CHAPTER IV

Mode of Uptake of Solid Hydrocarbons by Microorganisms.
Introduction

In the previous Chapters, investigations on the mode of microbial uptake of volatile, semi-volatile and non-volatile liquid \( \mu \)-alkanes have been presented. In this Chapter, studies on the mode of microbial uptake of solid \( \mu \)-alkanes are described.

Solid hydrocarbons are harder to be dispersed in the aqueous medium due to physical rigidity of the hydrocarbon particles. Microbial uptake of insoluble solid hydrocarbons may take place either from the aqueous phase as solubilized/pseudosolubilized substrate or from the hydrocarbon phase by direct contact of cells with hydrocarbons particles. In the first case substrate particles of much smaller size than the cells (molecular to submicron size) which are homogenously distributed in the aqueous phase may interact with the cells. Substrate uptake will then be dependent on the rate of substrate solubilization, and substrate uptake may be in dynamic equilibrium with substrate solubilization. The problem has been dealt with in detail by Chakravarty et al. (1972, 1975).

In the later case, cells interact with substrate through adsorption on the surface of substrate particles larger in size than the cells. Availability of substrate surface area will then be a limiting factor for substrate uptake. Further, the rate of adsorption and desorption of cells will have an influence on growth and substrate uptake. In the case of liquid hydrocarbons, Verkooyen et al. (1980 a,b,c) made a
detailed analysis of this mode of interaction between cells and substrate particles. In the case of solid hydrocarbons, dispersion would be harder than liquid hydrocarbons and consequently substrate surface area may be considerably limited.

Chakravarty et.al. (1972) observed that the cells of a *Pseudomonas* species used by them did not usually adhere to the solid paraffin particles during cultivation and suggested that growth and substrate uptake took place primarily from the solubilized substrate. They hypothesized that a metabolite produced by the cells brought about increased solubilization of the substrate. Zilber et.al. (1979,1980), using a marine pseudomonad, however, demonstrated that more than 30% of the cells were bound to large n-tetracosane particles during the early exponential growth phase. By $^{32}$p pulse labelling experiments, they obtained evidence for cellular growth both on the surface of the paraffin particles and in aqueous medium.

Goswami et.al. (1983), demonstrated the uptake of sterols by an *Arthrobacter* species predominantly through adsorption of cells on the surface of sterol particles. In this Chapter using two species of yeast and one of bacterium evidences are presented for the microbial uptake of solid n-alkanes predominantly in solubilized form.
Materials and Methods

Organisms:

The following organisms which were used in these studies have already been described in the previous Chapters.
(a) Yeast strain *E. lipolytica* YM (natural mutant of Y-13).
(b) Yeast strain *E. lipolytica* JM-1 (UV mutant of YM).
(c) Bacterial strain *Pseudomonas* PJ-1 previously isolated in the Laboratory.
(d) Bacterial strain *Pseudomonas* RRL JT-7 was isolated in the Laboratory. This strain also forms fluorescent colonies on nutrient agar plates and is an aerobic and flagellated chemotroph.

The maintenance and cultivation of *Pseudomonas* RRL JT-7 was done according to similar procedure used for *Pseudomonas* PG-1. Biomass, residual hydrocarbon determinations and isolation of hydrocarbon emulsifying and solubilizing factor have been described in the previous Chapters.

Preparation of Emulsified Hydrocarbon:

The same method used for liquid alkane emulsification (Chapter III) was used except that emulsification of solid alkanes (0.5%) was performed after melting the paraffin prior to ultrasonication.

Solid alkanes used as substrates were finely powdered after chilling. For studies with different particle size alkane during growth of *Pseudomonas* RRL JT-7,
n-docosane powder was serially sieved through 150, 90 and 63 micron sieves, respectively, and used in the experiments. Unless otherwise stated, solid n-alkanes were used in fine powder after sieving through a 150 µm sieve.

Cell adsorption on hydrocarbon particles:

To study the adherent capacity of Pseudomonas PG-1 on hydrocarbon particles, the organism was grown on powdered n-tetracosane for 48-72 hours. The culture broth, which was in exponential growth phase, was kept standing in a separating funnel at 4°C for 1 hour or centrifuged at 2,500 rpm for 5 minutes and the upper paraffin layer was carefully separated from the lower aqueous layer and dry biomass and residual alkanes were estimated in both fractions.

Estimation of alkane solubilization rate:

Solubilization of hydrocarbons by the action of the isolated polymer was carried out by mixing 50 mL of the crude polymer (undialysed) solution (2 mg/mL) in conical flasks with 250 mg of hydrocarbons on the rotary shaker. Normal tetracosane and docosane were used in powdered form and n-tetradecane as liquid. Flasks were withdrawn at various intervals ranging from 0 to 10 min and contents were centrifuged followed by filtration through 0.45 µm Millipore membrane. The filtrate was extracted with hexane and hydrocarbons in the extract estimated according to procedure described previously.
To determine the hydrocarbon solubilizing capacity of the culture broth, *Pseudomonas* PG-1 and *E. lipolytica* YM were grown on *n*-tetraicosane and *n*-docosane powders respectively for 24 hours followed by addition of 4-5 mM EDTA to deactivate the hydrocarbon solubilizing activity. The broth was centrifuged followed by filtration through 0.45 μm Millipore membrane to obtain cell-free broth. To 40 mL of the cell-free broth in the conical flask was added Ca$^{2+}$ solution in equimolar amount to EDTA to activate the hydrocarbon solubilizing activity and hydrocarbon solubilization was determined under saturation condition as described earlier.

**Results and Discussion**

*Growth of Pseudomonas RRI, JT - 7 on Solid n-alkanes, effect of non-ionic surfactant and substrate particle size on growth and polymer production*:

Wegner (1967) described a solid hydrocarbon fermentation process in which solid paraffins dissolved in liquid hydrocarbon such as decane was used. Likewise, Miller and Johnson (1966 a,b) reported the growth of *Candida intermedia* and *C. lipolytica* on $C_{22}-C_{28}$ solid paraffins and paraffin wax dissolved in pristane and obtained biomass in high yield. These observations indicated that if the solid paraffins are well dispersed,
microbial uptake and utilization of the solid substrates was enhanced. Yamada and Yogo (1970) reported that when C_{25}-C_{37} paraffin was dispersed in the medium as emulsion, some bacterial and yeast strains readily grew on it. They further observed that when non-ionic surfactant Plysurf A 210G was added, a highly concentrated wax emulsion was obtained but the growth of microorganisms on it was slower. Amin et al. (1973) observed complete conversion of \( n \)-eicosane and 40-50\% conversion of slack wax and paraffin wax into biomass by solid hydrocarbon utilizing bacterial isolates when the substrate was presented in the form of emulsion in the growth medium.

Figure 4.1 shows the growth of *Pseudomonas* RRL JT-7 on \( n \)-eicosane, finely powdered \( n \)-docosane and \( n \)-tetracosane and the effect of addition of 20 mg\% non-ionic emulsifiers (Unisperse-P and Tween-60) to the growth medium. It can be seen that Unisperse-P improved the growth of the organism on \( n \)-C_{20} and \( n \)-C_{24} alkanes, however the stimulating effect of Tween-60 on growth is doubtful. The emulsifier obviously helped in the uptake of solid alkenes by the cells through formation of microscopic particles of the substrate which either provide larger surface area for the cells to attach or help in the solubilization into aqueous phase.

Figure 4.2 shows the effect of particle size of the solid substrate on growth of *Pseudomonas* RRL JT-7 on \( n \)-docosane. Growth was comparatively poor with 150 \( \mu \)m size \( n \)-C_{22} alkane particles, whereas smaller particle sizes of
63 μm and 90 μm definitely improved the growth. This perhaps could be expected as smaller particle size would provide larger surface area and aid in the substrate solubilization. As in the case of Pseudomonas PG-1, this strain also produced polymeric substance precipitable by cold acetone, which was very similar to the hydrocarbon ESF described elsewhere. It is seen that polymer production was improved by the smaller particle sizes of the substrate. The production of ESF-like polymer perhaps conveys that substrate solubilization may be an important mechanism in the substrate transfer to the cells.

**Growth of Pseudomonas PG-1 on Crude Wax:**

There are very few reports on the utilization of paraffin wax by microorganisms. Amin et al. (1973) reported the utilization of slack and paraffin waxes by an unidentified bacterial species and discussed the possibility of producing single cell protein from paraffin wax. Yamada and Yogo (1970) also reported biomass production from paraffin wax using yeast and bacterial species. Lonsane et al. (1973) reported that Schwanniomyces occidentalis could utilize slack wax and paraffin wax as the sole carbon source.

_Pseudomonas_ PG-1, a versatile bacterial strain capable of growth on a variety of hydrocarbons could utilize finely powdered crude wax (m.p. 57.6°C) for growth as shown in Fig. 4.3. As in the case of the growth of this organism on liquid n-alkanes (Chapter III), ESF polymer was also produced
during the cultivation of this organism on paraffin wax (Fig. 4.3). Production of the polymer increased along with the growth of the organism. It appears that paraffin wax is solubilized by the substrate solubilization system during growth of the organism on crude wax.

**Growth and mode of Uptake of n-docosane by E. lipolytica YH:**

Growth and substrate uptake by *E. lipolytica YH* cultivated on *n*-docosane powder (5 g/L) are shown in Fig. 4.4. As described in Materials and Methods the docosane powder after sieving through 150 μm sieve was used. A diux type of growth curve was obtained probably indicating the formation of metabolite(s) which was utilized in the latter period of growth. This characteristic of growth was confirmed by triplicate experiments. A linear type of growth was observed in the initial 24 hours of cultivation. The apparent absence of an exponential growth phase could be due to the comparatively large inoculum used in these experiments combined with the limited substrate transfer from the hydrocarbon phase. Using a very low inoculum concentration (ca. 1/1000th of the concentration used in the present experiment) and following the growth by viable cell count and $^{32}$P incorporation, Zilber et al. (1979, 1980), obtained a prolonged exponential growth of a *Pseudomonas* species on *n*-tetracosane with a doubling time of 2.1 hours. With such low inoculum, substrate transfer could be adequate
to maintain exponential growth of appreciable duration. For practical fermentation and from theoretical consideration, however, the more interesting part of the growth is when substantial biomass is formed and the substrate transfer is stressed. This was obtained in the present experiment as depicted in Fig. 4.4.

Substrate uptake started linearly in the initial 24 hours of growth after which uptake rate decreased sharply coincident with the termination of the first phase of growth. An initial linear growth rate of 37 mg/L h and substrate uptake rate of 71 mg/L h with a biomass yield factor of 0.52 were obtained (Table 4.1). The low biomass yield in the initial period probably indicates the accumulation of metabolite(s). After 5 days of cultivation biomass yield factor was increased to 0.9.

EDTA at 5 mM concentration strongly inhibited the growth of the organism on n-docosane powder (Fig. 4.4). This concentration of EDTA was shown previously to be a strong inhibitor of the growth of this organism on liquid n-alkane through inhibition of alkane solubilization (Reddy et al. 1982). Fig. 4.5 shows that when artificially emulsified and solubilized n-docosane (5x3 g/L) was used as substrate the inhibitory effect of EDTA was removed to a considerable extent though not completely. As expected, growth was found to be stimulated considerably by artificially emulsified and solubilized substrate in comparison to substrate powder and no diphase growth was observed possibly due to availability
of readily utilizable substrate which inhibited the utilization of metabolites.

These evidences indicate that the main mechanism of substrate transfer to cells is likely to be through solubilization of the solid alkane.

**Growth of *E. lipolytica* UM-1 on Solid *n*-alkanes:**

Growth of the mutant strain of yeast, *E. lipolytica* UM-1 on powdered *n*-docosane (5 g/L) is shown in Fig. 4.6. The strain had negligible growth on this alkane presented in the forms described. Addition of the surfactant, Unisperse-P at the level of 20 mg% to the medium did not promote growth even though increased emulsification of the substrate was expected during shaking incubation due to the presence of the emulsifier. However, when hydrocarbon emulsifying and solubilizing polymer (PG-1 ESF C22) isolated from the culture broth of *Pseudomonas* PG-1 grown on *n*-docosane, was added to the growth medium at the level of 25 mg%, appreciable growth of the organism was obtained on *n*-docosane powder (Fig. 4.6). The polymer was shown previously to have both hydrocarbon emulsifying and specific hydrocarbon solubilizing properties (*Reddy et al.* 1982, 1983). It appears that mere dispersion of the hydrocarbons in macroscopic drops or particles was not enough to stimulate growth but that hydrocarbon should be in solubilized/pseudosolubilized form (submicron particles) for the cells to be able to take up and utilize it. Apparently the mutant strain of yeast lacked
the capacity to produce hydrocarbon emulsifying and solubilizing compounds. When artificially emulsified and solubilized \( \text{n-docosane} \) (5 g/L) prepared through ultrasonication in the presence of Unisperse-P, were used as substrate, there was dramatic growth of the organism (Fig. 4.6) providing strong evidence for the above conclusion. It has been shown that such artificially emulsified and solubilized hydrocarbon not only contains appreciable amount of solubilized hydrocarbon (Table 4.2) but it can also generate increased amount of solubilized hydrocarbon during shaking incubation as shown in Fig. 4.7.

**Growth and substrate uptake by Pseudomonas PG-1 cultivated on \( \text{n-tetracosane} \):**

Figure 4.6 shows the characteristics of growth, substrate uptake and formation of ESF polymer by *Pseudomonas* PG-1 cultivated on \( \text{n-tetracosane} \) powder (5 g/L). Growth was exponential for 96 hours but with a very low specific growth rate (\( \mu \)) of 0.038 h\(^{-1}\). Substrate uptake and polymer production however, started linearly from the beginning of the incubation at comparatively higher rates but the production of the latter compound levelled off after 72 hours of cultivation. It is evident that in the initial 48 hours of cultivation most of the substrate taken up was converted into the polymer at the expense of cell formation and with the decrease in the production
of polymer in the latter period of cultivation, increased biomass formation occurred coincident with the late exponential phase. Similar behavior of this organism was previously observed during growth on pristane (Reddy et al. 1983). It was previously shown that the polymer produced by the organism consisted of an EDTA-insensitive hydrocarbon emulsifying factor and an EDTA-sensitive specific hydrocarbon solubilizing factor (Reddy et al. 1983). It is interesting to note that the production of this hydrocarbon solubilizing polymer started very early during incubation and paralleled with the substrate uptake perhaps indicating that hydrocarbon solubilization subserved substrate transfer to cells.

EDTA (4 mM) inhibited the growth of the organism on n-tetracosane powder almost completely (Fig. 4.8). EDTA inhibition was removed when artificially emulsified and solubilized n-tetracosane was used as substrate (Fig. 4.9). These results are consistent with the hypothesis of substrate uptake primarily from solubilized hydrocarbon. Growth was considerably stimulated when artificially emulsified and solubilized n-tetracosane was used as substrate.

Cells of Pseudomonas FG-1 did not adhere to big n-tetracosane particles during cultivation (Table 4.3). Hydrocarbon phase separated from the culture broth either by gravity separation or by centrifugation at modest speed did not contain any measurable amount of biomass though most of the residual hydrocarbon (98%) was separated in this phase. As very mild separation procedure was used, it was unlikely
that desorption of cells occurred to any appreciable extent during experimental manipulation. Microscopic examination also showed that big hydrocarbon particles were free from adhering cells. It is apparent that substrate transfer through cellular contact with big \( n \)-tetracosane particles is likely to be negligible at least with this organism.

**Alkane solubilization:**

To demonstrate the validity of alkane uptake primarily through solubilization, it is necessary to show that the rate of alkane solubilization can fully account for the maximum rate of substrate uptake by the cells. Fig. 4.10 shows the alkane solubilizing capacity of the cell-free culture broth obtained after cultivation of the organisms on the same \( n \)-alkane powder for 24 hours at which period maximum substrate uptake rate was recorded. EDTA was added to the culture broth immediately after cultivation was over, to stop any further solubilization of the substrate during processing of the broth. When all the residual substrate was removed by filtration through 0.45 \( \mu \)m Millipore filter, cell-free broth was activated by adding adequate amount of \( \text{Ca}^{2+} \). If the activation by \( \text{Ca}^{2+} \) was omitted, EDTA-treated broth showed negligible hydrocarbon solubilization. The procedure gave a low and reliable value of the steady state level (zero minute value) of solubilized substrate in the broth. If this was not followed, a comparatively high level of solubilized substrate in the
broth was generally obtained making it difficult to compute solubilization rate.

As shown in Fig. 4.10 n-docosane solubilization by the culture broth of *E. lipolytica* YH under constant saturation conditions increased along with the increase in the period of shaking. Alkane solubilization rate calculated from the initial slope of the curve was found to be 31.6 mg/Lh which was greater than the observed maximum substrate uptake rate of 71 mg/Lh (Table 4.1). The solubilization of n-tetracosane by the culture broth of *Pseudomonas* PG-1 increased linearly for the initial 3 min of shaking after which rate of solubilization decreased. Alkane solubilization rate was 75 mg/Lh which was much greater than the observed maximum substrate uptake rate of 40 mg/Lh. As the mixing was done in the same manner as it was done for cultivation (rotary shaker, 120 rpm), the measured hydrocarbon solubilization rates may be considered to correspond very nearly to the actual solubilization rates occurring during cultivation. The results indicate that hydrocarbon solubilization can fully account for the actual substrate uptake taking place during the growth of the organism on solid alkane powder. The comparatively low steady state level of solubilized hydrocarbon in the culture broth indicates that the solubilized substrates were actually taken up by the cells.

Fig. 4.10 also shows hydrocarbon solubilizing capacity of the crude polymer (undialysed) isolated from the culture broth of *Pseudomonas* PG-1 cultivated on
\( \text{n-tetracosane powder. The crude polymer obtained by acetone precipitation contained a high amount of salt with an ash content of } 52\%. \text{ After exhaustive dialysis against distilled water for 36 hours, } 32\% \text{ of the original crude isolate could be recovered as non-dialyzable substance which did not show hydrocarbon solubilizing activity. But after activation by treatment with } 1 \text{ mM } \text{Ca}^{2+}, \text{ activity could be restored almost to the original level of the crude isolate.}

\text{As shown in Fig. 4.10 the crude polymer was associated with appreciable amount of } \text{n-tetracosane (zero min value) probably absorbed in it, but it was not saturated with the hydrocarbon as shown by the increasing solubilization during shaking in the presence of the hydrocarbon. A linear solubilization curve for } \text{n-tetracosane was obtained during the initial 5 min of shaking giving a solubilization rate of } 31.5 \text{ mg/Lh. The specificity of hydrocarbon solubilization by the polymer is shown by the low level of solubilization of } \text{n-docosane and negligible solubilization of } \text{n-tetradecane (Fig. 4.10).}

\text{Summary and Conclusion :}

\text{Using two species of yeast and one of bacterium evidences have been obtained for the microbial uptake of solid alkane powders primarily through a substrate solubilization mechanism. As described in Chapter III, demons-}

tration of the binding of cells to hydrocarbon particles is not sufficient evidence for major substrate uptake directly from the substrate particles as advanced by various investigators (Zilber et al. 1979, 1980), specially when substrate solubilization at an adequate rate could be demonstrated. Solubilized alkane will be available to both bound and free-cells and therefore $^{32}$p incorporation by both fractions of cells as demonstrated by Zilber et al. (1980) in pulse labelling experiment could be explained on the basis of substrate uptake through solubilization.

EDTA, a strong inhibitor of hydrocarbon solubilization by the cells inhibited the growth of these organisms on alkane powder and the inhibition could be removed by supply of artificially solubilized alkane. One of the yeast strains which was a mutant incapable of growing on solid alkane powder and also on liquid alkane could grow very well on artificially solubilized alkanes. It was demonstrated that solid alkane solubilization rate during microbial growth could satisfactorily account for the maximal alkane uptake rate actually observed during growth. The specificity of solubilization for the solid alkane used as growth substrate was demonstrated.
TABLE 4.1

Rates of growth, substrate uptake and substrate solubilization by E. lipolytica YM and Pseudomonas PG-1 cultivated on n-docosane and n-tetracosane powder (<150 μm) respectively.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Maximum growth rate, mg/Lh (dx/dt)\textsuperscript{max}</th>
<th>Specific growth rate, h\textsuperscript{-1} (μ)</th>
<th>Maximum substrate uptake rate, mg/Lh (ds/dt)\textsuperscript{max}</th>
<th>Biomass yield factor, y (dx/dt)\textsuperscript{max}</th>
<th>Substrate solubilization rate by cell-free broth, mg/Lh</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. lipolytica YM</td>
<td>37</td>
<td>-</td>
<td>71</td>
<td>0.52</td>
<td>81.6</td>
</tr>
<tr>
<td>Pseudomonas PG-1</td>
<td>-</td>
<td>0.038</td>
<td>40</td>
<td>-</td>
<td>75.0</td>
</tr>
</tbody>
</table>

TABLE 4.2

Size distribution of n-docosane particles in emulsified preparation by ultrasonication in the presence of 20 mg/L Unisperse-P. Alkane particles were separated by serial filtration through Millipore membrane of various pore sizes.

<table>
<thead>
<tr>
<th>Alkane particle size μm</th>
<th>n-docosane mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.45 - &lt;0.80</td>
<td>33.2</td>
</tr>
<tr>
<td>&lt;0.10 - &lt;0.45</td>
<td>16.3</td>
</tr>
<tr>
<td>&lt;0.025 - &lt;0.10</td>
<td>7.0</td>
</tr>
<tr>
<td>&lt;0.025</td>
<td>0.5</td>
</tr>
</tbody>
</table>
TABLE 4.3
Adherent capacity of cells of *Pseudomonas* PG-1 to *n*-tetracosane particles

<table>
<thead>
<tr>
<th>Period of cultivation h</th>
<th>Top hydrocarbon layer</th>
<th>Bottom layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bound cells g/L</td>
<td>Hydrocarbon g/L</td>
</tr>
<tr>
<td>Gravity separation for 90 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Nil</td>
<td>3.21</td>
</tr>
<tr>
<td>72</td>
<td>Nil</td>
<td>3.06</td>
</tr>
<tr>
<td>Centrifugation at 2500 rpm for 5 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Nil</td>
<td>3.24</td>
</tr>
<tr>
<td>72</td>
<td>Nil</td>
<td>3.08</td>
</tr>
</tbody>
</table>
EFFECT OF EMULSIFIERS 20 mg % \* GROWTH OF PSEUDOMONAS RRL J T-7 ON HYDROCARBON POWDERS (2 %)

![Graph showing the effect of emulsifiers on the growth of Pseudomonas RRL J T-7 on hydrocarbon powders.](image-url)
GROWTH OF PSEUDOMONAS RRLJT-7 ON N-DOCOSANE POWDERS AND POLYMER PRODUCTION

![Graph showing growth of Pseudomonas RRLJT-7 on N-docosane powders and polymer production.](image-url)
BIOMASS AND POLYMER
PSEUDOMONAS PG-1 GROWTH AND POLYMER FORMATION ON CRUDE-WAX POWDER.

CRUDE WAX M.P. 54.6°C
△ POLYMER
● BIOMASS

PSEUDOMONAS PG-1 GROWTH AND POLYMER FORMATION ON CRUDE-WAX POWDER.
GROWTH OF *E. lipolytica* YM ON *n*-DOCOSANE POWDER (≤ 150 μm).

---

**Figure 4.4.**

- Biomass g/L vs. Time (HOURS)
- Substrate Uptake g/L vs. Time (HOURS)
- Growth, Control
- Growth + 5 mM EDTA

Derivatives:
- $(\frac{ds}{dt})_{\text{max}}$
- $(\frac{dx}{dt})_{\text{max}}$
GROWTH OF *E. lipolytica* YM ON *n*-DOCOSANE EMULSIFIED WITH 20 mg% UNISPERSE-P BY ULTRASONICATION

(Arrows indicate addition schedule of emulsified substrate -250 ml in 10 ml)

![Graph showing biomass growth over time with and without EDTA](attachment:image.png)
**Fig. 4.6:** CULTURE OF *E. lipolytica* UM-1 ON *r*-DOCOSANE (0.5 mg%)
EFFECT OF SHAKING INCUBATION ON
GENERATION OF SOLUBILIZED ALKANE
IN EMULSIFIED ALKANE PREPARATION
GROWTH OF PSEUDOMONAS PG-1 ON n-TETRACOSANE POWDER (<150μm).

**Fig. 4.2**

GROWTH OF PSEUDOMONAS PG-1 ON n-TETRACOSANE POWDER (<150μm). (5 g/L).
GROWTH OF PSEUDOMONAS PG-1 ON EMULSIFIED n-TETRACOSANE (ULTRASONICATED) 5 g/L
Fig. 4.10.
ALKANE SOLUBILIZATION BY CELL-FREE BROTH AND POLYMER

HYDROCARBON SOLUBILIZATION mg/L

MIN

E. lipolytica YM BROTH
n-C 22
PSEUDOMONAS PG-1 BROTH
n-C 24
n-C 22, POLYMER
n-C 14, POLYMER