

Experimentation and Evaluation of Growth Kinetics Parameters for MEK Biodegradation

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Abstract

Biodegradation processes utilizes the microorganisms to biologically degrade Volatile Organic Compounds (VOCs) in waste air streams. In the present study, the acclimatized mixed culture is developed for the removal of methyl ethyl ketone (MEK). The biodegradation of MEK is studied for the initial concentration of MEK ranging from 200-700 mg/l. The maximum specific growth rate is 0.297 hr^{-1} which is achieved at 400 mg/l of initial concentration of MEK. The study incorporates the various kinetic models such as Monod's and Haldane's model available for biodegradation of MEK. The experimental data is well fitted with the Haldane's model ($R^2 = 0.87$) as compare to the Monod's model ($R^2 = 0.522$) as a result of self-inhibition which occurred because of excess substrate (MEK) concentration. The biodegradation rate follows the three half order kinetics and the kinetic parameters are calculated.

Keywords: Biodegradation; Mixed Culture; Inhibition; MEK; Specific Growth Rate.

1. Introduction

The advent of rapid industrialization has led to the release of pollutants in the atmosphere. The release of pollutants has contaminated air, groundwater and soil which led to the severe health problems. The exposure to VOCs can lead to various acute health problems such as headaches, nausea, dizziness, irritation of eyes, nose, throat, etc. The over exposure of some VOCs can also lead to chronic diseases such as cancer, damage to liver, kidney and central nervous system (Deshusses, 1994). MEK which is one such volatile organic compound is also regarded as a major pollutant released by various industries such as paint and coating industries, gas-oil, petrochemical and electronic industries. It is considered to be a highly toxic chemical (Mitchell, 1992). The annual production rate of MEK exceeds around 600,000 tonnes per year (Deshusses and Hamer, 1993). As MEK is used as a solvent in some of the industries, they are present in both concentrated and dilute waste gas streams. The concentrated streams are recovered and recycled because of the economical point of view while the dilute gaseous waste streams are subject to treatment because emissions of such compounds can lead to above mentioned health problems (Deshusses and Dunn, 1993). Hence, there is an immense

need of appropriate, efficient and cost effective treatment process for the removal of MEK from the dilute waste streams of above mentioned industries.

There are various techniques such as incineration, combustion, adsorption, absorption, ozonation, etc which are used in industries for tackling this problem (Rene et al., 2005; Deshusses et al., 1995; Lu et al., 1994; Babu and Raghuvanshi, 2004). These techniques suffer from additional fuel requirement, cost of big equipments and also suffer from the problem of disposal of secondary pollutants which get generated during the treatment process (Oh et al., 1994; Rene et al., 2005; Zarook and Baltzis, 1994). The disadvantages of conventional techniques mentioned above lead to the advent of cost effective and newer method and biofiltration is one such technique (Deshusses et al., 1995; Zarook et al., 1997; Babu and Raghuvanshi, 2006). It is the technique for the removal of VOCs by microbial degradation and is well established in the literature (Dechusses et al., 1996; Zarook et al., 1994). Geophen et al. (1997) studied the effect of unsteady state conditions of biooxidation of MEK and MIBK of liquid phase culture.

To understand the biofiltration process completely and to design the biofilter for industrial processes, a detailed kinetic study of biodegradation of VOCs is required. The kinetic data available in the literature is not sufficient to understand the kinetics of the biodegradation of MEK using mixed culture.

Hence the present study focuses on the detailed kinetic study for the biodegradation of MEK, which is one of the widely used solvent. It involves the development of acclimatized mixed culture for the biodegradation of MEK. The effect of initial concentration of MEK ranging from 200-700 mg/l is studied. The obtained experimental data is validated with the various kinetic models reported in the literature for biodegradation.

2. Materials and Methods

2.1. Materials

MEK used in the study is of analytical grade and is purchased from CDH Mumbai. The various nutrient media used is of analytical grade and is purchased from CDH and Merck, India.

2.2. Preparation of Media

The media, Mineral Salt Medium (MSM) is prepared which has the following composition (in g/l): K_2HPO_4 – 0.8, KH_2PO_4 – 0.2, $CaSO_4 \cdot 2H_2O$ – 0.05, $MgSO_4 \cdot 7H_2O$ – 0.5, $(NH_4)_2SO_4$ – 1.0, $FeSO_4$ – 0.01 in distilled water. 100 ml of MSM is taken in 250 ml Erlenmeyer flask and is autoclaved. Stock glucose solution is prepared by dissolving 10 g of glucose in 100 ml distilled water.

2.3. Microorganisms Culture

The microbial mixed culture is obtained from the Municipal Sewage Treatment Plant, BITS Pilani and acclimatized with MEK as the carbon source in a MSM. The sludge is kept for settling for almost 3-4 hours in cool place (away from sunlight). 10 gm

of settled sludge is taken and thoroughly mixed with 100 ml of distilled water. The shaking is carried out gently and then sludge is allowed to settle. 50 ml of supernatant is taken and centrifugation is carried out for 2 minutes at 10,000 rpm in the Centrifuge. The pellet which is achieved after the centrifugation is further used for the microbial growth and supernatant is discarded.

2.4. Immobilization Procedure

The immobilization is carried out in laminar hood chamber. The autoclaved MSM solution is added with 1 ml of stock glucose solution to obtain 1000 ppm of glucose concentration. Then 1 μ l of MEK is added to maintain 8 ppm concentration of MEK. After that, a loop full of sludge which is obtained after centrifugation is added. The solution is then kept in the rotary shaker at 37⁰ C for around 48 hours. After obtaining sufficient microbial culture, the glucose utilizing culture is acclimatized with MEK by slowly increasing MEK concentration and decreasing the concentration of glucose in the MSM. This is carried out by a series of transfers at 48 hour intervals for a period of more than 3 weeks to obtain a final well acclimatized mixed culture grown in MEK. The final enrichment culture showed extensive growth in MEK which prove that MEK is biodegradable compound as reported in the literature (Bridie et al., 1979; Price et al., 1974).

3. Biodegradation Study

The biodegradation of MEK is studied for a concentration range of 200-700 mg/l individually in 250 ml Erlenmeyer flasks. In these experiments, 100 ml of MSM is autoclaved and added with 5 ml of pre cultured suspension and fixed amount of MEK to maintain required concentration. Then it is kept in a rotary shaker at 150 rpm. The temperature is maintained 37⁰ C throughout the inoculation process. Flasks are sealed with stoppers to minimize VOCs loss. Samples are collected at regular intervals and are analyzed for biomass and residual MEK concentration. The optical density (OD) of the microbial culture is measured at 540 nm with respect to MSM using UV-VIS Spectrophotometer (Model 119- Systronics, India). Samples are centrifuged at 10000 rpm for 2 minutes to separate biomass and supernatant (aqueous MEK solution). Dry weight of biomass is obtained and concentrations of MEK in aqueous samples are measured using a gas chromatograph (Model 5700 series, Nucon Engineers, India). The temperature of injection port, detector and oven is maintained at 150⁰ C, 150⁰ C and 200⁰ C, respectively. Nitrogen is used as the carrier gas. Fig. 1 shows the calibration curve between optical density and biomass dry weight.

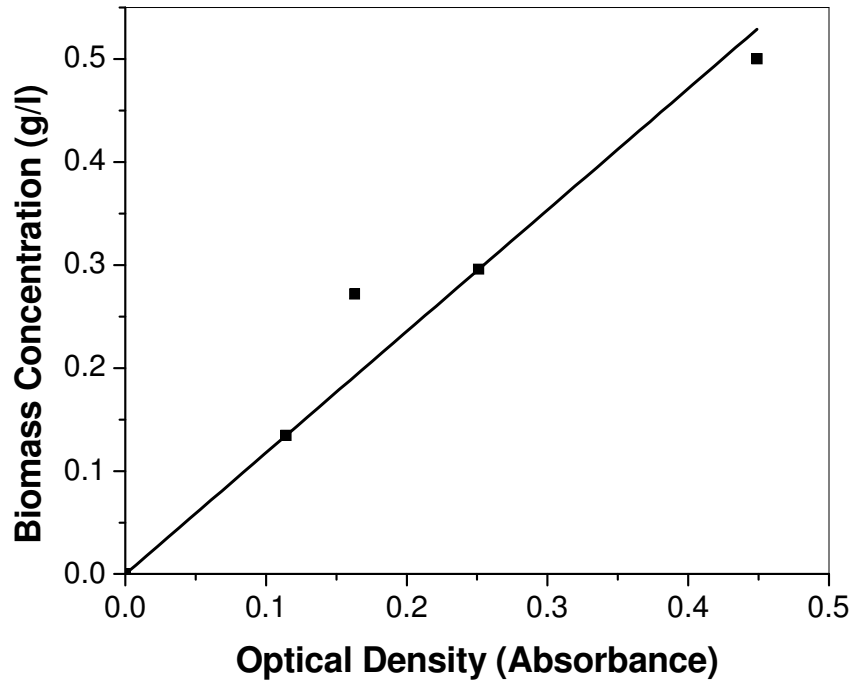


Fig. 1 Calibration Plot between biomass growth and optical density (absorbance)

4. Results and Discussion

In present study, biodegradation kinetics of MEK is being studied for the initial concentration range of 200-700 mg/l. The obtained results for biomass concentration and residual MEK concentrations are used to obtain biodegradation kinetics and rate kinetics.

4.1. Effect of Initial Concentration

The Fig 2 shows the change in MEK concentration with time. It is clear from the figure that the time taken by mixed culture to degrade MEK is dependent upon initial concentration. If initial concentration is increased from 200 to 700 mg/l of MEK, the time for degradation of MEK increases from 15 to 30 hours. The MEK concentration is decreasing with time which shows that MEK is being consumed by the microbes as they utilize MEK as a carbon source (Deviny et al., 1999).

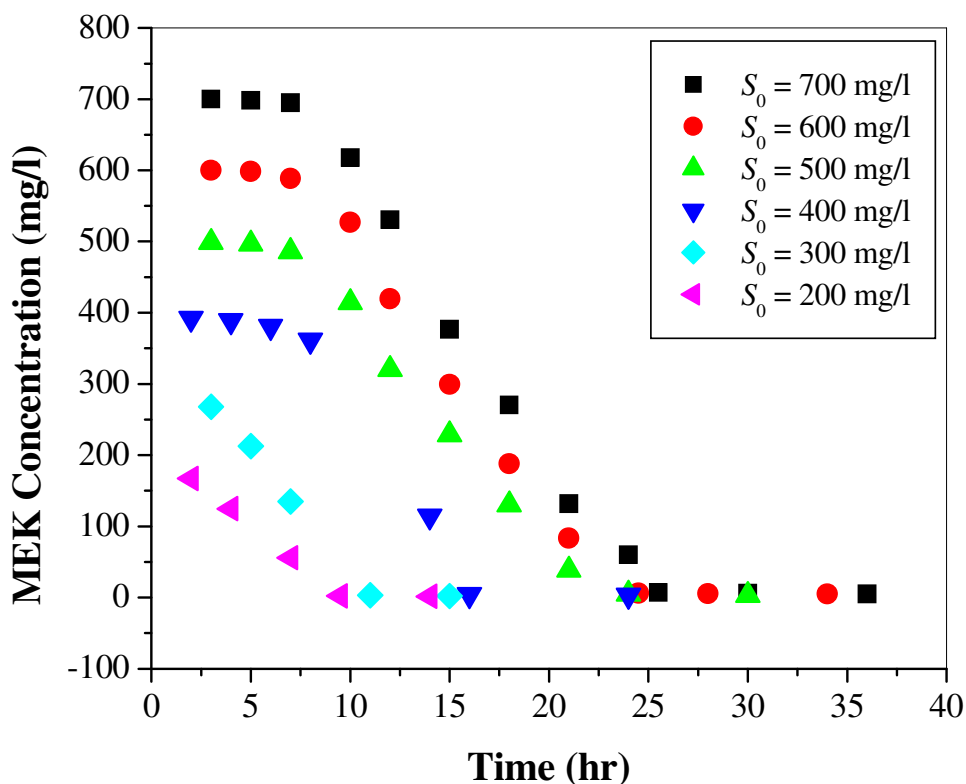


Fig. 2 Residual MEK concentration Vs time for different initial MEK concentration.

4.2. Biomass Dry weight Vs Time

Fig. 3 shows the change in biomass concentration with respect to time. Biomass concentration is increasing with time which shows that MEK is degraded by microbes. In Fig.3, growth curve for biomass can be categorized in phases namely, lag, log, stationary and death phase. Initially, there is no increment in the biomass concentration with time giving the lag phase. In log phase, the biomass concentration increases exponentially and after some time there is no increment in biomass concentration which indicates the stationary phase. An exponential decrease in biomass concentration is observed during death phase (Bailey and Olis, 1986). The maximum biomass concentrations are 0.3686, 0.451, 0.5, 0.475, 0.462 and 0.451 g/l for initial MEK concentrations 200, 300, 400, 500, 600 and 700 mg/l, respectively.

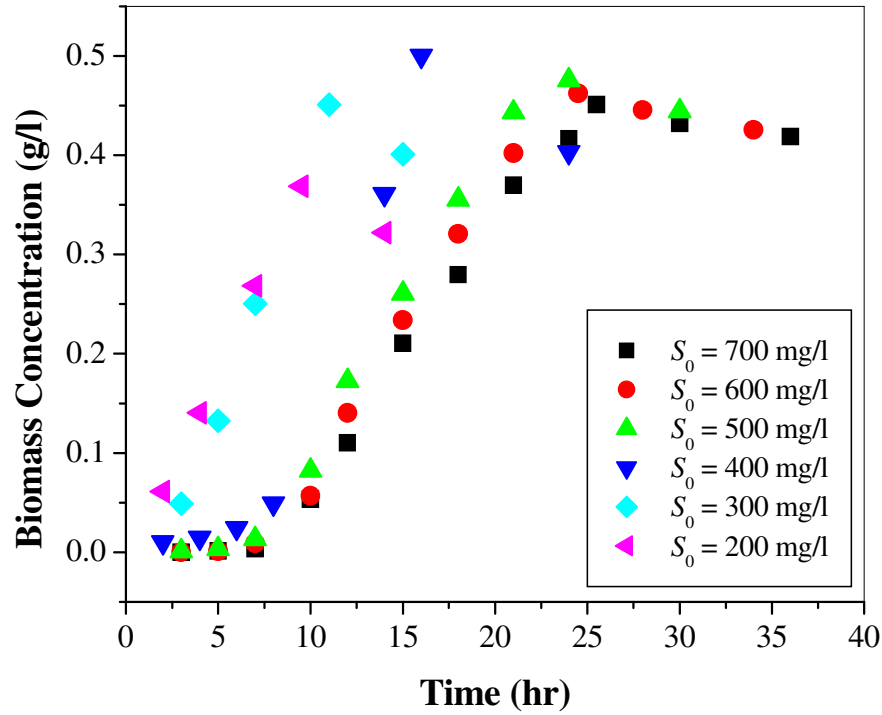


Fig. 3 Change in biomass Concentration with respect to time for different initial MEK concentration

4.3. Specific Growth rate

The obtained biomass concentration at various initial MEK concentration are used to calculate specific growth rate which is given by Eq 1.

$$\mu = \frac{1}{x} \frac{dS}{dt} \quad (1)$$

where, μ = specific growth rate (hr^{-1}), x = biomass concentration (g/l) at time t (hr), S = substrate (MEK) concentration (mg/l), t = time (hr).

The specific growth is determined by excluding the data for death phase. The plot between specific growth rates versus initial MEK concentration is given in Fig. 4. It is clearly observed from Fig. 4 that the specific growth rate increases from 0.230 to 0.297 hr^{-1} when initial MEK concentration increases from 200 to 400 mg/l. After 400 mg/l, specific growth rate starts to decrease. As initial MEK concentration is increased from 500 - 700 mg/l, the specific growth rate decreases from 0.286 to 0.265 hr^{-1} . The decrease in specific growth rate with increase in substrate (MEK) concentration is a result of self-inhibition which occurred because of excess substrate concentration (Saravanan et al., 2006; Deshusses, 1994). The obtained specific growth rate is found to be well with in the range as reported in the literature (Saravanan et al., 2006; Deshusses, 1994; Okpokwasili and Nweke, 2005; Banerjee et al., 2005).

The kinetic behavior which is obtained by carrying out above analysis can be modeled using various kinetic models such as Monod's, Haldane, Han-Levenspiel as available in the literature (Kovar and Egli, 1998; Okpokwasili and Nweke, 2005). The

significant biodegradation kinetic parameters such as maximum growth rate (μ_m), half saturation constant (K_s) and substrate inhibition constant (K_I) are calculated for different kinetic models (Kovar and Egli, 1998; Klecka and Maier, 1985; Simkins and Alexander, 1984). These important kinetic parameters of biodegradation kinetics are useful in the design of large scale biofilter.

Monod's Model

As can be seen from Fig. 4, the specific growth rate varies with the substrate concentration. The relationship between specific growth rate and substrate concentration was proposed by an empirical equation proposed by Monod in 1942.

$$\mu = \mu_m \frac{S}{K_s + S} \quad (2)$$

Since, it is difficult to measure μ_m , it is found by linearizing the Monod equation:

$$\frac{1}{\mu} = \frac{K_s}{\mu_m} \left(\frac{1}{S} \right) + \frac{1}{\mu_m} \quad (3)$$

By plotting ($1/\mu$) versus ($1/S$), Monod constants μ_m and K_s are obtained as given in Table-1. The value of correlation coefficient ($R^2 = 0.522$) showed that the present data does not confirm well to the Monod model. The Monod's model only describes the dependence of biodegradation rate on the biomass concentration (Okpokwasili and Nweke, 2005). When a substrate (MEK) biodegradation exhibits self inhibition, the Monod model fails. In such cases substrate inhibition is considered by incorporating the substrate inhibition constant in Monod's Model. Among the various substrate inhibition models, Haldane's model is widely used (Sokol, 1986; Tang and Fan, 1987).

Haldane's Model

Haldane model was originally proposed for substrate inhibition in 1968. According to Haldane model, the specific growth rate can be represented by eqn. (4).

$$\mu = \frac{\mu_{\max} S}{K_s + S + (S^2 / K_I)} \quad (4)$$

The model equation is non linear and it is solved by using non linear regression method using Origin-6. The obtained values of important parameters for Haldane model are given in Table-1. Fig. 4 shows the fit of Monod and Haldane models with the experimental results. The value of correlation coefficient ($R^2 = 0.87$) showed that the present data confirm well to the Haldane model as compared with the Monod model ($R^2 = 0.522$).

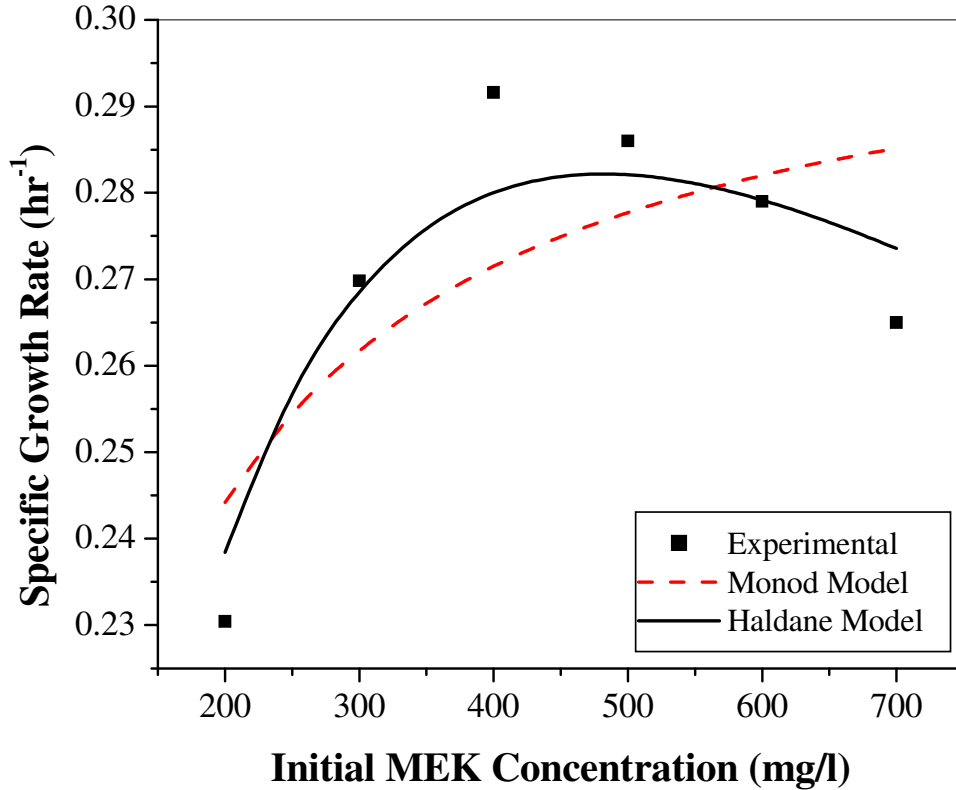


Fig. 4 Specific growth rate for different Initial MEK Concentration

4.4. Biodegradation Rates

In biodegradation processes, the substrate concentration which changes with time can be described by zero order, first order and second order rate kinetics. In biodegradation processes biomass is produced simultaneously as substrate concentration decreases (Brunner and Frocht, 1984). The first order and second order kinetics are not widely preferred as they suffer from the limitation that it does not take into account the biomass growth. Therefore for biodegradation processes generally three half order kinetic is used (Brunner and Frocht, 1984). The model is based on the assumption of first order model with the introduction of an additional term for explaining the biomass formation. The rate of MEK degradation is given as:

$$\frac{dS}{dt} = -k_1S - aES \quad (5)$$

Where k_1 is the proportionality constant per unit time, E is cell concentration and a is the proportionality constant per unit of biomass concentration per unit time. The final mathematical form of this equation is given as

$$Y = -k_1 - \frac{k_2t}{2} \quad (6)$$

where

$$k_2 = aE/t \quad (7)$$

$$Y = \frac{1}{t} (\ln(S_0 - P + k_0 t) / S_0) \quad (8)$$

$$P = S_0 (1 - e^{-k_1 t - (k_2 t^2)/2}) + k_0 t \quad (9)$$

k_0 and S_0 are zero order rate constant and substrate concentration at zero time, respectively. The equation contains four unknown parameters and is highly non linear. In this equation, s_0 and k_0 can be obtained by the zero order kinetics and k_1 and k_2 is found by plotting Y against t which gives a straight line. The intercept and slope of straight line is gives k_1 and k_2 respectively. The zero order and three half order kinetic constants are listed in the table below.

Table- 1 Monod`s and Haldane`s Kinetic Constants

Monod Kinetic Model			Haldane Kinetic Model			
Constants		Correlation Coefficient (R^2)	Constants			Correlation Coefficient (R^2)
μ_m (hr^{-1})	K_s (mg/l)		μ_m (hr^{-1})	K_s (mg/l)	K_I (mg/l)	
0.3052	50.377	0.5222	0.509	193.590	1198.506	0.875

Table-2 Parameters of three half order kinetic model at different initial MEK concentration

S No	Initial MEK Concentration (mg/l)	k_0	k_1	$k_2 \times 10^5$	R^2
1	200	14.630	0.01308	220.0	0.878
2	300	23.846	0.00606	90.12	0.922
3	400	22.227	0.00446	46.05	0.816
4	500	22.953	0.00255	20.78	0.854
5	600	24.445	0.00209	15.38	0.835
6	700	27.209	0.00179	12.55	0.830

5. Conclusions

The present study is focused on the growth and biodegradation kinetics of acclimatized mixed culture for MEK. It shows that the time required for utilizing MEK using mixed culture increases by increasing the initial concentration of MEK. Biodegradation of MEK occurred at all concentrations. The specific growth rate is found at all initial concentrations of MEK. The maximum specific growth rate is obtained at 400 mg/l of initial MEK concentration. Substrate inhibition occurred for more than 400 mg/l of MEK concentration. The substrate inhibition is explained using Haldane`s model. The kinetic parameters evaluated for Monod and Haldane kinetic models. Biodegradation rate kinetics is calculated using three half order kinetic model which is well suitable for biodegradation processes.

Nomenclature

a proportionality constant, l/g h

E	cell concentration, g/l
k_1	three half order rate constant
k_2	three half order rate constant
k_0	zero order rate constant
K_I	substrate inhibition constant, g/l
K_s	half saturation constant, g/l
S	substrate (MEK) concentration, mg/l
S_0	initial substrate concentration, mg/l
t	time, h
x	biomass concentration, g/l
Greek	
μ	specific growth rate, hr ⁻¹
μ_m	maximum specific growth rate, hr ⁻¹

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