# GROWTH AND DESULFURIZATION KINETICS OF RHODOCOCCUS ERYTHROPOLIS IGTS8 ON DIBENZOTHIOPHENE AND PETROLEUM FRACTIONS

BY

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## 1-Abstract

The growth Kinetics of *Rhodococcus erythropolis* IGTS8 on dibenso-thiophene, DBT, of different initial concentrations as well as on two petroleum fractions namely untreated and hydrodesulfurized gasoline and gas oil have been investigated in batch cultures.

Using dibenzothiophene as a substrate, the specific growth rates were found to decrease with increasing initial substrate concentration. The removal of dibenzothiphene from culture media was found to follow first order kinetics. The reaction rate constant, k, decreased with increasing substrate concentration.

The decrease of both specific growth rate and reaction rate constant with increasing substrate concentration suggested substrate inhibition. The growth rate on untreated gasoline as well as on hydrodesulfurized gasoline gave nearly the same specific growth rates of 0.067 $h^{-1}$ while growth on gas oil gave a higher specific growth rate of 0.1$h^{-1}$.

## 2- Introduction

The concept of microbial desulfurization is not new, BDS has been reported since 1935 and much work has been done in order to understand the biochemistry of microbial attack on organic sulfur compounds and the mechanisms of BDS reactions (Malik, 1978). The first U.S. patents were issued in the 1950s. This technology has not been commercialised because the biological systems has lacked reaction kinetics and specificity required for commercial application. There have been several reports over the last 20 years of successful lab scale "microbial desulfurization" that was of limited commercial interest. The problem was that although they desulfurized the oil, they also consumed most of it. (Monticello, 1994).

BDS is a process that is being developed based on naturally occurring bacteria that can remove organically bound sulfur from petroleum without degrading the fuel value of the product. (Monticello, 1993). The use of microorganisms for the BDS of high sulfur coals and oils has attracted attention as a potential pre -combustion technology for the reduction of the organo- sulfur content of fossil fuels. (Finnerty, 1993).

A number of organisms have been shown to be able to metabolize DBT to CO2 and biomass with the destruction of carbon- carbon bonds, but this reduces the fuel value. The mechanism for removal of the sulfur atom from DBT by IGTS8 is the most extensively studied example of microbial mediated thiophene ring cleavage. To a large part, Energy Biosystems Corporation (EBC) also concentrated in their studies on *Rhodococcus* IGTS8 to develop a commercial scale for biodesulfurization process (Monticello, 1994).The Strain IGTS8 (ATCC53968) is able to

extract sulfur from a variety of Organosulfoxides and sulfones (Kayser *et al.*,1993). Further more, the organism is able to remove organic sulfur from petroleum and soluble coal- derived materials without decreasing the caloric value of those substrates (Kilbane and Jackwski, 1992).

The metabolism of *Rhodococcus* IGTS8 is under aerobic conditions requiring oxygen with the pH of about 6 to 7, and a temperature of about 28$^{o}$C to 32˚C. The higher temperature rang result in faster metabolism, however, the microorganisms are known to not tolerate temperatures in the order of 37 $^{o}$C (Kilbane, 1992).

Wang *et al.,(*1996) Studied the kinetics in batch and fed-batch cultures of *Rh erythropolis* N1-36. In flask cultures, production of the desulfurization product, monohydroxybipheniel (OH-BP), was maximal at pH 6.0. Batch and glucose –fed –batch cultures of N1-36 having either DBT or $DBTO_2$ as the sole sulfur source where used to establish the kinetics of both growth and desulfurization.

The specific exponential growth rate (**μ)** of batch cultures with 100 μM DBT was 0.153 $hr^{-1}$ , while the corresponding rate with 100 μM $DBTO_2$ as the sulfur source was 0.180 $hr^{-1}$. Stationary phase typically occurred after approximately 40 hr of growth.

The standard conditions for kinetic analysis in bioreactors were as follows: temperature, 30 °C, agitation speed 300 rpm, and aeration 1.5 vol./vol./min, the pH was initially 6.0.

Folsom *et al.* (1999) used *Rh. erythropolis* I-19 to desulfurize alkylated dibenzo -thiophenes (Cx-DBTs) found in a hydrodesulfurized middle- distillate petroleum fraction 1850 ppm of total sulfur (MD 1850) in batch culutre. He found that the initial desulfurization rates of DBT and MD 1850 by I-19 were 5.0 and 2.5 μmol g $DCW^{-1}$ $min^{-1}$.

Extensive BDS resulted in a 67% reduction of total sulfur 1850 to 615 ppm S. Shifting zero to first-order desulfurization kinetics were observed when MD-1850 was diluted with hexadecane. Apparent saturation rate constant and half- saturation rate constant values were calculated to be 2.8 μmol g $DCW^{-1}$ $min^{-1}$ and 130 ppm, respectively. The water to oil ratio (w/o) used (3:1), pH 7.5, agitation 1000 rpm, temperature 30 °C, and dissolved oxygen (40%) of air saturation.

Setti *et al.,* (1999) studied the DBT biodesulfurization activity using *Rhodococcus rhodochrous* IGTS8 in batch conditions. Growth of Rh. IGTS8 on 1% (w/v) glucose and 200 ppm DBT as the sole carbon and sulfur sources respectively was monitored with a turbidimeter and the values reported as turbidity units, the stationary phase was reached after 150 hr and the lag phase was close to 48 hr.

The specific growth rate (**μ**) and product formation coefficient of the strain were found close to 0.061 $h^{-1}$ and 2.65 g HBP produced / g dry biomass respectively. Also, the maximum specific rate of HBP production of the strain was close to 0.7 g HBP produced /g of dry biomass $^{-1}$

$h^{-1}$. The conditions for kinetic analysis were as follows: temperature 30 °C, agitation speed 200 rpm, and pH 6.5.

Biodesulfurization of two different crude oils (Sand Flat and Van Texas) with total sulfur contents between 1 and 2% (wt/wt) was demonstrated in 1- lit batch stirred reactors using wild type *Rhodococcus* sp. IGTS8. Analysis of the crudes oil reveal significant (43-99%) desulfurization of DBT and substituted DBTs. The total sulfur content of the van Texas oil was reduced from 3.8 to 3.2% in 6 days of treatment with IGTS8. The rate of conversion of DBT species measured after a

period of 48 h, was 10.18 mg total DBT $gDCW^{-1}$ for the sand flat crude oil and 9.46 mg total DBTs $gDCW^{-1}$ for the van Texas crude.

The reactor was kept at pH 7.5, 30 °C, 800 rpm and aerated with room air at a rate of 0.2 standard lit $min^{-1}$ (Kaufman *et al.*,1999).Two types of diesel oils, middle distillate unit feed (MDUF) and light gas oil (LGO) were treated with resting cells of *Gordona* strain CYKS1 for 12 hr in batch culure. The total sulfur content significantly decreased, from 0.15% (wt/wt) to 0.06% (wt/wt) for MDUF and from 0.3% (wt/wt) to 0.25% (wt/wt) for LGO.The specific desulfurization rates of MDUF and LGO were 5.3 and 4.7 μmol of S.g $DCW^{-1}$, respectively.

The specific rates of desulfurization of DBT and DBTO2 by the resting cells of *Gordona* strain were 8.9 and 17.9 μmol of S. g $DCW^{-1}$. $h^{-1}$, respectively.The *Gordona* strain showed maximum growth (2.7 g $DCW/lit^{-1}$) at 100 hr of cultivation. When pH decreased from 7.4 to 5.9 desulfurization activity and cell growth decreased about 23% (Rhee *et al.,* 1998).

## 3. Basic Growth Kinetics

Considering growth of microorganisms in a batch culture, it may be assumed that the growth rate is proportional to cell concentration (X).

$$\frac{dx}{dt} = \mu x \qquad \textbf{(1)}$$

where the proportionality constant ( is known as the specific growth rate ( $h^{-1}$). Rearranging and integrating equation (1)

$$\int_{xo}^{x} \frac{dx}{x} = \int_{o}^{t} \mu \, dt \qquad \textbf{(2)}$$

at time t=0, X= $X_o$ then

$$\ln \frac{x}{x_\circ} = \mu t \qquad \textbf{(3)}$$

If the growth rate can be described by equation (3) then the microorganism is said to be growing in the exponential phase. In this phase if ln $X/X_o$ is plotted versus time, a straight line would result with a slope equals to μ (Wang et al., 1979). Plotting experimental growth data in the form of natural logarithm of cell concentration versus time will often yield a straight line over a large portion of the curve, as shown in fig.(2). In the range from $t_1$ to $t_2$ the curve is linear, and this is the region of exponential growth.

Three other regions can be identified between $t_o$ and $t_1$, there exists a period of cell adaptation or lag phase and beyond $t_2$ there is a region where the growth is limited by the lack of a particular substance, the number of new cells is equal to the number of dying cells, thus a stationary phase till $t_3$. In many cases cells stop multiplying and die after $t_3$ because of nutrient's depletion, toxic effects or cell aging.

Substrate consumption rate may be assumed to be proportional to the amount of substrate available according to equation (4).

$$-r = \frac{dS}{dt} = -kS \qquad \textbf{(4)}$$

or

$$\frac{dS}{S} = -kdt \qquad \textbf{(5)}$$

Integrating from time, t = 0 to time t, then;

$$\ln \frac{S}{S_\circ} = -kt \qquad \textbf{(6)}$$

Where for a constant volume reactor, k, is the first order velocity constant ($h^{-1}$). A plot of ln $S/S_o$ versus time would yield a straight line if first order kinetics holds.

## 4.Materials and methods

### 4.1. Microorganism

*Rhodococcus erythropolis* IGTS8 (ATCC 53968) was obtained from the American Treasury Culture Collection.

### 4.2. Media

#### 4.2.1.Basal Slat medium (BSM)

A defined BSM consisted of:2.44 g of KH2PO4, 5.57 g of Na2HPO4, 2.0 g of NH4CL, 0.2 g of MgCl2.6H2O, 0.001g of CaCl2.6H2O, 0.001 g of FeCl3.6H2O, 0.004 g of MnCl2.4H2O, 6.4 ml of Glycerol. Completed by distilled deionised water to one litre. Heat was used to completely dissolve the salts and to produce a homogenous solution. pH of medium was adjusted to7.0. by NaOH if necessary. (Piddington *et al.,* 1995) and (Patel *et al.,* 1997).

BSM was used to assay bacteria for their ability to desulfurize the sulfur compound.

### 4.2.2.Luria Broth Medium (LB)

A defined LB medium consisted of 1% Bactotryoptone , 0.5% yeast extract and 1% NaCl. were dissolved in 1 liter of distilled deionized water (Denome, *et al*(1994)):

## 4.3. Chemicals

### 4.3.1.Sulfur Compound

- Dibazothiphene (DBT)

### 4.3.2.Petroleum Fractions; (supplied by Cairo Oil Refining Co.)

-Gasoline (two types of gasoline were used; hydrodesufurized and untreated gasoline)
-Solar (the solar used was not subjected to HDS treatment)

### 4.3.3. Other Chemicals

- Ethyl alcohol (95% purity): used for dissolving DBT.
- Ethyl acetate: used for extraction of sulfur from the culture media.
- Concentrate HCL: used to acidify the culture before extraction.
- NaOH: used to adjust the pH of the mixture.
- Paraffin Oil (PO): used for dissolving DBT.

## 4.4. Equipment

- pH meter model 3071.
- Shaker (with Temperature control)
- Autoclave
- Mixer
- Centrifuge
- Oven
- Heater
- Separation funnel
- Spectrophotometer (UNICAM.5625 UV/VIS, England)
- High Performance Liquid Chromatography (Model Waters 600E equipped with UV Waters 2487 dual wavelength detector)
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## 4.5. Exprimental Procedure

A set of experiments have been done to follow up the growth of Rhodococcus IGTS8 on sulfur containing compounds and to monitor the depletion of DBT in the culture mixture.

### 4.5.1. Preparation of Cell Culture.

The procedure followed here is adopted from (Denome et al., 1994).

- *Rh. erythropolis* IGTS8 iwas heavily inoculated in LB and incubated for 24 hours at 130 rpm and 30 $^{o}$C.
- Cells were pelted by centrifugation at 5000 rpm for 15 minutes.
- The supernatant was removed and the pellets were washed three times in sterilized BSM and resuspended in sterilized fresh BSM.

- Washed cells were used to inoculate sterilized fresh BSM containing the test substrate (sulfur compounds) under sterile conditions (the inoculation size was 5% v/v).
- The cells that have been incubated in LB medium were used in DBT experiments.
- In case of gasoline and solar experiments the LB inoculated cells were activated by suspending the washed cells in BSM containing (0.1%v/v DBT) and incubated overnight at 130 rpm and 30 $^{o}$C, then it was ready for inoculation (inoculation size was 5% v/v).

### 4.5.2. Growth experiments

1- DBT dissolved in paraffin oil and in ethyl alcohol was supplied to BSM at concentrations of (1, 3, 6, 8 $gl^{-1}$) and (1, 3, 6$gl^{-1}$) respectively. Two replicates were used for each concentration and for each sulfur compound.

2- BSM containing the sulfur compounds were sterilized in autoclave for 15 minutes and left to cool.

3- Cold BSM containing sulfur compound was inoculated by (5%v/v) cells that were previously prepared.

4- Non- inoculated BSM was used as blank

5- The culture was incubated at 30$^{o}$C for (4-7) days in a shaking bath incubator at 200 rpm. The above steps were based on studies carried out by (Demone *at al*., 1994).

6- Growth was followed up by withdrawing samples from the cell suspension at the prescribed time then analyzed by a spectrophotometer (UNICM.5625 UV/VIS, England) by measuring the optical density (OD) at 600 □m.

7- pH of the culture was monitored by pH meter model (3071).

8- In case of gasoline and gas oil, oil to water ratio was 1:3

### 4.5.3. Following up sulfur removal

1. The culture that was prepared and incubated was acidified by 0.1N HCL to stop the biological reaction and then extracted with 60 ml of ethyl acetate three times. This is just for the case of DBT in ethyl alcohol..
2. In case of DBT in paraffin oil there were two phases and could be easily separated.
3. HPLC Model Waters 600E equipped with a UV Waters 2487 dual wavelength detector (Set at 254nm) was used for following up the depletion of DBT during the BDS process, using reversed phase Novapak C18 4 meum, 3.9X150nm column. as described by (Denome *et al.*,1994).
4. The concentration of DBT remaining in culture at any time relative to initial DBT concentration ($S/S_o$) is proportional to peak area of DBT at that time relative to the peak area at zero time ($A/A_o$) a sample curve of HPLC is shown in fig.(1)**.**

## 5- Results and Discussions

### 5.1.Growth of *Rh. Erythropolis* IGTS8 on DBT

Dibenzothiophane and its derivatives are known to be the most recalcitrant sulfur compounds found in crude oil and petroleum fractions. That is why this compound has been repeatedly been chosen by researchers asa model sulfur compound to investigate the effectiveness of

sulfur removal methods. In this work growth of *Rh.erthropolis* on DBT as well as its consumption were investigated.

At room temperature DBT is a solid substance in the form of needle crystals that are insoluble in aqueous media but are soluble in paraffin oil and ethyl alcohol.

These two solvents were used for solubilization of DBT. DBT dissolved in alcohol separates as powder as soon as it is mixed with BSM. While on the other hand, it stays in the oil phase in case of using paraffin oil. Follow up of optical density as a measure of growth when using ethyl alcohol was not possible and erratic results were obtained because the separated DBT in the form of powder interfered with the microorganism cells. But the follow up of the extent of desulfurization using HPLC analysis was possible as will be described here below.

However, in the case of using paraffin oil as a solvent, it was possible to follow up growth using optical density measurement. Data for growth on DBT of concentrations (1, 3, 6 and 8 $gl^{-1}$) are shown in Figures (3 a, b, c, d). Specific growth rates calculated from the plot of ln $X/X_o$ versus time according to equation (3) and using initial growth rates were (0.044, 0.026, 0.025 and 0.02 $h^{-1}$) for concentrations of (1, 3, 6 and 8 $gl^{-1}$) respectively.

This shows a decrease in specific growth rate with increasing substrate concentration as shown in fig.(4) which shows that growth is substrate inhibited. Setti et al.(1999) reported a specific growth value of 0.061$h^{-1}$ for Rh. IGTS8 grown on 200ppm DBT in a batch culture and Wang et al.(1996) reported a value of 0.153 $h^{-1}$ for cultures containing DBT concentration of 100 μ Mole $l^{-1}$. These findings are in line with the present results as DBT concentrations utilized in the present work are much higher than those of Setti et al.(1999) and Wang et al.(1996). To the

authors' knowledge no data on the effect of DBT concentration on growth of *Rh.erythropolis* has been published.

## 5.2. DBT Utilization Kinetics By *Rhodococcus Erythropolis* IGTS8

The depletion of DBT from the culture media with time as monitored by HPLC analysis and expressed as ln $S/S_o$ versus time is shown in figures (5 a, b, c and 6 a, b, c, d ) for DBT dissolved in ethyl alcohol and in paraffin oil respectively. The logarithmic relative concentrations show linear relationships with time suggesting first order substrate utilization kinetics.

Using Rh. erythropolis I-19 strain, Folsom et al. (1999) observed first order desulfurisation kinetics for a middle distillate petroleum fraction diluted with hexadecane. For DBT dissolved in ethyl alcohol the slope of the lines (k) decreases with increasing substrate concentration. The best fitted slopes are (0.0194, 0.0134 and 0.0047 ) for initial substrate concentrations of (1, 3 and 8 $gl^{-1}$) respectively. Showing a clear suppression effect due to increasing DBT concentration in the culture media.

In case of DBT dissolved in paraffin oil, the obtained slopes were (0.0043, 0.0131,0.0080 and 0.0071) for DBT concentrations of (1, 3, 6 and 8$gl^{-1}$) respectively. The reaction rate velocity constant (k) increased with increasing substrate concentrations from (1 to 3 $gl^{-1}$) then decreased with further increase in substrate concentration.

Here again suppression of reaction rate due to increasing substrate concentration is manifested, but, after an initial enhancement of the reaction rate due to the increase of DBT concentration from (1 to 3 $gl^{-1}$). This can be due to the hydrophilic nature of the microorganism (it stays

in the aqueous phase ) and the hydrophobic nature of the solute ( it is contained in the oil droplets), the reaction takes place at the oil water interface.

Increasing solute concentration beyond a certain limit enough for monolayer formation would not increase its bioavailability. This coupled with the inhibition effect of substrate, as it is clear from growth data, would lead to the observed behavior.

### 5.3. Growth of *Rh. Erythropolis* IGTS8 on Sulfur in Petroleum Fractions

Growth of DBT activated *Rh.erythropolis* was investigated on two types of petroleum fractions, namely gasoline and untreated gas oil. Two types of gasoline were investigated; untreated gasoline and hydrodesufurized gasoline.

The growth curves for both types of gasoline (Figs. 7, 8 ) are nearly identical. They are characterized by a lag period, which is slightly longer for the case of untreated gasoline followed by a sharp increase in relative cell concentration in the logarithmic phase then a slightly humped curve in the stationary phase.

The hump is more pronounced in the case of untreated gasoline, this may be because of the multiplicity of sulfur compounds in the untreated gasoline relative to that of the treated gasoline. It is surprising to find that the growth rate in both types is nearly identical,(0.068 and 0.067 $h^{-1}$) for the untreated and the hydrodesulfurized gasoline respectively, i.e. the total initial sulfur concentration had no effect on growth rate.

Growth of *Rh. erythropolis* on gas oil is shown in Fig.( 9 ). Here growth starts immediately after inoculation with no lag time.

It is known that sulfur compounds in this fraction are mainly DBT and its derivatives and since *Rh .erythropolis* was activated on DBT, then the necessary enzymes for metabolizing sulfur compounds in gas oil have already been synthesized in the cell body before inoculation. That is why there is no lag time. The calculated specific growth rate was $0.1h^{-1}$.

## 6.Conclusion

In this work growth kinetics of *Rh. erythropolis* bacteria has been studied on dibenzo- thiophene of different concentrations, ranging between 1 and $0.8gl^{-1}$. The specific growth rate was found to decrease with increasing substrate concentration. The utilization of DBT was found to follow first order kinetics with the reaction rate velocity constant also deceasing with increasing DBT initial concentration.

These findings on growth rate and substrate utilization rate are in line with published data. They both indicate substrate inhibition with increasing DBT concentration. Growth kinetics of the same microorganism on hydrodesulfurized and untreated gasoline gave nearly same specific growth rate of 0.067 and 0.068 $h^{-1}$ respectively. The specific growth rate on gas oil was slightly higher ($0.1h^{-1}$). It was interesting to notice that the specific growth rates are of the same order for both petroleum fractions and the model sulfur compound.

## 7-References

Denome, S. A., Stanley, D. C., Olson, E. S. and Young, K. D. (1993),"Metabolism of Dibenzothiophene and naphthaline in

pseudomonas strain: Complete DNA sequence of upper naphthalene catabolic pathway", Journal of Biotechnology,176: 2158-2164.

Finnerty,W. R. (1993),"Organic sulfur biodesulfurization in non-aqueous media".Fuel,72(12):1631-1634.

Folsom, B. R., Schieche, D. R.,Digrazia,P. M.,Werner,J. and Palmer, S. (1999),"Microbial desulfurization of alkylated dibenzothiophenes from a a hydrodesulfurized middle distillate by RH- erythropolis I-19". Applied and Environmental Microbiology, 65(11): 4967-4972.

Kaufmann, E, N., Borole, A. P., Shong, R., J. L. and Juengest, C. (1999). Short communication sulfur specifity in the bench-scale biological desulfurization of crude oil by Rh.IGTS8". J.Chem. Technol. Biotechnol. 74;1000-1004.

Kayser, K. L., Bielaga, B. A. Jackowskyi,k. Odusan, O. and Kilbane, J.(1993),"Utilization of organosulfur compounds by axenic and mixed cultures of rh. rhodochorous IGTS8", 139: 3123-3129.

Kilbane, J. J. (1992),"Mutant microorganisms useful for cleavage of organic C-S bonds", U. S. Pat. 5,104,801.

Kilbane, J. J.and Jachowaski, K. (1992),"Biodesulfurization of water-soluble coal-derived material by Rh. rodochorous IGTS8", Biotech. Bioeng.40: 1107-1114.

Malik, K. A. (1978),” Microbial removal of organic sulfur from crude oil and the environment, some new prespective”, Process Biochem., 35: 10-12.

Montcello,D. J. (1993),” Biocatalytic desulfurization of petroleum and middle distillates”, Environmental Progress, 2:39-45.

Montcello,D. J. (1994),” Biocatalytic desulfurization, the biorefining of petroleum”, Hydrocarbon Processing,2: 39-45.

Petal, S. B., Kilbane, J. J. and Webster, D. A. (1997),”Biodesulfurization of dibenzothiophene in hydrophobic mediaby Rh. sp. Strain IGTS”, J. Chem Technol.,69:100-106.

Piddington, C. S., Kovcevich, B. R. and Rambosek, J. (1995), “Sequence and molecular chracterization of DNA region encoding the dibenzothiophene encoding operon of RH. sp. Strain IGTS8”, Applied and Environmental Microbiology, 61(2): 468-475.

Rhee, S. K., Chang J. H. Chang, Y. K. and chang, H. N. (1998),”Desulfurization of dibenzothiophene by a newly isolated Gordona strain CYKS1”, Applied and Environmental Microbiology, 64(6): 2327-2331.

Setti, L., Farinelli, P., Martino, S. D. Frassinetti, S., Larnzarini, G. and Pifferi,P. G. (1999),” Developments in destructive and non-destructive pathways for selective desulfurization in oil-biorefining processes”, Appl. Microbiol. Biotech. 52: 111-117.

Wang, D. C., Coony, C. L., Dounill, P., Humphrey, A. E. and Lilly, M. D. (1979),” Fermentation and enzyme technology”,Jhon Wiley& Sons,Inc., New York.

Wang, P. and Krawic, S. (1996),” Kinetic analysis of desulfurization of dibenzothiophene by Rh,erythropolis in batch and fed-batch cultures”, Applied and Environmental Microbiology, 62(5):1670-1675.

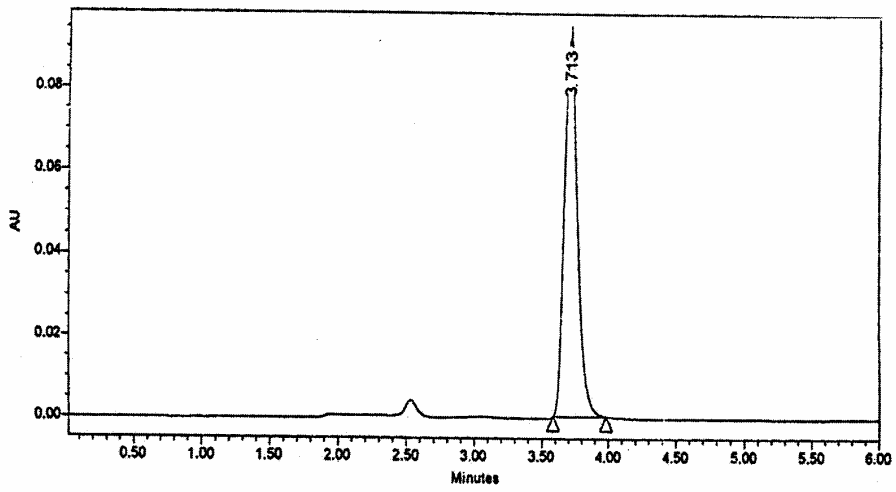


**Fig (1): Sample HPLC plot for determination of peak area of DBT remaining in culture media.**

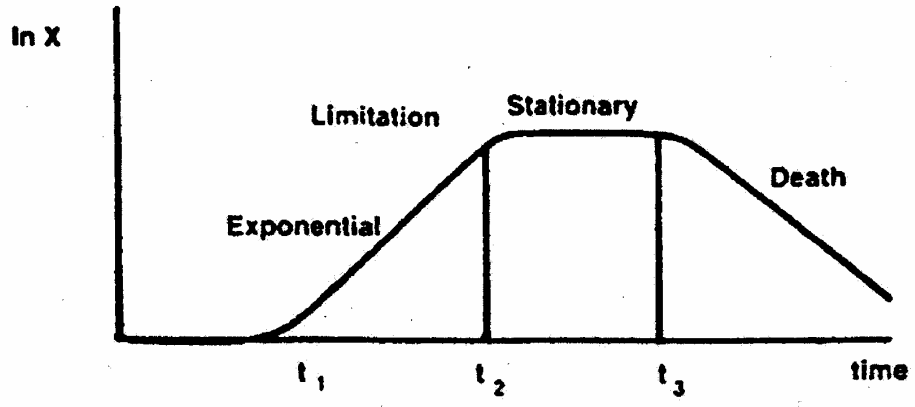


**Figure (2): Biomass concentration during batch growth.**

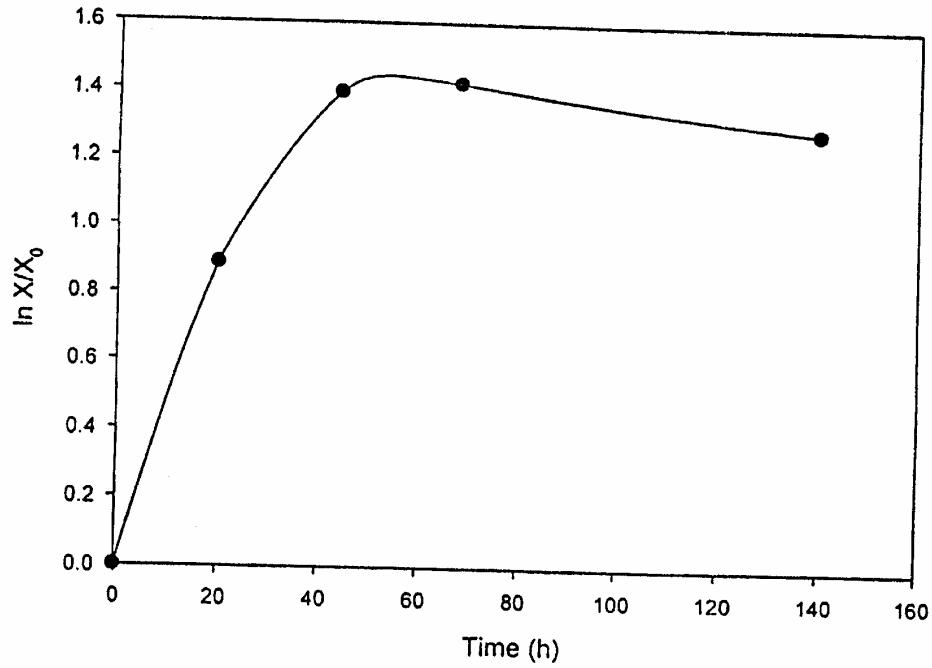


**Figure (3.a): Relative cell concentration vs time (h) for 1 g $l^{-1}$ DBT dissolved in paraffin oil**

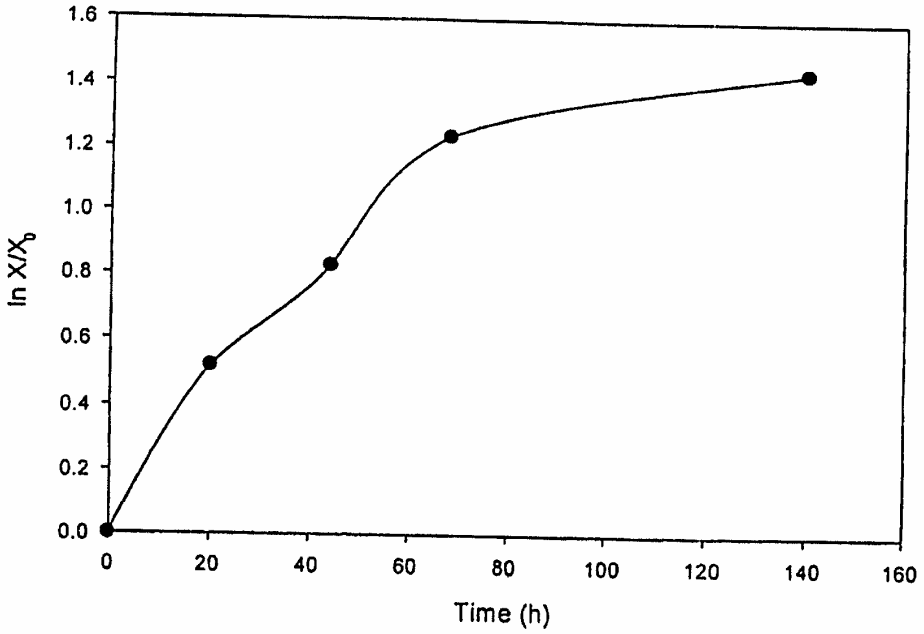


**Figure (3.b): Relative cell concentration vs time (h) for 3 g $l^{-1}$ DBT dissolved in paraffin oil**

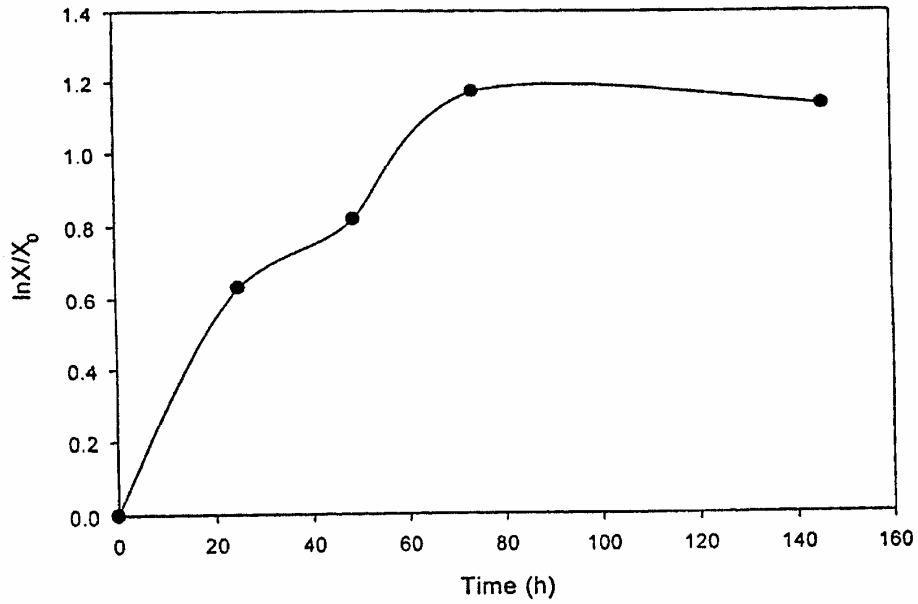


**Figure (3.c): Relative cell concentration vs time (h) for 6 g $l^{-1}$ DBT dissolved in paraffin oil**

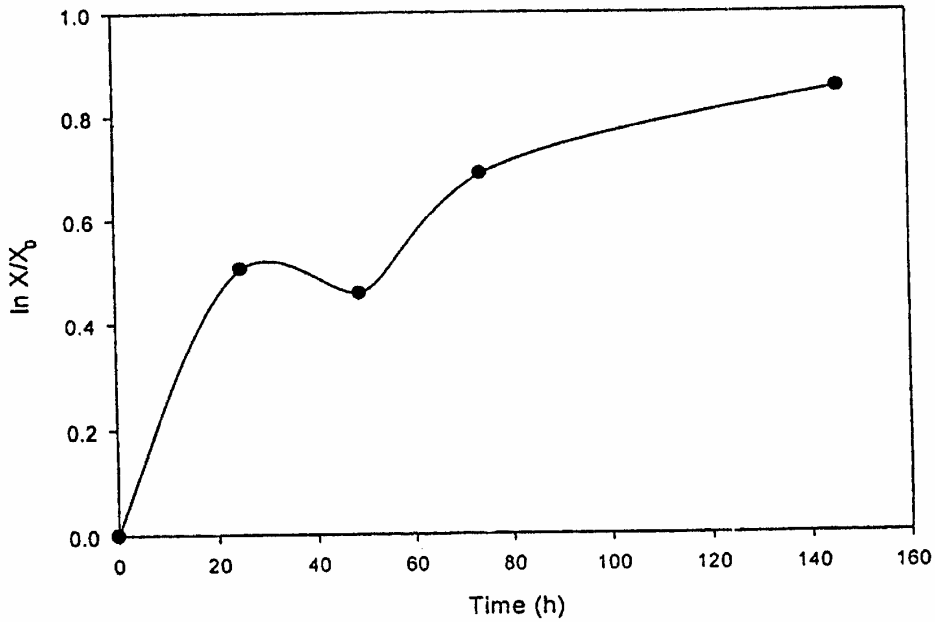


**Figure (3.d): Relative cell concentration vs time (h) for 8 g $l^{-1}$ DBT dissolved in paraffin oil**

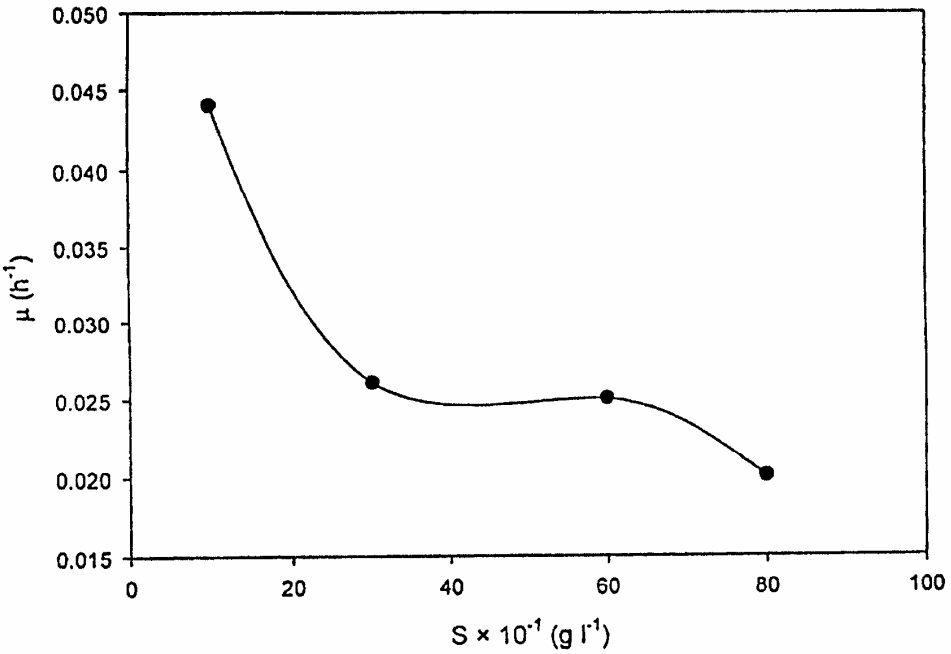


**Figure (4): Specific growth rate vs substrate concentration for DBT dissolved in paraffin oil.**

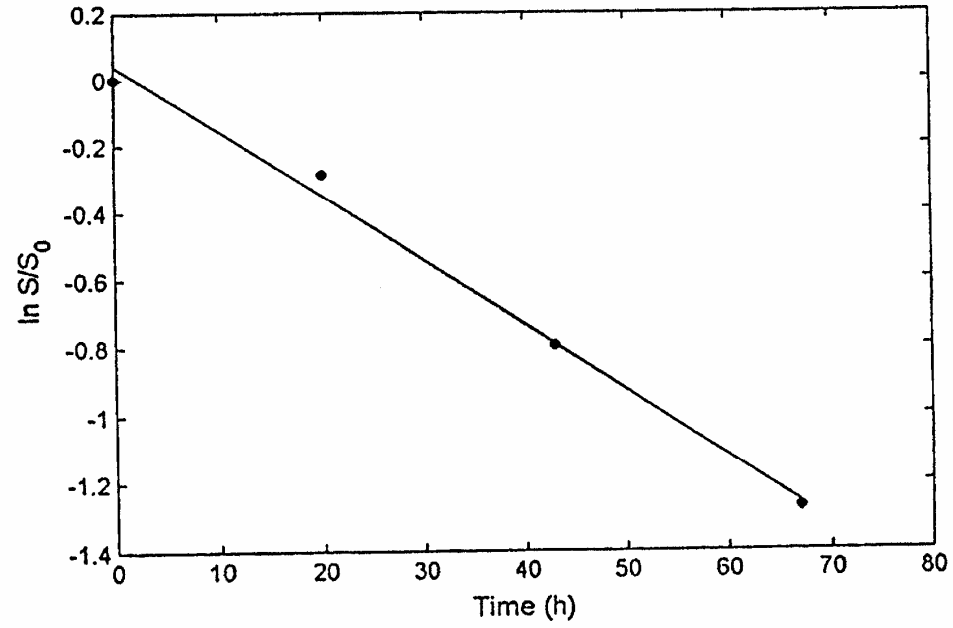


**Figure (5.a): Relative substrate concentration vs time (h) for 1 g $l^{-1}$ DBT dissolved in Ethyl Alcohol**

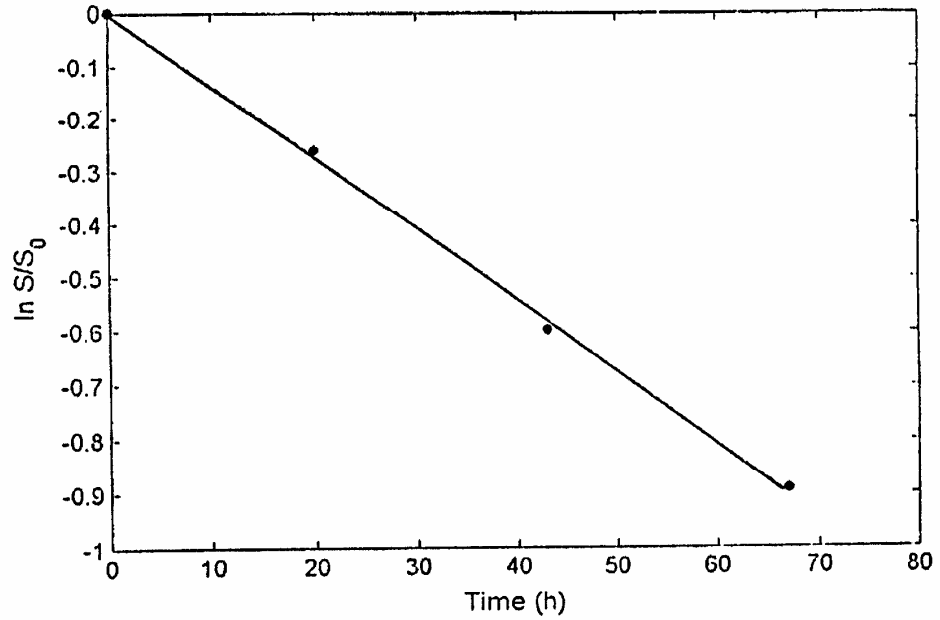


**Figure (5.b): Relative substrate concentration vs time (h) for 3 g $l^{-1}$ DBT dissolved in Ethyl Alcohol**

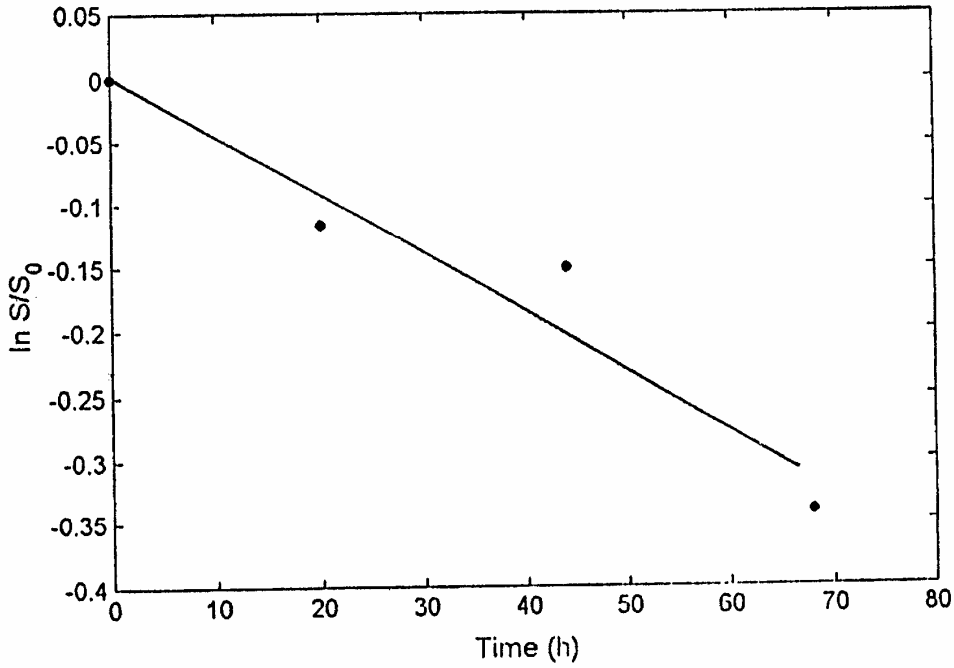


**Figure (5.c): Relative substrate concentration vs time (h) for 8 g $l^{-1}$ DBT dissolved in Ethyl Alcohol**

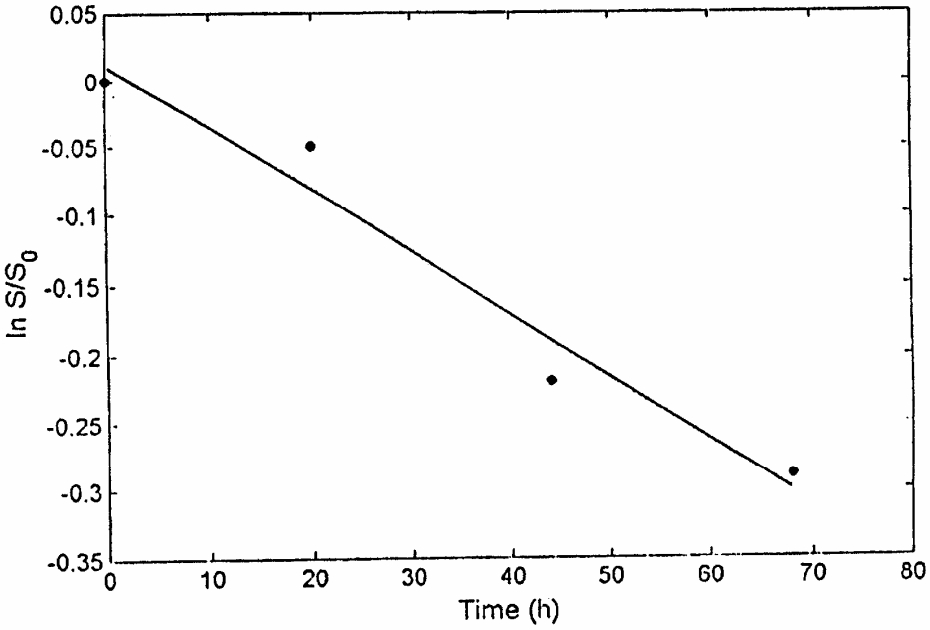


**Figure (6.a): Relative substrate concentration vs time (h) for 1 g $l^{-1}$ DBT dissolved in paraffin oil.**

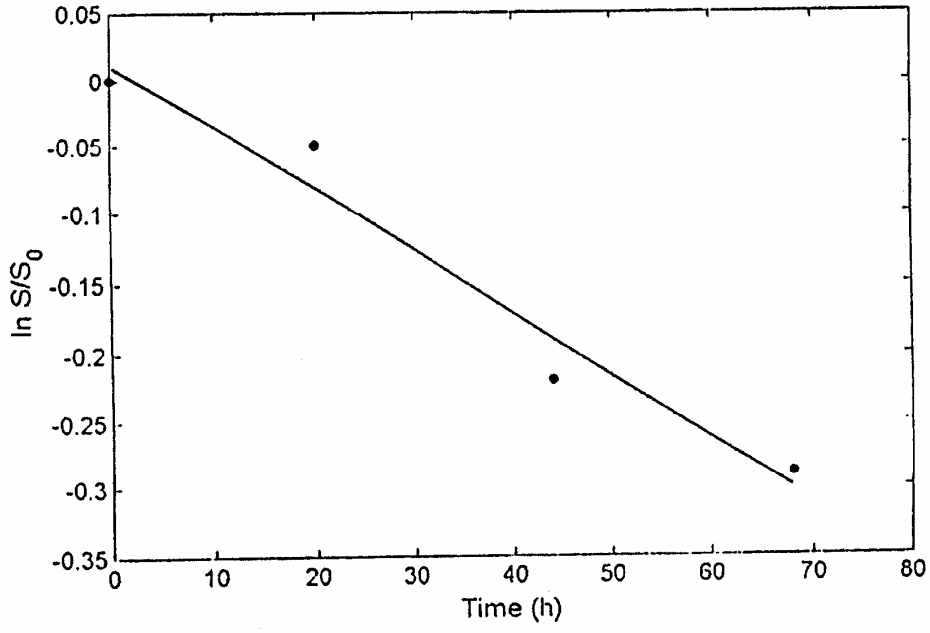


**Figure (6.b): Relative substrate concentration vs time (h) for 3 g $l^{-1}$ DBT dissolved in paraffin oil.**

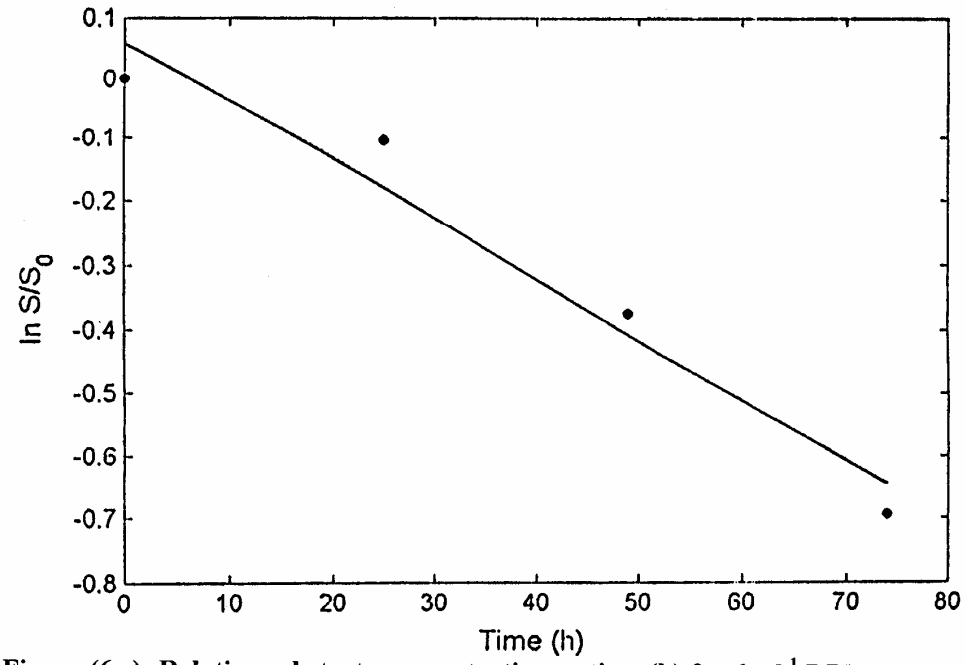


**Figure (6.c): Relative substrate concentration vs time (h) for 6 g $l^{-1}$ DBT dissolved in paraffin oil.**

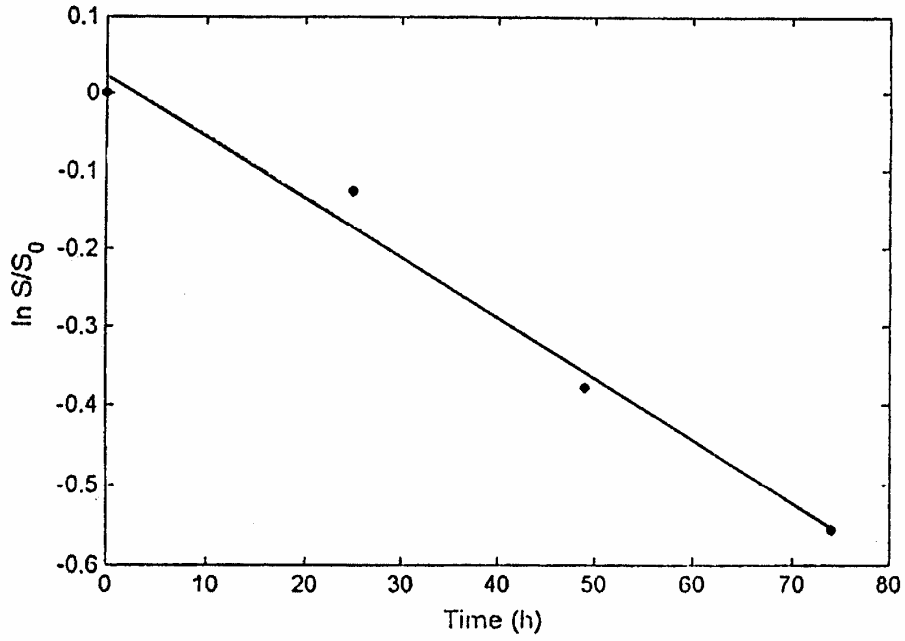


**Figure (6.d): Relative substrate concentration vs time (h) for 8 g $l^{-1}$ DBT dissolved in paraffin oil.**

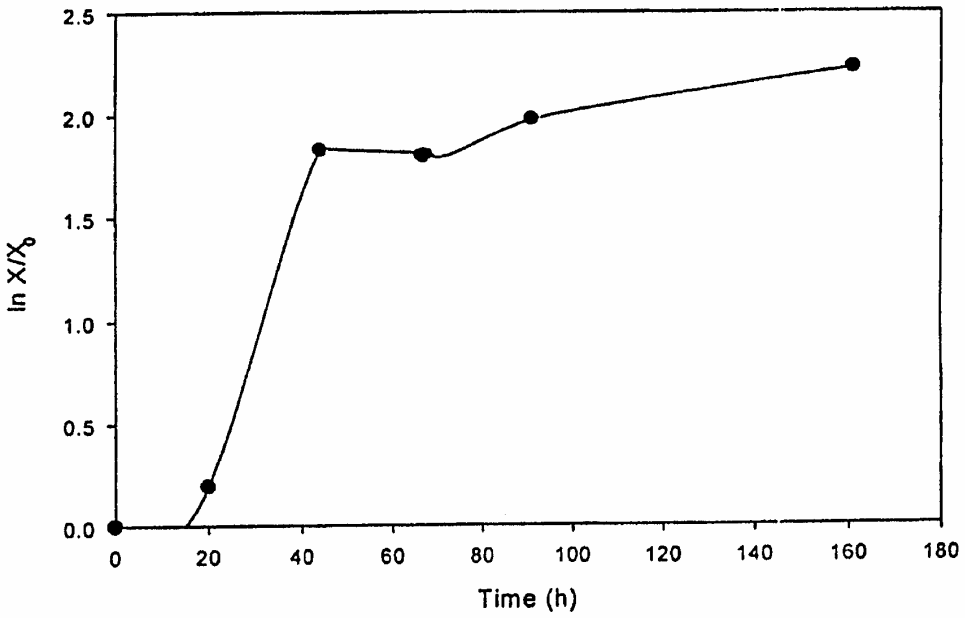


**Figure (7): Relative cell concentration vs time (h) for untreated gasoline .**

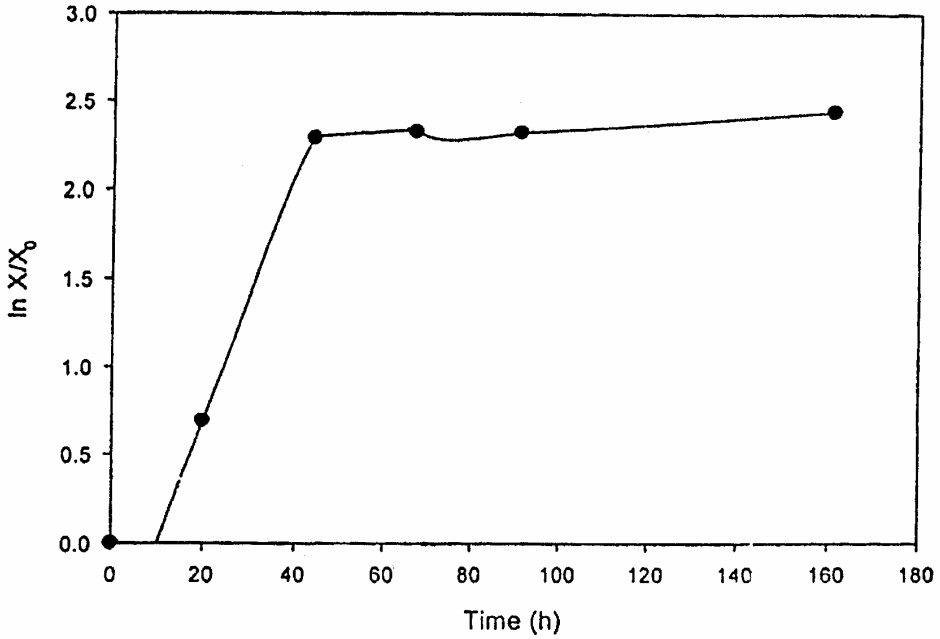


**Figure (8): Relative cell concentration vs time (h) for hydrodesulfurized gasoline**

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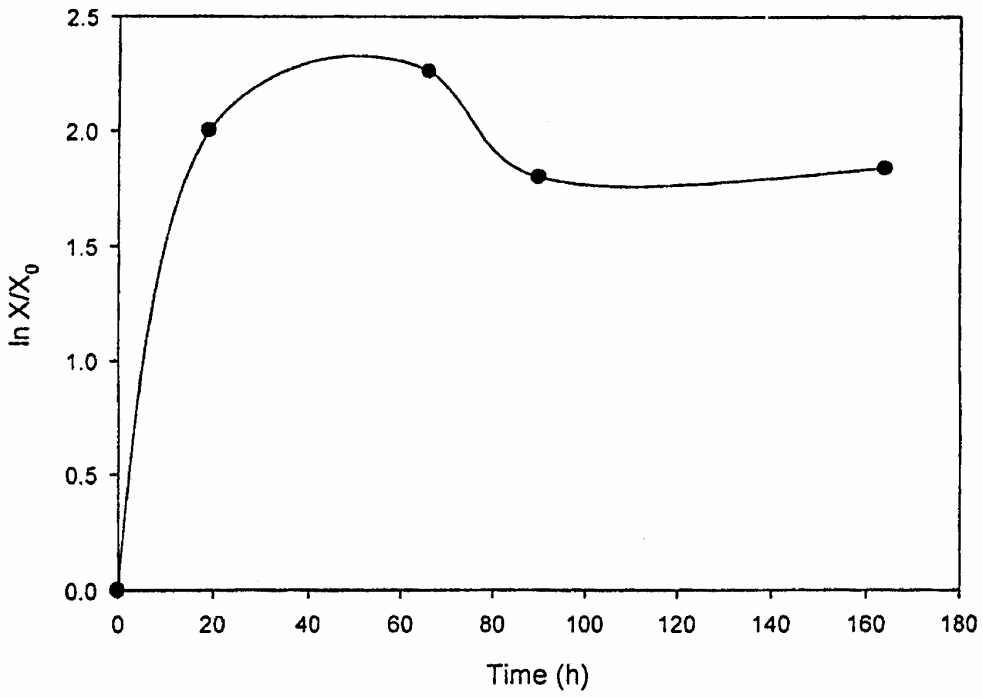


**Figure (9): Relative cell concentration vs time (h) for untreated gas oil .**