3. Growth of P. javanicum on Semidefined Medium

3.1 Introduction

These experiments were designed to determine the optimum fermentation conditions for biomass production and lactose utilisation by P. javanicum in a CTF. Fermentation conditions at which the maximum rate of biomass production occurs, might not necessarily coincide with those permitting maximum lactose utilisation. Wendorff, Amundson and Olsen (1970), in their study of the lactase activity of S. fragilis grown on whey, concluded that optimum conditions for lactase production (and hence lactose utilisation) differed considerably from those optimum for growth of the yeast. Results obtained with P. javanicum cultivated on semi-defined media could be used to predict the behaviour of the mould when grown on dairy effluents, and thus would be of use in designing a commercial plant.

Dilution rate, temperature and pH value were considered to be the most crucial factors requiring investigation.

Dilution Rate (Exp. 3.4.1)

Since dairy effluents can vary considerably, both in quality (concentration) and quantity (that is, volume produced), it was necessary to determine the range of dilution rates at which the system could be operated and provide data about the lactose utilisation and biomass production rates which might be expected at each dilution rate. It should then be possible to select the most appropriate dilution rate for any particular milk effluent.

To this end, dilution rates ranging from $0.024h^{-1}$ to $9.2h^{-1}$ were studied. The experiment was performed in two parts: 3.4.1.1. Low Dilution Rate ($0.024h^{-1}$ - $0.4h^{-1}$); and 3.4.1.2. High Dilution Rate ($0.23h^{-1}$ - $9.2h^{-1}$).

Temperature (Exp. 3.4.2)

Temperature exerts a considerable influence upon the metabolism of microorganisms and therefore it was of prime importance to determine both the overall temperature range of P. javanicum (that is, the upper and lower limits for growth) and the temperature(s) at which biomass production and lactose utilisation are maximised. Moreover, heating/cooling can comprise a large proportion of the operating costs of a fermentation system: economic considerations may, therefore, require the system to be operated at a temperature different from the optimum. Thus it was necessary to determine the effects that this might have on productivity and hence profitability.

pH Value (Exp. 3.4.3)

Analogous to temperature, the pH value of the environment has a great influence on the metabolism of microorganisms. Most enzyme systems have been shown to possess well-defined pH profiles; they have upper and lower limits of activity but exhibit a precise optimal value at which enzyme activity is maximal. In addition, the value of the medium pH may affect growth by changing the availability of certain nutrient ions (Rowley and Bull, 1973). Mukherjee et al (1975) reported that "the mycelial growth of P. javanicum van Beijma was at its maximum at pH 5.0 whereas at pH 6.5 growth was at its minimum". Thus a small change in pH

value caused a large change in the growth rate of the mould. In this investigation, the effects of pH on P. javanicum were studied in three types of experiment: 3.4.3.1. surface culture; 3.4.3.2. batch submerged culture; and 3.4.3.3. in the CTF.

Two fermentation parameters which were not investigated were substrate concentration and dissolved oxygen concentrations. All these experiments were performed in conditions of excess substrate in order to measure the maximum rates of lactose utilisation and biomass production (under a defined set of environmental conditions). The effects of dissolved oxygen tension in the CTF have already been extensively investigated. Stockbridge (1979) reported that above a critical superficial gas velocity the fermenter liquid was saturated with dissolved oxygen, even though the centres of the flocs could be oxygen-limited. This resulted from diffusion limitations and could not be overcome by increasing the air flow rate (this is discussed later). Therefore, as with substrate concentration, these experiments were performed in oxygen saturated conditions. On the industrial scale excess oxygen is not desirable because of the high operating costs. However, since aeration is greatly affected by "scale-up" in tower fermenters (Pannell, 1976), results obtained in a 5 litre volume fermenter would not be applicable to a large scale vessel. Orazem and Erickson (1979) have reported that experiments in small-scale tower fermenters should be performed at high v.v.m. values in order to keep superficial gas velocities constant during tower scale-up. Thus an aeration rate of 2.5 v.v.m. was selected for these experiments.

Fermentations were not performed in the order in which they are presented. Experiment 3.4.1.1. (the effects of low dilution rate, $0.024h^{-1} \leqslant D \leqslant 0.4$) was investigated first, and carried out at a temperature of $35^{o}C$. As a result of the second experiment (3.4.2. the effects of temperature) $30^{o}C$ was selected for the remaining fermentations. The investigation of the effects of pH (Experiment 3.4.3.3.) was performed at a dilution rate of $1.0h^{-1}$ because this dilution rate appeared to be the most appropriate for the fermentation of dairy factory effluents, which will probably require pH adjustment for efficient treatment (see Chapter 4).

3.2 Materials

Continuous fermentations were performed in the 5 litre Tower fermenter described in section 3.4 (Fermenter B, Fig 5). Media are given in Appendix 1.

The effects of various salts upon the growth of P. javanicum van Beijma have been investigated by Lockwood, Ward, May, Herrick and O'Neill (1934) and their findings are summarised in Table 11.

In these experiments, trace elements and vitamins were supplied in the form of yeast extract and the tap water used to make up the volume. P. javanicum has been reported to give progressively decreasing mycelial production in batch culture when $MgSO_4.7H_2O$ is present in concentrations greater than 0.5 $g.l^{-1}$ (Lockwood et al, 1934) and this level was not, therefore, exceeded during fermentations. Lockwood et al also noted that the range of concentrations 0.3 - 1.2 $g.l^{-1}$ of KH_2PO_4 supported the greatest mycelial growth (concentrations between 0.15 - 0.3 $g.l^{-1}$ gave the greatest fat yields). The sodium rather than the potassium salt of dihydrogen orthophosphate was used in the concentration range 0.25 - 1.0 $g.l^{-1}$, the lowest concentration used in the high dilution rate experiment (3.4.1.2) when the rapid liquid throughput required the use of more dilute medium. During fermentations involving the use of non-autoclaved medium (Experiments 3.4.1.2. and 3.4.3.3) batches of medium were prepared as necessary by diluting the required nutrients with cold tap water and adjusting to volume with iced tap water. By careful cleaning of the containers after use, non-autoclaved medium could be kept at room temperature for periods of up to 24 hours without serious bacterial or yeast contamination.

Table 12

The Effects of Various Salts on the Metabolism

of *P. javanicum* van Beijma

After Lockwood et al, 1934

Salt	Concentration	Effect	Salt	Concentration	Effect
$NaNO_3$ *	100 mg.l^{-1}	None	$LiNO_3$	20 mg.l^{-1}	Toxic
$RbNO_3$	"	"	$CuSO_4$	"	"
$CsNO_3$	"	"	$AgNO_3$	"	"
$AuCl_3$ *	"	"	$CdCl_2$	"	"
Ba-gluconate	"	"	$HgCl_2$	"	"
Sr-gluconate	"	"	$TI(NO_3)_3$	"	"
$Be(NO_3)_2$	"	"	$Na_3VO_4.16H_2O$	"	"
$Sc(NO_3)_3$	"	"	$TaF_5.2KF$	"	"
$Y(NO_3)_3$	"	"	$Na_3B\ O_4$	"	"
$LaCl_3$	"	"	$Na_3A_5O_4$	"	"
$Al_2(SO_4)_3$	"	"	$K_2Cr_2O_7$	"	"
$Ga(NO_3)_3$	"	"	$Pd\ Cl_2$	"	"
$InCl_3$	"	"	$O_5Cl_4.2kCl$	"	"
$Zr(NO_3)_4$	"	"	$NaPtA_6$	"	"
$Ce(SO_4)_2$ *	"	"	NaF	"	"
$SnCl_2$ *	"	"			
Pb-gluconate *	"	"	$ZnSO_4$	100 mg.l^{-1}	slightly
$Th(NO_3)_4$	"	"	Na_2MoO_4	"	toxic
$NaUO_4$	"	"	Na_2WO_4	"	"
$MnCl_2$	"	"			
Ni-gluconate	"	"			
$Co(NO_3)_2$	"	"			
$UO_2(C_3H_3O_2)_2$	"	"			
$RuCl_3$	"	"			
$RhCl_3$	"	"			
$IrCl_4.2kCl$	"	"			
NaCl	"	"			
NaBr	"	"			
KI	"	"			
H_3BO_3	"	"			

* saturated solutions of these salts were used.

3.3 Methods

3.3.1 Methods used in Experiments 3.4.1.1, 3.4.1.2, 3.4.2, and 3.4.3.3.

Lactose and Ammonium Nitrogen were determined as described in section 2.4.2.

Biomass crude protein content was determined by the microkjeldahl method (Markham, 1942). 20 - 40 mg. of dried biomass were digested with 1.0 cm^3 of concentrated analar sulphuric acid and approximately 20 mg. of catalyst (potassium sulphate: copper sulphate: selenium, 32:5:1). The digested sample was transferred to a Markham Still and the determination continued as for ammonium nitrogen (section 2.4.2). The crude protein content was expressed as percentage by weight of dried sample, and was calculated from the following equation:

$$\% \text{ crude protein} = \frac{0.0875V}{W}$$

where V = volume of titre in cm^3

W = weight of sample in g.

Phosphate was measured using a Technicon Autoanalyser (Technicon Corporation, Tarrytown, New York). Liquid samples were acidified with hydrochloric acid and stored at 4° C prior to analysis, during which the phosphate concentration was determined by the reduction of phosphomolybdic acid with aminonaptholsulphonic acid. Phosphate concentration was expressed as g. phosphate per litre sample ($g.l^{-1}$). Phosphate utilisation (ΔP) was calculated from the following equation:

$$\Delta P = P_2 - P_1$$

where P_2 = medium phosphate concentration

P_1 = fermenter liquid phosphate concentration

Minerals

Calcium, Magnesium, Sodium and Potassium ion concentrations were determined by Atomic Absorption Spectroscopy. Analyses were performed in the Geology Department, University of Aston in Birmingham, on liquid samples which had been stored frozen prior to examination. Mineral utilisations were calculated as was phosphate utilisation and were expressed as mg. per litre (ΔM) or mg. per hour (dM)

where $M = Ca^{++}$, Mg^{++}, Na^{+} or K^{+}.

Fat

Biomass fat content was determined by the soxhlet extraction procedure. This method was used by Delaney, Kennedy and Walley (1975) to measure the fat content of S. fragilis biomass cultivated on lactose permeate. Between 1.0 and 3.0 g of dried finely-ground biomass was weighed into a soxhlet thimble, the fat extracted into petroleum ether (boiling range $40^{o} - 60^{o}$), and measured by weighing. Fat content was expressed as percentage by weight of dried biomass.

Start-up

Fermenter start-up was accomplished as described in section 2.4.2.

Sample Collection

Sample collection and calculation of fermentation characteristics were performed as described in section 2.4.2.

Lactose and nitrogen utilisation and biomass production rates were expressed as grams per hour because this enabled the various data collected at different dilution rates to be compared. For example, the dilution rate at which maximum lactose removal occurred could be selected. Data from experiments performed at constant dilution rate, such as the effects of temperature and pH, were presented in the more usual form of grams per litre in order that they might be compared with previous workers' findings.

pH Value

Fermenter broth pH was uncontrolled and allowed to attain its "natural" level except in Experiment 3.4.3.3. which investigated the effects of fermenter broth pH upon the growth of *P. javanicum.*

3.3.2. Methods used in Experiment 3.4.3.1.

The agar-medium (Appendix 1) was dispensed in 100 cm^3 volumes into conical flasks, each of which was then adjusted to a specified pH by the addition of either concentrated orthophosphoric acid or 10 M. sodium hydroxide. The flasks were autoclaved at 103.5 kilopascals for 15 minutes and 5 petri plates poured per flask. Each plate was innoculated with a disc of sporulating mycelium, cut using a sterile 0.5 cm diameter cork-borer from a master plate of *P. javanicum.* The discs were placed centrally on each petri plate and these were incubated at 30°C for five days. The diameter of the area of mycelial growth was measured in two dimensions and the results expressed as the mean of the ten diameter measurements made for each pH value.

3.3.3. Methods used in Experiment 3.4.3.2.

Four 150 cm^3 conical flasks containing 100 cm^3 of medium (Appendix 1) were prepared for each pH value to be tested and the pH value adjusted with concentrated orthophosphoric acid or 10 M sodium hydroxide before autoclaving at 103.5 kilopascals for 15 minutes. Three flasks of each pH value were innoculated with 1.8 x 10^8 spores of P. javanicum dispersed in a sterile solution of detergent("Triton X": distilled water; 3 drops: 100 cm^3). The fourth flask served as a control. All flasks were incubated at 30°C and 100 rpm in an orbital incubator (Gallenkamp Ltd.,) for five days. The mycelium was harvested by filtering on to tared Whatman No.1 filter paper, dried to constant weight at 105°C and the biomass weight calculated. The final pH values of the filtrates were measured. Means and standard deviations of the three recplicates were calculated for each pH value.

3.4 Results

Morphology

P. javanicum under all experimental conditions tested except at pH 4.0 to 5.0 grew in the form of spherical flocs (pelleted form); at no time did the morphology become filamentous as has been reported for A. niger cultivated on sucrose medium in the CTF (Stockbridge, 1979). When cultivated at pH 4.0 to 5.0 (Exp. 3433) the morphological form was that of "feathery-flocs" (approximately 0.4 x 0.2 cm in size) which have previously been described by Pannell (1976).

Under most conditions, the flocs were approximately 0.1 cm in diameter, hairy and cream-coloured. When the dilution rate was raised above $2.0h^{-1}$ (Exp. 3412) or the temperature lowered below $20^{o}C$ (Exp. 342) the flocs became progressively larger (up to 1.0 cm in diameter at $D \geqslant 4.0h^{-1}$; 0.7 cm in diameter at $T \leqslant 17^{o}C$). These large flocs were hollow-centred, with hairy outer and smooth inner surfaces. At $D = 9.271h^{-1}$ the biomass washed out of the fermenter within 48 hours.

Above $35^{o}C$ the morphology was different from the three forms described above. When cultivated at $37^{o}C$ sporulation was initiated; the flocs became smooth-surfaced and the mycelium was more highly branched. Upon examination by light microscope structures resembling phialides were visible. This morphology prevailed for four days until repeated compressor failure disrupted the fermentation. When the air supply was resumed the mould required several days to recover completely (Temperature held at $35^{o}C$), but once the temperature was returned to $37^{o}C$, the mould again initiated sporulation - phialides were formed. The initiation of sporulation at $37^{o}C$ was not, therefore, an artifact but indicated the upper temperature limit for vegetative

growth of this strain of P. javanicum. It was noteworthy that during compressor falure, the organism survived for several days in the fermenter with no air or medium supply and was observed to recommence growth immediately the air and substrate supplies were resumed, thus demonstrating the mould's tolerance of adverse environmental conditions.

At 37°C the biomass also changed from its normal cream colour to olive-green and then grey-black. The pigment was associated with the mycelium and not released into the fermentation broth which upon filtration was observed to be a clear primrose/yellow colour.

When the temperature was raised to 38°C, the mould was killed. After six days at this temperature, biomass production (X_E) and growth rate (μ) had both diminished to near zero (X_E = 0.003g.l^{-1}; μ = 0.00004h^{-1}). Samples of mould subcultured onto modified buffered yeast agar (Appendix 1) failed to grow, thus demonstrating that the mould was dead, although some metabolic activity remained.

The lowest pH value which permitted growth of the mould during Experiment 3.4.3.3 was 3.3, at which the morphology was of the normal form described above. When the pH value was lowered to 2.0 the mould washed out of the fermenter within 24 hours. At pH 7.0 the flocs were spherical and the same size as flocs produced at pH 3.3, but were less hairy - that is, the morphology became slightly inhibited. The biomass produced at both pH 5.0 and 7.0 was "slimy" to touch and was less easy to filter when harvested than was the biomass produced at lower pH values. At pH 7.0 the fermenter walls became fouled by the mould attaching to the glass, growing in a mat up to 2.0 cm thick. At the most alkaline pH value investigated, pH 8.0, the fermenter was

heavily infected with bacteria after only two days. Few flocs remained and these were encrusted with bacteria. The experiment was, therefore, not continued.

During Experiment 3.4.2, P. javanicum produced various pigments at different temperatures. The gre-black colour of the mycelium at $37^{o}C$ has already been described, but the colour of the biomass also changed at low temperature - at 17°, 20° and $25^{o}C$ and the flocs were a pale yellow/primrose colour. The low temperature pigment was associated with the mycelium and not released into the fermenter broth whereas at $37^{o}C$ the yellow pigment was found to be in the fermenter liquid while the mycelium was a darker colour.

Experiments 3.4.11 and 3.4.12. The Effect of Dilution Rate upon P. javanicum in the CTF.

For tables of results see Appendix 4 (Tables 23 and 24).

Results obtained in Experiment 3.4.11 showed that as the dilution rate was raised from $0.024h^{-1}$ to $0.4h^{-1}$, lactose utilisation, growth and biomass production rates increased almost linearly (Figs. 7, 8 and 9). These trends continued at higher dilution rates - the three rates increased until maxima were reached at a dilution rate between $4.0h^{-1}$ and $5.0h^{-1}$ (Exp. 3.4.12) after which they declined to zero at $D = 9.271h^{-1}$ when washout occurred (Figs. 10, 11 and 12).

Phosphate and mineral utilisation rates also increased as the dilution rate was raised from $1.0h^{-1}$ to $5.0h^{-1}$ (Fig. 13) but when D was further increased to $7.0h^{-1}$, the ions differed in their utilisation patterns. That is, sodium and calcium utilisation continued to rise, while phosphate utilisation remained constant and potassium and magnesium utilisation declined.

Nitrogen utilisation (dN) increased to a maximum as the dilution rate was raised from $0.2h^{-1}$ to approximately $3.0h^{-1}$, after which dN declined slightly (Fig. 14). There was a corresponding increase in biomass crude protein content as the dilution rate was raised from $0.2h^{-1}$ to $1.0h^{-1}$, but at dilution rates above $1.0h^{-1}$ both X_E and X_F crude protein content remained constant at 47 - 50% (Fig 17). The fermenter biomass concentration (X_F) declined with increasing dilution rate until approximately $5.0h^{-1}$ (at which $D \mu max$ occurred) after which X_F increased slightly until $D = 7.0h^{-1}$. The fat content of X_F decreased steadily (to 0.83% at $D = 7.0h^{-1}$) while the X_E fat content increased to a maximum of approximately 4.0% over this range of dilution rates (Fig. 20).

Experiment 3.4.2. The Effect of Temperature upon P. javanicum in the CTF.

For table of results see Appendix 4 (Table 25).

Unlike dilution rate, there was no single temperature at which lactose utilisation, growth rate, and biomass production were maximal. Lactose utilisation (Fig. 22) was greatest at $25^{o}C$, diminishing at higher temperature but increasing again as the temperature reached $37^{o}C$. When the temperature was reduced below $25^{o}C$, lactose utilisation decreased almost linearly (with temperature). Temperatures below $14^{o}C$ were not investigated because biomass production and growth rate were almost zero.

Growth rate increased rapidly between 14°C and 17°C, but above 17°C the increase in growth rate continued less rapidly, until a maximum was reached at 35°C (Fig.23). Above this temperature the growth rate declined and was zero at 38°C. Thus the temperature optimum for growth of P. javanicum in the CTF was close to the thermal death point of the organism.

Little variation in biomass production (X_E) occurred within the temperature range 17°C - 37°C (Fig. 19). Consequently, minimal attemporation would be required when P. javanicum is used for SCP production if the climate of the site was such that ambient temperatures fell within this range. At the extremes of temperature (14°C and 37°C) the fermenter biomass concentration (X_F) was higher than the optimum range used (Fig. 24).

The crude protein content of the fermenter biomass (X_F) was slightly lower than that of the effluent biomass (X_E) (Fig.18). Crude protein was at its maximum at 20°C, while nitrogen utilisation was maximal at 25°C.

Results obtained in experiment 3.4.2 indicated that there was no correlation between the fat content of the effluent biomass (X_E) and the temperature at which the biomass was cultivated. The X_E fat content varied between 2.0% and about 7.0% over the temperature range 17°C to 35°C, averaging approximately 4.5%. The fermenter biomass (X_F) fat content was at a maximum at approximately 25°C (Fig. 25), declining at temperatures to either side of 25°C.

Experiments 3.4.3.1 and 3.4.3.2. The Effect of pH in Surface and Batch Submerged Culture.

For tables of results see Appendix 4 (Tables 26 and 27).

In surface culture the growth rate of P. javanicum was greatest between pH values 4.0 and 7.0 (Fig26). Above pH 7.0 growth diminished rapidly and was zero at pH 9.0. The range of pH values between which maximum growth occurred was displaced towards the acid region in batch submerged culture - pH 2.0 to pH 5.0 (Fig.27). As in surface culture, when the pH was raised above this range, growth of P. javanicum decreased, although a small amount of biomass was produced at pH 9.0. In all flasks, except for those initially adjusted to pH 8.0 and 9.0, the growth of the mould had resulted in a broth pH value below 2.7.

Experiment 3.4.3.3. The Effect of pH upon P. javanicum in the CTF.

For table of results see Appendix 4 (Table 28).

Lactose utilisation, growth rate and biomass production

(X_E) were all maximal at pH 4.0. Both μ and X_E decreased rapidly at pH values to either side of 4.0, but lactose utilisation decreased only slightly as the pH value of the fermenter broth was raised from 4.0 to 7.0 (Fig.28, 29 , and 30). The fermenter biomass concentration (X_F) varied with pH in a manner similar to that of X_E, although there was no sharp increase in X_F to form a maximum (Fig.30).

Phosphate utilisation increased slightly as the pH value was raised from 3.3, to a maximum at pH 5.0 and thereafter declined slightly. Nitrogen, potassium, calcium and magnesium utilisations all decreased to minima as the pH was raised from 3.3 to 4.0 and then increased as the pH rose to 7.0. Sodium utilisation was different from these - it increased rapidly over the entire pH range (that is, from pH 3.3 to 7.0) (Figs. 16 , 31).

Biomass crude protein content (both X_E and X_F) remained relatively constant at approximately 46% between pH values 3.3 and 5.0, thereafter rising to approximately 54% at pH 7.0 (Fig. 19). Effluent biomass (X_E) fat content increased slightly as the pH was raised from 3.3 to 5.0, but then increased sharply to approximately 17% at pH 7.0. The fermenter biomass (X_F) fat content increased to a maximum of approximately 2.4% as the pH was raised from 3.3 to 4.0, but thereafter decreased. At pH 7.0 no fat was detected in the X_F biomass (Fig 21).

3.5. Discussion

Morphology

P. javanicum, under all experimental conditions except at pH 4.0 and 5.0, grew in the form of spherical flocs (pelleted form). These varied in size from less than 0.1 cm to 1.0 in diameter. These findings agree with those of Pannell (1976) who reported that the morphology of A. niger when cultivated in the CTF on sucrose medium was always pelleted except at extremely low dilution rate ($D = 0.008h^{-1}$). This dilution rate was not investigated in the present study. Pannell suggested that the filamentous morphology exhibited by A. niger at $D = 0.008h^{-1}$ was produced from pieces of mycelium broken from flocs during their long residence time in the fermenter. He advanced the additional hypothesis that dilution rate exerts the greatest influence upon morphology and reported that nutrient concentrations had negligible effects upon morphology. This is in contrast to the findings of Broderick (1980), who also cultivated A. niger in the CTF on a sucrose medium, that pH exerts a greater influence upon morphology than does dilution rate. The present results, however,

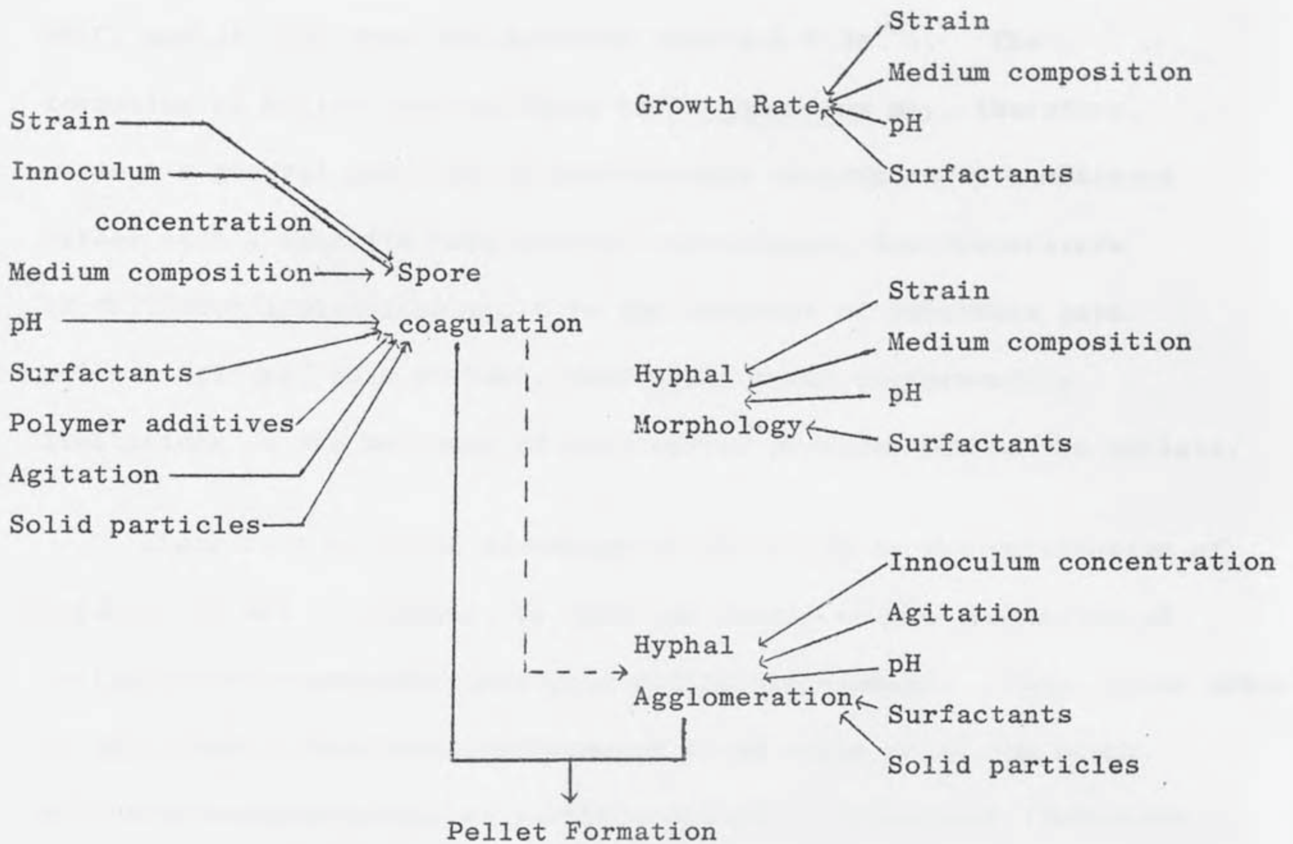
suggest that morphology will be determined by a combination of these influences.

Several authors have discussed colony (floc) formation in submerged culture (Whitaker and Long, 1973; Cocker, 1975; Metz, 1976; Metz and Kossen, 1977). Cocker and Greenshields (1977) concluded that colony formation may occur in a variety of ways, depending on initial innoculum size, environmental conditions such as agitation, medium constituents and upon biochemical and physiological parameters of the organism. The inter-relationships between these factors and their influences upon pellet (floc) formation and structure are demonstrated in Figs.32 and 33 .

Oxygen is usually considered to be the limiting nutrient for pellet growth (Pirt, 1966). Phillips (1966) showed that the specific oxygen consumption of pellets (flocs) decreases with increasing pellet size. Atkinson and Ur-Rahman (1979) suggested that diffusion limitations on the transport of substrate through flocs may occur. These authors state that the density, water content, viable/nonviable cell ratio, and possibly the internal environment within the flocs may vary with floc size. Phillips (1966), with Penicillium chrysogenum, obtained results which indicated that oxygen was supplied to the pellet interior by simple molecular diffusion and that the apparent ctitical oxygen tension increased rapidly with increasing pellet radius. He suggested that it might, therefore, be relatively easy to maintain a highly aerated medium, but not so easy to maintain an adequate oxygen supply to the pellet interior. Oxygen depletion in the centre of the pellets will lead to autolysis, and eventually the formation of a hollow centre. This phenomenon has been observed by many authors, including Phillips (1966)

Fig.32

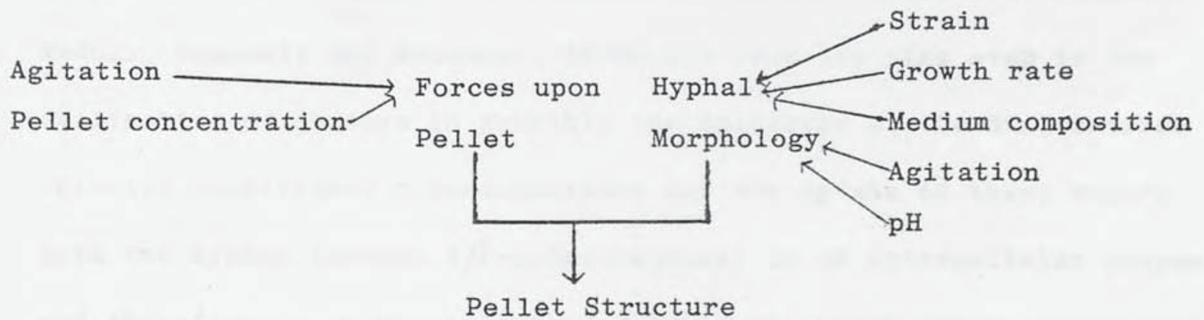
Diagram Representing Factors Influencing Pellet Formation



After Metz and Kossen, 1977

Fig.33

Diagram Representing Factors Influencing Pellet Structure



After Metz and Kossen, 1977

Trinci and Righelato (1970), Kobayashi and Suzuki (1972), Cocker (1975), Metz et al (1977), and occurred under certain conditions during this study (at dilution rates of $4.0h^{-1}$ and higher when the temperature was $30^{o}C$, and at $14^{o}C$ when the dilution rate was $0.2h^{-1}$). The formation of hollow-centred flocs by P. javanicum may, therefore, reflect a general response to unfavourable environmental conditions, rather than a specific response to, for example, low temperature. If diffusion limitations apply to the movement of substrate into pellets (flocs), then probably there will occur corresponding limitations on the movement of biochemical products out of the pellets.

There is a possible advantage to the cells in the retardation of metabolites and exoenzymes, as this may result in the production of optimal physicochemical conditions within the biomass. This latter effect is analogous to the large differences in pH value which can occur within an unbuffered enzyme particle due to a diffusional limitation on the egress of product (Atkinson and Ur-Rahman, 1979).

This phenomenon may be contributing to the morphology of P. javanicum observed during these experiments. Since galactose supports the same maximum growth rate as glucose (Anderson, Longton, Maddix, Scammell and Solomons, 1975) the rate-limiting step in the utilisation of lactose is probably the splitting of the disaccharide into its constituent monosaccharides and the uptake of these sugars into the hyphae. Lactase (β-galactosidase) is an extracellular enzyme, and therefore it is possible that sufficiently high enzyme concentrations for growth occur only when the mould grows in the form of flocs in which the enzyme molecules may be entrained.

When cultivated at pH 4.0 and 5.0, the observed morphology of P. javanicum (that is, "feathery flocs"), although not the spherical form found under all other conditions, was nevertheless a pelleted type of structure within which enzyme molecules could be trapped. P. javanicum has been observed to grow in a filamentous morphology when cultivated with sucrose as the sole carbon-source (Camberwell, 1980), all other medium constituents and fermentation conditions comprising those which produced a pelleted (floc) morphology with lactose. It could be that the particular type of floc found in this work is determined by the nature of the carbon source and the biochemical attributes required by the mould in order to utilise it.

The spherical flocs formed by P. javanicum increased in size as the dilution rate was raised (and also as the temperature was lowered). This probably occurred in response to the increased superficial liquid velocity through the fermenter. Pannell's (1976) explanation for the decrease in X_F with increasing dilution rate proposed the existence of an internal biomass recycle within the fermenter. This represented a balance between the two opposing forces of upward movement carrying mould out of the fermenter and gravity causing sedimentation of the flocs. The second factor is dependent on floc mass and therefore this mass must become larger when the superficial liquid velocity increases in order to maintain the balance and prevent washout. The overall result is an increase in the average mass (and hence size) of individual flocs retained in the fermenter.

The increase in floc size at low temperature may have resulted from the low growth rate, which contributed to the production of larger flocs rather than to the formation of new flocs. These large flocs were

so dense that the superficial liquid velocity at $D = 0.2h^{-1}$ was insufficient to wash them out of the fermenter and therefore X_F became extremely high and X_E rather small.

The feather type of floc observed at pH 4.0 to 5.0 was of the same structure as a transient morphology of A. niger described by Pannell (1976), however, in the case of P. javanicum this structure was stable.

The usual colour of P. javanicum biomass was ivory-cream. This changed to grey-black at 37°C when sporulation was initiated. At this temperature the mould also released an unidentified yellow pigment. Stockbridge (1979) reported the production of a similar pigment by A. niger at 40°C and noted that such pigment production has been associated with the protection of proteins at high temperature. It seem feasible, therefore, that the yellow pigment produced at 37°C may also be implicated in this function. In both instances, the temperature at which pigment release occurred was close to the thermal death point of the mould. P. javanicum also produced a low temperature pigment which might be associated with the protection of protein-function at the lower temperature limit for growth.

The morphology of P. javanicum (that is, pelleted form) would confer considerable cost advantages to SCP production using this organism. The biomass could be economically harvested on a large scale by simple filtration or sedimentation methods. The effectiveness of these techniques has been demonstrated when harvesting A. niger biomass from Palm Oil effluent (Greenshields, 1978[b]). The morphology of biomass produced under almost all conditions (except those which caused the formation of hollow-centred flocs) would be suitable for

harvesting by filtration methods such as squeeze-pressing.

Lactose utilisation

Wendorff et al (1970) found the temperature optimum for the lactase from S. fragilis to be 28°C, enzyme activity diminishing rapidly to either side of this temperature. They reported that temperature was critical for maximum enzyme production. Thus lactase from a yeast possessed a similar temperature optimum to that found for lactose utilisation by P. javanicum.

When discussing single-cell-protein production in addition to effluent treatment it can be more relevant to consider lactose utilisation relative to biomass production(Qs). When data obtained in Experiment 3.4.2 are expressed in this form (Fig.34), it is clear that the process was almost equally efficient at any temperature between 20°C and 30°C. Special circumstances necessitate the elimination of the upper and lower temperatures investigated because Qs was rendered artificially high. At 14°C X_E was almost zero and at 37°C, when sporulation was initiated, X_E was small combined with an increase in lactose utilisation.

Experiment 3.4.2 demonstrated, therefore, that P. javanicum, in addition to possessing a wide temperature range for survival, could be cultivated in a CTF at any temperature between 20°C and 30°C with no drop in efficiency of lactose utilisation relative to biomass production. This attribute confers great flexibility upon a commercial system, which thus could be operated with a minimal amount of attemperation.

The investigation of the effect of dilution rate ($0.203h^{-1}$ to

$9.271h^{-1}$) was performed at $30^{o}C$, within the range where maximum productivity occurred (Exp. 3.4.1.2). The results agreed with those of Pannell and Greenshields (1976), who reported that although biomass concentration within the fermenter (X_F) decreased with increasing dilution rate, the potential for overall nutrient utilisation increased because the productivity of the biomass is proportional to the nutrients removed and dependent upon the growth rate. Pannell (1976) also stated that the tower fermenter may have a lower efficiency of conversion of carbohydrate to biomass when carbon-limited than has a conventional STR because a mould growing in this type of fermenter has a higher maintenance energy coefficient. This derives from the lower growth rate of a mould in a tower fermenter and the internal biomass recycle maintaining higher cell concentrations in the fermenter even in conditions of nutrient limitation. Low efficiency of conversion of carbohydrate to biomass, in some circumstances, can be a disadvantage of this type of fermentation system, but in others is a positive advantage. When treating effluents which are variable in their carbohydrate content, for example dairy effluents, the artificially high fermenter biomass concentration allows the system to be operated carbon-limited when the medium feed is at its lowest "average-lactose-content" but with the capacity to respond rapidly if that lactose concentration rises. There would be little or no lag phase, since the organism need not increase in quantity in order to take advantage of the extra nutrients. If the lactose concentration in the medium feed fell again, the system could return to carbon-limited operation.

Apart from the initial rapid increase in lactose utilisation from zero at pH=2.0 to $3.728g.l^{-1}$ at pH=4.0, pH had little effect on

lactose utilisation, that is over the range 4.0 to 7.0 there was only a slight decrease in lactose utilisation.

The majority of investigations of pH optima for lactase activity have been performed with enzymes isolated from yeasts. These optima vary with species, for example, *Saccharomyces fragilis* pH 4.0 - 4.7 (Wendorff et al, 1970) and *Saccharomyces lactis* pH 6.5 - 6.8 (Kosikowski et al, 1973). The pH optimum may also vary according to the form of the enzyme, for example, Weetall et al (1974) reported that the pH optimum for activity of "lactase-M" was between 3.0 and 4.0 when the enzyme was immobilised on a gel support and between 4.0 and 5.0 when the enzyme was soluble. The latter (soluble enzyme) pH value corresponds to the pH value at which lactose utilisation by *P. javanicum* was at a maximum.

Experiment 3.4.3.3 demonstrated, therefore, that a tower fermentation system treating a dairy effluent could be operated at any pH value within the range 4.0 to 7.0 with almost equal efficiency of lactose utilisation and hence BOD reduction.

Growth Rate

The growth rate of *P. javanicum* in the CTF varied, as the dilution rate was increased, in manner which has also been observed to occur when *A. niger* is cultivated on sucrose (Pannell, 1976) and *S. Thermophile* is cultivated on cellulose (Anzoulatos, 1980) in the CTF. This pattern of growth probably applies, therefore, to all flocculent filamentous fungi when cultivated in the CTF, because three different species (two mesophiles and one thermophile) cultivated on three different substrates (two disaccharides and one polysaccharide) exhibited the same behaviour.

One of three possible reasons for this pattern of growth is that as the liquid throughput is increased potentially toxic waste products are removed and fresh substrate supplied to the vicinity of the cells and they are able, therefore, to grow more rapidly. A further contributory factor could be that excess mould is being continuously removed, effectively increasing the volume of the fermenter and allowing more "space" for the remaining mould to grow more rapidly.

A reason for the decrease in growth rate above μmax may be linked with the morphology of the organism. As described previously, at high dilution rate the mould grew in the form of older, large dense flocs. One of the consequences of mycelial aging is that cross walls are laid down, and older hyphae become vacuolate (Burnett, 1968; Fencl, 1978). Biochemical activity tends to be confined to the growing hyphal tips, and thus the proportion of biochemically active regions of the floc will decrease as it becomes older. The total metabolic activity of all the biomass in the CTF will, therefore, decline as the age of the mycelium increases, leading to the observed decline in substrate utilisation and growth rates.

A further reason for the observed growth pattern of P. javanicum can be based on the following model:

(1) Lactose utilisation is inhibited in the presence of a more readily available carbon source, for example, glucose (Kobayashi and Suzuki, 1972). This occurs by two mechanisms. Firstly, the lac-operon is repressed in the presence of glucose, thus preventing the synthesis of enzyme molecules, and secondly feedback inhibition of lactase occurs in the presence of galactose

thus stopping the activity of already formed enzyme molecule (Metzler, 1977).

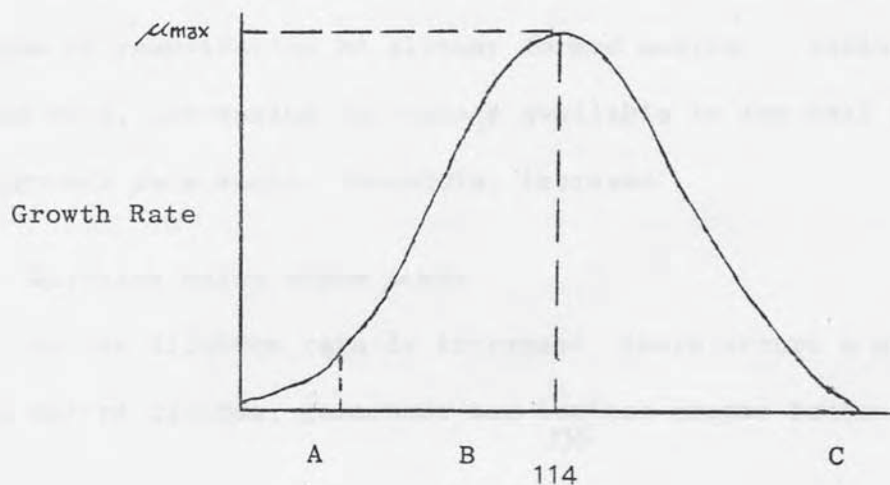
(2) It is assumed that P. javanicum can utilise glucose and galactose equally well. Anderson et al, (1975) have reported the growth rate of another filamentous fungus (A Fusarium spp.) to be the same when either glucose or galactose comprise the carbon course.

(3) It is assumed that the rates of uptake and assimilation of glucose and galactose are constant regardless of the external conditions. The rate limiting step(s) for utilisation of lactose, and hence growth, will primarily occur outside the cell. That is, during events leading to and including the hydrolysis of lactose rather than during the catabolism of glucose and galactose within the cell.

The model is more clearly demonstrated by division of the growth pattern into three phases (Fig.35):

(A) At extremely low dilution rate; (B) At dilution rates up to the point where μmax occurs; (C) At dilution rates above μmax. Conditions of excess nutrient supply apply throughout.

Fig.35 Model of Growth in a CTF



(A) Extremely low Dilution Rate

The lac-operon is induced, β-galactosidase (lactase) synthesised and secreted. Hydrolysis of lactose is initiated, but unless the hydrolysis products (glucose and galactose) are rapidly taken up by the cells, inhibition of both enzyme activity and synthesis of new enzyme will occur. Since there is some liquid flow, albeit slow, through the fermenter, this inhibition will eventually be removed, when the glucose and galactose molecules have either washed out or been taken-up by the cells. The enzyme molecules will also be washed out and therefore the mould must synthesise more, in order to resume utilisation of the lactose which is being continuously supplied. Thus lactose utilisation would occur in a "stepwise" fashion, although it would be difficult to observe this because the induction/repression events would average out when considering the total fermenter contents. The lag phases between repression and induction would result in a slow rate of lactose utilisation and hence the observed low growth rate.

(B) Dilution Rate up to μmax

As dilution rate increases, the hydrolysis products will either be taken up by the cells, or washed out, before inhibition can occur. Thus the mould can continuously produce lactase and the enzyme molecules will be fully induced. Utilisation of lactose would be limited by the rate of uptake and not by the rate of synthesis of new enzyme or reactivation of already formed enzyme. Lactose utilisation would rise, increasing the energy available to the cell for metabolism. The growth rate would, therefore, increase.

(C) Dilution Rates above μmax

As the dilution rate is increased, there occurs a point at which wash out of glucose, galactose and lactase enzyme becomes significant

in reducing the growth rate. Hydrolysis products are removed before they can be assimilated and the rate of hydrolysis declines because the enzyme molecules are washed out. Consequently lactose utilisation and growth rate are reduced.

The three phases described above would not be discrete entities. There would be a gradual transition from one to the next, resulting in the observed steady increase and then decrease in both lactose utilisation and growth rate.

In order to provide supporting evidence for the above model it would be necessary to demonstrate the existence of the transitory states in which lactase activity and synthesis are inhibited. The period of these states would tend to zero as growth rate rises to μmax. It might be possible to demonstrate this type of growth if the fermenter could be operated with synchronous growth. Pannell (1976) has reported that the CTF can be used for this purpose, but such experiments would be outside the scope of a project aimed at SCP production.

The growth of P. javanicum rose as temperature was increased because the rates of enzyme reactions are directly proportional to temperature . As temperature rises beyond the optimum for the organism there occurs a point where thermal denaturation of cellular proteins becomes significant and therefore growth rate begins to decline. Guy and Bingham (1978) report that the rate of reaction of lactase from S. lactis was at its maximum at 35°C (incubation time 1 hour), and decreased to either side of that temperature. Incubation for 1 hour at 40°C and above caused complete inactivation of the enzyme. Thus the temperature optimum for the isolated lactase from a yeast was the same as that observed for lactose utilisation by P. javanicum in

the CTF.

Several authors have reported that growth rate is a function of pH (Muzychencho, Mascheva and Yakovleva, 1974; Brown and Reddington, 1975) and this was confirmed in these experiments.

P. javanicum was unable to grow at pH 2.0 in the CTF. This was in contrast to the findings of Experiment 3.4.3.2 that the mould grew in submerged batch culture at pH 2.0 and the observation made during Experiment 3.4.4.1 (low dilution rate) that it also grew well when the fermenter broth pH value was 2.4. Consequently, there must occur between 2.0 and 2.4 a pH value at which mould growth in the CTF is inhibited.

In submerged batch culture growth occurred within the range 2.0 to 9.0 and was at its greatest between pH values 2.0 and 5.0. Growth of P. javanicum caused a lowering of the culture liquid pH value. This phenomenon was also noted by Lockwood et al (1934). These authors reported that the growth of P. javanicum van Beijma in "shake flask batch cultures" occurred over the range of initial pH values 2.1 - 7.9, the final pH values approaching 2.5 and concluded that the range of pH values 3.1 - 6.8 was favourable for growth and metabolism of the fungus. Moreover in their experiments no growth occurred at pH 1.1. This was also confirmed in the present study.

In the CTF, the maximum growth rate of P. javanicum was measured at pH 4.0, where lactose utilisation was also maximal. Mukherjee and Chaudhuri, (1975) found the mycelial growth in submerged culture of P. javanicum was at its maximum at pH 5.0, whereas at pH 6.5 growth was at its minimum, the overall range permitting vegetative growth

of their strain of P. javanicum being 4.5 to 9.0. The strain isolated and studied in this project was, therefore, slightly different from their strain, since both the pH optimum for growth and the overall range in which growth occurred were displaced towards the acid region. Results obtained in batch submerged culture (Exp. 3.4.3.2) suggest that this strain of P. javanicum might be able to grow in the CTF at pH values more alkaline than 7.0, but to demonstrate this it would be necessary to perform the experiment with sterile media. Technical difficulties in sterilising large volumes (100 litres per day) of media made this impossible.

Effluent Biomass Concentration.

Conditions contributing to an increase in growth rate also tended to result in an increase in X_E, as was demonstrated in Experiment 3.4.1.2 (high dilution rate). When biomass concentrations (X_E and X_F) are plotted against dilution rate the resulting curves (Fig. 12) are similar to those found by Pannell (1976) growing A. niger on a sucrose medium. The similarity extends beyond the "shape" of the curves to the precise biomass concentrations measured for each dilution rate. This could represent a pattern to be expected of any flocculent filamentous fungus when cultivated on a disaccharide in the CTF. The Penicilli and Aspergilli are considered to be fairly closely related taxonomically (Barron, 1968) and their similarity of behaviour in the CTF may, therefore, merely be a result of that relationship.

Biomass production varied only slightly over the temperature range 17°C to 37°C, although biomass production relative to lactose utilisation was greatest between 20°C and 30°C. Summer temperatures in the UK rarely rise above 30°C and thus it should be possible to

operate a commercial tower fermenter without external heating/cooling, although this would be affected by the substrate temperature. Heating might be required when the ambient temperature fell below 20°C, but again this would be dependent upon the substrate temperature. Since heating/cooling can provide a large proportion of the operating costs of a commercial fermentation system, the results of these experiments suggest that SCP production using P. javanicum would be more economic than most other organisms.

Since biomass production was optimal at pH 4.0, a tower fermentation system should be operated in the range of pH values 3.5 to 4.5 in order to maximise SCP production. This is more narrow than the range in which maximum lactose utilisation occurred (pH 4.0 to 7.0) and therefore the operators could select the preferred option from (1) operating the fermenter with fine control of the pH value in order to achieve maximum biomass production, although this would be at the expense of higher costs because of the greater requirement for a precise control system, acid and alkali etc; or (2) operating the fermenter with a lesser degree of pH control and thus sacrificing some biomass productivity for reduced running costs. Both options would give the same efficiency of effluent treatment, (that is, lactose removal) providing the appropriate pH value was maintained. Current prices (May, 1980) for technical grades of acid (Table 13) demonstrate that significant economies in operating costs could be made if the second option was selected.

Table 13

Current Prices for Technical Grades of Acid

Acid	Price per tonne	
Nitric	£124.33	ex I.C.I.
Phosphoric	£301.50	ex Zinchem
Hydrochloric	£59.66	ex I.C.I.
Sulphuric	£40.96	ex I.C.I.

source: M J Andrews
Albright & Wilson Ltd.,
Albright & Wilson House,
Hagley Road, West Oldbury,
Warley, West Midlands, B68 ONN

Fermenter Biomass Concentration

The decrease in X_F as the dilution rate was raised from $0.2h^{-1}$ to approximately $5.0h^{-1}$ may have resulted from the internal biomass recycle. Pannell (1976) suggested that at any particular dilution rate the CTF is capable of physically supporting a strictly limited biomass concentration. Over this range of dilution rates the quantity of effluent biomass (dX_E) increased and therefore a smaller quantity of fermenter biomass was producing a larger amount of biomass which was shed from the fermenter. This resulted from the higher growth rate at these dilution rates. The increase in X_F at dilution rates $5.0h^{-1}$ to $7.0h^{-1}$ may result from the morphology of the mould, as described in the morphology section (p.108).

In a previous study Pannell (1976) noted the appearance of a concentration gradient within the fermenter at dilution rates above $1.46h^{-1}$. Samples collected from an upper port contained higher biomass concentrations than did samples collected from a lower port.

Thus the tower fermenter was exhibiting characteristics of a multistage fermenter or pipeflow reactor with partial biomass recycle. He suggested that the lower and upper regions of the tower fermenter were equivalent to the first and second stages respectively of a multistage tower fermenter and that the concentration gradient was formed as a result of better gas distribution and hence higher gas holdup in the upper portion of the fermenter (particularly at high dilution rates). His experiments demonstrated that greater gas hold-up led to a drop in the apparent density of the culture medium, this in turn permitting greater floc sedimentation. The apparent biomass concentration would, therefore, be increased.

In the present study, all fermenter biomass samples were collected from a port situated in the lower region of the fermenter, although Figure 12 exhibits characteristics of both curves obtained by Pannell. If the X_F concentration at $D=4.0h^{-1}$ is anomalous and can be ignored (solid line, Fig. 12), the curve resembles that obtained by Pannell for lower port samples, but if this measurement reflects the true situation at that dilution rate (dotted line, Fig. 12), the curve is more like that obtained for upper port samples by Pannell. "Wall effects" would have exerted a greater influence in this experiment than in Pannell's, because the fermenter volume was smaller and the surface area to volume ratio higher. "Slugging" of the air may have occurred to greater degree, and the consequent larger gas hold-up resulted in the elevated biomass concentration. Thus at dilution rates above $4.0h^{-1}$ the whole fermenter was exhibiting characteristics peculiar to the second stage of a multistage tower fermenter.

The high X_F concentration observed at both the upper and lower temperature limits of P. javanicum probably resulted from the

morphology of the organism at those temperatures. Experiment 3.4.2 was performed at a dilution rate of $0.2h^{-1}$, and it is possible that if the dilution rate was higher, X_F would not have increased to such a degree. At $37^{o}C$ sporulation was initiated. Any growth which occurred contributed to an increase in floc mass via the formation of a highly branched mycelium and phialides rather than to X_E. The decrease in X_F observed as the temperature was raised from $17^{o}C$ to $35^{o}C$ was in contrast to the findings of Stockbridge (1979). He reported that when A. niger was cultivated on sucrose in the CTF X_F rose with increasing temperature to a maximum at $40^{o}C$, followed by a sharp drop.

Biomass Crude Protein Content

Andrews (1980) has reported that the crude protein content of Penicillium oxalicum cultivated on Palm Oil effluent in the CTF was a reliable indicator of the true protein content, although the crude protein will, of course, be a higher figure than that of true protein. Biomass protein content is of great importance when producing a single cell protein because it is the major determinant of product quality and hence retail value. Soya bean meal is commonly used as a protein-quality-standard and contains approximately 45% crude protein (McDonald, Edwards and Greenhalgh, 1973). Consequently, P. javanicum biomass produced at any dilution rate above 1.0h (Exp. 3.4.1.2); any temperature between $17^{o}C$ and $24^{o}C$ (Exp. 3.4.3), and at pH 3.3 to 7.0 (Exp. 3.4.3.3) was of a higher crude protein content than is soya bean meal.

The temperature range in which P. javanicum biomass contained more than 45% crude protein could be extended if the dilution rate was raised.

That is, Exp. 3.4.1.2 demonstrated that the biomass crude protein content was considerbly lower when the mould was cultivated at $D=0.2h^{-1}$ compared with that of biomass from dilution rates above $1.0h^{-1}$. If the effect of temperature was investigated at higher dilution rate, the crude protein content of the biomass would be expected to rise.

Nitrogen Utilisation

Nitrogen is an essential nutrient for living cells and therefore sufficient quantities of a nitrogen source must be supplied in the fermenter medium in order to permit maximum utilisation of the carbon source. However, too much nitrogen is undesirable because discharge of the excess can generate a secondary effluent problem. Moreover, economic considerations suggest that only the minimum quantity of nitrogen suppliments should be so employed. Therefore the critical quantities of nitrogen used by P. javanicum in the CTF under varying conditions of dilution rate, pH and temperature were determined.

Nitrogen utilisation was greatest at 25°C, the temperature at which lactose utilisation and growth rate were also maximal. This was in contrast to the crude protein content which was highest at the lower temperature of 20°C. An analogous situation prevailed in Exp.3.4.3.3 when nitrogen utilisation was at a minimum at pH 4.0, although the biomass crude protein content remained relatively constant between pH 3.3 and 5.0. This offers the possibility of making considerable economies in the use of nitrogen supplements since the fermenter can be operated at pH 4.0 to produce a product of high protein content but yet with a reduced requirement for nitrogen. Similarly, Exp. 3.4.1.2 demonstrated that the fermenter could be operated with

reduced nitrogen requirements without affecting the biomass crude protein content if the dilution rate was maintained above $3.0h^{-1}$ (see Fig. 14).

Under certain conditions, for example at pH 3.3 and pH 5.0, when a larger amount of nitrogen was used by the mould without the crude protein content rising, the biomass must have increased in non-protein-nitrogen content.

Biomass Fat Content

Fat is commonly used in animal diet formations as an additional source of energy. Consequently, if microbial biomass destined for animal feed contains appreciable quantities of fat, the market value of the product will be raised (Greenshields, 1978[a]). Ratledge and Hall (1977) investigated the continuous production of microbial lipid, and reported that the constitution of the medium needs to be high in carbon and low in nitrogen so that the cultures grow permanently deprived of nitrogen. Lipid accumulation occurs when the growth rate of the organism is kept below its maximum. P. javanicum has previously been used for the purposes of fat production, although these studies do not appear to have been resumed after World War II. Lockwood et al, (1934) investigated fat production by P. javanicum van Beijma cultivated on glucose in batch shake flasks and their results are presented in Table 14.

Table 14

Fat Content of P. javanicum Cultivated on Glucose

initial pH	% Fat
2.1	4.7
3.1	17.5
3.9	22.6
5.0	20.0
6.2	23.9
6.8	23.4
7.9	19.3

After Lockwood et al, 1934.

Although these results were obtained in batch culture, it is apparent that the fat content was greatest at pH 6.2, and rose rapidly over a short range of pH values (2.1 to 3.1) from 4.7% to 17.5%. The maximum at pH 6.2 corresponds to the rapid increase in effluent biomass fat content which was observed between pH values 5.0 and 7.0 in the present study. Lockwood et al (1934) reported the composition of the fat extracted from P. javanicum van Beijma to be 30.8% saturated fatty acids, 60.8% unsaturated fatty acids and 2.0% unsaponifiable material. The fatty acids were a mixture of oleic, stearic, palmitic, linoleic, tetracosanic acids. Results of analyses performed by Ward and Jamieson (1934) of the oil (fat) from P. javanicum are presented in Table 15. These authors reported that the fat was composed of palmitic, stearic, tetracosanic, oleic and α and β -linoleic acids, in addition to glycerol and a small quantity of unsaponifiable matter.

Table 15

Physical and Chemical Characteristics of the Oil from P. javanicum van Beijma

Solidification point, ^{o}C	6-7
Melting point ^{o}C	about 15
Specific gravity ($25^{o}/25^{o}$)	0.9145
Refractive index (25°)	1.4680
Acid value	10.6
Saponification value	191
Iodine value (Hanus)	84.0
Reichart-Meissl value	0.3
Acetyl value	10.7
Unsaponifiable matter, %	2.00
Saturated acids (corrected), %	30.8
Unsaturated acids (corrected),%	60.8
Melting points of mixed saturated acids	52.5
Mean molecular weight of saturated acids	272

After Ward and Jamieson, 1934

Ward, Lockwood, May and Herrick (1935) found that the mycelium of P. javanicum vanBeijma could contain as much as 41.5% fat, depending on culture conditions, although this was, of course, in batch culture. The highest level of effluent biomass fat measure in these experiments was 16.91% at pH 7.0. The biomass was "slimy" to touch and difficult to filter when harvesting, which may have resulted from the high fat content.

There appeared to be no correlation between fat content of the effluent biomass and temperature. Biomass produced between 17°C and 35°C contained between 2% and 7% fat.

In contrast, the fermenter biomass fat content appeared to be maximal at c.25°C. The effluent biomass fat content increased and the fermenter biomass fat content decreased as the dilution rate was raised from $0.2h^{-1}$ to $7.0h^{-1}$.

These analyses were performed by the soxhlet extraction procedure, which has been reported to give low readings (Tacon, 1979) and these results should, therefore, be taken as minimum quantities of fat.

Phosphate and Mineral Utilisation

Utilisation by *P. javanicum* of phosphate and four mineral ions were investigated in order to determine the required quantities of these nutrients for maximum productivity. This would enable economies to be made in medium preparation and therefore reduce the operating costs of a commercial plant. Unfortunately it was not possible to analyse samples collected under all fermentations conditions investigated. These results should, therefore, be taken as indicators of trends and guides to required phosphate and mineral levels, rather than as absolute requirements of *P. javanicum* in the CTF.

As might have been expected, phosphate and mineral utilisation rates increased as the growth rate rose to a maximum. These mineral ions are required for 'cellular' biochemical activities (Fiedler, 1959; Kinghorn and Pateman, 1977) and phosphate is intimately

involved in energy metabolism (Metzler, 1977). Thus as the metabolic rate increases (leading to a higher growth rate) demand for these nutrients will also increase. When the growth rate declined, phosphate utilisation diminished slightly (Exp. 3.4.3.3) or remained constant (Exp. 3.4.1.2). Similarly, potassium and magnesium utilisation rates declined after μmax, but sodium and calcium utilisation rates continued to rise. The increased demand for these may have resulted from less efficient cycling of them within the cell as floc size and hence age increased.

3.6 Summary and Conclusions

One of the major economic advantages of the tower fermenter is that it can be operated septically. Pannell and Greenshields (1976) reported that contamination of the fermenter by yeasts and/or bacteria was minimised because these generally tend not to form floc structures and consequently were rapidly washed out. They further suggested that contamination washout was assisted by flotation in the small amount of froth usually present at the liquid/air interface at the outlet of the fermenter. In addition, the low pH resulting from growth of the fungus usually inhibits the growth of yeasts and bacteria. Fungi can usually be grown at a pH far lower than that suitable for most bacteria (Rowley and Bull, 1973), and Stockbridge (1979) stated that low pH was the major factor allowing nonaseptic operation of the fermenter.

Results obtained in these investigations demonstrated that a combination of both dilution rate and pH maintained the fermenter in a state relatively free from contamination when using nonsterile media. The fermenter broth remained "clean" at dilution rates as low as $0.4h^{-1}$ providing the pH value was sufficiently acidic (in practice between 2.0 and 3.5) or at higher pH (up to pH 7.0) providing the dilution rate was at least $1.0h^{-1}$. At pH 7.0 and a dilution rate of $1.0h^{-1}$ the fermenter was slightly infected, but not sufficiently to affect the efficiency of biomass production or lactose utilisation, whereas at pH 8.0 the fermenter was so badly infected that fermentation effectively ceased. In one continuous fermentation lasting more than 4000 hours using nonsterile media, significant contamination by other microorganisms was not observed. During this period P. javanicum survived repeated air

failure, including several days when the mould remained in the fermenter with no air or fresh medium supply. Upon resumption of these, the mould recommenced growth immediately, thus demonstrating its tolerance of adverse environmental conditions. This tolerance renders P. javanicum more valuable to industry because it will be able to survive temporary plant failure, or mishandling by poorly trained personnel.

During all fermentations it was noted that while at "steady-state" measured parameters, for example lactose utilisation, did not remain constant relative to time. Observed values appeared to oscillate about a mean value, the period of these oscillations being approximately 5 days. For this reason, once "steady-state" was attained, at least five consecutive days measurements were averaged to give the mean values presented in the tables of results. One unavoidable corollary of this was that the standard deviations calculated for these means were large, but it was felt that a better representation of the true situation was given by data including the whole period of the oscillation, rather than selecting data in order to give a small standard deviation.

It was noted that with increasing dilution rate the fluctuations became wider, and therefore the standard deviations of the sample means larger, although the period of the oscillations remained the same. Several factors may account for this. For example, although the "rate-setting" of the Baron Yemm media pump was not altered, the medium flow rate and hence dilution rate was observed to be variable from day to day. This may have been caused by a gradual stretching of the silicon rubber tubing, or may simply derive from a slightly unreliable pump. Variations in medium flow rate caused variations in

the supply of fresh substrate to the mould which must have affected its growth rate as well as affecting the rate of removal of excess biomass from the tower. Thus all the parameters measured during the fermentations would have included some errors derived from the variable medium flow rate.

Wall growth is a well-documented problem of laboratory fermentation, but occurred only once during this study, at pH 7.0. The attachment of mould to the walls appeared to be via a pH-dependent adhesive substance since the biomass became detached almost immediately when the pH value was lowered to 3.0 on completion of the experiment.

Lactose utilisation was maximal and varied only slightly between pH 4.0 and 7.0. A temperature between 20°C and 30°C and a dilution rate of 5.0h^{-1} permitted maximum lactose utilisation relative to biomass production. As might be expected, the optimum conditions for growth rate were close to those for lactose utilisation, since the organism is dependent upon the utilisation of the sugar for growth. Maximum growth rate was measured at pH 4.0, a dilution rate of 4.0h^{-1} and 35°C, although growth rates measured between 25°C and 35°C varied only slightly. Biomass production was greatest when the fermenter was operated at a dilution rate between 4.0h^{-1} and 5.0h^{-1}, a temperature between 17°C and 35°C and a pH of 3.5 to 4.5.

Biomass (X_E) crude protein content increased as the pH value became less acid, rising from 48% at pH 3.3 to 54% at pH 7.0. It was maximal at a temperature of 20°C, but appeared to vary only slightly with dilution rate, remaining relatively constant between 47-50% at dilution rates of 1.0h^{-1} or above.

Biomass (X_E) fat content was greatest at high dilution rate, and neutral pH value. Temperature had little effect on fat content, which ranged between 2% and 7%, averaging 4.5%. The overall range of biomass (X_E) fat contents measured in this study was 0.49% (Exp. 3.4.1.2) to 16.91% (Exp. 3.4.3.3) and the mean was 4.0%.

Conclusions

1. The wide temperature range of P. javanicum especially its tolerance of low temperatures allows more flexibility in operation and permits the "working temperature" to be selected by economic considerations rather than being restricted by organism constraints.

2. The wide pH range over which efficient lactose utilisation occurs also confers flexibility to the system. The fermenter may be operated with a high degree of Ph control, maximising biomass production or with a lesser degree of pH control, sacrificing some biomass production for reduced running costs without affecting the efficiency of lactose removal.

3. The fermenter can be operated septically with nonsterile media providing a combination of sufficiently acid pH value and rapid dilution rate is maintained. (In practice pH < 3.5 when $0.4h^{-1} \leqslant D \leqslant 1.0h^{-1}$; and pH $\leqslant 7.0$ when $D \geqslant 1.0h^{-1}$).

4. The artificially high fermenter biomass concentration maintained by the fermenter allows a more rapid response to an increase in substrate concentration.

5. Biomass (X_E) produced at pH values between 3.3 and 7.0 and at all dilution rates greater than or equal to $1.0h^{-1}$ contained a higher crude protein content than does soya bean meal.

6. The mean fat content of *P. javanicum* biomass (X_E) was 4%.

7. At dilution rates $4.0h^{-1}$ and above the fermenter may have been exhibiting characteristics of the second stage of a multistage tower fermenter.

8. The fermenter walls remained free of wall growth under all conditions except at pH 7.0.

Fig 7 <u>The Effect of Dilution Rate upon Lactose Utilisation by P.javanicum in a C.T.F. (data from table 23)</u>

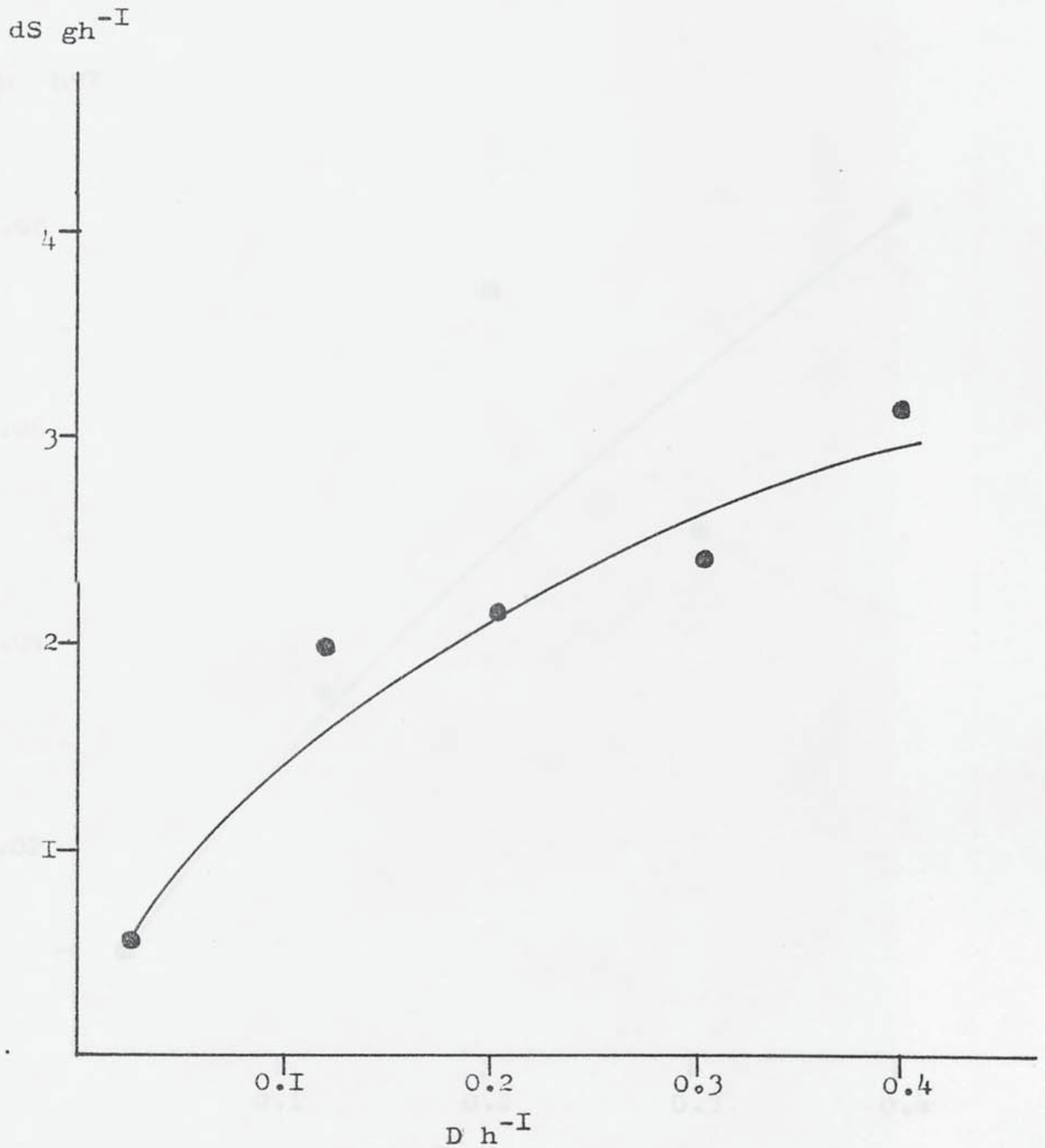


Fig 8 The Effect of Dilution Rate upon Growth Rate of P.javanicum in a C.T.F. (data from table 23)

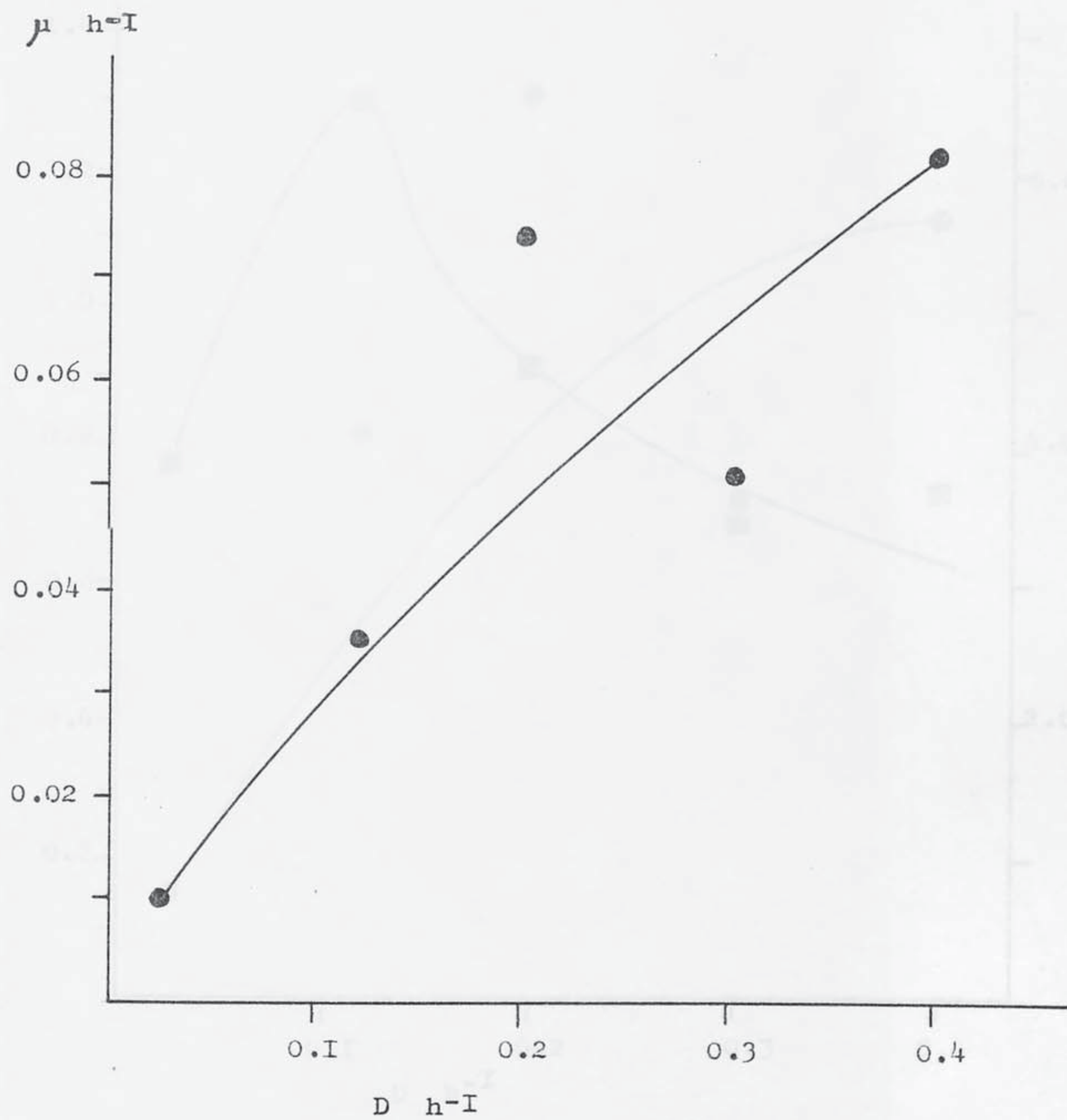


Fig 9 The Effect of Dilution Rate upon Biomass Production by P.javanicum in a C.T.F. (data from table 23)

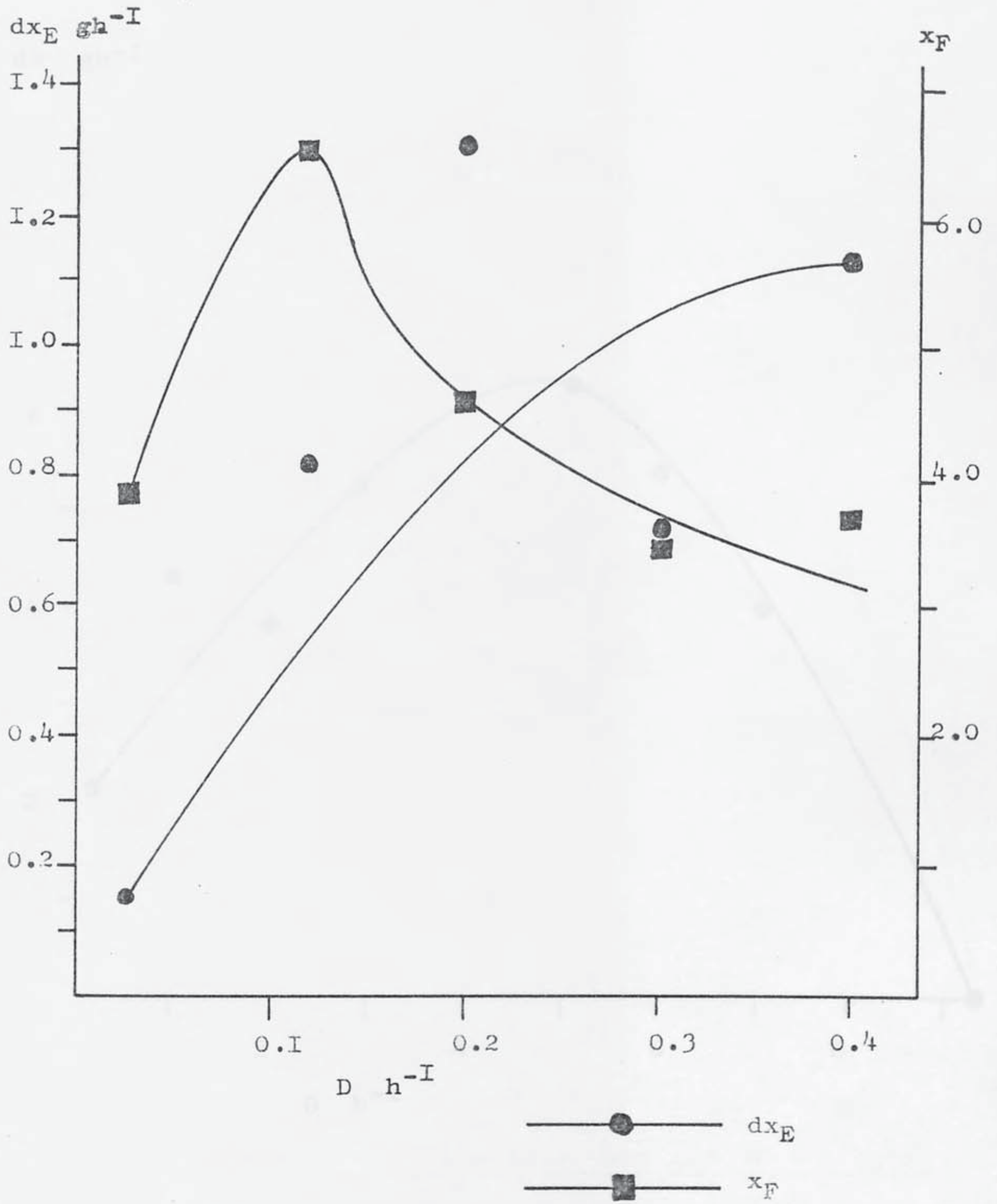
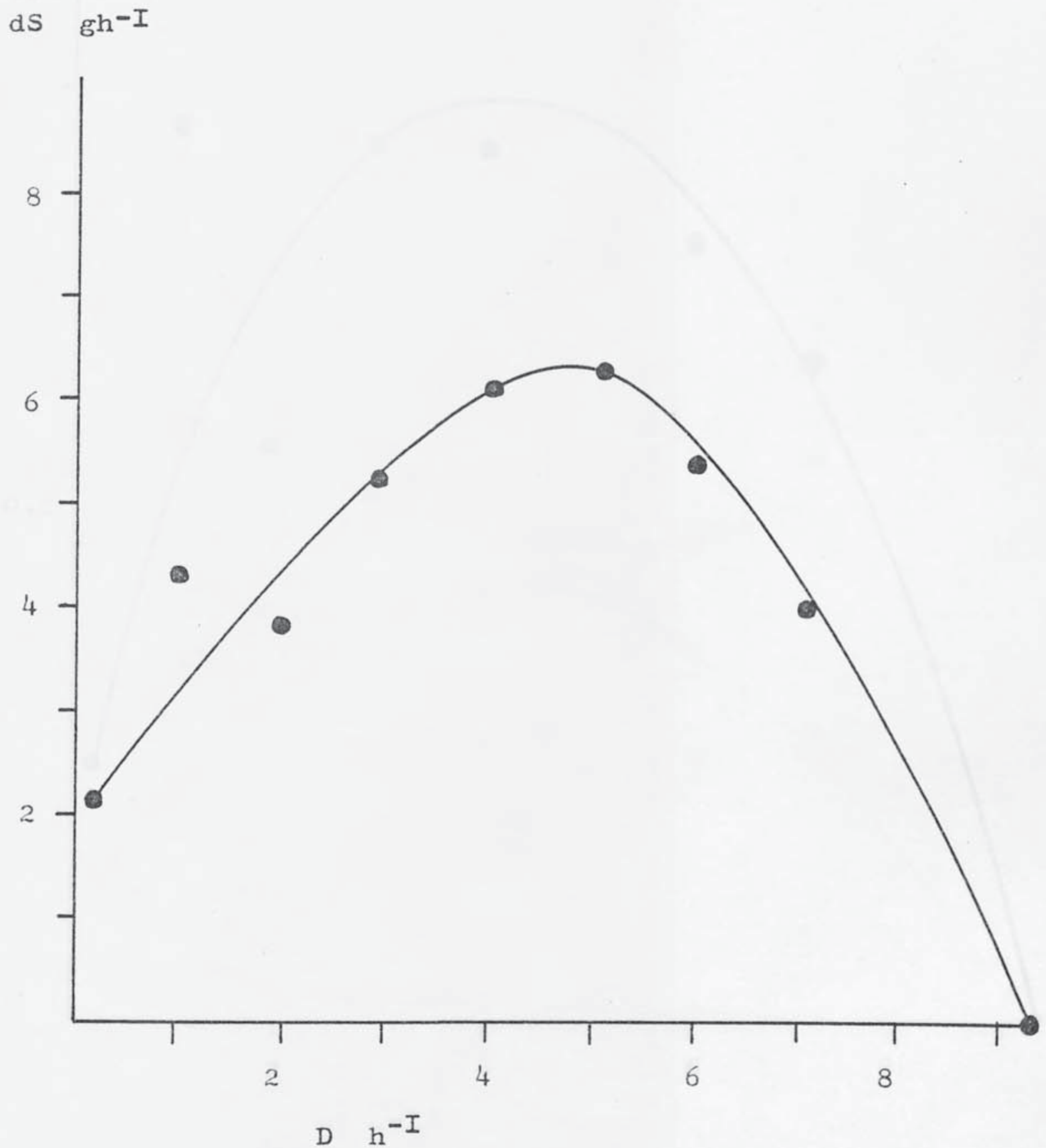


Fig 10 The Effect of Dilution Rate upon Lactose Utilisation by P.javanicum in a C.T.F. (data from table 24)



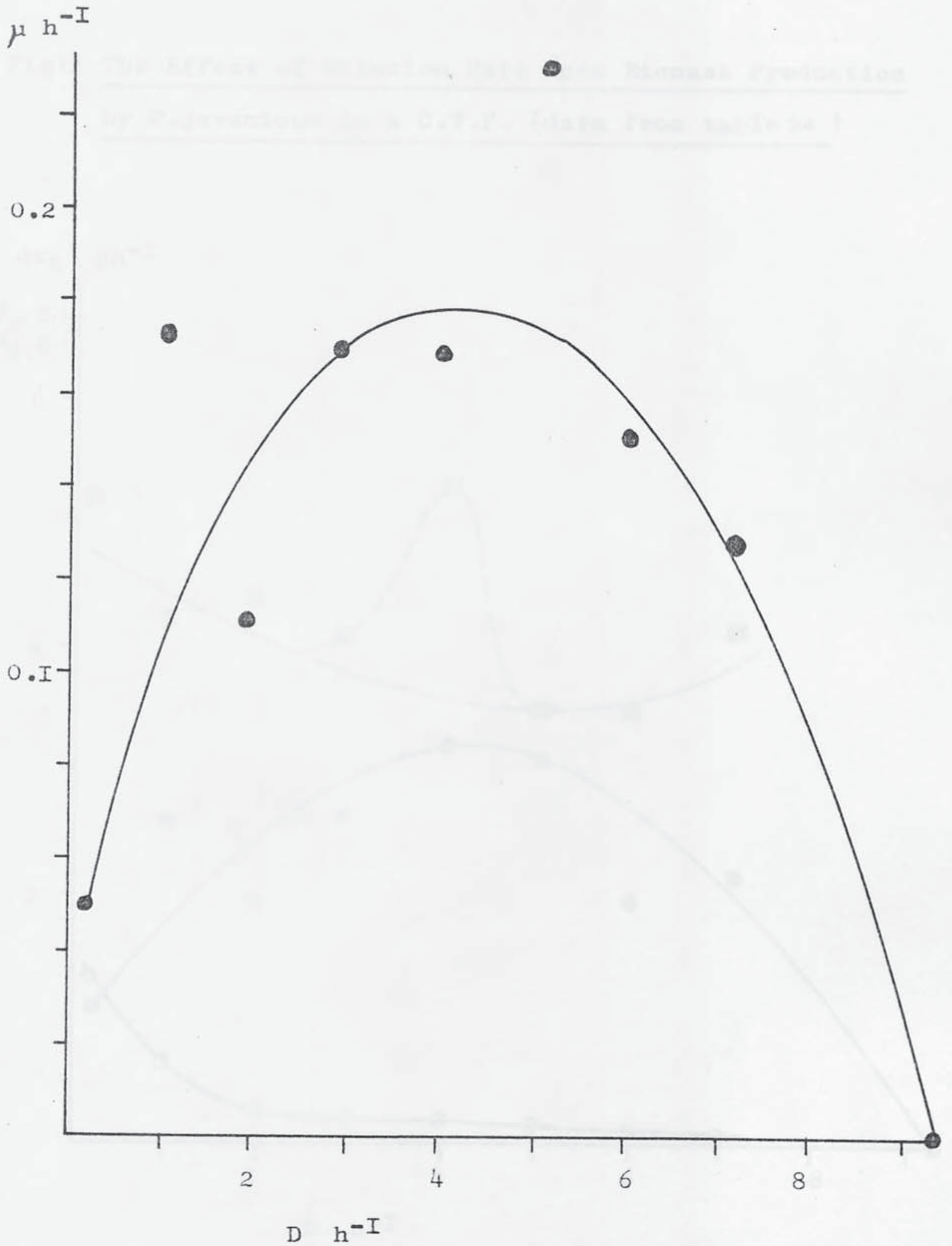
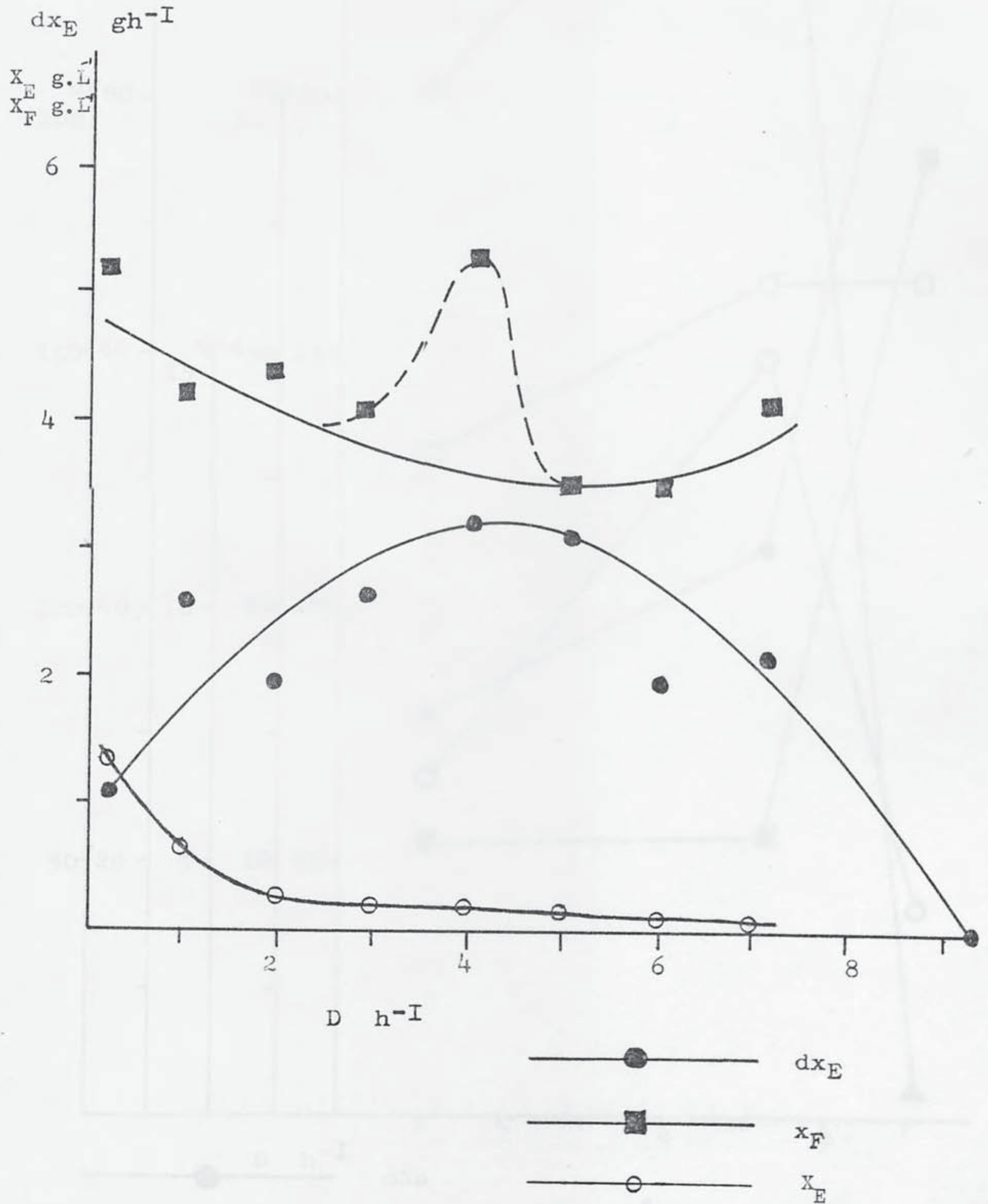


Fig 11 The Effect of Dilution Rate upon the Growth Rate of P. javanicum in a C.T.F. (data from Table 24)

Fig12 The Effect of Dilution Rate upon Biomass Production by P.javanicum in a C.T.F. (data from table 24)



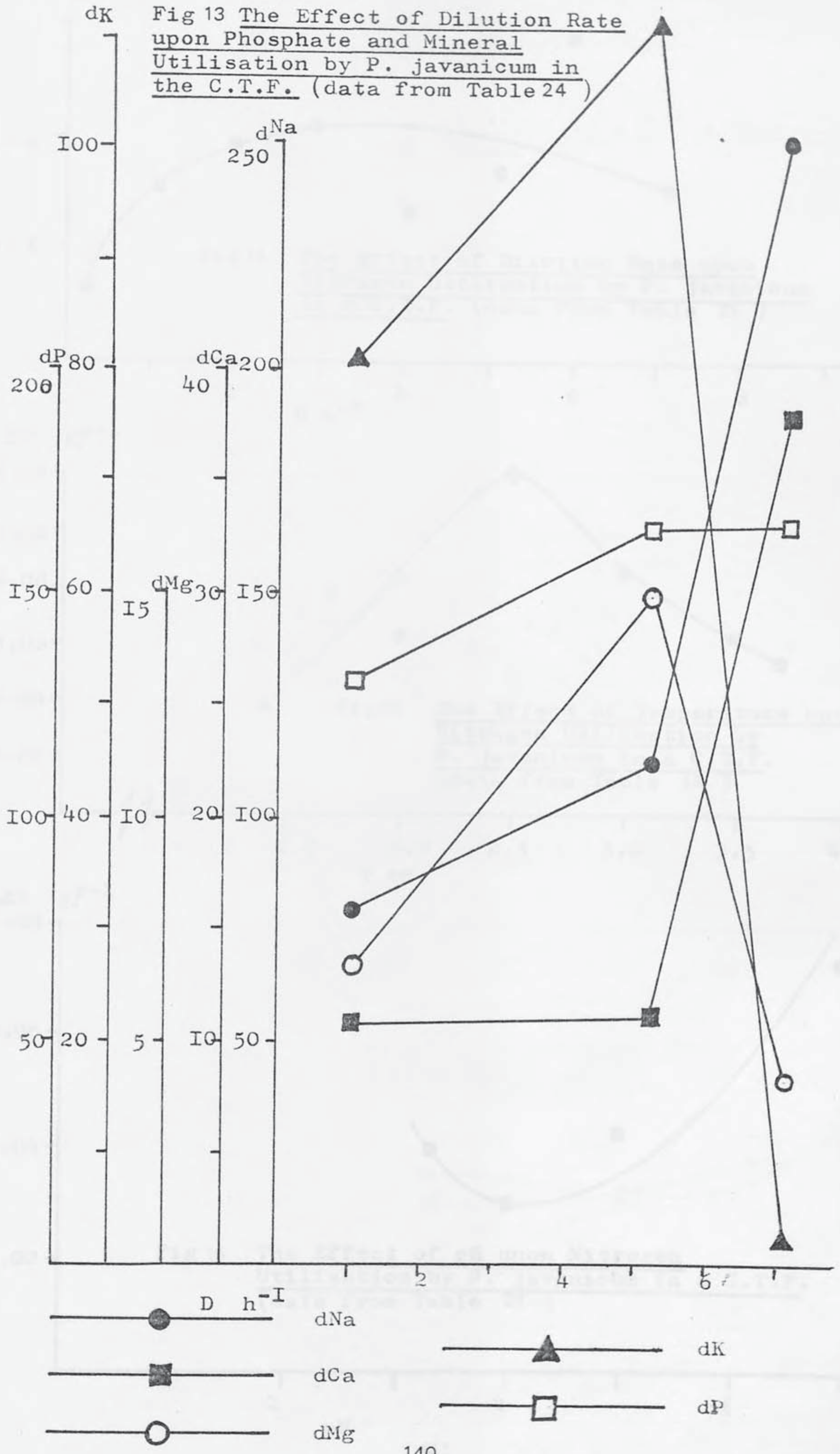


Fig 13 The Effect of Dilution Rate upon Phosphate and Mineral Utilisation by P. javanicum in the C.T.F. (data from Table 24)

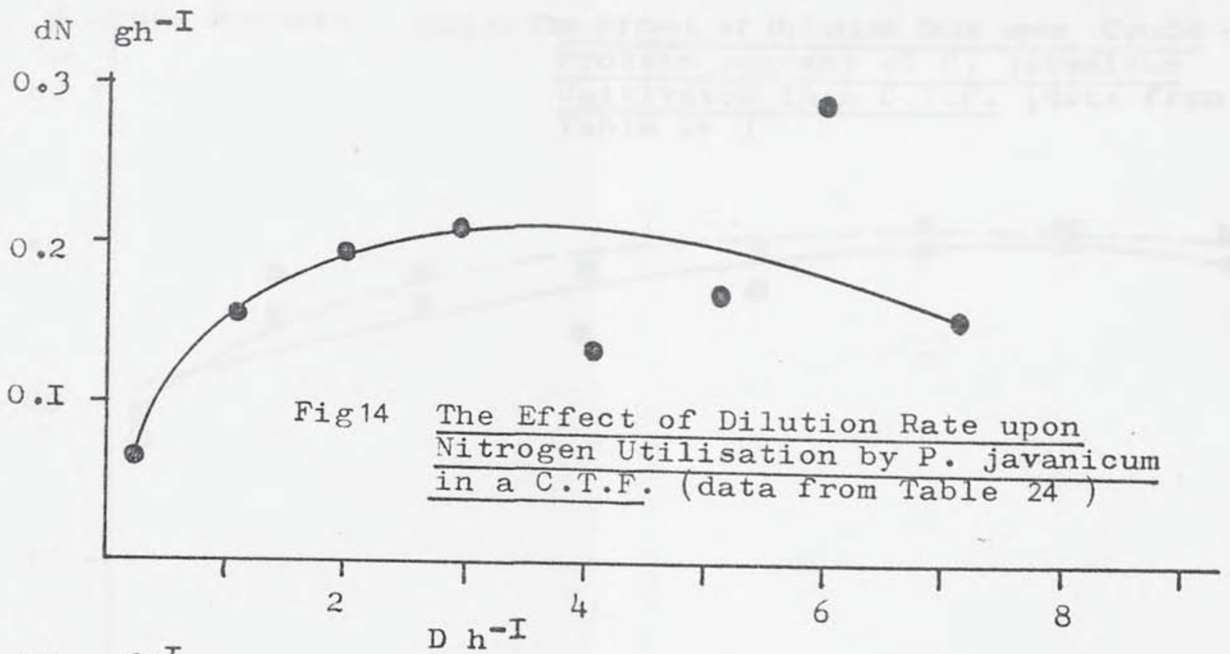


Fig 14 The Effect of Dilution Rate upon Nitrogen Utilisation by P. javanicum in a C.T.F. (data from Table 24)

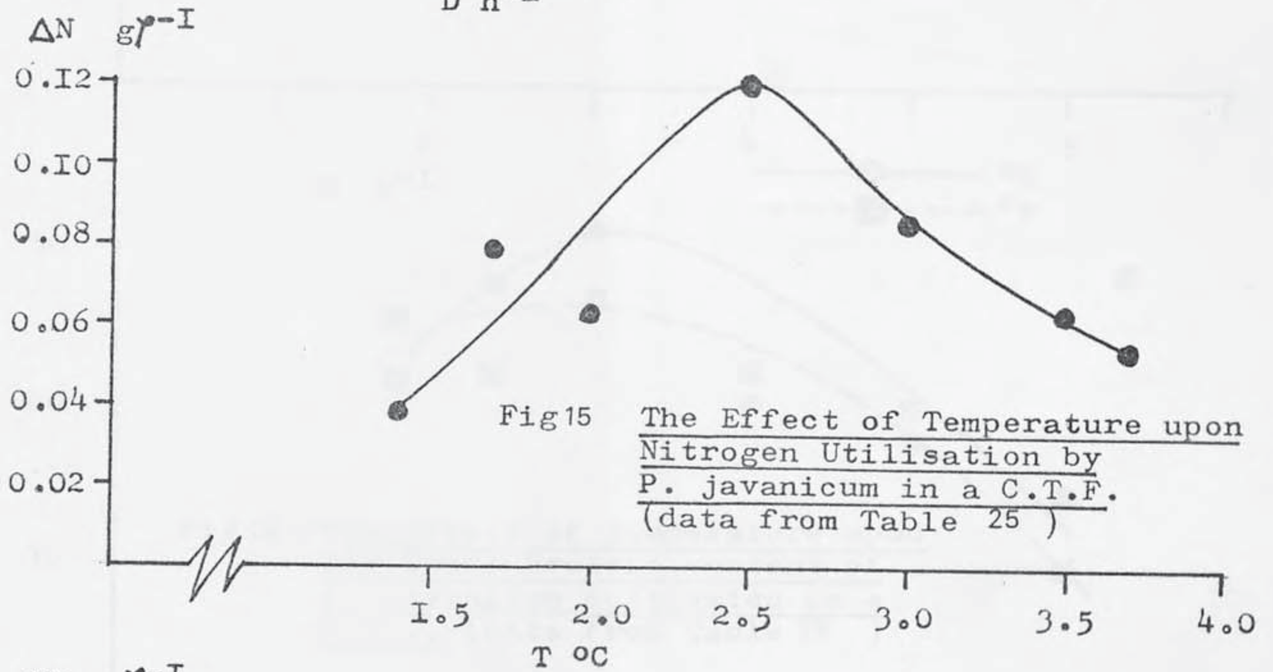


Fig 15 The Effect of Temperature upon Nitrogen Utilisation by P. javanicum in a C.T.F. (data from Table 25)

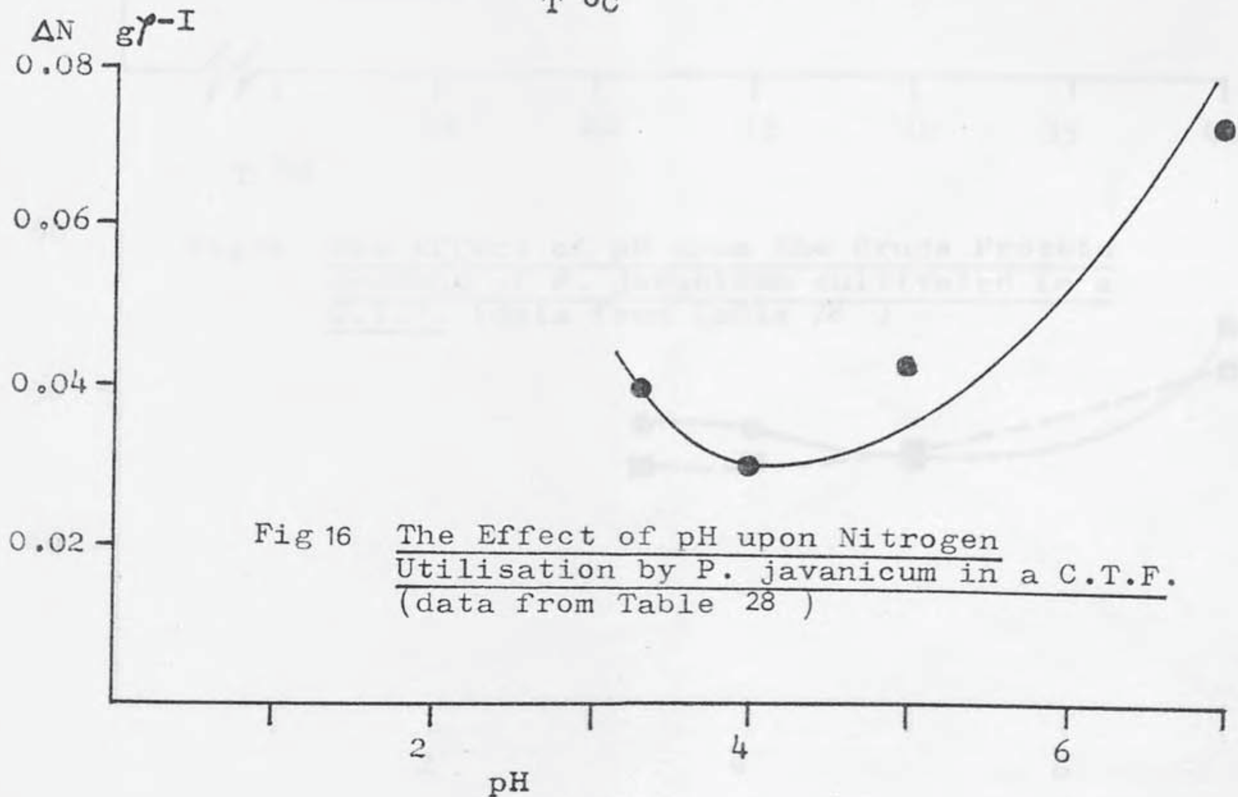


Fig 16 The Effect of pH upon Nitrogen Utilisation by P. javanicum in a C.T.F. (data from Table 28)

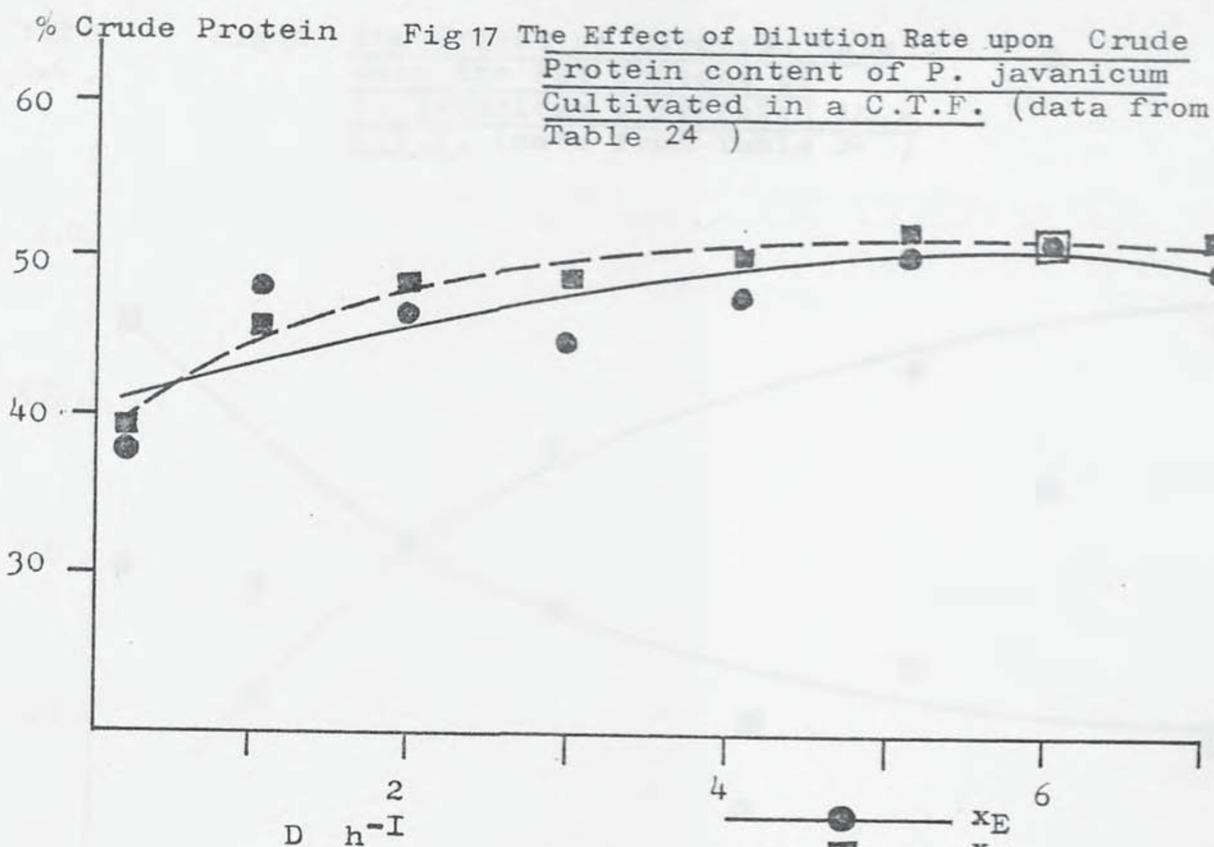


Fig 17 The Effect of Dilution Rate upon Crude Protein content of P. javanicum Cultivated in a C.T.F. (data from Table 24)

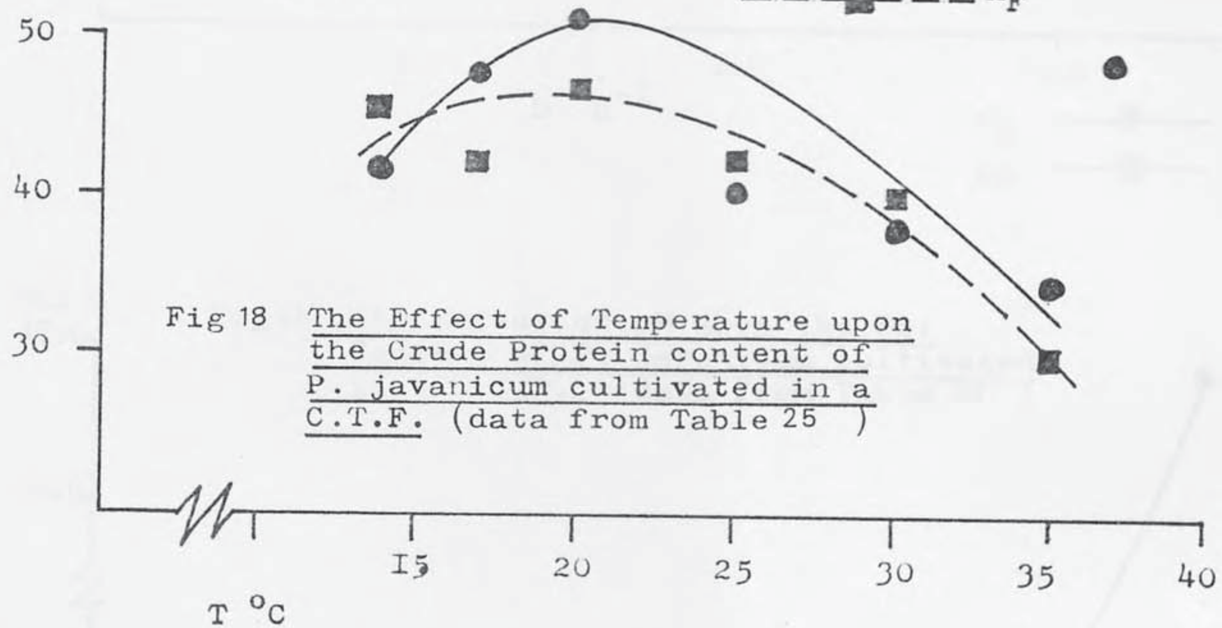


Fig 18 The Effect of Temperature upon the Crude Protein content of P. javanicum cultivated in a C.T.F. (data from Table 25)

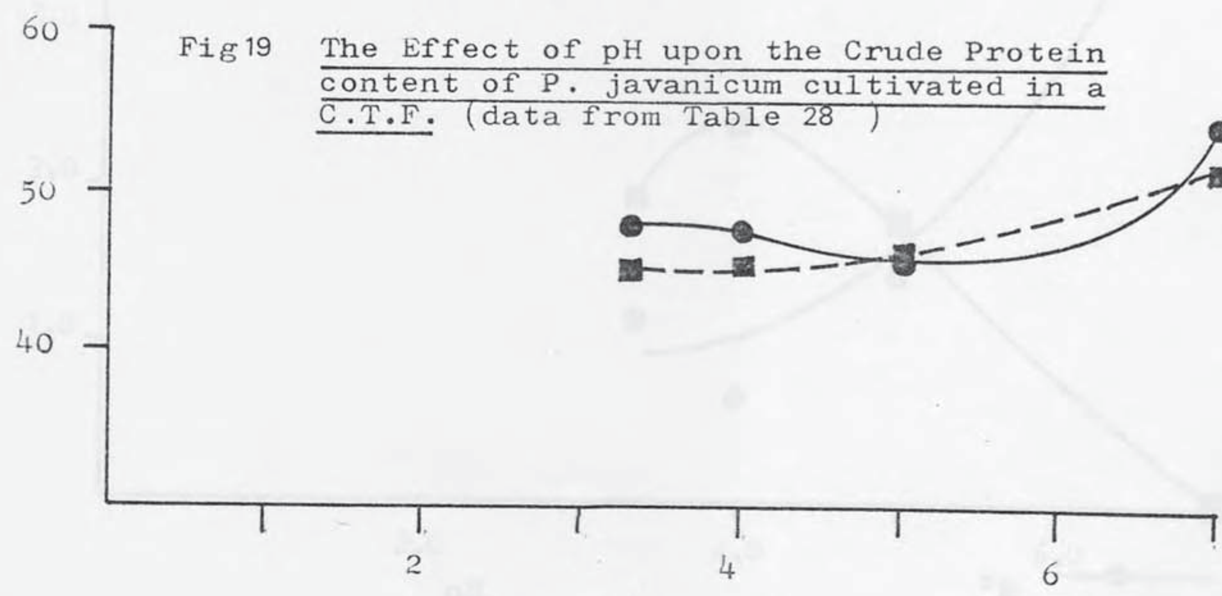


Fig 19 The Effect of pH upon the Crude Protein content of P. javanicum cultivated in a C.T.F. (data from Table 28)

Fig 20 The Effect of Dilution Rate upon the Fat Content of P. javanicum cultivated in a C.T.F. (data from Table 24)

FAT %

5.0

4.0

3.0

2.0

1.0

2.0 4.0 6.0

D h^{-1}

x_E

x_F

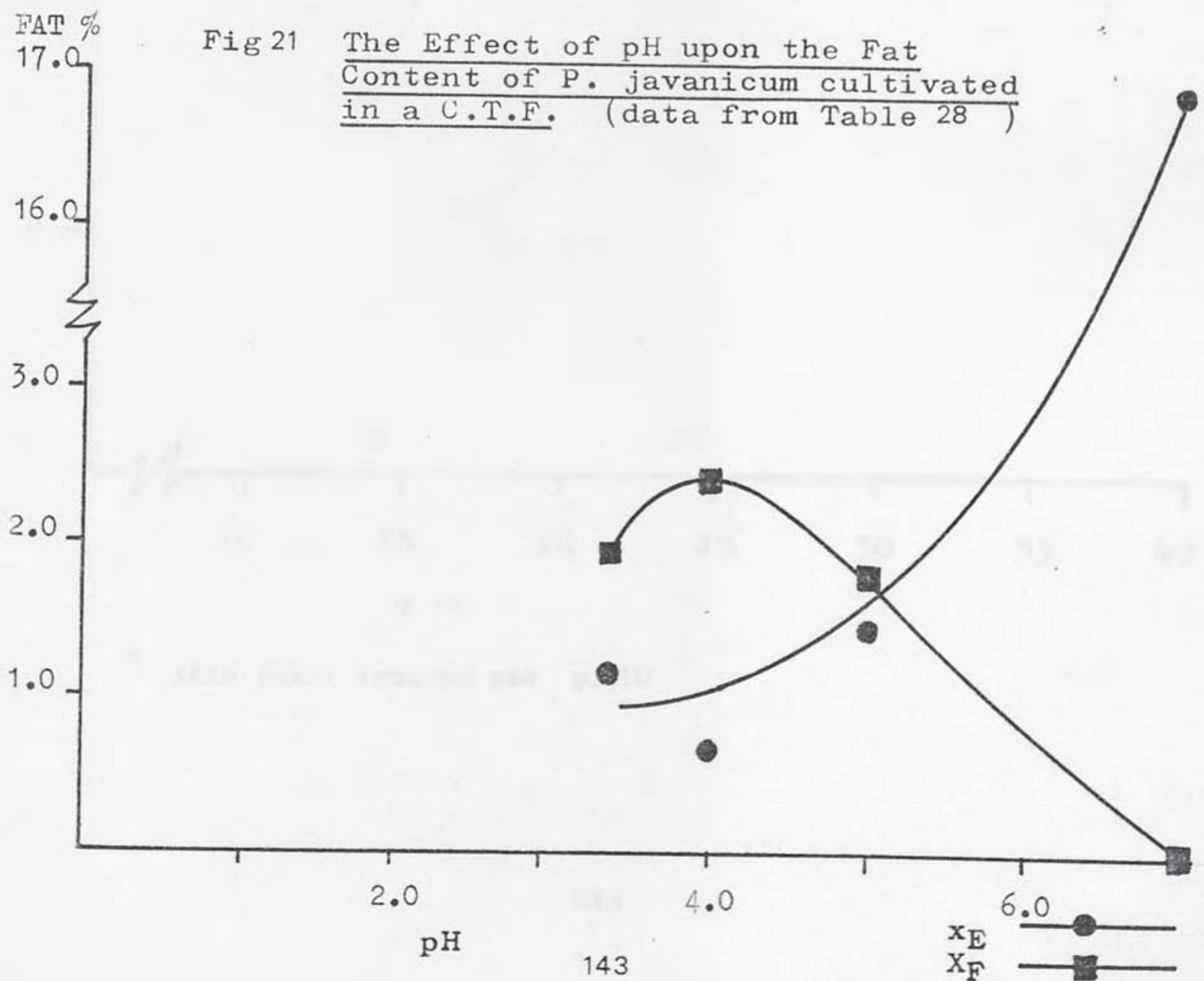
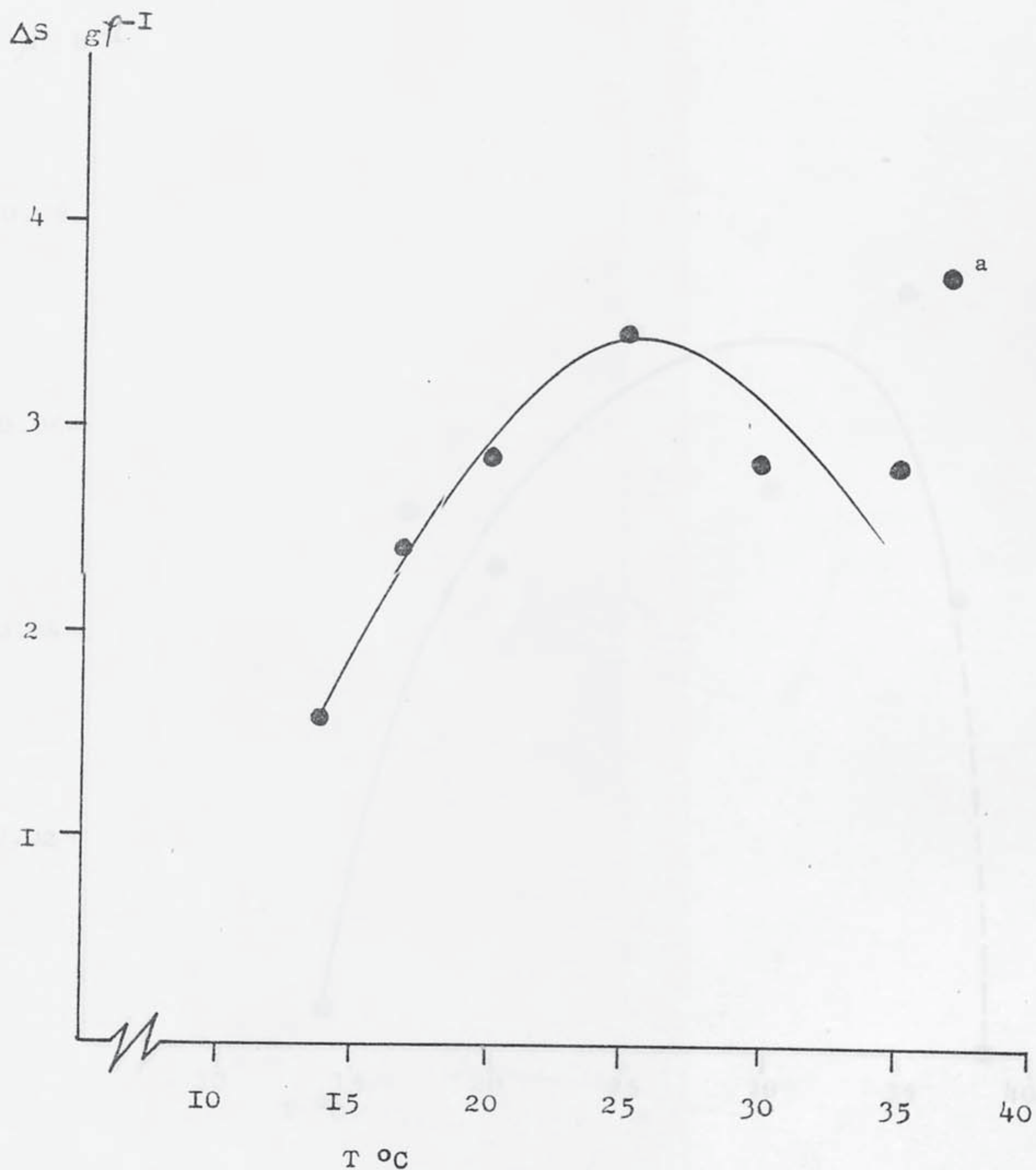


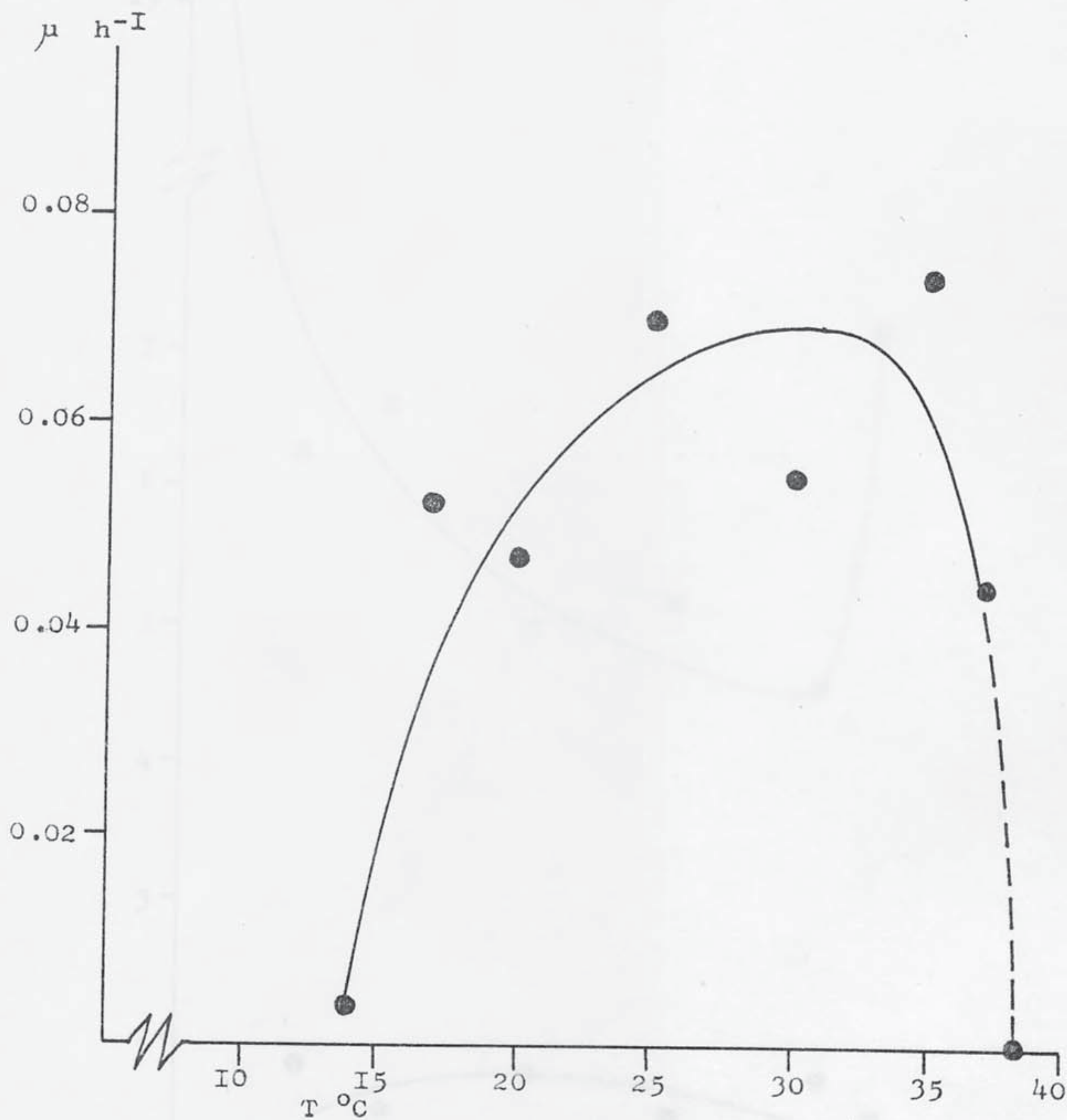
Fig 21 The Effect of pH upon the Fat Content of P. javanicum cultivated in a C.T.F. (data from Table 28)

Fig 22 The Effect of Temperature upon Lactose Utilisation by P. javanicum in a C.T.F. (data from Table 25)



[a] this point ignored see p.110

Fig 23 The Effect of Temperature upoh Growth Rate of P. javanicum in a C.T.F. (data from Table 25)



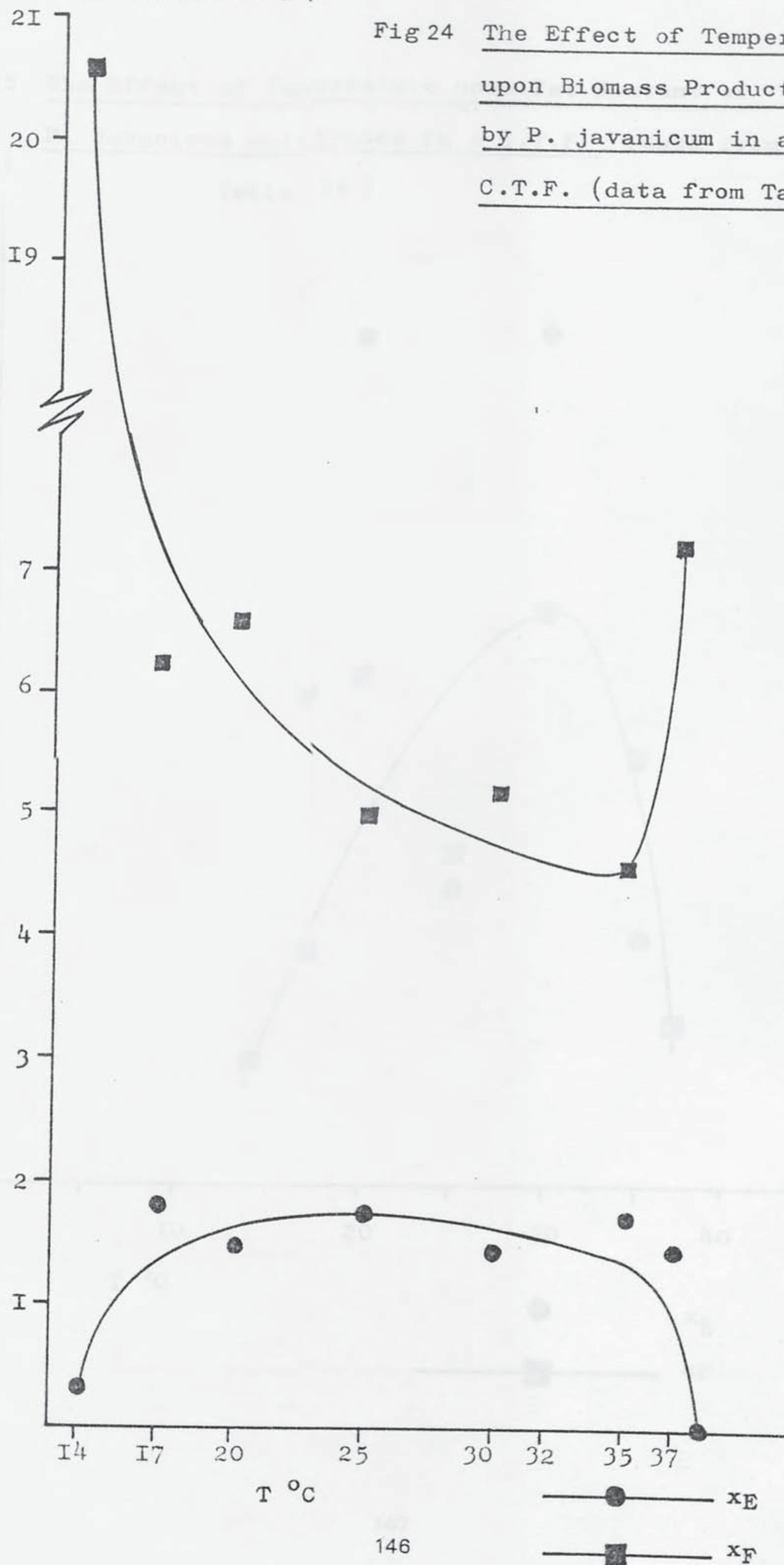
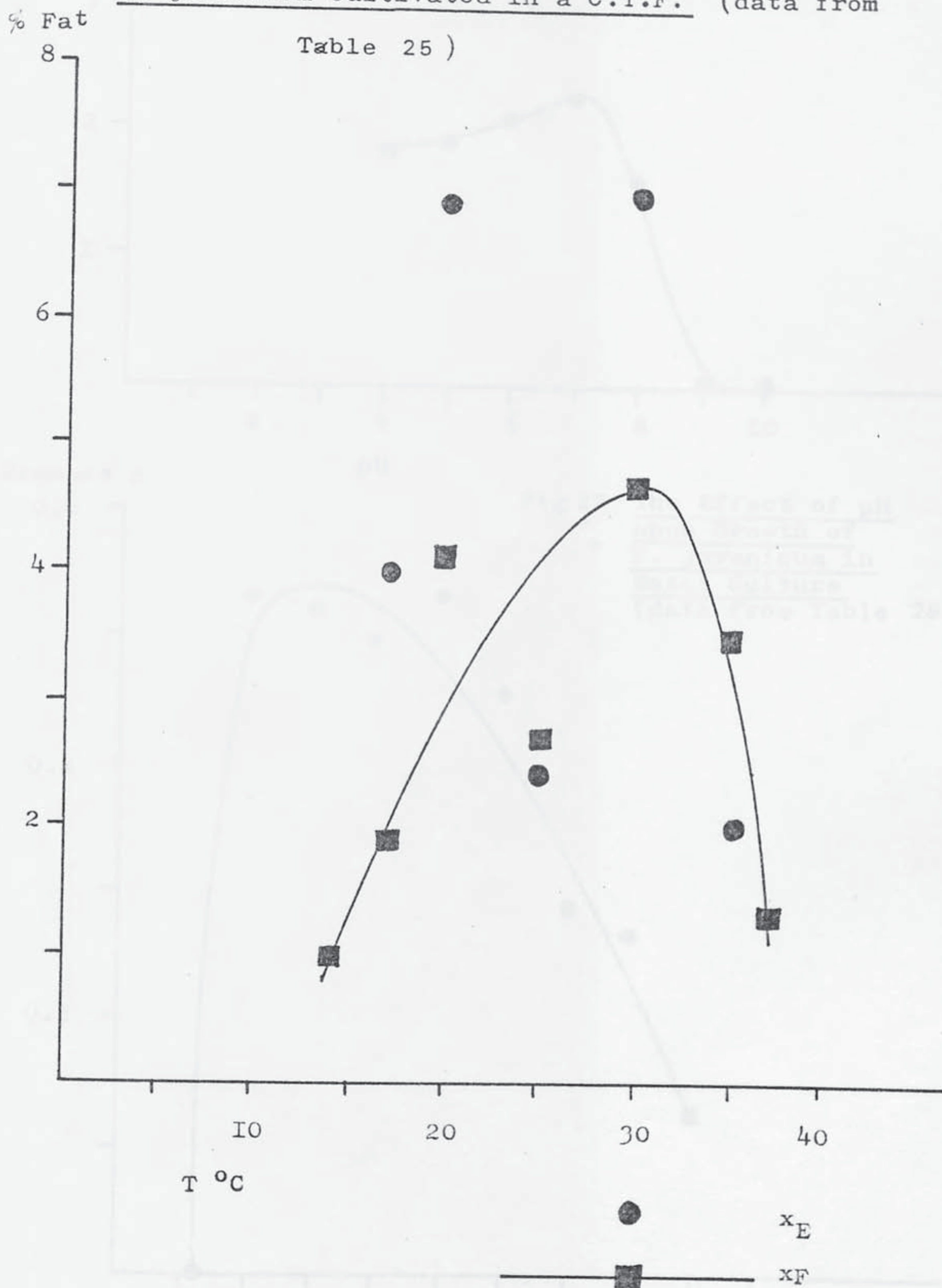


Fig 24 The Effect of Temperature upon Biomass Production by P. javanicum in a C.T.F. (data from Table 25)

Fig 25 The Effect of Temperature upon Fat Content of P. javanicum cultivated in a C.T.F. (data from Table 25)



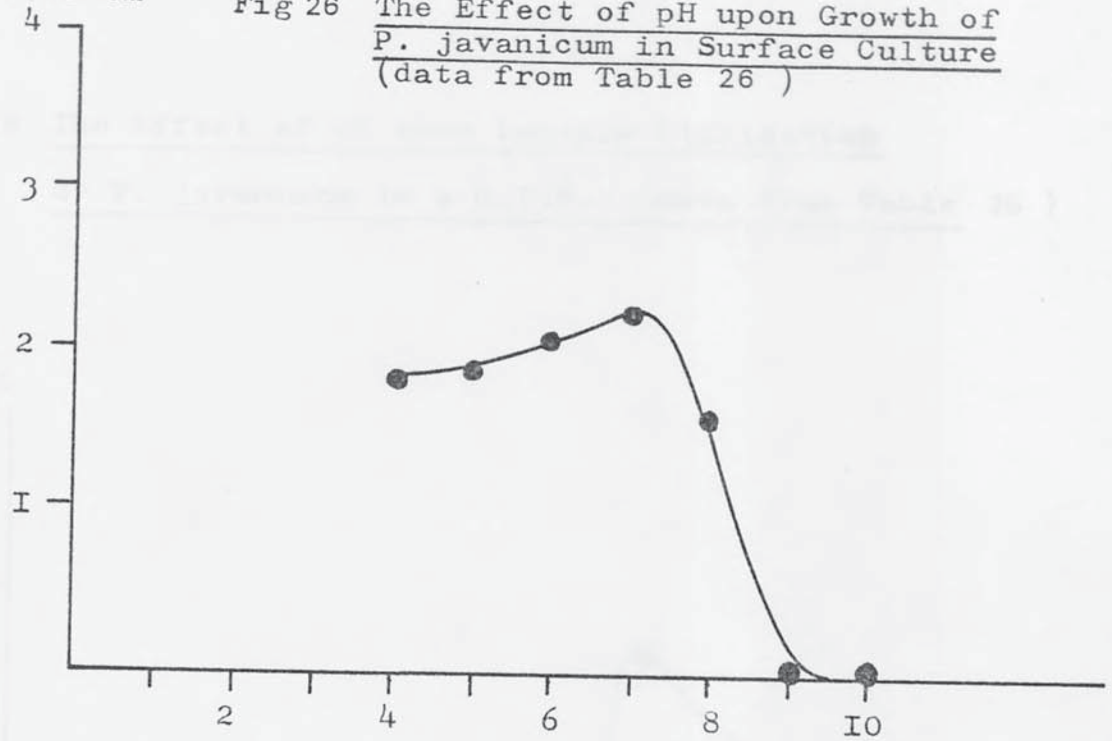


Fig 26 The Effect of pH upon Growth of P. javanicum in Surface Culture (data from Table 26)

pH

Biomass g

0.6

0.4

0.2

2 4 6 8 IO

pH

Fig 27 The Effect of pH upon Growth of P. javanicum in Batch Culture (data from Table 28)

Fig 28 The Effect of pH upon Lactose Utilisation by P. javanicum in a C.T.F. (data from Table 28)

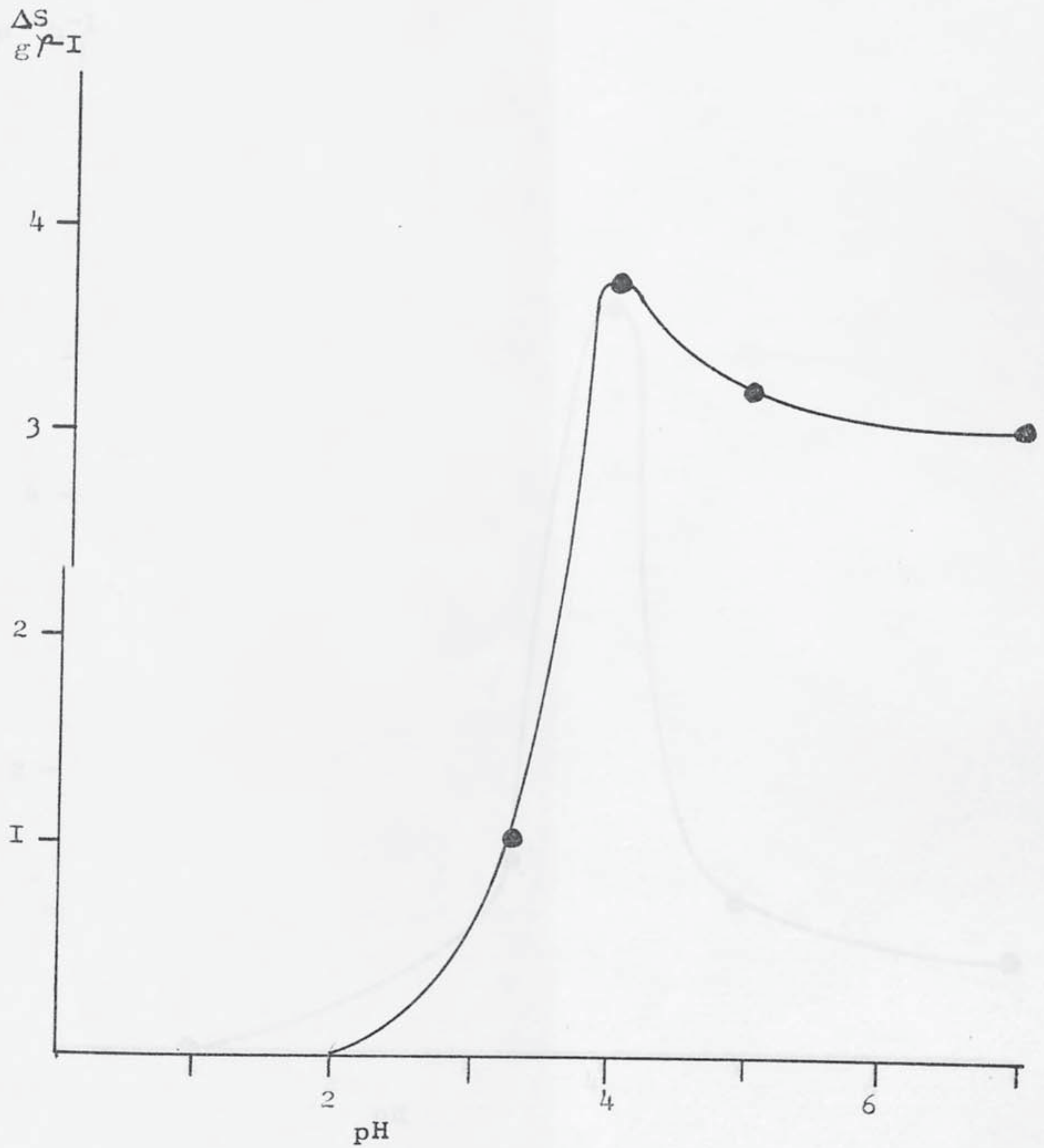


Fig 29 The Effect of pH upon Growth Rate of P. javanicum in a C.T.F. (data from Table 28)

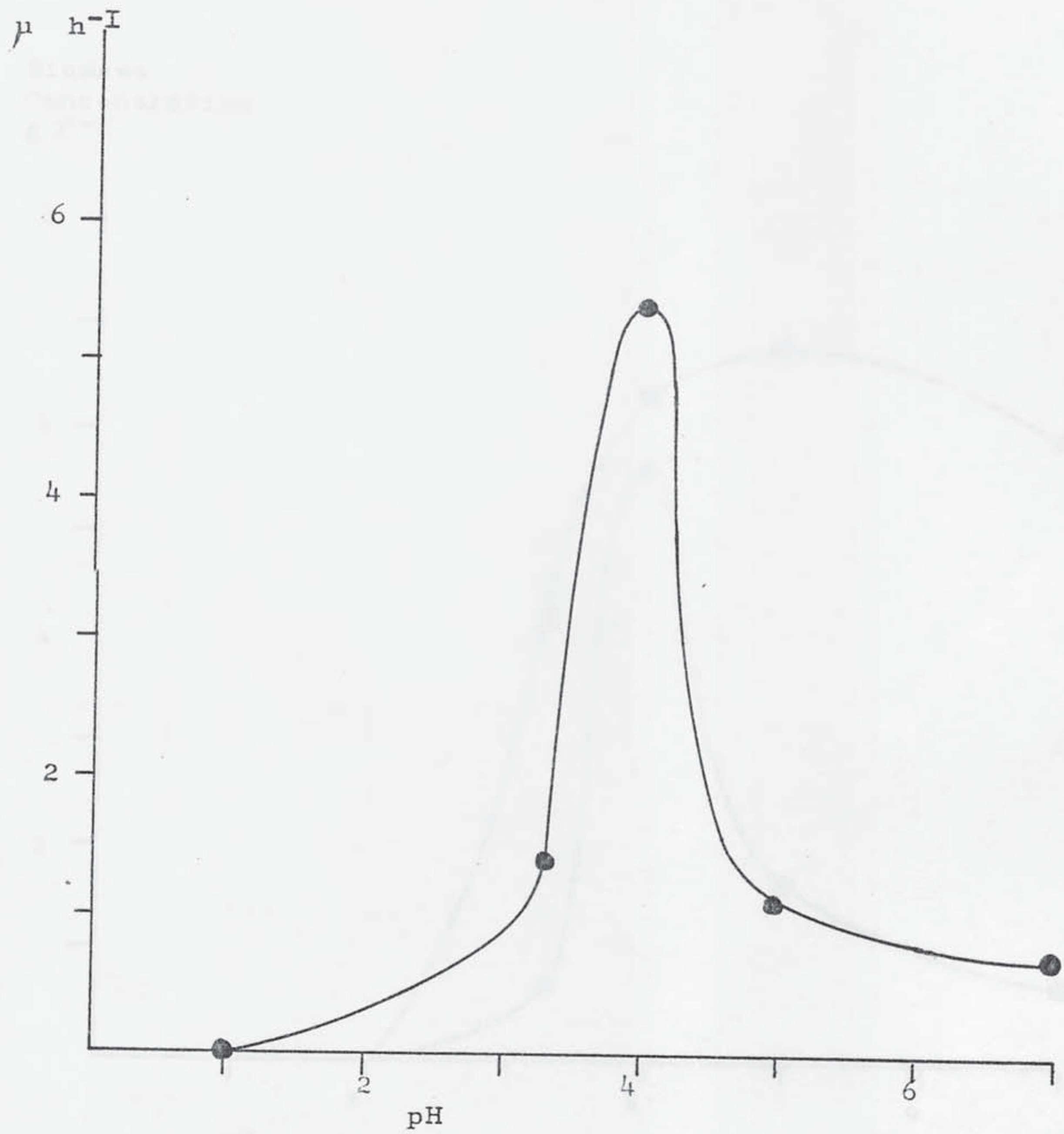
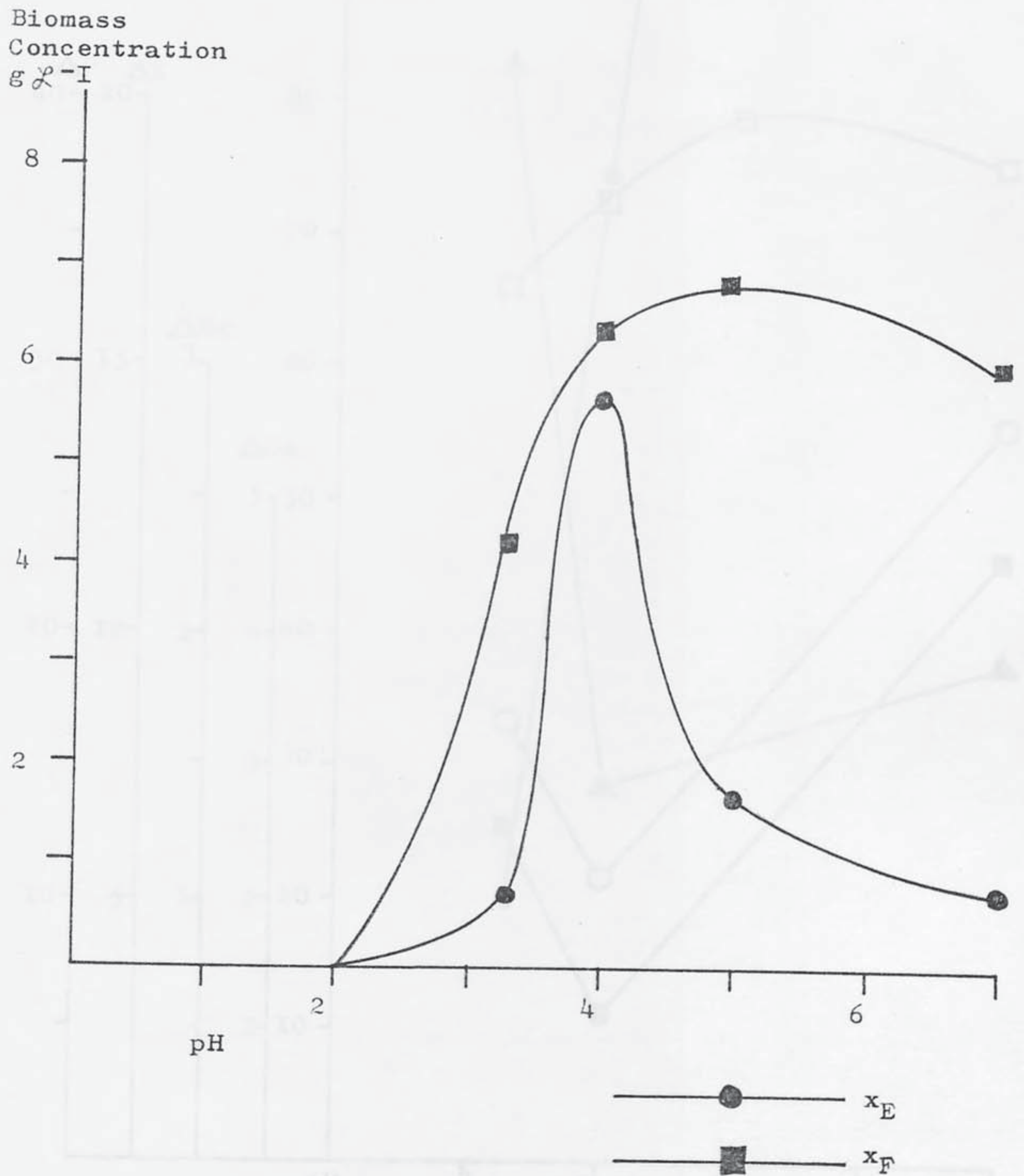


Fig 30 The Effect of pH upon Biomass Production by P. javanicum in a C.T.F. (data from Table 28)



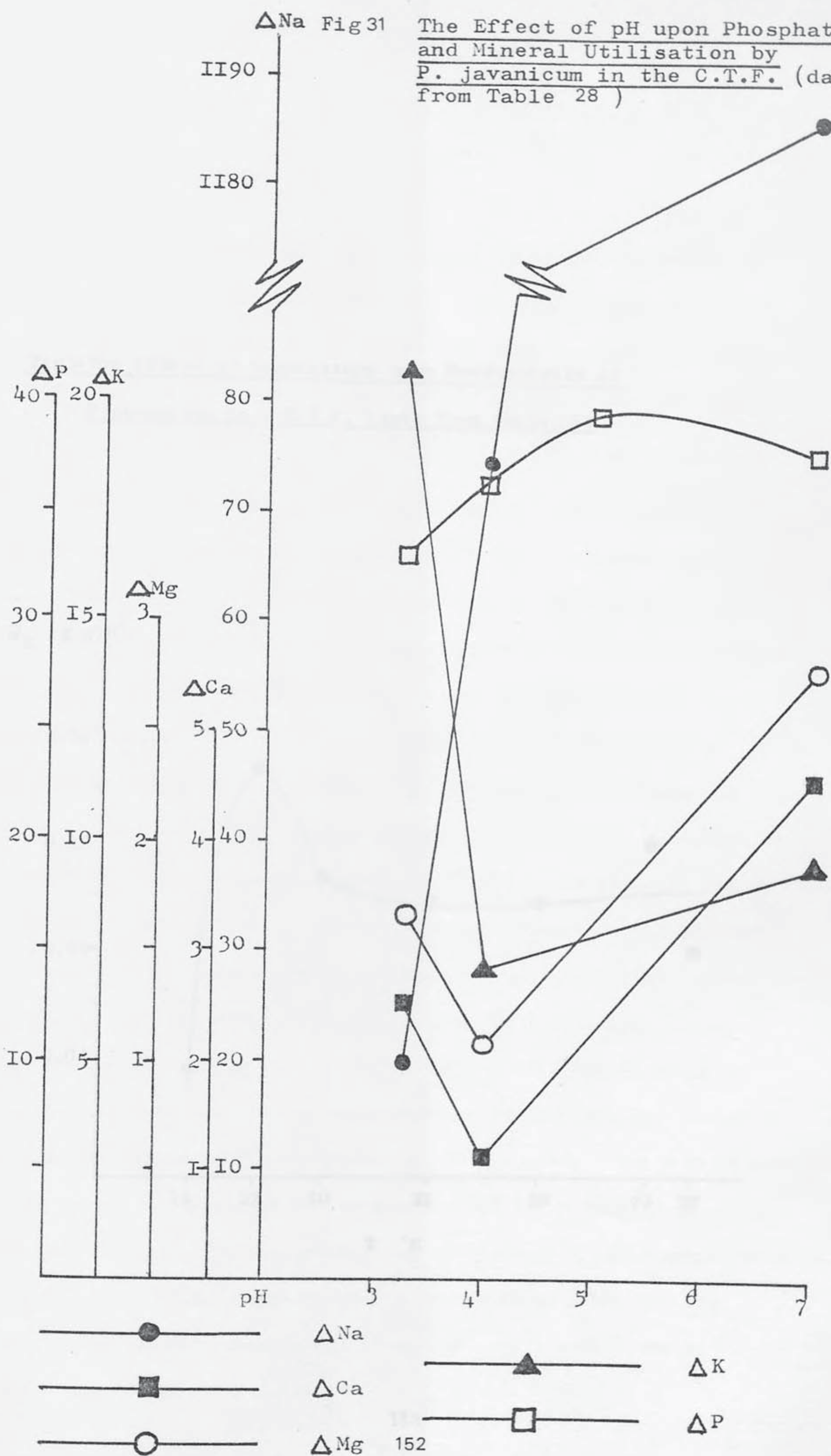
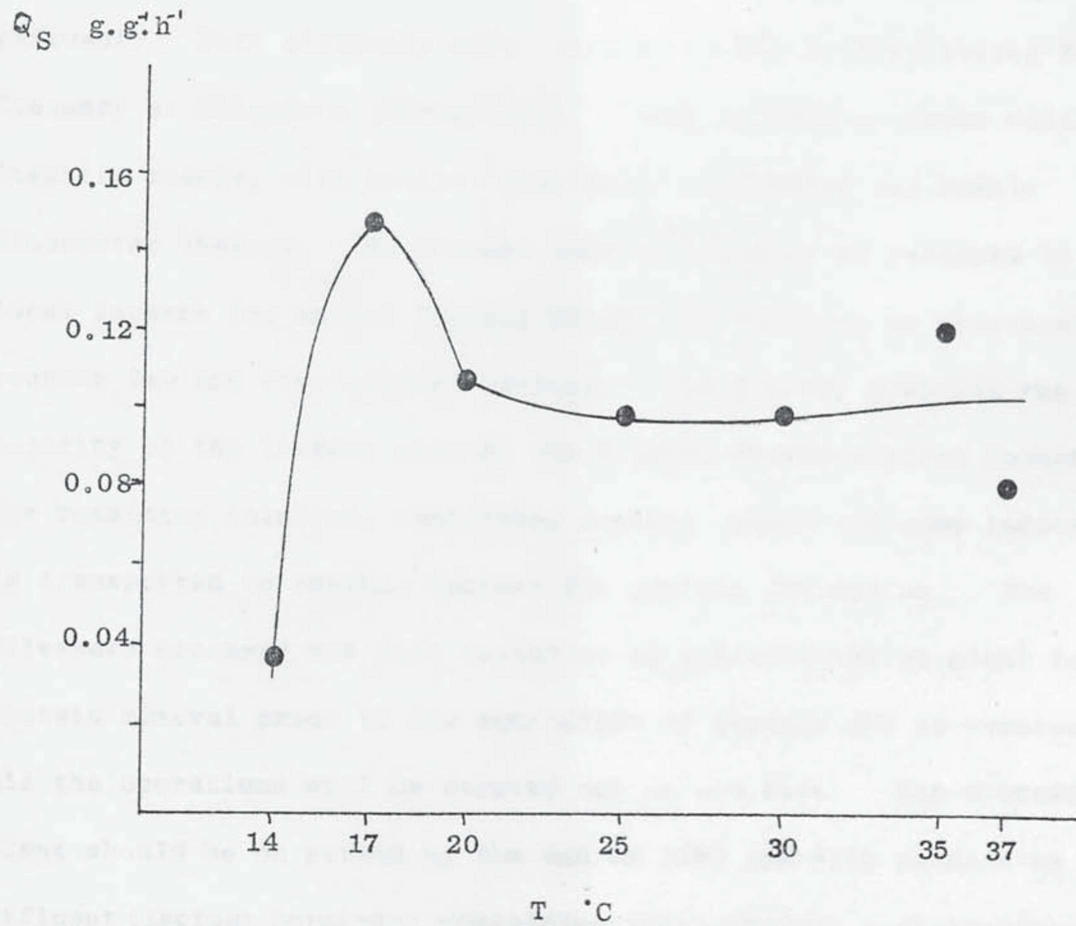


Fig 31 The Effect of pH upon Phosphate and Mineral Utilisation by P. javanicum in the C.T.F. (data from Table 28)

Fig34 The Effect of Temperature upon Productivity of *P. javanicum* in a C.T.F. (data from table 25)



4. Dairy Effluents

4.1 Introduction

These experiments were performed to determine the operating characteristics of the tower fermenter when P. javanicum is cultivated on dairy effluent. From this data an effluent treatment system could be designed with appropriate specifications for expected yield of biomass and treatment efficiency.

Two types of dairy effluent were chosen: defatted Cheshire cheese whey, providing an example of a "strong" effluent; and Whole Factory Effluent (WFE), an example of a large volume dilute effluent. Both effluents were supplied by the Milk Marketing Board Creamery at Ellesmere (Shropshire). This factory produces mainly Cheshire cheese, with smaller quantities of Cheddar and Double Gloucester cheeses. At present some of the whey is returned to local farmers for animal feeding whilst the majority is processed to recover fat and crystallised lactose. The factory supplies the majority of the lactose used by the British Pharmaceutical Industry. The remaining solution, containing protein, salts and some lactose, is transported to another factory for protein extraction. The Ellesmere creamery has just installed an ultrafiltration plant for protein removal prior to the extraction of lactose and so eventually all the operations will be carried out on one site. The ultrafiltration plant should be on stream by the end of 1980 and will produce an effluent (lactose permeate) containing approximately 4-5% lactose plus mineral salts. The creamery has, at present, no plans for the utilisation of the permeate and intends to pay a contractor to remove and "dump" it.

This permeate would make an admirable substrate for fermentation and initially it was intended to include it in this project, but unfortunately installation of the ultrafiltration plant fell almost a year behind schedule and thus the effluent was not available.

WFE derives from washings and spillages, although management are attempting to reduce the latter to a minimum. It is a dilute solution of whole milk, whey, detergent and sodium hydroxide used in cleaning operations. At present WFE is treated using in sequence an ICI Flocor tower, an activated sludge unit, trickle filters and lagooning to produce an effluent of acceptable standards of purity before discharge into a natural waterway. This treatment system is, however, working at or near the upper limit of its capacity and any future increase in either volume or concentration of the effluent will necessitate its upgrading.

A tower fermenter could be installed either to provide complete treatment of WFE, or more appropriately as a "roughing treatment" to lower the organic load before it enters the existing treatment system. Thus it was necessary to establish whether WFE is suitable for fermentation by P. javanicum in the tower fermenter and if so, what level(s) of treatment efficiency could be achieved.

4.2 Materials and Methods

Whey samples were collected after defatting, prior to the addition of formalin which is used by the creamery as a preservative. Samples were collected weekly and stored at 4°C until required. Providing the storage time did not exceed 6-7 days, no "souring" of the whey was observed. Fermentations were performed in the 5 litre tower fermenter (Fig. 5) previously described. The fermenter was operated, samples collected and analyses performed as described in Chapters 2 and 3. During whey fermentations the fermenter pH was monitored, but not controlled. Media (Appendix 1) were prepared as described in section 3.2, but with the substitution of liquid whey for lactose. During initial experiments it was observed that P. javanicum was utilisating part of the whey-protein nitrogen in addition to the inorganic nitrogen supplied in the medium, and thereafter medium and effluent nitrogen concentrations were determined by the microkjehdahl procedure (5.0cm^{3} samples were digested with 2cm^{3} of concentrated sulphuric acid).

WFE was collected from a sample point at the inlet to the existing effluent treatment system and was stored at 4°C until used. Within the periods of storage required for these experiments (no longer than 4 days) no observable deterioration in quality occurred. Analysis of a sample collected prior to the start of the experiments showed the effluent lactose concentration to be approximately 1.5g.l^{-1} and therefore WFE was fed directly to the fermenter, without dilution, at a dilution rate of 1.0h^{-1}. Results achieved with semidefined media (Chapter 3) demonstrated that the fermenter could not be operated at dilution rates lower than this if the medium lactose concentration was no greater than 1.5g.l^{-1}. Additional nutrients supplied with the

effluent in the medium are given in Appendix 1. During the first effluent fermentation, the pH was merely monitored and not controlled, but during the second it was controlled as described in Chapter 2. Fermentation operation, sample collection and analyses were performed as described in Chapters 2 and 3. In both whey and WFE fermentations, fermenter temperature was maintained at 30°C, with an aeration rate of 2.5 v.v.m.

4.3 Whey Fermentation Results

For a table of results see Appendix 4 (Table 29).

Morphology

At the lowest dilution rate investigated ($0.4h^{-1}$) the mould grew in the form of loose, almost filamentous, flocs approximately 0.2-0.3 x 0.1cm in size. As the dilution rate was raised ($0.5h^{-1}$ - $1.0h^{-1}$) the flocs became spherical and "hairy", that is mycelial filaments radiated out from a central region and were approximately 0.3cm in diameter. Above $1.0h^{-1}$, the flocs became somewhat inhibited, with dense centres and varied between 0.2 and 0.3cm in diameter.

It proved impossible to operate the fermenter at dilution rates outside the range $0.4h^{-1}$ - $1.6h^{-1}$; at the lower limit the fermenter was heavily infected, whilst the fermenter contents completely washed out within 48 hours at a dilution rate of $3.0h^{-1}$.

Copious foam was produced at the lower dilution rates investigated ($0.4h^{-1}$ and $0.5h^{-1}$) and also at the higher dilution rate of $1.6h^{-1}$, but was not of any significance when the dilution rate was $1.0h^{-1}$. Accompanying the production of foam was the presence of a heavy bacterial and yeast contamination.

Analyses

After a slight drop between dilution rates $0.4h^{-1}$ and $0.5h^{-1}$, lactose utilisation (dS) increased as the dilution rate was raised to $1.6h^{-1}$ (Fig. 36). Maximum growth rate was observed at $D=1.0h^{-1}$, the growth rate diminishing at dilution rates to either side of $1.0h^{-1}$ (Fig. 37). Therefore the pattern of growth of P. javanicum on whey

was similar to that observed on semidefined media, except that the range of dilution rates over which growth on whey took place was reduced compared with that of semidefined media.

Biomass production (dX_E) showed a steady decrease as the dilution rate was increased (Fig. 38). This trend was also observed on semi-defined media, although the actual concentrations of biomass produced from whey were higher than those from semidefined media, for example at $D=1.0h^{-1}$, $dX_E = 3.880g.h^{-1}$ (whey) compared with $dX_E = 2.622g.h^{-1}$ (semidefined medium). Fermenter biomass concentration (X_F) decreased slightly from $D=0.4h^{-1}$ to $0.5h^{-1}$, remained relatively constant until $D=1.0h^{-1}$ and thereafter increased rapidly to its highest concentration at $1.6h^{-1}$.

The X_E crude protein content was approximately 48% and did not vary appreciably with dilution rate, but X_F crude protein content increased slightly from 44% to 48% as the dilution rate was raised from $0.4h^{-1}$ to $1.6h^{-1}$. Nitrogen ultisation (dN) was at a minimum whereas utilisation of the four mineral ions were at maxima at $1.0h^{-1}$. Phosphate utilisation declined from a dilution rate of $0.4h^{-1}$ to $0.5h^{-1}$, but then increased rapidly as the dilution rate was raised to $1.0h^{-1}$. Unfortunately, samples collected at $1.6h^{-1}$ were lost, and therefore it was impossible to determine whether phosphate utilisation continued to rise (Figs. 39, 41, 42).

The fat contents of X_E and X_F biomass were not identical - X_E consistently contained a higher proportion of fat than did X_F. There was a steady increase in the X_E fat content as the dilution rate was increased. In contrast the X_F fat content remained constant from $0.4h^{-1}$ to $1.0h^{-1}$, but then increased as the dilution rate was raised

to $1.6h^{-1}$. X_E fat content ranged between 1.28% and 2.86%, the mean being 2.05% whilst X_F fat content ranged between 0.89% and 1.70%, averaging 1.09% (Fig. 40).

4.4 Whey Fermentation Discussion

Morphology

The morphology of P. javanicum when cultivated on Cheshire whey was similar to that exhibited by the mould when grown on a semi-defined medium. The formation of flocs (pellets) was discussed in Chapter 3. The advantage of pelleted growth is that harvesting is made much more simple and cheap. Sedimentation or filtration can be used, and the centrifugation techniques developed for harvesting yeast SCP's would not be required.

Lactose Utilisation

Lactose utilisation by P. javanicum from Cheshire whey was greatest at a dilution rate of $1.6h^{-1}$. However, by this dilution rate the fermentation was heavily infected and the appearance of the fermenter contents suggested that the mould was not particularly healthy. A dilution rate of $1.6h^{-1}$ would not, therefore, be the best choice for a commercial system, which would be better operated at $1.0h^{-1}$, at which dilution rate significant contamination did not occur.

These results were in contrast to those found with semidefined media, on which maximum lactose utilisation occurred between $4.0h^{-1}$ and $5.0h^{-1}$. Both the upper and lower limits of dilution rate at which the fermenter could be operated on whey appeared to be determined by the level of foaming. It should, therefore, be possible to operate

the fermenter outside the dilution rate range used in these experiments providing the contamination level is reduced. This could be achieved by protein removal, thus reducing foaming, or by sterilisation.

Lactose utilisation at a dilution rate of $1.0h^{-1}$ was higher on whey than on semidefined media ($6.062g.h^{-1}$ compared with $4.302g.h^{-1}$). Extra growth factors supplied by the whey may have enable P. javanicum to grow more rapidly. Milk contains potassium, calcium, sodium, magnesium, chloride and phosphate ions, and although part of the calcium and phosphate are retained in the cheese, the remainder are present in whey in virtually the same quantities as in whole milk. Guy and Bingham (1978) reported that the activity of Saccharomyces lactis lactase was accelerated slightly by the potassium and magnesium ions, whereas it was significantly depressed by sodium and calcium ions. Apart from calcium, the semidefined medium upon which P. javanicum was cultivated contained appreciable quantities of these ions, but they were supplied in greater amounts in the whey medium which contained the extra whey minerals (potassium $181.00mg.l^{-1}$: $62.07mg.l^{-1}$; Magnesium $12.40mg.l^{-1}$: $4.16mg.l^{-1}$) Assuming that the lactase activity of P. javanicum is affected by these ions in the same manner as that of S. lactis, then it is possible that the positive effects of higher levels of potassium and magnesium may have compensated for the negative effects of sodium and calcium, leading to a greater lactose utilisation.

One other experimental condition which differed slightly between the whey and semidefined media fermentations, was the fermenter-broth pH, which was uncontrolled in both cases. For example, at $D=1.0h^{-1}$, on whey the pH value was 4.1, but on semidefined media it was 3.3.

The whey medium may have possessed more buffering capacity, and thus the pH value may not have been lowered by the growth of P. javanicum to such an extent as occurred with the semidefined medium. This pH difference could explain the observed difference between lactose utilisation rates in the two media, since it was found in Exp. 3.4.3.3 (the effects of pH upon the growth of P. javanicum in semidefined media) that lactose utilisation was increased by a factor of three when the pH was raised from 3.3 to 4.1. The higher lactose utilisation measured in a whey medium could, therefore, be a result of the slightly less acid pH value.

Growth Rate

A similar pattern of growth was observed when P. javanicum was cultivated on both whey and a semidefined medium, although the range of dilution rates over which the mould was able to grow was reduced. At a dilution rate of $1.0h^{-1}$, the growth rate on whey was $0.259h^{-1}$, compared with $0.174h^{-1}$ on the semidefined medium.

Effluent Biomass Concentration

The more rapid growth rate of P. javanicum on whey compared with semidefined medium was also reflected in a higher rate of biomass production. Biomass production was maximal at a dilution rate of $0.4h^{-1}$, but at this rate the fermenter was heavily infected, whereas at a dilution rate of $1.0h^{-1}$, when biomass production was only slightly reduced, infection was not a problem. Consequently on the industrial scale it would be preferable to operate the fermenter at $1.0h^{-1}$, sacrificing some productivity for a more stable fermentation. At this dilution rate 14.175g. (dry weight) of biomass was produced from

1 litre of whey, which is somewhat lower than the yields reported for yeasts from whey, for example 22.3g per litre by Kluveromyces fragilis (Terra, 1976). Alternatively, the yield can be expressed as 1.134g (dry weight) of biomass produced from 1.480g of whey lactose, a conversion efficiency of 76.62% (the conversion efficiency in semidefined media at the same dilution rate was 60.95%). If the total whey lactose supplied to the mould is used to calculate the yield, the conversion efficiency is much lower - 34.56% (1.134g. biomass from 3.281g. lactose).

It is well recognised that yields from filamentous fungi are lower than yields from yeasts, but lower productivity may be offset by the economic advantages of cheaper harvesting (Solomons, 1975; Pannell, 1976).

Fermenter Biomass Concentration

Fermenter biomass concentrations were of the same order as concentrations measured at corresponding dilution rates on semidefined media. This provides support for the hypothesis advanced by Pannell (1976) that the tower fermenter can physically retain a defined quantity of biomass.

Growth rate of the mould was higher on whey compared with semi-defined media, and therefore the extra growth contributed to the observed increase in effluent biomass production (dX_E). The curve for X_F relative to dilution rate (Fig. 38) is of the same shape as the equivalent portions of Fig. 12 (dotted line) and the fermenter may, therefore, have been exhibiting characteristics of the second stage of a multistage tower fermenter. Alternatively, the rise in X_F at $D=1.6h^{-1}$ may have resulted from the morphology of the mould. The

flocs were of approximately the same diameter as those produced at lower dilution rates, but possessed dense centres and were observed to sediment more rapidly when removed from the fermenter and placed in a cylinder of spent medium; thus their mass was larger. The internal biomass recycle theory discussed in Chapter 3 predicts that if floc mass increases to the point where the liquid velocity is insufficient to carry the flocs out of the fermenter, the biomass concentration within the fermenter will rise. Therefore, the apparent increase in fermenter biomass concentration may have been a direct result, not of the higher dilution rate, but rather of the floc morphology which prevailed at that dilution rate.

Biomass Crude Protein Content

There was almost no variation in biomass (X_E) crude protein content over the range of dilution rates investigated. The crude protein content was approximately 48%, and thus higher than that of soya bean meal.

Nitrogen Utilisation

It was noteworthy that although the biomass crude protein content remained relatively constant, the nitrogen utilisation rate varied considerably, therefore under some conditions a larger proportion of the nitrogen being removed from the medium was not being incorporated into protein. Since only protein nitrogen was analysed it is possible that the remainder of the nitrogen was incorporated into non-protein nitrogenous substances such as chitin in the cell wall.

At a dilution rate of 1.0h^{-1} nitrogen utilisation was at a minimum without affecting the biomass crude protein content. This will

confer economic advantages to a commercial fermentation system because savings can be made on added medium nitrogen.

The large error calculated for dN at D=0.4h^{-1} probably results from the effects of the heavy contamination. The infecting microorganisms were probably utilising some of the nitrogen and thus contributed to the observed results. However, because their population was not necessarily stable, the amount of nitrogen utilised by them was somewhat variable.

Biomass Fat Content

The observed rise in biomass (X_E) fat content as the dilution rate increased corresponded to an increase in lactose utilisation.

The increase in X_F fat content which occurred between 1.0h^{-1} and 1.6h^{-1} accompanied a drop in the growth rate of P. javanicum. Two factors may have contributed to this. Firstly as growth slows, the average age of each floc will increase and therefore more fat can be deposited in the cells. Secondly, when the biomass is growing rapidly, the carbon source will be used to provide energy and materials for growth, but as growth slows, there will be less demand for these and they can be converted into storage materials - fat.

P. javanicum when cultivated on whey at D=1.0h^{-1} contained a slightly higher percentage of fat compared with biomass produced on semidefined medium (2.42% : 1.14%). It should be remembered that these figures represent the lowest proportion of fat to be expected because of the analytical method employed. Previous work with P. javanicum produced high mycelial fat contents in batch culture (Lockwood et al, 1934; Ward et al, 1934; Ward et al, 1935,

Fink, Haehn and Hoerburger, 1937) but it was not continued because the growth rate of the mould was considered to be too low. The strain of *P. javanicum* used in this project attained growth rates comparable to those of another filamentous fungus (*A. niger*) in the CTF (Pannell, 1976; Stockbridge, 1979). The mould may, therefore have used its supplies of metabolic energy, precursors etc. to grow rapidly rather than to produce a reserve carbon store.

Phosphate and Mineral Utilisation

Utilisation rates of all four mineral ions followed the same pattern: they were maximal at the dilution rate where μmax occurred. Mineral concentrations were higher in the whey than in the semidefined medium because of the extra whey minerals. Utilisation rates of the four minerals at $D=1.0h^{-1}$ were also considerably higher on whey compared with semidefined medium, for example, calcium utilisation rates were $69.67mg.h^{-1}$ and $10.31mg.h^{-1}$ respectively. Higher medium mineral concentrations may have stimulated the greater rate of lactose utilisation, resulting in an increase in growth rate and hence demand for the ions.

Unfortunately, samples from only three dilution rates were available for phosphate analysis, and so these results should be interpreted only as indicators of a possible trend which is that phosphate utilisation from whey medium follows the same pattern as on semidefined medium.

4.5 Whey Fermentation Summary and Conclusions

During fermentations some difficulties with foaming were encountered. This problem has been noted previously when fermenting

whey - Perzow (1974) observed that vigorous aeration (2.5 v.v.m) of whey produced a heavy foam, necessitating the use of some type of antifoam system. Kountz (1956) also reported that foaming caused operational problems when whey was fermented in a mechanically stirred system at pilot-plant scale. He suggested that excessive agitation or aeration was one of the causes. Proteins are good foaming agents, and consequently the agitation of whey proteins in the tower fermenter produced a foam. This foaming could be controlled by operating the fermenter with only slight lactose excess, that is sufficient whey was added to the medium such that the effluent contained only a small amount of unused lactose. In practice, this was achieved by ensuring that the medium whey concentration did not exceed 10% (volume per volume). This imposed a lower limit on the dilution rate at which the fermenter could be operated without a foam-control system. At the lower dilution rates which were investigated ($0.560h^{-1}$ and $0.409h^{-1}$) foaming was a serious problem, but at $1.0h^{-1}$ with a medium whey concentration of 8%, foaming was of no significance. This was not the case at more rapid medium flow rate; at $D=1.643h^{-1}$ it was necessary to incorporate an antifoaming agent in the medium. The use of chemical antifoams in industrial fermentations is undesirable, not only because of the increased operating cost, but also because their presence causes reduced oxygen transfer and may lead to the fermentation becoming oxygen-limited (Morris, 1973). Thus a dilution rate of $1.0h^{-1}$ would be the most practicable choice for a commercial tower fermentation system producing SCP from whey.

At this dilution rate the growth rate was at a maximum. Lactose utilisation was greatest at $1.6h^{-1}$, and biomass production at $0.4h^{-1}$, but at both these dilution rates problems with foaming and contamination arose, consequently some lactose utilisation and biomass production

must be sacrificed when the fermenter is operated at $1.0h^{-1}$.

At this dilution rate P. javanicum produced 14.2g. (dry weight) of biomass from 1 litre of whey. The biomass contained 48% crude protein and (at least) 2.4% fat and thus should find a ready market as an animal feed.

Operation at $1.0h^{-1}$ would allow economies to be made in the use of nitrogen supplements, because nitrogen utilisation was at a minimum at this dilution rate.

In summary therefore, a commercial tower fermentation system for the cultivation of P. javanicum on whey to produce SCP should be operated at a dilution rate of $1.0h^{-1}$.

A more detailed analysis of the biomass produced at this dilution rate is given in Chapter 5.

4.6 Whole Factory Effluent Fermentations

Results and Discussion

Two attempts, both unsuccessful, were made to cultivate P. javanicum on whole factory effluent (WFE) in a laboratory scale tower fermenter. In the first fermentation WFE plus mineral salts (Appendix 1) gave a pH of 7.1 and thus the fermenter was operated with no pH control. The experiment was initiated by substituting the WFE-medium for semidefined medium (Appendix 1, Exp. 3.4.3.3), both supplied at a dilution rate of $1.0h^{-1}$, once the mould was established in the fermenter. It was impossible to perform "start-up" from spores because this requires aseptic operation. This was not possible when fermenting such large volumes of a dilute lactose solution. Over the first 48 hours the fermenter biomass concentration gradually decreased, and by 72 hours the mould had completely washed out of the fermenter. The WFE medium lactose concentration was $2.5gl^{-1}$ and therefore carbon-limitation was not considered to be the cause of washout.

Apart from the possibility that the WFE contained substances inimical to the growth of P. javanicum, a more likely explanation of washout was that the pH was insufficiently acidic for good growth of the mould. Consequently, for the second attempt at fermentation of WFE the pH control system of the fermenter was adjusted to control the pH at 5.0, this representing a compromise between economising on the use of acid and selecting the optimum pH value (determined on semi-defined media) for growth, lactose utilisation and biomass production by P. javanicum. Start-up was accomplished in the same manner as in the first fermentation. Unfortunately in this fermentation the mould washed out within 48 hours, probably because the fermentation was

carbon limited - this particular batch of WFE contained only $0.153g.l^{-1}$ lactose.

The main problem hindering the fermentation of WFE appears, therefore, to be its variability. Three batches collected at approximately weekly intervals were analysed for lactose concentration and pH value, the results of which are presented in Table 16.

Table 16

Composition of Whole Factory Effluent

Batch	pH	Lactose concentration, $g.l^{-1}$
1	10.1	1.571
2^{a}	7.1	2.500
3^{b}	8.5	0.153

a used in first fermentation

b used in second fermentation.

The high pH of batch 1 resulted from operator error at the creamery - a "slug" of hydroxide had been permitted to enter the effluent treatment system. This happens fairly regularly at the creamery, despite efforts by management to restrict its occurrence since the resulting high pH value of the effluent causes reduced operating efficiency of the existing effluent treatment system.

High pH value would make it mandatory for pH control to be used if the effluent was to be treated in a tower fermenter. This could be accomplished via direct addition of acid into the fermenter, or into a balance tank prior to entry into the fermenter. The problem of variable feed lactose concentration might be overcome by installing a high degree

of control over the medium flow rate (dilution rate). Thus if the lactose concentration dropped the dilution rate could be increased rapidly, and vice-versa, ensuring supply of sufficient lactose to the fungus. If it was considered undesirable to reduce the dilution rate below a certain point, the same effect could be achieved by recycling part of the spent WFE from the fermenter outlet. This would require a high degree of automatic control over pumps, switching, etc., The main impediment would be continuously or semicontinuously monitoring the lactose concentration. The phenol-sulphuric method used in this study requires 25 minutes to perform, approximately 20 minutes of which is waiting for the colour to develop. Enzyme assays can be more rapid, but still are not particularly amenable to automation. Depending upon the type of effluent and conditions prevailing at a creamery, it might be possible to perform the analysis at regular times throughout the day - perhaps once every 2 or 4 hours - and then to adjust the dilution rate manually. If these two objectives of pH and lactose concentration control within the fermenter could be achieved, it might be possible to successfully ferment WFE in a tower fermenter. This was impossible to test in the laboratory because of the logistical difficulties of collecting sufficient volumes of effluent.

Fig 36 The Effect of Dilution Rate upon Lactose Utilisation by P. javanicum Cultivated on Whey in a C.T.F.

(data from Table 29)

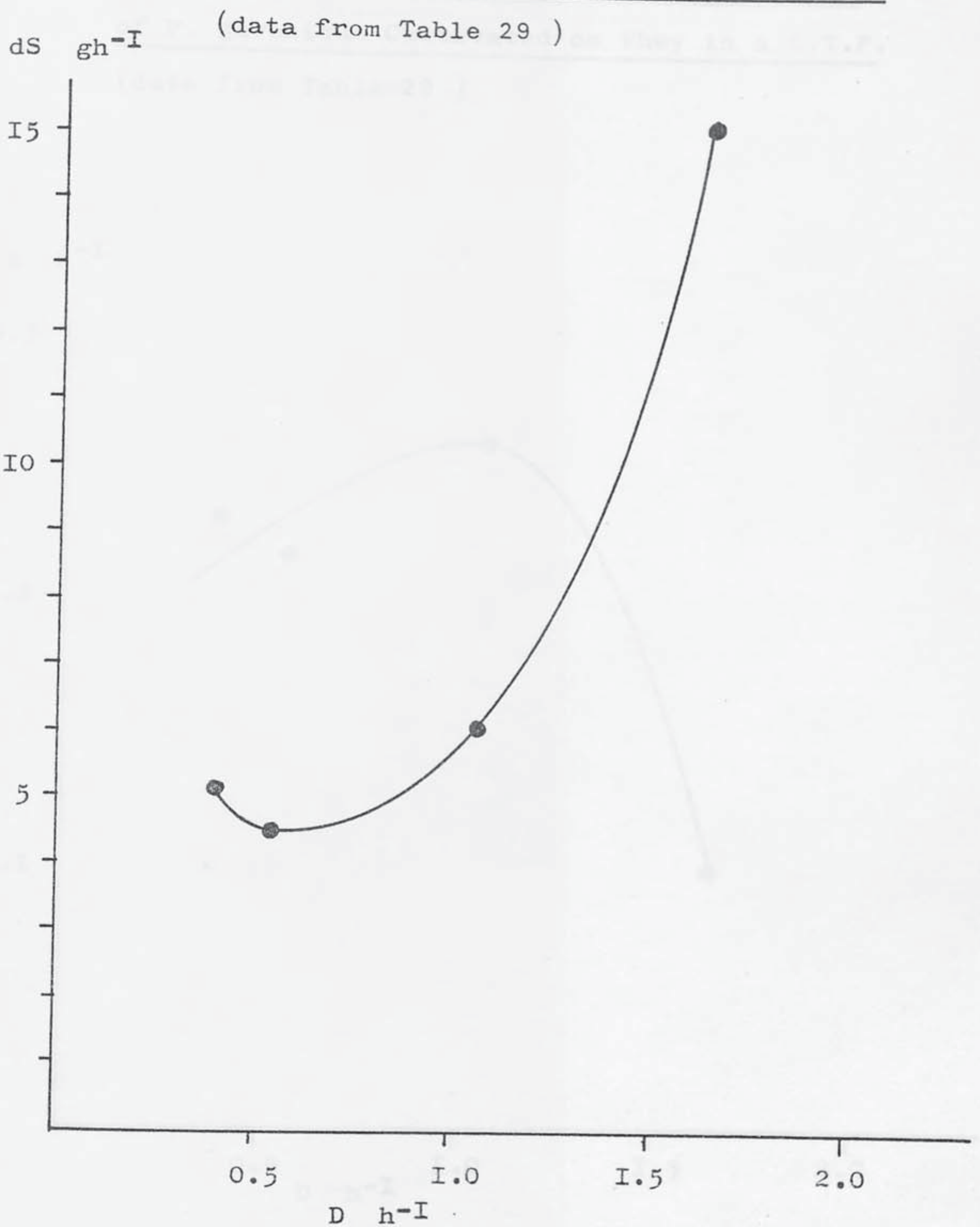


Fig 37 The Effect of Dilution Rate upon Growth Rate of P. javanicum Cultivated on Whey in a C.T.F. (data from Table 29)

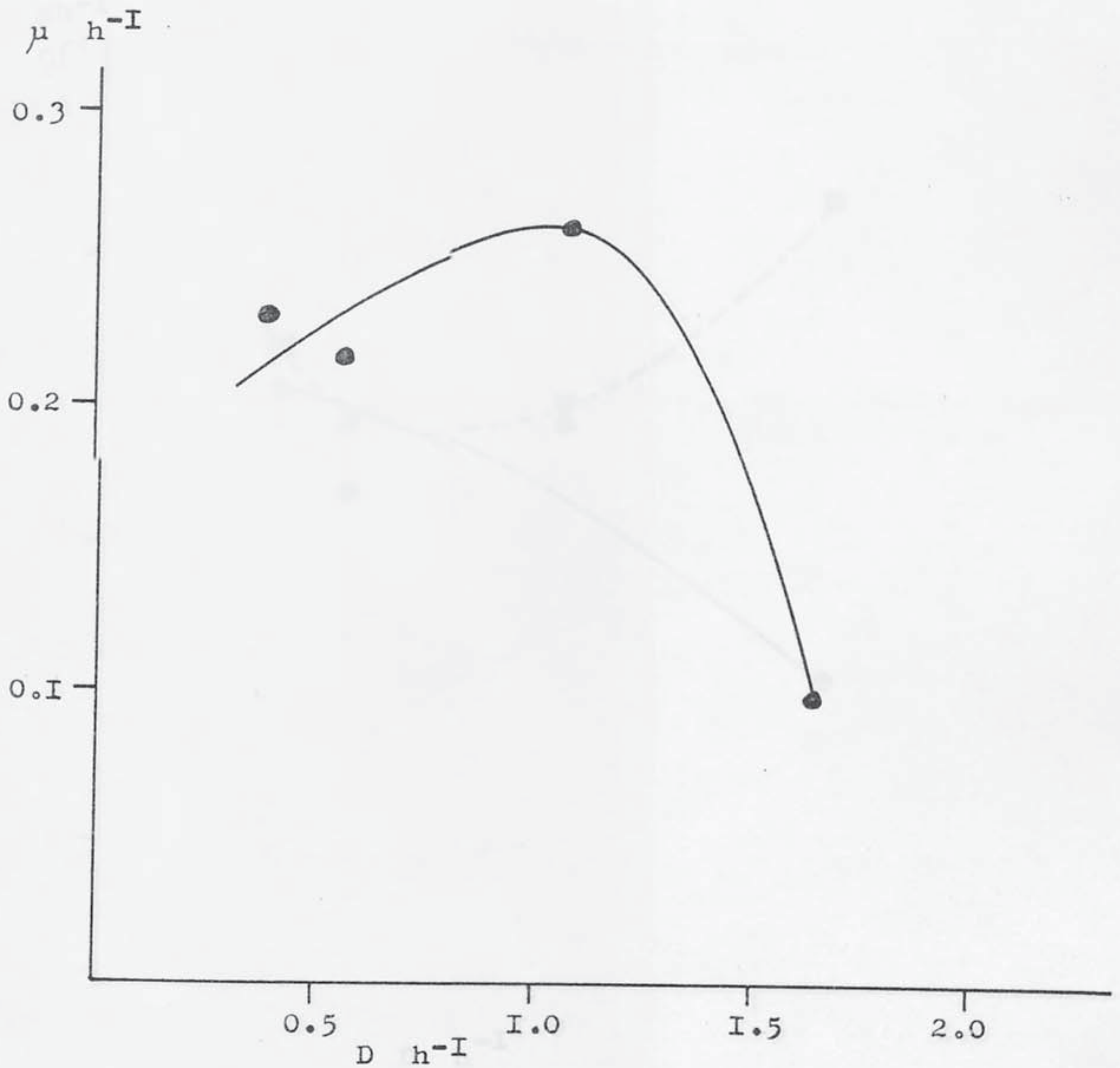


Fig 38 The Effect of Dilution Rate upon Biomass Production by P. javanicum Cultivated on Whey in a C.T.F.

(data from Table 29)

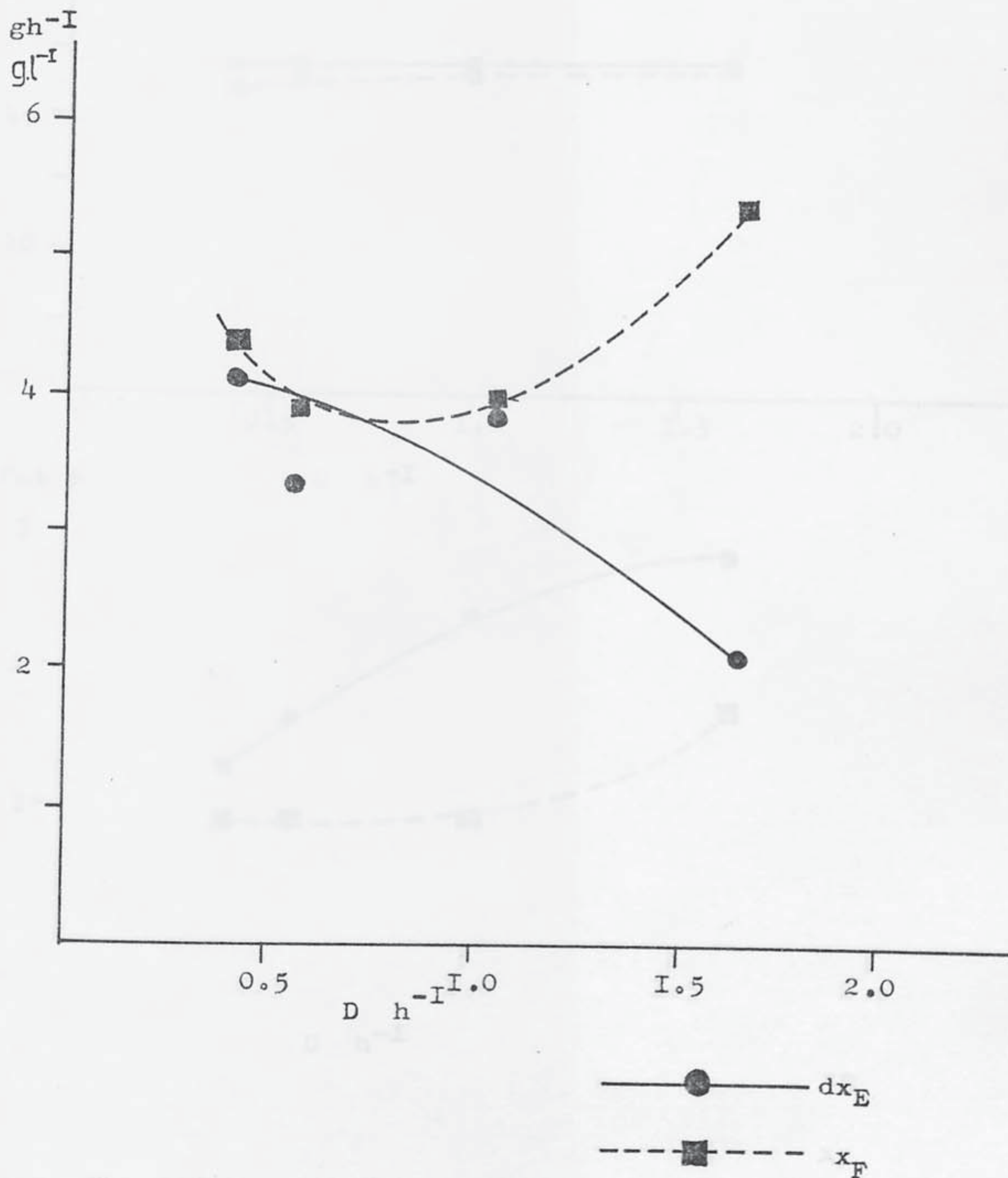
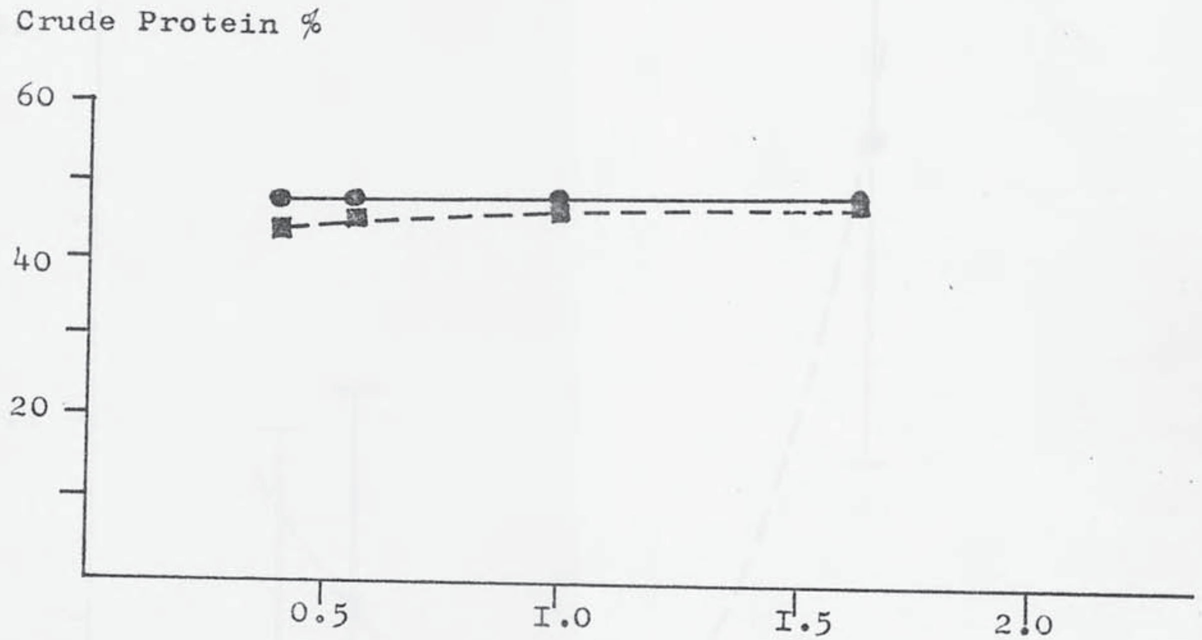


Fig 39 The Effect of Dilution Rate upon the Crude Protein Content of P. javanicum Cultivated on Whey in a C.T.F. (data from Table 29)



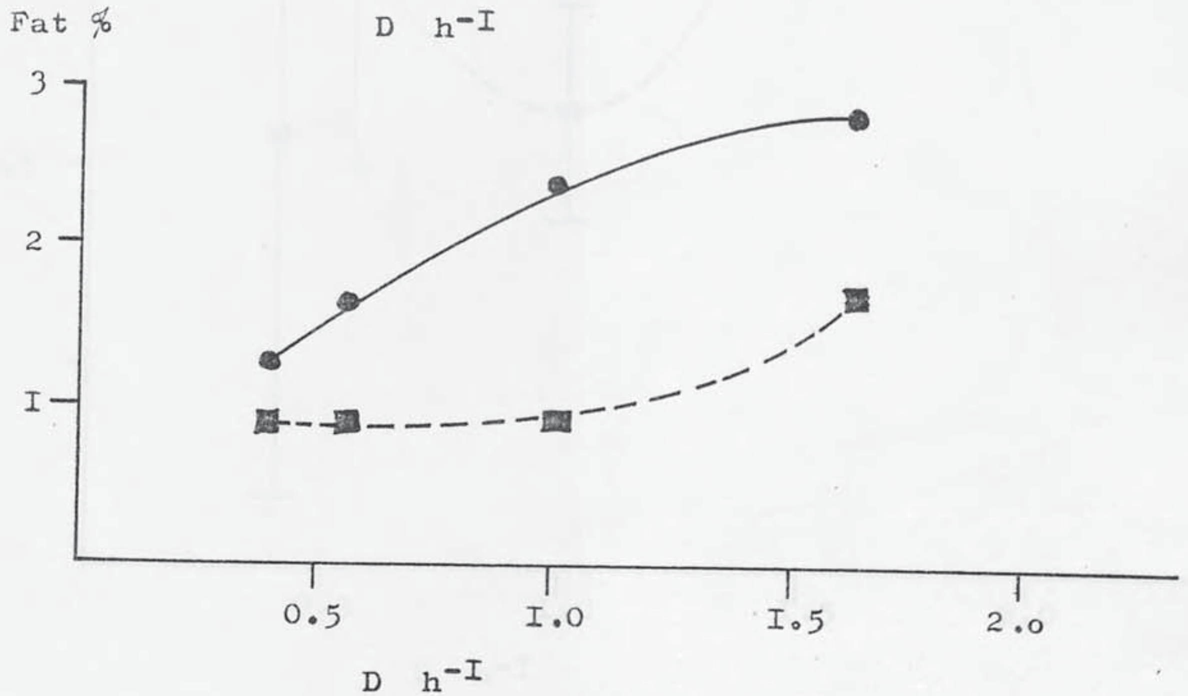


Fig 40 The Effect of Dilution Rate upon the Fat Content of P. javanicum Cultivated on Whey in a C.T.F. (data from Table 29)

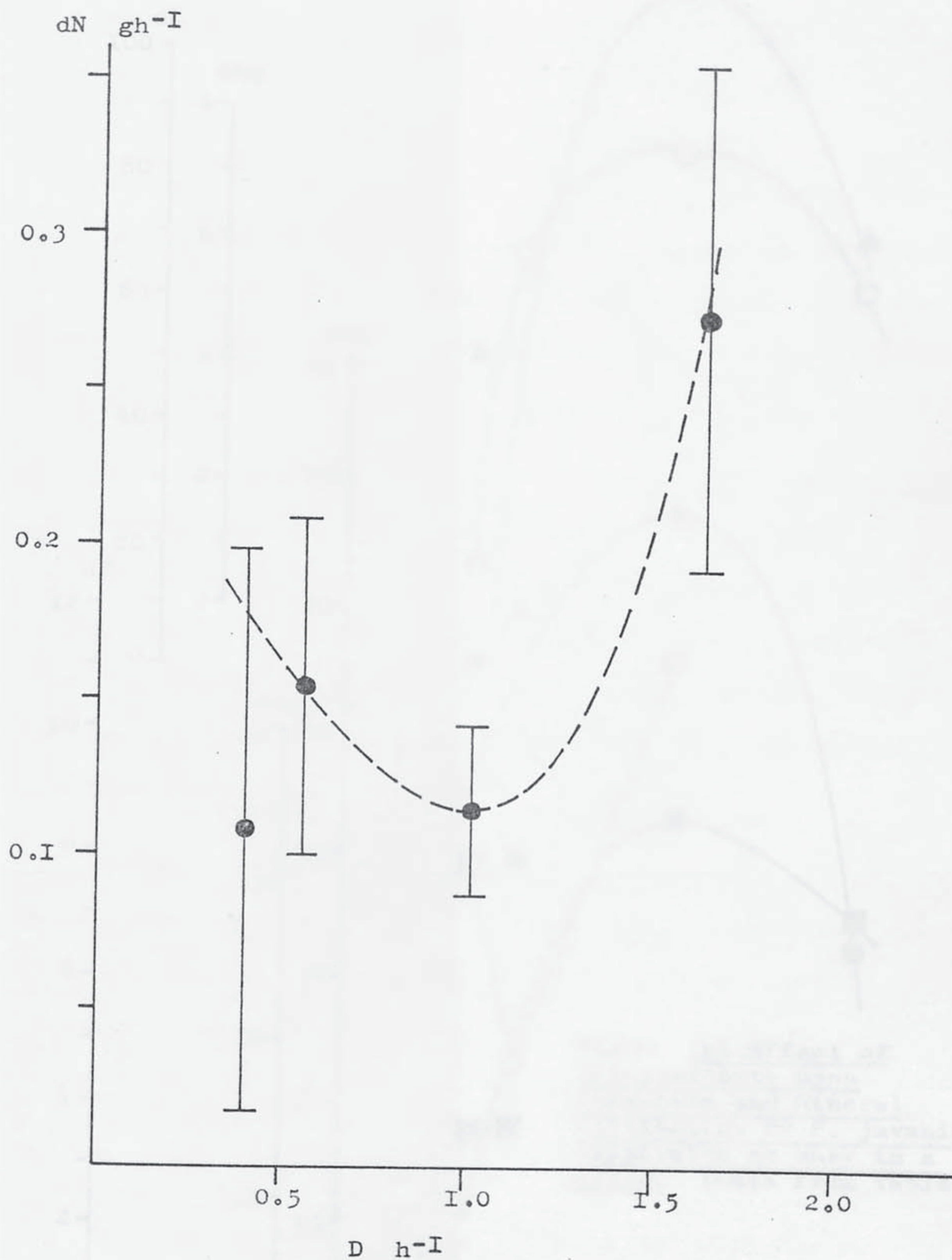
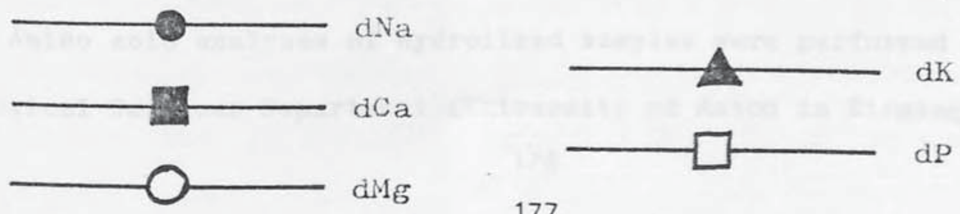


Fig 41 The Effect of Dilution Rate upon Nitrogen Utilisation by P. javanicum Cultivated on Whey in a C.T.F. (data from Table 29)

Fig 42 The Effect of Dilution Rate upon Phosphate and Mineral Utilisation by *P. javanicum* Cultivated on Whey in a C.T.F. (Data from Table 29)



5. Proximate Analysis of *P. javanicum* Biomass

5.1. Introduction

A product destined for sale as an animal feed must be of a certain minimum nutritional value before animal feed manufacturers will consider its purchase. The market value of the product will be determined by many factors, including availability, cost of manufacture etc., but principally by its protein content.

Both crude protein and true protein (that is, amino acid content) are commonly-used determinants of SCP quality. Biomass crude protein content, together with fat content, was routinely measured during experiments (Chapters 3 and 4) but economic considerations precluded regular determination of the amino acid content. Samples of biomass produced only under four different regimens were analysed for amino acid content.

Two other biomass constituents were examined - fibre and ash. These analyses were performed on biomass produced from Cheshire whey "effluent" at a dilution rate of $1.0h^{-1}$, which was shown to be the most appropriate choice of dilution rate for a commercial tower fermentation system (Chapter 4).

5.2. Materials and Methods

Finely-ground dried *P. javanicum* biomass (pooled X_E samples) produced during experiments described in Chapters 3 and 4, was stored in air-tight containers until examination.

Amino acid analyses of hydrolised samples were performed in the Biological Sciences Department (University of Aston in Birmingham)

using a Locarte amino acid analyser (sodium citrate buffer system). Peak areas were estimated by the following equation:

$$A = h.c$$

where A = peak area

h = peak height

c = peak width at half height.

The amino acid concentration was calculated by comparison with a standard curve, and was expressed as grams amino acid per 100grams dried biomass (g/100g) or grams amino acid per 16grams nitrogen (g/16N). Analyses were performed on 4 biomass samples - two produced from semidefined media ($D = 0.2h^{-1}$, $T = 35^{o}C$; $D = 1.0h^{-1}$, $T = 30^{o}C$) and two produced from whey ($D = 0.5h^{-1}$, $T = 30^{o}C$; $D = 1.0h^{-1}$, $T = 30^{o}$).

Fibre analysis was performed by Aynsome Laboratories Ltd., (Grange-over-Sands, Cumbria) in duplicate and the result was expressed as % of dried biomass.

Ash was determined by the following procedure: weighed dried samples (c.5g) were placed in tared crucibles, incinerated at $500^{o}C$ for 24 hours, then cooled to room temperature in a dessicator before weighing. The quantity of ash was calculated from the weights and the mean ash content of 3 replicate samples expressed as % of dried biomass.

5.3. Results and Discussion

Tables of Results are given in Appendix 4 (Tables 30 and 31)

The crude protein content of P. javanicum biomass produced from Cheshire whey at a dilution rate of $1.0h^{-1}$ was 48.13% (dry

weight). This was higher than the crude protein content of soya bean meal which has been used for comparison purposes during this project. Meyrath and Bayer (1979) give the crude protein contents of six yeast SCP's produced from whey (three S. fragilis products, three Candida intermedia). These range between 32% and 55% according to the harvesting method, but mostly are between 47% and 50%. Thus P. javanicum at 48.13% contains approximately the same proportion of crude protein as do these yeast products.

When considering animal feeds, protein quality (that is amino acid content) can be of more significance than protein quantity (crude protein content). Anderson et al (1975) compared the amino acid profiles of 15 microorganisms (including 2 strains of Penicillium chrysogenum-notatum) and concluded that although the protein content might vary between organisms, the amino acid profiles were surprisingly similar. Differences that did exist could, of course, be most important for the nutritional value of the SCP. These authors added that this was especially true for the sulphur amino acids, as these are usually first-limiting.

Consequently, the high methionine content of P. javanicum (Table 31) renders the biomass of this organism potentially most valuable for animal (or even human) feeding. When cultivated on Cheshire whey at $D = 1.0h^{-1}$ the biomass contained 2.9g/16N methionine. This is considerably higher than methionine levels reported by Meyrath and Bayer (1979) for 16 yeast or bacterial single cell proteins produced from whey. The Food and Agriculture Organisation of the United Nations (UNFAO) recommended level for the methionine content of an SCP is 2.2g/16N (Anon, 1975; Meyrath and Bayer,

1979), which is lower than the methionine content of P. javanicum. Indeed, the methionine content of P. javanicum approaches that of whole egg (3.1g/16N). which is used by the UNFAO as a reference protein (Delaney et al, 1975).

The profile of the remainder of the amino acids (excluding tryptophan and cysteine) was scattered when compared with other whey-grown SCP's (Table 17 and Fig.43) that is, levels of some amino acids were higher, others lower and some almost identical.

Part of the amino acid content of P. javanicum biomass may have derived from whey proteins. These could have been precipitated by the acidic environment of the fermenter, trapped within the flocs, and then harvested with the biomass. When the amino acid profile of biomass produced at the same dilution rate ($1.0h^{-1}$) on semi-defined medium (Table 31) is examined, however, it can be seen that although the total true protein content is slightly lower, the relative proportions of the amino acids appear identical to the whey derived biomass. The observed amino acid profile is, therefore, that of P. javanicum biomass and does not reflect whey-proteins from the medium. The methionine level of biomass produced on semi-defined medium (2.5g/16gN) is still higher than the UNFAO pattern and all the single cell proteins listed in Table 17.

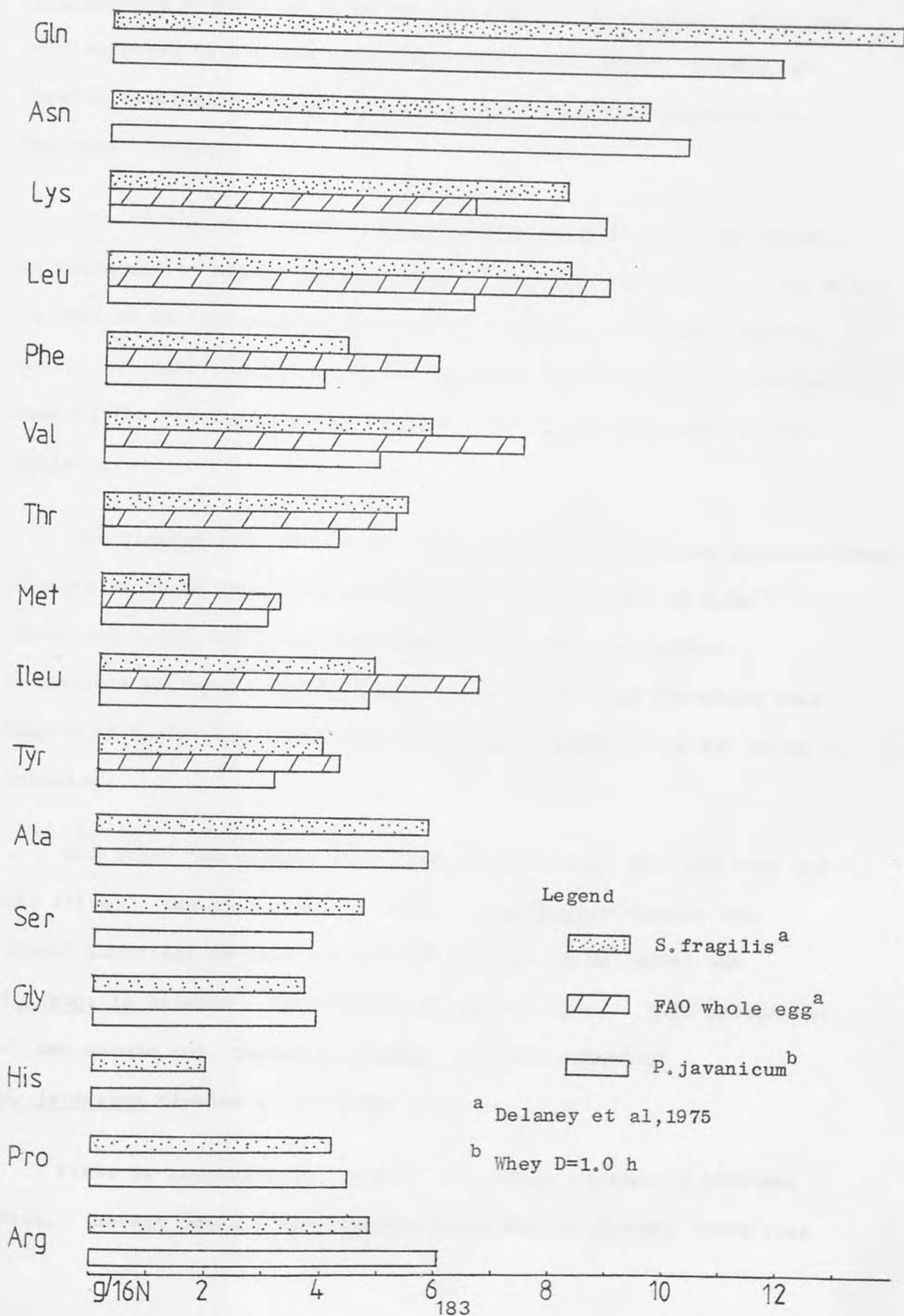
The total true protein content of biomass produced from whey at $D = 1.0h^{-1}$ was 41.65g/100g, even without data for tryptophon and cysteine which were not analysed. Thus both the true protein and crude protein contents (at 41.65+%) and 48.13% respectively) are extremely similar to those reported by Delaney et al (1975) for their yeast (S. fragilis) SCP produced from whey ultrafiltrate (lactose

Table 17. <u>Amino Acid Composition of Biomass Produced from Whey.</u>

After Meyrath and Bayer , 1979

Amino acid (g/16g nitrogen)	F.A.O. pattern	Amundson (1967)		Aitken et al. (1967)		Bern-stein and Everson (1973)	Blanchet and Biju-Duval (1969)	Delaney et al. (1975)	Schulz & Oslage (1975)	Skupin et al. (1974)	Sura-zynski et al. (1967)	Surazynski et al. (1968)		Vienna-UKM process Bayer & Meyrath (1976)	Wasserman (1961)		
		Yeast	Yeast whey protein	Tricho-spororon culaneum	Saccharo-myces fragilis	Saccharo-myces fragilis	Saccharo-myces fragilis	Saccharo-myces fragilis	Candida krusei Lacto-bacillus bulgaricus	Propioni-bacterium shermanii	Yeast protein prepara-tion	Candida pseudo-tropicalis	Saccharo-myces lactis	Candida inter-media	Saccharo-myces fragilis	Strepto-coccus faecalis	Escheri-chia coli
Lysine	4.2	11.1	10.4	8.41	11.14	6.9	6.65-7.3	8.0	7.6	7.98	8.86	7.4	7.7	6.7	11.14	7.2	3.8
Histidine	-	4.0	3.0	2.47	3.98	2.1	1.2-2.25	2.0	2.1	-	1.82	1.7	2.0	-	3.98	1.3	1.2
Threonine	2.8	6.5	7.5	4.69	5.57	5.8	5.2- 5.9	5.3	4.8	5.46	5.28	3.6	4.9	5.7	5.57	3.3	3.1
Cystine/Cysteine	-	-	3.8	-	-	-	0.95-1.2	1.7	1.6	-	1.01	1.4	1.0	1.7	-	-	-
Valine	4.2	7.8	7.4	5.69	5.72	5.4	5.3-6.95	5.6	5.0	5.63	6.50	6.5	6.3	5.3	5.72	4.5	6.0
Methionine	2.2	1.6	2.0	1.97	1.57	1.9	1.2-1.4	1.5	1.7	1.44	2.29	1.8	1.4	1.3	1.57	1.9	2.0
Isoleucine	4.2	6.0	6.1	4.44	5.05	4.0	4.45-6.45	4.8	4.6	5.04	-	-	-	5.1	5.05	4.1	4.3
Leucine	4.8	9.6	11.0	7.15	-	6.1	7.45-7.65	8.1	7.5	10.36	14.48	-	-	7.8	-	4.7	8.3
Tyrosine	2.8	3.4	4.1	4.21	4.57	2.4	-	3.9	-	2.51	4.44	4.8	4.0	-	4.57	2.2	-
Phenylalanine	2.8	5.1	4.4	7.42	5.05	2.8	3.75-4.3	4.2	-	3.65	5.65	4.5	4.5	4.0	5.05	2.8	2.8
Tryptophan	1.4	-	2.3	-	-	-	1.3-1.35	1.7	-	-	1.69	1.5	1.5	-	-	-	-
Proline	-	-	-	4.21	-	-	-	4.2	-	-	-	3.5	4.0	3.7	-	2.6	-
Glycine	-	-	-	5.94	4.24	-	-	3.7	3.6	-	-	5.1	4.9	3.1	4.24	4.6	-
Arginine	-	7.4	5.7		7.37	-	4.8-5.0	4.9	-	4.77	-	-	-	3.6	7.37	3.4	4.2
Aspartic acid	-	-	-	8.9	10.4	-	-	9.4	4.2	-	-	-	-	9.8	10.4	7.6	-
Serine	-	7.0	6.8	5.45	5.21	-	-	4.7	-	-	-	4.8	4.3	5.1	5.21	1.9	-
Glutamic acid	-	-	-	12.37	13.34	-	-	13.8	-	16.24	-	11.5	11.0	14.7	15.24	10.1	-
Alanine	-	-	-	7.91	7.21	-	-	5.8	-	-	-	7.3	6.6	5.2	7.21	5.7	-

Fig 43Comparison of Three Amino Acid Profiles.



permeate) (true protein = 41.8% ; crude protein = 49.5%). Consequently, in terms of both protein quantity and quality, P. javanicum biomass is comparable to that of S. fragilis, which has been approved by the FDA as a human and animal feed. Indeed, as regards methionine content, P. javancium is markedly superior to the yeast product.

The nutritional value of P. javanicum biomass must, of course, be confirmed by animal feeding trials; the amino acid content can only be used as an indicator of nutritional quality. Such experiments would also test the safety of the product, but would require a time span of the order of years, and thus were beyond the scope of this project.

The biomass fat content was measured routinely during fermentations. Biomass produced from Cheshire whey at a dilution rate of $1.0h^{-1}$ contained 2.42% fat. As described previously, the soxhlet extraction procedure tends to give low readings, and therefore this figure of 2.42% should be taken as a minimum quantity of fat to be expected.

The other two biomass constituents which were analysed were ash and fibre. The proportion of ash in P. javanicum biomass was almost identical to that reported by Delaney et al (1975) for S. fragilis biomass - 7.57% and 7.6% respectively. This proportion of ash should not, therefore, hinder the development of P. javanicum biomass as an animal feed.

Fibre is necessary to the diet of animals because it provides bulk. Recent reports have implicated a lack of dietary fibre with

an increased incidence of colon cancer in humans. Too much fibre in an SCP is, however, undesirable because it reduces the proportions of nutritional components such as protein. The level of fibre in P. javanicum biomass was acceptable by both these criteria, being neither too high to affect the protein content nor too low to make additions of fibre necessary.

The composition of a soybean product used as a protein supplement in commercial swine diets is presented in Table 30. The overall composition of P. javanicum biomass is almost identical to the soybean product; although proportions of certain amino acids are lower, others (in particular methionine and lysine) are present in larger quantities.

In summary therefore, P. javanicum biomass is potentially a valuable animal feedstuff. Sale of the biomass may render effluent treatment economic, or even profitable especially if negative accounting procedures are applied - that is, the present costs of effluent disposal are subtracted from the cost of biomass production. Andrews (1980) has discussed this topic in greater detail. It is not elaborated on here because this project was designed to establish the technical feasibility of SCP production from dairy effluents in the CTF and not necessarily the economic viability of such a process.

untreated to natural waterways. Present and potential processes for the utilisation of dairy waste waters and process byproducts were discussed in Chapter 1.

The most important dairy process byproduct is whey, derived from either cheese, or (especially in New Zealand) casein manufacture. Processes wherein whey is fermented to produce yeast SCP are well developed (Wasserman, 1960[a,b,c], 1961, 1962; Perzow, 1974; Delaney et al, 1975; Vananuvat et al, 1975[a,b]; Burgess, 1977; Lane, 1977; Meyrath et al, 1979) but little work has been performed using filamentous fungi to ferment whey. The use of filamentous fungi for SCP production has been discussed in Chapters 1 and 2 and it was concluded that these organisms may be superior to yeasts for this purpose, principally because their structure facilitates harvesting.

The experiments performed in this project were designed firstly to select a species of filamentous fungus able to grow in the CTF with lactose as the sole carbon source. P. javanicum van Beyma was chosen from a total of sixty-one different species isolated from a variety of sources (Chapter 2). This mould, which has been studied in the past for fat production, did not produce any antibacterial substances when cultivated in the CTF. A rigorous examination of the literature, including a computer search, did not produce any references relating to it being toxic or pathogenic.

The effects of three fermentation parameters - dilution rate, temperature, fermenter broth pH value - on the growth of P. javanicum on a semidefined medium in the CTF were investigated (Chapter 3). Lactose utilisation was greatest when the temperature was between 20°C and 30°C, the pH value between 4.0 and 7.0, and the dilution rate

between $4.0h^{-1}$ and $5.0h^{-1}$.

When P. javanicum was cultivated in the CTF on whey (Chapter 4) the range of dilution rates over which the mould was able to grow was reduced. Both the upper ($1.6h^{-1}$) and lower ($0.4h^{-1}$) limits appeared to be determined by the degree of infection within the fermenter, and this in turn was affected by the incidence of foam production. It should be possible to cultivate P. javanicum at dilution rates outside this range if the fermenter is operated aseptically, but this will, of course, increase the operating costs. Foaming and/or contamination by other microorganisms did not cause any problems at a dilution rate of $1.0h^{-1}$, providing the whey concentration in the medium did not exceed 10% (vol : vol). Thus $1.0h^{-1}$ would be the best choice of dilution rate for a commercial fermentation system.

At this dilution rate ($1.0h^{-1}$) P. javanicum utilised approximately 90% of the lactose supplied to it. It should, therefore, be possible to achieve high levels of reduction of BOD or Chemical Oxygen Demand (COD) when treating dairy effluents since the majority of their BOD/COD derives from lactose. Moreover, P. javanicum utilised approximately 40% of the whey protein, hence reducing the concentration of another contributor to the total BOD/COD. A calculated estimate of the BOD reduction to be expected after this treatment is 74% (see Appendix 5).

When cultivated at a dilution rate of $1.0h^{-1}$, P. javanicum produced 14.2g. (dry weight) of biomass from 1 litre of Cheshire whey. This yield was lower than reported yields for yeast SCP produced from whey, but reduced productivity may be offset by the cheaper harvesting possible for filamentous fungal SCP.

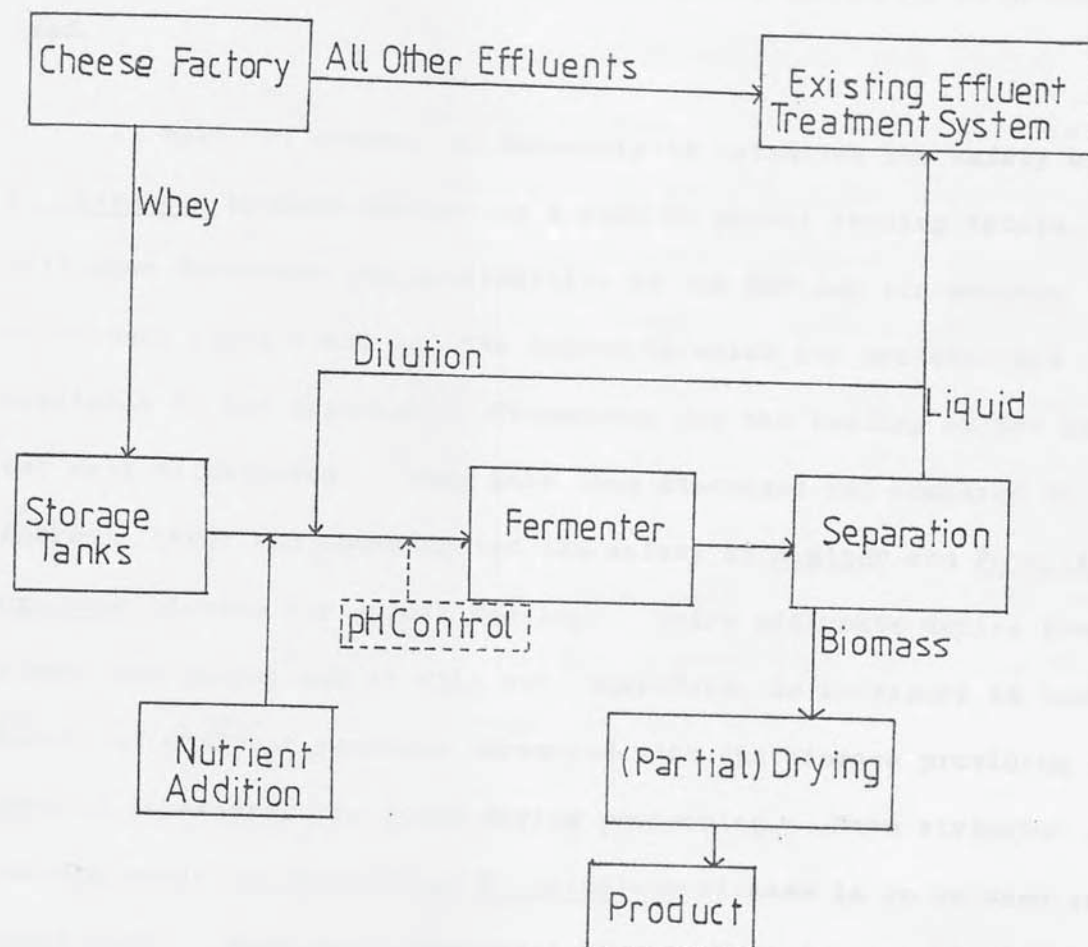
If data obtained with the five-litre tower fermenter can be extrapolated to predict the performance of P. javanicum in a commercial-scale fermenter, some estimate of expected yields can be arrived at. Meyrath et al (1979) used the figure of 20,000 litres of whey per day when calculating the costs for various fermentation processes. Using this volume, the maximum quantity of P. javanicum biomass which could be produced at a dilution rate of $1.0h^{-1}$ would be 283.5 kg. per day. In order to process 20,000 litres of whey per day at this dilution rate, a fermenter of circa 10,000 litres in volume would be required. This volume is estimated by assuming that the same liquid through-put rate (20 per day in the five-litre fermenter) would be required for the large-scale fermenter. Since the whey would be diluted by one-tenth (to prevent foaming) the total liquid volume would be 200,000 litres and hence the required fermenter volume approximately 10,000 litres.

To date, the largest tower fermenter which has been installed commercially is the 20,000 litre fermenter operated by Dunlop Estates Behad, Gomali Mill, Negri Sembilan, W. Malaysia (Greenshields, 1978[b]; Andrews, 1980) to treat Palm Oil effluent. Few problems would be anticipated, therefore, when constructing and commissioning a 10,000 litre fermenter. It would, however, be advisable to confirm the results obtained in the five-litre fermenter at, say, the 100 litre scale before proceeding to a commercial scale plant.

A flow diagram for a proposed commercial scale tower fermentation system treating whey is presented in Fig. 44 .

Fig. 44.

Proposed Whey Fermentation System.



A detailed costing for the commercial system would be beyond the scope of this project, but such a scheme, for the continuous fermentation of Palm Oil Effluent, is given by Frances Harmon Ltd., (1979).

P. Javanicum biomass produced at a dilution rate of $1.0h^{-1}$ from whey contained a minimum of 2.4% fat, and 48% crude protein. True protein amounted to at least 41% (tryptophan and cysteine not determined) which is almost identical to S. fragilis biomass true protein. Moreover,

the methoionine content of P. javanicum was markedly superior to other whey derived SCP's, approaching the level of whole egg protein. Thus P. javanicum biomass should find a ready market as an animal feed.

It will, of course, be necessary to establish the safety of P. javanicum biomass for use as a feed by animal feeding trials. These will also determine the palatability of the SCP and its protein efficiency ratio (that is, the degree to which its proteins are available to the animals). Procedures for the testing of new foods are well established. They have been discussed and compared by Andrews (1980) who investigated the safety of A.niger and Pencillium oxalicum biomass for animal feeding. Dairy effluents derive from a human food (milk) and it will not, therefore, be necessary to test the safety of effluent residues harvested with the biomass providing no harmful substances are added during processing. More stringent safety testing would be required if P. javanicum biomass is to be used for human food. Rank Hovis McDougall have conducted human feeding trials with their filamentous fungus derived SCP and no toxic or pathogenic effects have been observed (Solomons, 1980). It is quite possible, therefore, that P. javanicum biomass may also prove safe for human feeding.

Attempts to cultivate P. javanicum on Whole Factory Effluent in the laboratory-scale tower fermenter were unsuccessful. This was ascribed to the variability of WFE and suggestions by which it might be possible to treat this type of dairy effluent were discussed in section 4.6. Further work to test these suggestions would best be performed "on site" at the dairy factory.

The third type of dairy effluent which it was originally intended to include in this project was lactose permeate, which is the ultrafiltrate remaining after proteins are removed from whey. Yeast SCP and alcohol have been produced by fermentation from lactose permeate in the CTF in Ireland (Burgess, 1977). The composition of lactose permeate is approximately 4.5% lactose, 0.8% inorganic materials (minerals) plus some lower molecular weight nitrogenous compounds (Delaney et al, 1975) which closely resembles the semidefined medium used in this project (Chapter 3). It appears probable, therefore, that P. javanicum will be able to ferment lactose permeate (plus medium supplements) in the CTF, achieving yields of biomass and efficiencies of lactose removal comparable to those observed on semidefined media. Supplies of lactose permeate were not available and these experiments, therefore, await future investigation.

This project was principally aimed at the production of an animal feed protein while treating dairy effluents, but the biomass protein, and in particular its high methionine content, indicates more potential as a human food. P. javanicum biomass could be produced in the continuous tower fermenter from dairy or other food processing effluents in third world countries which frequently suffer shortages of high quality protein.

Appendix 1 Media

Chemicals used to make up media used in chapters 2(preliminary tower fermentations) 3 and 4 were supplied by the following companies :

Fisons Scientific Apparatus,Loughborough,Leics.

Lactose,S.L.R.

Sodium dihydrogen orthophosphate,S.L.R.

Magnesium sulphate,S.L.R.

B.D.H. Chemicals ltd.,Poole,Dorset

Potassium chloride, laboratory reagent

Various sources

Ammonium sulphate, technical grade

Bovril, Burton-on-Trent

Yeast extract,low salt,food grade

I.C.I .ltd.

Silicones (Silicon based antifoam)

Chapter 2 (Isolation and Batch Fermentation) Media.

Modified Buffered Yeast Agar.

Lactose	20.00g
$(NH_4)_2SO_4$	0.72g
$NH_4H_2PO_4$	0.26g
Yeast extract	5.00g
Agar	12.00g

Made up to 1 litre with distilled water and autoclaved at 103.5 kilopascals for 15 minutes.

Modified Buffered Yeast Broth

Lactose	20.00g.
$(NH_4)_2SO_4$	0.72g.
$NH_4H_2PO_4$	0.26g.
Yeast extract	5.00g.

Made up to 1 litre with distilled water and autoclaved at 103.5 kilopascals for 15 minutes.

Chapter 2 (Preliminary Tower Fermentations) Media.

MEDIUM		1	2	3	4	5	6	7	8	9	10	11	12
Pasteurised Milk	$cm^3.l^{-1}$	-	200.0	-	-	-	200.0	-	-	-	-	-	-
Dried Milk (Marvel)	$g.l^{-1}$	-	-	-	-	-	-	-	10.0	-	-	-	-
Sucrose	$g.l^{-1}$	-	-	-	-	-	-	-	-	10.0	-	-	-
Lactose	$g.l^{-1}$	20.0	-	10.0	10.0	10.0	-	10.0	-	-	10.0	5.0	5.0
$(NH_4)_2SO_4$	$g.l^{-1}$	0.72	0.72	0.72	2.0	4.0	4.0	4.0	4 0	2.0	2.0	2.0	1.0
NaH_2PO_4	$g.l^{-1}$	0.26	0.26	0.2	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
KCl	$g.l^{-1}$	-	-	-	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
$MgSO_4$	$g.l^{-1}$	-	-	-	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.25	0.1
CaCl	$g.l^{-1}$	-	-	-	0.05	0.05	0.05	0.05	0.05	-	-	-	-
Yeast extract	$g.l^{-1}$	1.0	-	1.0	0.5	0.5	0.5	1.0	1.0	0.5	0.5	0.5	0.5
Autoclaved		Yes	-	Yes	Yes	Yes	-	Yes	-	-	Yes	-	-
Prepared with iced tap water		-	Yes	-	-	-	Yes	-	Yes	Yes	-	Yes	Yes

Used in Experiment		3.4.1.1			3.4.1.2		3.4.2	3.4.3.1	3.4.3.2	3.4.3.3
		D=0.024	D=0.1,0.2	D=0.03,0.4	D=1.0,2.0	D=3.0 $\rightarrow$ 9.2				
Lactose	$g.l^{-1}$	10.0	6.0	4.0	2.0	1.0	5.0	10.0	10.0	4.0
$(NH_4)\ SO_4$	$g.l^{-1}$	3.0	2.0	2.0	1.0	0.5	1.0	2.0	2.0	1.0
$Na\ H_2\ PO_4$	$g.l^{-1}$	1.0	0.5	0.5	0.25	0.25	0.5	1.0	0.5	0.3
K Cl	$g.l^{-1}$	0.5	0.25	0.25	0.125	0.63	0.25	0.5	0.25	0.1
$Mg\ SO_4$	$g.l^{-1}$	0.25	0.1	0.1	0.05	0.025	0.1	0.1	0.1	0.04
Yeast extract	$g.l^{-1}$	1.0	0.5	0.5	0.5	0.25	0.5	1.0	2.5	0.3
Agar	$g.l^{-1}$	-	-	-	-	-	-	20.0	-	-
Autoclaved		Yes	Yes	Yes	-	-	Yes	Yes	Yes	-
Prepared with iced water		-	-	-	Yes	Yes	-	-	-	Yes

Chapter 4 Media

		Whey					W.F.E.
		D=0.4	D=0.5	D=1-0	D=1.6	D=3.0	
Whey	$cm^3.1^{-1}$	133	100	80	80	50	-
W.F.E.	1.1^{-1}	-	-	-	-	-	1.0
$(NH_4)_2 SO_4$	$g.1^{-1}$	1.5	1.2	1.0	1.0	1.0	1.0
$NaH_2 PO_4$	$g.1^{-1}$	0.5	0.4	0.3	0.3	0.3	0.3
K Cl	$g.1^{-1}$	0.2	0.16	0.1	0.1	0.1	0.1
$Mg SO_4$	$g.1^{-1}$	0.05	0.04	0.04	0.04	0.04	0.04
Yeast Extract	$g.1^{-1}$	0.5	0.4	0.3	0.3	0.3	0.3
Silicones	$cm^3.1^{-1}$	-	-	-	0.1	-	-
Prepared with iced tap water		Yes	Yes	Yes	Yes	Yes	-

Appendix 2 Chapter 2 Results.

Table 18 Milk Effluent Results.

Sample point	pH value	Isolate
Raw effluent	4.96	Aspergillus fumigatus
		Aspergillus terreus
		Aspergillus humicola
		Geotrichum sp.
		Ascomycete sp.
Activated sludge	4.95	Geotrichum sp.
		Trichoderma viride
		Trichoderma hamatum
Intermediate humus sludge	7.54	A. fumigatus
		A. terreus
		Aspergillus sydowi
		A. humicola
		T. viride
		T. hamatum
		Trichoderma koningii
Final humus sludge	7.80	
Roughing trickle filter mat		Geotrichum sp.
Final trickle filter mat		T. viride
		Trichoderma harzianum

Table 19 <u>Results Soil Isolations : December.</u>

	Control								2% Milk Solids								4% Milk Solids							
day	0		3		7		14		0		3		7		14		0		3		7		14	
Isolate	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.
Sterile Mycelium	100	80	20	40	80	40	40	40	40	100	40	40	40	40	20	20	20	80	80		40			
Zygorrynchus moelleri vuill.	100	80	100	100	80	100	100	100	100	100	100	100	40	100	100	100	100	100	100	100	60	100	40	100
Mucor hiemalis Wehmer	40	20	40		80	40	80	100				20	20	60	100	100			40	20	60	20	100	100
M.silvaticus Hagem	20										20													
M.fragilis Bainier			20																					
M.attenuatus Linnemann															20									
M.subtilissimus Oudemans																	20							
M.corticolus Hagem																		40						
M.jansseri Lendner																			20					
M.varians Povah																			20					
Mortierella exigua Linnemann			40	20																				
M.dichotoma Linnemann				40																				
M.candelabrum VanT.& Le Monnier				20							20						20			20		20		
M.alpina Peyroud																	40		20					
M.minutissima Van T.					20	20	40	20	40		40	40	60	20							20			
M.gemmifera M.Ellis							20								20								20	
M.marburgensis Linnemann								20																
M.humicola								20																
Hyalodendron sp.																								20
Rhizopus cohnii																	40	40						
Rhizopus sp.	20		40		20					20			20							20	20			
Syzygites sp.	40	20	20			20	40				20					20								
Trichocladium asperum Harz.		80		20				100								60				40				
T.opacum(Corda)Hughes								20																
Sepedonium sp .												20												
Paecilomyces sp.								40																
Humicola grisea								20																
Absidia repens Van T.																20								
Fusarium semitectum(Berk.&Rav.)	20						20																	
F.ventricosum Appel & Woll.			60	80			20					100		40		20	20		20	100	20	20		
F.tabacinum(Beyma)W.Gams				20																				20
F.nivale(Fr.)						20			20		20	100	20	20					20	40		20		
F.culmorum											40													
F.avenaceum																				40				
Aspergillus fumigatus						20													20					
Aspergillus sp.													20											
Gliocladium roseum(Link)Thom				20				20				20								60				
Penicillium sp.				20		60	20	80		20		100		60						80				
Trichoderma sp.				100								40								100				

Table 20 <u>Results Soil Isolations : February.</u>

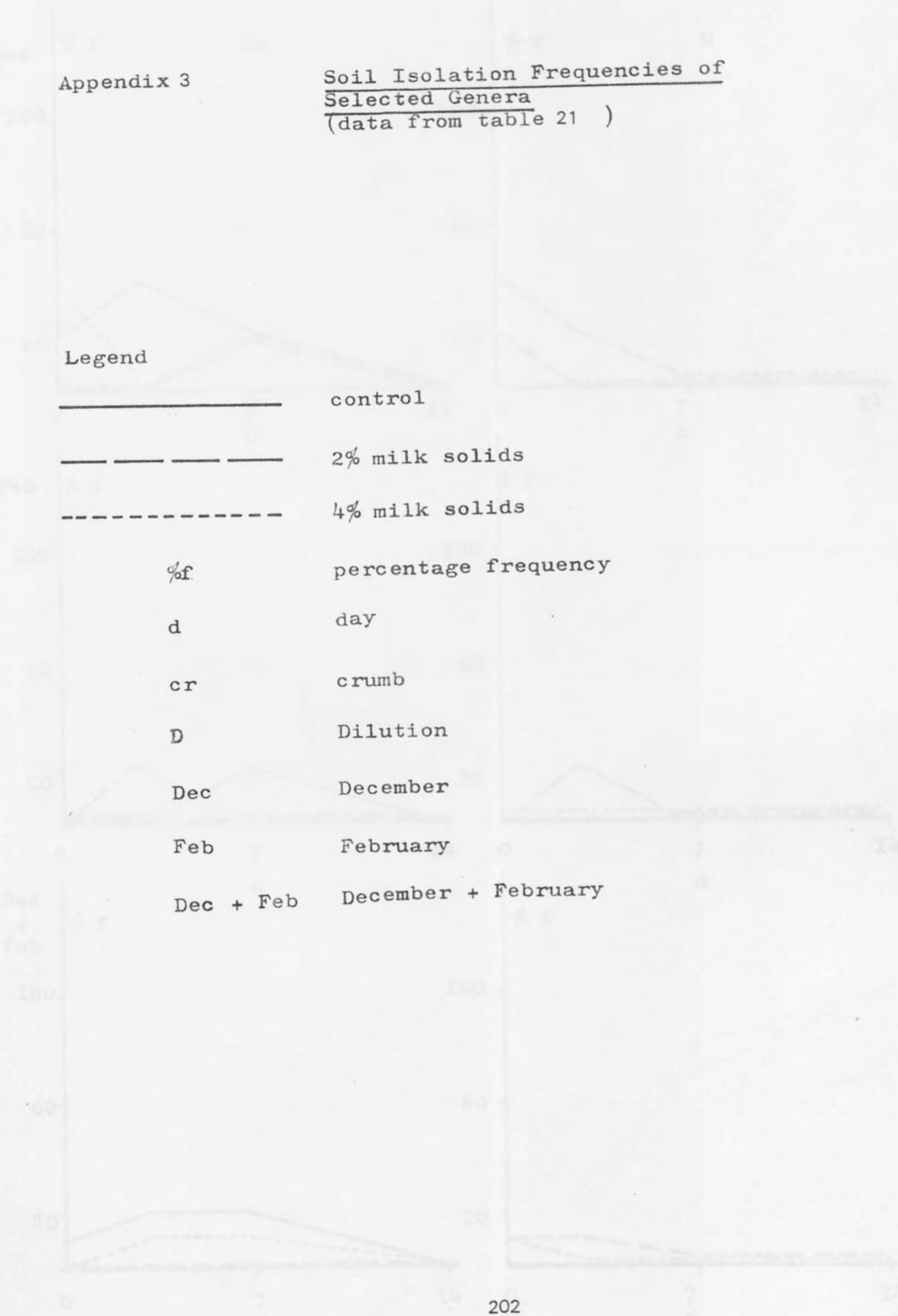
	Control								2% Milk Solids								4% Milk Solids							
day	0		3		7		14		0		3		7		14		0		3		7		14	
Isolate	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.	cr.	dil.
Sterile Mycelium	20	60	80	40	40	80	20		40	100	40	40	40	40		20	40	100	40	80	80	20		20
Zygorrhynchus moelleri Vuill.	100	100	100	100	80	100	100	60	80	100	40	100	100	100	80	100	20	100	40	60	100	40	20	100
Mucor hiemalis Wehmer	80	100	20	40	60	100	100	100	60	80	100	40	80	100	80	100	100	80	100	100	100	100	100	100
M.silvaticus Hagem			20																					
M.fragilis Bainier																60								
M.jansseri Lendner		20		20		20		40		40				40		20				20				
Mortierella elongata Linnemann																	60							
M.van tieghemi Bachman										20														
M.subtilissima Oudemans	60				20	40	20		80	40	40	80	40	100	20			20		20	60		20	
M.alpina Peyroud		20		100	40	40	20	40				40	40	20	80			80		40		80	60	
M.minutissima Van Tieghman						20	20						40		40	20							40	
M.gemmifera M.Ellis		20	20	20				60								60				60	20	20	40	60
M.marburgensis Linnemann				20						20		20												20
Syncephalis sp.														20										
Piptocephalis sp.								20																
Rhizopus cohnii					20						20	20												
Kickxella sp.																		20						
Syzygites sp.	80	40	60	40												20								
Trichocladium asperum Harz.	20	20	20	80		20				40					20	40								
T.canadense (Hughes)																20								
Sepedonium sp.			20							20														
Cephalosporium sp.				20																				
Humicola grisea		20	20					20		20						60								
Aspergillus fumigatus																20								
Fusarium ventricosum		20	20		60	60	40	20		20				60							40	40		
F.tabacinum(Beyma)W.Gams		40	40		40	20	80	20			20	60	20	20	20	20					20		40	20
F.nivale(Fr.)		20	20	40							20				20						40			
F.culmorum(W.G.Smith)Sacc.	20	20																					20	
F.stoveri Booth Comb.nov.				20						20										40				
F.dimerum												40												
Gliocladium roseum(Link)Thom		80	20	40		20		20		20				60		80		40						
Verticillium albo-atrum		20																20						
Ascomycete sp.				40								20		40										
Trichoderma viride(Rifai)								20							20	60								
T.hamatum (Rifai)														40		40					20			
Penicillium javanicum																20								

Table 21 Results Selected Genera:Percentage Frequency of Isolation.

Genus	day	December Control cr.	December Control dil.	December 2% cr.	December 2% dil.	December 4% cr.	December 4% dil.	February Control cr.	February Control dil.	February 2% cr.	February 2% dil.	February 4% cr.	February 4% dil.	December+February Control cr.	December+February Control dil.	December+February 2% cr.	December+February 2% dil.	December+February 4% cr.	December+February 4% dil.
Mucor	0	20	20			20	40	80	100	80	100	100	80	60	60	40	50	60	60
	3	60		20		80	20	40	60		40	100	100	50	30	10	20	90	60
	7	80	40	20	60	60	20	60	100	80	100	100	100	70	70	50	80	80	60
	14	80	100	100	100	100	100	100	100	80	100	100	100	90	100	90	100	100	100
Mortierella	0			40		60	20	60	20	80	80	60	100	30	10	60	40	60	60
	3	40	80	40	40		20	20	100	60	100	40	80	30	90	50	70	20	50
	7	20	20	60	20	20	20	60	100	80	80	80	100	40	60	70	50	50	60
	14	60	40	20		20		60	100	100	80	100	80	60	70	60	40	60	40
Rhizopus	0	20			20	40	40							10			10		10
	3	40					20				20	20		20			10	10	
	7	20		20		20		20						20				10	
	14																		
Trichocladium	0		80					20	20		40			10	50		20		
	3		20				40	20	80					10	50				20
	7						20						10						
	14		100		60					20	60		20		50	10	60		10
Gliocladium	0								80		20		40		40		10		20
	3		20		20		60	20	40				60	10	30		10		30
	7								20				60		10				30
	14								20		80				10		40		
Penicillium	0				60				100	40	100		40		50	20	80		20
	3		20		100		80	20	100		100		100	10	60		100		40
	7		60		80		100		100		100		100	10	100		90		100
	14	20	100		80		40	40	20	40	60		40	30	60	20	70		40
Fusarium	0	20		20		20		20	60		20			20	30	10	10	10	
	3	60	100	60	100	40	100	80	60	20	80	40	40	70	80	40	90	40	70
	7		20	20	60	20	40	80	60	20	40	80	80	40	40	20	50	50	60
	14	20			20		20	100	40	40	20	40	20	60	20	20	20	20	20
Trichoderma	0																		
	3		100		40		100								50		20		50
	7								20	20	100		20		10	10	50		10
	14								20	20	60				10	10	30		

Appendix 3

Soil Isolation Frequencies of Selected Genera
(data from table 21)



Legend

————————	control
— — — —	2% milk solids
- - - - - - - - - - - - -	4% milk solids
%f	percentage frequency
d	day
cr	crumb
D	Dilution
Dec	December
Feb	February
Dec + Feb	December + February

Fig I Rhizopus spp

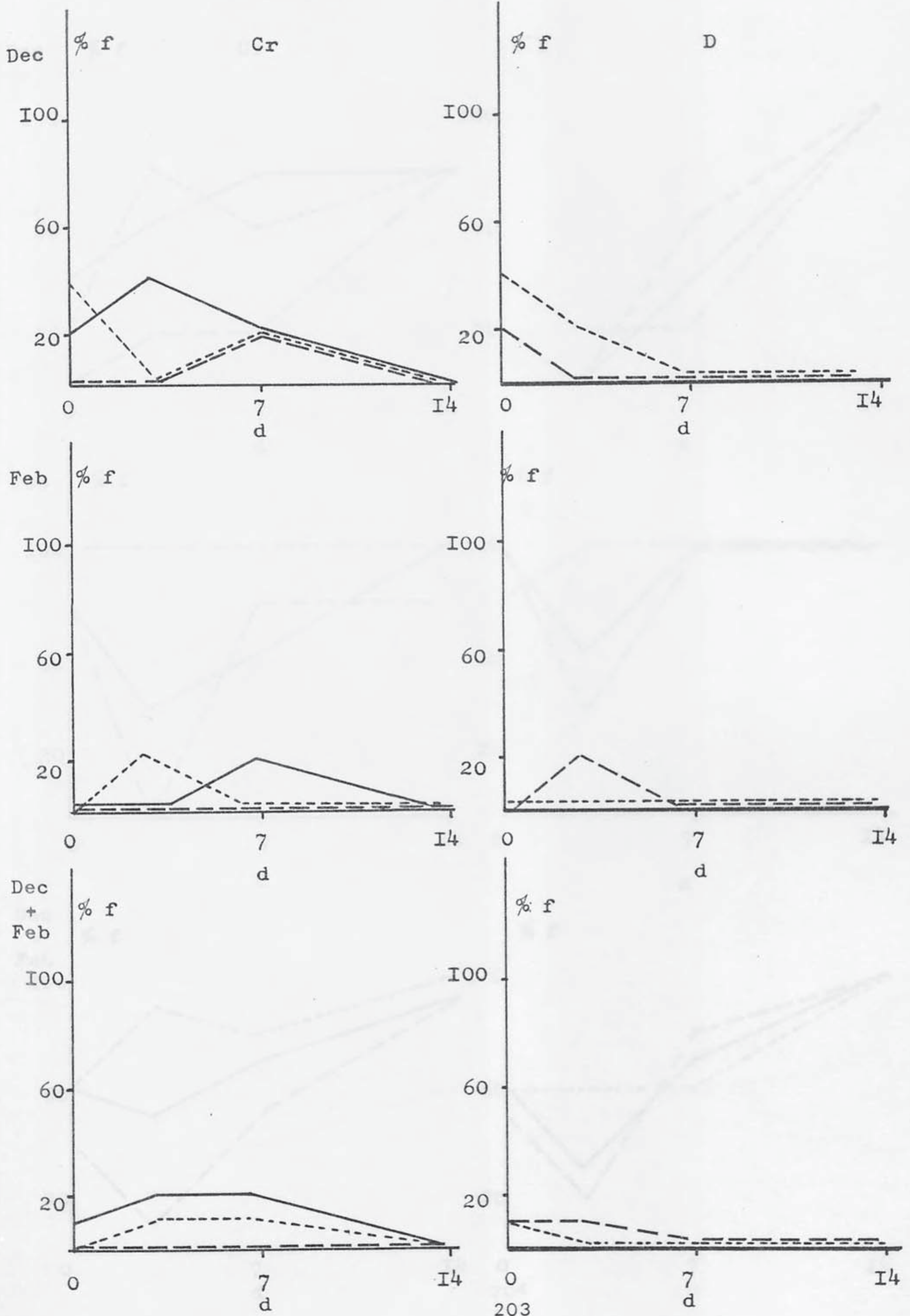


Fig 2 Mucor spp

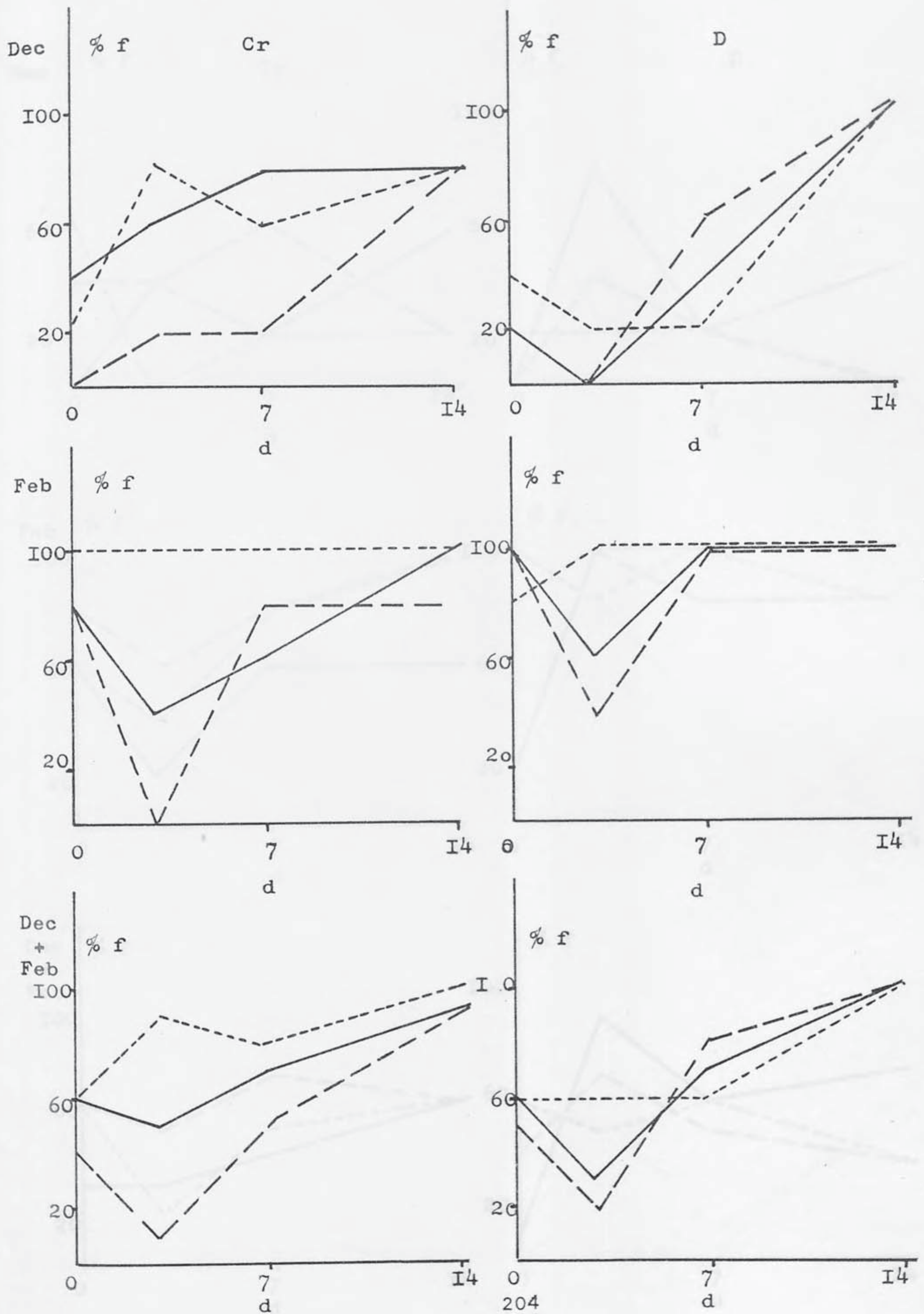


Fig 3 Mortierella spp

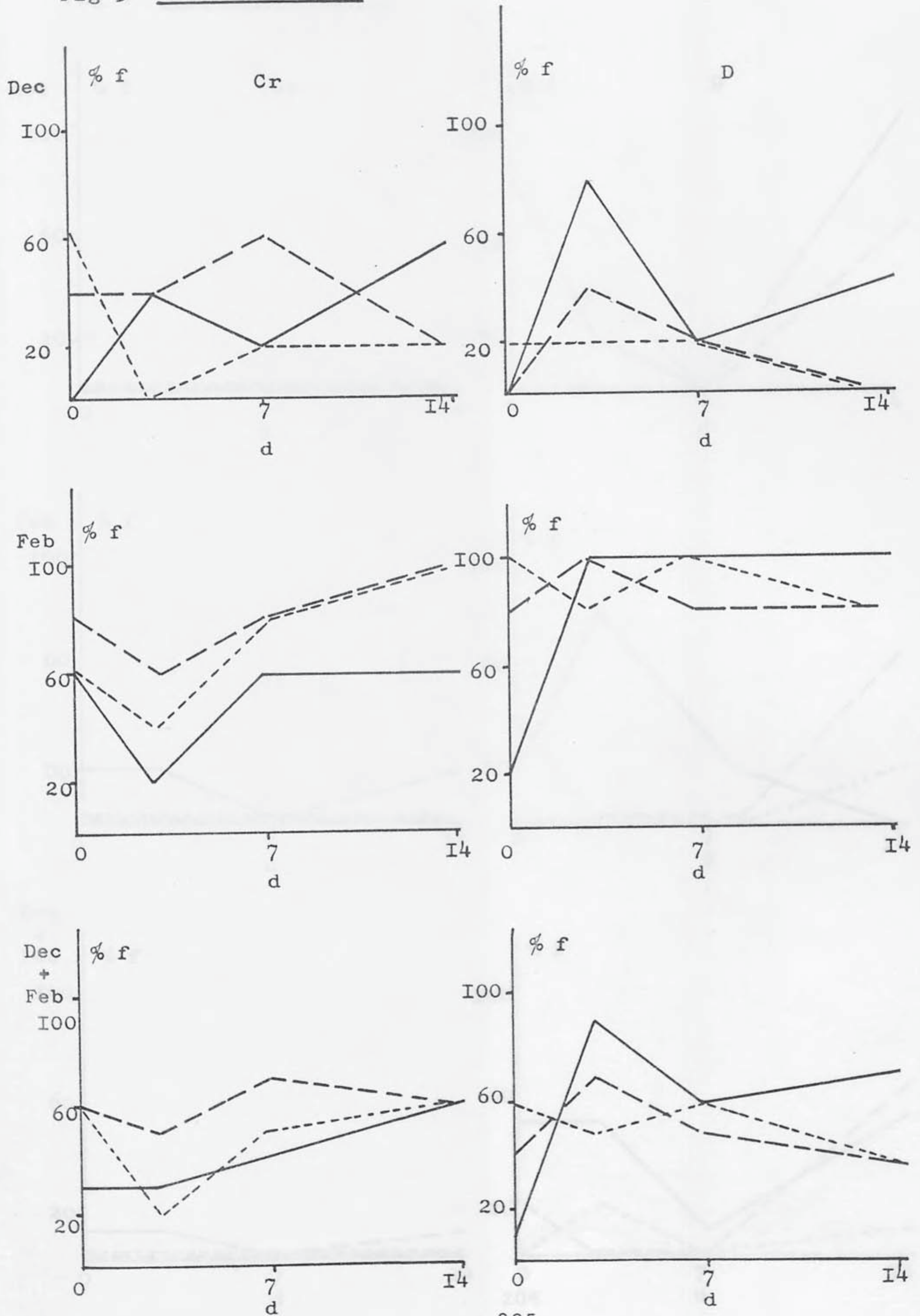


Fig 4 Trichocladium spp

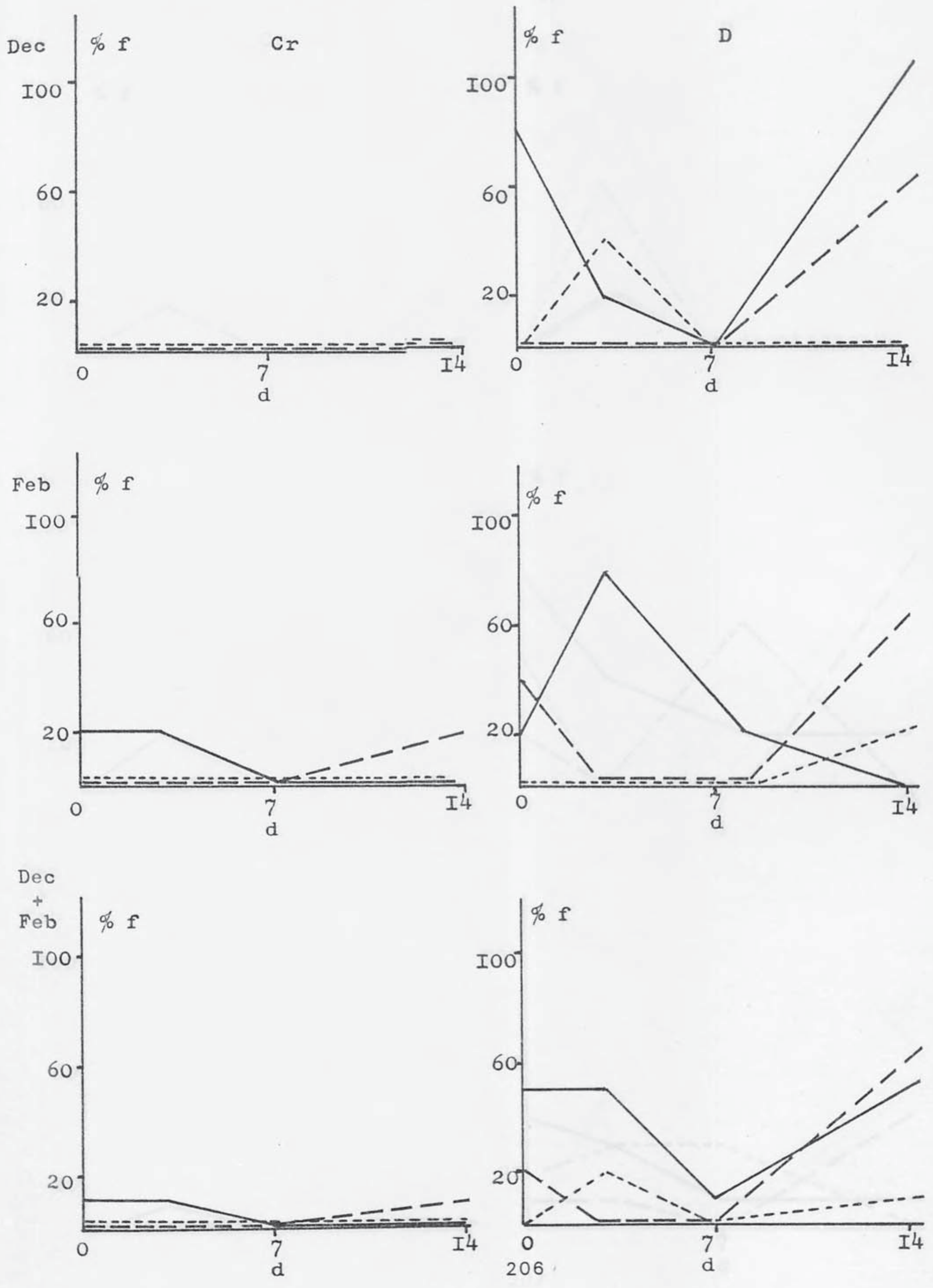


Fig 5 Gliocladium spp

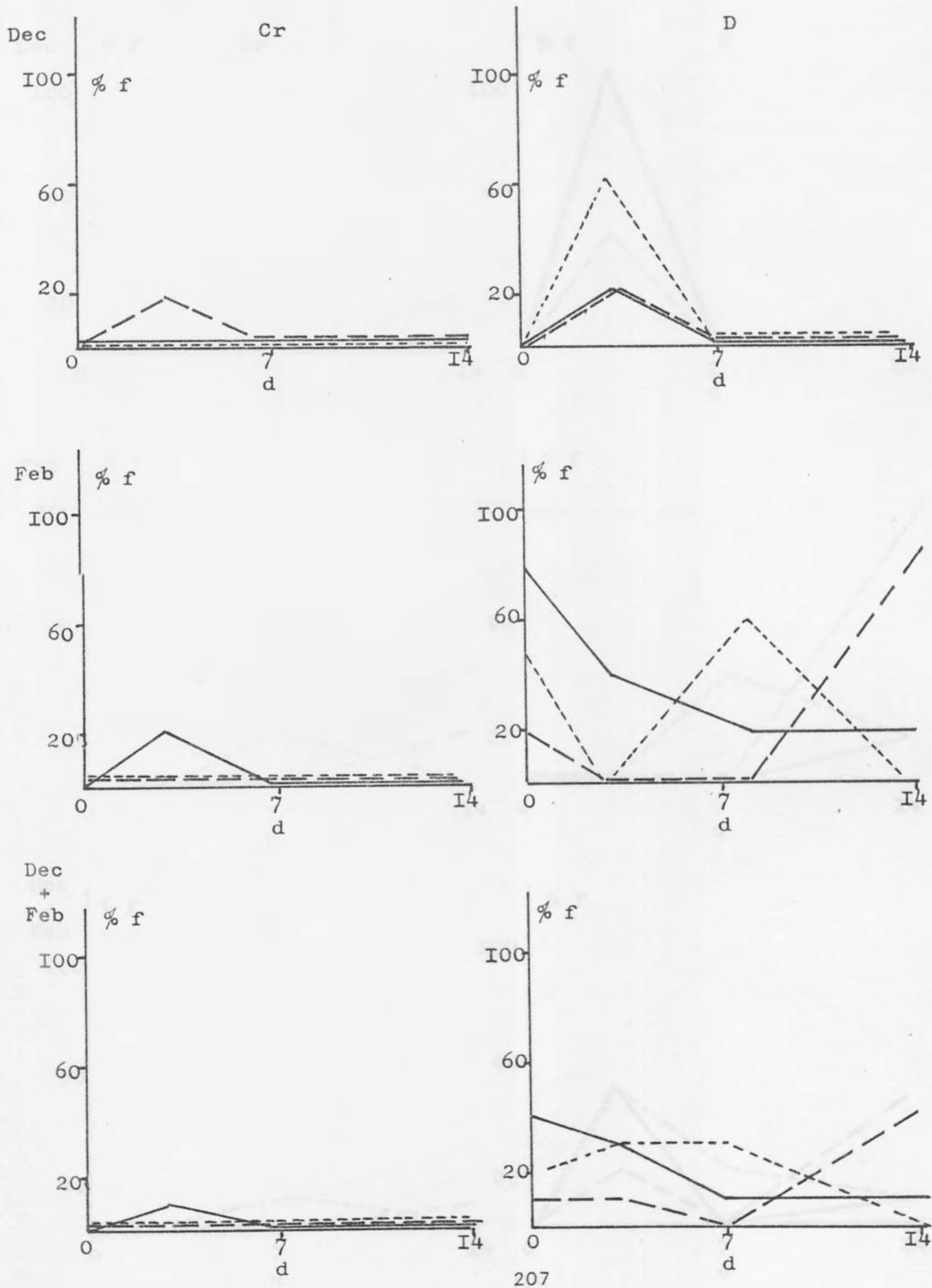


Fig 6 Trichoderma spp

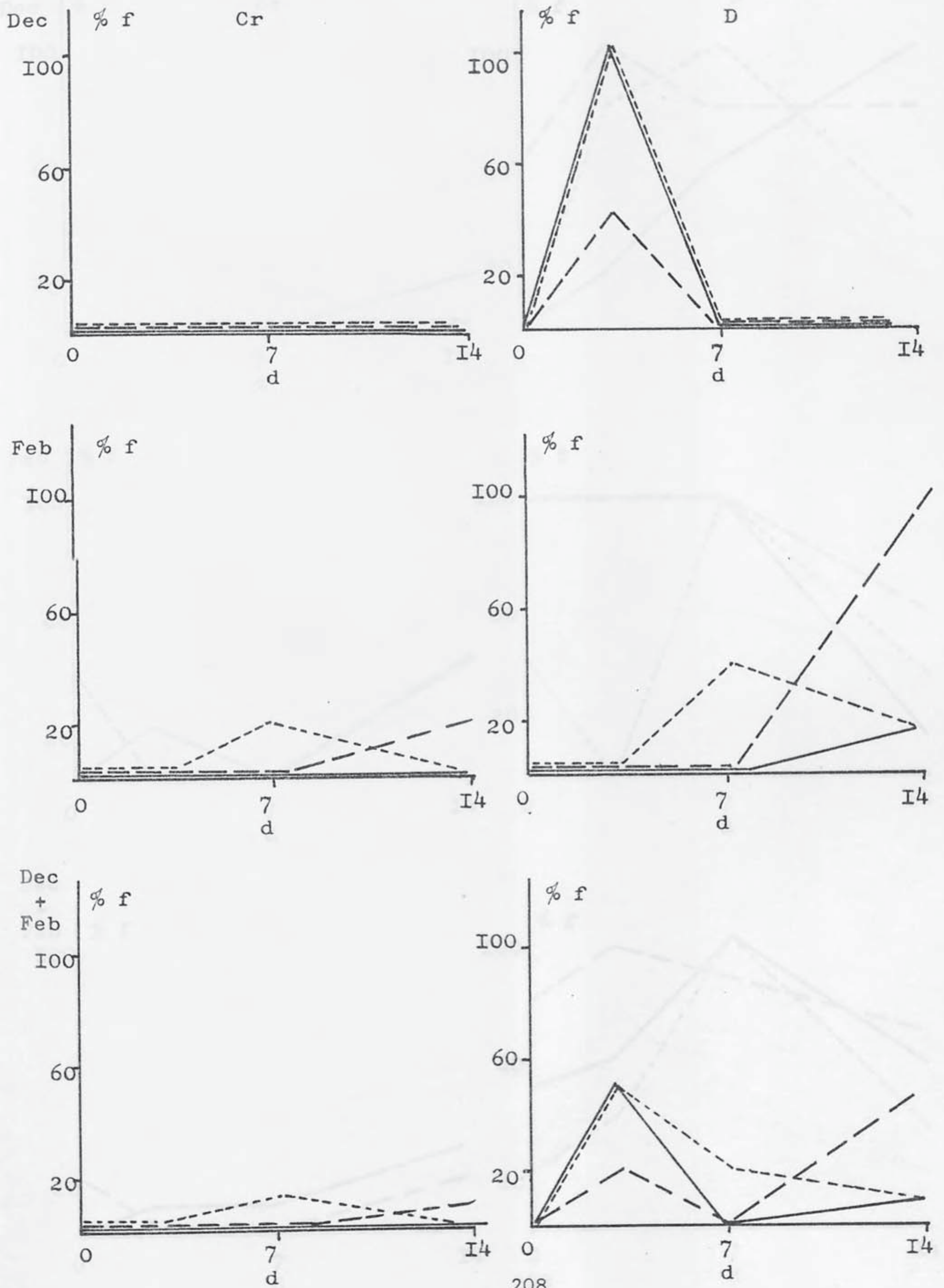


Fig 7 Penicillium spp

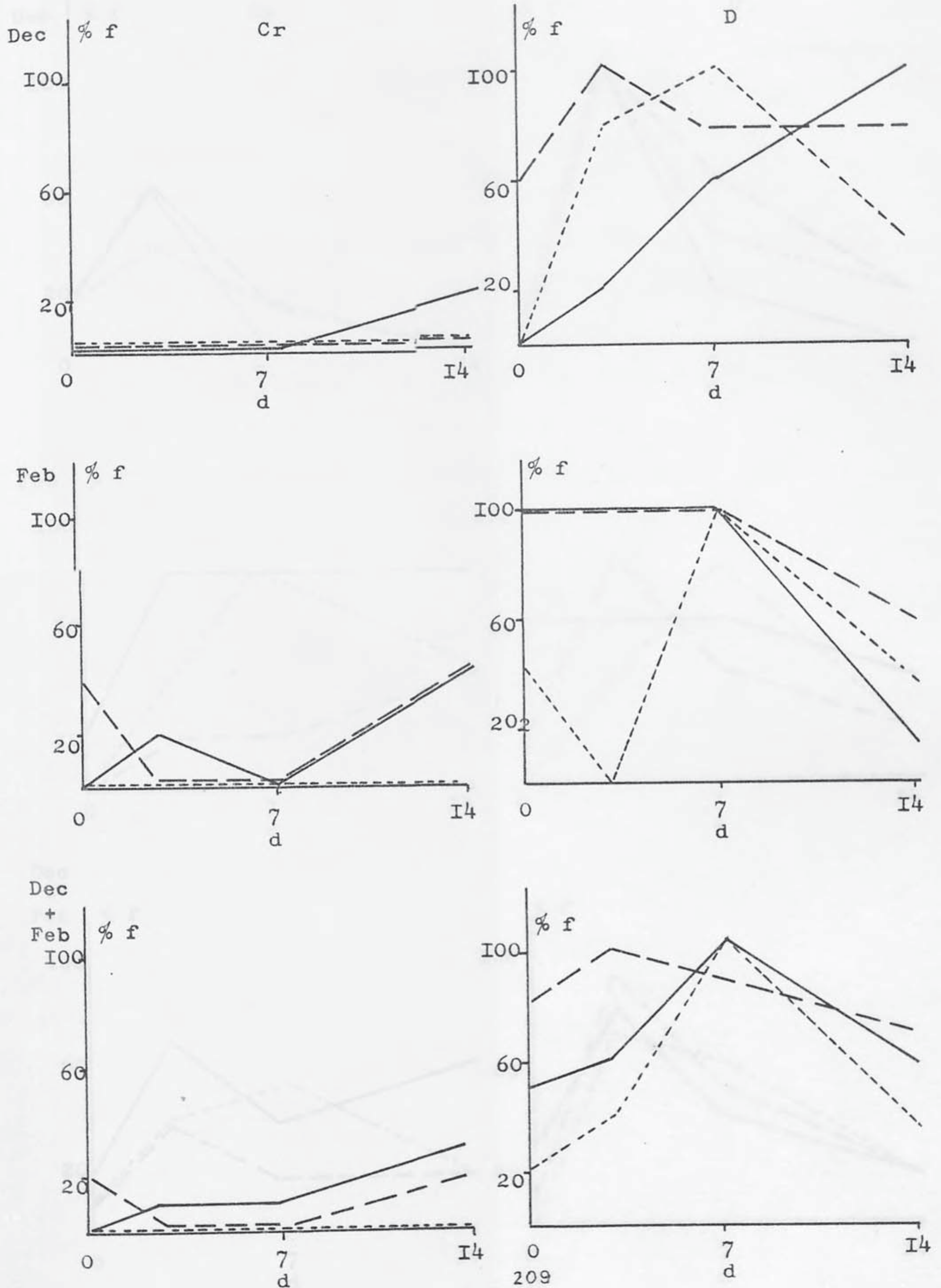
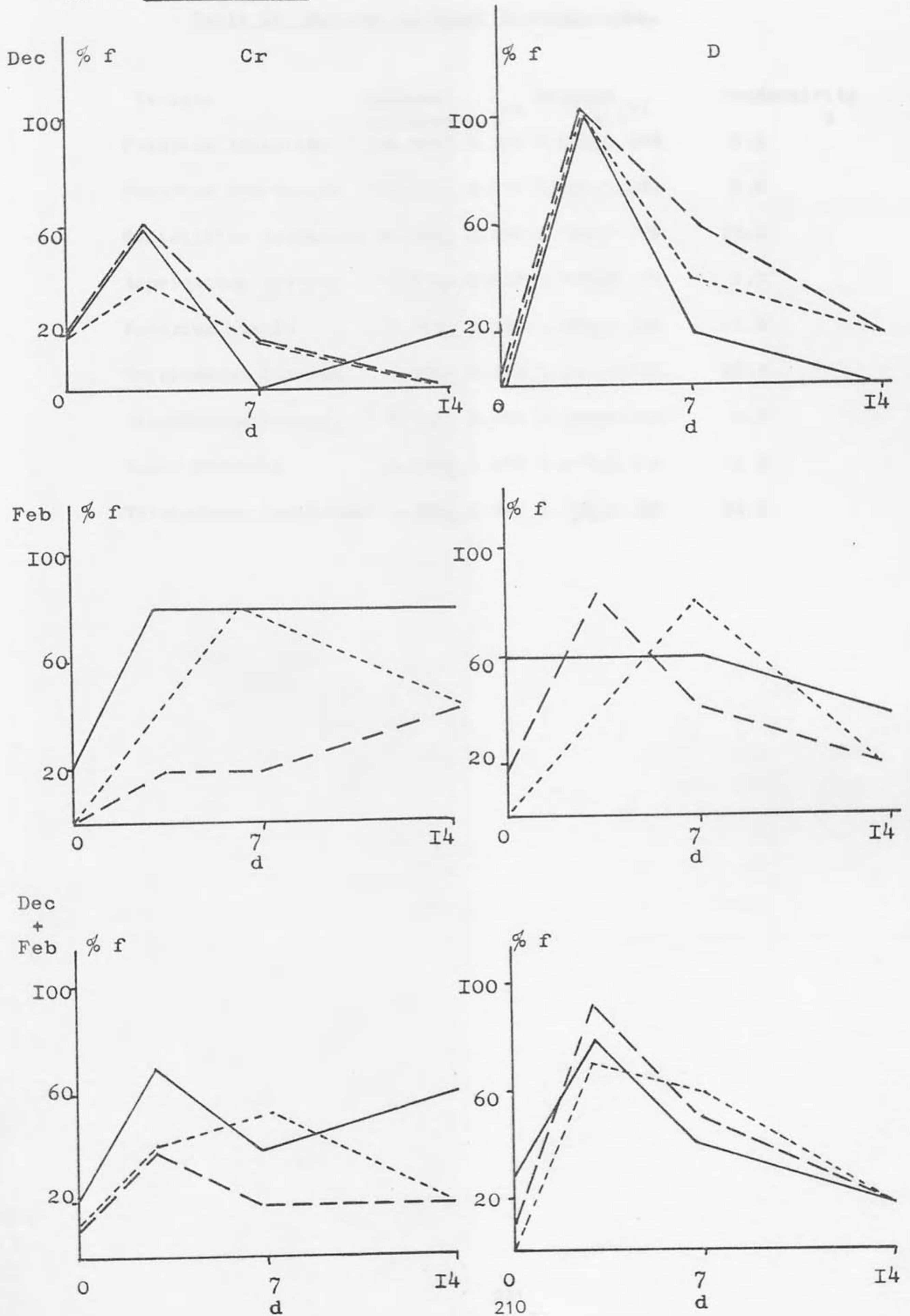


Fig 8 Fusarium spp



Appendix 4. Results Batch and C.T.F.Fermentations.

Table 22 Results of Batch Fermentations.

Isolate	Lactose utilised $g.l^{-1}$	Biomass $g.l^{-1}$	Productivity %
Fusarium tabacinum	16.440± 5.464	0.035±0.004	0.2
Fusarium semitectum	16.410± 4.575	0.100±0.021	0.6
Penicillium javanicum	16.225± 5.800	2.785±0.379	17.2
Aspergillus terreus	10.930± 0.915	1.060±0.179	9.7
Fusarium nivale	7.155± 0.390	1.805±0.139	25.2
Trichoderma hamatum	6.070± 0.304	1.240±0.075	20.4
Gliocladium roseum	5.915± 0.524	0.165±0.025	2.8
Mucor hiemalis	4.630± 0.275	0.675±0.110	14.8
Trichoderma harzianum	4.595± 0.795	1.145±0.055	24.9

Table 23 The Effect of Dilution Rate (Low Rate) upon P. javanium in a C.T.F.

D	h^{-1}	0.024	0.121	0.201	0.305	0.400
	s.d.	0.001	0.006	0.005	0.002	0.014
Air	v.v.m	2.5	2.5	2.5	2.5	2.5
T	^{o}C	35	35	35	35	35
pH		2.4	2.4	2.5	2.9	2.9
n		5	5	5	5	6
dX_E	$g.h^{-1}$	0.146	0.812	1.310	0.726	1.132
	s.d.	0.025	0.301	0.445	0.156	0.327
X_F	$g.l^{-1}$	3.849	6.522	4.583	3.646	3.742
	s.d.	0.123	0.084	0.735	0.043	0.274
μ	h^{-1}	0.010	0.035	0.074	0.051	0.081
	s.d.	0.001	0.012	0.021	0.015	0.019
dS	$g.h^{-1}$	0.524	1.967	2.158	2.400	2.981
	s.d.	0.025	0.395	0.142	0.240	0.301
dN	$g.h^{-1}$	0.009	0.036	0.064	0.059	0.080
	s.d.	0.001	0.009	0.001	0.013	0.005

Table 24 The Effect of Dilution Rate (High Rate) upon *P. javanicum* in a C.T.F.

D	h^{-1}	0.203	1.063	1.974	2.928	4.088	5.162	6.002	7.107	9.271
	s.d.	0.008	0.037	0.082	0.073	0.110	0.119	0.306	0.170	-
Air	v.v.m	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
T	°C	30	30	30	30	30	30	30	30	30
pH		2.6	3.3	3.7	3.9	4.0	4.4	4.3	4.1	-
n		5	8	5	5	5	5	5	5	-
X_E	$g.l^{-1}$	1.390	0.649	0.265	0.237	0.206	0.160	0.086	0.080	-
	s.d.	0.319	0.278	0.089	0.029	0.060	0.052	0.018	0.020	-
dX_E	$g.h^{-1}$	1.072	2.622	1.988	2.637	3.200	3.138	1.961	2.161	-
	s.d.	0.246	1.122	0.667	0.321	0.931	1.019	0.409	0.540	-
X_F	$g.l^{-1}$	5.214	4.228	4.403	4.120	5.308	3.503	3.500	4.157	-
	s.d.	0.521	0.795	0.203	0.560	0.451	0.231	0.396	0.287	-
μ	h^{-1}	0.055	0.174	0.119	0.169	0.165	0.235	0.153	0.136	-
	s.d.	0.016	0.096	0.040	0.009	0.049	0.079	0.051	0.029	-
dS	$g.h^{-1}$	2.187	4.302	3.811	5.241	6.121	6.297	5.383	4.051	-
	s.d.	0.242	0.895	1.044	0.409	1.163	0.608	1.303	1.000	-
dN	$g.h^{-1}$	0.066	0.161	0.195	0.205	0.137	0.169	0.290	0.154	-
	s.d.	0.012	0.017	0.011	0.066	0.008	0.080	0.132	0.026	-
crude	X_E %	37.84	47.93	46.37	44.67	47.80	50.02	50.81	49.66	-
protein	X_F %	37.97	45.08	48.18	47.90	49.31	50.91	50.81	51.76	-
Fat	X_E %	2.00	1.14	2.01	2.72	0.49	3.30	5.49	3.59	
	X_F %	3.50	1.88	2.01	1.70	1.01	1.43	2.56	0.83	
dP	$mg.h^{-1}$		130.04				165.39		165.60	
d Ca	$mg.h^{-1}$		10.31				10.91		38.35	
dMg	$mg.h^{-1}$		6.75				14.99		4.32	
dNa	$mg.h^{-1}$		80.59				124.56		251.16	
dK	$mg.h^{-1}$		80.90				111.61		2.70	

Table 25 The Effect of Temperature upon P. javanicum in a C.T.F.

T	°C	14	17	20	25	30	35	37	38
D	h^{-1}	0.207	0.199	0.207	0.196	0.203	0.201	0.218	0.200
	s.d.	0.003	0.003	0.003	0.019	0.008	0.005	0.016	0.002
Air	v.v.m	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
pH		3.2	2.8	2.6	2.5	2.6	2.5	2.5	-
n		5	5	5	5	5	5	5	7
X_E	$g.l^{-1}$	0.293	1.781	1.495	1.771	1.390	1.715	1.431	-
	s.d.	0.001	0.138	0.065	0.058	0.319	0.377	0.347	-
X_F	$g.l^{-1}$	20.612	6.278	6.616	4.992	5.214	4.583	7.239	-
	s.d.	0.103	0.819	0.767	0.514	0.526	0.215	0.810	-
μ	h^{-1}	0.003	0.056	0.047	0.070	0.055	0.074	0.044	-
	s.d.	0.001	0.019	0.003	0.008	0.016	0.011	0.008	-
ΔS	$g.l^{-1}$	1.575	2.392	2.844	3.486	2.835	2.826	3.787	-
	s.d.	0.659	0.250	0.253	0.146	0.314	0.723	0.454	-
ΔN	$g.l^{-1}$	0.037	0.079	0.062	0.120	0.086	0.062	0.054	-
	s.d.	0.009	0.012	0.003	0.003	0.001	0.027	0.012	-
Qs	$g.g^{-1}h^{-1}$	0.039	0.148	0.109	0.100	0.100	0.122	0.082	-
Crude protein	X_E %	41.06	47.55	50.11	40.01	37.84	34.67	48.41	-
	X_F %	45.05	42.77	46.84	42.04	37.97	30.06	-	-
Fat	X_E %	trace	4.00	6.90	2.46	6.96	2.00	trace	-
	X_F %	0.99	1.90	4.15	2.71	4.69	3.50	1.34	-

Table 26 The Effect of pH upon the Growth of *P. javanicum* in Surface Culture

pH	Diameter /cm
4.0	1.82 ± 0.13
5.0	1.85 ± 0.17
6.0	2.05 ± 0.23
7.0	2.22 ± 0.17
8.0	1.56 ± 0.22
9.0	no growth
10.0	no growth

Table 27 The Effect of pH upon the Growth of *P. javanicum* in Batch Submerged Culture

Initial pH	Final pH	Biomass /g
1.2	1.2	no growth
2.0	2.2	0.529 ± 0.048
3.0	2.4	0.520 ± 0.070
4.0	2.4	0.496 ± 0.026
5.0	2.4	0.533 ± 0.038
6.0	2.4	0.453 ± 0.042
7.0	2.6	0.291 ± 0.091
8.0	3.7	0.268 ± 0.043
9.0	5.3	0.126 ± 0.009

Table 28 The Effect of pH upon P. javanicum in a C.T.F.

pH		2.0	3.3	4.0	5.0	7.0	8.0
D	h^{-1}	1.041	1.063	1.003	0.976	1.018	1.045
	s.d.	-	0.037	0.070	0.019	0.036	0.016
Air	vvm	2.5	2.5	2.5	2.5	2.5	2.5
T	^{o}C	30	30	30	30	30	30
n		1	8	5	5	5	2
X_E	gl^{-1}	-	0.649	5.687	1.644	0.706	-
	s.d.		0.278	0.610	1.195	0.215	
X_F	gl^{-1}	-	4.228	6.379	6.864	5.714	-
	s.d.		0.795	0.820	0.422	1.410	
μ	h^{-1}	-	0.174	0.904	0.233	0.132	-
	s.d.		0.096	0.150	0.162	0.044	
ΔS	gl^{-1}	-	1.065	3.728	3.423	3.044	-
	s.d.		0.222	0.480	0.263	0.892	
ΔN	gl^{-1}	-	0.040	0.030	0.043	0.073	-
	s.d.		0.004	0.009	0.009	0.028	
crude protein	X_E %	-	47.93	47.85	45.86	54.33	-
	X_F %	-	45.08	45.58	46.03	52.90	-
Fat	X_E %	-	1.14	0.68	1.38	16.91	
	X_F %	-	1.88	2.39	1.80	0.00	
ΔP	$mg.l^{-1}$	-	33.19	36.80	39.86	37.82	-
ΔCa	$mg.l^{-1}$	-	2.55	1.13	-	4.06	
ΔMg	$mg.l^{-1}$	-	1.67	1.07	-	2.79	
ΔNa	$mg.l^{-1}$	-	19.95	74.40	-	1185.90	
ΔK	$mg.l^{-1}$	-	20.77	7.10	-	9.55	

Table 29 The Effect of Dilution Rate upon Growth of P. javanicum on Cheshire Whey in a C.T.F.

D	h^{-1} s.d.	0.409 0.083	0.560 0.025	1.066 0.022	1.643 0.076	3.000 -
T	^oC	30	30	30	30	30
Air	v.v.m	2.5	2.5	2.5	2.5	2.5
pH		3.8	4.0	4.1	4.1	-
n		6	5	5	5	-
dX_E	$g.h^{-1}$ s.d.	4.092 2.741	3.366 1.588	3.880 1.346	2.121 1.273	-
X_f	$g.l^{-1}$ s.d.	4.399 1.258	3.944 0.987	3.922 0.219	5.399 2.163	- -
μ	h^{-1} s.d.	0.235 0.150	0.216 0.045	0.259 0.070	0.100 0.032	- -
dS	$g.h^{-1}$ s.d.	5.075 1.192	4.501 1.516	6.063 0.800	15.189 4.401	-
dN	$g.h^{-1}$ s.d.	0.108 0.085	0.154 0.047	0.114 0.025	0.274 0.068	- -
crude protein	X_E % X_F %	47.73 44.07	48.10 46.04	48.13 47.22	48.77 48.51	- -
Fat	X_E % X_F	1.28 0.89	1.63 0.89	2.42 0.90	2.86 1.70	- -
dP	$mg.h^{-1}$	78.00	45.63	115.92	-	-
dCa	$mg.h^{-1}$	10.85	15.07	69.67	90.28	-
dMg	$mg.h^{-1}$	1.06	12.12	29.81	29.22	-
dNa	$mg.h^{-1}$	86.72	84.69	274.64	204.78	-
dK	$mg.h^{-1}$	70.56	8.51	449.64	469.50	-

Table 30 Composition of P. javanicum Biomass compared with a Commercial Swine Protein Supplement (Soybean)

		P. javanicum (dry)[a]	Soybean (dry)[b]
Crude protein (N x 6.25)	%	48.13	48.7
True protein[c]	%	>41.65	-
Fat (ether extract)	%	2.42	5.2
Fibre	%	8.15	6.7
Ash	%	7.57	6.3
Arginine	%	2.93	2.89
Glycine	%	1.89	2.78
Histidine	%	1.01	1.22
Isoleucine	%	2.26	3.11
Leucine	%	3.09	4.00
Lysine	%	4.19	3.00
Methionine	%	1.38	0.89
Phenylalanine	%	1.83	2.33
Threonine	%	1.97	1.89
Tyrosine	%	1.50	1.56
Valine	%	2.31	2.44

a Biomass produced from Cheshire Whey in C.T.F. at $D = 1.0h^{-1}$

b data from table 8, National Academy of Sciences, 1968

c Cysteine and Tryptophan not determined.

Table 31 The Amino Acid Spectrum of *P. javanicum* biomass produced in a C.T.F.

	Semidefined Medium				Cheshire Whey			
Fermentation conditions	$D=0.2h^{-1}$, $T=35^oC$ [b]		$D=1.0h^{-1}$, $T=30^oC$		$D=1.0h^{-1}$, $T=30^oC$		$D=0.5h^{-1}$, $T=30^oC$	
Amino Acid [a]	g/100g	g/16N	g/100g	g/16N	g/100g	g/16N	g/100g	g/16N
Asparagine	3.213	7.427	3.797	7.922	4.849	10.075	3.852	8.008
Threonine	1.477	3.414	1.767	3.687	1.968	4.089	1.708	3.551
Serine	1.472	3.403	1.634	3.409	1.844	3.831	1.441	2.996
Glutamine	3.382	7.818	4.370	9.117	5.629	11.695	4.330	9.002
Proline	-	-	1.737	3.624	2.186	4.542	1.765	3.669
Glycine	1.331	3.077	1.722	3.593	1.889	3.925	1.553	3.229
Alanine	2.664	6.158	2.636	5.500	2.779	5.774	2.342	4.869
Valine	1.646	3.805	1.813	3.783	2.306	4.791	1.842	3.830
Methionine	0.471	1.089	1.197	2.497	1.384	2.876	1.151	2.393
Isoleucine	1.306	3.019	1.766	3.685	2.262	4.700	1.756	3.651
Leucine	2.133	4.931	2.534	5.287	3.093	6.426	2.426	5.044
Tyrosine	0.668	1.544	1.284	2.679	1.501	3.119	1.300	2.703
Phenyalanine	1.682	3.888	1.481	3.090	1.832	3.806	1.481	3.079
Histidine	1.052	2.432	0.814	1.698	1.006	2.090	0.750	1.559
Lysine	2.273	5.254	3.099	6.466	4.188	8.701	3.047	6.335
Arginine	1.301	3.007	2.841	5.927	2.935	6.098	1.957	4.069
Total amino acid [a]			34.49		41.65		32.70	
crude protein	43.26		47.93		48.13		48.10	

[a] Cysteine and Tryptophan not determined

[b] biomass from exp 3.4.1.1

Appendix 5

Estimated reduction in BOD of whey after treatment in a Continuous Tower Fermenter.

The average BOD's of the three main constituents of whey, lactose, protein and fat are 0.65, 1.03, and 0.89 g. per g. whey respectively (Harper, Blaisdell and Groskopf, 1971). Liquid whey contains 5.1% lactose, 0.9% protein and 0.3% fat (Table 3). Thus it can be calculated that 100g. of whey would have a BOD of approximately 4.509g. (lactose = 3.315g; protein = 0.927g; fat 0.267g). If it is assumed that 1g. is equivalent to $1cm^3$ of whey this is equivalent to a BOD of $45090mg.l^{-1}$ (lactose = 33150; protein = 9270; fat = 2670) which is within the range of theoretical BOD for whey calculated by Wix and Woodbine (1958[a]) - 40,000 to 60,000 ppm. Therefore it is assumed that the BOD of whey is 45,090 $mg.l^{-1}$ of which lactose contributes $33,150mg.l^{-1}$ and protein $9270mgl^{-1}$.

When these figures are reduced by 90% (lactose) and 40% (protein) the remaining BOD is $11547mgl^{-1}$ (lactose = $3315mg.l^{-1}$; protein = $5562mg.l^{-1}$; fat = $2670mg.l^{-1}$) which gives an overall BOD reduction of approximately 74%.[a]

[a] assumes fat not removed.

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