulum used.

The charging wort (commercial term for the malt alcoholic medium) was obtained from Beechams (Food and Drinks) Ltd., Worcester. As described in paragraph 4.3. the charging wort was produced by a process similar to that used for beer from malted barley wort.

The ethanol concentration was 48.3 $g.l^{-1}$ and the residual acid concentration in terms of acetic acid was 4.7 $g.l.^{-1}$.

The size of the fermenter used was 3.105 l.

The inoculum used was obtained from the vinegar producing tower fermenter of Hammond's Co. Ltd., Barrowford, Lancs.

6.3.2. EFFECT OF AERATION RATE ON THE PRODUCTION OF MALT VINEGAR IN TOWER FERMENTER

Five different rates were chosen for testing. The choice was limited by the foam production at 0.5 v/v/m (Greenshields, 1972). The aeration rates were 0.1, 0.2, 0.3, 0.4, 0.5 v/v/m.

The temperature used was 30°C (Greenshields, 1976).

At the temperature at 30°C, no loss of ethanol due to evaporation was noticed. A double system of condensers was used. (Paragraph 2.2.4.3.) The fermentation was running in batch mode.

6.3.2.1. Effect of aeration rate on the bacterial growth rate and product formation rate

The aeration for the production of malt vinegar was determined by the fact that at rates higher than 0.5 v/v/m (s.g.v. 0.67 cm.sec^{-1}) the surface tension properties permitted the production of foam (i.e. a greater volume of air than liquid in a specific area). That was the factor which limited the amount of oxygen which could be supplied to maintain an efficient fermentation.

From experiments with water-filled fermenter, was found that the bubble size was larger than that in the malt medium. The bubble size is a factor which, as explained in paragraph 2.2.4.2., affects the gas transfer rate from air to liquid. The smaller bubbles the greater the air/liquid interface and the better the gas transfer.

In figures (6.3.1) and (6.3.2) is shown the development of bacterial dry weight concentration and acetic acid concentration, respectively.

A useful observation is that the lag phase duration in high aeration rates is shorter. In table 6.3.1. are included values of the lag phase at different aeration rates. Between 0.2 and 0.3 v/v/m aeration rates, there was no difference in the duration of lag phase (Method of Lodge and Himshelwood, 1943).

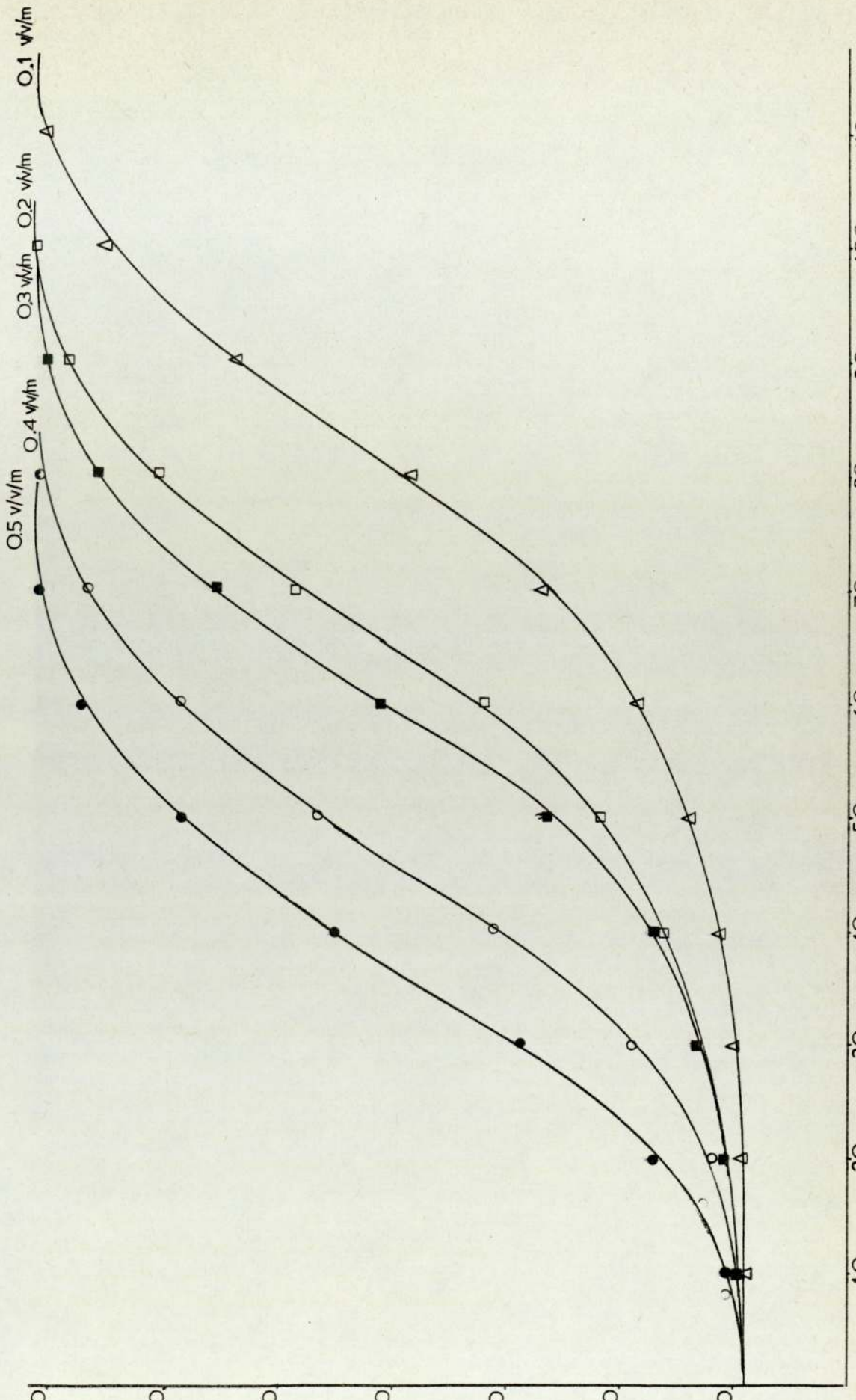


Figure 6.3.1. Acetic acid concentration versus time in batch malt vinegar fermentation.

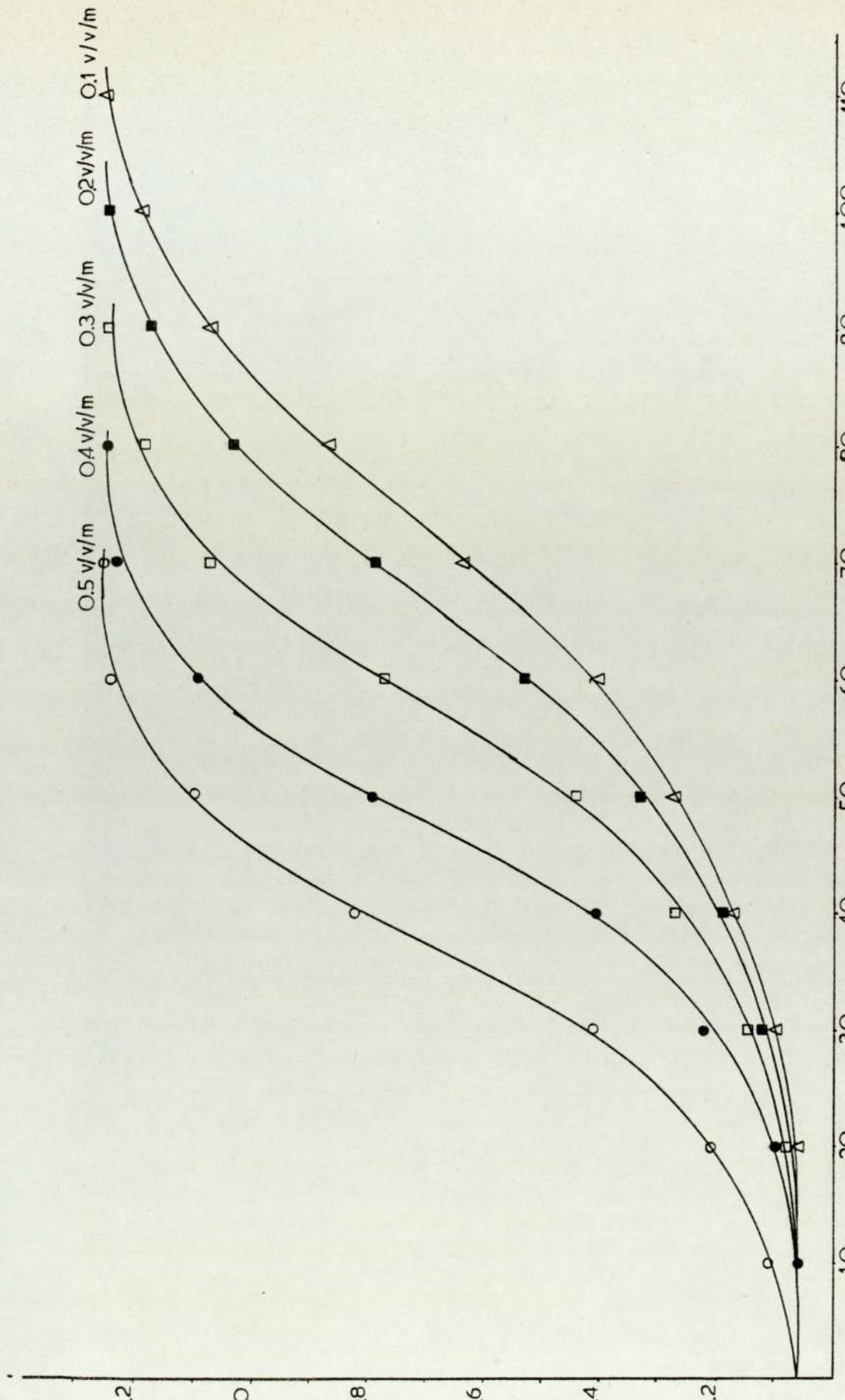


Fig. 6.3.2. Biomass concentration versus time in batch malt vinegar fermentation. Temperature 29°C

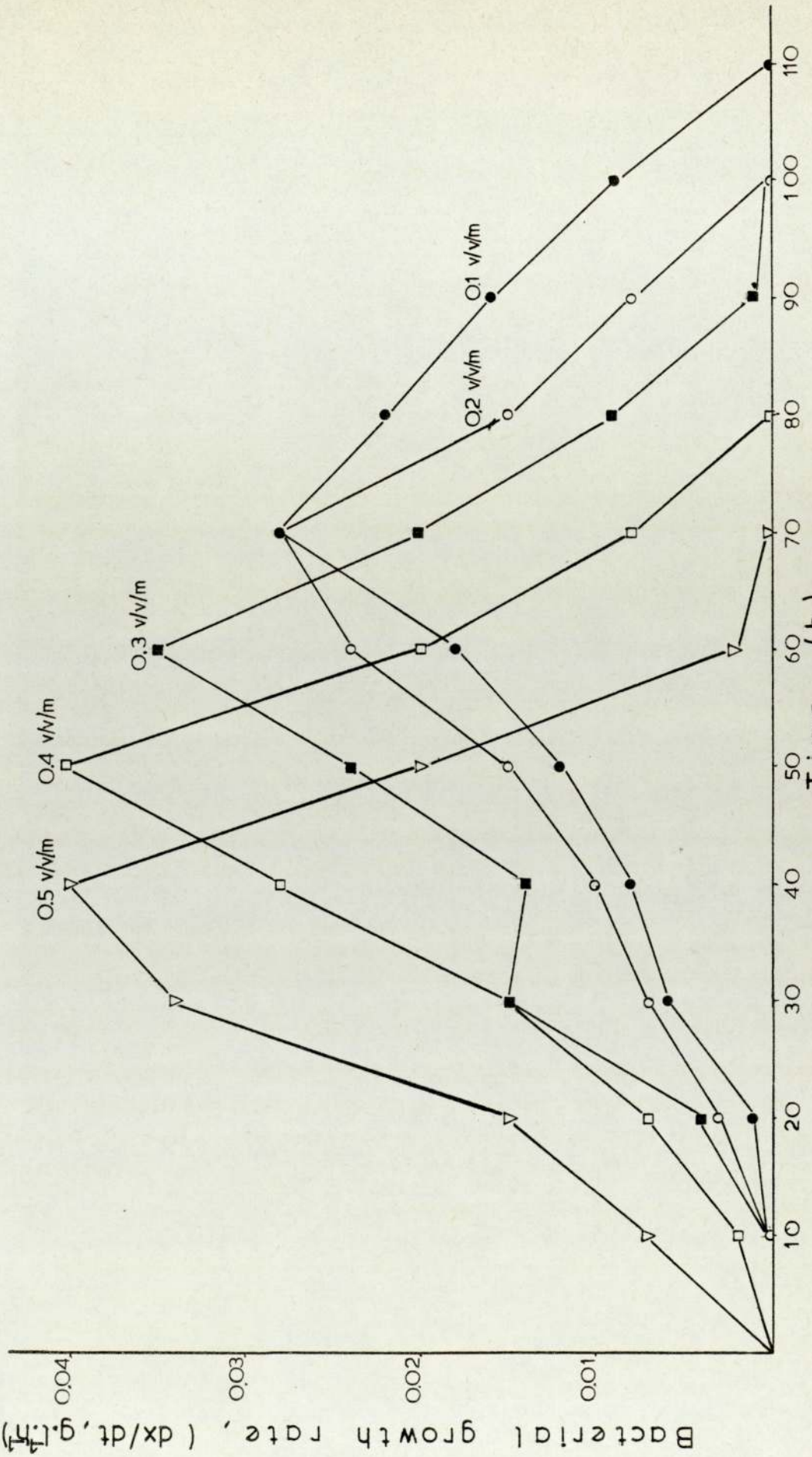


Fig. 6.3.3. Bacterial growth rates dx/dt versus time for different aeration rates. Temperature 28°C

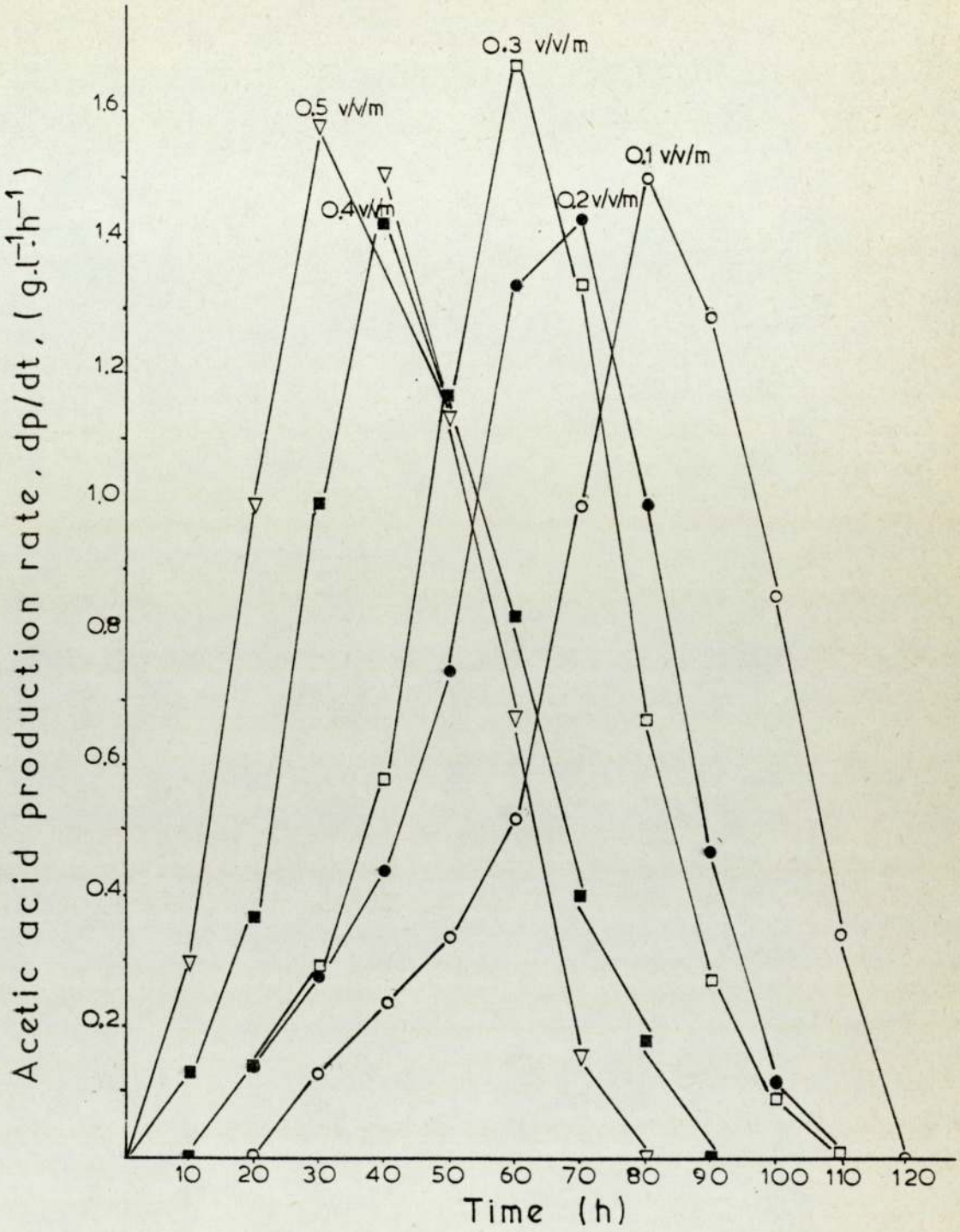


Figure 6.3.4. Product formation rate versus time for different aeration rates.
Malt vinegar.
Temperature 28°C

Aeration rate v/v/m	s.g.v. $cm.sec^{-1}$	Duration of lag phase (h)
0.1	0.13	20
0.2	0.27	16
0.3	0.40	16
0.4	0.55	12
0.5	0.67	16

Table 6.3.1. Effects of aeration rate on the duration of the lag phase (Lodge and Hinshelwood method, 1943). Malt vinegar. Temperature 30°C Total acid 67.7 $g.l^{-1}$.

From the figures (6.3.1) and (6.3.2), the values of instantaneous bacterial growth rate $\frac{dx}{dt}$ and the instantaneous product formation rate, $\frac{dP}{dt}$, were calculated and plotted in figures (6.3.3.) and (6.3.4). At aeration rates of 0.1 and 0.2 v/v/m (s.g.v. 0.13, 0.27 $cm.sec^{-1}$) the maximum growth rate occurred at the 70th hour with value of 0.024 $g.l^{-1}.h^{-1}$.

As the aeration rates increased, to 0.3 v/v/m, the maximum growth rate reached the value of 0.035 $g.l^{-1}h^{-1}$ at the 60th hour.

The aeration rates of 0.5 and 0.4 v/v/m did not affect the value of the maximum growth rates but had considerable difference on the time of their appearance. At the aeration of 0.4 v/v/m, the maximum growth rate appeared at the 50th hour whilst at the aeration of 0.4 v/v/m appeared at the 40th hour. This means that, although the bacterial concentration per unit of time did not change with more intensive aeration, the development of the whole fermentation process was achieved in

a shorter period (table 6.3.2). The product formation followed a similar pattern to that of the bacterial growth. At more intensive aeration rates, the time of appearance of the maximum acid formation rate was shorter than in less intensive aeration rates (Table 6.3.2.)

Aeration rate v/v/m	s.g.v. cm. sec^{-1}.	Time for maximum growth rate (h)	Time from maximum product formation rate (h)	Maximum growth rate $g.l^{-1}h^{-1}$	Maximum acetic acid formation rate $g.l^{-1}h^{-1}$
0.1	0.13	70	80	0.024	1.5
0.2	0.27	70	70	0.024	1.43
0.3	0.40	60	60	0.035	1.67
0.4	0.55	50	40	0.04	1.43
0.5	0.67	40	30	0.04	1.58

Table (6.3.2.) Effect of aeration rate on the bacterial growth rate and the acetic acid formation rate.
Malt vinegar. Batch culture.
Temperature 30°C. Total acid 67.7 $g.l^{-1}$.

6.3.2.2. Relationship between specific product formation rate and specific growth rate.

In malt vinegar fermentations, the relationship between specific product formation rate $K = \frac{1}{X}.\frac{dP}{dt}$ and specific growth rate (μ) has been shown to be linear. Figure (6.3.5.) Values of K were plotted against values of μ for different conditions of aeration rate. It has been found that the relationship $K = a\mu+b$ was valid with high probability to be true (p 0.001). In table 6.3.3, there are results of the regression analysis of the equation $K = a\mu+b$ for different

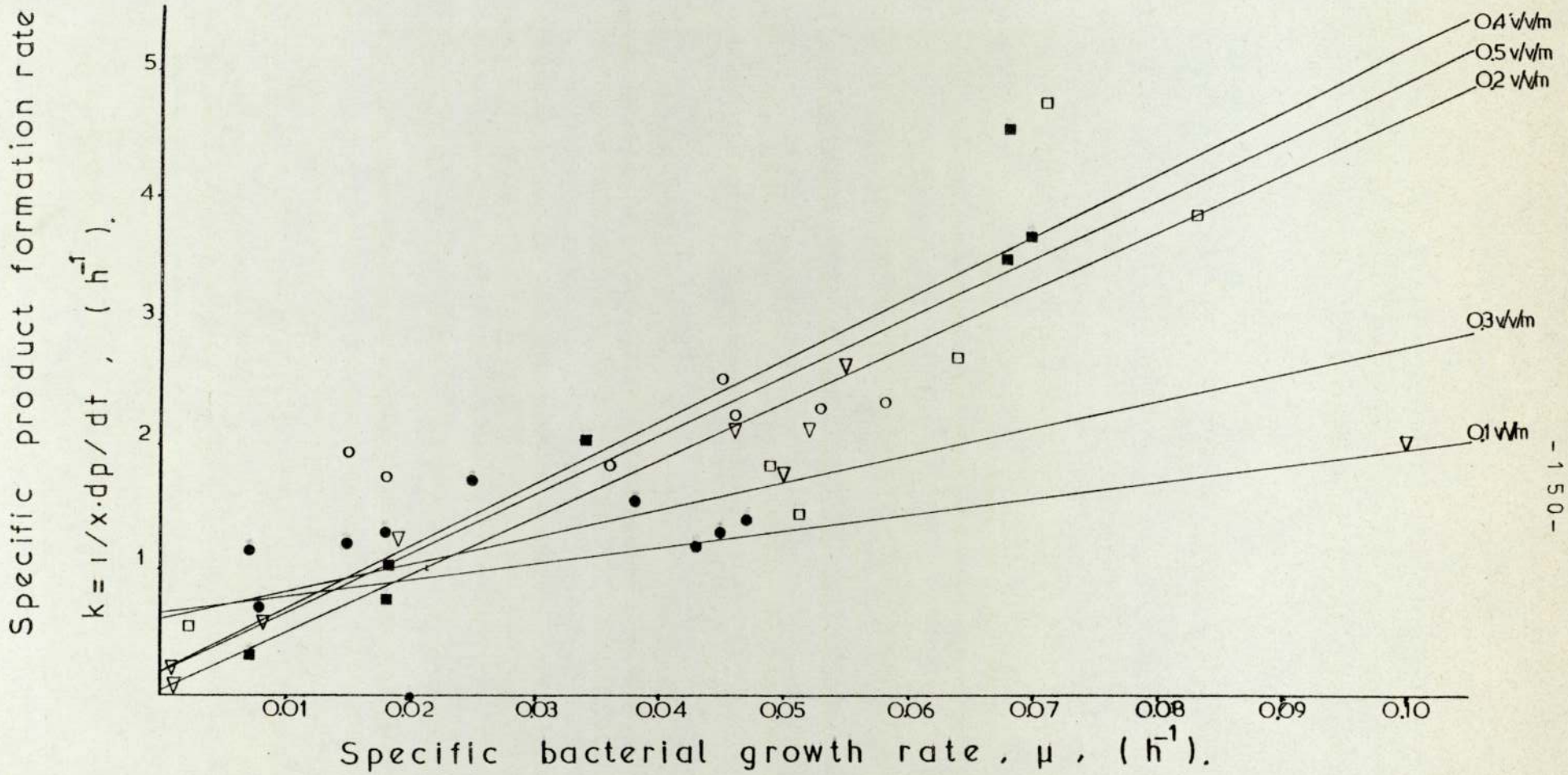


Figure 6.3.5. Relationship between specific product formation rate, K, and specific growth rate, μ, in batch fermetnations.

Malt vinegar. Temperature 28°C

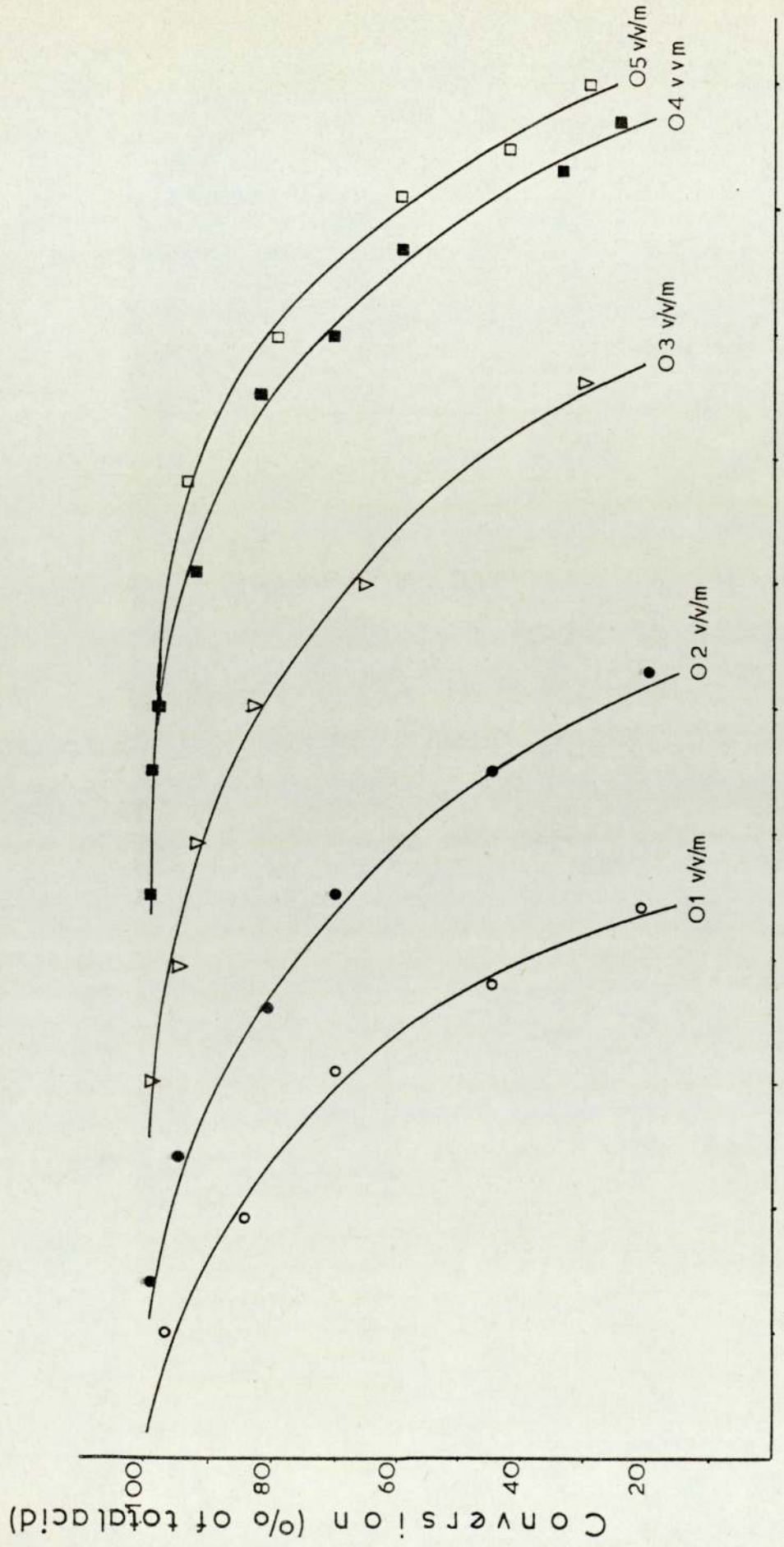


Figure 6.3.6. Ethanol conversion versus dilution rate at different conditions of aeration.
Malt vinegar. Temperature 28°C

aeration rate conditions. Values of the experimental parameters (a, b,) are also included.

Aeration v/v/m	s.g.v. $cm.sec^{-1}$	a	b (h^{-1})	r	p	Degrees of freedom
0.1	0.13	13.34	0.64	0.561	0.100	9
0.2	0.27	48.67	0.004	1.000	0.001	8
0.3	0.40	22.50	0.63	0.840	0.001	8
0.4	0.55	47.57	0.17	0.936	0.001	8
0.5	0.67	47.00	0.065	0.942	0.001	6

Table (6.3.3.) Regression analysis of K on μ. Values at a and b (experimental parameters) from the equation $K = a\mu+b$ (Divies, 1973). Malt vinegar batch fermentation. Temperature 30°C. Total acid 67.7 $g.l^{-1}$

6.3.3. Continuous tower fermentation

6.3.3.1. Effect of dilution rate on the ethanol coversion to acetic acid.

The conversion of ethanol to acetic acid was limited by the aeration rate and by the dilution rate. As the dilution rate was increasing the conversion ability of ethanol to acetic acid was diminishing for the same aeration rate. Additionally, the conversion was reduced when the aeration rate reduced. (Figure 6.3.6.)

For the first phenomenon, i.e. the dilution rate effect on the conversion, the reasons are based on the washing out of the biomass which is the main cause for the production of acid. The acetic acid being partially growth associated product, and partially biomass concentration product any change in the biomass

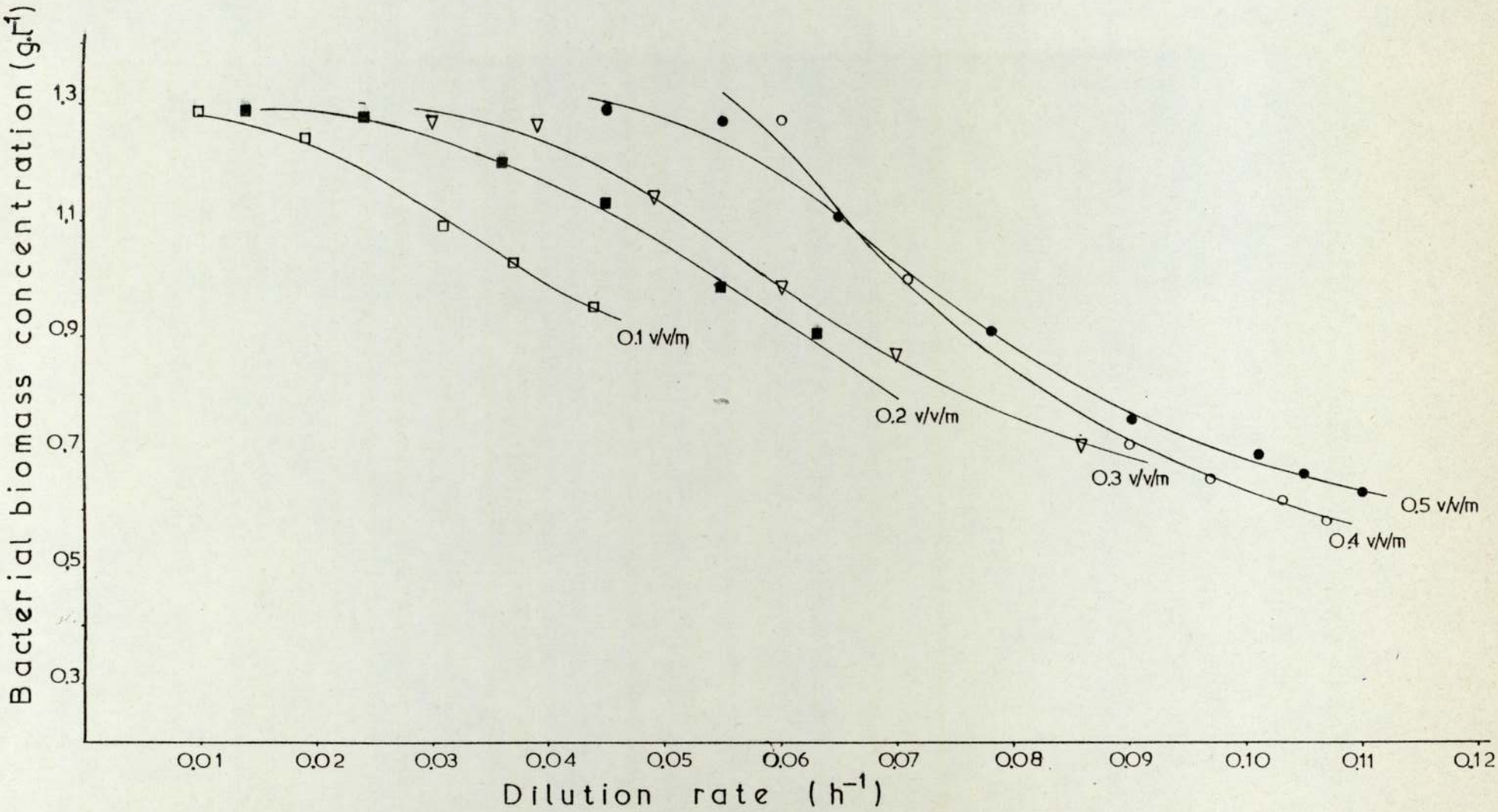


Figure 6.3.7. Bacterial biomass concentration in the fermenter, X_F, versus dilution rate, D, at different conditions of aeration. Malt vinegar. Temperature 28°C.

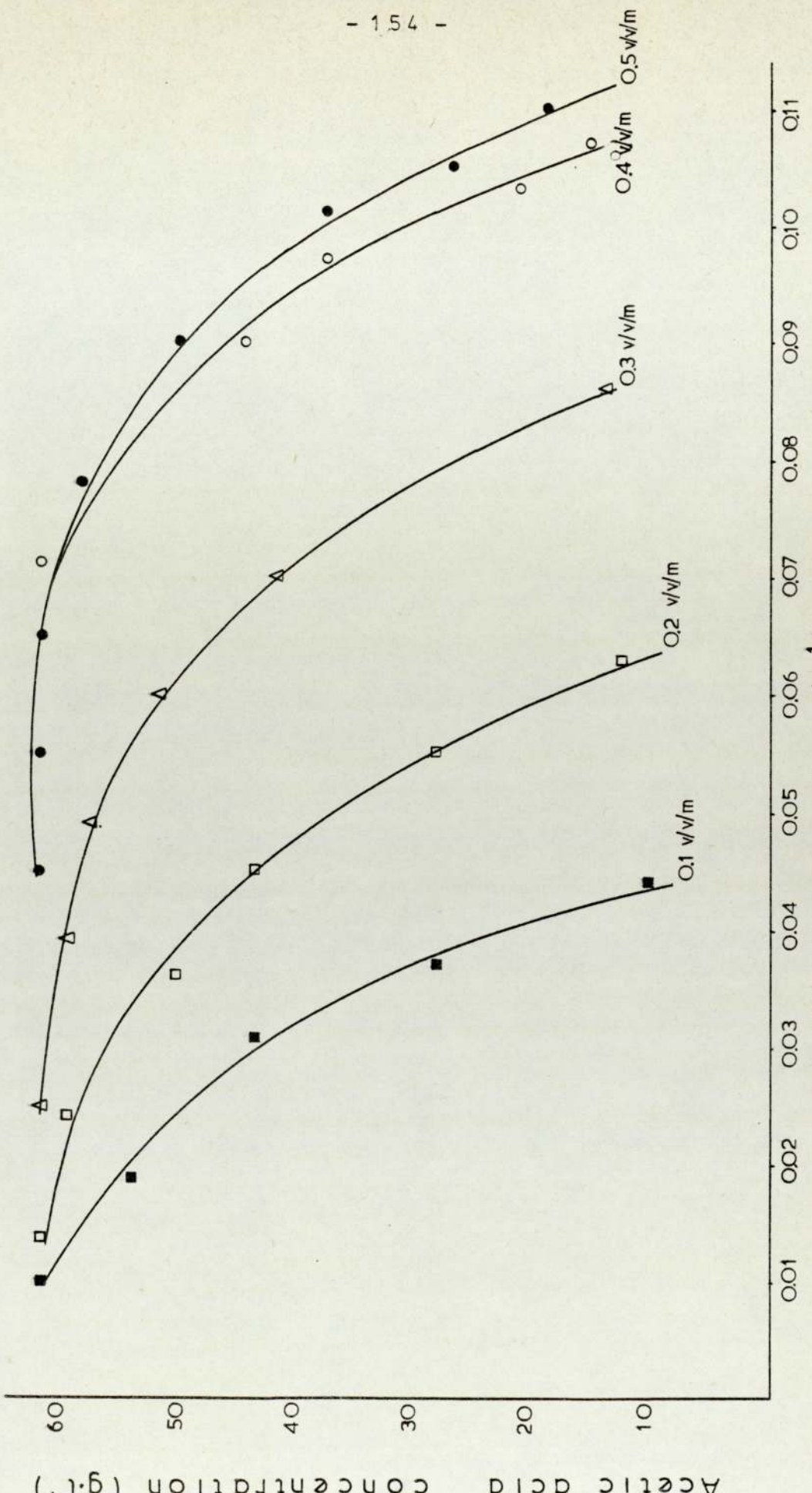


Figure 6.3.8. Acetic acid concentration versus dilution rate at different conditions of aeration. Malt vinegar. Temperature 28°C.

concentration causes change in the product formation and consequently in the conversion of ethanol and acetic acid.

6.3.3.2. Effect of dilution rate on the concentration of biomass dry weight and acetic acid in the fermenter

In Figures (6.3.7) and (6.3.8), values of biomass dry weight concentration and acetic acid concentration in the fermenter are plotted against dilution rate values.

In malt vinegar fermentation, the aeration rate played an important role in the kinetics of the biomass concentration and acid concentration in the fermenter. The dilution rate being a limiting factor of the fermentation can be proved by the phenomenon that when all the other parameters being constant, small changes of it causes substantial changes in the biomass concentration in the fermenter (X_F). (Figure 6.3.7).

The profiles of the plots of X_F against D indicate that in the C.T.F. the acetic acid bacteria, remain in the fermenter in high concentration at high dilution rates.
This comes in contrast to the C.S.T.R. where there is a value in which most of the bacteria are washed out.

The plot of acetic acid concentration against dilution rate gives another picture which is more similar to C.S.T.R. kinetics. The reason is that the factor weight which exists in the solid phase of the fermentation, it does not apply to the liquid phase. (In ideal C.S.T.R. the weight does not insert as a distribution factor because of its homogeinity.)

The aeration rate is another limiting factor for the malt vinegar fermentations. The bubble size and the foam formation

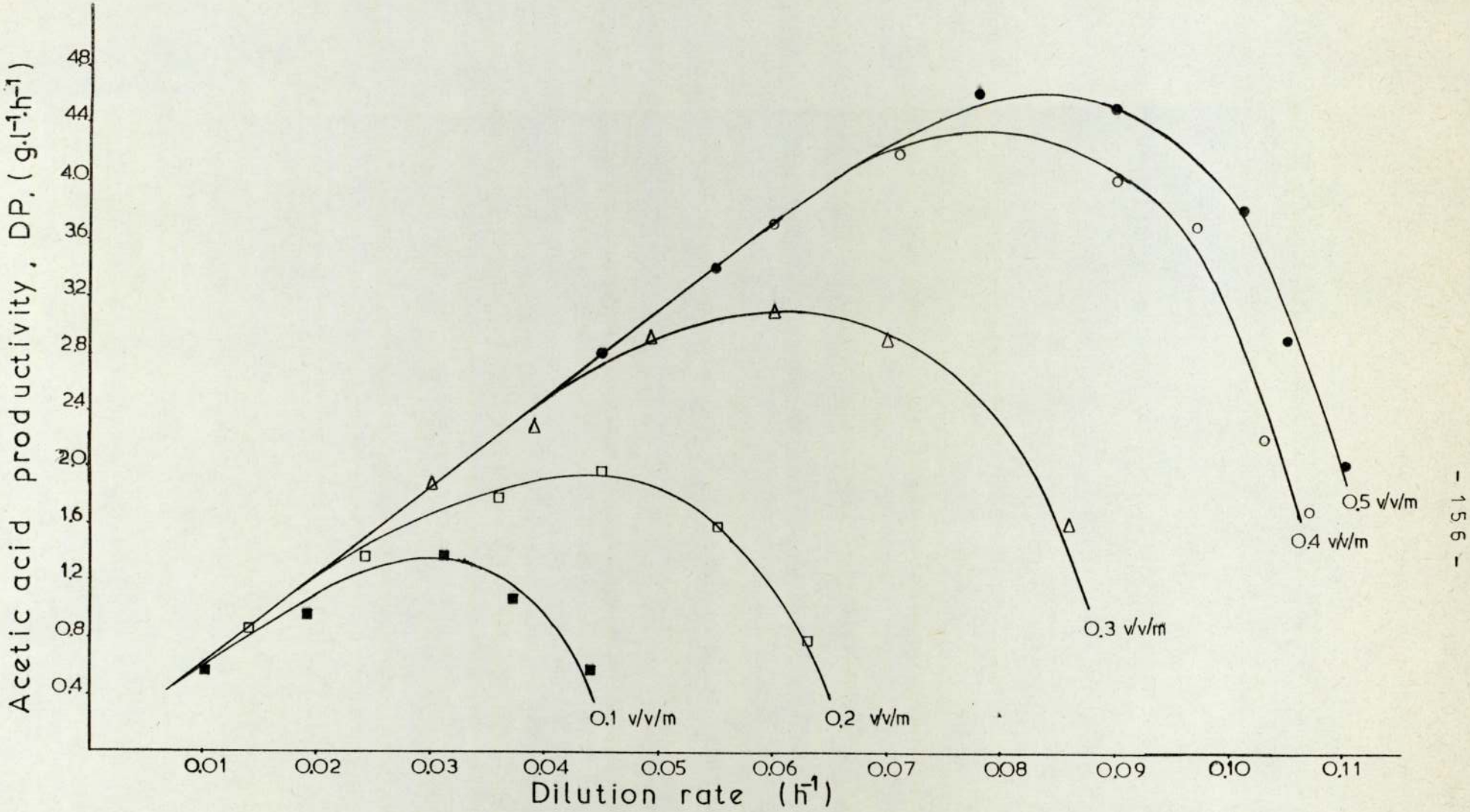


Figure 6.3.9. Productivity lines for the production of malt vinegar at different aeration rates. Temperature 28°C.

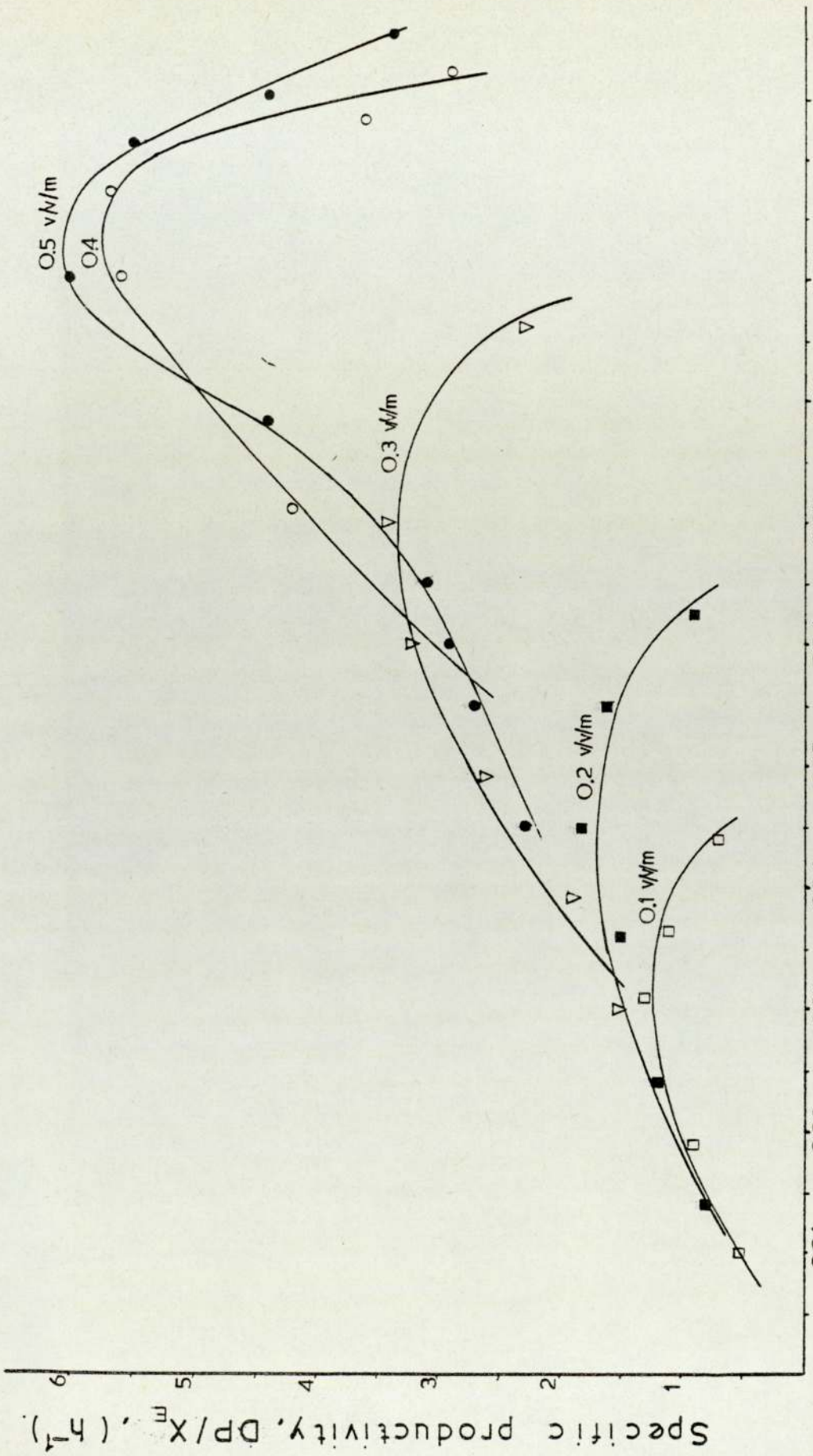


Figure 6.3.10. Specific productivity versus dilution rate. Malt vinegar. Temperature 28°C

affect the oxygen transfer rate from the gas phase to the liquid one (see also paragraph 6.3.2.1).

6.3.3.3. Effect of dilution rate on the productivity (D.P.) at the C.T.F.

For aeration rates higher than 0.4 v/v/m, the productivity of system did not increase as much as when the aeration rate was increasing from 0.1 to 0.4 v/v/m.

The superficial gas velocity following the aeration rate and being independent of the fermenter size provides a better means of comparison of aeration between two fermenters. Therefore, the aeration rates were also converted to superficial gas velocities. In table (6.3.4) values of the maximum productivities and maximum specific productivities are included, for the different superficial gas velocities (s.g.v.)

Aeration rate (v.v.m.)	s.g.v ($cm.sec^{-1}$)	D.P max ($g.l^{-1}h^{-1}$)	D.P max/X_E (h^{-1})
0.1	0.13	1.4	1.3
0.2	0.27	2.0	1.8
0.3	0.40	3.1	3.4
0.4	0.55	4.2	5.7
0.5	0.67	4.6	6.0

Table (6.3.4.) Values of productivity and specific productivity of C.T.F.

Malt vinegar continuous tower fermentation

Temperature 30°C

Total acid 67.7 $g.l^{-1}$

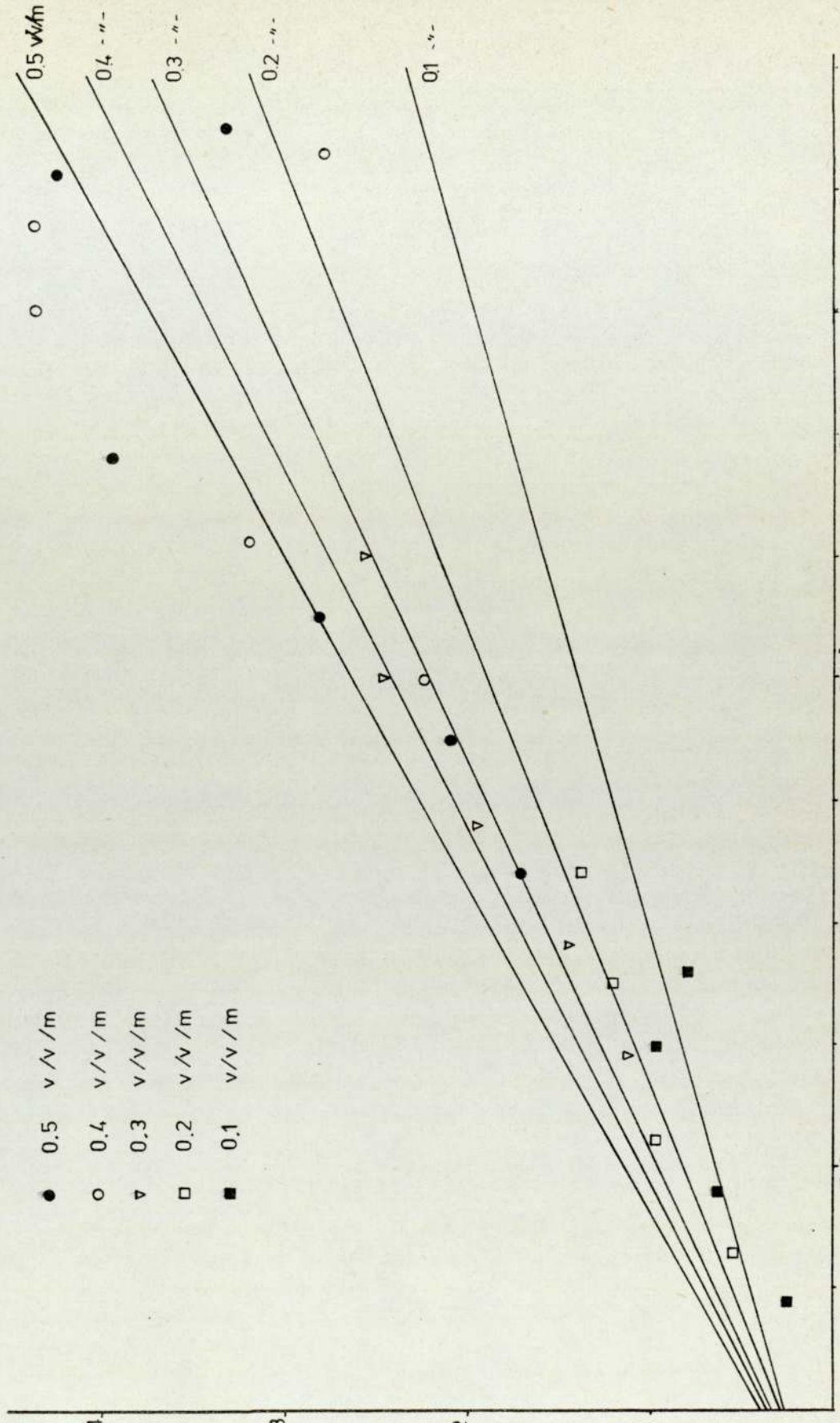


Figure 6.3.11. Yield studies. Determination of Y_G and m. Malt vinegar. Temperature 30°C.

Figures (6.3.9 and (6.3.10) show the change of productivity and specific productivty for the different aeration rates.

As the aeration rate increased, both the productivity and specific productivity increased. This is another reason which proves that the aeration is one of the limiting factors on the malt vinegar fermentation.

The increase of the productivity and specific productivity with the increasing dilution rate indicates that in the tower the dilution rate is another limiting factor for the production of malt vinegar.

6.3.3.4. Determination of the true growth yield, Y_G, and the maintenance energy coefficient, m

Values of the specific substrate utilization rate q_s were plotted against the specific growth rate, μ, figure 6.3.11. The results are shown in Table 6.3.5.

Aeration rate v/v/m	Y_G $g.g^{-1}$	m $g.g^{-1}.h^{-1}$
0.1	0.053	0.27
0.2	0.038	0.30
0.3	0.032	0.34
0.4	0.029	0.37
0.5	0.027	0.40

Table 6.3.5. Values of Y_G and m at different aeration rates. Malt vinegar. Temperature 30°C. Total acidity 67.7 $g.l^{-1}$.

The maintenance energy coefficient increased as the aeration rate was increased while the temperature and the total acidity remained constant. The true growth yield shows a different pattern. With the aeration increasing the specific

gas/liquid interfacial area in increased. (Schügerl et al, 1977). Consequently the dissolved oxygen is increasing and this could minimize the constraints on the maintenance energy of the bacteria in the culture, and as a result cause a lower meintenance energy.

For fuller understanding and explanation of the phenomenon, further investigation is necessary.

SPIRIT VINEGAR

6.4. PRODUCTION OF SPIRIT VINEGAR IN CONTINUOUS TOWER FERMENTER

6.4.1. Medium preparation and size of fermenter used.

After dilution and supplementation with NH_4SO_4 and yeast extract, the final media had the following composition (Table 6.4.1):

	A $g.l^{-1}$	B $g.l^{-1}$
Ethanol	45.00	55.90
Acid (in terms of acetic acid)	2.00	2.30
Total Nitrogen	0.25	0.27
NH_4.Nitrogen	0.21	0.22
Total reducing sugars	1.90	2.10
Yeast extract	0.10	0.12

Table 6.4.1. Final composition of media used for the production of spirit vinegar. The pH was for medium A=4.3 and for medium B=4.2.

Addition of 1 $g.l^{-1}$ each of KH_2PO_4 and $MgSO_4$ was made according to Alian, (1963) medium. The size of fermenter used was 3.100 litres (Section 2.2.4.3).

6.4.2. Preliminary fermentation

A preliminary fermentation was conducted with the purpose of determining the maximum aeration rate which could possibly be used without excess of foam production. Also, the behaviour of the system was indicated by the preliminary fermentation.

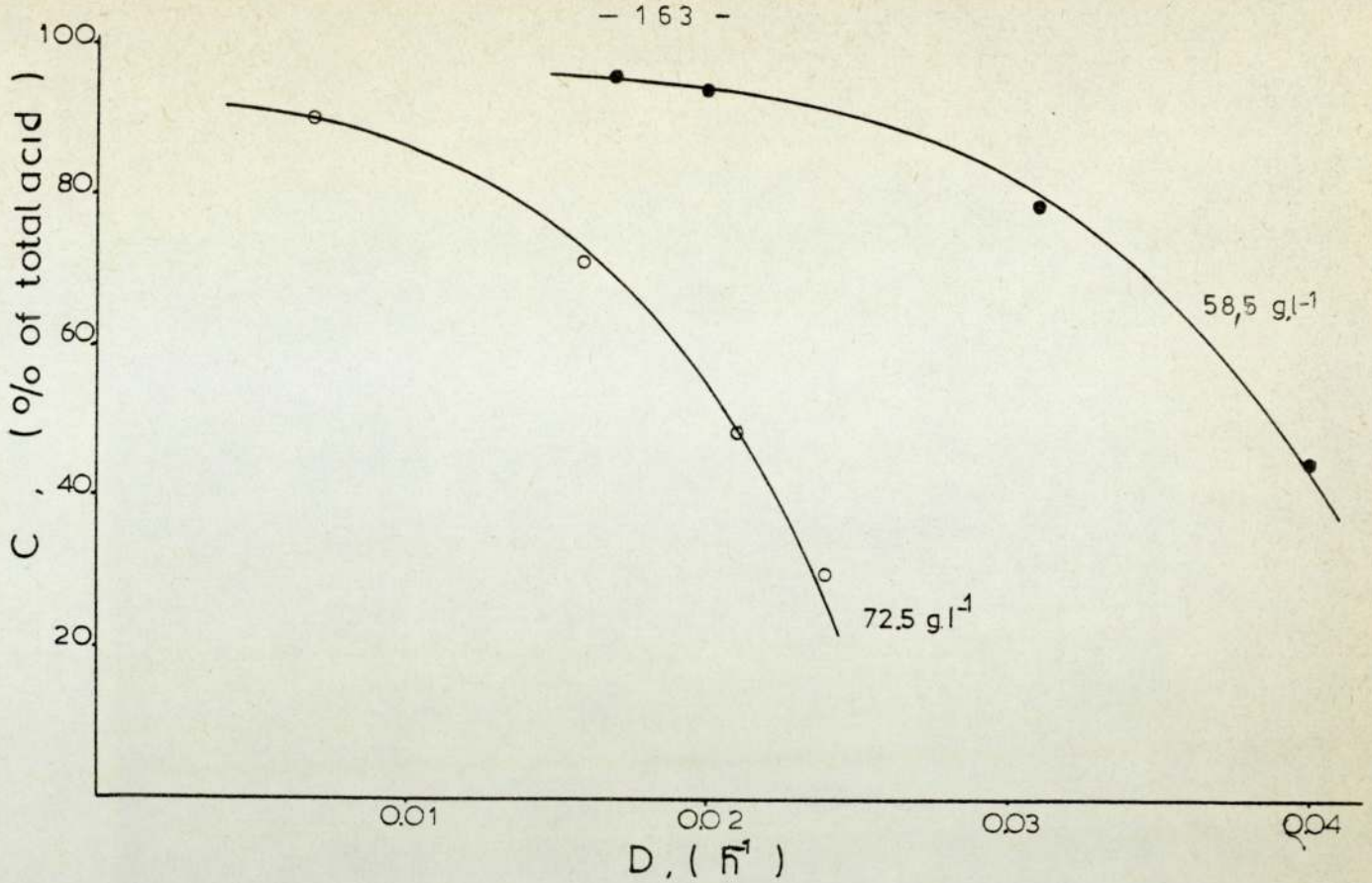


Figure 6.4.1. Ethanol conversion ability versus dilution rate for different substrate concentrations spirit vinegar.

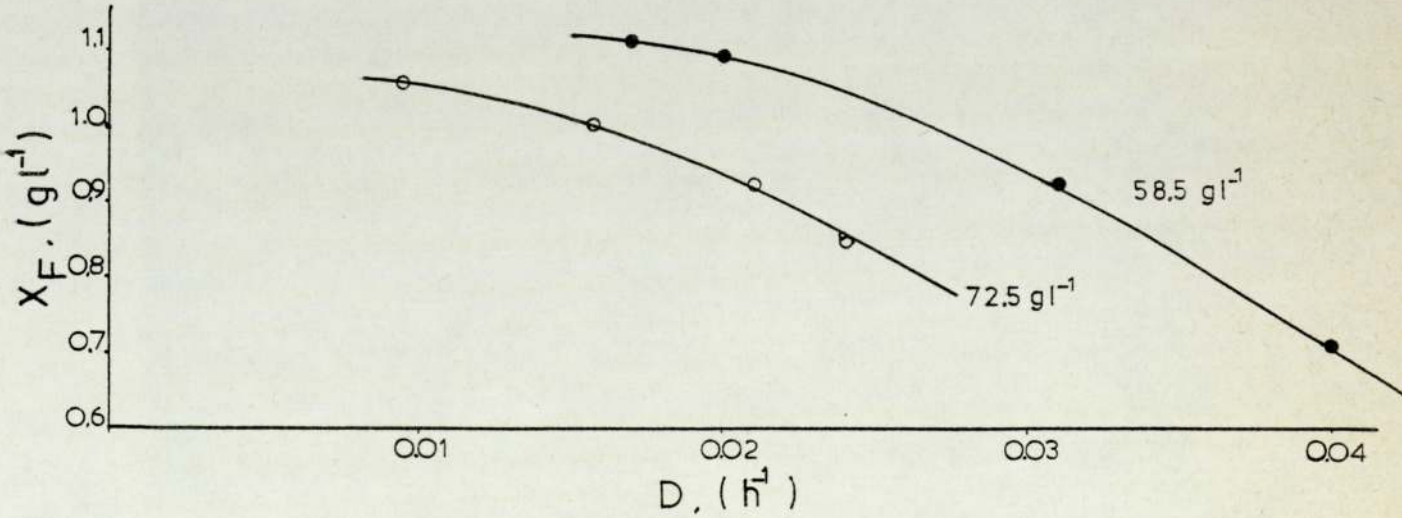


Figure 6.4.2. Biomass concentration versus dilution rate for two different substrate concentrations.
Spirit vinegar.

The aeration of 0.5 v/v/m (s.g.v. 0.8 cm sec^{-1}) was found to be the maximum aeration rate at which the system did not foam so the use of antifoam was not necessary.

During the initial batch fermentation in which the medium reached the point to start the continuous process, use of antifoam (0.04g.l^{-1}) was necessary because the system showed a tendency to foam. The inoculum used was from malt vinegar tower. The long lag phases observed (15 to 30 days) were the reason for not experimenting the system in batch mode.

6.4.3. EFFECT OF TOTAL ACID CONCENTRATION ON THE PRODUCTION OF SPIRIT VINEGAR IN TOWER FERMENTER.

6.4.3.1. Effect of dilution rate on the ethanol conversion to acetic acid at different total acid concentrations

Figure 6.4.1. is representative of the effect of dilution rate on the conversion of ethanol to acetic acid (media: A,B).

The ability to oxidise ethanol seems to be higher for the total acidity of 58.5 g.l^{-1} rather than that for 72.5 g.l^{-1}. The conversion C, at an acidity of 58.5 g.l^{-1} reached the value of 96% and after extrapolation, the value of 98% for dilution rates of 0.017 h^{-1} and 0.008 h^{-1}, respectively.

In contrast, at total acidity of 72.5 g.l^{-1}, the maximum conversion ability was 90% at dilution rate of 0.007 h^{-1}.

This phenomenon could be explained (see also section 6.1.3. and 6.2.3, 6.3.3) as an inhibition of ethanol and acetic acid in the growth of the bacteria of acetic acid.

6.4.3.2. Effect of dilution rate on the biomass concentration in the fermenter and on the bacterial specific growth rate.

In paragraph (6.4.3.1.) was described that the conversion ability of ethanol to acetic acid is greatly affected by the

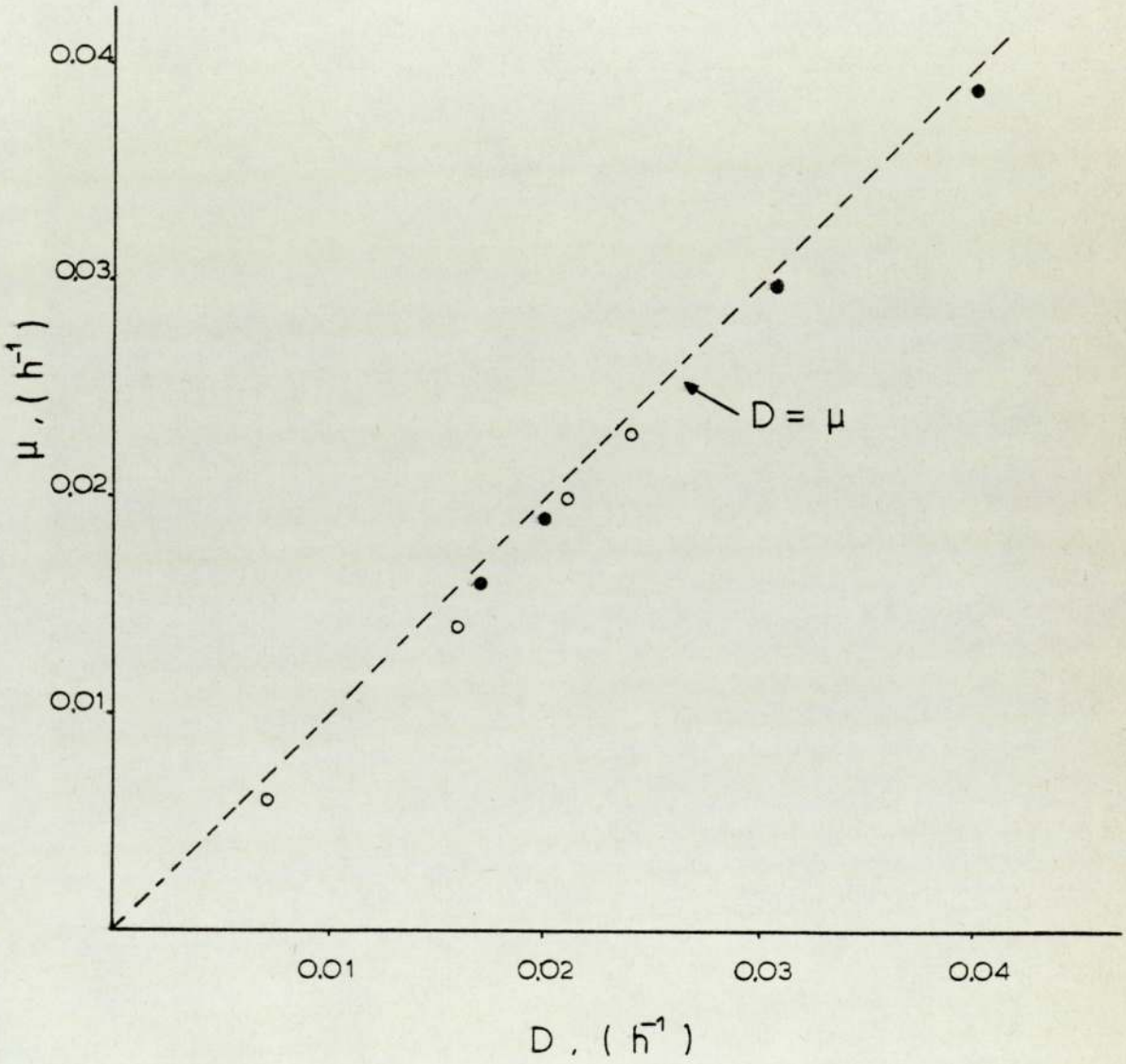


Figure 6.4.3. Relationship between specific bacterial growth rate, μ, and dilution rate, D, in tower fermentation for the production of spirit vinegar.
The broken line corresponds to conventional stirred reactor conditions (μ = D)

o 72.5 g l⁻¹
• 58.5 g l⁻¹

dilution rate and the total acidity. The concentration of the bacteria in the fermenter was affected by the parameter acidity. (Figure 6.4.2.) The phenomenon was expected because both the ethanol and the acetic acid act as inhibitors (paragraph. 6.4.3.1) The death rate of bacteria at high acidities increases and consequently the remaining bacteria, at the dynamic equilibrium, at which a steady-state occurs, exhibit a strong adaptation to the high acidity and a higher oxidation ability (Alian, 1963).

In the case of spirit vinegar the concentration of bacteria X_f, in the fermenter at acidity 58.5 $g.l^{-1}$ was higher than at acidity of 72.5 $g.l^{-1}$ (Figure 6.4.2.) At the acidity of 57.5 $g.l^{-1}$, X_F was 1.0 $g.l^{-1}$ at dilution rate of 0.025 h^{-1}, whilst for the same concentration of bacteria the dilution rate of 0.015 h^{-1} was necessary to be applied. The pattern of the wash out of the bacteria is affected by the tower geometry, the weight of the bacteria and the recycling phenomena (See also paragraph 6.1.)

The same reason affects the ratio $\frac{X_E}{X_F}$ where X_F is the concentration of bacteria which were in the effluent stream. Consequently, the value of μ was greatly affected, as μ is dependant upon the product $\frac{X_E}{X_F}.D.$ (Section 6.1.3.2.1.)

In figure (6.4.3.) the broken line represents the relationship $\mu=D$ in ideal chemostat conditions. The values of D were always higher than the values of μ because $X_E < X_F$

6.4.3.3. Effect of dilution rate on the acetic acid, and ethanol concentration in the fermenter.

The concentration of the acetic acid in the tower fermenter was affected by the dilution rate following a similar pattern of that of a chemostat.

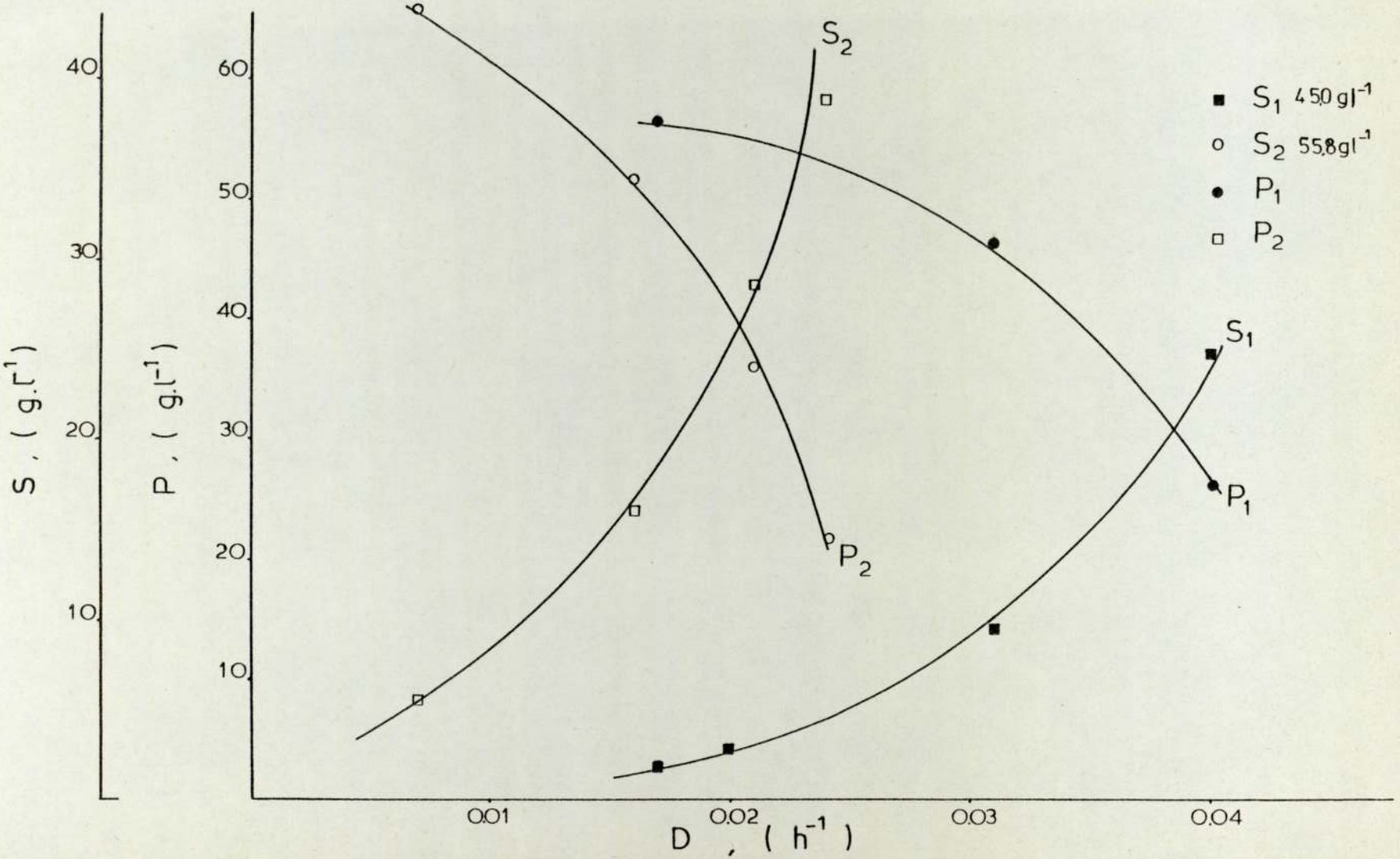


Figure 6.4.4. Relationship between substrate, S, product, P, concentration in the fermenter versus dilution rate. Spirit vinegar.

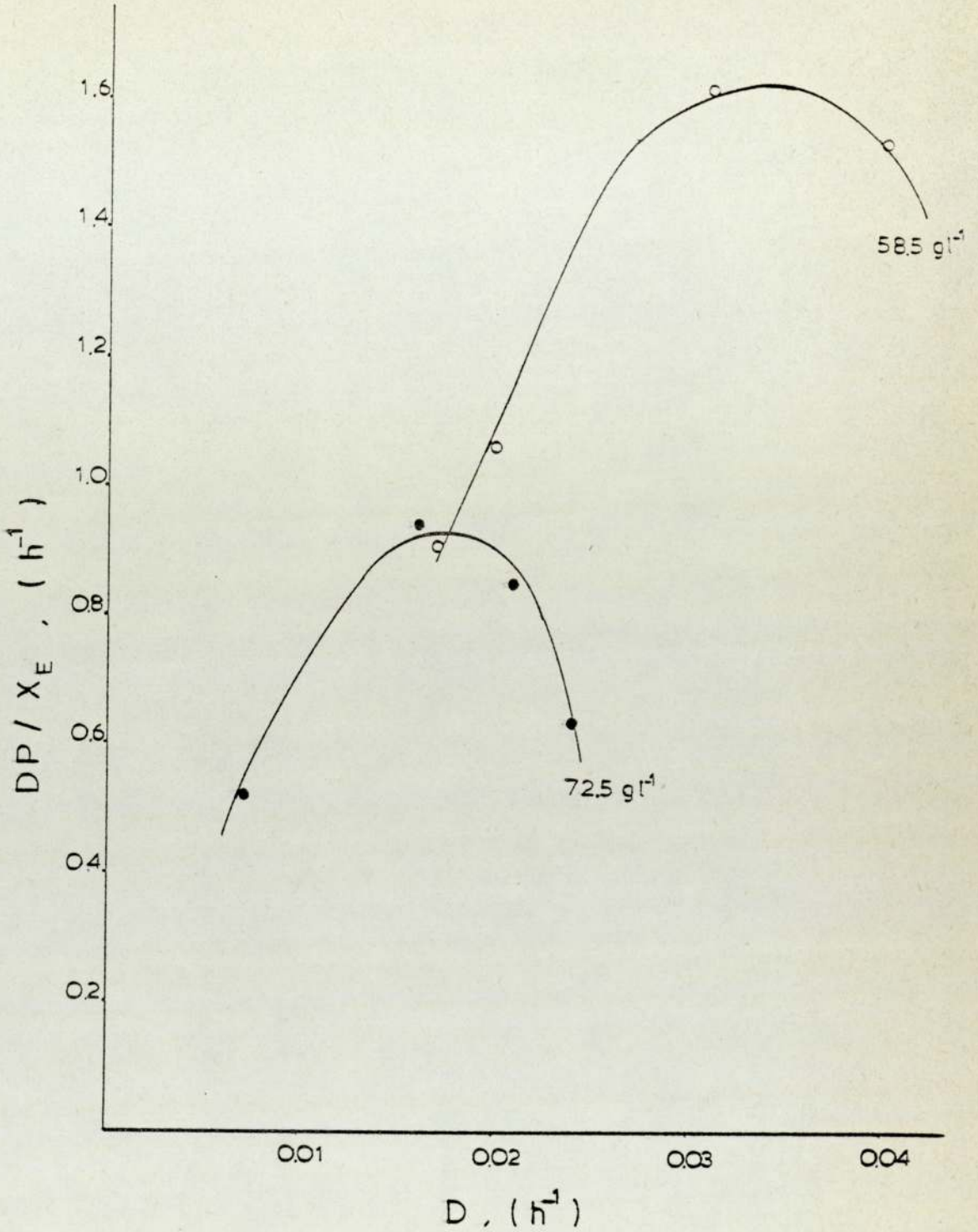


Figure 6.4.5. Specific productivity versus dilution rate for the production of spirit vinegar for two different substrate concentrations.

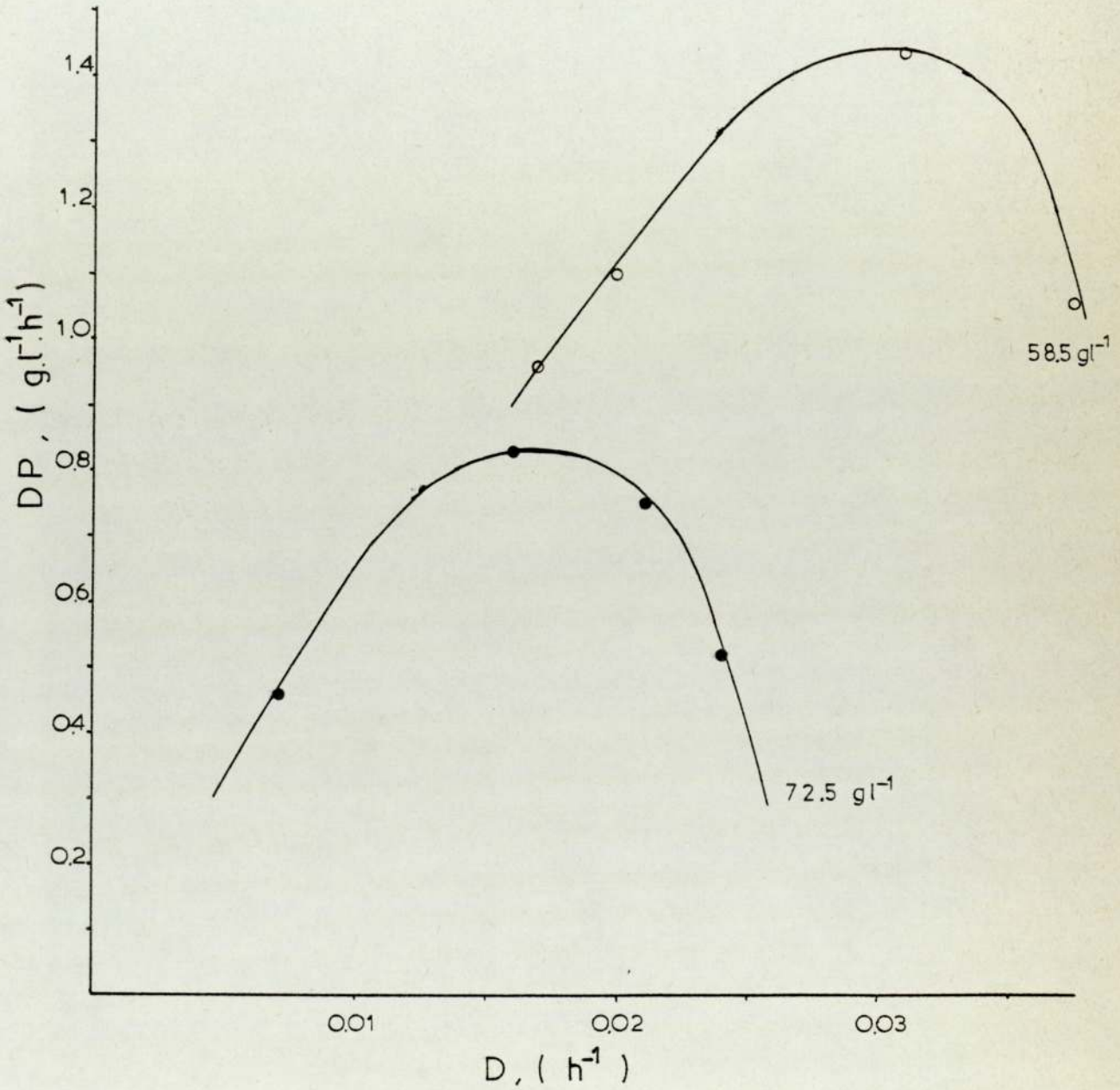


Figure 6.4.6. Productivity versus dilution rate at two different substrate concentrations.
Spirit vinegar continuous fermentation.

The substrate concentration was also governed by the dilution rate. In product formation cases, the effect of the tower fermenter geometry is low. In contrast with the kinetics of the biomass concentration in which the C.T.F. geometry effect is high (paragraph 6.4.3.2.), the acetic acid concentration almost follows the C.S.T.R. kinetics. A reason could be that, in both product and substrate concentration kinetic patterns, the weight is not involved.

When the total acidity was 58.5 $g.l^{-1}$, the fermenter could maintain concentrations of ethanol lower than that with 72.5 $g.l^{-1}$ total acidity. The acetic acid concentration also at the acidity of 58.5 $g.l^{-1}$ was decreased at lower rates than that with 72.5 $g.l^{-1}$ total acidity, when the dilution rate was increasing. (Figure 6.4.4.). An explanation of the phenomenon has been given in paragraph 6.2.3.

6.4.3.4. Effect of dilution rate on the acetic acid productivity and specific productivity of C.T.F.

The acetic acid productivity when the total acidity was 58.5 $g.l^{-1}$ exhibited higher values rather than that at an acidity of 72.5 $g.l^{-1}$. At acidity of 58.5 $g.l^{-1}$ and temperature 30°C the maximum acid productivity was 1.44 $g.l^{-1}.h^{-1}$. At acidity of 72.5 $g.l^{-1}$ the maximum productivity was 0.83 $g.l^{-1}.h^{-1}$. The aeration rate in both cases was kept constant, 0.5 v/v/m. (Figure 6.4.6, Table 6.4.2.).

The specific productivity, i.e. the productivity per biomass unit was following a similar pattern to that of productivity. The maximum specific productivity for total acidity 58.5 $g.l^{-1}$ was 1.63 h^{-1} and for total acidity of 72.5 $g.l^{-1}$ was 0.93 h^{-1} (Figure 6.4.5, table 6.4.2.)

Total acidity ($g.l^{-1}$)	Max. productivity ($g.l^{-1}.h^{-1}$)	Max Spec. Productivity (h^{-1})
58.5	1.44	1.63
72.5	0.83	0.93

Table 6.4.2. Values of maximum producitivty and maximum specific productivity at different total acidities.
Spirit vinegar.
Aeration rate 0.5 v/v/m (s.g.v. 0.8 $cm.sec^{-1}$).
Temperature 30°C.

Discussion on the effect of the acidity on the productivity and specific productivity is given in sections (6.1.3.2.3) and (6.1.3.2.4).

6.4.3.5. Yiels studies: Determination of the bacterial true growth yield, Y_G, and the maintenance energy coefficient, m.

The true growth yield, Y_G, of ethanol per g. of bacterial dry weight and the maintenance energy coefficient m (g of ethanol per g of bacterial dry weight per h) were determined from the plot of specific substrate utilization, q_s, against specific growth rate μ (Figure 6.4.7) equation $q_s = \frac{\mu}{Y_S} + m$ (Pirt, 1975).

The values of q_s and m are shown in Table 6.4.3. When the acidity was increased both m and Y_G were increased. Discussion is given in paragraph 6.2.3.4.

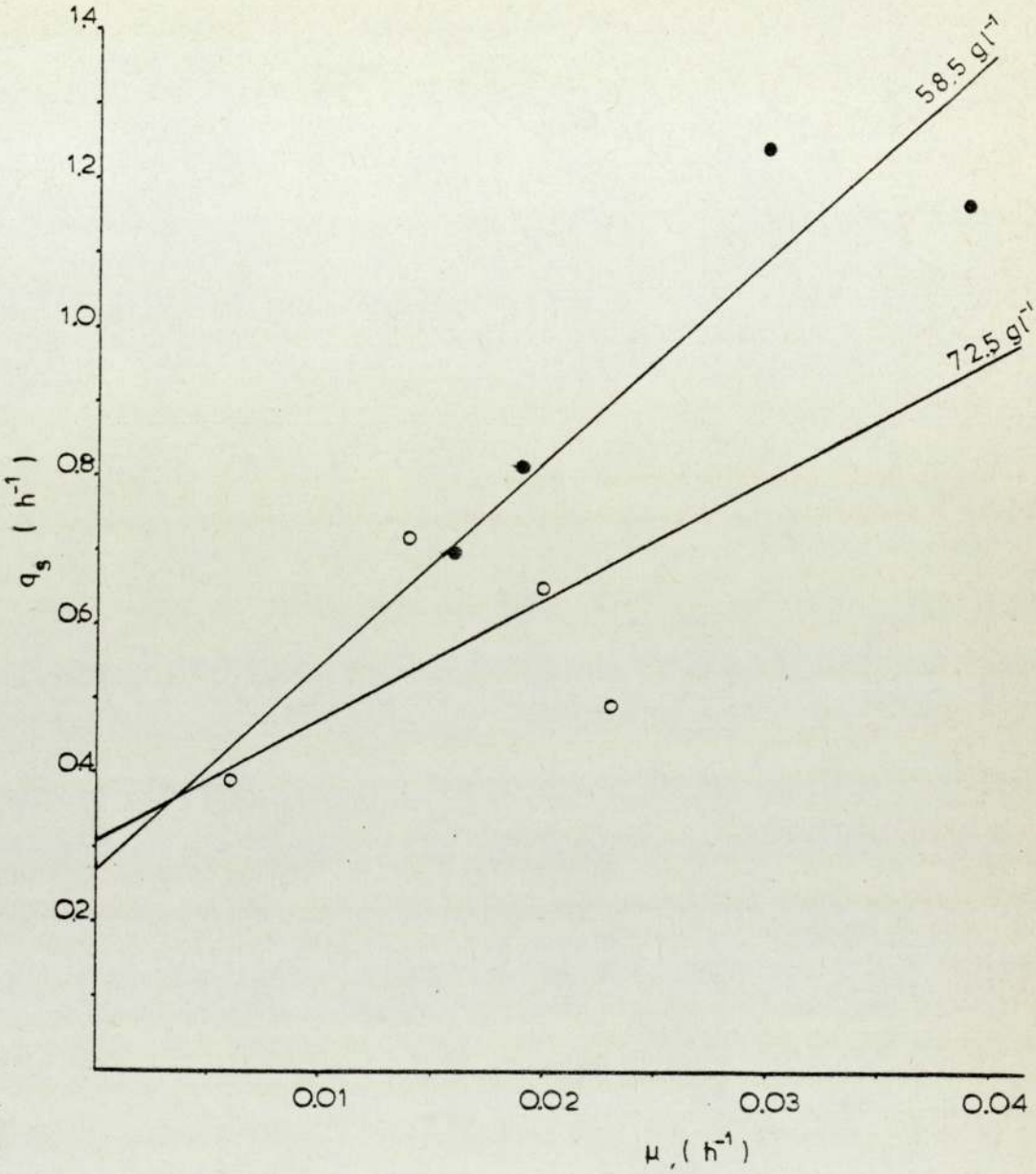


Figure 6.4.7. Yield studies Determination of Y_G and m.

Spirit vinegar

Temperature 30°C.

Total acid $g.l^{-1}$	Y_g $g.g^{-1}$	m $g.g^{-1}.h^{-1}$
58.5	0.04	0.27
72.5	0.06	0.31

Table 6.4.3. Values of Y_G and m at different total acid concentrations. Spirit vinegar. Aeration rate 0.5 v/v/m Temperature 30°C

6.4.4. EFFECT OF TEMPERATURE ON THE PRODUCTION OF SPIRIT VINEGAR IN C.T.F.

6.4.4.1. Effect of dilution rate on the biomass, acetic acid and ethanol concentration at the temperature of 35°C

At the temperature of 35°C the loss of ethanol due to evaporation was 0.90 $g.l^{-1}h^{-1}$. This phenomenon affected the overall activity of the system. The initial substrate concentration was 45.0 $g.l^{-1}$. After the subtraction of the substrate concentration which was evaporated from the initial the remaining substrate concentration for reeding was 44.1 $g.l^{-1}$. Hence a direct effect was the reduction of the total acidity.

Another effect of the temperature of 35°C was on the biomass concentration in the fermenter X_F. A comparison between the graphs of Figures 6.4.2. and 6.4.8. would show that in the temperature of 35°C the biomass concentration in the fermenter was higher than that at 30°C. For instance at

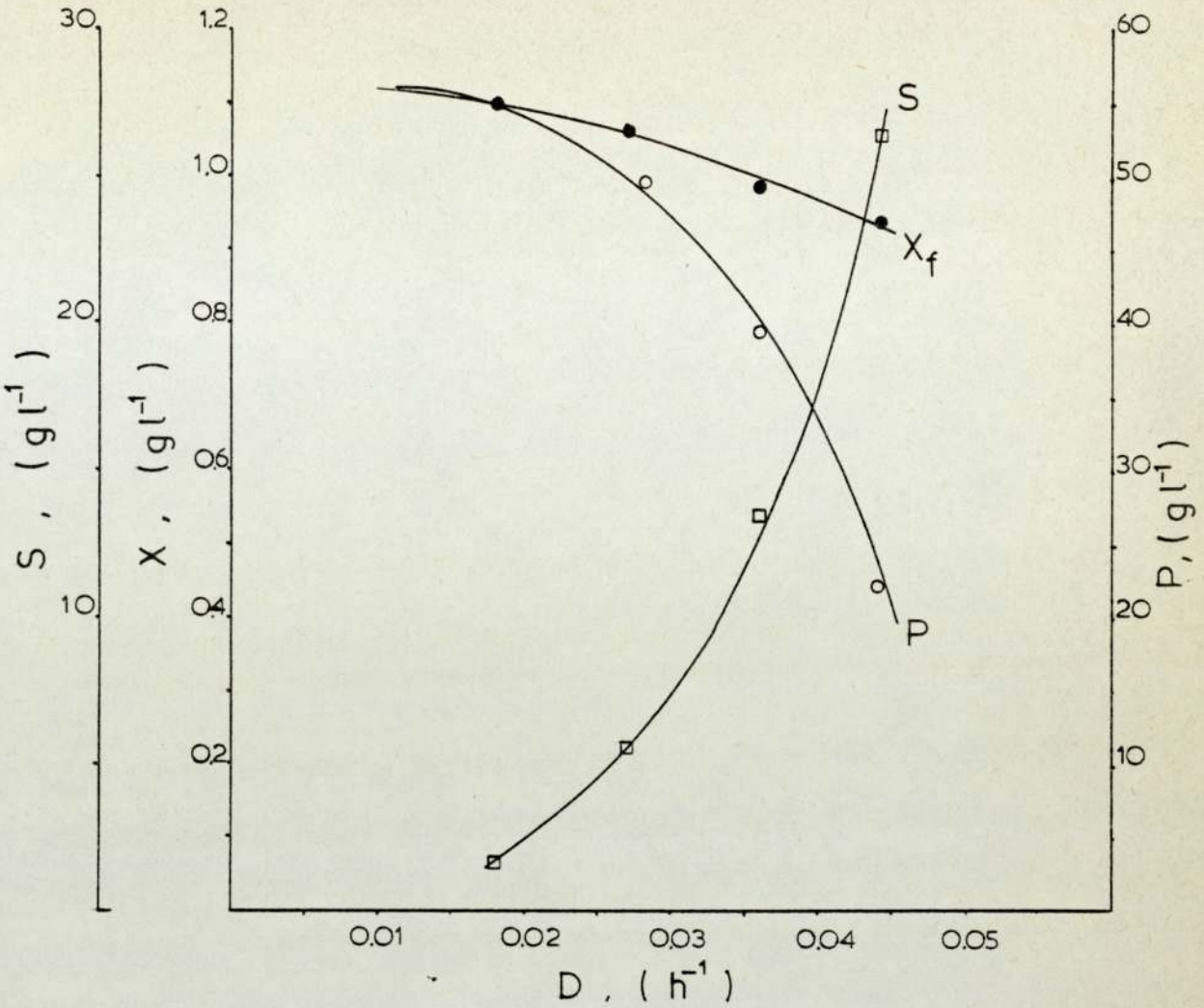


Figure 6.4.8. Relationship between dilution rate and substrate concentration, S, bacterial dry weight concentration X and Acetic acid concentration, P, in the fermenter.
Spirit vinegar.
Temperature 35°C, Total acid 55.8 $g.l^{-1}$.

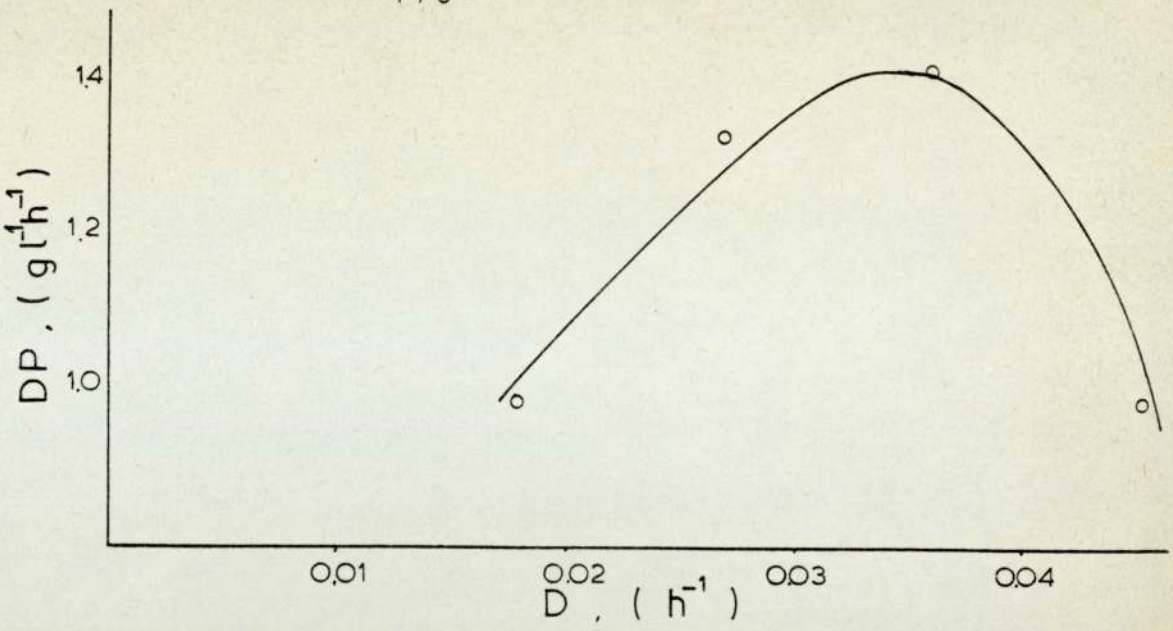


Figure 6.4.9. Productivity versus dilution rate.
Spirit vinegar. Temperature 35°C.
Total acid concentration 55.8 g.l^{-1}.

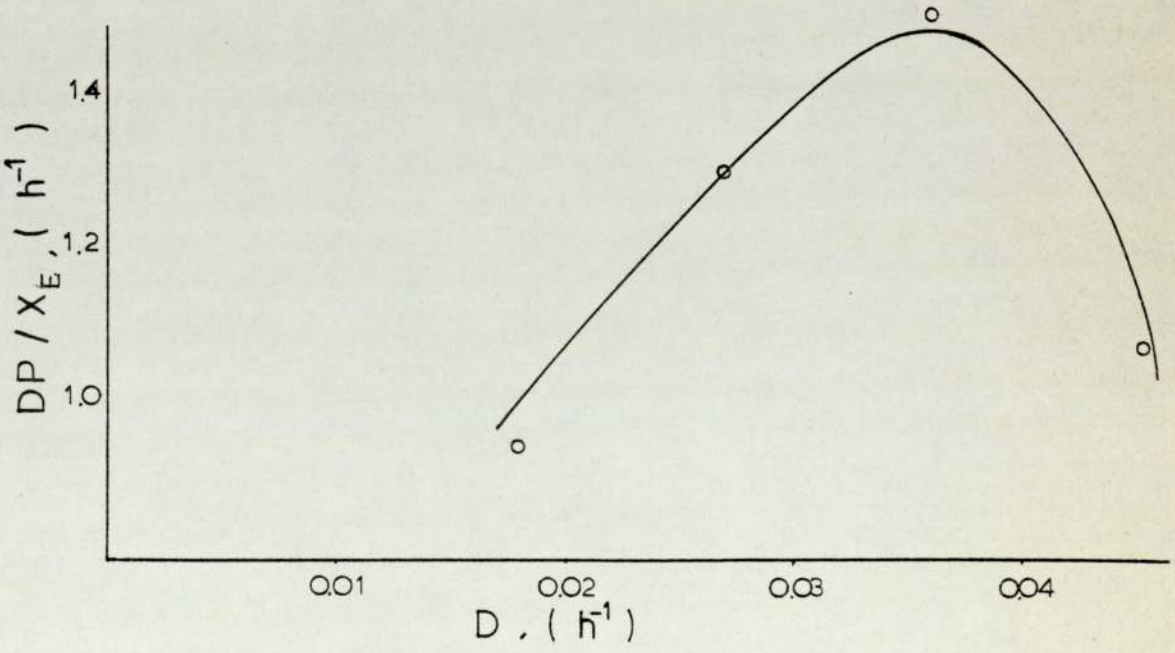


Figure 6.4.10. Specific productivity versus dilution rate.
Spirit vinegar.
Temperature 35°C. Total acid 55.8 g.l^{-1}

dilution rate of 0.04 h^{-1} at the temperature of 35°C the biomass concentration in the fermenter was 0.96g.l^{-1} whilst at 30°C was 0.70 g.l^{-1}. At dilution rates less than 0.02 h^{-1} the differences in biomass concentration in the fermenter at the two temperatures tested, were not substantial. (Figures 6.4.3 and 6.4.8).

No considerable changes on the pattern of product wash out were observed for the two temperatures tested.

6.4.4.2. Effect of dilution rate on the acetic acid productivity D.P. and specific productivity DP/X_E at the temperature of 35°C.

Figures (6.4.9 and 6.4.10) show that the productivity and the specific productivity at the temperature of 35°C, their maxima were 1.37 g.l^{-1}.h^{-1} and 1.45 h^{-1} respectively. Although the high temperature favoured the growth of the acetic acid bacteria, the losses due to the increased evaporation lowered the output of the product.

In comparison studies between the temperatures of 30°C and 35°C, the productivity at the first temperature found to be higher than that of the second one. (Figure 6.4.6.)

The specific productivity was found to follow similar patterns. At temperature of 30°C the maximum productivity found to be 1.45 h^{-1} (Figure 6.4.10). The maximum specific productivity at 35°C was as mentioned at paragraph (6.4.3.4), 1.63h^{-1}. The phenomenon is based, again, on the same reasons as above mentioned for the productivity. The loss of substrate and product due to their evaporation caused the lowering of the specific productivity at 35°C.

WINE VINEGAR

6.5. PRODUCTION OF WINE VINEGAR IN CONTINUOUS TOWER FERMENTER

6.5.1. Medium preparation and size of fermenter used.

The medium was 'white' wine and it was obtained from J.H. Swift Wines Ltd.(Nelson Lane, Warwick, CV4 5JB). A chemical and microbiological analysis was made to determine the amount of ethanol, nitrogen, reducing substances and acid present in the medium (Table 6.5.1).

Ethanol	92.10 $g.l^{-1}$
Acid (as acetic acid) ..	4.50 $g.l^{-1}$
Medium total Nitrogen ..	0.43 $g.l^{-1}$
Medium NH_4-Nitrogen	0.38 $g.l^{-1}$
Total reducing substances.	6.70 $g.l^{-1}$
Solids	0.00 $g.l^{-1}$
pH	5.10

Table 6.5.1. Results of chemical analysis of wine medium

The microbiological analysis shows that no microbial contamination occurred during storage. The medium was diluted to concentrations of total acid (a) 67.1 $g.l^{-1}$ and (b) 89.0 $g.l^{-1}$.

Yeast extract (oxoid) was added in the media a and b in amounts of 1.0 $g.l^{-1}$ and 1.2 $g.l^{-1}$ respectively.(Alian et al,1963a).

The fermenter size used was 3.1 l and the configuration of it was similar to that described in paragraph 2.2.4.3.

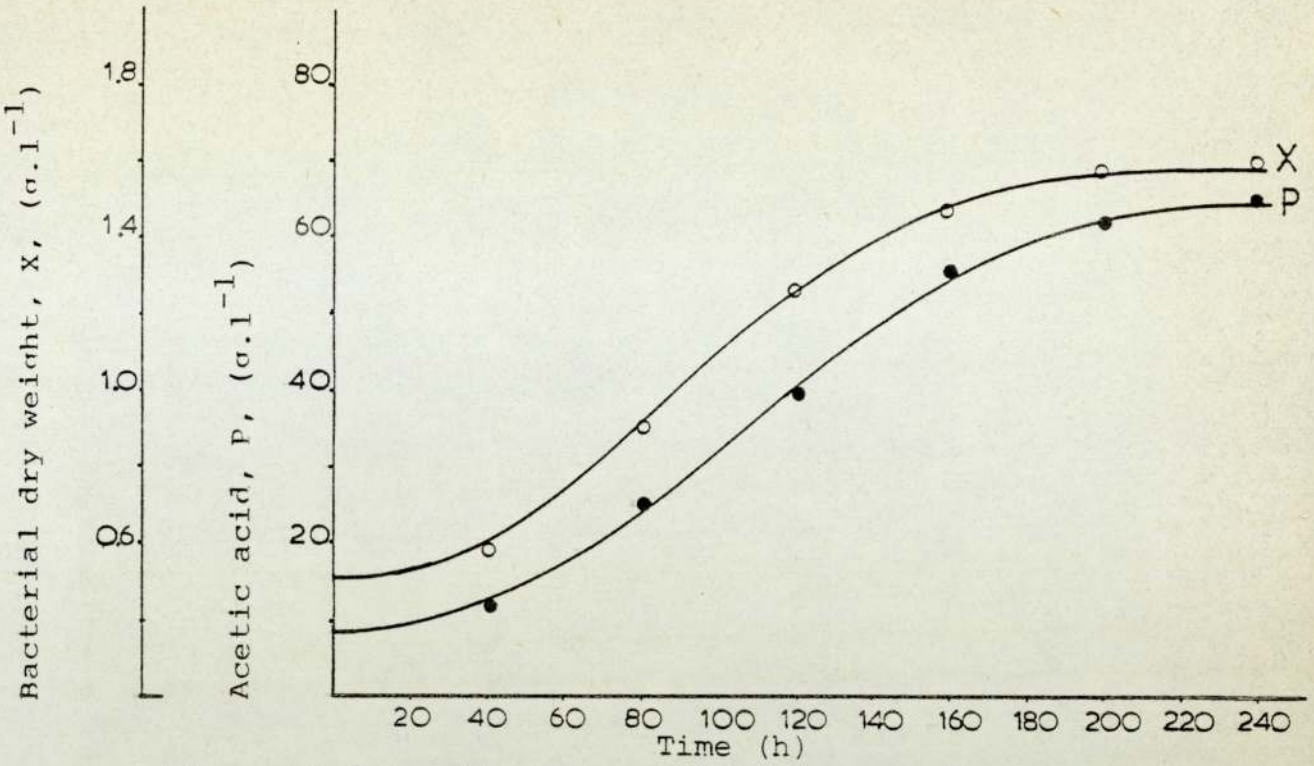


Figure 6.5.1. Wine vinegar batch tower fermentation Temperature 30°C Aeration rate 0.7 v/v/m

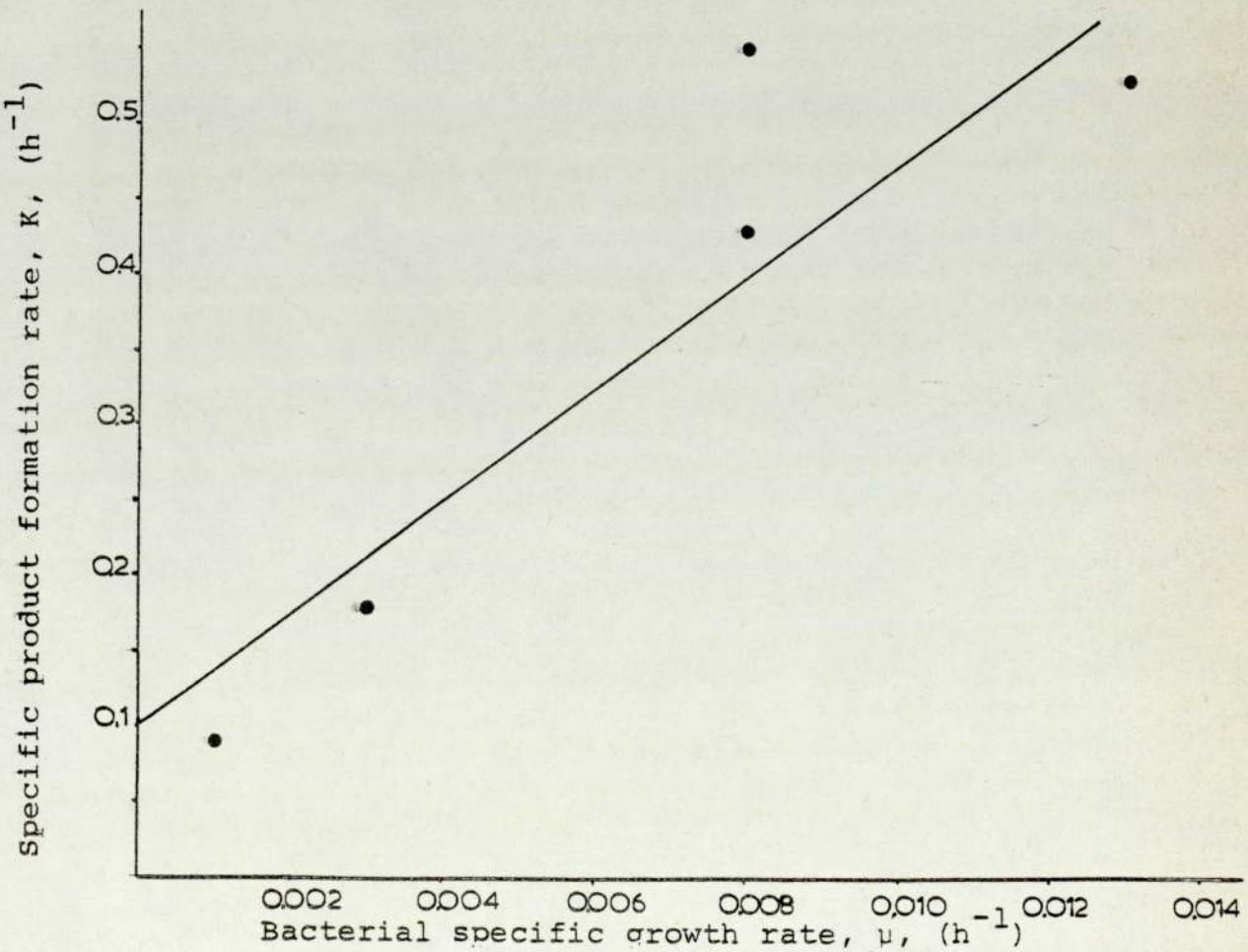


Figure 6.5.2. Relationship between specific product formation rate, K, and bacterial specific growth rate, μ, in wine vinegar batch fermentation.

6.5.2. Preliminary fermentation

A preliminary fermentation had shown that the maximum aeration rate was 0.7 v/v/m.

With 0.7 v/v/m the system only produced foam during the lag phase. The use of antifoam was kept in the minimum and only in the beginning of the fermentation. The inoculum used was an active culture of Acetobacter from the malt vinegar tower acetifier. (50 ml of inoculum to 2.5 l of medium). The temperature was 30°C.

6.5.3. Relationship between specific product formation rate (K), and specific bacterial growth rate (μ) in batch wine vinegar fermentation

2.5 l of medium with 67.1 $g.l.^{-1}$ total acid concentration was subjected to a batch tower fermentation in order to determine the relationship between specific acid formation rate and specific bacterial formation rate, (Figure 6.5.1.) The results of the regression analysis show that there is linear relationship between specific product formation rate, K (g at acid formed per g of bacteria per h) and specific growth rate μ (h^{-1}). (Figure 6.5.2.) The relationship $K = a\mu+b$ was valid with probability p = 0.1 and r = 0.715 with 4 degrees of freedom. The experimental parameters a and b were 37 and 0.1 h^{-1} respectively.

The instantaneous product formation rates $\frac{dp}{dt}$ ($g.l^{-1}.h^{-1}$) and bacterial growth rates $\frac{dx}{dt}$ ($g.l^{-1}.h^{-1}$) were obtained from Figure 6.5.1.

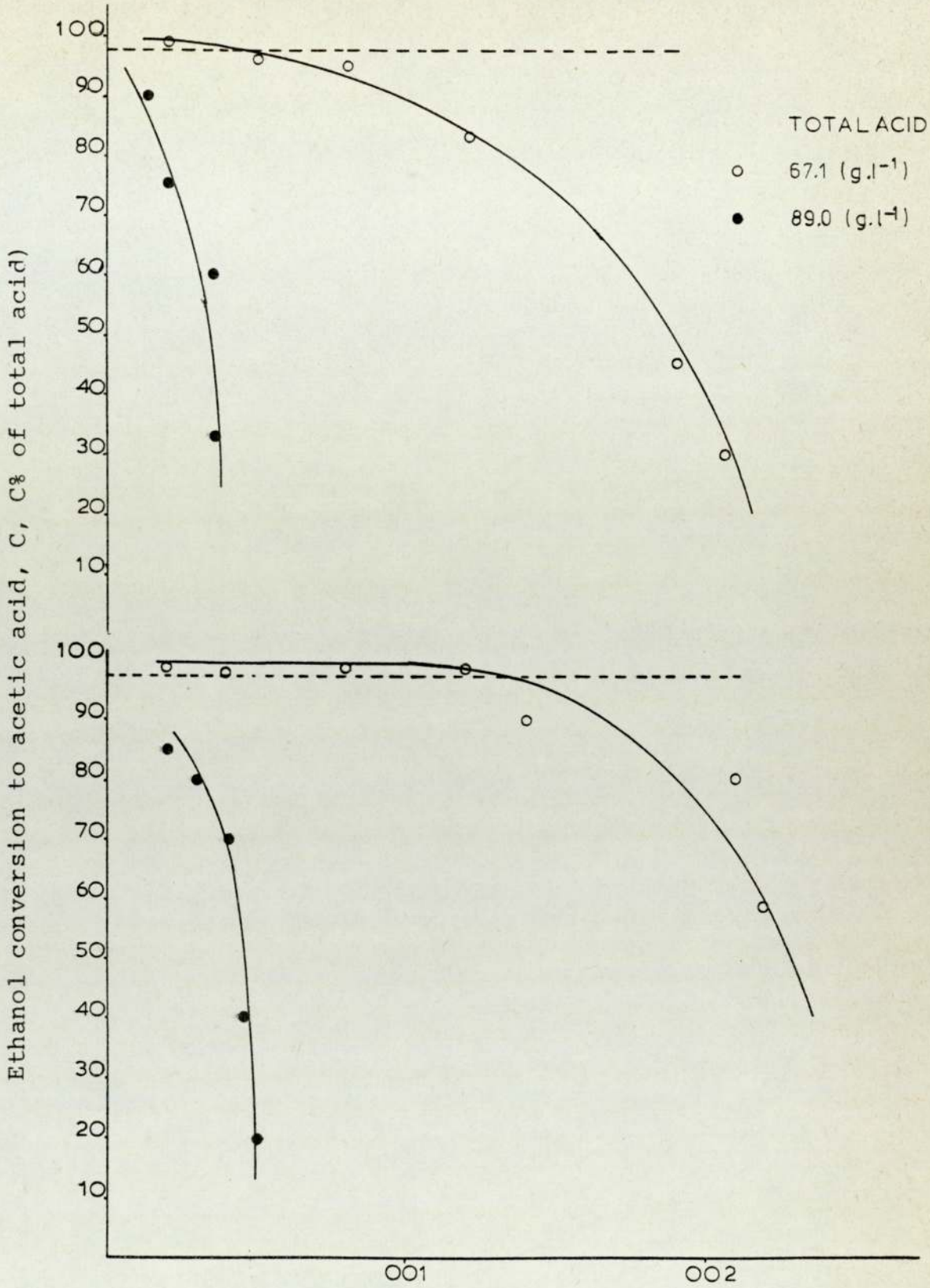


Fig. 6.5.3. Ethanol conversion to acetic acid at different dilution rates at temperatures a) 32°C, b) 34°C. The broken lines at 97.5% conversion level are the operation lines.
Substrate wine.

6.5.4. EFFECT OF TEMPERATURE ON THE PRODUCTION OF WINE VINEGAR IN CONTINUOUS TOWER FERMENTER

6.5.4.1. Effect of dilution rate on the ethanol conversion to acetic acid in continuous tower fermentation

Continuous acetification of the wine medium with total acidities 67.1 and 89.0 $g.l^{-1}$ at temperatures 32°C and 34°C show the following results (Table 6.5.2):

Total acid ($g.l^{-1}$)	Temperature (°C)	Dilution rate with 97.5% conversion (h^{-1})
67.1	32	0.0045
67.1	34	0.0135
89.0	32	0.0002
89.0	34	0.0006

Table 6.5.2. Effects of temperature and total acidity on the ethanol conversion ability.
Wine vinegar.
Aeration rate 0.7 v/v/m

Figures (6.5.3a and 6.5.3b) Explanations are given in paragraphs 6.1.3.2.1. and 6.2.4.2.2.

6.5.4.2. Effect of dilution rate on the acetic acid, P, bacterial dry weight concentration X_F and pH in the fermenter (substrate wine)

When the dilution rate increased, the pH of the medium in the fermenter increased from 2.4 to 3.0. (Figures 6.5.4.c, 6.5.5.c). This was expected because the acid concentration in the fermenter at high dilution rates was low. At the temperature of 32°C the acid concentration in the fermenter was lower than that at a temperature of 34°C for the same dilution rates (Figures 6.5.4.a, 6.5.5.a). A similar pattern followed by the bacterial dry weight concentrations in the fermenter.

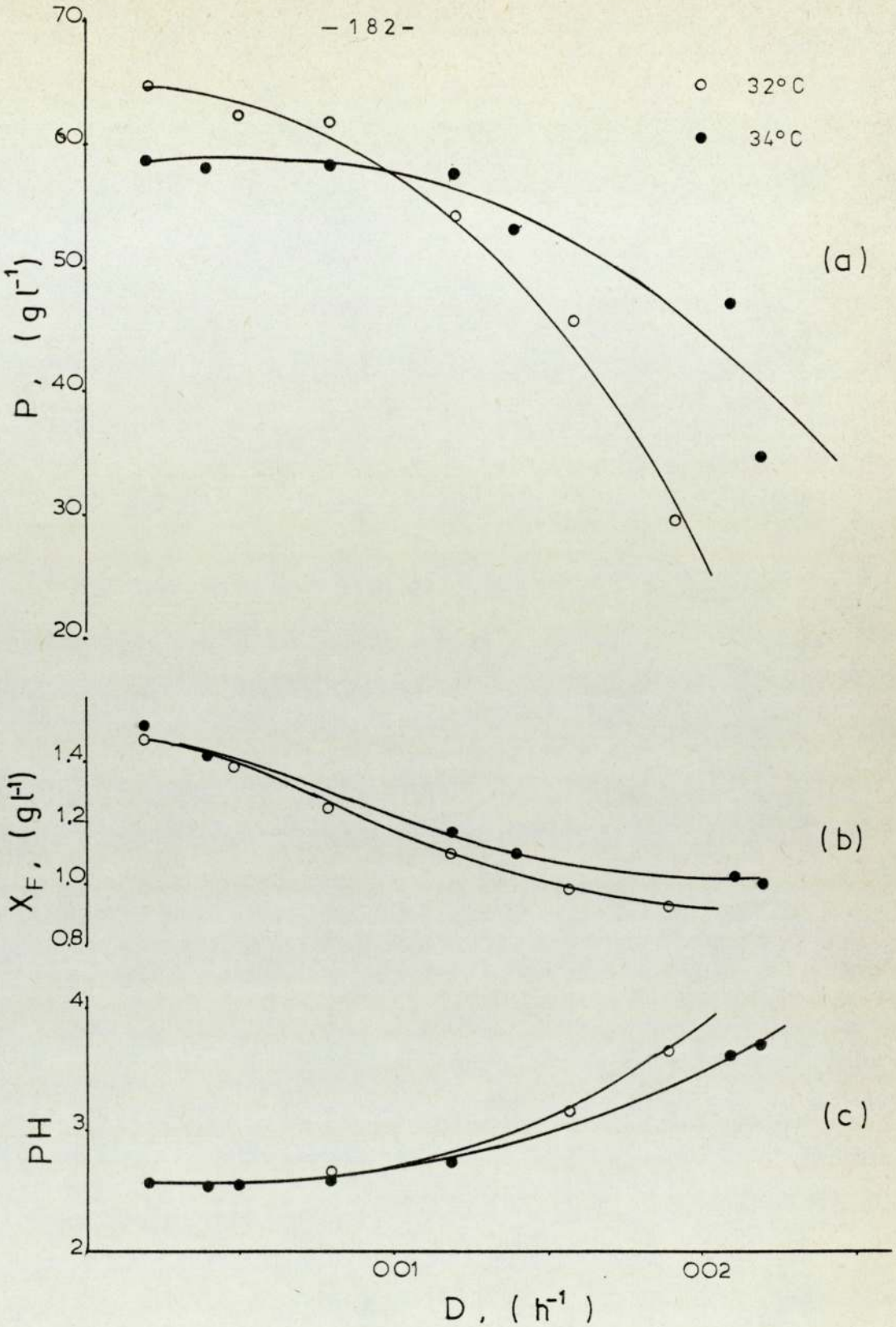


Figure 6.5.4. Effects of dilution rate, D, on acid concentration, bacterial dry weight concentration in the fermenter and medium pH for different temperatures. Substrate wine. Total acidity 67.1 g.l^{-1}

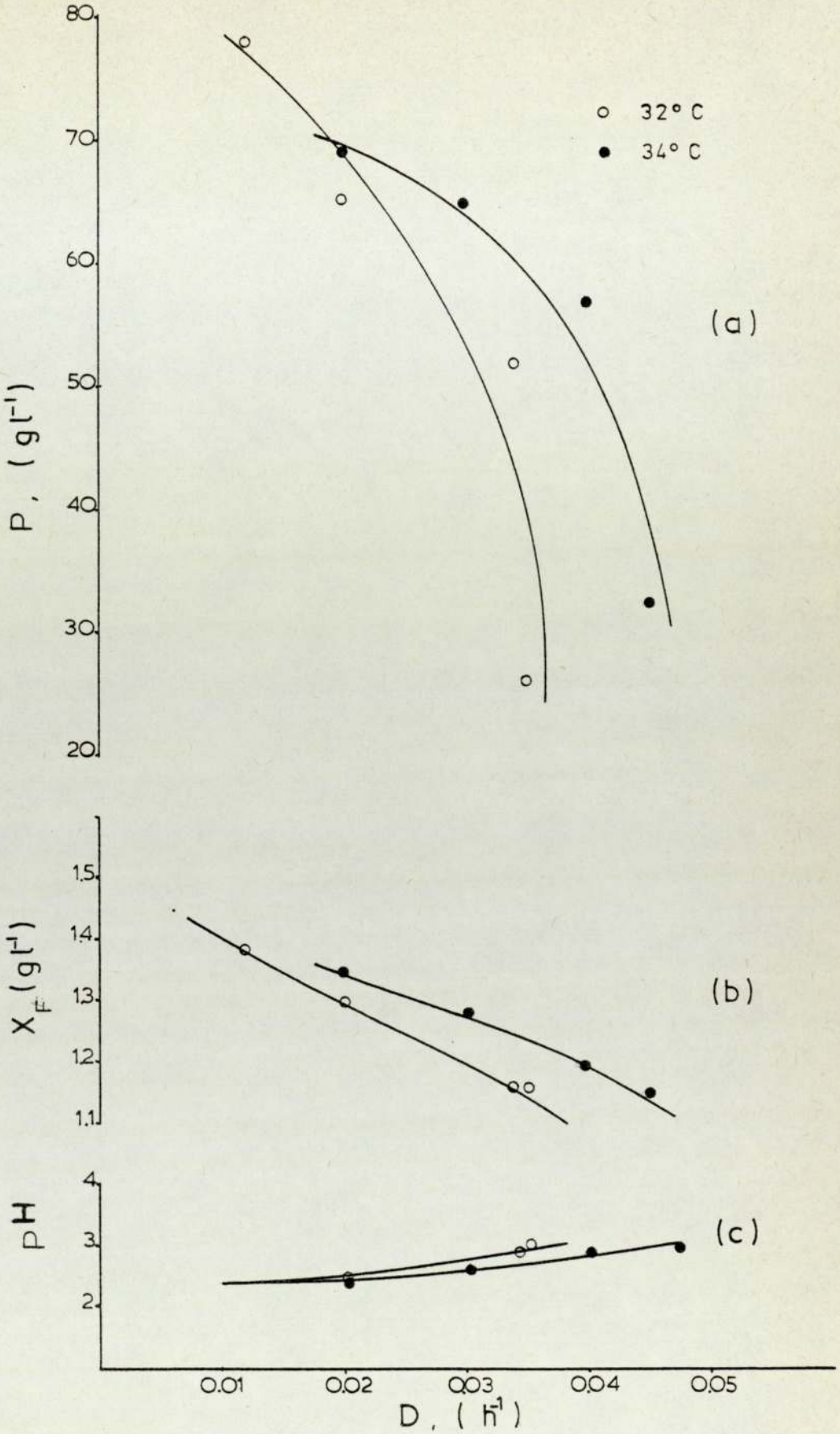


Figure 6.5.5. Effects of dilution rates, D, on acetic acid, P, concentration, bacterial dry weight concentration in the fermenter X_F and medium pH at different temperatures. Substrate wine. Total acid 89.0 $g.l^{-1}$.

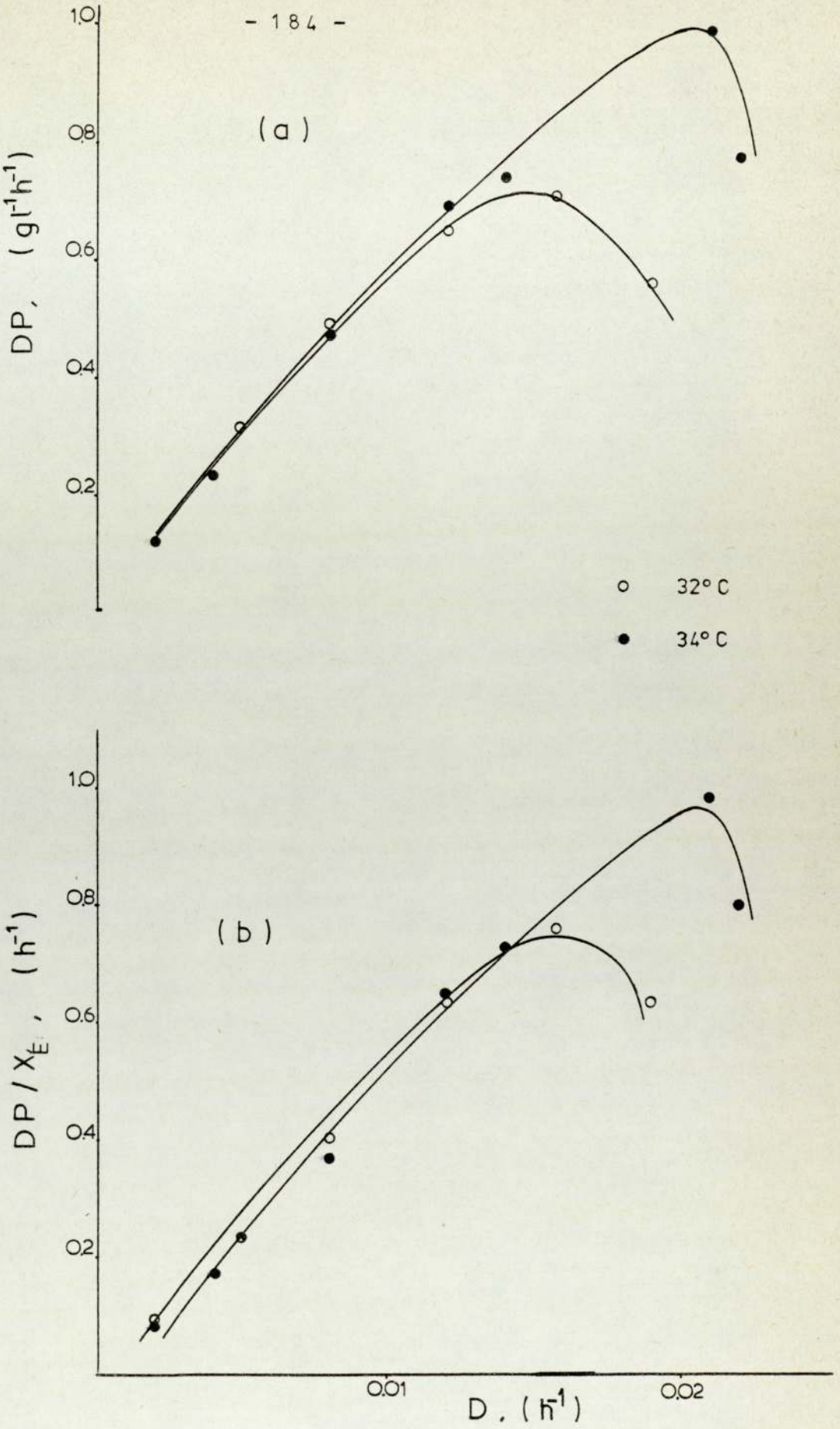


Figure 6.5.6. Relationship between productivity, DP, Specific productivity DP/X_E and dilution rate at different temperatures. Substrate wine. Total acid 67.1 $g.l^{-1}$

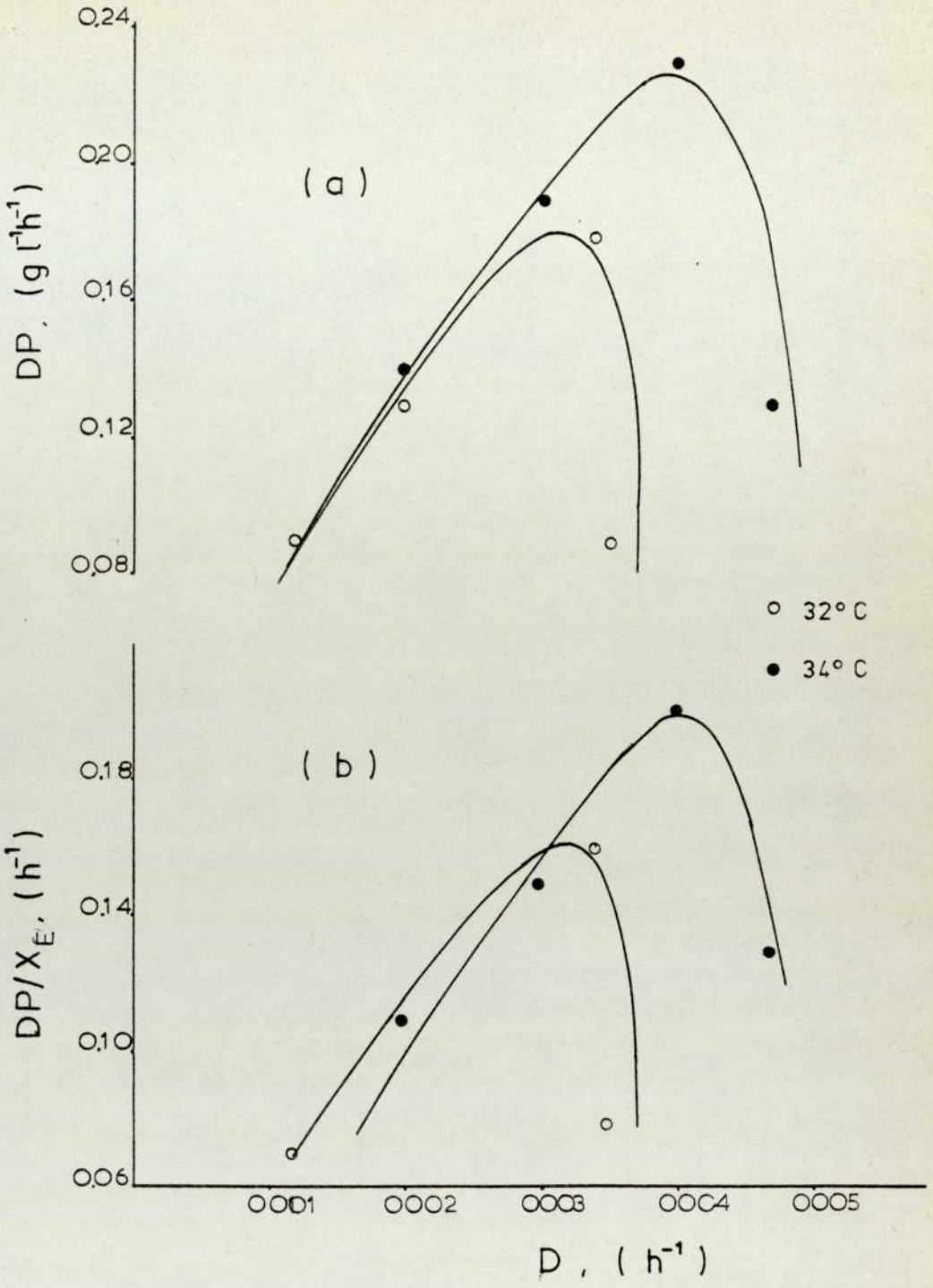


Figure 6.5.7. Relationship between productivity DP, specific productivity, DP/X_E and dilution rate D at 32°C and 34°C. Substrate wine. Total acid 89.0 $g.l^{-1}$

At high dilution rates, the dry weight concentrations were lower than at high dilution rates (Figures 6.5.4b and 6.5.5.b.).

6.5.4.3. Effects of dilution rate on acid productivity and specific productivity, at different temperatures and acidities.

The decrease of temperature from 34°C to 32°C and the increase of the medium total acid concentration brought a dramatic fall to both acetic acid productivity and specific productivity in the wine vinegar fermentation (Figures 6.5.6.a and b and 6.5.7.a and b).

In table 6.5.4. values of the maximum productivities and maximum specific productivities are compared at temperatures of 32°C and 34°C. Discussion is given in paragraphs 6.1.3.2.3. and 6.1.3.2.4.

Temperature (°C)	Total acid ($g.l^{-1}$)	Max. productivity ($g.l^{-1}.h^{-1}$)	Max. Spec. Productivity
32	67.1	0.710	0.760
32	89.0	0.180	0.160
34	67.1	0.990	0.980
34	89.0	0.226	0.198

Table 6.5.4. Effects of temperature and medium total acidity on acid productivity and specific productivity in wine vinegar continuous tower fermentation.
Aeration rate 0.7 v/v/m.

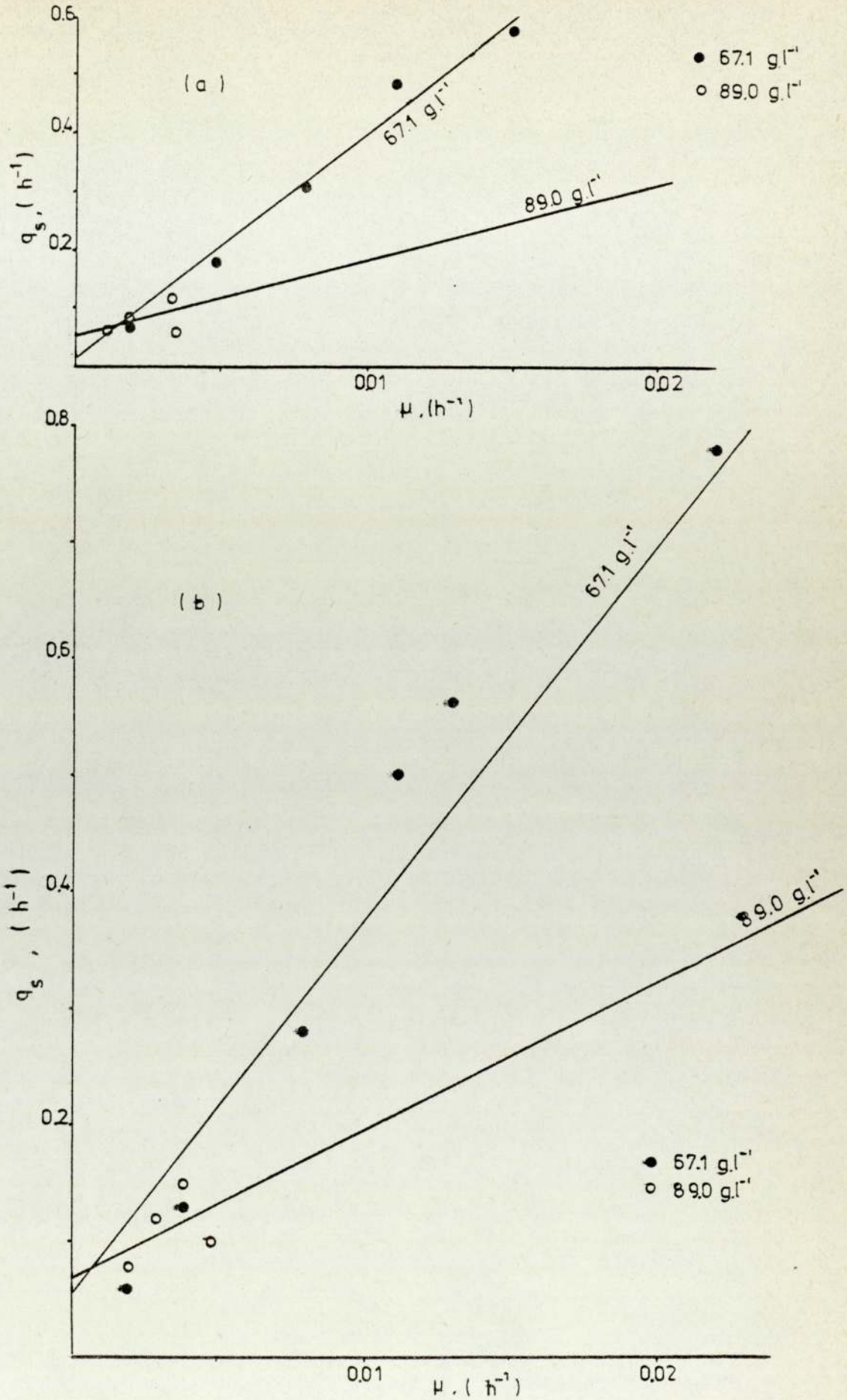


Figure 6.5.8. Yield studies. Determination of Y_G and m. a) 32°C, b) 34°C
Substrate wine.

6.5.4.4. Yield studies: Determination of the true growth yield, Y_G, and the maintenance energy coefficient, m in wine vinegar fermentation

The true growth yield Y_G and the maintenance energy coefficient m were determined by plotting values of specific substrate utilization rage, q_s, against values of specific growth rate, μ. (Figures 6.5.8a and b). The results are shown in Table 6.5.4).

Temperature ^oC	Total acidity $g.l^{-1}$	Y_G $g.g^{-1}$	m $g.g^{-1}.h^{-1}$
32	67.1	0.025	0.01
32	89.0	0.076	0.05
34	67.1	0.029	0.06
34	89.0	0.080	0.07

Table 6.5.4. Values of Y_G and m at different conditions of temperature and medium total acid concentration

Wine vinegar fermentation

Aeration rate 0.7 v/v/m

The true growth yield and the maintenance energy coefficient were increased when the temperature and the total acid increased. Discussion is given in paragraph 6.2.4.2.4.

6.6. Pilot scale production of vinegar

The application of the tower fermentation system commercially was first attempted with malt vinegar by Hammonds Vinegar Co. Ltd., Barrowford, Lancs. Successful continuous production of malt vinegar was made in a 3000 l capacity tower for several months at 6 and 8.5% w/v acetic strength. However, the project was not taken to full-scale due to management changes in the company. Subsequently, two tower fermentation systems were constructed for the production of cider vinegar by Aspall Cider at Debenham, Suffolk. The construction material was polypropylene. The towers were of 300 l and 1500 l volumetric capacity. These were provided with perforated plates with 2 mm holes fitted at the bottom of the towers. The air was supplied from two air compressors (Atlas Copco), specially installed and operated in tandem. Two peristaltic pumps (Byo 800, Baron Yemm Developments Ltd., Watford, Hertfordshire) supplied medium with the aid of a silicone tubing fitted to ports 10 cm above the perforated plates. The towers consisted of two parts. The tubular part which was lower, covered with a water jacket, and the expansion which was above the tubular section. The medium exit was fitted in the expansion part and the air exited at the top of the towers. At the top of each tower the air exit was connected to an all-glass condenser and from there to the open air.

Temperature attemporators were also provided to regulate the temperature at the condensers and to the water jackets of the towers. The temperature was monitored by means of external thermometers.

6.6.1. Medium

The medium was cider which was prepared from apple juice naturally fermented in vats.

The alcohol content of the cider varied from batch to batch but was in the range 4.2 to 5% by weight.

Analysis for nitrogen and phosphate for the media was made by the laboratories of Long Ashton Cider Research Station. The analysis gave nitrogen nil. Consequently the medium was supplemented with 0.1 g.l^{-1} of ammonium sulphate. Yeast extract was also added for the preparation of inoculum for the 3 l fermenter. Subsequently the cider was acetified without the addition of salts.

6.6.2. Preparation of the towers

The towers were thoroughly cleaned with the use of 0.6% by weight of detergent (Pyroneg, Diversey Ltd.). The cleaning procedure was made by filling towers with water and detergent forthe first time. After six hours the towers were emptied and refilled with tap water. This procedure was repeated four times for each fermenter. The towers during the cleaning process were aerated (0.04 v/v/m aeration rate).

6.6.3. Inoculum preparation

6.6.3.1. Preliminary work

Samples of cider were brought to the laboratories of The University of Aston in Birmingham and were subjected to preliminary fermentations. The fermentations took place in 9 shake flasks (250 cc) at three different temperatures: 27, 29 and 31°C. The cultures under 29°C and 31°C had the shortest lag phase than those at 27°C. The cultures under 29°C

and 31°C gave positive indications of acid production at the 27th and 32nd hour respectively, from the time of inoculation. The cultures at 27°C started at the 46th hour. The inoculum used was taken from stock cultures of Acetobacter in cocoa 'sweatings' medium.

For the purpose of the production of large size of inoculum a 3 l batch tower fermentation system was prepared. The medium used was Aspall Cider and as inoculum the contents of the shake flasks. The acid content of the inoculum was 4.5% by weight. The aeration rate was 0.3 v/v/m and the temperature 29°C.

Under the above preferred conditions the culture did not foam and the acid production commenced after four hours from the time of inoculation.

6.6.3.2. Inoculation of the 300 l tower fermenter

A 3 l tower fermenter was constructed and set up next to the 300 l fermenter, in a manner that the exit of the 3 l fermenter was higher than the middle port of the 300 l fermenter. 2 l of cider medium was introduced by the peristaltic pump to the 3 l fermenter. The inoculum was the already prepared culture at the University laboratories. The inoculum was transferred in a flask which was continuously oxygenated from an oxygen cy linder during transit. 0.5 l of the culture was used for inoculation of the 3 l fermenter. The temperature was 29°C and the aeration rate 0.3 v/v/m.

Six hours later the fermentation showed acid concentration of 4.2% by weight. At the same time a continuous fermentation of vinegar started in the 3 l fermenter. The

dilution rate was set up at 0.02 h^{-1}. The exit of the fermenter was connected with the middle port of the 300 l tower fermenter. By this technique, all the output of the 3 l fermenter entered the 300 l fermenter continuously which was already charged with 100 l of cider. The aeration rate of the 300 l fermenter was 0.4 v/v/m and the temperature 29°C.

The process of continuous inoculation laster 24 hours. After this period of time the process was terminated because it was established that the culture in the 300 l fermenter had started producing acid.

6.6.4. Semicontinuous operation of the 300 l tower fermenter

When the acid concentration in the fermenter reached the value of 3.5% by weight, the tower was filled with 150 l of cider. A cycle of discharging and refilling the tower commenced as figure 6.6.I. shows.

The process was repeated for 20 consecutive days. There were instances at which 50% of the contents were discharged at values of ethanol conversion lower than the theoretical maximum values. This was due to lack of continuous ethanol concentration monitoring and an immediate requirement commercially for cider vinegar at 4.5% acetic acid. The early discharge of vinegar was also made to avoid the over-oxydation of acetic acid to carbon dioxide and water. During the exponential phase, because the oxidation of ethanol is an exothermic reaction, the temperature in the water jacket was decreased to 10°C.

This problem of loss of unfermentable ethanol has been

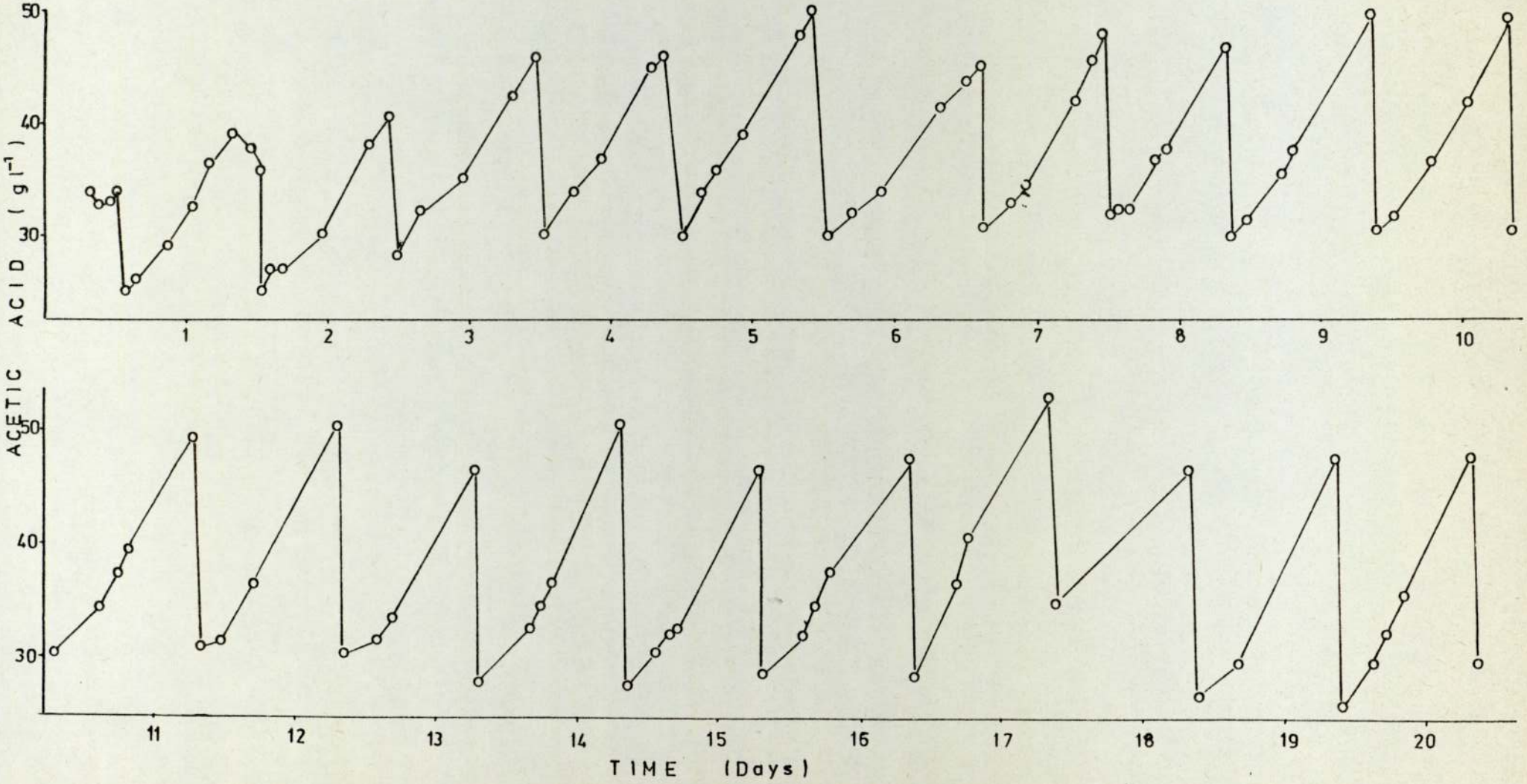


Figure 6.6.1. Semicontinuous production of cider vinegar in 300 l fermenter

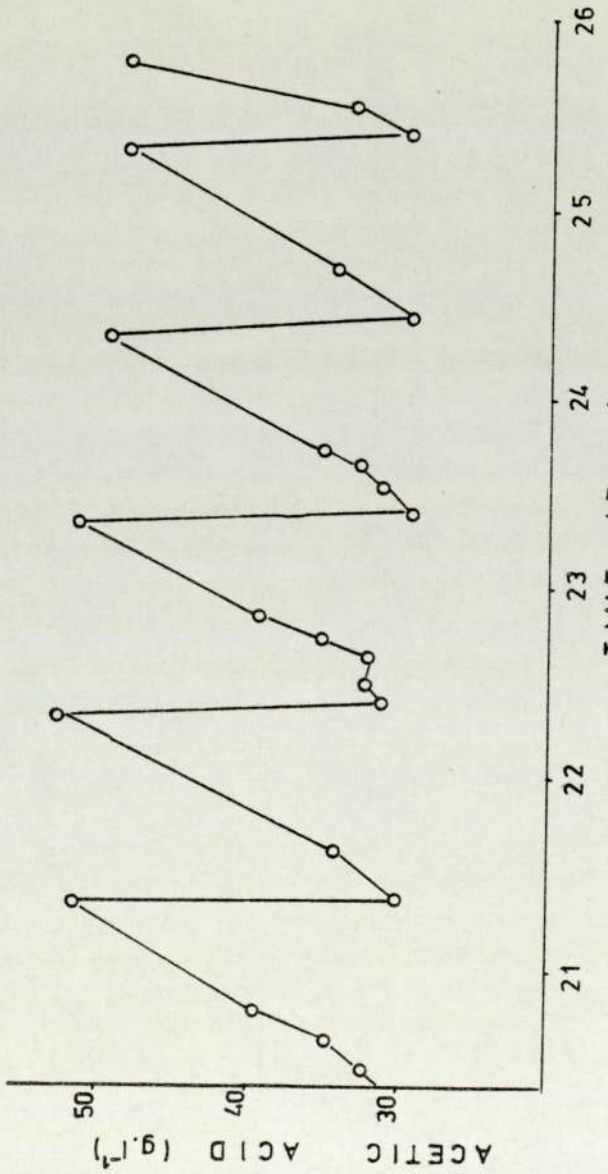


Figure 6.6.I. Continued.

solved by the Frings Acetator with the provision of a continuous ethanol concentration monitoring apparatus.

To overcome these problems, the technique of operation was changed from semicontinuous to continuous.

6.6.5. Continuous production of cider vinegar in 300 l tower fermenter

After the last semicontinuous cycle, shown in Figure 6.6.1, the acid concentration of the fermenter was 2.5% by weight. It was filled with 7.6% by weight total acidity cider. After 23 hours, when the acid concentration was 6.2% by weight, cider started to enter the 300 l fermenter with the help of a peristaltic pump at a feed rate of 2.2 $l.h^{-1}$ or dilution rate of 0.008 h^{-1}. As the results show, the acid concentration was increased, from 6.2% to 7.4% by weight in seven days. At that time the dilution rate was increased to 0.012h^{-1} (feed rate 3.3 $l.h^{-1}$). The productivity of the tower also increased from 0.50 $g.l^{-1}.h^{-1}$ to 0.90 $g.l^{-1}.h^{-1}$. After two days the dilution rate reached the value of 0.014 h^{-1} and the productivity 1.04 $g.l^{-1}.h^{-1}$. Any further increase of dilution rate caused decrease of productivity. Successful continuous acetification was then continued for commercial purposes and is still operating at present.

6.6.6. Semicontinuous vinegar production in 1500 l tower fermenter.

200 l of cider medium were already introduced to a 1500 l tower fermenter. The aeration rate was 0.4 v/v/m and the temperature was 29°C. Continuous inoculation from the 300 l fermenter commenced which lasted 24 hours. Figure 6.6.2

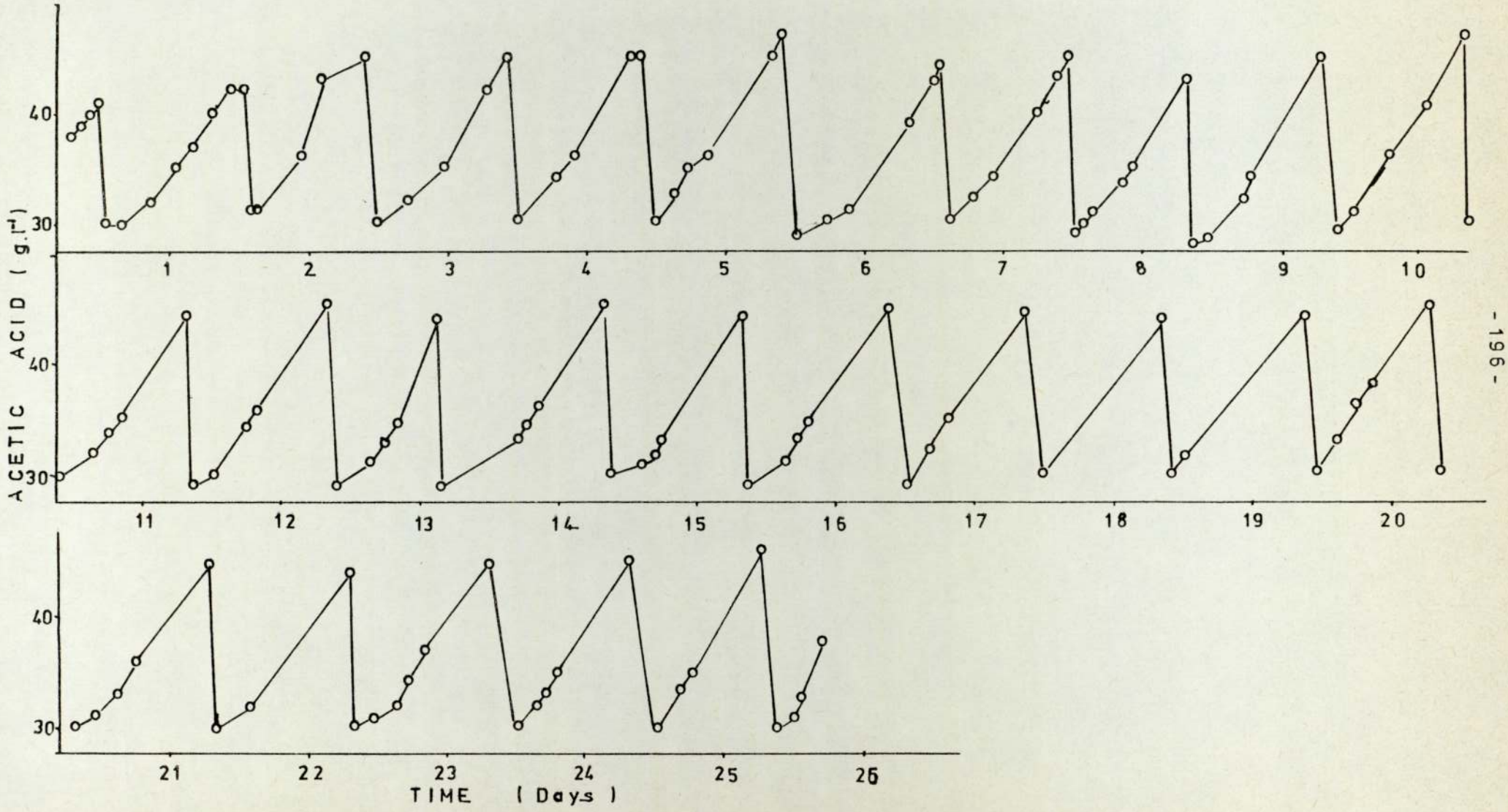


Figure 6.6.2. Semicontinuous production of cider vinegar in 1500 l fermenter.

shows the cycles of the semicontinuous process. The total acidity of the medium was 6.2% by weight.

6.6.7. Continuous production of vinegar in 1500 l tower fermenter.

During the last cycle of the semicontinuous process when the acid concentration was 4.5% by weight with the help of a peristaltic pump medium was introduced to the feed rate of 10 $l.h^{-1}$ ($D = 0.008\ h^{-1}$). The productivity of the system at this dilution rate was 0.4 $g.l^{-1}.h^{-1}$. After four days the acid concentration increased to 6% by weight and the productivity reached the value of 0.48 $g.l^{-1}.h^{-1}$. Successful continuous commercial production of cider vinegar has continued to the present time.

6.6.8. General discussion about the production of cider vinegar in pilot scale tower fermenters

Generally, the operation of the two pilot scale tower fermenters did not present any major problems during their operation.

The semicontinuous process proved to be more sensitive to human errors. For example, the discharge and refilling time of the towers varied from cycle to cycle. Also, the time of temperature change from 29°C to 10°C and vice-versa during certain phases of the fermentation - these operations were concluded during a particularly sever winter and the input cider was often very cold.

These variations were caused by the non automative operation of the system.

The continuous operation did not present any problems.

The operation during continuous mode demanded only the establishment of the optimum dilution rate, temperature and aeration rate and the system remained stable for more than four months. The productivity of the system was found to be higher in continuous fermentation than that in semicontinuous fermentation. For the 300 l tower fermenter at continuous operation the productivity was 1.04 $g.l^{-1}.h^{-1}$. At semi-continuous operation the range was of 1.02 $g.l^{-1}.h^{-1}$. For the 1500 l tower fermenter at continuous operation the productivity was 0.48 $g.l^{-1}.h^{-1}$ at semicontinuous operation was in the range of 0.92 $g.l^{-1}.h^{-1}$.

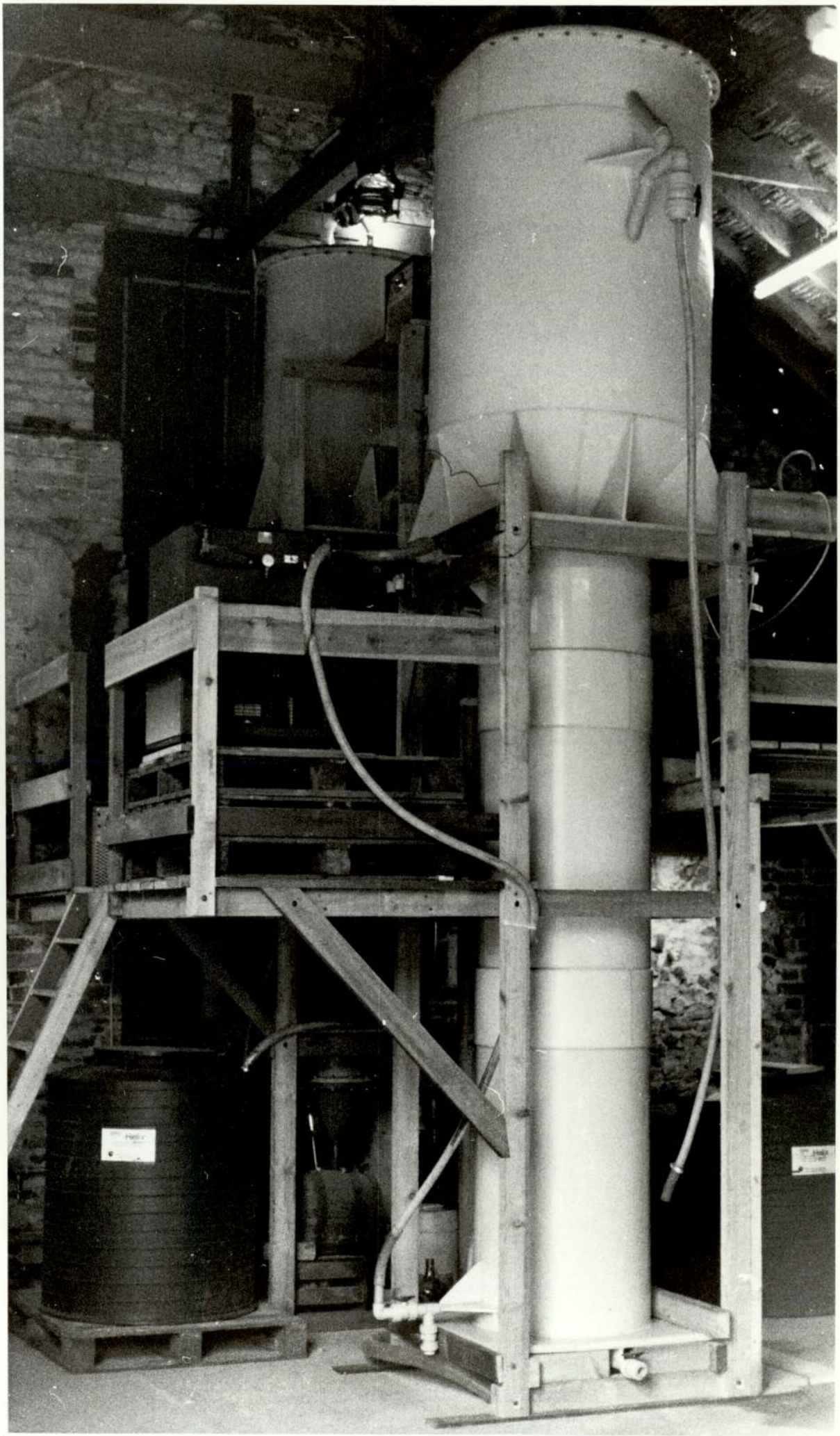
The difference in productivities between the two towers could be explained as a result of different oxygen transfer rates between gas/liquid interfacial areas and by the different aspect ratios of the towers. In the 1500 l fermenter the air bubbles after a certain height became larger due to lower hydrostatic pressure as they travel alongs the tower. This causes lower oxygen transfer rates from gas to liquid phase. Another interesting point is that the perforated plate produces larger bubbles than the porous plate. At the laboratory scale 3 l tower fermenter the air distributor was porous plate (porosity 2). Therefore the results from the laboratory fermenter had no value for predicting pilot scale fermentations with perforated plates. This is the reason of applying different (higher) aeration rates for the pilot scale fermentations than that used for the laboratory scale fermentations.

The expansion chambers of the towers caused an efficient foam desruction by their physical shapes. No foam problems were observed during the semicontinuous or the continuous

operation.

The power input for the 300 l fermenter was calculated as 0.24 Kw/Kg of ethanol converted during the continuous operation and 0.27 Kw/Kg of ethanol converted for the 1500 l fermenter operating in continuous mode.

Plate 6.1. Pilot scale tower fermenters for the production of cider vinegar



SECTION 7

7. DISCUSSION AND CONCLUSIONS

7.1. General

In product-formation fermentations other than biomass, the behaviour of biomass in the fermenter is considered only when the product is growth or partially growth-associated. The acetic acid is partially growth and partially biomass associated. This conclusion was obtained from our experiments for all the substrates tested (except cider where there have been no measurements of biomass concentration). The equation $K = a\mu + b$ has proved to be valid with high probabilities for the substrates tested, k being the specific product formation rate and μ the specific growth rate and a, b are experimental parameters. The conclusion came to an agreement with Divies (1973) results working with Acetobacter in defined medium. In continuous tower fermentations the product wash-out follows a different pattern from that of the bacterial biomass. The wash-out pattern of the product is approaching that of a C.S.T.R. The reason is that there is no sedimentation of the product.

The specific productivity of the acetic acid is found to be linear at low dilution rates. At high dilution rates the linearity disappears. Divies (1973) found the linearity of the specific productivity with the dilution rate but he has worked at low dilution rates. At high dilution rates the washout of the bacteria does not permit high bacterial concentrations in the fermenter. The linearity at high dilution rates could be possibly justified with adaptation of the bacteria and increase of their productivity. This could not apply in the acetic acid fermentation because

it has been proved to be growth and biomass concentration associated.

The acetic acid tower fermentation is found to be limited by four parameters:

1. Total acidity
2. Dilution rate
3. Aeration rate
4. Temperature

Generally the fermentation is affected by both the substrate and the product. Both substances ethanol and acetic acid act as growth inhibitors to the acetic acid bacteria. The dilution rate found to be a limiting factor, because when it was changing, the bacterial biomass concentration was also changed.

The aeration rate and temperature were found to affect the fermentation in many ways.

1) With aeration and temperature increasing the growth rate of the bacteria was increased as a consquence the acetic acid formation was increased as well.
2) A negative effect of the temperature is the substrate and product evaporation which occurred when the temperature was increased, particularly above $30^{o}C$.
3) The increase in aeration rate brought an increase in the productivity. The reason for this is the increase of the gas-liquid interfacial area.
4) The higher productivity with increasing the temperature could be explained by change of the cellular membrane

(Hunter and Rose, 1972).

5) With the temperature increasing, the maintenance energy coefficient was increased. An explanation could be that with increasing the temperature, the metabolic activities of the cell are increased, consequently the energy for maintenance is increased.

Another negative effect of the aeration is the production of foam. If the addition of antifoam is to be avoided, the aeration must stay under control because of the possible loss of substrate and bacterial population.

The expansion of the tower on the top helps the destruction of the foam by physical methods but its activity is limited.

An improvement of the tower fermenter with the addition of side arms for medium recycling could have a positive effect on the foam desctruction.

7.2. Discussion about cocoa 'sweatings' vinegar production in C.T.F.

From the temperature experiment the following conclusions were resulted:

1. Increase of temperature from 23 to 35°C resulted in an increase of the bacterial growth rate (dx/dt) and product formation rate (dP/dt) from 0.025 $g.l^{-1}h^{-1}$ to 0.044 $g.l^{-1}h^{-1}$ and 0.80 $g.l^{-1}.h^{-1}$ to 1.20 $g.l^{-1}.h^{-1}$ respectively.
2. Increase of temperature from 23°C to 35°C resulted in an increase in the productivity (DP) and specific productivity (DP/X_E) from 1.0$g.l^{-1}.h^{-1}$ to 1.7 $g.l^{-1}h^{-1}$ and 0.83 h^{-1} to 1.60 h^{-1} respectively.
3. The specific bacterial growth rate (μ) showed a linear

relationship with the specific product formation rate (K) (paragraph 6.1.3.1.2).

4. The maintenance energy coefficient (m) showed an increase with the temperature increasing (Table A.1.3).

5. The output of the acetic acid was strongly influenced by temperatures higher than 30°C because of the substrate and product evaporation.

From the experiment on the elucidation of the optimum medium aeration rate the following conclusions were reached:

1. Increase of the aeration rate from 0.2 to 0.7 v/v/m resulted in an increase of bacterial growth rate and product formation rate from 0.026 to 0.035 $g.l^{-1}h^{-1}$ and from 0.92 to 1.00 $g.l^{-1}.h^{-1}$ respectively.

2. The productivity and specific productivitv increased with an increase of aeration rate from 0.2 to 0.6 v/v/m as follows:
DP: from 1.6 $g.l^{-1}h^{-1}$ to 2.7 $g.l^{-1}h^{-1}$, and
DP/X_E: from 1.52 h^{-1} to 2.37 h^{-1}.

7.3. Discussion about molasses vinegar production in C.T.F.

From the concentration experiment the following conclusions were reached:

1) The dilution rate, D, has a more pronounced effect at acidities rather than it does in low acidities of the medium, because in high acidities a small change of the dilution rate gives rise to a gross change in the bacterial concentration in the fermenter, X_F.

2) At low acidities (54.3 $g.l^{-1}$ and 56.2 $g.l^{-1}$ total acid) the maximum productivity is higher (1.95 and 1.35 $g.l^{-1}h^{-1}$ respectively) than at high acidities (paragraph 6.2.3.3.)

3) At low dilution rates, the relationship between specific

productivity DP/X_E and dilution rate, D, is almost linear. In higher dilution rates (higher than 0.03 h^{-1} for 54.3 and 58.2 $g.l^{-1}$ total acidities and higher than 0.045 h^{-1} in 68.3 and 84.6 $g.l^{-1}$ total acidity) the linearity disappears (paragraph 6.2.3.3.)

From the temperature experiment the following conclusions were reached:

1) In temperatures higher than 30°C there was loss of ethanol due to evaporation.

2) The duration of the batch culture till the stationary phase was longer than that at 33°C (paragraph 6.2.4.1.1.)

3) The relationship between specific product formation rate, K, and the specific bacterial growth rate, μ, is linear (paragraph 6.2.4.1.2.)

4) The conversion of ethanol to acetic acid found to be faster at the temperature of 33°C than at 28°C and 30°C (paragraph 6.2.4.2.1, Table 6.2.6.)

5) The acetic acid productivity at the temperature of 33°C found to be higher than at 28°C and 30°C (table 6.2.7.)

6) The maintenance energy coefficient was higher as the temperature increased.

From the aeration rate experiment, the following conclusions were reached:

1) With aeration rate of 0.4 v/v/m the conversion of ethanol to acetic acid was faster than that with 0.3 and 0.2 v/v/m. As it was explained in paragraph 6.2.5.1, the excess of air which is not utilized by the bacteria is used for increase of the gas-liquid interfacial area and for agitation purposes.

2) The productivity found to be higher with aeration rate of 0.4 v/v/m than that of 0.2 and 0.3 v/v/m (see table 6.2.10),

(paragraph 6.2.5.3.)

Generally, the molasses vinegar production could improve achieving higher productivities if the medium for the alcoholic fermentation is optimized.

7.4. Discussion about malt vinegar production in C.T.F.

1) The lag phase in batch tower fermentation was shorter in high dilution rates than that in low aeration rates.

2) At more intensive aeration rates than 0.1 v/v/m the time of appearance of the maxima of growth rates and product formation rates was shorter (table 6.3.2.)

3) The relationship between specific product formation rate, K, and specific growth rate has been shown to be linear (paragraph 6.3.2.1.2.)

4) The conversion of ethanol to acetic acid was limited by the aeration rate and by the dilution rate (paragraph 6.3.2.2.1.)

5) The maximum of acetic acid productivities 4.6 $g.l^{-1}h^{-1}$ and specific productivity 6.0 h^{-1} occurred when the aeration rate was 0.5 v/v/m.

7.5. Discussion about spirit vinegar production in C.T.F.

From the aeration rate experiment, the following conclusions were reached:

1) The ability of ethanol oxidation is higher for total acidity of 58.5 $g.l^{-1}$ rather than for 72.5 $g.l^{-1}$. This phenomenon has been explained as inhibition of ethanol and acetic acid in the growth of the bacteria of acetic acid.

2) The substrate and bacterial concentrations were affected by the dilution rate (paragraph 6.4.3.3.)

3) At acidity of 58.5 $g.l^{-1}$ and temperature 30°C the

maximum acid productivity was 1.44 $g.l^{-1}h^{-1}$. At the acidity of 72.5 $g.l^{-1}h^{-1}$ the maximum productivity was o.83 $g.l^{-1}h^{-1}$. This figure confirms the product inhibition and substrate inhibition or product and substrate inhibition at the fermentation (paragraph 6.4.3.4.)

From the temperature experiment, the following conclusions were reached:

1) The substrate evaporation rate at 35°C of 0.90 $g.l^{-1}h^{-1}$ affected the overall activity of the system (paragraph 6.4.4.1.)

2) At the temperature of 30° the biomass concentration in the fermenter was lower than that at 35°C.

3) The acetic acid productivity was higher at 35°C than that at 30°C they were 1.45 $g.l^{-1}h^{-1}$ and 1.37 $g.l^{-1}h^{-1}$ respectively.

7.6. Discussion about wine vinegar production in C.T.F.

1) The relationship $K = a\mu+b$ was valid with probability $p = 0.1$ and $r = 0.715$ with 4 degrees of freedom (where K is the specific product formation rate and μ the specific bacterial growth rate, $a = 10$ and $b = 0.286\ h^{-1}$ being experimental parameters.)

2) Conversion of 97.5% was achieved only when the concentration of total acid was 67.1 $g.l^{-1}$ at the temperature of 34°C. With all other conditions tested the dilution rate at the level of 97.5% conversion was lower.

3) The maximum productivity was achieved when the temperature was 34°C and the total acid concentration was 67.1 $g.l^{-1}$.

Optimization of the medium for the alcoholic fermentation could help to achieve better productivities.

With the study of the kinetics of the fermentation of the five substrates, the tower fermenter proved to be an efficient

7.7. Discussion on all Substrates used

The production of different kinds of vinegar using tower fermentation system was found to be similar or better than that of existing systems. Table 7.1 shows the productivity, dilution rate, fermentation condition of temperature, aeration rate and total acid concentration for the different substrates used, at the level of 97.5% of conversion efficiency.

Although vinegar is an old process only inadequate comparative data exist on the relative productive capacities of the different methods of production referred to in Chapter 2. From the existing data, it could be obtained that the tower appears to have certain advantages from the old processes, such as the Orleans andQuick methods. Their production rates are slower than that of the tower production rates. The cost effective and time consuming use of the packing materials on which these fermentation systems are using, make the tower superior over these systems. The same reason apply for the Frings circulating generator which operates on the packing principle. The tower among the modern submerged culture fermentation systems appears to have certain advantages and disadvantages.

The main advantages of the tower system are that the fermenter is simple, cheap to construct, has no moving parts, it is fully continuous requiring little attention and maintenance. From the pilot scale cider vinegar was found to be stable in steady-state. There were no difficulties for scale up from laboratory scale to pilot scale. The input power was calculated as being in the range of 0.24-0.27 Kw/Kg of ethanol converted. In the Frings type acetifier

(Acetator) the input power was between 0.30 to 0.35 Kw/Kg of ethanol converted.

The cost of its construction is cheaper than the Frings Acetator and Chemap Vinegator because the construction material is polypropylene, and does not require mechanical stirring devices for agitation purposes.

The disadvantages are based on the nature of bubble column bioreactors. It is oxygen limiting and as a consequence is unable to oxidize high substrate concentration mashes. In table 7.1. it is shown that the maximum total acidity that the tower could oxidize was 75.0 $g.l^{-1}$. The higher the substrate concentration the lower the productivity of the tower system at 97.5% conversion efficiency, which is considered to be economical (Greenshields, 1977).

The plastic acetifying tower fermenters could have a considerable potential for village-level technology and provide the useful product of vinegar to great advantage in the developing world whose economic and expertise are limited and where food is scarce and requires storage. The surface fermentation which is referred to, is also a non-expensive method for the production of vinegar. The advantage of the tower system lies on the fact that the surface fermentation is sensitive to the medium flow rates. A small change to the flow rate could result in reducing the conversion efficiency in surface fermentation. This problem finds solution only with close attention of the system. Another drawback of the surface fermentation is the necessity of cleaning the surfaces from bacterial film on the expense of productive time. The lack of temperature

Type of vinegar	D h^{-1}	DP $g.l^{-1}.h^{-1}$	^{o}C	Aer.rate v/v/m	Total acid $g.l^{-1}$
Wine	0.013	0.70	34	0.7	67.1
Spirit	0.017	0.95	30	0.5	58.5
Cocoa 'sweat-ings'	0.035	1.80	31	0.7	50.7
Molasses	0.035	2.00	30	0.3	54.3
Malt	0.075	4.60	30	0.5	67.7
Cider* (300 l)	0.014	1.04	29	0.4	61.0
Cider* (1500 l)	0.008	0.48	29	0.2	75.0

Table 7.1 Parameters at which 97.5% or higher Conversion efficiencies have been observed for different types of vinegar.

* Pilot scale measurements

control also gives rise to the problem of ethanol and acetic acid loss due to evaporation.

In the tower fermentations tried, the malt, molasses and cocoa sweatings vinegar production was found to have the higher productivities among the other substrates.

The optimization of the alcoholic fermentation medium for theproduction of vinegar charging wort would prove helpful. Also, the measurement of redox potential during the fermentation could be beneficial in the production control. The use of air enriched with oxygen and measurement of the $K_L a$ at the top and bottom of the tower which could reduce the cost and increase the productivity are recommended for future work.

LIST OF ABBREVIATIONS AND SYMBOLS

a, b,	Experimental parameters
c	Conversion
CFCC	Continuous Fermentation Culture Collection
D	Dilution rate
g	gram
h	hour
K	Specific product formation rate
K_La	Oxygen transfer coefficient
l	litre
m	maintenance energy coefficient
max	maximum
mm	millimetre
μ	Specific growth rate
N	Normality
P	Product concentration
q_s	Specific substrate utilization rate
r	regression coefficient
S	substrate concentration
S.G.V.	Superficial gas velocity
t	time
v/v/m	volume per volume per minute
X	biomass dry weight concentration
X_E	Biomass dry weight concentration in the effluent stream
X_F	Biomass dry weight concentration in the fermenter
Y_G	True growth yield coefficient
i.d.	internal diameter
Z	Amount of vinegar produced

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APPENDICES

Table A.1.1. Batch fermentation data. Temperature experiment

Cocoa sweatings vinegar

Aeration rate 0.2 v/v/m (s.g.v. 0.10 $cm.sec^{-1}$)

Time	23°C			28°C			31°C			35°C		
	x	p	s	x	p	s	x	p	s	x	p	s
h	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$	$g.l^{-1}$
0	0.16	19.0	38.4	0.16	19.0	38.4	0.16	19.0	38.4	0.16	19.0	38.4
8	0.16	19.0	38.4	0.18	19.5	38.2	0.20	20.5	36.4	0.23	22.0	35.3
16	0.18	19.5	38.0	0.19	20.0	37.6	0.25	23.0	34.5	0.27	24.5	32.6
24	0.22	22.0	36.1	0.28	23.5	35.1	0.38	28.5	30.3	0.46	31.5	26.5
32	0.31	25.0	33.8	0.42	26.5	32.8	0.50	33.5	26.5	0.57	36.0	22.2
40	0.38	27.5	31.9	0.59	32.0	28.6	0.76	41.0	20.7	0.95	45.5	14.2
48	0.56	32.5	28.0	0.77	35.0	26.3	1.05	49.5	14.2	1.21	55.0	8.1
56	0.73	37.0	24.6	0.99	42.5	20.6	1.27	57.5	7.5	1.39	58.5	3.5
64	0.92	44.0	19.0	1.15	50.0	14.7	1.42	64.0	2.3	1.45	61.0	1.5
72	1.13	51.0	13.9	1.30	58.0	8.6	1.46	65.0	1.6	1.48	61.5	0.4
80	1.24	58.5	8.1	1.37	69.0	5.5	1.48	66.0	0.8	1.48	62.0	0.0
88	1.36	64.0	3.9	1.46	66.5	2.1	1.48	66.5	0.0	1.48	62.0	0.0
96	1.43	67.0	1.6	1.48	69.2	0.0	1.48	66.5	0.0	1.48	62.0	0.0
106	1.48	69.0	0	1.48	69.2	0.0	1.48	66.5	0.00	1.48	62.0	0.0

Table A.1.2. <u>Temperature experiment. Calculated results of Figures</u> 6.1.1., 6.1.3.

<u>Cocoa sweatings</u>

	23°C				28°C				31°C				35°C			
Time	$\frac{dN}{dt}$ $g.l^{-1}.h^{-1}$ 10^{-3}	$\frac{dP}{dt}$ $g.l^{-1}.h^{-1}$	μ h^{-1}	K h^{-1}	$\frac{dN}{dt}$ $g.l^{-1}.h^{-1}$ 10^{-3}	$\frac{dP}{dt}$ $g.l^{-1}.h^{-1}$	μ h^{-1}	K h^{-1}	$\frac{dN}{dt}$ $g.l^{-1}.h^{-1}$ 10^{-3}	$\frac{dP}{dt}$ $g.l^{-1}.h^{-1}$	μ h^{-1}	K h^{-1}	$\frac{dN}{dt}$ $g.l^{-1}.h^{-1}$ 10^{-3}	$\frac{dP}{dt}$ $g.l^{-1}.h^{-1}$	μ h^{-1}	K h^{-1}
0	0	0	0	0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0
10	1	0.08	0.006	0.5	3	0.14	0.018	0.82	7	0.34	0.034	1.6	9	0.45	0.039	2.0
20	5	0.27	0.28	1.5	10	0.27	0.044	1.17	12	0.50	0.040	1.7	17	0.56	0.049	1.6
30	10	0.36	0.037	1.4	17	0.50	0.046	1.35	20	0.71	0.053	1.2	26	0.80	0.046	1.4
40	15	0.47	0.042	1.2	24	0.62	0.040	1.05	35	1.00	0.047	1.4	44	1.20	0.048	1.3
50	22	0.57	0.037	1.0	26	0.78	0.031	0.94	35	1.06	0.031	0.9	23	0.78	0.018	0.6
60	25	0.80	0.030	1.0	22	0.92	0.020	0.87	20	0.67	0.015	0.5	9	0.21	0.006	0.2
70	22	1.00	0.021	0.9	17	0.89	0.014	0.71	6	0.32	0.004	0.2	3	0.08	0.002	0.1
80	15	0.73	0.012	0.6	11	0.60	0.008	0.43	0	0.00	0.000	0.0	0	0.04	0.00	0.0
90	10	0.48	0.007	0.4	0	0.34	0.008	0.24	0	0.00	0.000	0.0	0	0.00	0.00	0.0
100	6	0.25	0.004	0.2	0	0.00	0.008	0.00	0	0.00	0.000	0.0	0	0.00	0.00	0.0
110	0	0.00	0.000	0.0	0	0.00	0.008	0.00	0	0.00	0.00	0.0	0	0.00	0.000	0.0

Table A.1.3 Cocoa vinegar continuous tower fermentation.
Steady state data. Temperature experiment.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	No of Days
0.005	49.7	0.8	98.0	3.70	0.004	1.43	1.15	0.17	0.3	0.26	3
0.013	48.2	1.9	95.1	3.70	0.012	1.40	1.29	0.37	0.6	0.47	3
0.023	40.6	7.8	80.1	3.70	0.021	1.35	1.23	0.58	0.9	0.73	3
0.038	25.5	19.4	50.3	3.70	0.038	1.21	1.21	0.61	1.0	0.83	2
0.040	20.4	23.3	40.2	3.75	0.040	1.20	1.20	0.52	0.8	0.67	2

Temperature 23°C

Aeration rate 0.2 v/v/m

Fermenter active volume 0.95 l

Total acid 50.7 $g.l^{-1}$

Residual acid 18.5 $g.l^{-1}$

Table A.1.4 Cocoa vinegar continuous tower fermentation.

Steady state data. Temperature experiment.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	No of Days
0.012	49.4	0.5	98.8	3.70	0.010	1.45	1.21	0.38	0.6	0.49	3
0.020	48.0	0.8	98.0	3.70	0.018	1.44	1.30	0.57	1.0	0.82	3
0.025	46.5	2.7	93.0	3.70	0.023	1.41	1.30	0.69	1.2	1.03	3
0.040	38.5	8.8	77.2	3.70	0.038	1.34	1.27	0.93	1.5	1.39	2
0.053	30.8	14.7	61.7	3.75	0.053	1.24	1.24	1.01	1.6	1.52	2
0.060	23.3	21.2	45.8	3.80	0.060	1.17	1.17	0.92	1.4	1.37	2

Temperature $31^{o}C$

Aeration rate 0.2 v/v/m

Fermenter active volume 0.95 l

Substrate evaporation rate 0.07 $g.l^{-1}h^{-1}$

Total acid 50.7 $g.l^{-1}h^{-1}$

Residual acid 18.5 $g.l^{-1}$

Table A.1.5 Cocoa vinegar continuous tower fermentation.

Steady state data. Temperature experiment.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	No of Days
0.012	47.0	0.5	98.5	3.70	0.011	1.46	1.34	0.32	0.6	0.45	3
0.020	46.8	0.7	98.1	3.70	0.018	1.45	1.31	0.55	0.9	0.78	3
0.030	44.8	2.2	93.9	3.70	0.028	1.41	1.32	0.78	1.3	1.08	3
0.046	36.8	8.4	77.9	3.70	0.044	1.34	1.28	1.01	1.7	1.52	2
0.055	30.1	13.5	63.1	3.70	0.055	1.27	1.27	1.00	1.7	1.60	2
0.065	18.7	22.3	39.2	3.85	0.065	1.18	1.18	0.79	1.2	1.09	2

Temperature $35^{o}C$

Aeration rate 0.2 v/v/m

Substrate evaporation rate $0.3 g.l^{-1}.h^{-1}$

Fermenter active volume 0.95 l

Total acid 50.7 $g.l^{-1}$

Residual acid 18.5 $g.l^{-1}$

Table A.1.6 Cocoa sweatings vinegar
Batch fermentation data
Aeration rate experiment

	0.2 v/v/m		0,5 v/v/m		0.7 v/v/m	
Time (h)	$\bar{X}$ $g.l^{-1}$	$\bar{P}$ $g.l^{-1}$	$\bar{X}$ $g.l^{-1}$	$\bar{P}$ $g.l^{-1}$	$\bar{X}$ $g.l^{-1}$	$\bar{P}$ $g.l^{-1}$
0	0.16	19.0	0.16	19.0	0.16	19.0
8	0.18	19.5	0.17	20.0	0.18	20.0
16	0.19	20.0	0.21	21.0	0.22	22.0
24	0.28	23.5	0.32	24.0	0.43	26.5
32	0.42	26.5	0.49	28.5	0.66	31.0
40	0.59	32.0	0.65	37.0	0.94	37.5
48	0.77	35.0	0.90	42.0	1.14	48.5
56	0.99	42.5	1.04	49.5	1.30	54.5
64	1.15	50.0	1.22	56.5	1.40	62.5
72	1.30	58.0	1.34	62.5	1.43	65.5
80	1.37	62.0	1.40	65.5	1.44	68.0
88	1.46	66.5	1.45	69.2	1.44	69.2
96	1.48	69.2	-	-	-	-

Temperature 28°C

Table A.1.7. Cocoa sweatings vinegar.

Calculated results from batch fermentation data.

Aeration rate experiment. Temperature 28°C

	0.2 v/v/m				0.5 v/v/m				0.7 v/v/m			
Time (h)	dx/dt $g.l^{-1}.h^{-1}$	dp/dt $g.l^{-1}.h^{-1}$	μ (h^{-1})	K (h^{-1})	dx/dt $g.l^{-1}.h^{-1}$	dp/dt $g.l^{-1}.h^{-1}$	μ (h^{-1})	K (h^{-1})	dX/dt $g.l^{-1}.h^{-1}$	dp/dt $g.l^{-1}.h^{-1}$	μ (h^{-1})	K (h^{-1})
0	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00
10	0.003	0.14	0.018	0.82	0.006	0.14	0.035	0.82	0.007	0.18	0.040	1.06
20	0.010	0.27	0.044	1.17	0.013	0.37	0.050	1.42	0.020	0.54	0.065	1.74
30	0.017	0.50	0.046	1.35	0.020	0.80	0.047	1.86	0.035	0.77	0.060	1.32
40	0.024	0.62	0.040	1.05	0.026	0.89	0.039	1.33	0.034	1.00	0.036	1.06
50	0.026	0.78	0.031	0.94	0.024	0.89	0.026	0.96	0.019	1.00	0.016	0.84
60	0.022	0.92	0.020	0.87	0.020	0.87	0.018	0.76	0.011	0.78	0.008	0.58
70	0.017	0.89	0.014	0.71	0.014	0.71	0.011	0.54	0.005	0.50	0.004	0.35
80	0.011	0.60	0.008	0.43	0.006	0.43	0.004	0.30	0.000	0.23	0.000	0.16
90	0.000	0.34	0.000	0.24	0.000	0.12	0.000	0.08	0.000	0.00	0.000	0.00
100	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00

Table A.1.8. Cocoa sweatings vinegar.

Determination of a, b from the equation $K = a\mu+b$

Regression analysis of K on μ (Bishop, 1966)

Aeration rate v/v/m	$\bar{x}$	Σx	Σx^2	$\bar{y}$	Σy	Σy^2	Σdy	Σdx^2	Σdy^2	$\Sigma dxdy^2$	n	r	p	a	b (h^{-1})
0.2	0.025	0.221	0.008	0.843	7.58	7.35	0.23	0.003	0.966	0.044	8	0.817	0.01	14.67	0.48
0.5	0.026	0.230	0.009	0.896	8.07	9.81	0.285	0.003	2.57	0.079	8	0.900	0.001	26.34	0.21
0.7	0.029	0.230	0.011	0.889	7.11	8.21	0.004	0.004	1.89	0.087	7	0.995	0.001	21.65	0.26

K = x

μ = y

Temperature 28°C

Table A.1.9. Cocoa vinegar continuous tower fermentation.
Steady state data. Aeration rate experiment.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}.h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	Number of Days
0.012	50.0	0.5	98.6	3.70	0.010	1.43	1.19	0.39	0.6	0.50	3
0.020	49.7	0.8	98.0	3.70	0.019	1.38	1.31	0.58	1.0	0.76	3
0.025	47.2	2.7	93.1	3.70	0.023	1.36	1.25	0.72	1.2	0.96	3
0.040	39.0	9.0	76.9	3.70	0.038	1.25	1.19	1.01	1.6	1.35	2
0.053	30.9	15.2	61.0	3.75	0.050	1.11	1.05	1.20	1.6	1.52	2
0.060	23.4	21.0	46.2	3.90	0.060	1.05	1.05	1.03	1.4	1.34	2

Temperature 28°C
Aeration rate 0.2 v/v/m
Fermenter active volume 0.95 l
Total acid 50.7 $g.l.^{-1}$
Residual acid 18.5 $g.l^{-1}$

Table A.1.10. Cocoa vinegar continuous tower fermentation.
Steady-state data. Aeration rate experiment.

D (h^{-1})	P ($g.l^{-1}$)	S ($g.l^{-1}$)	C (%)	pH	μ (h^{-1})	$\bar{X}_F$ ($g.l^{-1}$)	$\bar{X}_E$ ($g.l^{-1}$)	q_s (h^{-1})	DP ($g.l^{-1}.h^{-1}$)	$\frac{DP}{\bar{X}_E}$ (h^{-1})	Number of Days
0.016	49.7	0.8	97.4	3.70	0.015	1.44	1.35	0.45	0.8	0.59	3
0.028	49.1	1.2	96.8	3.70	0.025	1.38	1.23	0.86	1.4	1.14	3
0.035	48.2	1.9	95.1	3.70	0.034	1.33	1.29	1.00	1.7	1.32	3
0.045	43.2	5.8	85.2	3.70	0.044	1.23	1.20	1.24	1.9	1.58	2
0.053	40.6	7.8	80.1	3.70	0.050	1.18	1.11	1.49	2.2	1.98	2
0.062	33.0	13.6	65.1	3.75	0.062	1.09	1.09	1.44	2.1	1.98	2
0.069	20.4	23.3	40.2	3.80	0.069	1.06	1.06	1.02	1.4	1.32	2

Temperature 28°C
Aeration rate 0.5 v/v/m
Fermenter active volume 0.92 l
Total acid 50.7.$g.l^{-1}$
Residual acid 18.5 $g.l^{-1}$

Table A.1.11. Cocoa vinegar continuous tower fermentation.
Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}.h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	No. of days
0.010	50.0	0.5	98.6	3.70	0.008	1.48	1.18	0.33	0.5	0.42	3
0.025	49.7	0.8	98.0	3.70	0.024	1.41	1.35	0.71	1.2	0.89	3
0.036	49.3	1.1	97.2	3.70	0.035	1.35	1.31	1.04	1.8	1.37	3
0.050	48.2	1.9	95.1	3.70	0.046	1.25	1.15	1.61	2.4	2.09	2
0.067	40.6	7.8	80.1	3.75	0.067	1.14	1.14	1.83	2.7	2.37	2
0.074	33.0	13.6	65.1	3.80	0.074	1.10	1.10	1.70	2.4	2.18	2

Temperature 28°C
Aeration rate 0.7 v/v/m
Fermenter active volume 0.90 l
Total acid 50.7 $g.l^{-1}$
Residual acid 18.5 $g.l^{-1}$

Table A.2.1. Molasses vinegar continuous tower fermentation
Steady-state data. Concentration experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}.h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	number of days
0.001	74.1	8.1	87.6	3.45	0.0009	0.91	0.82	0.07	0.07	0.09	4
0.002	60.0	18.9	70.9	3.45	0.0010	0.85	0.43	0.21	0.12	0.28	4
0.003	30.5	41.6	36.1	3.60	0.0020	0.83	0.55	0.13	0.09	0.16	4
0.005	16.9	52.1	20.0	3.75	0.0030	0.60	0.36	0.18	0.08	0.23	4

Temperature 28°C

Aeration rate 0.4 v/v/m

Total acid 84.6 $g.l^{-1}$

Fermenter active volume 3 l

Table A.2.2. Molasses vinegar continuous tower fermentation
Steady-state data. Concentration experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.003	66.5	1.4	97.4	3.45	0.002	1.37	0.92	0.17	0.2	0.22	3
0.012	51.7	12.8	75.6	3.45	0.010	1.17	0.61	0.78	0.6	0.61	3
0.017	35.5	25.2	52.0	3.50	0.014	0.81	0.67	0.69	0.6	0.90	3
0.020	22.3	35.4	32.7	3.65	0.018	0.53	1.48	0.71	0.5	1.04	3

Temperature 28°C

Aeration rate 0.4 v/v/m

Total acid 68.3 $g.l^{-1}$

Fermenter active volume 3 l

Table A.2.3. Molasses vinegar continuous tower fermentation
Steady-state data. Concentration experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.005	56.8	1.1	97.6	3.45	0.003	1.32	0.79	0.28	0.3	0.38	3
0.018	55.3	2.2	95.0	3.45	0.016	1.20	1.07	0.71	1.0	0.94	3
0.029	48.3	7.6	83.2	3.50	0.027	0.94	0.88	1.22	1.4	1.59	3
0.035	34.9	17.9	60.1	3.55	0.033	0.78	0.74	1.27	1.2	1.62	2
0.040	17.4	37.4	30.0	3.85	0.039	0.68	0.66	0.81	0.7	1.06	2

Temperature 28°C

Aeration rate 0.4 v/v/m

Total acid 58.2 $g.l^{-1}$

Fermenter active volume 3 l

Table A.2.4. Molasses vinegar continuous tower fermentation

Steady-state data. Concentration experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}.h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.016	53.5	0.6	98.5	3.45	0.015	1.14	1.07	0.61	0.9	0.84	3
0.022	53.0	1.0	97.6	3.45	0.020	1.11	1.01	0.89	1.2	1.19	3
0.035	49.7	3.5	91.5	3.50	0.034	0.94	0.91	1.47	1.7	1.87	3
0.041	46.8	5.8	86.2	3.50	0.039	0.83	0.79	1.86	1.9	2.41	2
0.052	36.5	13.7	67.2	3.67	0.052	0.70	0.70	2.08	1.9	2.71	2
0.058	11.4	32.9	21.0	3.95	0.058	0.30	0.30	1.69	0.6	2.0	2

Temperature 28°C

Aeration rate 0.4 v/v/m

Total acid 54.3 $g.l^{-1}$

Fermenter active volume 3 l

Table A.2.5. Molasses vinegar. Batch fermentation data
Temperature experiment.

	28°C		30°C		33°C	
t h	X $g.l^{-1}$	P $g.l^{-1}$	X $g.l^{-1}$	P $g.l^{-1}$	X $g.l^{-1}$	P $g.l^{-1}$
0	0.22	7.0	0.22	7.0	0.22	7.0
10	0.23	13.0	0.22	8.0	0.26	11.0
20	0.26	21.0	0.32	18.0	0.45	19.0
30	0.47	34.5	0.50	33.0	0.70	34.5
40	0.73	44.5	0.87	42.5	0.99	45.0
50	0.97	49.0	1.03	50.0	1.12	51.0
60	1.11	52.5	1.17	54.0	1.13	51.1
70	1.17	53.5	1.18	54.3	-	-
80	1.17	54.0	-	-	-	-

Aeration rate 0.4 v/v/m

Table A.2.6. Molasses vinegar. Calculated results from batch fermentation (figures 6.2.9, 6.2.10) Temperature experiment.

	28° C				30°C				33° C			
t h	$\frac{dx}{dt}$ $g.l^{-1}.h^{-1}$	$\frac{dp}{dt}$ $g.l^{-1}h^{-1}$	μ h^{-1}	K h^{-1}	$\frac{dx}{dt}$ $g.l^{-1}.h^{-1}$	$\frac{dp}{dt}$ $h.l^{-1}h^{-1}$	μ h^{-1}	K h^{-1}	$\frac{dx}{dt}$ $g.l^{-1}.h^{-1}$	$\frac{dp}{dt}$ $g.l^{-1}.h^{-1}$	μ h^{-1}	K h^{-1}
0	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00	0.000	0.00
10	0.003	0.20	0.015	0.91	0.004	1.00	0.017	4.35	0.009	0.50	0.034	1.85
20	0.010	0.67	0.036	2.39	0.013	1.17	0.041	3.66	0.020	1.25	0.047	2.91
30	0.023	1.20	0.052	2.73	0.028	1.34	0.053	2.53	0.031	1.50	0.043	2.08
40	0.029	1.13	0.040	1.55	0.027	0.88	0.033	1.06	0.020	0.78	0.020	0.79
50	0.002	0.77	0.021	0.79	0.020	0.50	0.019	0.47	0.008	0.27	0.007	0.24
60	0.009	0.46	0.005	0.41	0.007	0.21	0.006	0.78	0.000	0.00	0.000	0.00
70	0.005	0.20	0.004	0.17	0.000	0.06	0.000	0.05	-	-	-	-
80	0.000	0.05	0.000	0.04	-	-	-	-	-	-	-	-

Table A.2.7. Molasses Vinegar batch fermentation

Regression analysis of K on μ

(Equation $K = a\mu + b$)

Temperature ^{o}C	$\bar{x}$	Σx	Σx^2	$\bar{y}$	Σy	Σy^2	Σxy	Σdx^2	Σdy^2	$\Sigma dxdy$	n	r	p	a	b
28	0.019	0.177	0.006	1.00	8.99	17.22	0.332	0.003	8.23	0.155	8	0.986	0.001	51.6	0.20
30	0.021	0.169	0.006	1.54	12.3	40.1	0.400	0.002	21.2	0.140	7	0.680	0.05	70.0	0.07
33	0.022	0.151	0.006	1.12	7.87	16.9	0.307	0.003	8.05	0.137	6	0.882	0.01	45.67	.12

Aeration rate 0.4 v/v/m

$x = \mu$ $K = y$

Table A.2.8. Molasses vinegar. Continuous tower fermentation
Steady-state data. Temperature experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.020	53.6	0.5	98.7	3.45	0.019	1.15	1.09	0.75	1.07	0.98	3
0.030	53.3	0.8	98.2	3.45	0.029	0.93	0.90	1.36	1.60	1.78	3
0.040	50.5	2.9	93.0	3.50	0.039	0.80	0.78	1.99	2.02	2.59	3
0.052	42.9	8.8	79.0	3.55	0.051	0.55	0.54	3.17	2.23	4.13	3
0.063	30.4	18.4	56.0	3.60	0.063	0.40	0.40	3.67	1.92	4.80	3
0.069	19.1	27.1	35.0	3.90	0.069	0.35	0.35	2.89	1.32	3.77	2

Temperature 30°C
Aeration rate 0.4 v/v/m
Total acid 54.3 $g.l^{-1}$
Fermenter active volume 3 l

Table A.2.9. Molasses vinegar. Continuous tower fermentation
Steady-state data. Temperature experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}.h^{-1}$	DP h^{-1}	Number of days
0.018	53.3	0.5	98.7	3.45	0.019	1.12	1.06	0.69	0.96	0.91	3
0.027	53.0	0.8	98.2	3.45	0.026	0.97	0.93	1.18	1.43	1.54	3
0.043	49.7	3.3	92.0	3.50	0.041	0.70	0.67	2.45	2.14	3.19	3
0.056	39.4	11.2	72.0	3.50	0.056	0.43	0.43	3.94	2.21	5.14	3
0.065	30.9	17.8	57.3	3.60	0.065	0.34	0.34	4.53	2.01	5.91	2
0.067	13.6	31.1	25.2	3.95	0.067	0.22	0.22	3.18	0.91	4.14	2

Temperature 33°C
Aeration rate 0.4 v/v/m
Total acid 54.3 $g.l^{-1}$
Fermenter active volume 3 l
Substrate evaporation 0.03 $g.l^{-1}.h^{-1}$

Table A.2.10. Molasses vinegar. Continuous tower fermentation.

Steady-state data. Aeration rate experiment.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.019	53.2	0.85	98.0	3.45	0.018	1.13	1.07	0.72	1.01	0.94	3
0.025	52.9	1.10	97.4	3.45	0.024	1.10	1.06	0.96	1.32	1.25	3
0.043	46.2	6.22	85.1	3.55	0.041	0.70	0.67	2.27	1.99	2.97	3
0.057	33.6	15.9	61.9	3.55	0.057	0.46	0.46	3.19	1.92	4.17	3
0.064	16.9	28.8	31.1	3.85	0.064	0.34	0.34	2.44	1.08	3.18	2

Temperature: 30°C

Aeration rate: 0.3 v/v/m

Total acid: 54.3 $g.l^{-1}$

Fermenter active volume: 3.02 l

Table A.2.11. Molasses vinegar. Continuous tower fermentation

Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.016	53.2	0.85	98.0	3.45	0.015	1.12	1.05	0.62	0.85	0.81	3
0.028	51.0	2.50	93.9	3.50	0.026	0.98	0.91	1.20	1.43	1.57	3
0.033	46.2	6.22	85.1	3.55	0.032	0.92	0.97	1.21	1.53	1.58	4
0.050	35.8	14.19	65.9	3.60	0.049	0.57	0.56	2.45	1.79	3.20	3
0.059	24.4	22.94	44.9	3.65	0.058	0.43	0.42	2.63	1.44	3.43	2
0.061	16.4	29.15	30.2	3.85	0.061	0.38	0.38	2.02	1.00	2.63	3

Temperature: $30^{o}C$

Aeration rate: O2 v/v/m

Total acid 54.3 $g.l^{-1}$

Fermenter active volume: 3.05 l

Table A.3.1. Malt vinegar batch fermentation results. Aeration rate 0.1 v/v/m Temperature 28°C

Time (h)	$\bar{x}$ $g.l^{-1}$	$\bar{p}$ $g.l^{-1}$	p^H
0	0.06	9.0	4.20
10	0.06	9.0	4.20
20	0.06	9.0	4.20
30	0.10	10.0	3.95
40	0.17	11.0	3.85
50	0.28	14.0	3.50
60	0.40	18.5	3.50
70	0.64	27.0	3.45
80	0.87	38.5	3.05
90	1.07	58.0	2.85
100	1.19	60.0	2.75
110	1.25	70.0	2.70

Table A.3.2. Calculated results from figures 6.3.1, 6.3.2.

Time (h)	dx/dt $g.l^{-1}.h^{-1}$	dp/dt $g.l^{-1}.h^{-1}$	μ h^{-1}	K h^{-1}
0	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
20	0.001	0	0.017	0.00
30	0.006	0.13	0.080	1.30
40	0.008	0.24	0.047	1.41
50	0.012	0.34	0.043	1.21
60	0.018	0.52	0.045	1.30
70	0.024	1.00	0.038	1.56
80	0.022	1.50	0.025	1.72
90	0.016	1.29	0.015	1.21
100	0.009	0.86	0.008	0.72
110	0	0.34	0	0.27

Table A.3.3. Malt vinegar batch fermentation results. Aeration rate 0.2 v/v/m Temperature 28°C

Time (h)	$\bar{x}$ ($g.l^{-1}$)	$\bar{p}$ ($g.l^{-1}$)	pH
0	0.06	9.0	4.20
10	0.06	9.5	4.20
20	0.08	10.5	4.15
30	0.12	12.5	3.85
40	0.19	16.0	3.75
50	0.33	22.0	3.50
60	0.53	32.0	3.40
70	0.78	58.5	2.95
80	1.03	60.5	2.75
90	1.19	68.5	2.70
100	1.24	71.0	2.70

Table A.3.4. Continued. Calculated results from Figures 6.3.1, 6.3.2.

Time (h)	dx/dt ($g.l^{-1}.h^{-1}$)	dp/dt ($g.l^{-1}.h^{-1}$)	μ (h^{-1})	K (h^{-1})
0	0.000	0.00	0.000	0.00
10	0.000	0.00	0.000	0.00
20	0.003	0.14	0.038	1.75
30	0.007	0.28	0.058	2.34
40	0.010	0.44	0.053	2.32
50	0.015	0.75	0.046	2.27
60	0.024	1.34	0.045	2.53
70	0.028	1.43	0.036	1.84
80	0.015	1.00	0.015	0.97
90	0.008	0.47	0.007	0.40
100	0.000	0.12	0.000	0.10

Table A.3.5. Malt vinegar batch fermentation results. Aeration rate 0.3 v/v/m Temperature 28°C

Time (h)	$\bar{x}$ ($g.l^{-1}$)	$\bar{p}$ ($g.l^{-1}$)	pH
0	0.06	9.0	4.20
10	0.06	9.5	4.20
20	0.08	10.5	3.95
30	0.14	13.0	3.60
40	0.27	14.5	3.40
50	0.44	21.5	3.25
60	0.77	41.0	3.00
70	1.07	55.5	2.95
80	1.18	65.5	2.85
90	1.24	70.0	2.70
100	1.24	71.0	2.70

Table A.3.6. Continued. Calculated results from figures 6.3.1, 6.3.2.

Time (h)	dx/dt ($g.l^{-1}.h^{-1}$)	dp/dt ($g.l^{-1}.h^{-1}$)	μ (h^{-1})	K (h^{-1})
0	0.000	0.00	0.000	0.00
10	0.000	0.00	0.000	0.00
20	0.004	0.14	0.050	1.75
30	0.015	0.29	0.107	2.07
40	0.014	0.58	0.052	2.15
50	0.024	1.17	0.055	2.66
60	0.035	1.67	0.046	2.17
70	0.020	1.34	0.019	1.25
80	0.009	0.67	0.008	0.57
90	0.001	0.27	0.001	0.22
100	0.000	0.09	0.000	0.07

Table A.3.7. Malt vinegar batch fermnetation results. Aeration rate 0.4 v/v/m Temperature 28°C

Time (h)	$\bar{x}$ ($g.l^{-1}$)	$\bar{p}$ ($g.l^{-1}$)	pH
0	0.06	9.0	4.20
10	0.06	10.0	3.95
20	0.10	12.0	3.80
30	0.22	19.0	3.70
40	0.41	31.0	3.75
50	0.79	46.5	3.35
60	1.09	58.5	2.80
70	1.23	66.5	2.75
80	1.24	71.0	2.70

Table A.3.8. Calculated results from Fig. 6.3.1, 6.3.2.

Time (h)	dx/dt ($g.l^{-1}h^{-1}$)	dp/dt ($g.l^{-1}.h^{-1}$)	μ (h^{-1})	K (h^{-1})
0	0.000	0.00	0.000	0.00
10	0.002	0.13	0.034	2.08
20	0.007	0.37	0.070	3.68
30	0.015	1.00	0.068	4.55
40	0.028	1.43	0.068	3.49
50	0.040	1.17	0.051	1.48
60	0.020	0.83	0.018	0.77
70	0.008	0.40	0.007	0.33
80	0.000	0.18	0.000	0.15

Table A.3.9. Malt vinegar batch fermentation results.

Aeration rate 0.5 v/v/m. Temperature 28°C

Time (h)	$\bar{x}$ ($g.l^{-1}$)	$\bar{p}$ ($g.l^{-1}$)	pH
0	0.06	9.0	4.20
10	0.11	10.5	3.95
20	0.21	17.0	3.80
30	0.41	19.0	3.55
40	0.82	45.0	3.20
50	1.10	58.5	2.90
60	1.24	67.5	2.85
70	1.25	71.0	2.70

Table A.3.10 Calculated results from Fig. 6.3.1, 6.3.2.

Time (h)	dx/dt ($g.l^{-1}.h^{-1}$)	dp/dt ($g.l^{-1}.h^{1-}$)	μ (h^{-1})	K (μ^{-1})
0	6.000	0.00	0.000	0.00
10	0.007	0.30	0.064	2.73
20	0.015	1.00	0.071	4.76
30	0.034	1.58	0.083	3.85
40	0.040	1.50	0.049	1.83
50	0.020	1.13	0.018	1.03
60	0.002	0.67	0.002	0.54
70	0.000	0.15	0.000	0.12

Table A.3.11. Malt vinegar batch fermentation

Regression analysis of K on

(Equation $K = \alpha\mu + b$) $x = \mu$, $y = k$

Aeration rate v/v/m	$\bar{x}$	Σx	Σx^2	$\bar{y}$	Σv	Σv^2	Σxv	Σdx^2	Σdy^2	$\Sigma dxdy$	n	r	p	a	b (h^{-1})
0.1	0.032	0.318	0.015	1.07	10.7	14.28	0.407	0.005	2.83	0.067	10	0.561	0.1	13.34	0.64
0.2	0.033	0.298	0.013	1.61	14.52	29.97	0.627	0.003	6.55	0.146	9	1.000	<0.001	48.67	0.004
0.3	0.036	0.328	0.022	1.44	12.91	25.70	0.695	0.010	7.18	0.225	9	0.840	0.001	22.5	0.63
0.4	0.041	0.287	0.19	2.12	14.86	49.65	0.942	0.007	18.10	0.333	7	0.936	0.001	47.57	0.17
0.5	0.035	0.248	0.014	1.71	11.98	32.96	0.657	0.005	12.46	0.235	7	0.942	0.001	47.00	0.065

Table A.3.12. Malt vinegar continuous tower fermentation. Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	Number of days
0.01	61.7	1.0	97.9	3.05	0.009	1.29	1.76	0.27	0.6	0.5	4
0.019	53.6	7.2	85.1	3.05	0.018	1.24	1.18	0.66	1.0	0.9	3
0.031	44.1	14.5	70.0	3.10	0.030	1.09	1.06	0.99	1.4	1.3	3
0.037	28.5	26.5	45.2	3.10	0.036	1.03	1.00	0.81	1.1	1.1	3
0.044	13.3	38.2	21.2	2.15	0.043	0.95	0.93	0.48	0.6	0.7	3

Temperature 28°C

Aeration rate 0.1 v/v/m

Fermenter active volume 3.0 l

Table A.3.13. Malt vinegar continuous tower fermentation
Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_E$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	Number of days
0.014	62.2	0.6	98.7	3.05	0.013	1.29	1.20	0.56	0.9	0.8	4
0.024	59.9	2.4	95.1	3.05	0.022	1.27	1.16	0.95	1.4	1.2	3
0.036	51.0	9.2	81.0	3.05	0.035	1.20	1.17	1.20	1.8	1.5	3
0.045	44.1	14.5	70.0	3.10	0.044	1.13	1.11	1.37	2.0	1.8	4
0.055	28.5	26.5	45.2	3.10	0.055	0.98	0.98	1.23	1.6	1.6	3
0.063	12.7	38.7	20.2	3.15	0.063	0.90	0.90	0.68	0.8	0.9	3

Temperature 28°C

Aeration rate 0.2 v/v/m

Fermenter active volume 3.05 l

Table A.3.14. Malt vinegar continuous tower fermentation

Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	$\bar{X}_F$ $g.l^{-1}$	$\bar{X}_F$ $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{\bar{X}_E}$ h^{-1}	Number of days
0.030	62.3	0.5	98.9	3.05	0.029	1.28	1.24	1.16	1.9	1.5	3
0.039	59.9	2.4	95.1	3.05	0.038	1.26	1.23	1.46	2.3	1.9	3
0.049	58.1	3.8	92.3	3.05	0.048	1.14	1.12	1.95	2.9	2.6	4
0.060	52.3	8.2	83.0	3.10	0.060	0.98	0.98	2.46	3.1	3.2	3
0.070	41.7	16.4	66.2	3.15	0.070	0.87	0.87	2.57	2.9	3.4	3
0.086	19.1	33.8	30.3	3.20	0.086	0.71	0.71	1.78	1.6	2.3	3

Temperature 28^{o}C

Aeration rate 0.3 v/v/m

Fermenter active volume 3.0 l

Table A.3.15. Malt vinegar continuous tower fermentation
Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.060	61.7	1.0	97.9	3.00	0.060	1.27	1.27	2.24	3.7	2.9	4
0.071	58.6	3.4	93.0	3.05	0.071	1.00	1.00	3.19	4.2	4.2	3
0.090	44.8	14.0	71.2	3.05	0.090	0.71	0.71	4.36	4.0	5.6	3
0.097	37.9	19.3	60.2	3.05	0.097	0.65	0.65	4.34	3.7	5.7	4
0.103	21.5	31.9	34.1	3.10	0.103	0.61	0.61	2.79	2.2	3.6	3
0.107	15.9	36.2	25.2	3.15	0.107	0.58	0.58	2.25	1.7	2.9	4

Temperature 28°C

Aeration rate 0.4 v/v/m

Fermenter active volume 2.95 l

Table A.3.16. Malt vinegar continuous tower fermentation
Steady-state data. Aeration rate experiment

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.045	62.4	0.5	99.0	3.05	0.044	1.29	1.26	1.71	2.8	2.3	4
0.055	62.4	0.5	99.0	3.05	0.055	1.27	1.27	2.07	3.4	2.7	4
0.065	61.7	1.0	97.9	3.05	0.065	1.10	1.10	2.80	4.0	3.6	4
0.078	59.2	2.9	94.0	3.10	0.078	0.91	0.91	3.89	4.6	4.4	3
0.090	50.4	9.7	80.0	3.10	0.090	0.75	0.75	4.64	4.5	6.0	4
0.101	37.8	19.3	60.0	3.10	0.101	0.69	0.69	4.24	3.8	5.5	3
0.105	27.2	27.5	43.2	3.15	0.105	0.66	0.66	3.32	2.9	4.4	4
0.110	19.1	33.8	30.3	3.20	0.110	0.63	0.63	2.56	2.1	3.4	3

Temperature 28°C.

Aeration rate 0.5 v/v/m

Fermenter active volume 2.90 l

Table A.4.1. Spirit vinegar continuous tower fermentation

Steady-state data.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.018	55.0	1.76	96.0	2.70	0.017	1.10	1.04	0.73	0.99	0.95	3
0.027	49.7	5.90	86.8	2.75	0.026	1.06	1.02	1.01	1.34	1.31	3
0.036	39.6	13.58	69.1	2.80	0.034	0.99	0.94	1.16	1.43	1.52	3
0.044	22.4	26.81	39.1	2.90	0.043	0.94	0.92	0.82	0.99	1.08	3

Temperature 35°C

Aeration rate 0.5 v/v/m

Fermenter active volume 2.95 l

Total acid 55.8 $g.l^{-1}$

Substrate evaporation rate 1.2 $g.l^{-1}.h^{-1}$

Table A.4.2. Spirit vinegar continuous tower fermentation

Steady-state data

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.017	56.2	1.8	96.1	2.70	0.016	1.11	1.05	0.70	0.96	0.91	3
0.020	55.0	2.7	94.0	2.76	0.019	1.09	1.04	0.81	1.10	1.06	3
0.031	46.3	9.4	79.2	2.75	0.030	0.92	0.89	1.24	1.44	1.62	3
0.040	26.4	24.7	45.1	2.85	0.039	0.71	0.69	1.17	1.06	1.54	3

Temperature 30°C

Aeration rate 0.5 v/v/m

Fermenter active volume 2.95 l

Total acid 58.5 $g.l^{-1}$

Table A.4.3. Spirit vinegar continuous tower fermentation
Steady-state data

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	p^H	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}.h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.007	65.3	5.5	90.1	2.70	0.006	1.05	0.90	0.39	0.46	0.52	3
0.016	51.6	16.1	71.2	2.75	0.014	1.00	0.88	0.72	0.83	0.94	3
0.021	35.6	28.4	49.1	2.80	0.020	0.92	0.88	0.65	0.75	0.85	3
0.024	21.8	39.0	30.1	2.85	0.023	0.85	0.82	0.49	0.52	0.63	3

Temperature 30°C

Aeration rate 0.5 v/v/m

Fermenter active volume 2.95 l

Total acid 72.5 $g.l^{-1}$

Table A.5.1. Wine vinegar continuous tower fermentation. Steady-state data

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}g^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.0012	78.2	6.7	90.0	2.40	0.0011	1.38	1.27	0.06	0.09	0.07	3
0.0020	65.2	16.6	75.1	2.45	0.0019	1.30	1.24	0.08	0.13	0.11	4
0.0034	52.1	26.7	60.0	2.90	0.0033	1.16	1.13	0.12	0.18	0.16	4
0.0035	26.3	46.6	30.3	3.00	0.0034	1.16	1.13	0.06	0.09	0.08	4

Temperature 32°C Aeration rate 0.7 v/v/m

Substrate evaporation rate 0.21 $g.l^{-1}.h^{-1}$

Total acid 89.0 $g.l^{-1}$

Fermenter active volume 3 l

Table A.5.2. Wine vinegar continuous tower fermentation. Steady state data.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F g.l-1	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.0020	68.9	9.3	85.1	2.55	0.0019	1.35	1.28	0.08	0.14	0.11	3
0.0030	64.8	12.4	80.2	2.60	0.0029	1.28	1.24	0.12	0.19	0.15	3
0.0040	56.7	18.6	70.1	2.90	0.0038	1.19	1.13	0.15	0.23	0.20	4
0.0047	32.4	37.3	40.1	3.0	0.0046	1.15	1.13	0.10	0.15	0.13	3

Temperature 34°C

Aeration rate 0.7 v/v/m

Substrate evaporation rate 0.81 $g.l^{-1}$ (in terms of acetic acid)

Total acid 89.0 $g.l^{-1}$

Fermenter active volume 3 l

Table A.5.3. Wine vinegar continuous tower fermentation. Steady-state data.

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.0020	64.4	0.5	98.9	2.55	0.0019	1.46	1.39	0.07	0.13	0.09	4
0.0050	62.4	2.0	96.0	2.55	0.0048	1.38	1.33	0.18	0.31	0.23	4
0.0080	61.7	2.6	94.8	2.65	0.0079	1.24	1.23	0.31	0.49	0.40	4
0.0120	54.0	8.4	83.2	2.80	0.0110	1.10	1.01	0.49	0.65	0.64	3
0.0157	45.5	15.0	70.0	3.15	0.0150	0.98	0.94	0.58	0.71	0.76	4
0.0190	29.3	27.4	45.1	3.65	0.0180	0.92	0.87	0.49	0.56	0.64	3

Temperature 32°C Aeration rate 0.7 v/v/m

Total acid evaporation rate 0.21g.$l^{-1}h^{-1}$

Fermenter active volume 31

Table A.5.4. Wine vinegar continuous tower fermentation. Steady-state data

D h^{-1}	P $g.l^{-1}$	S $g.l^{-1}$	C %	pH	μ h^{-1}	X_F $g.l^{-1}$	X_E $g.l^{-1}$	q_s h^{-1}	DP $g.l^{-1}h^{-1}$	$\frac{DP}{X_E}$ h^{-1}	Number of days
0.002	58.4	0.5	99.0	2.55	0.0019	1.50	1.43	0.06	0.12	0.08	4
0.004	57.7	0.9	97.8	2.55	0.0038	1.42	1.35	0.13	0.23	0.17	3
0.008	58.4	0.5	99.0	2.60	0.0079	1.30	1.28	0.28	0.47	0.37	3
0.012	57.7	0.9	97.8	2.75	0.0110	1.16	1.06	0.50	0.69	0.65	4
0.014	53.1	4.5	90.0	2.90	0.0130	1.10	1.02	0.56	0.74	0.73	3
0.021	47.2	9.1	80.0	3.10	0.0200	1.02	0.97	0.78	0.99	1.02	3
0.022	34.8	18.6	59.0	3.20	0.0210	1.00	0.96	0.61	0.77	0.80	3

Temperature 34°C

Aeration rate 0.7 v/v/m

Substrate evaporation rate 0.81 $g.l^{-1}h^{-1}$

Total acid 67.1 $g.l^{-1}$

Fermenter active volume 3 l

Table A.6.1. Cider vinegar semicontinuous fermentation in 300 l Tower fermenter

Z = Amount of vinegar produced (% by volume)

Time (h)	P $g.l^{-1}$	Z (%)	Time (h)	P $g.l^{-1}$	Z (%)	Time (h)	P $g.l^{-1}$	Z (%)
0	34.0		107	30.0		229	50.0	45
2	33.0		111	34.0		230	31.0	
4	33.0		113	36.0		234	32.0	
5	34.0		118	39.0		241	38.0	
7	25.0	33	128	48.0		247	42.0	
9	26.0		130	50.0	50	254	49.5	45
14	29.0		133	30.0		256	31.0	
19	32.5		137	32.0		261	35.0	
22	36.5		142	34.0		265	38.0	
26	39.0		153	41.5		267	40.0	
29	38.0		157	44.0		278	50.0	50
31	36.0		159	45.0	45	280	31.5	
32	25.0	33	160	30.5		283	32.0	
35	27.0		165	33.0		289	37.0	
37	27.0		168	34.5		304	51.0	50
44	30.0		176	42.0		310	32.0	
52	38.0		179	46.0		313	34.0	
55	40.5	40	181	48.0	45	329	47.0	50
57	28.0		183	32.0		330	28.5	
61	32.0		185	32.5		339	33.0	
68	35.0		186	32.5		343	35.0	
76	42.5		191	37.0		346	37.0	
80	45.0	40	193	38.0		359	51.0	50
82	30.0		203	47.0	45	361	28.0	
87	34.0		204	30.0		366	31.0	
92	37.0		207	31.5		369	32.5	
101	45.0		214	35.5		370	33.0	
104	46.0	40	216	38.0		384	47.0	50

Table A.6.1. Continued

Time (h)	P ($g.l^{-1}$)	Z (%)	Time (h)	P ($g.l^{-1}$)	Z (%)
385	29.0		564	31.0	
392	32.5		569	32.0	
395	35.0		572	35.0	
398	38.0		575	39.0	
412	48.0	50	587	51.0	50
414	29.0		588	29.0	
421	37.0		591	31.0	
423	38.5		593	32.0	
437	53.0	50	596	35.0	
438	35.0		610	49.0	50
458	47.0	50	611	29.0	
459	27.0		618	34.0	
465	30.0		633	48.0	50
482	48.0	50	634	29.5	
483	27.0		638	33.0	
488	30.0		644	48.0	
491	32.5		658	62.0	
495	36.0		Continuous operation		
506	48.0	50			
508	30.0				
512	32.0				
516	34.5				
520	38.0				
538	51.5	50			
539	30.0				
545	34.0				
563	52.5	50			

Table A.6.2. Cider vinegar continuous fermentation in 300 l tower fermenter

Time (h)	D h^{-1}	P $g.l^{-1}$
0	0.008	62.0
4	"	61.0
7	"	64.0
9	"	62.0
12	"	64.0
26	"	62.0
29	"	66.0
31	"	67.0
34	"	68.0
50	"	68.0
54	"	68.0
58	"	68.0
73	"	68.0
82	"	68.0
93	"	70.0
97	"	70.0
105	"	71.0
110	"	72.0
136	"	73.0
142	"	72.5
156	0.012	74.0
163	"	73.0
185	0.014	74.0

Table A.6.3. Cider vinegar semicontinuous fermentation in 1500 l Tower fermenter

Z = Amount of vinegar produced (% by volume)

Time (h)	P ($g.l^{-1}$)	Z (%)	Time (h)	P ($g.l^{-1}$)	Z (%)	Time (h)	P ($g.l^{-1}$)	Z (%)
0	38.0		103	45.0	45	213	32.0	
2	39.0		105	30.0		215	34.0	
4	40.0		108	32.5		232	45.0	45
6	41.0	33	111	35.0		234	29.0	
8	30.0		114	36.0		237	31.0	
10	30.0		125	45.0		244	36.0	
15	32.0		127	47.0	50	251	40.5	
20	35.0		129	29.0		257	47.0	45
23	37.0		134	30.0		259	30.0	
28	40.0		138	31.0		265	32.0	
31	42.0		148	39.0		268	33.5	
32	42.0		153	43.0		270	35.0	
33	42.0	33	155	44.0	45	281	44.0	56
35	31.0		157	30.0		283	29.0	
37	31.0		162	32.0		286	30.0	
45	36.0		166	34.0		292	34.0	
48	43.0		174	40.0		294	35.5	
56	45.0	45	178	43.0		307	45.0	45
58	30.0		180	45.0	45	309	29.0	
64	32.0		182	29.0		315	31.0	
69	35.0		184	30.0		318	32.5	
76	42.0		186	31.0		322	34.5	
80	45.0	45	191	33.5		328	44.0	45
82	30.0		193	35.0		330	29.0	
88	34.0		202	40.5	45	343	33.0	
92	36.0		204	28.0		345	34.5	
102	45.0		206	28.5		347	34.6	

Table A.6.3. Continued

Time (h)	P ($g.l^{-1}$)	Z (%)	Time (h)	P ($g.l^{-1}$)	Z (%)
358	45.0	45	523	36.0	
360	30.0		536	45.0	45
366	31.0		538	30.0	
368	31.5		543	32.0	
370	33.0		560	44.0	45
384	44.0	45	562	30.0	
386	29.0		565	31.0	
393	31.0		569	32.0	
395	33.0		571	34.0	
398	35.0		574	37.0	
412	45.0	45	585	45.0	43
414	29.0		590	30.0	
418	32.0		593	32.0	
421	35.0		595	33.0	
435	45.0	45	597	35.0	
438	30.0		609	45.0	43
459	44.0	45	613	30.0	
461	30.0		617	33.0	
464	32.0		619	35.0	
485	44.0	40	631	46.0	43
487	30.0		633	30.0	
492	33.0		636	31.0	
496	36.0		639	32.5	
499	38.0		643	38.0	
509	45.0	45	655	45.0	
511	30.0		Continuous operation		
515	31.0				
519	33.0				

Table A.6.4. Cider vinegar continuous fermentation in 1500 l fermenter

Time (h)	P ($g.l^{-1}$)	D (h^{-1})
0	45.0	0.008
7	46.5	"
21	52.0	"
27	53.0	"
30	54.0	"
44	56.0	"
49	56.5	"
54	57.0	"
70	59.0	"
79	58.0	"
90	60.0	"
94	60.0	"
123	60.0	"
145	60.0	"