CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 SCREENING OF YEAST STRAINS

4.1.1. Screening of Yeast Strains

Among the hydrocarbon assimilating microorganisms, Yeast was found suitable for SCP production from liquid alkanes (C_{14}-C_{20}) because of its higher yield coefficient (0.8 to 1.0) as compared to that of bacteria and fungi (Rehm and Reiff, 1981). Bacteria generally produce oxidised derivatives of the assimilated hydrocarbons (Rehm and Reiff, 1981). Moreover yeasts have got the advantages of being larger in size and posses lower concentration of nucleic acid compared to bacteria (Soloman, 1985). Yeasts belonging to the same genera but of different species were used in Single Cell Protein production and for microbial dewaxing of gas oil (Litchfield, 1979; Moo Young, 1976; Cheipigo, 1969; Katrush, 1979; Kockert, et.al., 1976). Among the hydrocarbon assimilating yeasts (Table 2.1) *Candida* genus has been used widely for dewaxing of petroleum fraction as well as SCP production and has been tested extensively for acceptability as protein substitute in cattle feed (Shukla, et.al., 1979; Parnerkar, 1983). Based on these favourable aspects, yeast strains *Candida tropicalis* and *Candida lipolytica* had been primarily choosen for the present study.

The adaptation potentiality of the strains were evaluated from the growth of the strains by measuring the optical density (O.D.) of the aqueous medium at different times (Table 4.1.1).

Comparatively higher sp. growth rate of the strain *C.tropicalis* (0.11 h^{-1}) was observed than that of *C.lipolytica* (0.06 h^{-1}) (Fig. 4.1.1).

The lag phase of *C.tropicalis* was 18 hrs. whereas *C.lipolytica* could grow only after 20 hrs. This shows that the induction of enzyme system of *C.tropicalis* was faster than *that of C.lipolytica* in the presence of heavier petroleum fraction (A) Chepigo, 1968, observed that *C.lipolytica* could grow efficiently on n-paraffin in the carbon range of C_{12}-C_{21} whereas the carbon range of C_{14}-C_{24} was suitable for maximum growth of *C.tropicalis*.

It is also evident from the Figure 4.1.1 that higher growth of *C.tropicalis* was observed (optical density, -0.341 compared to that of *C.lipolytica* (optical density, -1.0) at the end of growth phase (25th hr.).

Based on these results the yeast strain, *C.tropicalis* was selected for further studies on microbial dewaxing of vacuum distillates.

TABLE 4.11 SCREENING OF THE YEAST STRAINS ON VACUUM DISTILLATE SAMPLE (A).

Time, hr.	*C-tropicalis*		*C-lipolytica*	
	O.D.	ln O.D.	O.D.	ln O.D.
16.0	0.36	-1.00	0.245	-1.40
17.	0.36	-1.00	0.245	-1.40
18	0.39	-0.940	0.245	-1.40
19	0.40	-0.910	0.245	-1.40
20	0.45	-0.798	0.260	-1.34
20.5	0.48	-0.730	0.270	-1.30
21	0.52	-0.653	0.290	-1.24
22	0.55	-0.590	0.300	-1.20
22.5	0.60	-0.500	0.315	-1.15
23	0.62	-0.478	0.325	-1.12
24	0.69	-0.358	0.330	-1.10
25	0.71	-0.342	0.340	-1.08
26	0.71	-0.342	0.340	-1.08

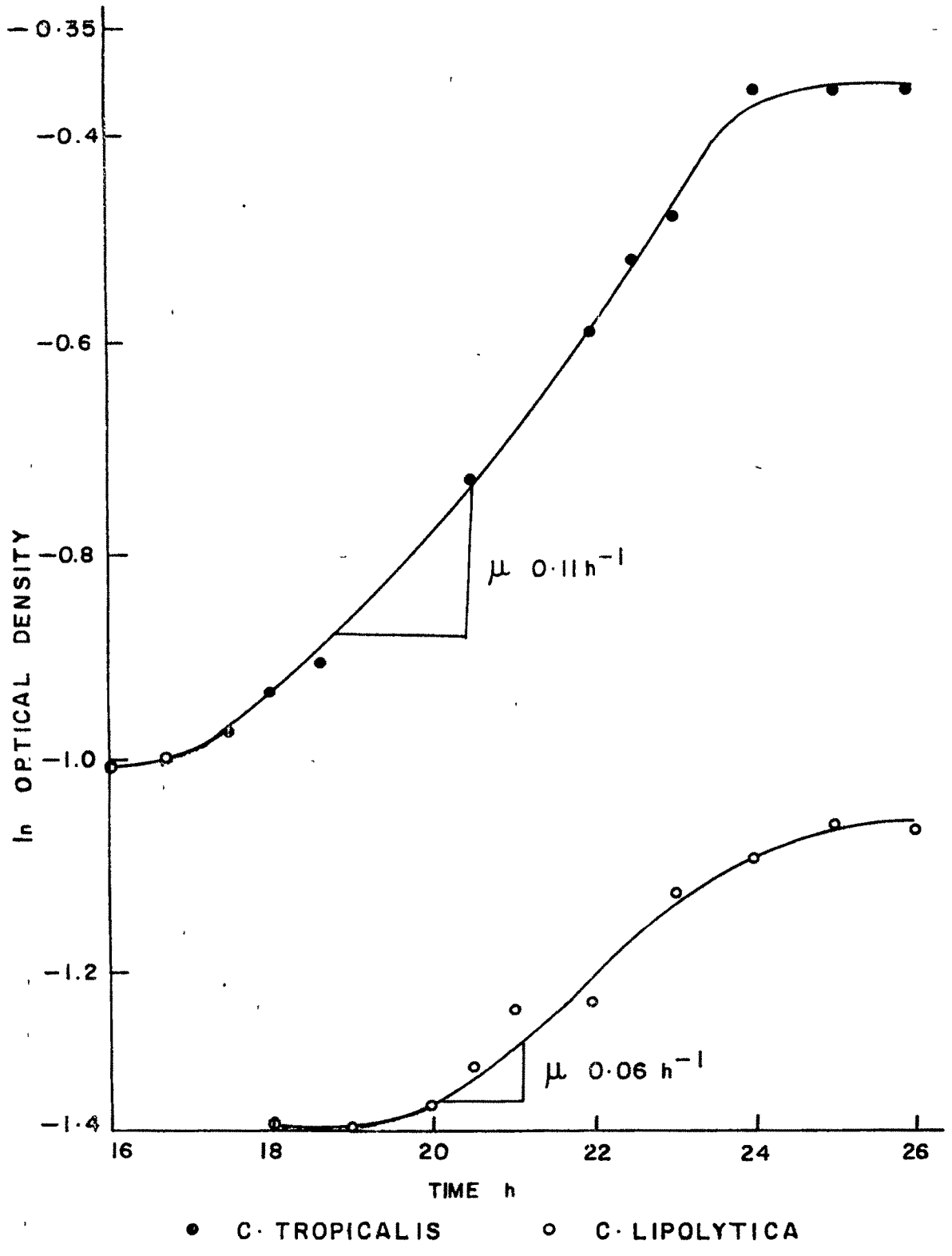

Fig.4.1.1.Growth of yeast strains in shake flasks

4.1.2 Adaptation of *C.tropicalis* on vacuum distillate

Prior exposure of a microbial strain to any petroleum fraction plays an important role in increasing the hydrocarbon oxidizing potential of the organism and this phenomenon is known as adaptation (Spain and Veld, 1983). There are three inter related mechanisms as given below by which adaptation can occur:

a) Induction and/or depression of specific enzymes.

b) Pheno type genetic changes which result in new metabolic capabilities.

c) Selective enrichment of organism (s) which are capable of transforming the components of a petroleum fraction of interest.

Selective enrichment of the organism has been widely observed in studies of petroleum degradation in the environment as reported by Colwell and Walker, 1977; Atlas, 1981; Floodgate, 1984; Bossert and Bartha, 1984.

Shake Flask Studies :

Earlier studies (4.1.1) indicated that the yeast strain *C.tropicalis* possesed the inducible enzyme systems which supported the growth of the strain on high boiling petroleum fraction (400-490^{o}c) containing more polyaromatic hydrocarbons (PAHs) along with saturates with more branched and cyclic compounds as compared to *n-paraffin* (C_{14}-C_{10}) and gas oil fraction. Moreover

the physical state of the hydrocarbon selected for dewaxing was semi solid in nature.

Bauer and Capone, 1988 provided evidence for "Cross-acclimation" of mixed microbial culture, in which exposure to one compound affects metabolism rate of a compound of similar structure.

The adaptation capacity of the yeast, *C. tropicalis* was tested in the shake flask culture and the sub culturing of the strain was done. The study provided the observation that there are three distinct stages of interactions between the growing cells and the semi solid heavier fraction..The three phases as observed under microscope are shown in Photographs (Fig. 4.1.2). The microscopic observation was made at a fixed magnification (X45) for all the cases.

Phase I: Attachment of yeast cells with the large semi solid oil fragments

Phase II Formation of oil drops smaller in size than the earlier phase due to emulsification of the semi solid oil fragments. Yeast cells completely covered the surface of the oil droplets.

Phase IIIA pseudo solubilized state of oil/water emulsion containing majority of yeast cells in free state. The oil/cell droplets were found smaller in size than that of Phase II. Hydrocarbon layers had disappeared and got uniformly distributed throughout the culture broth in the flask.

The three distinct phases of adaptation of yeast on heavier petroleum fraction and physical change of the hydrocarbon in the shake flasks are shown in photograph 4.1.3.

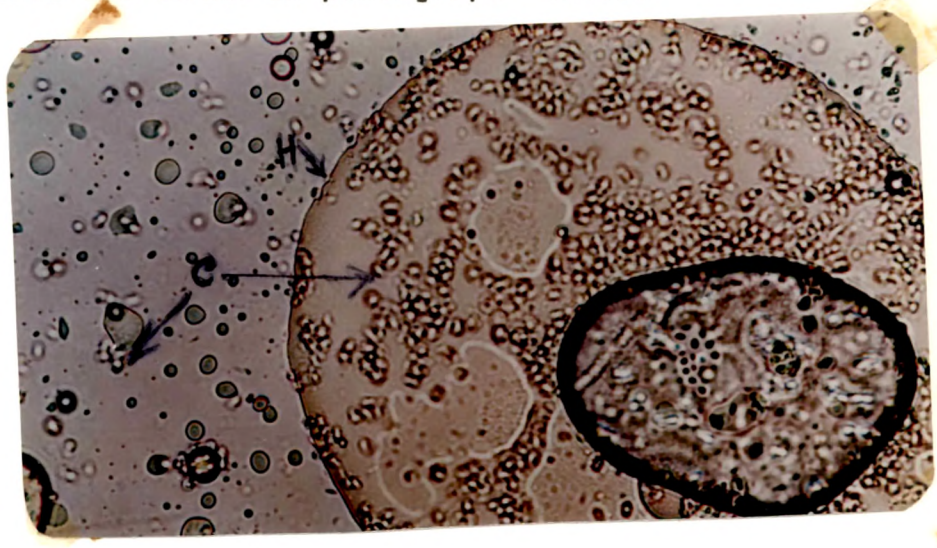

Fig. 4.1.2 Phase I Hydrocarbon fragment (H) , Yeast cells (c)

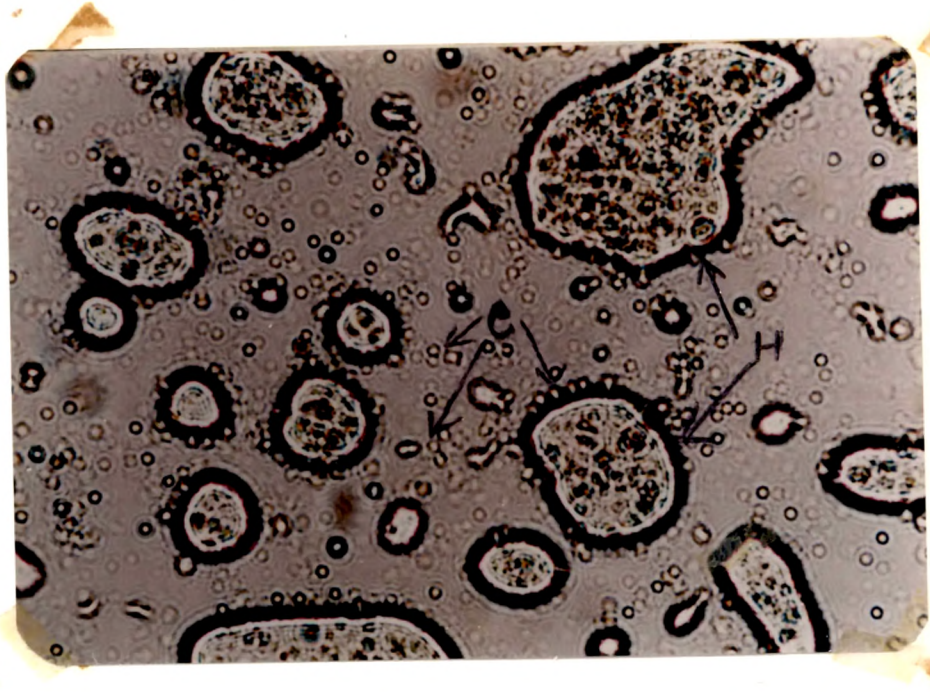

Fig. 4.1.2 Phase II Hydrocarbon droplets (H) , Yeast cells (c)

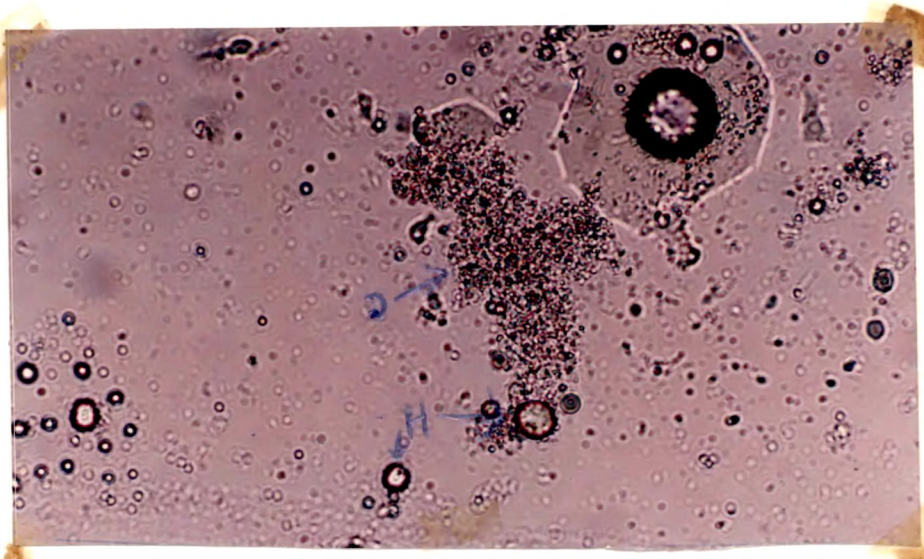

Fig 4.1.2 Phase III Oil in water emulsion
Hydrocarbon droplets (H), yeast cells (c).

Fig.4.1.3. Physical examination of shake flasks for phase I (PI), Phase II (PII) and Phase III (PIII)

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4.2 OPTIMISATION OF GROWTH PARAMETERS

4.2.1. Effect of temperature on microbial growth

Microbial growth and product formation comprises of a complex series of enzymatic reactions which like chemical reactions, are influenced by temperature. Growth of any microbial population can be described by the equation (4.2.1)

$$\frac{dx}{dt} = \mu x - Kx \quad \text{---------} \quad 4.2.1$$

$$\text{or,} \quad \frac{1}{x}\cdot\frac{dx}{dt} = \mu - K \quad \text{----} \quad 4.2.2$$

where μ is the growth rate, K is the death rate and x is the cell concentration. Thus the observed Sp. growth rate, 1/x.dx/dt is a balance of growth and death of the population of cells. The growth of the culture predominates when $\mu >> K$ during exponential growth phase. As both μ and K are temperature dependent, the specific growth rate of any organism changes with the change of growth temperature due to cumulative effect of rate constant, μ and K.

Most of the hydrocarbon assimilating yeasts exploited for SCP process development, are classified as mesophilic (30-40^{o}c) organisms (Litchfield, 1977).However, biodegradation of petroleum was reported even in extreme conditions. Huddleston and Cresswell, 1976 observed petroleum biodegradation in soil at -1.1^{o}c in contrast to the existence of thermophillic alkane utilising bacteria as reported by Klug & Markovetz, 1967.

In order to find the effect of temperature on specific growth of the yeast strain *C.tropicalis*, growth temperature of the batch culture was varied from 30 to 42°c using n.parafins (C_{14}-C_{20}) and vacuum distillate (C_{22}-C_{40}) as carbon substrates. The experimental conditions e.g., pH, initial substrate concentration, aeration and agitation rate were kept same during each batch operation.

The yeast strain maintained a growth phase in the temperature from 30°c to 36-37°c and the declined growth phase prevailed in the short span of 37°c to 42°c when death rate, K >> growth rate of the cells as shown in table 4.2.1.

The strain has exhibited the maximum specific growth rate of 0.38 h^{-1} at 35-36°c on n.paraffins (C_{14}-C_{18}) and a specific growth rate of 0.27 h^{-1} at 36-37°c on heavier petroleum fraction (C_{22}-C_{40}) . The higher specific growth rate of the strain on short chain liquid alkanes (C_{14}-C_{18}) was observed as compared to that on longer chain alkanes (C_{22}-C_{40}) in the mesophillic range of temperature (Fig.4.2.1). Dostalek et.al., 1968 observed the similar behavier with *C.lipolytica* grown on n.alkanes of carbon chain length of C_{10}-C_{14} and C_{18}-C_{22} present in different gas oil fractions.

Activation Energy :

Temperature dependance of sp. growth rate, μ and death rate K of microorganism obey the Arrhenius correlation (Eq. 4.2.3), as described by Pirt, 1975.

$$\mu = A \cdot e^{-E/RT} \quad \text{----------4.2.3}$$

or, $$\ln \mu = \ln A - E/R.1/T \quad \text{------4.2.4}$$

where R is the gas constant (1.98 cal/mole ^{o}K), T is the temperature (^{o}K), A is a constant dependent on the frequency of formation of activated complex of the enzymes and substrate involved in hydrocarbon metabolism and E is the activation energy (K J/ mole). The calculated values of natural logarithm of Sp. growth rate, (ln μ) and 1/T are given in Table 4.2.2. Activation energy for growth and death phases of *C.tropicalis* growing on both the petroleum fractions were calculated from the slope of the plot ln μ v/s 1/T as shown in Fig. 4.2.2.

Higher energy of activation (67.7 KJ/mole) for growth of the strain on vacuum distallate (A) was observed as compared to activation energy (58.5 KJ/mole) on n.paraffins (C_{14}-C_{18}) which indicated that inspite of similar metabolic pathway followed by the strain for assimilation of short and long chain alkanes, higher activation energy was required by the strain for growth on long chain alkanes (C_{22}-C_{40}). Goma et.al., 1981 have established a direct correlation on energy of activation and maintenance energy of the cells and observed that higher percentage of carbon substrate was used for cell maintenance when the growth temperature was increased.

The uniform slope of the plot (Fig. 4.2.2) either for growth and death phases indicated the absence of any shift of metabolic process due to activation or repression of enzymes with the

increase of temperature. However, similar phenomenon was observed with the growth of E. Coli on glucose when growth temperature was increased beyond 25°c (Pirt, 1975).

Higher activation energy of declined growth i.e., 605 KJ/mole (higher alkanes) and 247 KJ/mole (n.paraffins) was observed as compared to their respective energy of activation for growth (Fig. 4.2.2) because of the denaturation of enzymes proteins associated in the hydrocarbon metabolism and low molecular diffusion of hydrocarbon across the cell membrane (Wang et.al., 1979).

The Table 4.2.3 shows that the gradual decrease of cellular yield from 0.12 to 0.06 on heavier hydrocarbon was observed when the temperature was increased from 34°c to 42°c due to higher demand of maintenance energy of cells with the increase of temperature. (Pirt, 1975; Topiwala & Sinclair, 1972). However, there was an increase of cellular yeild from 0.05 to 0.12 when temperature was increased from 30°c to 34°c perhaps due to lower maintenance energy requirement in this range of temperature.

The dewaxing of vacuum distillate (A) was observed maximum in the temperature range of 34°c to 37°c as indicated by the decrease of pour point of the oil fraction from 48°c to 18°c (Fig.4.2.3), However, temperature beyond the range 34-37°c was unfavourable because of decreasse of cellular yield.

The optimum growth and dewaxing temperature at 37°c would be favourable for lower operating cost of the process as higher cooling efficiency can be achieved by circulating tap water (25-30°c) in the fermentor.

TABLE 4.2.1 EFFECT OF TEMPERATURE ON SP. GROWTH RATE

Temperature, ^{o}C	Sp. growth rate, h^{-1}	
	Vacuum distillate(A) (C_{22}-C_{40})	n.paraffins (C_{14}-C_{18})
30	0.15	0.27
31	0.17	0.30
32	0.18	0.32
33	0.20	0.34
34	0.22	0.375
35	0.24	0.38
36	0.26	0.38
37	0.27	0.30
38	0.26	0.24
39	0.16	0.16
40	0.06	0.12
42	-	-

TABLE 4.2.2 DETERMINATION OF ACTIVATION ENERGY

Temperature (T) ^{o}K	1/T	ln Sp. growth rate, h^{-1}	
		Vacuum distillate(A) (C_{22}-C_{40})	n.paraffin (C_{14}-C_{18})
303	3.30	-1.90	-1.3
304	3.28	-1.83	-1.2
305	3.27	-1.77	-1.14
306	3.26	-1.61	-1.07
307	3.25	-1.52	-0.98
308	3.24	-1.43	-0.96
309	3.23	-1.35	-0.96
310	3.22	-1.31	-1.20
311	3.21	-1.34	-1.43
312	3.20	-1.83	-1.83
313	3.19	-2.80	-2.12

TABLE 4.2.3 THE EFFECT OF TEMPERATURE ON YIELD OF BIOMASS AND POUR POINT OF OIL

Temperature, ^{o}C	Yield of boiomass Y $\frac{\text{gm of biomass}}{\text{gm of oil fract.}}$	Pour point of dewaxed oil, ^{o}C
31	0.05	27
33	0.11	24
34	0.12	21
36	0.11	18
37	0.10	18
38	0.08	21
39	0.05	27
40	0.02	36

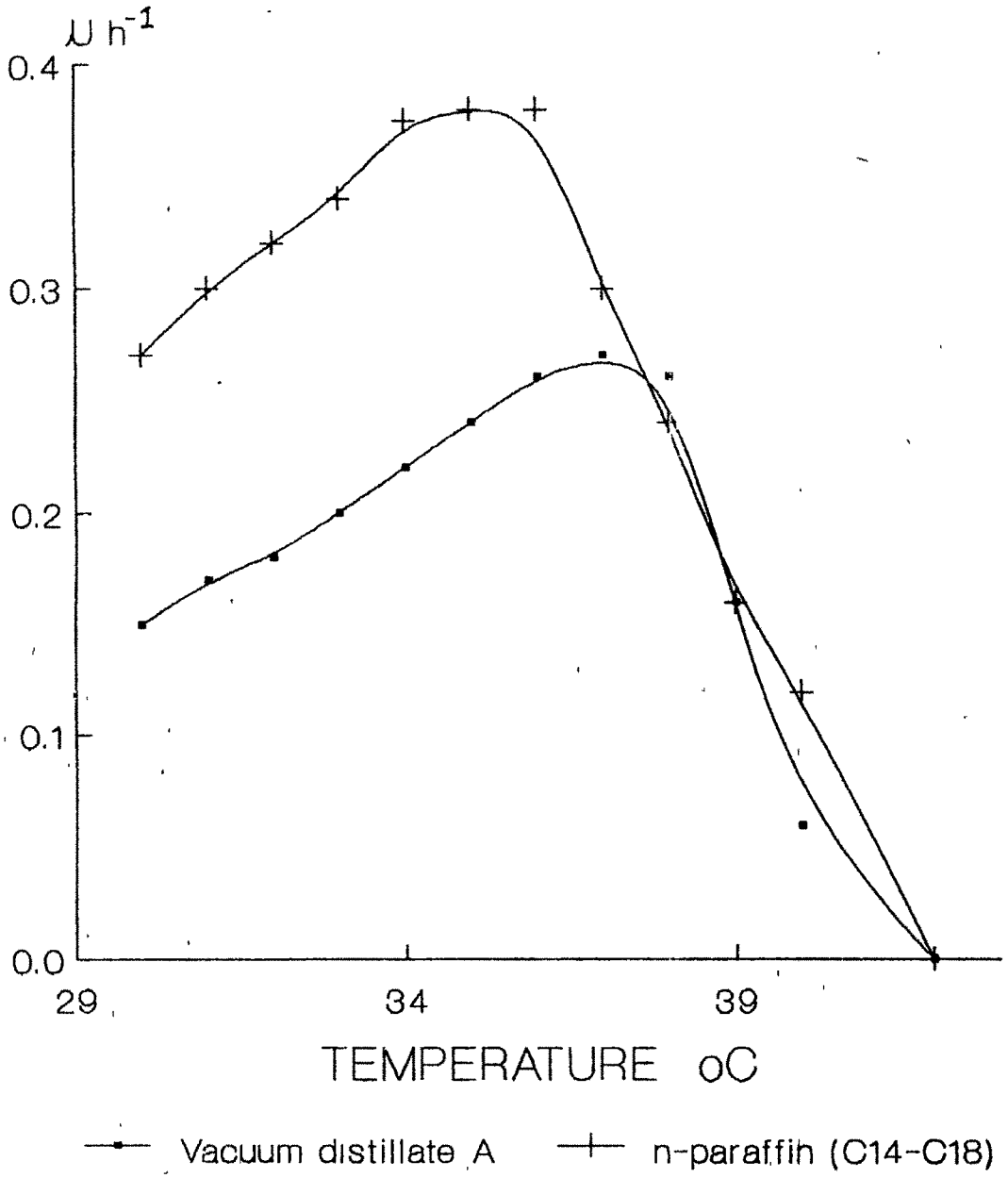

FIG .4.2.1 EFFECT OF TEMPERATURE ON SP. GROWTH RATE ($\lambda U\ h^{-1}$).

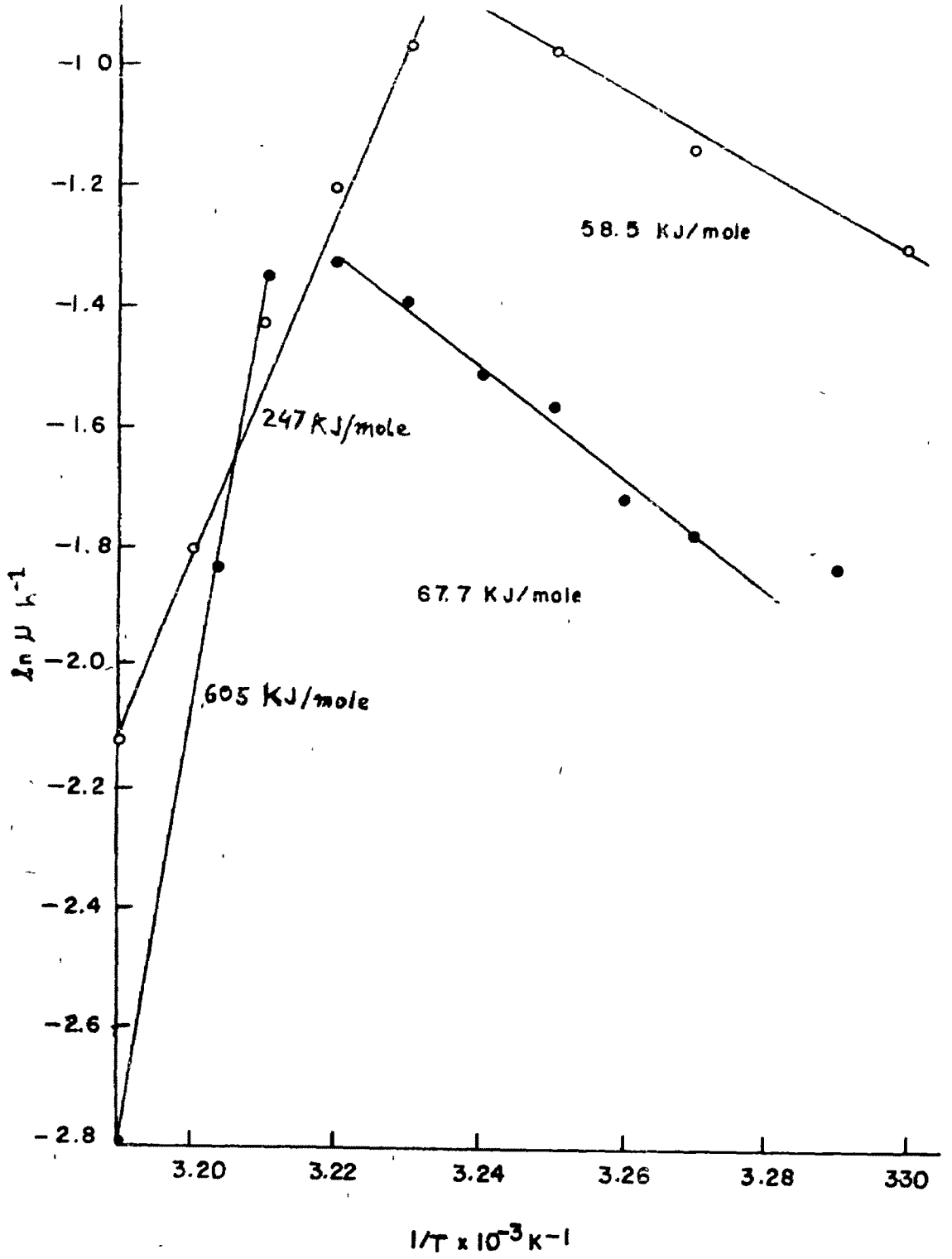

Fig.4.2.2.Activation energy of the strain on Sample A'(●) and n paraffin (O).

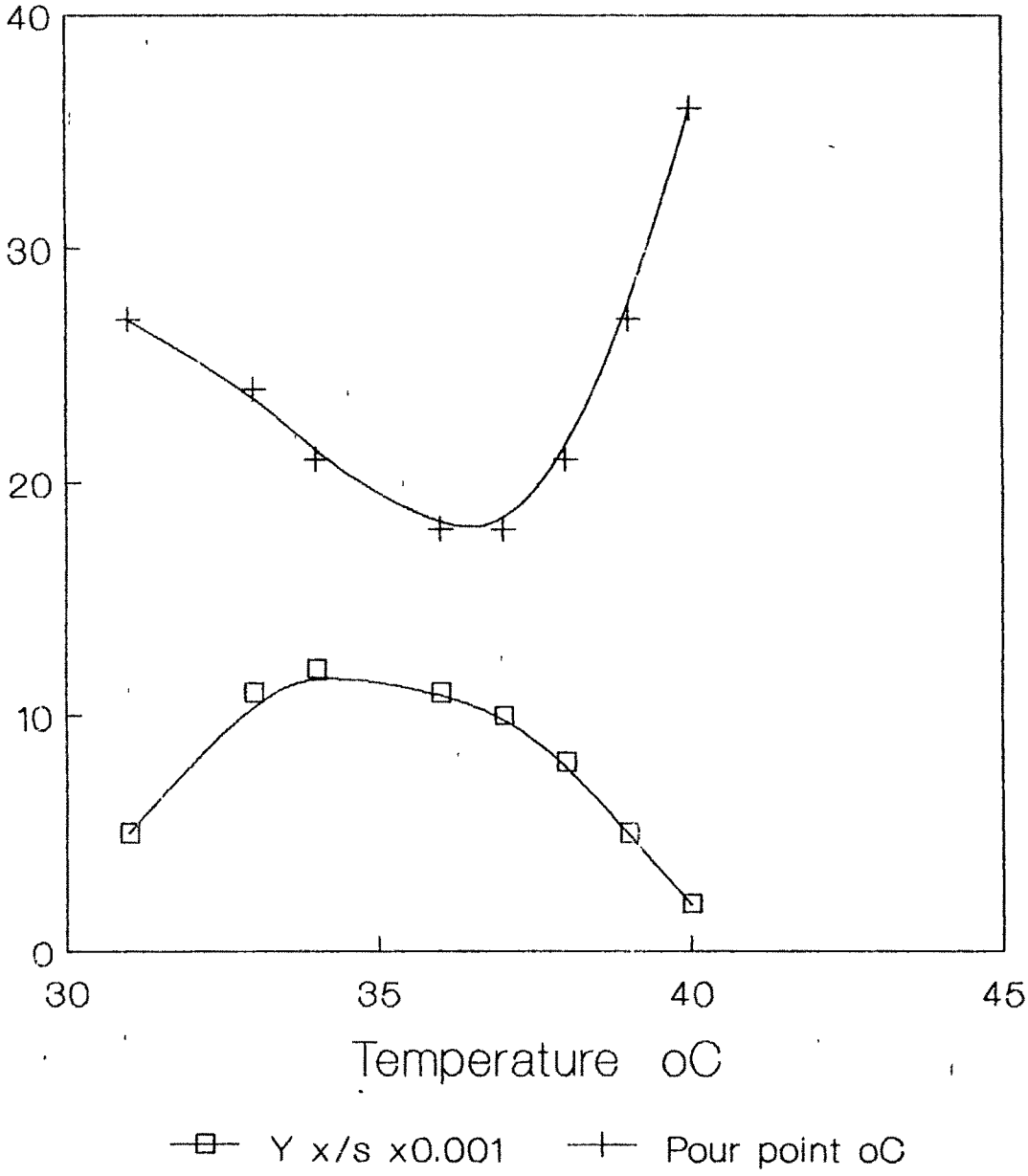

Fig. 4.2.3. Biomass yield and pour pt. at different temperature

4.2.2 EFFECT OF P^H ON MICROBIAL GROWTH

Most heterotrophic bacteria favour a pH near neutral range (6-7) with fungi being more tolerant of acidic condition i.e., pH 4-6 (Atlas, 1988). Yeast also grows in the acidic range and the yeast based SCP processes were operated in the pH range of 3 to 5.5 (Table 2.5). Among the yeast strains, *C.tropicalis* is more tolerant to the acidic conditions than *C.lipolytica*. Hydrogen ion concentration (H^+) has a very sharp effect on growth of microorganism because large difference between intracellular and the extracellular pH across the cell membrane causes deactivation of membrane bound enzymes.

Hence the control of pH is an important criteria in most of the fermentation processes. H^+ concentration in the medium is controlled either by using phosphate buffer (Na_2HPO_4/KH_2PO_4) which is applied in shake flask culture, or by using automatic pH control system.

pH of the medium generally changes during fermentation for several reasons. Verkooyen et.al., (1980) and Huth (1990) have demonstrated that when ammonia salt is used as the source of nitrogen, the pH value decreased due to the assimilation of $R\text{-}NH_3^+$ ion as nutrient and leaving H^+ in the medium.

On the contrary, if nitrate is the nitrogen source, then hydrogen ions are removed from the medium to reduce the $NO_3^=$ to $R\text{-}NH_3^+$ and the pH tends to rise. Prokop et.al., (1971) and Huth (1990) demonstrated that growth of *C.lipolytica* on gas oil was proportional to the cumulative consumption of 10% NaOH per litre of fermentor broth maintained at constant pH. Growth of yeast,

C.tripicalis on vacuum distillate (A) was associated with the decrease of pH of the medium containing $(NH_4)_2.SO_4$ as nitrogen source. When the fermentation experiments were carried out in MICROGEN, 5.0 L fermentor turning off the pH control system, pH of the culture broth decreased at the rate of 0.2 pH unit per hour from the preset value of pH 4.0 In order to study the effect of pH on the Sp. growth rate of the strain, batch experiments were conducted with constant inoculun size at different pH value automatically controlled by the addition of 1 N NH_4OH in the fermentor. The results are summarised in Table 4.2.4. It was observed that the strain could grow in wide range of pH 3.5 to 5.5 and attained a maximum Sp. growth rate 0.27 h^{-1} in the range of pH 4 to 4.5 as illustrated in Fig. 4.2.4.

The pour point of the dewaxed oil obtained at the end of batch growth was found minimum i.e., 18°c in the pH range 4 to 4.5. Hence the dewaxing of the vacuum distillate was observed maximum in this range of pH as there may be inhibitory effect of pH of the fermentor broth on membrane bound oxygenase enzymes beyond the range 4-4.5 of pH.

Gale and Epps, 1942 showed that change in pH from 5 to 8 during the growth of E.Coli on Caseine hydrolysate inducted the activity of different enzymes and he suggested that the amount of the enzymes that are regulated during growth so as to maintain a constant activity and compensate for the effect of medium pH value.

However, the optimum range of pH (4 to 4.5) for growth of *C.tropicalis* made it advantageous for the development of dewaxing

process as the acidic range does not favour the growth of undesirable bacteria and fungal strains.

TABLE 4.2.4. Effect of pH on growth rate of yeast strain

pH value	Pour pt. of dewaxed oil, ^{o}c	Sp. growth rate, h^{-1}
3.0	30	0.22
3.5	24	0.23
4.0	18	0.265
4.5	18	0.27
5.0	21	0.24
5.5	33	0.18

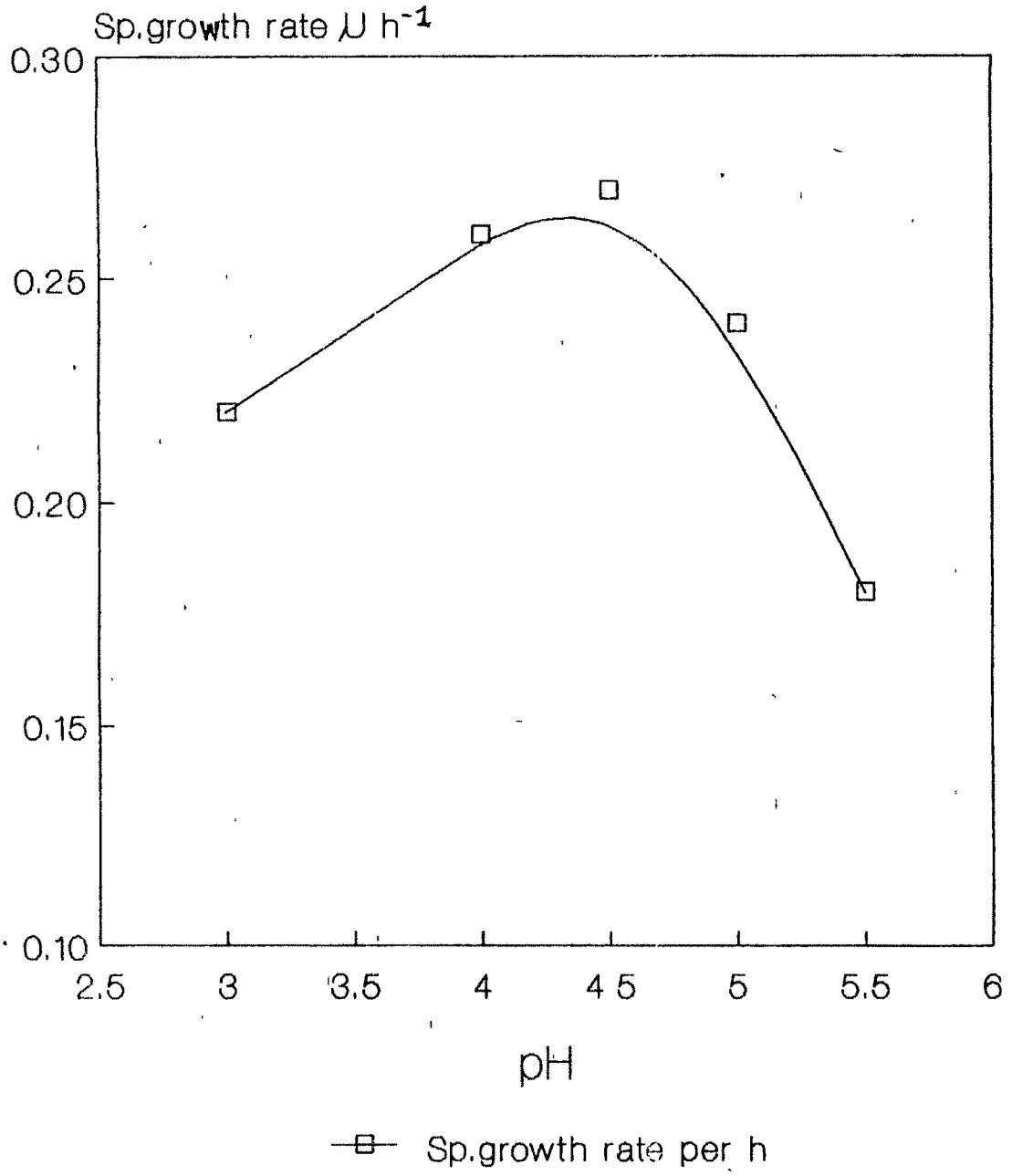

Fig.4.2.4 EFFECT OF pH ON SP.GROWTH RATE

4.2.3 Effect of oil concentration on dewaxing :

The relationship between substrtate concentration and Sp. growth rate was established by Monod, 1949 as follows :

$$\mu = \frac{\mu m \cdot S}{Ks + S} \qquad \text{----------4.3.1}$$

where μ is the specific growth rate and μ_m is the maximum specific growth rate, S is the substrate concentration, Ks is the constant equivalent to substrate concentration when Sp. growth rate is half of its maximum value. The plot of S v/s μ showed that max. Sp. growth rate μ_m remains independent of substrate concentration. When S>>Ks which is normally observed during logarithmic growth phase of most of the batch culture (Bailey & Oillis, 1986). The specific growth rate μ becomes a function of the substrate concentration when substrate concentration remains low i.e., S<10 Ks .This situation arises during stationary phase of the culture (Wang et.al., 1979).

The critical value of Ks is obtained by lineweaver-burk plot ($1/\mu$ v/s 1/s) applying continuous culture technique. It became very difficult to obtain the Ks value of *C.tropicalis* in a batch culture with vacuum distillates. It was possible, however, to employ initial rate kinetics at a low substrate concentration for the determination of Ks value. This method could not be applicable in the present study of dewaxing heavier petroleum fraction because of its heterogeneous semi solid characteristic. The fragments of the oil fraction in the culture broth, as observed in

Fig. 4.1.2, posed the problem of dispersion during fermentation. Consequently, the continuous cultivation of the strain could not be applied for the dewaxing of heavier petroleum fraction.

However, Ks value of the strain for liquid paraffins and gas oil was determined by continuous culture techniques (Saini et.al.) and it was observed comparatively higher Ks value(0.08 $g.l^{-1}$) for gas oil than that of other microorganism-substrate system e.g., 0.025 g/l^{-1} for *S.cerevisiae* on glucose as reported by Wang et.al., 1979.

In order to study the effect of oil concentration on dewaxing of vacuum distillate, batch experiments conducted in 1.5 l glass fermentor at optimum temperature, 37°c and pH 4, aeration 1.5 vvm and with the agitation rate of 1400rpm. The substrate concentration in the fermentor was varied from 10 g/l to 100 g/l.

The effect of higher substrate concentration on the pour point of the dewaxed oil along with the yield of biomass and dewaxed oil are summarised in Table 4.2.5.

The oil concentration upto 40 g/l did not have any inhibitory effect on the growth of biomasss by assimilating the higher alkanes as indicated by a constant yield of 0.11 gm biomass per gm of vacuum distillate and 55 gm of dewaxed oil per 100 g of vacuum distillate. The pour point of the dewaxed oil remained same at 18°c. A decrease in biomass yield by increase in the oil conc. been reported by Dostalek et.al., 1968. In the present stydy increasing the oil concentration from 40g/l to 100 g/l resulted in a decrease in the biomass yield from 0.11 to 0.008 and poor dewaxing was observed from the pour point rise of dewaxed oil

(42°c) as illustrated in Fig. 4.2.5.

Consequently the increase in productivity of dewaxed oil with the increase of substrate concentration during batch dewaxing of vacuum distillate for 70 h was observed (Table 4.2.5). The decrease of yield of biomass indicated the possible limitation of substrate availability to the yeast cells due to improper dispersion of heterogeneous hydrocarbon fraction. The assimilation of long chain alkanes occured at rate which exceeds of the rate of hydrocarbon emulsification and was a function of hydrocarbon surface area available for physical attachment of cells (Nakahara et. al., 1977; Wang, at.al., 1972). Munk et.al. (1969) found that biomass concentration was poor at high concentration of gas oil and also reported the increase of freezing point of the oil from -20°c to 10°c. Laine, 1975 studied the relationship between productivity and gas oil concetration in the fermentor. They observed that productivity increased linearly upto 3.8 $g.l^{-1}.h^{-1}$ by increasing the gas oil concentration upto 20% and beyond which the oil became continuous phase which affected the productivity sharply.

TABLE 4.2.5. EFFECT OF OIL CONCENTRATION ON DEWAXING OF VACUUM DISTILATE `A`

Oil concentration	Yield factor Biomass	dewaxed oil	Pour pt. ^{o}c	Productivity $g.l^{-1}h^{-1}$
10	0.11	55	18	0.078
20	0.11	55	18	0.157
30	0.11	53	18	0.235
40	0.10	54	18	0.314
50	0.08	60	21	0.428
70	0.06	68	27	0.66
100	0.008	80	39	1.14

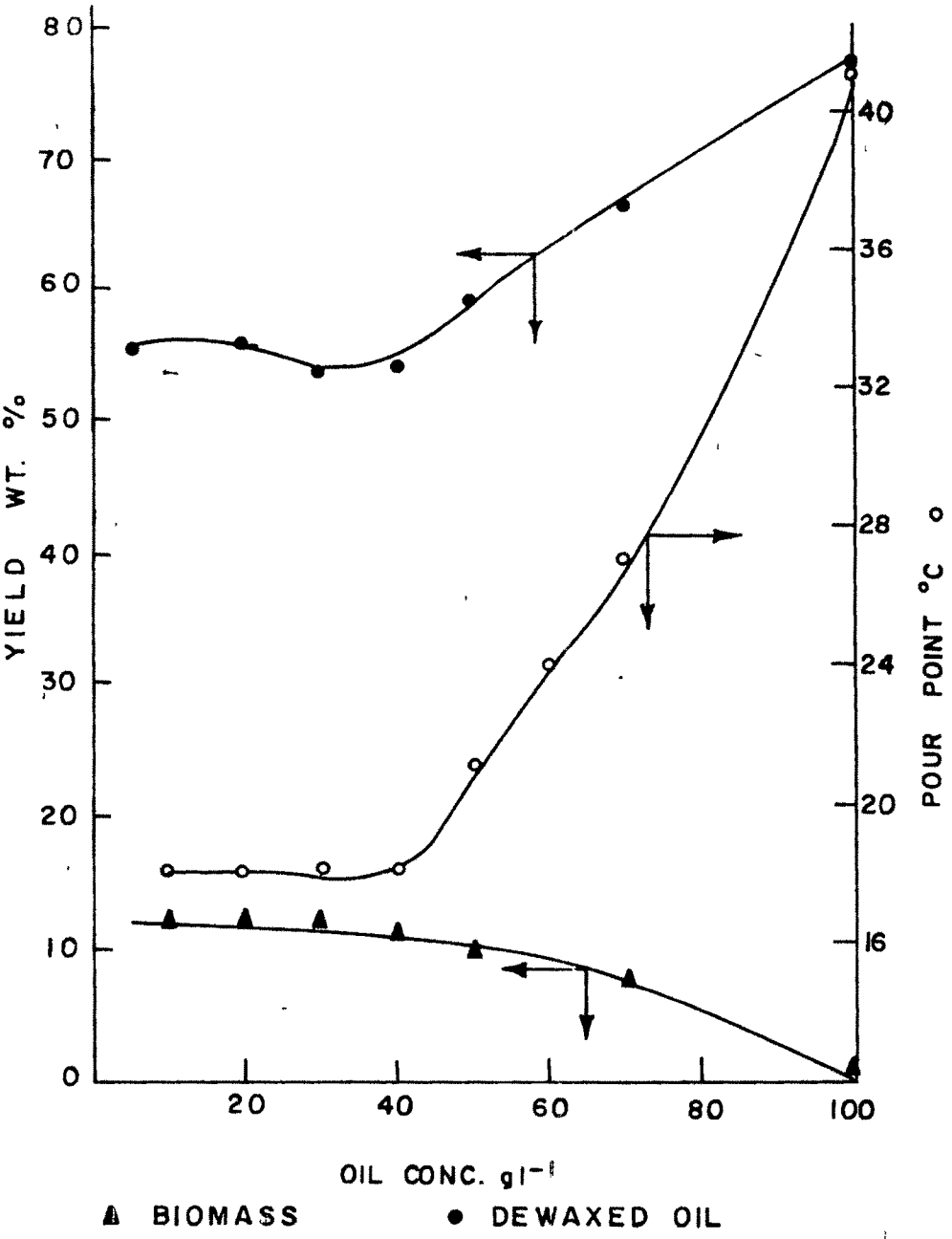

Fig. 4.2.5. Effect of oil concentration on dewaxing of vacuum distillate'A'

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4.3 GROWTH STUDIES

4.3.1. Dewaxing by batch operation :

During microbial dewaxing the n alkanes present in the petroleum fraction are assimilated by the microbes to synthesise biomass. Therefore , dewaxing is expected to be a growth associated phenomenon. The kinetics of such reactions, in a batch operation , depend not only on specific growth rate (μ) of the strain but also on lag phase and the changes in the product during stationary phase. The productivity of the system can be enhanced by switching over to single stage chemostat operation if the reaction is purely growth associated. In order to confirm the association of growth and dewaxing , the dewaxing rate of both the feed stocks namely, vacuum distillate 'A' (b.p. 400-490 ^{0}C) and 'B' (b.p. 350-500^{0}C) was studied in batch fermentation runs conducted in 1.5 l and 5 l capacity fermentors. The fermentors were operated at optimised process conditions i.e; pH 4.0, temperature 37^{0}C , substrate concentration 40 g.l^{-1} and rpm 1000 to 1400. The growth related data obtained in these runs were used to evaluate μ and yield coefficients

The growth curve of the strain along with the pour point of the dewaxed oil in various phases of the curve is shown in Fig.4.3.1. and Fig. 4.3.2. for feed stock 'A' and feed stock 'B' respectively. The following observations are made:

1. Lag phase : The lag phase is the period of adaptation of a microbe which involves synthesis or repression of enzyme needed for efficient utilisation of nutrient for the growth (Wang et al,1979 ; Bailey and Ollis ,1986). During this phase negligible growth takes place . Duration of lag phase depends upon many

factors which include change of environment, change of substrate, and the affinity of the cells for attachment to the hydrocarbon droplets. In one of our earlier studies (Adhikari,et al .1990) it has been determined that if hydrocarbon fraction is used as substrate the lag phase is elongated because the lighter fraction extracts lipid from cell wall and hence hampers the capacity of cells to attach themselves to hydrocarbon droplets.

As illustrated in the figures , duration of lag phase for feeds stocks `A' and `B' is 14 hours and 8 hours respectively. These periods are much less than observed in shake flasks (Fig. 4.1.1). This is due to the fact that the operating conditions are effectivily controlled in the fermentors.

Sample `A' has a solidification temperature of $48^0$C and is a semisolid mass where as sample `B' is liquid at the fermentation temperature . The emulsification of `B' is therefore easier than `A' which may be one of the reason for longer lag phase on sample `A'.

2.Growth phase : After the completion of lag phase growth phase starts in which cell mass increases exponentially as per equation 4.3.1.

$$\frac{dx}{dt} = \mu . x \qquad \ldots\ldots\ldots \qquad 4.3.1.$$

Where $\frac{dx}{dt}$ is rate of increase in biomass at time t when biomass concentration is x. Equation 4.3.1 when integrated between boundray conditions :

$t = t_1$, $x = x_0$

$t = t_2$, $x = x_t$

one can get ,

$$\mu = \frac{\ln x_t - \ln x_0}{t_2 - t_1} \qquad 4.3.2.$$

A plot between x or any other directly proportional parameter versus t would yield a straight line during growth phase, the slope of the line would equal to μ

The total duration of exponential growth phase for sample 'A' is 28 hours , the μ ,however is not constant throughout the exponential phase. At 22 nd. hours ,it has changed from 0.27 h^{-1} to 0.11h^{-1}. The higher growth rate in first part of experimental phase may be due to assimilation of lower chain alkanes as a suitable substrate to the strain in preference to long chain alkanes. It is well established that specific growth rate is a function of carbon range of petroleum substrate, highest being the carbon range of C_{14}-C_{23} (Ballerini ,1969; Dostalek et al, 1968). Earlier same strain has been reported to have specific growth rate of 0.38 h^{-1} on C_{14}-C_{18} and 0.31 h^{-1} on C_{19}, n-paraffins (Sista,1989).

c) Stationary phase - During the stationary phase of batch cultivation i.e; 20 hrs after the log phase for sample'A' and 10 hrs after the log phase for the sample 'B' respectively, a little change (3^0C) of pour point was observed for sample 'A' only. Hence dewaxing of vacuum distillates was associated with growth of the

TABLE 4.3.1 DEWAXING OF VACUUM DISTILLATES (A & B) BY BATCH CULTIVATION OF *C.tropicalis*

Time hr	∑ Alkali vol. ml 'A'	'B'	ln ∑ alkali vol. ml 'A'	'B'	Pour point °C 'A'	'B'
6	1.22	1.1	0.2	0.1	48	30
8	1.22	1.1	0.2	0.1		
10	1.22	1.22	0.2	0.2	48	30
12	1.22	2.22	0.2	0.8		
14	1.37	3.74	0.32	1.32		
16	2.46	6.68	0.90	1.90	48	21
18	3.96	11.47	1.38	2.44		
20	6.18	15.18	1.82	2.72		
22	9.98	20.08	2.3	3.0	45	18
24	12.18	28.22	2.5	3.34		
26	14.88	37.34	2.7	3.62		
28	18.17	45.60	2.90	3.82	42	12
30	22.20	48.42	3.10	3.88		
32	27.11	49.40	3.30	3.90	-	8
34	31.20	49.40	3.44	3.90		
36	40.40	49.4	3.7	3.90	39	4
38	45.6	49.4	3.82	3.90		
40	54.6	49.4	4.0	3.90	36	0
44	65.36	49.4	4.18	3.90	-	0
46	66.68	49.4	4.20	3.90		
50	72.40	49.40	4.28	3.90	30	0
55	73.7		4.30			
60	73.3		4.3		24	
65	73.3		4.3			
70	73.3		4.3		18	
90	73.3		4.3		15	

strain . An increase of pH from 4 to 5.5 was observed during this period perhaps due to the endogeneous metabolism of the cells.

4.3.2 Yield of biomass :

The final cell concentration was determined at the end of the batch cultivation and the yield of biomass was reported in Table 4.3.2 based on the initial oil concentration in the fermentors.

TABLE 4.3.2. YIELD OF BIOMASS ON VACUUM DISTILLATES

Oil sample	oil conc. g/l	Final cell conc. g/l	Yield of biomass cell conc/ oil conc.
A	40	4.5	0.11
B	40	3.0	0.07

Yield of biomass from 'A' was observed higher than that of sample 'B' due to higher composition of saturates present in 'A' (40%) as compared to sample 'B' (25%) The yield of biomass on heavier fraction was observed lower than that of gas oil containing same percentage of saturates but of shorter chain length of n. alkans.

4.3.2 Yield of dewaxed oil :

Yield of dewaxed oil obtained after completion of batch experiments was determined as shown in Table 4.3.3.

TABLE 4.3.3. YIELD OF DEWAXED OIL

Oil sample	initial oil conc. x g/l	final oil conc. y g/l	yield x/y %
A	40	22	55
B	40	28	70

Higher yield and lower pour point of the oil sample `B' was observed as compared to sample `A' may be due to assimilation of short chain alkanes which were present quantitatively higher in sample `B' as compared to sample`A'.This is also evident from the initial pour point of the oil samples (Table 3.1.2.).

Warth ,1960; and Sergeinko,1965 reported that structurally symetric saturate hydrocarbons exhibit lower melting point as compared to their unsymetrical isomers. Hence assimilation of shorter alkyl chains attached with branched and cyclic saturated hydrocarbons may have yielded structurally symetrical compounds higher in quantity in sample `B'. Whereas assimilation of longer alkyl chains attached with the similar types of compounds may have yielded structurally symetrical compounds lesser in quantity in sample `A'.

4.3.2 Dewaxing by semicontinuous operation :

With a view to increase the productivy of dewaxed oil, a semicontinuous operation was conducted in a 5 l fermentor maintaining the same operating conditions as fixed in the batch operation. One litre of culture fluid was renewed with equal volume of fresh medium and 30 g of fresh oil sample `A' was added in the fermentor in every 24 hours. The pour point of the dewaxed oil, cell concentration and dewaxed oil concentration was analysed at the end of each cycle .The results are summarised in the Table 4.3.2.

A pseudo steady state was observed within 48 hrs of operation as illustrated in Fig. 4.3.3.

It was observed that yield of dewaxed oil was higher (75 %) by the semicontinuous operation as compared to yield of dewaxed oil in batch process (55 %) . Pour point of the dewaxed oil was little lower (15 ^{0}C) than that of obtained by batch operation (18^{0}C). Higher productivity of the dewaxed oil were obtained in the semi -continuous operation as compared to batch operation (Table 4.3.3.).No change of pH was observed in the semicontinuous operation due to absence of stationary phase.

TABLE 4.3.4. DEWAXING OF VACUUM DISTILLATE (A) BY SEMICONTINUOUS OPERATION

Time hr	Cell conc.g/l initial	final	Oil conc.g/l initial	final	Yield of oil	pour point 0C
0	1.8	-	32	-	-	18
24	3.8	1.6	29.2	22	76	27
48	2.0	0.8	26.0	18	70	15
72	2.3	1.0	26	20	77	15
96	2.4	1.0	25	18	72	15
120	2.0	0.8	24	17	74	15
144	2.0	0.9	26	19	70	15
168	2.0	0.8	-	18	70	15

TABLE 4.3.5. BATCH AND SEMICONTINUOUS OPERATION

Dewaxing parameters	Batch	Semicontinuous
Time of operation hr.	70	24
Final cell conc. g/l	4.5	4.5
Yield of dewaxed oil %	55	70
Productivity g/l.h	0.235	0.875
Pour point of dewaxed oil ^0C	18	15

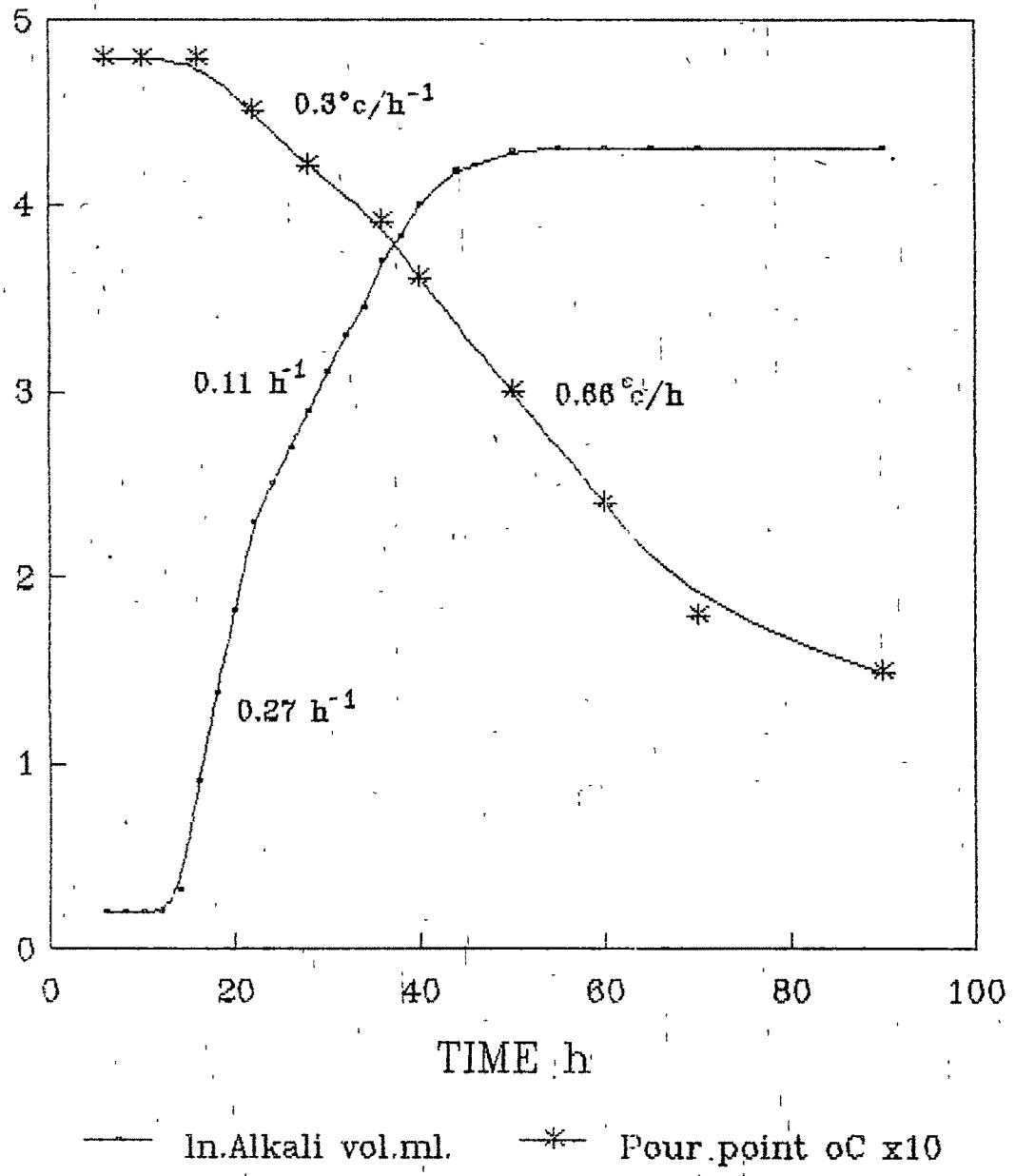

Fig. 4.3.1. Dewaxing of sample 'A' by C.tropicalis.

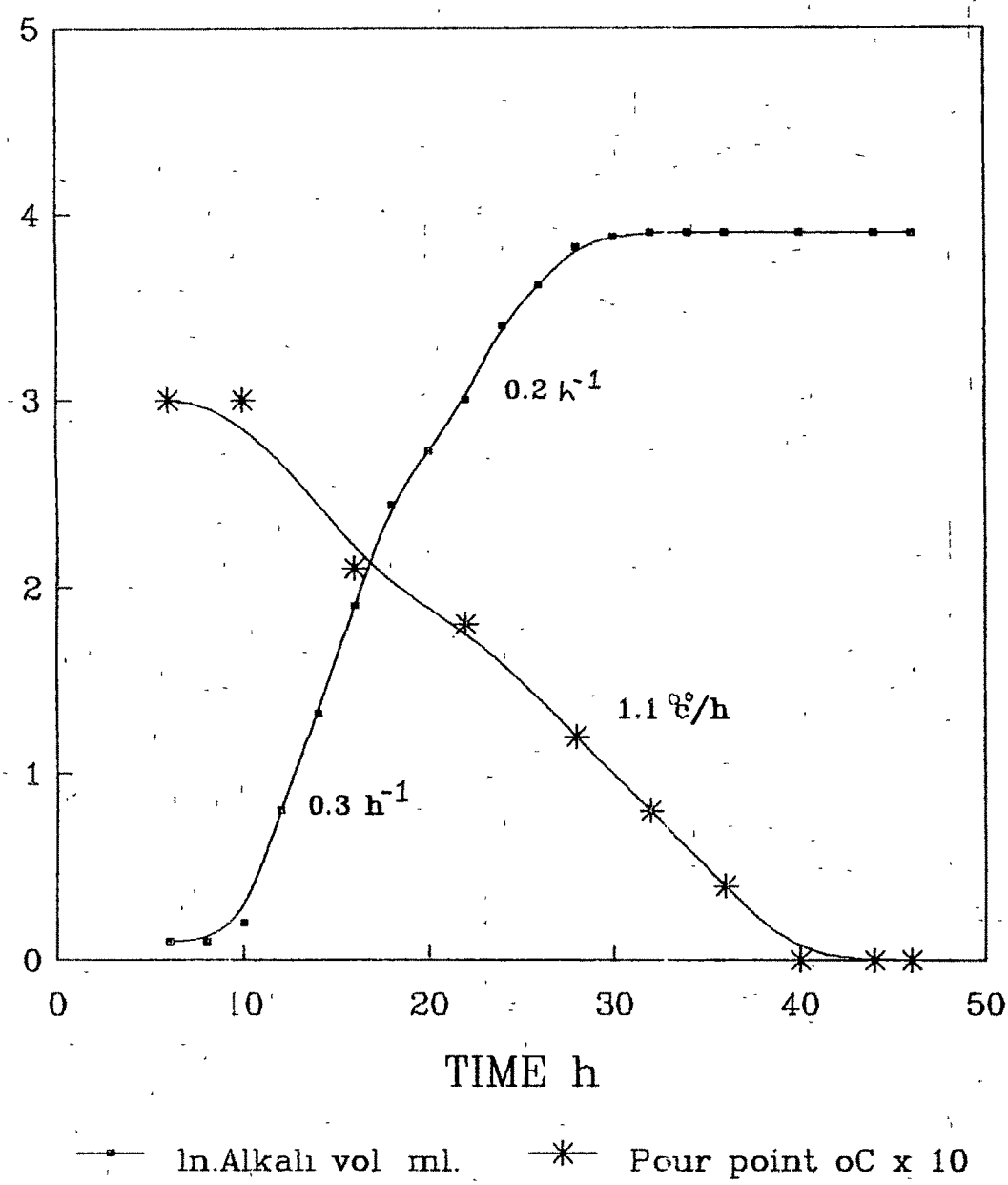

Fig. 4.3.2. Dewaxing of sample 'B' by C.tropicalis.

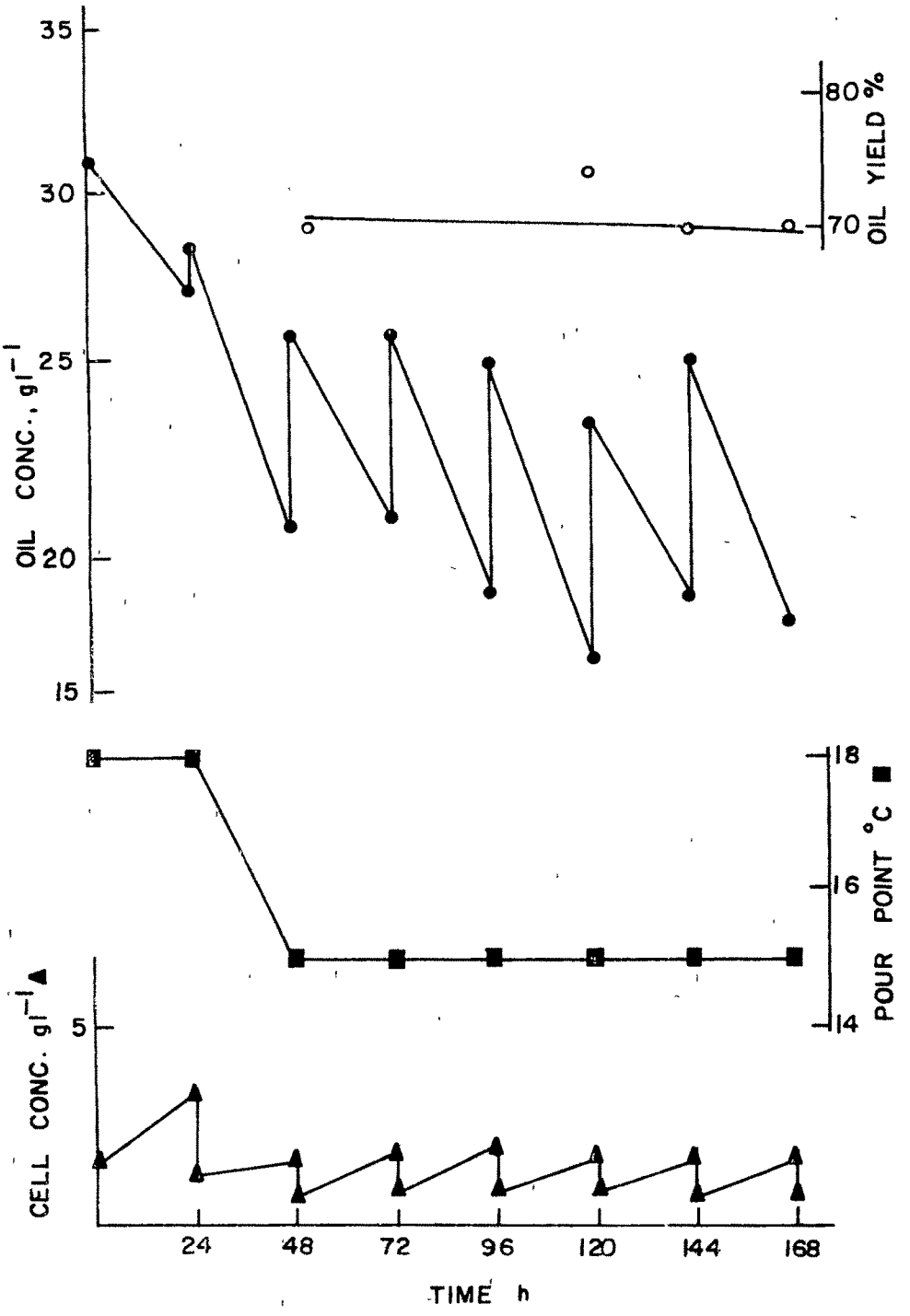

Fig.4.3.3.Dewaxing of vacuum distillate sample 'A' by semicontinuous operation

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4.4 SCALE UP STUDIES

Scaling up is a procedure whereby large-scale reactor is designed on the basis of the experimental results obtained in the laboratory scale reactor. Although the environmental parameters e.g; pH, temperature etc, are independent of scale ratio of bioreactors, but the kinetic behavior of microorganism sometimes controlled by the transport phenomena which is dependent on scale of operation (Taguchi et al,1968; Wang and Fewkes,1977; Hattori et al, 1974).

The overall growth rate of microorganism is dependent on the physico-chemical environment and the hydrodynamic state in the immediate vicinity of the organism. Einsele et. al., 1973, reported that oxygen transfer into the flocks containing, o/w emulsion and cells becomes limited under diminished turbulence although the dissolved oxygen in the vessel was maintained above the critical value.

The desired state of homogenity in a fermentor containing heterogeneous substrate depends on rheological properties of the culture broth, reactor configuration and impeller's Reynolds number as observed by Braur, 1973; Roles, 1974; Charles, 1978. In order to determine the optimum operating parameters related to oxygen transfer and hydrocarbon dispersion which was needed for maximum growth rate of the strain, the following studies were conducted.

4.4.1 Rheological property of the culture broth :

Blanch and Bhavaraju, 1976 reported that fermentation broth, in general, behaves as non-Newtonian fluid They determined the coefficient of power law fluid from the following correlations, described by Calderbank, et. al., 1967.

$$\tau = K.\dot{\gamma}^{n} \qquad 4.4.1$$

$$\ln \tau = \ln K + n \ln \dot{\gamma} \qquad 4.4.2$$

Where τ is the shear force , dynes.

$\ln \dot{\gamma}$ is the shear rate , sec^{-1}

K is the fluid constistancy factor, dyne-sec/ cm^2

n is the flow behavior index

The rheological characteristics of the culture fluid containing o/w emulsion droplets and yeast cells were determined by measuring the shear rate and shear force of the fluid collected at different interval during microbial dewaxing of heavier petroleum fraction. The results are summarised in Table 4.4.1.

The pseudoplastic character of the culture fluid was observed during initial growth phase (24 to 40 h) and then behaved as newtonian fluid at the end of growth phase (54 to 70 h) as evident from the Fig. 4.4.1.

It was observed that the Power law curve has been changed towards linear from 24 h to 70 h indicating the change of reheological behavior of the fluid from non Newtonian to Newtonian. The value of flow behavior index (n) and consistancy co-efficient (K) were determined from the slope and intercept respectively from the plot of $\ln \tau$ Vs $\ln \dot{\gamma}$.

The value of n was observed to ncrease fron 0.62 to 0.97 while the value of K was observed to decrease from 1.1 to 0.16 during the fermentation indicating the change of rheological

behavior from Non Newtonian to Newtonian fluid. Thomson et. al., 1980, also observed change of n and K of culture fluid during Xanthan and Pullulan fermentation.

The apparant viscosity (μ_a) of the broth was determined using the correlation described by Schugerl, 1981 for non-Newtonian fluids as following :

$$\mu_a = \frac{K}{\left\{ 11.\ N \right\}^{1-n}} \left[\frac{3n+1}{4n} \right]^{n} \qquad 4.4.3.$$

The value of K and n were assumed as 1.1 and 0.62 for the study because these values correspond to maximum value of apparent viscosity (μ_a) at 24 h . It was observed that viscosity of the culture fluid was
incresed from 10 cp to 48 cp during growth phase (24 to 40 h) and then decreased gradually to 15 cp at the end of the fermentation (Fig. 4.4.2). The increase of viscosity of the culture fluid during growth phase was observed due to the emulsification of the heavier petroleum fraction and the viscosity decreased in the latter part of the fermentation perhaps due to assimilation of highly viscous long chain n.paraffins as compared to their smaller homologes. Consequently the pour point of the oil also decreased faster in the later phase of the batch experiment.

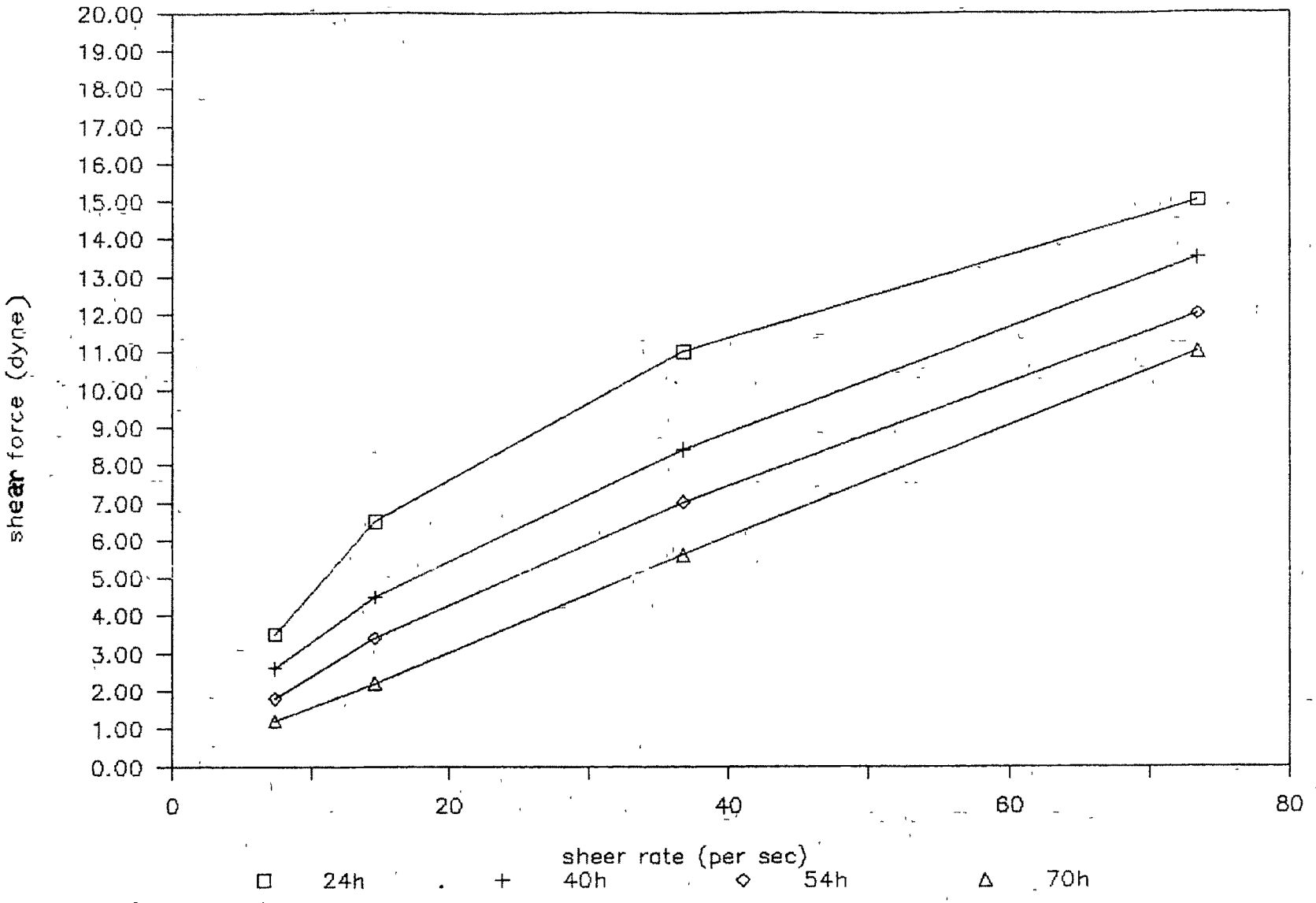


Fig 4.4.1. Power law curve of the culture fluid .

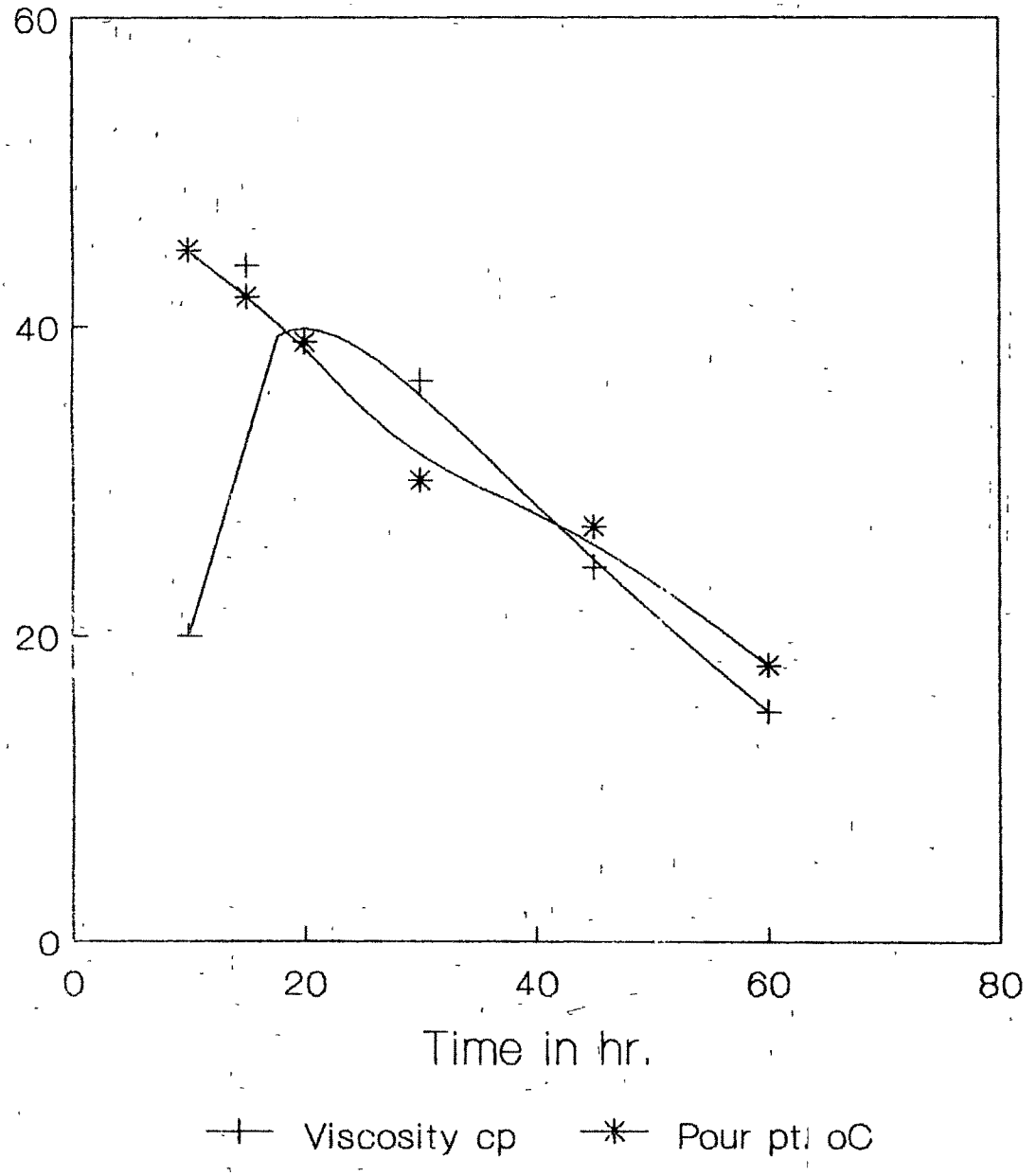


FIG.4.4.2. CHANGE OF VISCOSITY OF BROTH AND POUR POINT WITH MICROBIAL GROWTH

4.4.2 Oxygen transfer rates :

Hydrocarbon fermentation is highly aerobic process. Growth of microorganisms are likely to be inhibited if the concentration of dissolved oxygen in the medium falls below certain critical levels. Typical value of critical dissolved oxygen level (0.15 ppm) for yeast was reported by Finn, 1967. The critical dissolved oxygen concentration for the yeast, *C.tropicalis* was reported 0.5 ppm (Moo Young et. al., 1971). Einsele et. al., 1973, observed that dissolved oxygen value which was maintained at 30% of its saturation value did not become a limiting factor for the growth of *C.tropicalis* when 4% hydrocarbon was used as carbon source. In spite of low value of critical dissolved oxgen concentration, Mimura et. al., 1973, observed that high oxygen transfer rate i.e., 150-200 m. moles/l.hr. was required for a cell concentration of 15-20 g/l when yeast *C.petrophilum* was grown on hydrocarbon.

Oxygen transfer rate is an intrinsic property of a bioreactor and design of any bioreactor should provide the rate of oxygen transfer to the cells equal to the oxygen uptake rate of the strain.

In order to determine the optimum oxygen transfer rate (OTR) to achieve the maximum growth rate of the yeast *C.tropicalis* on heavier petroleum fraction (OTR) was determined in three fermentors were determined by sulphite oxidation method as described by Mimura et. al., 1973.

$$OTR = K_L a \ (C^* - C_L) \qquad 4.4.4$$

Where K_La = oxygen transfer coefficient

C^* = saturation concentration oxygen at 37^oc

C_L = dissolved oxygen in bulk of liquid

As the catalytic (Cu^{++}) oxidation rate of sulphite is higher than the oxygen uptake rate of yeast (12 m. mole/g. h.), reported by Einsele et. al.,1973, $C^* >> C_L$ in sulphite oxidation system (Wang et. al., 1979).

Hence one can determine the maximum oxygen transfer co-efficient, Kla from the equation 4.4.5 as follow:

$$K_La = \frac{OTR}{C^*} \qquad 4.4.5$$

Ideally, oxygen transfer rate should be measured directly in biological system but it could not be done in the present system as the transient response of the dissolved oxygen probe was slow in presence of heavier hydrocarbons.

However, a common strategy for study of oxygen transfer rate was followed by sulphite oxidation method which approximates bioreaction conditions.

Hence the oxygen transfer capacity of three fermentors at different agitation rate was calculated from the values of sulphite oxidation rate.

The OTR values were determined by varying the aeration rate in each fermentor but keeping the agitation rate constant in the respective fermentors.

The results are summarised in Table 4.4.2.

TABLE 4.4.2 OXYGEN TRANSFER CAPACITY AT DIFFERENT AERATION AND AGITATION SYSTEM

Parameters	Aeration rate vvm	Fermentors Gallankamp A	Glass fermentors B	C
Oxygen transfer rate, m. moles/l.h.	0.66	41	80	50
	1.0	120	100	67
	1.5	140	110	70
Agitation rate, rpm		600	1450	1650
$\frac{d_r}{D_t}(d_r)$ *		0.46	0.39	0.32
$\frac{h_r}{D_t}(h_r)$ *		0.15	0.25	0.20

TABLE 4.4.3 KL_a VALUES AT DIFFERENT AERATION RATE

Aeration rate vvm	Kla vaues Min^{-1} Fermentors A	B	C
0.66	2.85	5.57	3.48
1.0	8.36	6.97	4.67
1.5	9.76	7.66	4.88

The dissolved oxygen saturation concentration (C^*) in the liquid phase was observed 7.65 ppm at the operating temperature 37°C. Hence, the values of K_La were calculated from the OTR values at different aeration rate in each fermentor (Table 4.4.3.).

Results showed that maximum oxygen transfer capacity (OTR) and the corresponding Kla values are obtained in Gallankamp fermentor (A) beyond 1 vvm aeration rate. The high agitation rate in fermentor B and C did not provide higher Kla perhaps due to short channeling or low residence time distribution of air bubbles in the fermentor B & C as compared to fermentor A. This is evident from the Table 4.4.2 that higher the ratio of strirrer dia to vessel dia (dr^*) higher the K_La value would be obtain in the fermentor at a particular aeration and agitation rate. Similar observation was reported by Mehta and Sharma, 1971.

The effect of oxygen transfer rate (OTR) on the sp. growth rate of the yeast, *C.tropicalis* on heavior petroleum fraction was determined by conducting batch experiments in the said fermentors at the aeration rate of 1 vvm, agitation rate and other parameters were kept same as maintained during experiments for determination of oxygen transfer rates.

Specific growth rate (h^{-1}) of the strain was found higher (0.27 h^{-1}) in fermentor B which has the lower of oxygen transfer rate (100 m. mole/l. h.) as compared to fermentor (A) which has the maximum oxygen transfer rate (120 m. mole/l. h.). The results are shown in Fig. 4.4.3.

The following observations were noted from oxygen transfer studies:

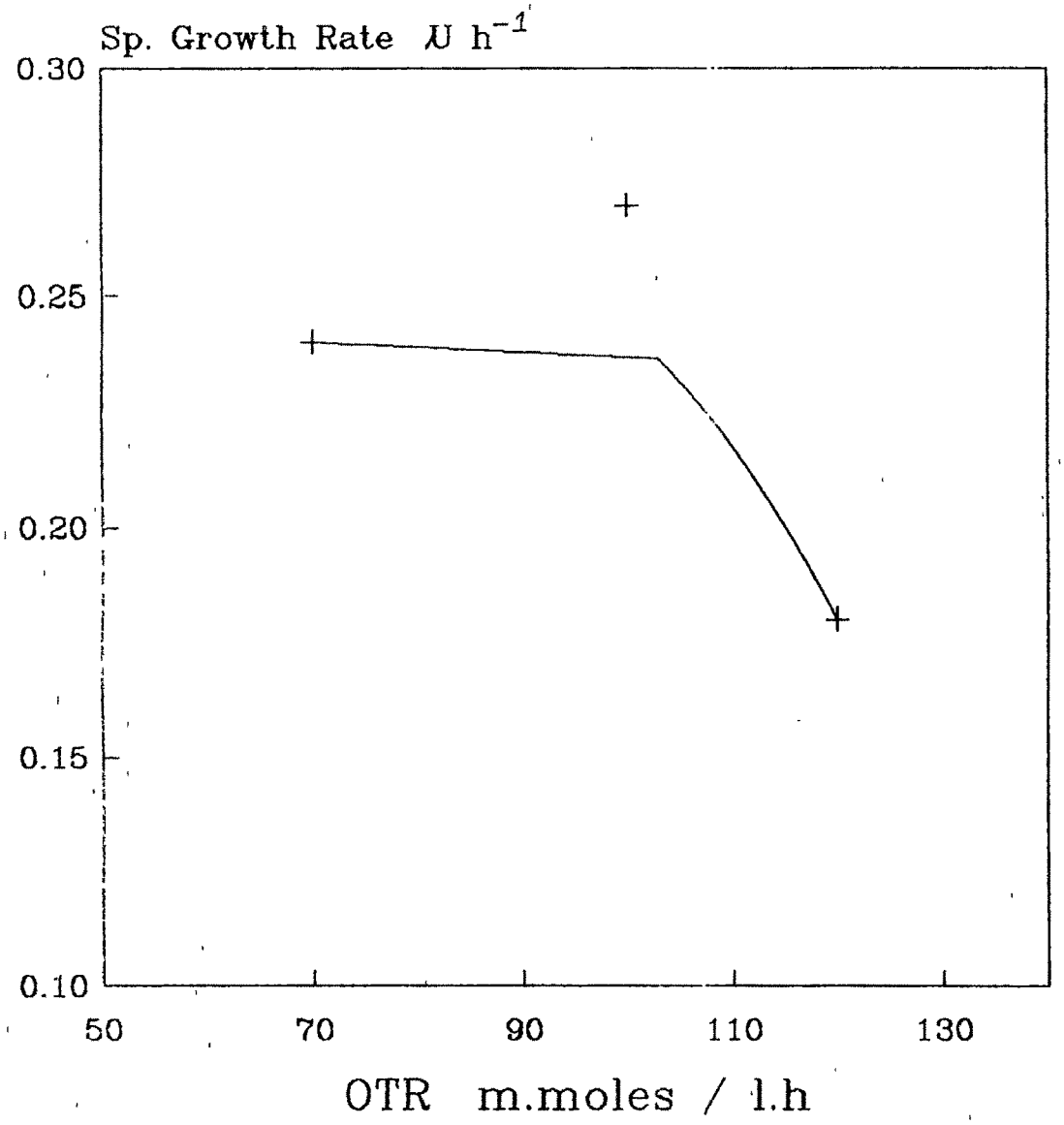


Fig.4.4.3. Sp. Growth rate at different OTR

a) The growth rate of the yeast strain on heavier hydrocarbons was not limited upto a OTR of 100 m. moles/l. h.

b) Apart from the effect of OTR/Kla of the fermentors on growth of the strain, better dispersion of air bubbles and hydrocarbon droplets maintained in the fermentors B and C may have enhanced the sp. growth rate of the strain on heavier hydrocarbon.

4.4.3 Impellers Reynold No. (NRe_I) :

The state of turbulance in a stirred tank fermentor is measured by Impellers Reynolds No. (NRe_I). It is defined by Calderbank et. al., 1979, for non-Newtonian fermentation fluid. The values of impellers Reynolds No. were calculated based on the different values of co-efficients (n and K) of power law equation (4.4.1) obtained during microbial dewaxing of heavier hydrocarbons (Sample A), as shown in Table 4.4.4.

$$N_{ReI} = \frac{d_r^{2} \cdot N^{2-n} \cdot \rho}{0.1.K} \left[\frac{n}{6n + n} \right]^{n} \qquad 4.4.6.$$

Where ρ is the density of the culture browth

N is the rotational speed of impeller in Sec^{-1}.

d_r is the dia of the impeller

n flow behaviour index

K fluid consistancy factor

TABLE 4.4.4 IMPELLERS REYNOLDS NO OF FERMENTORS DURING FERMENTATION PERIOD

Time hr	Broth density g/ml	K dyne-sec/cm^2	n	$Re_I \times 10^3$		
				Fermentors		
				A	B	C
			N^*	7.5	24.16	27.5
24	0.9927	1.10	0.62	1.32	5.57	6.67
40	0.9927	0.64	0.71	1.60	6.08	7.19
54	0.9927	0.36	0.82	1.86	6.22	7.24
70	0.9927	0.16	0.97	2.35	6.59	7.52

N^* Rotational speed (N) of the agitator in Sec^{-1}.

The impeller Reynolds No. was increased in each fermentor due to lowering of apparent viscosity (μ_a) of the broth during the period of fermentation. However, higher impellers Reynolds No. maintained in fermentors B and C might have provided better dispersion and effective diameter of hydrocarbons droplets which enhanced the sp. growth rate of the strain. Although the Reynolds number in the fermentors were maintained in the turbulant regine ($>10^3$), specific growth rate of the strain was observed higher in the fermentor operated at higher Reynolds number as shown in Fig. 4.4.4. The above results also confirmed the influence of hydrodynamic state of the fermentation fluid on specific growth

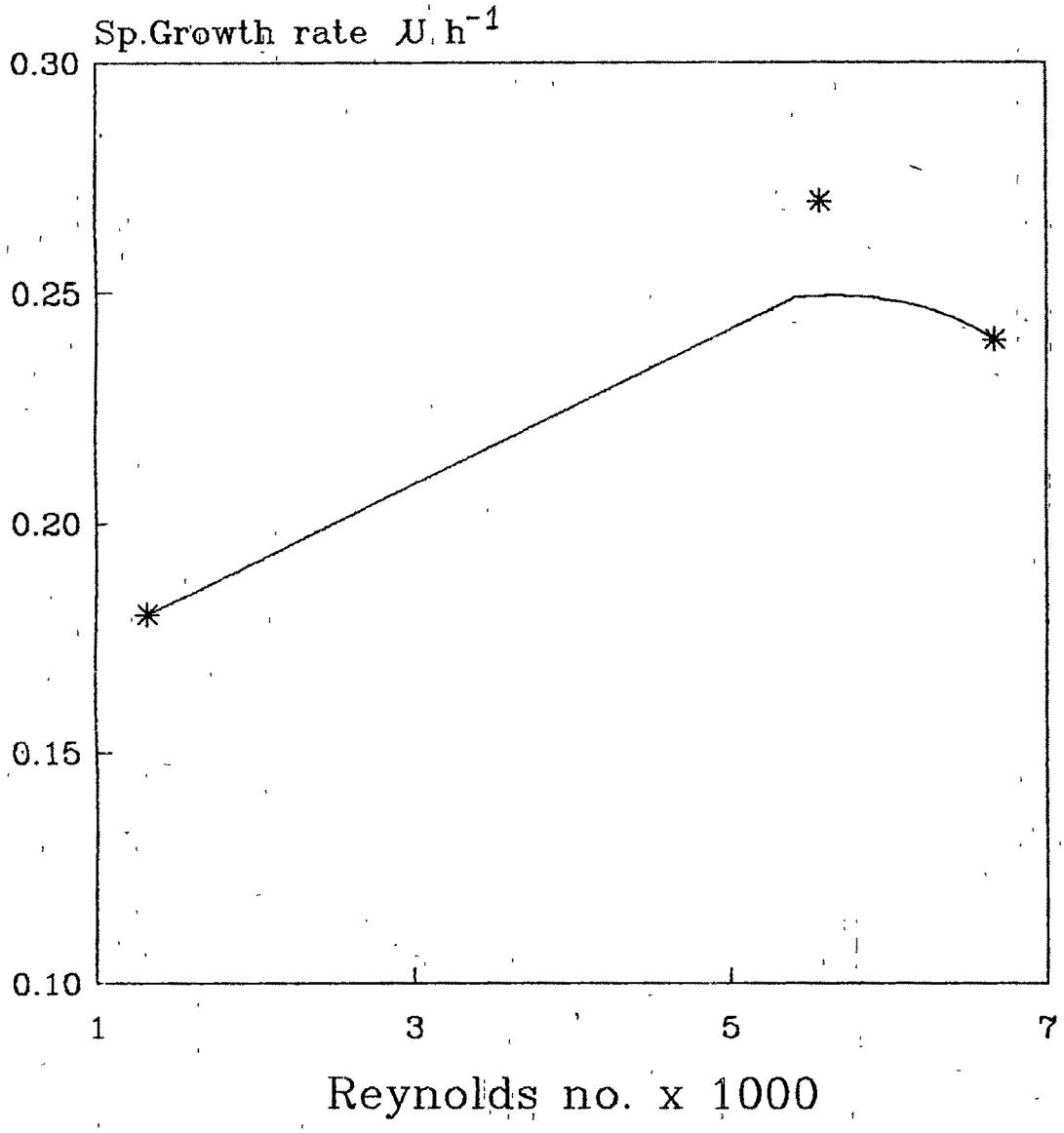


Fig. 4.4.4 Sp.Growth rate at different Reynolds number

rate of the strain during dewaxing and a minimum level of N_{Re} is needed to be maintained ($6x10^3$) to achieve the maximum sp. growth rate. Sp. growth rate decreased when the fermentation conducted beyond the impellers Reynolds number $6x10^3$ perhaps due to higher shear effect on the hydrocarbon flocks.

4.4.4 Power per unit volume :

The size of enengy required for maintaining the desired state of homogenety in a fermentor is determined by the power input per unit volume of the system (p/v). Thus, nearly similar hydrodynamic situations can be maintained if p/v is kept constant during scaling up the process. Van't Riet, 1979, reported that constant p/v would be ideal scale up criteria for any dispersion limited process.

Power drawn by the agitator exclusively for mixing the culture broth during batch dewaxing operation was calculated in two fermentors (B and C) by the Swenburn test method described in section 3.2.4.

The net power drawn by the agitator for mixing the system was calculated from the difference of power drawn for an empty and the charged culture vessel of the fermentor as following

i)	Armature current (no load)	=	0.04
ii)	Field voltage (DC), V	=	210
	Initial temperature, ^oc	=	30
	Temperature rise, ^oc	=	12
	Co-efficient of expansion (Cu)	=	0.00427 cm/^oc

Initial armature resistance (ohms) = 40

Initial field resistance (ohms) = 2000

Final armature resistance (ohms) = 41.8

Final field resistance (ohms) = 2090.8

TABLE 4.4.5 CALCULATION OF NET POWER INPUT PER UNIT VOLUME (P/V) OF CULTURE FLUID

Fermentor capacity l	rpm	Power output Empty	Filled	Net power (P) drawn by agitator w	P/V w/l
	810	23.0	26.99	3.99	2.66
1.5	1400	23.0	34.28	11.27	7.51
	2250	23.0	47.22	24.22	16.14
	620	25.27	25.98	0.71	0.14
5.0	1125	28.95	33 06	4.11	0.82
	1650	37.23	52.02	14.78	2.95

The power output of empty fermentor (1.5 l) capacity was constant with the increase of armature voltage whereas there was rise of power output in empty 5.0 l. fermentor was observed with the increased of rpm (Table 4.4.6). The increase of power output in the empty vessel (5.0 l.) was due to the frictional resistance associated with the agitation system fitted in 5.0 l. fermentor.

Effect of power input on sp. growth rate of the strain :

The effect of power iput per unit volume (p/v) on sp. growth rate of the strain was obtained from the batch experiments conducted in 1.5 l. and 5.0 l. fermentors. The results are shown in Table 4.4.7.

TABLE 4.4.6 EFFECT OF P/V ON SP. GROWTH RATE

Fermentor capacity l.	rpm	p/v w/l	Sp. growth rate h^{-1}
	500	3	0.24
1.5	1400	7.5	0.27
	2250	11.4	0.25
	620	0.14	0.12
5.0	1120	0.82	0.17
	1650	2.95	0.24

The results indicated that a max. sp growth of 0.27 h^{-1} could be achieved by maintaining a maximum p/v of 7.5 w/l. However, an optimum value, could be achieved by maintaining power input of 3 w/l in 1.5 and 5.0 l. fermentors (Fig 4.4.5.).

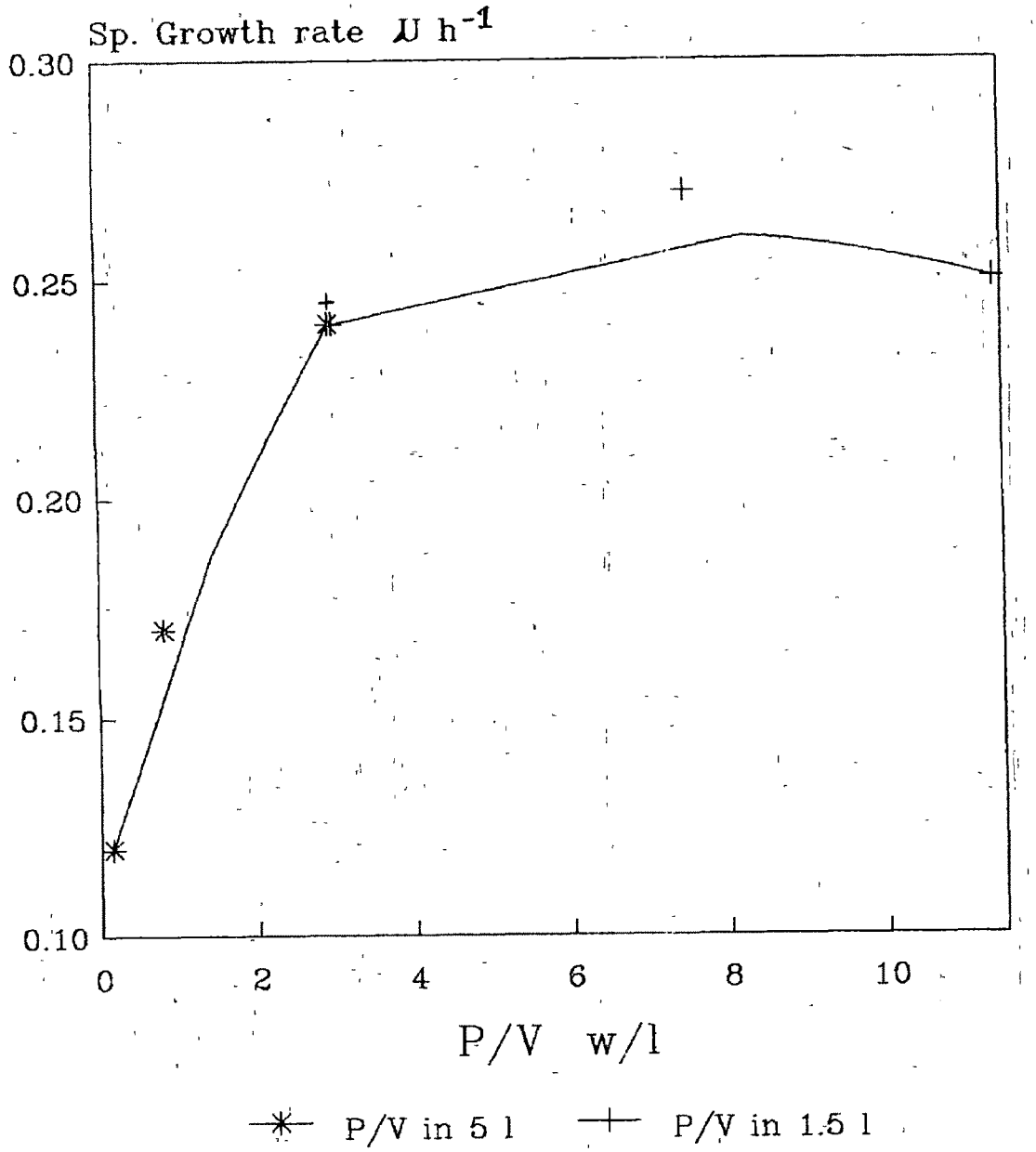


Fig.4.4.5. Effect of P/V on sp. growth rate

4.4.5. Scale up of bioreactor for dewaxing of heavier fraction :

In hydroarbon fermentation system, a sophisticated approach to scale up is required because the performance of large fermentor can be enhanced by the following rate controlling factors:

a) Oxygen mass transfer from air bubbles to the cells.

b) Uniform dispersion of hydrocarbon droplets or flocks.

Criteria for scaling up of a biological process :

The usual procedure of scale up is to fix one of the folowing criteria as the basis of design, however the choice of criterion will depend on the particular fermentation process under study.

1. Constant volumetric O_2 transfer rate , Kv_g .
2. Constant volumetric power input , P_g / V .
3. Constant impeller tip speed , v_{tip} .
4. Equal mixing time , t_M .

Einesele,1978 observed that majority of fermentation industries in Europe followed either of the first two criterion as a basis of scale up . These critarion are related and refered mainly to oxygen transfer rate and turbulence of the culture fluid as function of P/V provided the value of tip velocity of impeller i.e; $v_{tip} > 3$ $m.s^{-1}$ (Mooyoung and Blanch,1981).

In the present study of dewaxing , it was observed in the laboratory scale that sp. growth rate of *C.tropicalis* was the function of N , P/V , aeration rate which provided the oxygen transfer rate and dispersion of hydrocarbon flocks .

Based on the experimental results obtained in the 5.0 l fermentor basis of scale up cretaria was determined for the large scale dewaxing of lube fraction in a 1000 l stirred tank bioreactor.The selection of critaria would be such that operating conditions in the large fermentor should provide a hydrodynamic condition for attaining the sp.growth rate of 0.24 h^{-1} of the strain.

Similar physical properties of the fermented broth and dimentional ratio of the fermentors were assumed.

The operating parameters of the laboratory fermentor (5l) are given below :

1. Volume	$(V)_1$ m^3	= 0.005
2. Height of liquid	$(H_L)_1$ m	= 0.21
3. Dia of tank	$(D_t)_1$ m	= 0.175
4. Dia of impeller (turbine)	$(d_r)_1$ m	= 0.055
5. H_L / D_t		= 1.2
6. d_r/D_t		= 0.31
7.Oxygen transfer rate kg moles /m^3 h($Kv)_1$		= 0.07
8.Power per unit volume	$(P/V)_1$ w/l	= 3.0
9.Aeration rate	vvm	= 1

Scale up factor : 1000/ 5 = 200

1.Dia of large fermentor $(D_t)_2 = \left| \frac{4. V_2}{1.2 \Pi} \right|^{\frac{1}{3}}$ = 1 m

assuming $(H_L/D_t)_2$ = 1.2

2.Liquid Height $(H_L)_2$ = 1.2 .$(D_t)_2$= 1.2 m.

3.Dia of impeller $(d_r)_2$ = 0.31$(D_t)_2$ = 0.31 m

Assuming $(d_r / D_t)_2 = 0.31$

4. Aeration rate :

To maintain the same O_2 transfer rate (K_v) in the large fermentor, it was assumed by Aiba et al, 1969

$$K_v = \frac{\beta . H_L \quad Q^{2/3}}{10\ V\ d_B N_{sc}^{1/2}} \quad \ldots\ldots \quad 4.4.7.$$

Where Q = Volumetric air flow rate.

H_L = Height of liquid

V = Volume of liquid

d_B = Mean bubble dia

N_{sc} = Sherwood number

β = Emperical cofficient

As the physical properties of the broth would be same in large scale fermentor, the above equation can be simplified as following :

$$K_v \propto \beta\ Q/V\ H_L^{2/3}\ 1/d_B \quad \ldots\ldots \quad 4.4.8.$$

$$\text{or, } K_{v\,1} = \frac{(Q/v)_1}{(Q/v)_2} \cdot \frac{H_{L\,1}}{H_{L\,2}} \cdot \frac{d_{B\,1}}{d_{B\,2}} \quad \ldots\ldots \quad 4.4.9.$$

In order to maintain same bubble dia, $d_{B2} = d_{B1}$ it would be necessary to provide same air flow rate and sufficient power input in the fermentor such that the bubble could attain an equilibrium dia due to sufficient turbulence in the large fermentor (Bhavaraju et al,1978). It was also observed that increase of viscosity reduces the bubble coalescence.

It was observed that the viscosity of the broth increased up

to 40 cp during initial growth phase of the strain and it would be favourable for the system. But at the same time high turbulance (N_{Re}) and power input (P/V) in the large fermentor would be required to maintain same oxygen transfer rate i.e; $K_{v2} \simeq K_{v1}$.

Under such conditions one can get,

$$Q/V_2 = H_{L2}/H_{L1} \cdot Q/V_1 = \left| \frac{D_{t2}}{D_{t1}} \right|^{-2/3} \cdot Q/V_1 .$$

Aeration rate in large fermentor $Q/v_2 = (1/0.175)^{-2/3}.1$

$= 0.31$ vvm.

5. Superfacial gas velocity V_s :

$$V_{s2} = \frac{Q/V_2 . V_2 . 60.4}{\pi . D_{t2}^2} = 23.9 \text{ m/h} .$$

A) CONSTANT VOLUMETRIC OXYGEN TRANSFER RATE, Kv :

The oxygen transfer rate in the large fermentor after necessary hydrostatic pressure and height correction would be 0.057 kg moles / m^3 h.atm.

Cooper et al ,1944 and Moo young and Blanch ,1981 established the following correlation on oxygen transfer rate with power per unit volume (P/V) and superfacial gas velocity (V_s). This correlation is applicable to most aerobic fermentation process carried out in stirred tank bioreactors.

$$K_{vp} = a . (P_g/V)^x (V_s)^y \quad \ldots\ldots\ldots \quad 4.4.10.$$

Where K_{vp} = Oxygen transfer rate , kg.moles/ l. h. atm.

P_g/V = Gas power input per unit volume ,HP / m^3.

V_s = Superfacial gas velocity , m/ sec.

Hassan, 1977 obtained the value of the exponents x and y as 0.52 and 0.43 respectivily by using dissolved oxygen probe for the determination of $K_L a$ or K_v in a water-electrolyte system.

$$K_{vp} = a \cdot (P_g/V)^{0.52} (V_s)^{0.43} \quad .... \quad 4.4.11.$$

Substituting the experimental results (Table 4.4.5) obtained for 51 fermentor in the above correlation ,the value of the coefficient 'a' was obtained as 0.0115 .

6) Power requirement in the large fermentor :

$$K_{vp2} = 0.0115 \quad (P_g/V)_2^{0.52} \cdot (V_s)_2^{0.43} \quad ... \quad 4.4.12.$$

Therefore, $(P_g/V)_2$ = 1.57 HP/m^3 = 1.15 w/l .

Gas power requirement would be 1150 w for 1000 l fermentor.

Assuming non gas power requirement is 40% of the gas power requirement one can get, the motor power requirement for the large fermentor as,

$$P = P_g . 0.4 = 463 \text{ w.}$$

7) Agitator rpm :

Assuming culture broth in the fermentor in turbulent regime one can get,

$$P = \frac{6.\rho .N^3 .d_{r2}^5}{g_c} \quad .... \quad 4.4.13.$$

Where ρ = density of the broth kg/M^3

P = Power input kg.m/ sec

N = Rotational speed. rps

g_c = Gravitational const.

Rpm of the impeller of large fermentor was obtained by substituting the values in the above correlation.

$$\text{Thus , Rpm } N = \left[\frac{P.\ g_c}{6.\rho.d_r^{\ 5}} \right]^{1\backslash 3} \quad \ldots\ 4.4.14.$$

$$N_2 = \left[\frac{37.8 \times 9.81}{6 \times 927.7 \times [0.31]^5} \right]^{1/3} = 170 \text{ rpm} = 2.85 \text{ rps.}$$

8) Impellers Reynolds number (N_{Re}) :

Impellers Reynolds number of the large fermentor was calculated using the equation 4.4.6.as discussed earlier.

$$N_{Re} = \frac{(31)^2 \times (2.85)^{1.38} \times 0.9277}{0.1 \times 1.1} \left[\frac{0.62}{6 \times 0.62 + 2} \right]^{0.62}$$

$$= 8.6 \times 10^3.$$

9)Aeration number $N_a = Q_g / N.d_{r2}^{\ 3} = 6.12 \times 10^{-2}$.

10) $V_{tip} = \pi .N.d_{r2} = 277.55$ cm/sec = 2.77 m/sec.

B) CONSTANT POWER PER UNIT VOLUME (P/V) :

The concept of equal power per unit volume of culture fluid in different scale of fermentors is based on the assumption that the fermentation should be conducted in the turbulent regime(Bailey et al,1983).

In the turbulent regime, the power input is independent of N_{Re}.

So $P \propto N^3.d_r^{\ 5}$ when Power no = constant.

Hence one can get,

$$\frac{P_1}{P_2} = \frac{N_1^3 . dr_1^5}{N_2^3 . d_{r2}^5}$$

Assuming same power input , $P_1 = P_2 = 3$ w/l

$$N_2 = N_1 \left(\frac{d_{r1}}{d_{r2}}\right)^{2/5} = 1650 \left[\frac{0.055}{0.31}\right]^{0.6} = 585 \text{ rpm} = 9.75 \text{ rps}$$

1)Hence the rotational speed of the impeller in large fermentor ,

$$N_2 = 585 \text{ rmp}$$

2) Impellers Reynolds number $(N_{ReI})_2$:

$$N_{ReI} = \frac{961 \times 23.16 \times 0.9277}{0.11}\left[0.2521\right]$$

$$N_{ReI} = 4.7 \times 10^4$$

3) Tip velocity $V_{stip} = \Pi N_2 d_{r2} = \Pi \times 9.75 \times 32 = 9.50$ m/sec

4) Aeration number $N_a = 0.31/ 585 \times (0.31)^3 = 1.77 \times 10^{-2}$.

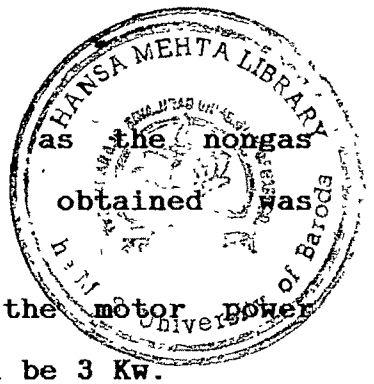
5) Oxygen transfer rate $K_{vp\ 2} = 0.0115.(P_g/V)^{0.52}.(V_s)^{0.43}$

$$= 0.0115 .(4)^{0.52}.(23.9)^{0.43}.$$

$$= 0.925 \text{ kg.moles/ l.h.atm.}$$

6) Motor power requirement :

$$(P_g/V)_1 = (P_g/V)_2 = 3 \text{ w/l}$$



Assuming gas power requirement would be same as the nongas power requirement ,because the aeration no obtained was comparatively low i.e; 1.7x 10^{-2} .

Hence $(P_g /v)_2=(P/v)_2$ = 3 w/l .Hence the motor power requirement in the large fermentor (1000 l) would be 3 Kw.

The operating parameters for lab scale (5l) and pilot scale (1000 l) data obtained by the two scale-up criteria are compared in Table 4.4.7.

TABLE 4.4.7. COMPARISON OF SCALE UP DATA

Parameters	Lab scale 5 l	Pilot scale 1 m^3 constant K_v	constant P/V
H_L*	1.2	1.2	1.2
d_r*	0.31	0.31	0.31
Aeration rate vvm	1	1	1
K_v m.moles /l.h.	70	57	748
$N_{Rel} \times 10^3$	6.67	8.6	40.7
V_s m/h	12.46	23.9	23.9
V_{tip} m/sec	4.75	2.77	8.55
$N_a \times 10^{-2}$	1.87	6.12	1.7
Motor power Kw	0.014	0.375	3.0

The results shown in Table 4.4.7. indicated that the following limitations would appear in the large fermentor if constant K_v criterion is followed .

1.Power per unit volume becomes substantially low as compared to small fermentor. This may decrease the sp.growth rate of the strain in large fermentor and poor dewaxing may occure as

observed in experimental results

2. Tip velocity of the impeller is less than the minimum requirement of 3 m/s . Hence low shear rate of the agitator would provide minimum dispersion of hydrocarbon flocks.

3.Impellers Reynolds number would be at the minimum limit of turbulent regime i.e; 10^3.

Based on the above observations it can be concluded that suitable criteria for scale up for the dewaxing of heavier petroleum fration would be CONSTANT POWER PER UNIT VOLUME (P / V).

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4.5 RECOVERY OF DEWAXED OIL

4.5 RECOVERY OF DEWAXED OIL

The culture broth was harvested after the completion of batch or semicontinuous operation. The harvested broth comprises of the two following immiscible phases :

a) Oil in water emulsion (O/W) phase containing biomass adhering to the oil droplets.

b) Aqueous phase containing part of biomass, medium constituents and negligible quantity of emulsified oil(0.1%).

The emulsified phase was separated from the aqueous phase in a separating funnel and subjected to solvent treatment for the recovery of dewaxed oil. Breaking of emulsion by physical forces e.g; centrifugation at 6000 rpm could not be achieved.

Laine,1975 reported that dewaxed gas oil was recovered from the culture broth by leaching operation in the BP process for the production of SCP from gas oil(Fig 2.1). However Ghose et al,1968 demonstrated that the release of yeast cells from the oil-water-yeast emulsion could be achieved by liquid-liquid extraction principle. They determined the phase equilibrium of gas oil-water-solvent system in triangular diagram but the true values of phase equilibrium could not be obtained due to the solubility of wax in the solvent when high boiling gas oil fraction(350-400^{o}C) was used for dewaxing.

The emulsified broth obtained after the fermentation was associated with strong emulsion of oil in water and yeast cells. In order to avoid the problem of wax dissolution of the heavier fraction(400-490^{o}C), the process of leaching was applied to extract the oil from the oil-water-biomass miscelles.

The principle of leaching process was found to be more appropriate for the recovery of dewaxed oil and the biomass than the principle of liquid liquid extraction in the present system. The design of extractor would also be simpler.

Three main steps are involved in the leaching operation:

1. Contact of liquid solvent with the solute(oil) to effect transfer of solute from the solid (Biomass) to the solvent.
2. Separation of oil-solvent solution known as overflow stream from the residual mass known as underflow stream.
3. Recovery of dewaxed oil from the overflow stream by distillation and recovery of Biomass by filteration of aqueous phase and subsequent drying and recovery of solvent by distillation from the underflow stream.

Ideal stage and equilibrium in leaching is defined as a stage from which the resulting solution is of same composition as the solution adhering to the solids leaving the stage.

The ideal stage of a leaching operation is described below.

4.5.1 COMPOSITION OF OIL EMULSION

The average composition of the emulsified broth obtained after the batch operation is given below.

Oil (dewaxed) % w/w - 23.12

Biomass % w/w - 1.70

Aqueous phase % w/w - 75.28

The Biomass (solid) along with the aqueous phase can be assumed as a single inert phase because the components are immiscible with the overflow stream.

Hence the composition of inert phase would be :

Biomass - 1.7 %

Aqueous phase - 75.3 %

4.5.2 CHOICE OF SOLVENT

The o/w emulsion obtained after the fermentation consists of a non polar oil phase , polar aqueous phase and yeast cells without significant polarity . So a mixture of polar and non polar solvents in appropriate ratio would be required for ideal separation of the components .

A mixture of acetone as polar solvent and petroleum ether (b.p.80-100^{o}C) with different proportion was used for the treatment of the emulsion to determine the optimum ratio of the mixture . The results are summarised in Table 4.5.1

TABLE 4.5.1 EFFECT OF SOLVENT COMPOSITION ON OIL RECOVERY IN SOLVENT PHASE

Emulsion g	Solvent Composition Acetone:Petrol.ether	Qty g	Oil recovery wt %	Remarks
40	3 :1	40	25	wax precipitate observed
40	1:1	40	30	
40	1:3	40	45	
40	1:7	40	32	Formation of emulsion

The data given in Table 4.5.1 indicated that the optimum composition is acetone : petroleum ether :: 1 : 3.

Low oil recovery and precipitation of wax was observed at higher acetone concentration whereas further emulsion formation was observed when low acetone concentration was present in the solvent.

4.5.3 OPTIMUM SOLVENT TO EMULSION RATIO

Recovery of oil in overflow stream was determined by treating the emulsion with solvent of optimised composition varying the emulsion to solvent ratio (Table 4.5.2)

The maximum recovery of oil(98.2%) in the overflow was observed at the solvent to emulsion ratio of 1 : 2.45. However the solvent to emulsion ratio of 1 : 2 would be optimum to get an oil recovery of 96.5 % (Fig 5.1).

TABLE 4.5.2 EFFECT OF SOLVENT/ EMULTION RATIO ON RECOVERY OF OIL

Emulsion g	solvent g	solvent / emulsion	recovery in overflow w / w %	
			oil	solvent
10.12	12	1.18	66	34
10.19	21	2.10	96.5	62
10.20	15	1.47	75.5	48.2
10.21	25	2.45	98.2	75

4.5.4 DETERMINATION OF NUMBER OF STAGES REQUIRED FOR RECOVERY OF DEWAXED OIL.

From the table 4.5.3 it can be observed that the composition of the immiscible phases obtained after solvent treatment and rigorous mixing was different. Hence the system deviated from the ideal stage in the leaching operation. However practical overall stage efficiency is a more feasible criteria in addition to the number of ideal stages to obtain the number of stages for given leaching operation (Prabhudesai, 1979).

The following assumptions are made for fixing the parameters in the operation.

1) The dewaxed oil as solute (A) .
2) The biomass and aqueous phase as inert residue (B) .
3) Solvent mixture acetone and petroleum ether (1 : 3) as solvent (S) .
4) The overflow stream is a solution of oil and solvent.
5) The underflow stream is a mixture of biomass , water and solvent.
6) The inert residue in the underflow is insoluble in the overflow stream. The wt.of solution retained by unit wt.of inert is denoted as K.

Number of stages were determined by graphical method as described by Bradger , 1955 and Prabhudesai , 1979. The temperature during the leaching operation was maintained at $30^{o}C$. The experimental data and calculation of composition of the overflow and underflow streams at diferent solvent compositions are illustrated in Table 4.5.3 and Table 4.5.4.

TABLE.4.5.3.COMPOSITION OF DIFFERENT STREAMS IN THE LEACHING OPERATION

Emulsion g	solvent/ emulsion	Overflow stream oil g	solvent g	wt.frac. oil ,g	Underflow stream oil g	solvent g	inert g	K g/g
10.12	1.18	1.57	4.1	0.271	0.79	7.9	7.79	1.1
10.12	1.47	1.73	7.23	0.193	0.56	7.76	7.85	1.0
10.19	2.10	2.22	14.5	0.135	0.08	6.98	7.84	0.9
10.21	2.45	2.31	18.75	0.10	0.04	6.25	7.86	0.8

TABLE 4.5.4. DETERMINATION OF UNDERFLOW COMPOSITION

Experimental data oil in overflow wt frac Y_s	K	Underflow stream per g of inert oil,g $k.y_s$	solvent,g $k(1-y_s)$	underflow,g $k+1$	Wt. fraction oil/ underflow $\frac{k.y_s}{k+1}$	solvent/ underflow $\frac{k(1-y_s)}{k+1}$
0.0	0.6	0.0	0.6	1.6	0.0	0.375
0.1	0.8	0.08	0.72	1.8	0.04	0.40
0.135	0.9	0.121	0.778	1.9	0.063	0.41
0.193	1.05	0.202	0.847	2.05	0.098	0.413
0.217	1.1	0.298	0.801	2.1	0.141	0.381

4.5.5 DETERMINATION OF TERMINAL COMPOSITION

BASIS :

1. 1000 kg feed containing 230 kg oil and 770 kg inert per hour
2. Solvent composition , Acetone : Petroleum ether :: 1:3
3. Desired oil concentration in overflow stream = 60 %
4) Recovery of oil in overflow stream = 90 %

a) Feed composition :

Wt fraction oil in feed is 0.23

b) Overflow feed :

Pure solvent mixture of Acetone and petroleum ether of 1 : 3 was added at a solvent to feed ratio of 2 : 1. Hence feed rate of solvent = 1000 x 2 = 2000 kg / hr. The feed point is (0,0.23) as there is no solute present in the solvent.

c) Overflow composition :

Solute in overflow discharge 230 - 23 = 207 kg

Amount of overflow solution V_1 = 207 / 0.6 = 345kg

Hence overflow composition :

		wt fraction
oil =	207 kg	0.375 (x_1)
solvent =	345 kg	0.625 (x_s)
	552 kg	

d) Underflow discharge composition

From the experimental data (Fig 5.2) it was found that wt fraction of oil in the overflow 0.375 ,corresponds to 1.22 gm of solution per gm of inert.

On the basis of 1000 kg of underflow feed / hr

oil in underflow feed = 230 kg / hr

oil free inert in the underflow feed = 770 kg

Underflow discharge contains 0.029 kg of oil / kg of inert .

From experimental data (Table 4.5.4) one can get by interpolation the value of K at Ys = 0

Hence by interpolation 0.029 kg of oil / kg of inert corresponds to $= 0.6 - \frac{0.029}{0.08} [0.6 - 0.72]$ kg of solvent/kg inert

$= 0.6 + 0.043 = 0.643$ kg of solvent / kg inert

Underflow discharge composition

		wt. Frac
Solute(oil)	0.029	0 0173
Solvent	0.643	0.384
Inert	1.000	0.598

Hence the discharge point composition (wt frac) is as following :

Oil = 0.173

Inert = 0.598

Solvent = 0.384

The terminal points are plotted in a right angle triangle S O A . The number of stages required for leaching of 90 % oil was determined graphically as described by Prabhudesai , 1979 and Bradger , 1955, as shown in Fig.4.5.3. The number of stages was found to equal to 2.

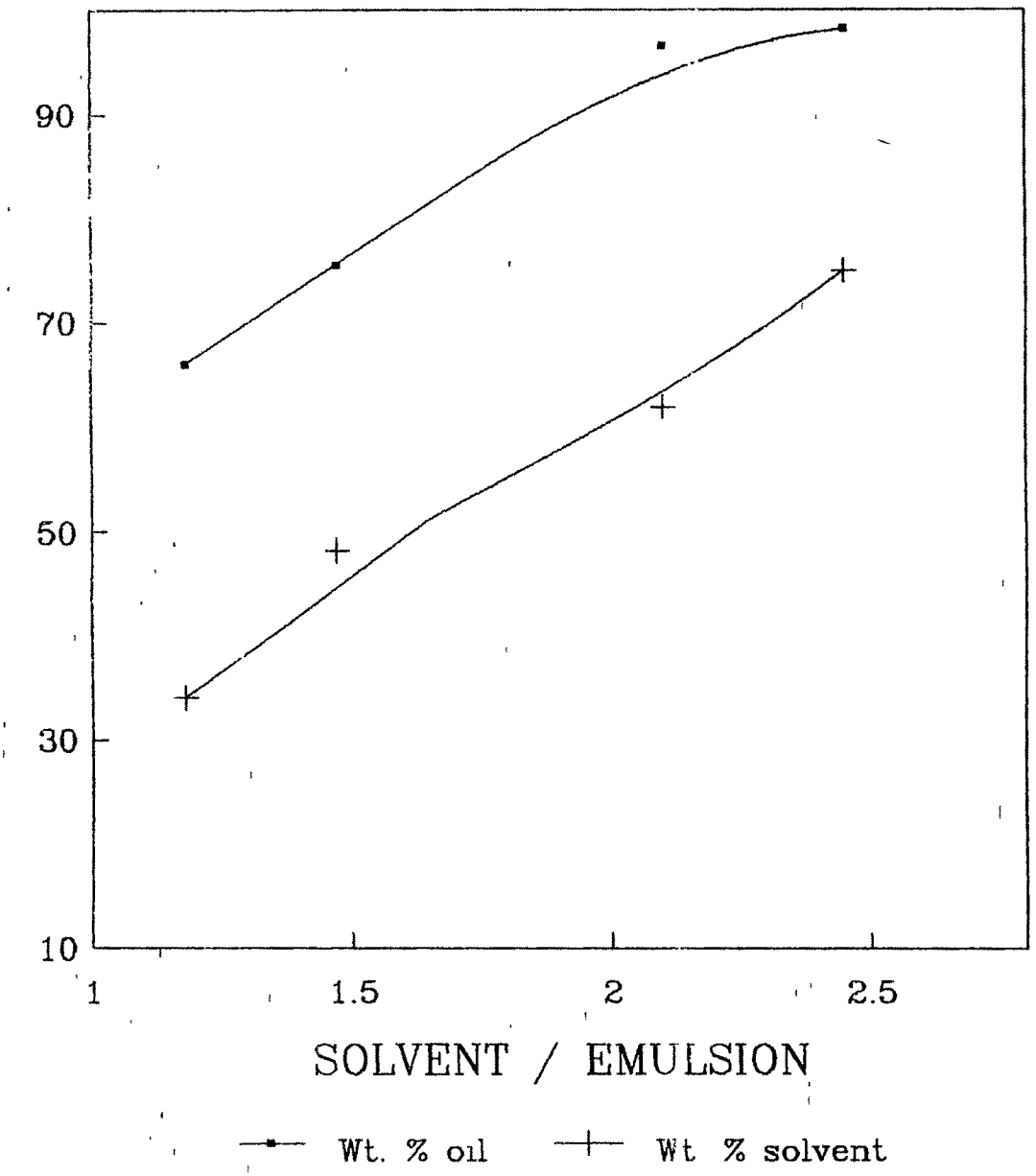

Fig.5.1 Effect of solvent/emulsion on oil recovery

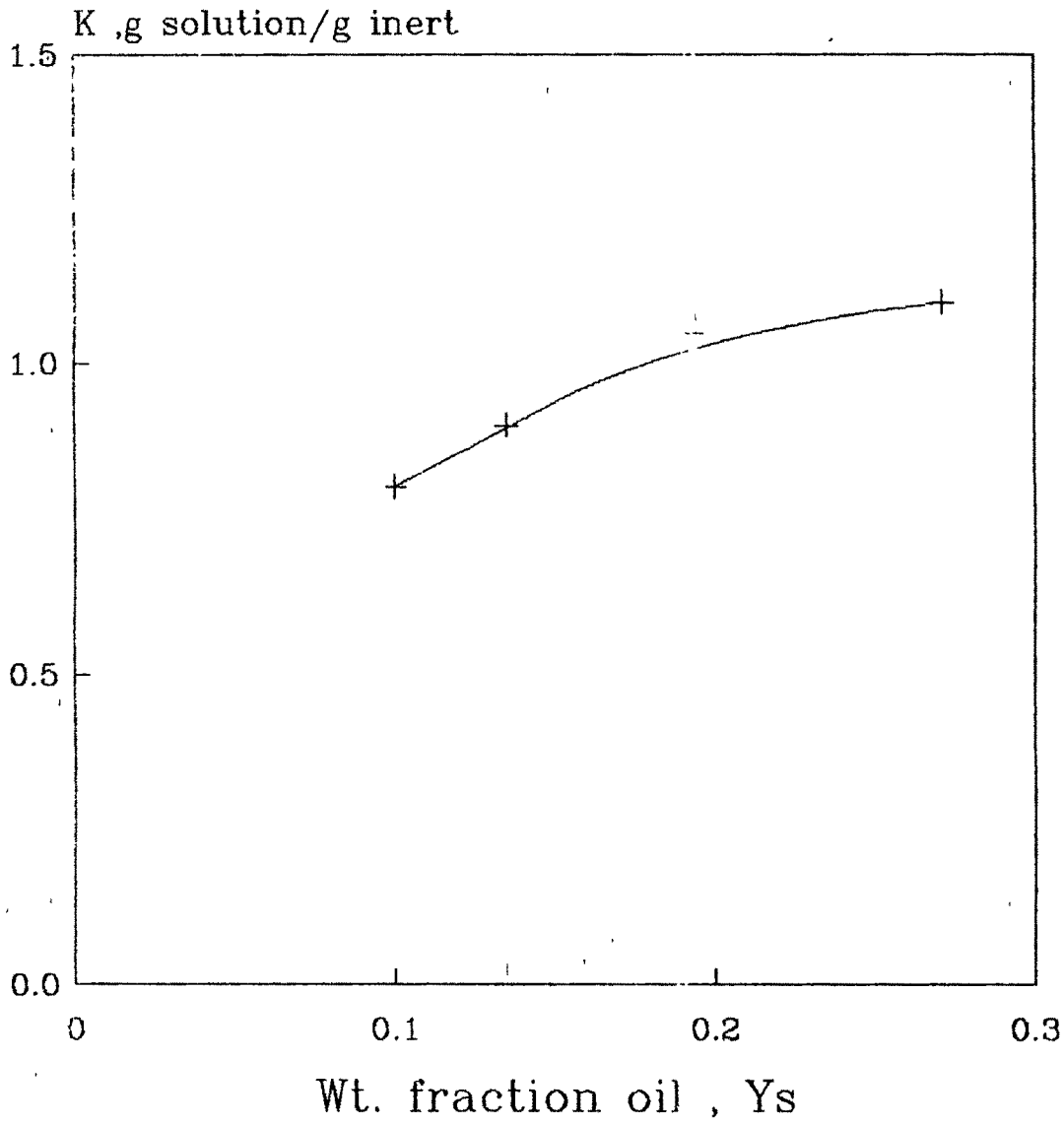

Fig.5.2. Retaintion value (k) at different oil conc.

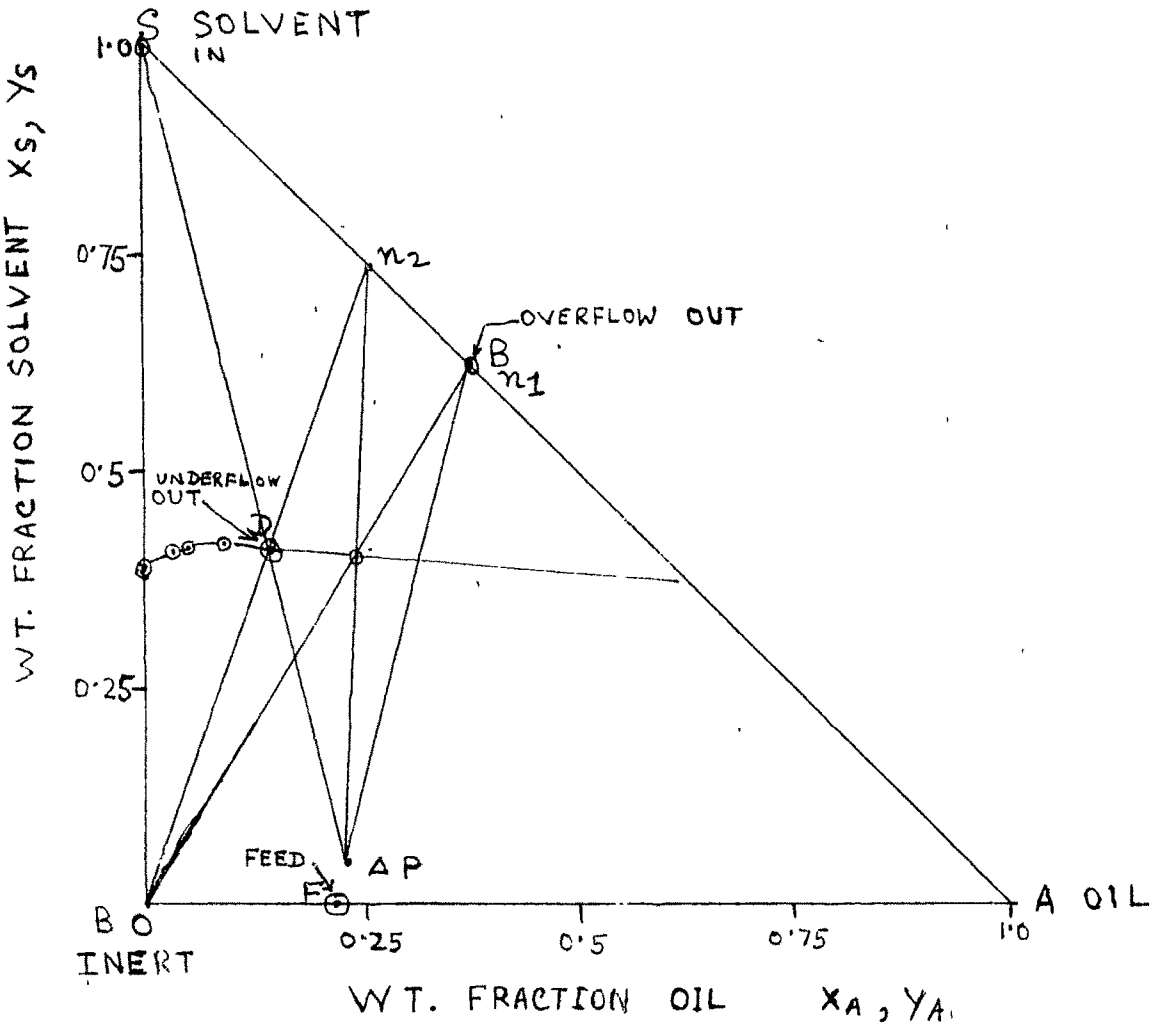

Fig.5.3.Graphical determination of stages required for leaching operation

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4.6 ANALYSIS OF DEWAXED OIL AND SCP

4.6.1 Analysis of dewaxed oil :

The dewaxed oil was made free from solvent by vacuum distillation. To find its suitability as lubricating oil, its physical properties were determined and were compared with the commercial dewaxed oil produced by Refineries following solvent extraction and solvent dewaxing method. Results are given in Table 4.6.1., Where the physical properties of the feed stocks used are also shown to reflect the net change in properties.

TABLE 4.6.1. PROPERTIES OF THE MICROBIAL AND SOLVENT EXTRACTED / DEWAXED OIL

Properties	Feed stocks Vacuum distillate		Microbial dewaxed oil		Commercial dewaxed oil	
	A	B	A	B	A	B
1.Pour Pt. ^0C	48	30	18	0	18	- 3
2.Density $_{20}{}^0C$ g/ml	0.9371	0.8806	0.9495	0.9165	0.9441	0.8994
3.Viscosity cS at 100 C	4	10	10	7.7	9	9.3
4.Viscosity Index	-	42	-	76	60	95

There was a net decrease of pour point of 30°C in both the feed stocks undergone microbial dewaxing. The pour point of the microbial dewaxed oil obtained from both the feed stocks is very well comparable with that of commercially produced dewaxed oil. However the viscosity index is lower in microbial dewaxed oil. The low value of viscosity index and increase of density of microbial

dewaxed oil indicated the deparaffinisation of the feed stocks by which aromatic content has gone up in the microbial dewaxed oil. The viscosity index, however can be improved either by solvent extraction or by hydrogenation.(Kockert et al, 1982).

In order to find the selectivity of the strain *C.tropicalis* on the different hydrocarbon components the feed stocks 'A' and the dewaxed oil were analysed by column chromatograph and mass spectrometer . The results of the analysis are shown in the Table 4.6.2.

TABLE 4.6.2. CHANGE OF HYDROCARBON COMPONENTS BY MICROBIAL DEWAXING

Oil sample		Hydroarbon components in dewaxed oil			
		saturates		Aromatics	
	g	g	wt%	g	wt%
Vacuum distillate	60	28.3	47	31.7	52.8
Microbial dewaxed oil	33	9.8	29.8	23.2	70.2

It can be seen from the Table 4.6.2 that 65.3 % of the saturates and 26.9 % aromatics present in the feed have been utilised during microbial dewaxing which has resulted in the net increase of aromatic content in dewaxed oil.The utilisation of some long chain alkyl aromatic hydrocarbon and the n.paraffins as determined by mass spectra analysis is shown in Table 4.6.3.

TABLE 4.6.3. CHANGE IN INDIVIDUAL HYDROCARBON TYPES IN VACUUM DISTILLATE 'A' DUE TO MICROBIAL DEWAXING

Hydrocarbon types	Decrease wt%
AROMATICS	
1. Alkyl benzene	39.78
2. Naphthene benzene	24.12
3. Naphthelene	38.0
4. Acenaphthelene	36.21
SATURATES	
1.n-paraffins	65.8
2.isoandcycloparaffins	nil

The results indicated the selectivity od the strain towards n-paraffinic hydrocarbons present in the vacuum distillate sample.

4.6.2.Analysis of biomass :

The dry yeast cells were obtained as by product from the underflow stream of the oil extraction unit as compared to slack wax produced in solvent dewaxing process. However the slack wax produced from vacuum distillate 'A' in Barauni Refinery could not find its market due to high oil content (30-40%).

n-Paraffin grown yeast cells (*C.tropicalis*) have been tested as SCP powder in cattle feed and the toxciological , nutritional and field trial experiments indicated its potentiality as a substitute of vegetable proteins. So vacuum distillate grown yeast cells (*C.tropicalis*) can be utilised as SCP in the cattle feed .

The proximate analysis of the strain grown on heavier hydrocarbon is compared with that of grown on n paraffin in Table 4.6.2. The composition of biomass obtained from n paraffin and vacuum distillates is quite comparable . Marginally lower protein and carbohydrate content and marginally higher lipid content were observed in vacuum distillate grown biomass.

The cost of production of SCP obtained as by product of the dewaxing process of lubricating oil fraction would be comparatively cheaper than cost of production of SCP from gas oil or n paraffin .

TABLE 4.6.4.COMPOSITION OF SCP FROM DIFFERENT PETROLEUM FRACTIONS

Composition	Vacuum distillate 'A' wt %	n paraffin wt %
Protein	52	59.9
Lipid	12	7.8
Carbohydrate	22	24.4
Ash	9	7.9
Water	5	8.0

As the composition of polycyclic aromatic hydrocarbons in the vacuum distillats are higher than that of gas oil or n paraffins,possibly some of the heavier aromatic hydrocarbons may be present in the biomass . To bring down the undesirable aromatic hydrocarbon content of the biomass to an acceptable limit as recomended by IUPAC 1978, through washing of biomass with suitable solvent system is required.