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Biodegradation kinetics of methyl iso-butyl ketone by acclimated mixed culture

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Abstract Methyl iso-butyl ketone (MIBK) is a widely used volatile organic compound (VOC) which is highly toxic in nature and has significant adverse effects on human beings. The present study deals with the removal of MIBK using biodegradation by an acclimated mixed culture developed from activated sludge. The biodegradation of MIBK is studied for an initial MIBK concentration ranging from 200–700 mg l^{-1} in a batch mode of operation. The maximum specific growth rate achieved is 0.128 h^{-1} at 600 mg $l^{-1}$of initial MIBK concentration. The kinetic parameters are estimated using five growth kinetic models for biodegradation of organic compounds available in the literature. The experimental data found to fit well with the Luong model ($R^2 = 0.904$) as compared to Haldane model ($R^2 = 0.702$) and Edward model ($R^2 = 0.786$). The coefficient of determination (R^2) obtained for the other two models, Monod and Powell models are 0.497 and 0.533, respectively. The biodegradation rate found to follow the three-half-order kinetics and the resulting kinetic parameters are reported.

Keywords Biodegradation · Acclimated mixed culture · Biomass · Methyl iso-butyl ketone · Growth kinetics · Inhibition · Rate kinetics

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Introduction

The widespread use of methyl isobutyl ketone (MIBK) as a solvent for manufacturing paints, rubbers, and pharmaceuticals, has led to its detection in air and ground water as a significant pollutant as it is considered to be a high priority toxic chemical (Mitchell 1992). It is partially soluble in water. MIBK is a flammable liquid and concentrations as low as 0.1 g m^{-3} in air is found to have significant effect on both humans and animals as given by the World Health Organization in 1990 and 1993 (WHO 1990, 1993). Prolonged exposure to MIBK results in headache, dizziness, narcosis, nausea, numbness in the fingers and toes, unconsciousness, and even death. MIBK vapor irritates the eyes, nose, and throat, and its contact with the skin causes skin irritation as given by Occupational safety and health hazards (OSHA) of US Department of Labor (1989). It does not bind well to soil and thus pollutes the groundwater. It has acute (short-term) and chronic (long-term) toxicity to the aquatic life as well.

The various techniques available for the removal of MIBK are adsorption, absorption, condensation, incineration, etc. The disadvantages of these techniques are generation of contaminated solid waste, separation of MIBK from solvents, and emission of other off gases (NOx) which require additional cost for the secondary treatment (Deshusses et al. 1995; Babu and Raghuvanshi 2004; Rene et al. 2005). The application of biological methods is considered to be a promising alternative to these traditional technologies for the removal of VOCs (Deshusses et al. 1995; Rene et al. 2005; Raghuvanshi and Babu 2009). Biodegradation is one of the useful techniques for the removal of organic chemicals (MIBK). Biodegradation has many potential advantages over other physical-chemical treatment methods such as low energy use, lower capital investment, low operating and maintenance cost. Certain previous studies have included the aerobic degradation of MIBK.

Quesnel and Nakhla (2006) have studied the removal kinetics of acetone and MIBK in a pilot scale activated sludge system. The analysis carried by them is discussed in terms of mixed liquor total suspended solids (MLTSS), hydraulic retention time (HRT) and solids retention time (SRT). The results are based on the single substrate removal which follows the first order biodegradation rate (obtained as 2.23 (day^{-1}) for the MIBK removal. This study has not evaluated the biokinetic constants using different growth kinetic models. Deshusses (1994) studied the biodegradation of MEK and MIBK by suspended cultures. He has fitted the biodegradation growth kinetic data with different Monod and Haldane growth kinetic models. This study has not incorporated Luong and Edwards growth kinetic models which are considered as inhibition models. Hence, the kinetic data available in the literature for biodegradation of MIBK are not sufficient to understand the kinetics of mixed culture.

The present study is focused on the detailed kinetic study for the biodegradation of MIBK (aerobic degradation), which is one of the widely used chemical in industries. The work involved the development of acclimated mixed culture for the biodegradation of MIBK. The effect of initial concentration of MIBK on biodegradation and effect of time on biomass concentration ranging from 200–700 mg l^{-1} is also studied. The information collected from these experimental studies are then used for the calculation of growth kinetic constants and the rate kinetic constants from different models as reported in the literature for biodegradation of organic pollutants.

Materials and methods

Materials

All the materials used (MIBK and various nutrient media) were of analytical grade Merck (India) brand.

Preparation of media

The media, Minimal Salt Medium (MSM), prepared has the following composition (in g l^{-1}): 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 was taken in 250 ml Erlenmeyer flask and was autoclaved. The pH of MSM obtained after autoclaving was 6.7. Stock glucose solution was prepared by dissolving 10 g of glucose in 100 ml distilled water.

Microorganism culture conditions

The source for the sludge obtained for enrichment of culture was the activated sludge part of Sewage Treatment Plant of BITS Pilani. The sludge was mixed thoroughly with water. It was allowed to settle for 3 h at room temperature (away from sunlight) in order to separate the supernatant and the sludge. This first settling was carried out in order to remove the dissolved impurities from sludge and hence the time given for this settling was more. The supernatant which include the dissolved impurities was discarded off and sludge was retained including microbial culture. Ten grams of sludge obtained from first settling was taken and again thoroughly mixed with 100 ml of distilled water in a beaker. The shaking was carried out gently and then sludge was allowed to settle for short time (1 min) in order to screen out the stones or other particles. The second settling was carried out to collect microbial culture in the supernatant. Fifty milliliters of supernatant was then taken in a 50 ml centrifuge tube. The centrifugation was carried out for 2 min at 10,000 rpm at 4°C in a Centrifuge (Remi Cooling Centrifuge, India). After centrifugation for 2 min, a clear pellet was obtained.

The liquid was not readily poured off as doing so can mix up the pellet obtained and the liquid. The portion of the upper liquid was removed carefully from the top of the centrifuge tube without disturbing the pellet. Then the pellet was taken out with the help of loop and transferred in the 250 ml flask in an aseptic environment.

Enrichment procedure

The culture enrichment was carried out in the laminar hood chamber. A loop full of sludge obtained after centrifugation was added to 100 ml of MSM. The solution was then kept in rotary shaker at 37°C for 48 h. The growth of microbial culture was measured by optical density value in a UV–VIS Spectrophotometer (Model 119-Systronics, India) having the cuvett of path length 10 mm. The value of optical density obtained for microbial culture was 0.1 which was an indicative of sufficient microbial culture. The enrichment of culture was carried out for a period of 18 days by decreasing the amount of glucose from 1,000 to 0 mg l^{-1} with a decrement of 200 mg l^{-1} in every 3 days and increasing MIBK concentration from 0 to 60 µl (corresponds to 480 mg l^{-1}) with an increment of 20 µl (corresponds to 160 mg l^{-1}) after first 3 days and then 10 µl (corresponds to 80 mg l^{-1} of MIBK concentration) from 4th to 18th day. The final acclimated culture was obtained only with 480 l^{-1} of MIBK (no glucose) which was then used for the biodegradation study. The final acclimated culture showed extensive growth in MIBK which substantiates the fact that MIBK is biodegradable compound as reported in the literature (Bridie et al. 1979; Price et al. 1974).

Biodegradation study

The biodegradation of MIBK was studied for a concentration range of 200–700 mg l^{-1} with the values of 200, 300, 400, 500, 600 and 700 mg l^{-1} individually in 250 ml Erlenmeyer flasks. In these experiments, 100 ml of MSM was autoclaved and added with 5 ml of acclimated mixed culture obtained from the enrichment procedure and known amount of MIBK to maintain the required concentration. The amount of MIBK added was 25, 38, 50, 63, 75 and 88 µl to maintain 200, 300, 400, 500, 600 and 700 mg l^{-1} of MIBK concentration, respectively. Flasks were sealed with stoppers to minimize VOCs loss. Then they were kept in rotary shaker which was maintained at 37°C and at 150 rpm throughout the biodegradation process. The samples were collected at different intervals for different concentrations ranging from 200 to 700 l^{-1} based on visual observation (turbidity). The flasks were briefly opened for taking out the samples in the laminar hood chamber. It was done in a very less time in order to minimize the VOCs loss. The loss of opening vials was calculated by conducting the experiments using the blank samples of 500–700 $l^{-1}$of initial MIBK concentration for initial time intervals (first two openings). The loss of MIBK was 0.0042, 0.0051 and 0.00568% (vol) for 500, 600 and 700 mg $l^{-1}$of initial MIBK concentration while first time opening. It was decreased in the second opening and obtained as 0.0031, 0.0039 and 0.0041% for 500, 600 and 700 mg $l^{-1}$of initial MIBK concentration. The values obtained for percentage loss seems to be negligible. So in this study, loss of VOCs was neglected during collection of the sample and hence in the analysis of final MIBK concentration in the samples. The time was not before 4 h for any sample and final time was decided by observing the constant value of biomass concentration for two or three consecutive samples.

Analytical techniques

The optical density (OD) of the microbial culture was measured at 540 nm with respect to MSM using UV–VIS Spectrophotometer (Model 119-Systronics, India). The path length for the optical density measurements is 10 mm. The same samples were then centrifuged at 10,000 rpm for 2 min to separate biomass and supernatant (aqueous microbial culture solution) (Saravanan et al. 2008). Dry weight of biomass was obtained from a known volume of microbial culture. The calibration curve was prepared in terms of optical density value vs biomass concentration. The concentrations of MIBK in aqueous samples (supernatant) were measured using a gas chromatograph (Model 5700 series, Nucon Engineers, India). The temperatures of injection port, detector and oven were maintained at 150, 150 and 200°C, respectively. Nitrogen was used as the carrier gas. All the experiments and measurements were carried out twice and the arithmetic averages were taken for calculations and data analysis.

In the present study, the linear and nonlinear growth kinetic models were fitted by method of linear and nonlinear least squares using a professional graphics software package ORIGIN (version 6). It uses the linear and nonlinear curve fit tool available in this software. It has a flexibility to build the user defined mathematical equations which has different constant values and can be used to fit the experimental data. The results obtained are useful to obtain the constants used in various models with error estimation in terms of coefficient of determination.

Results and discussion

In the present study, biodegradation kinetics of MIBK was studied for the initial concentration range of 200–700 mg l^{-1} using mixed culture. The effect of time for MIBK degradation was obtained. The effect of various parameters on biodegradation is discussed below.

Effect of initial MIBK concentration

Figure 1 shows the time profile of MIBK biodegradation for concentration ranging from 200–700 mg l^{-1} using the acclimated culture. It was observed that the mixed culture degraded MIBK in 13, 17, 23, 25, 27 and 29 h for 200, 300, 400, 500, 600 and 700 l^{-1} of initial MIBK concentration. The enrichment conditions (160–480 mg l^{-1}) are in the same order of magnitude as the conditions for biodegradation study (200–700 mg l^{-1}) of MIBK. The MIBK concentration was found decreasing with time showing the consumption of MIBK by the microbes as they utilize it as a carbon source for their growth (Devinny et al. 1999). As the initial MIBK concentration increased, the lag time taken by culture also increased. Longer lag time corresponds to an increase in initial MIBK concentration as reported for mixed culture (Saravanan et al. 2008) and is attributed to substrate inhibition. This indicates that the initial MIBK concentration has a significant effect on its degradation rate.

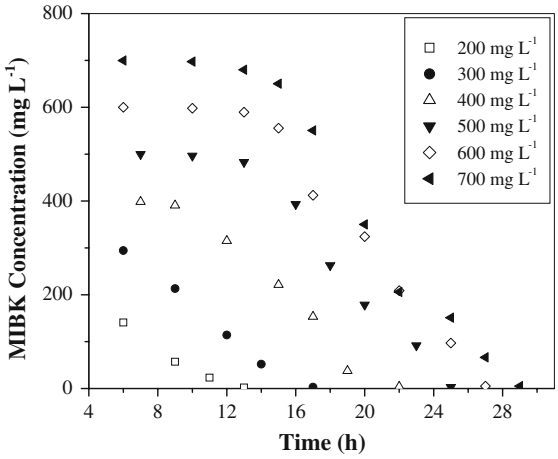

Fig. 1 Residual MIBK concentration vs time for different initial MIBK concentration

Effect of time on biomass concentration

Figure 2a shows the biomass concentration profile of the acclimated mixed culture at different times for initial MIBK concentration ranging from 200–700 mg l^{-1}. Biomass concentration was calculated using calibration curve (optical density vs. biomass concentration). Biomass concentration showed an exponential increase until complete depletion of MIBK took place. The maximum biomass concentrations were obtained as 0.172, 0.21, 0.257, 0.294, 0.286 and 0.28 l^{-1} for initial MIBK concentrations of 200, 300, 400, 500, 600 and 700 mg l^{-1}, respectively. The biomass concentration increased with increase in initial MIBK concentration from 200 to 500 mg l^{-1}. This indicated that MIBK was not showing any inhibitory effect on the microorganisms as shown by small lag phase (different phases are described in Fig. 2b for one specific concentration which is discussed below) during the growth. The obtained values of biomass concentration were 0.286 and 0.28 g l^{-1} for 600 and 700 mg l^{-1} of initial MIBK concentration which is lesser than the maximum biomass concentration obtained for 500 mg l^{-1} (0.294 g l^{-1}) of initial MIBK concentration. This indicated that above 500 mg l^{-1} of initial MIBK concentration, substrate inhibition effect was apparent.

Growth curve for MIBK can be categorized in phases namely; lag, log, stationary and death phase as shown in Fig. 2b for 600 mg l^{-1} of initial MIBK concentration. Initially, there was no increment in the biomass concentration with time giving the lag phase which is due to the reason that microbes take some time for acclimation to the new environment. In log phase, the biomass concentration was increased rapidly where most of the substrate (MIBK) was

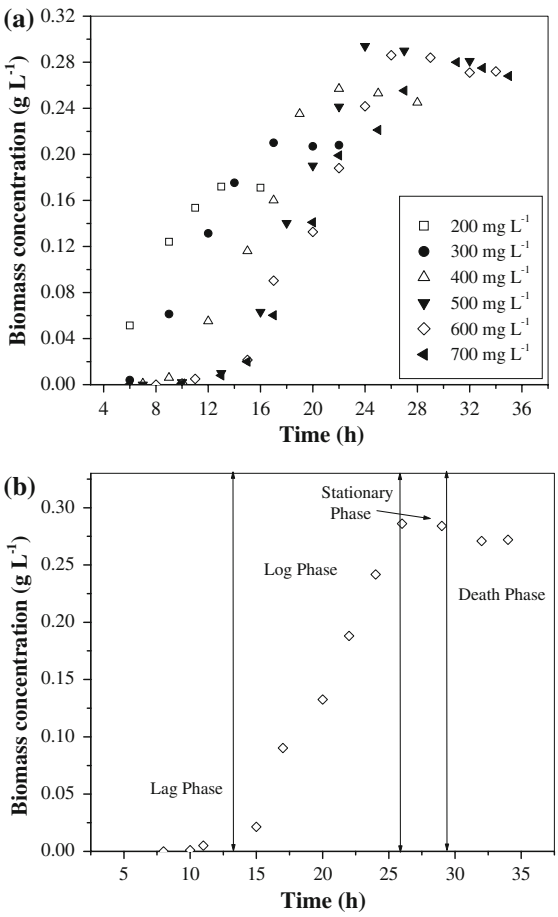

Fig. 2 **a** Change in biomass concentration with respect to time for different initial MIBK concentration. **b** Growth curve for MIBK biodegradation for 600 mg l^{-1} of initial MIBK concentration

utilized by the microbes for their growth. After that, there was no increment in biomass concentration which is the stationary phase of growth curve. After the stationary phase, there was a decrease in biomass concentration which lead to death phase. Similar trends were obtained for initial MIBK concentration of 200, 300, 400, 500, and 700 mg l^{-1} as can be seen from Fig. 2a.

Modeling the growth kinetics of the mixed culture in presence of MIBK

The contaminant degradation leads to the formation of biomass. As contaminant degradation is the result of the microbial activity, the kinetics of contaminant degradation is closely related to the kinetics of microbial growth. The obtained biomass concentrations and substrate concentration at different time intervals for various initial concentrations of MIBK were used to calculate specific growth rate and given by Eq. 1.

$$\mu = \frac{1}{x}\frac{\mathrm{d}x}{\mathrm{d}t} \tag{1}$$

where, μ is the specific growth rate (h^{-1}), x is the biomass concentration (g l^{-1}) at time t (h), and dt is change in time (h) for the change in biomass concentration, dx. After integration, Eq. 1 can be represented by Eq. 2:

$$\ln x = \ln x_0 + \mu t \tag{2}$$

where, x_0 is the initial biomass concentration (g l^{-1}) at $t = 0$. A linear least square method was used to calculate the specific growth rate using the data obtained for log phase at different initial concentration of MIBK. A plot of ln x versus t gives a straight line with ln x_0 as its intercept and μ as the slope. The lag phase and death phase data are not considered for the estimation of specific growth rate. The step size for ln x and t used to calculate specific growth rate in linear least square method at different initial MIBK concentration are listed in Table 1.

Table 1 Step size of ln x and t at each MIBK concentration for the determination of specific growth rate

S. no.	Initial MIBK concentration (mg l^{-1}) S_0 (mg l^{-1})	Value of specific growth rate (Experimental), μ (h^{-1})	Step size used in linear regression for ln x	Step size used in linear regression for time, t (h)
1	200	0.0816	0.02061	0.25263
2	300	0.09112	0.02878	0.31579
3	400	0.11628	0.05141	0.44211
4	500	0.12291	0.04658	0.37895
5	600	0.12788	0.04846	0.37895
6	700	0.09761	0.03699	0.37895

Carbon substrates are most often utilized by microorganisms simultaneously under the carbon and energy controlled environmental conditions. Since growth is a result of catabolic and anabolic enzymatic activities, these processes, i.e., substrate utilization or growth-associated product formation, can also be quantitatively described on the basis of growth models. The relationship between the specific growth rate (μ) of a population of microorganisms and the substrate concentration (S) is a valuable tool in biodegradation processes. This relationship is expressed by a set of empirically derived rate laws which are considered as theoretical models. Various theoretical models such as Monod kinetic model (Monod 1949), Powell kinetic model (Powell 1967), Haldane model (Andrews 1968), Luong model (Luong 1986) and Edwards model (Edwards 1970) are reported in the literature.

Monod model

During the last half century, the concepts of microbial growth kinetics have been dominated by the relatively simple empirical model proposed by Monod (Monod 1949). The Monod model differed from the classical growth models in the way that it introduced the concept of a growth-controlling (limiting) substrate. Monod model relate the specific growth rate (μ) to the concentration of a single growth-controlling substrate (S) via two parameters, the maximum specific growth rate (μ_{max}) and the substrate affinity constant (K_s) as given by Eq. 3.

$$\mu = \mu_m \frac{S}{K_s + S} \tag{3}$$

Eq. 3 has two unknown parameters (μ_m and K_s), which was linearized as given by Eq. 4 and was solved using the method of linear least squares.

$$\frac{1}{\mu} = \frac{K_s}{\mu_m}\left(\frac{1}{S}\right) + \frac{1}{\mu_m} \tag{4}$$

The graph was plotted between $(1/\mu)$ and $(1/S)$ by which the intercept of the line gave the value of μ_m and the slope gave K_s. The values of μ_m and K_s were obtained as 0.142 (h^{-1}) and 131.54 mg l^{-1}, respectively, and also reported in Table 2. The obtained value of coefficient of determination (R^2) was 0.497 which indicates that the present data did not conform well to the Monod model (Fig. 3). The predicted values of specific growth rate at different substrate concentration values are given in Table 3. The self-inhibition of MIBK was observed above the 600 mg l^{-1} of initial MIBK concentration as the specific growth rate was decreased above this concentration (Table 2). The value of K_s was much smaller than the lowest concentration used in this study which also indicated the self-inhibition of MIBK (Deshusses 1994).

Monod model is a simple model. But the model only describes the dependence of biodegradation rate on the biomass concentration (Okpokwasili and Nweke 2005). The limitation of classical Monod's equation is that it does not account for the fact that cells may need substrate or may synthesize product even when they do not grow. The original Monod equation was modified by Powell (1967) which takes into account of some of the limitation of Monod model.

Powell's model

The original Monod equation was modified by introducing the terms of maintenance, expressed as the threshold substrate concentration (S_{min}) and maintenance rate (m). This lead to the modified

Table 2 Growth kinetic parameters obtained from different growth models

S. no.	Model	μ_m (h^{-1})	K_s (mg l^{-1})	K_I (mg l^{-1})	S_m (mg l^{-1})	K	m	n	R^2
1	Monod	0.142	131.54	–	–	–	–	–	0.497
2	Powell	0.134	2.344	–	–	–	4.296	–	0.533
3	Haldane	2.452	5219.49	51.996	–	–	–	–	0.702
4	Luong	1.718	4052.11	–	762.193	–	–	0.377	0.904
5	Edward	1.298	3188.62	23818.57	–	3.145	–	–	0.786

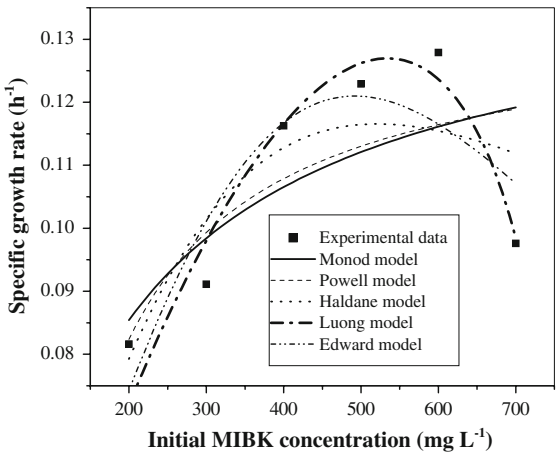

Fig. 3 Experimental and theoretically obtained specific growth rate for different growth kinetics models at different initial MIBK concentrations

equation proposed in various studies by Powell (1967) as given by Eq. 5:

$$\mu = (\mu_{\max} + m)\frac{S}{K_S + S} - m. \quad (5)$$

Kinetic constants could not be obtained accurately using graphical method because of three unknown parameters. So the three kinetic constants were obtained by log phase data using the data regression and were listed in Table 2. Table 3 shows the comparison between experimental data and Powell model predicted data (also shown in Fig. 3). The obtained maximum substrate concentration (μ_m), substrate affinity constant (K_s) and maintenance rate (m) were 0.137 h^{-1}, 2.344 mg l^{-1} and 4.296, respectively. The coefficient of determination (R^2) was obtained as 0.533 which indicates that the Powell models fits the experimental data slightly better than the Monod model. This may be due to the incorporation of maintenance rate in Powell model. The lower value of coefficient of determination (R^2) did not show the applicability of this model for explaining the growth kinetics data for biodegradation of MIBK.

Monod and Powell models do not consider the self inhibition effect which was exhibited during the (MIBK) biodegradation process. It was well established in earlier studies that if the substrate concentration is much higher than the affinity constant values, substrate inhibition models are better than the Monod model (Dapena-Mora et al. 2007). In this study, obtained substrate affinity constant (K_s) was 131.54 and 2.344 mg l^{-1} using Monod and Powell models, respectively, which were quite lesser than the initial MIBK concentration (200–700 l^{-1}).

As the affinity constant values obtained using the Monod and Powell models shows significant inhibition effect, it is important to get an accurate inhibition growth kinetic model to define the relationship between the specific growth rate and substrate concentration. In the present study, as acclimated culture was used for the biodegradation of MIBK, it is essentially required to test the data obtained for MIBK biodegradation with different inhibition growth kinetic models available in the literature so as to know which one is the best suited out of all the reported inhibition models. Various substrate inhibition models such as Haldane model (Andrews 1968), Luong model (Luong 1986) and Edward model (Edwards 1970) are applied and discussed in the following sections. These models can be used to describe the biodegradation of volatile compounds (MIBK) (Andrews 1968).

Table 3 Experimental and predicted values of specific growth rate using different growth kinetic models

S. no.	S_0 (mg l^{-1})	Exp. μ (h^{-1})	Value of predicted specific growth rate, μ (h^{-1})				
			Monod	Powell	Haldane	Luong	Edward
1	200	0.0816	0.08541	0.08236	0.07925	0.07204	0.07427
2	300	0.09112	0.09850	0.09954	0.10165	0.09750	0.1013
3	400	0.11628	0.1064	0.10801	0.11302	0.11665	0.11688
4	500	0.12291	0.11211	0.11310	0.1160	0.12620	0.12094
5	600	0.12788	0.11620	0.11650	0.1153	0.1230	0.11650
6	700	0.09761	0.11918	0.11889	0.11187	0.0984	0.1071

Haldane model

Methyl iso-butyl ketone biodegradaton was subjected to the self-inhibition as indicated in Monod and Powell models. In such cases, substrate inhibition is considered by incorporating the substrate inhibition constant (K_I) in Monod model. Among the various substrate inhibition models, Haldane model has been widely used (Sokol 1986; Tang and Fan 1987; Deshusses 1994; Peyton et al. 2002; Saravanan et al. 2008). Haldane model was originally proposed for substrate inhibition in 1968. According to Haldane model, the specific growth rate can be represented by Eq. 6.

$$\mu = \frac{\mu_{max} S}{K_s + S + (S^2/K_I)} \tag{6}$$

where, K_I is the substrate inhibition constant (mg l^{-1}).

The Eq. 6 was solved and bio-kinetic constants were obtained using the log phase data and listed in Table 2. Figure 3 shows the fit of Haldane model with the experimental results and predicted values of specific growth rate at different concentration are listed in Table 3. The value of coefficient of determination ($R^2 = 0.702$) showed that the present data fit reasonably well to the Haldane model as compared with the Monod model ($R^2 = 0.497$) and Powell model ($R^2 = 0.533$). For self-inhibitory compounds, there is a critical substrate concentration, S_{crt}, which is defined by Eq. 7, above which the substrate removal rate falls due to self-inhibitory effect (Tomei et al. 2004).

$$S_{crt} = \sqrt{K_s K_I}. \tag{7}$$

Critical MIBK concentration was obtained as 520.95 mg l^{-1}. At this critical MIBK concentration, the maximum specific growth rate which could be obtained was 0.1165 h^{-1}. The coefficient of determination ($R^2 = 0.702$) obtained for the Haldane model was not very satisfactory though it is better than that obtained using Monod and Powell models. Hence the data obtained for specific growth rate at different initial concentration of MIBK were also tested with other inhibition models.

Luong model

The inhibitory effect of substrate on biomass growth under batch conditions can also be represented by a mathematical expression which was proposed by Luong (1987). This model is based on certain assumptions which include no lag phase, organism death, endogenous respiration, substrate used for maintenance energy, or inhibition by products. The model incorporates a term given as S_m (mg l^{-1}) critical inhibitor concentration above which the growth is completely inhibited. The inhibitory effect of MIBK at higher concentration for mixed culture is given by Eq. 8.

$$\mu = \frac{\mu_m S}{K_s + S}\left(1 - \frac{S}{S_m}\right)^n \tag{8}$$

where S_m (mg l^{-1}) is the critical inhibitor concentration above which biodegradation stops, n is the positive integer in the Luong model.

The kinetic constants were obtained using log phase data and are listed in Table 2. Experimentally found specific growth rate at different initial MIBK concentration was fitted with Luong model and shown in Fig. 3. The obtained value of coefficient of determination ($R^2 = 0.904$) and comparison between the experimental and predicted value of specific growth rate (Table 3) indicated that the experimental results fit well to the Luong model as compared to Haldane model. The obtained value of maximum MIBK concentration at which the culture ceased to grow was 762.193 mg l^{-1} according to Luong model. The concentration of MIBK in a contaminated air is usually less than 500 mg l^{-1} and the critical exposure limit of MIBK is 100 mg l^{-1} (WHO 1990). As per the results obtained in this study, the culture can grow up to 762.193 mg l^{-1} which is higher than the MIBK concentration in air and critical exposure limit.

Edward model

Edwards (1970) discussed various reasons for the substrate inhibition which affects the microbial growth. The reasons for substrate inhibition could be the formation of intermediates, or products; changed activity of one or more enzymes; dissociation of one or more enzymes or formation of metabolic aggregates. The study also focused on the development of inhibition models which can explain the highly complicated nature of microorganisms in the degradation of organic compounds and can

suggest a mechanism of biodegradation at high substrate concentration. This study included the five different inhibition models which are derived from the Haldane model. However, in the present study one of the proposed a mathematical model is studied which describes the mechanisms causing substrate inhibition for a wider concentration range and is given by Eq. 9.

$$\mu = \mu_{\mathrm{m}} \frac{S}{S + K_{\mathrm{s}} + \left(\frac{S^2}{K_{\mathrm{I}}}\right)\left(1 + \frac{S}{K}\right)} \tag{9}$$

where K_{s} is the substrate affinity constant, K_{I} is the substrate inhibition constant (mg l^{-1}) and K is a positive constant which appear in Edward model. This model show an improvement over Haldane model with the incorporation of one term $[1 + (S/K)]$ in the denominator which includes a positive constant K. Higher value of K (3.145 mg l^{-1}) was obtained which indicate that Haldane model was not suitable as compare to Edward model. The experimental data for log phase at initial concentration ranging from 200 to 700 mg l^{-1} were used for estimating the kinetic parameters for the Edward model and listed in Table 2. The obtained value of coefficient of determination ($R^2 = 0.786$) indicated that the Edward model explains the mechanism of biodegradation of MIBK better than the Haldane model ($R^2 = 0.701$) but not as good as Luong model ($R^2 = 0.904$). Higher value of the fourth parameter, K (3.145 l^{-1}) was obtained which indicates that Haldane model was not suitable as compared to Edward model (Edwards 1970). Also the experimental and predicted value of specific growth rate as shown in Table 2. The difference in experimental and predicted values of specific growth is large which also conform that Edward model did not fit well as compared to Luong model (Table 3).

Based on the detailed analysis as discussed above, the Luong model explains the growth kinetics of MIBK degradation better than the Monod, Powell, Haldane and Edward models.

Biodegradation rate kinetics

In biodegradation processes, several kinetic approaches for describing the transformation of organic compounds (MIBK into biomass) by suspended microorganisms were being evaluated. The rate of disappearance of substrate was dependent on the substrate concentration. The substrate concentration which changes with time can be described by zero-order, first-order and second-order rate kinetics.

Robinson and Tiedje (1983) have discussed that Monod kinetics though deterministic are more suitable to continuous than to batch culture systems. This is due to the linear approximation ($\Delta s/\Delta t$) of the differential equation which becomes highly inaccurate and impractical as the time intervals between the measurements increases.

According to Paris et al. (1981), the second order kinetics depends either on the substrate concentration and biomass or substrate concentration and time. Larson (1980) has considered that the biomass concentration is directly proportional to time and arrived at the second order differential equation. The integrated form of the model equation produces a sigmoidal curve and does not allow for the metabolism without growth.

The kinetics proposed in earlier studies are Monod, first order and second order which have the limitation that they do not take into account the biomass growth. Therefore, it was found that both the first-order and second-order kinetics were not widely preferred and are not sufficient to explain the biodegradation rate kinetics. These conceptual and mathematical difficulties led to single deterministic model known as three-half-order kinetic model which was proposed by Brunner and Focht (1984). The model was based on the assumption of first-order model with the introduction of an additional term for explaining the biomass formation. The rate of MIBK degradation is given by Eq. 10.

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -k_1 S - aES \tag{10}$$

where k_1 is the proportionality constant (time^{-1}), E is the cell concentration and a is the proportionality constant (biomass concentration^{-1} time^{-1}). After integration and simplification, Eq. 10 reduces to Eq. 11 (Brunner and Focht 1984).

$$Y = -k_1 - \frac{k_2 t}{2} \tag{11}$$

where

$$k_2 = aE/t \tag{12}$$

$$Y = \frac{1}{t}(\ln(S_0 - P + k_0 t)/S_0) \quad (13)$$

$$P = S_0\left(1 - e^{-k_1 t - \left(k_2 t^2\right)/2}\right) + k_0 t \quad (14)$$

k_1 and k_2 were found by plotting Y against t which gave a straight line using the log phase data. The P is the rate of product formation (CO_2) which is directly related to the change in biomass concentration. As the three-half order model included the term to explain biomass formation which can be measured in term of P. k_0 and S_0 are zero-order rate constant and substrate concentration at zero time, respectively. The Eq. 14 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 which is represented by differential and integral form as given by Eqs. 15 and 16 respectively.

$$\frac{dS}{dt} = -k_0 \quad (15)$$

$$S = S_0 - k_0 t \quad (16)$$

The zero-order and three-half-order kinetic constants were evaluated and listed in Table 4 and the corresponding plots are shown in Figs. 4 and 5, respectively. The coefficient of determination obtained for zero-order kinetics was found in the range of 0.96–0.99 for various initial MIBK concentration values. The value of S_0 was found increasing from 179.07–1368.49 mg l^{-1} and k_0 was found increasing from 13.79–49.49 mg l^{-1} h^{-1}) except the values obtained for 500 and 600 mg l^{-1} of initial MIBK concentration.

The obtained value of three-half-order rate constants, (k_1 and k_2) were found decreasing with increase in the initial MIBK concentration. The obtained value of regression coefficient of determination ($R^2 = 0.937$–0.994) indicated that the three-half-order kinetic model is suitable to explain the MIBK biodegradation rate kinetics

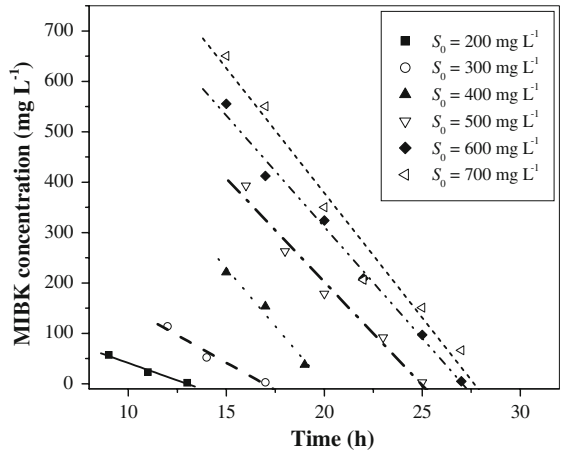

Fig. 4 Zero-order kinetics for biodegradation of MIBK at different initial MIBK concentrations

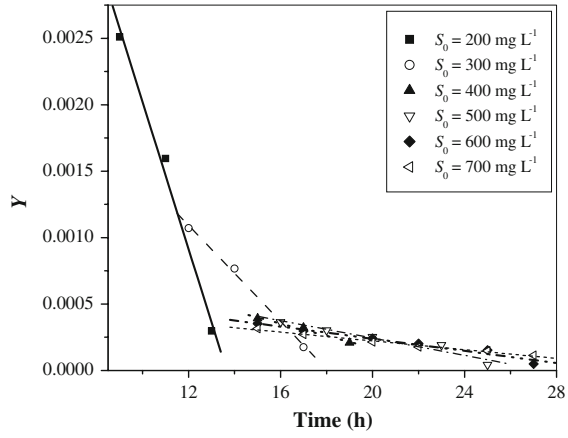

Fig. 5 Three-half-order kinetics for biodegradation of MIBK at different initial MIBK concentrations

Table 4 Parameters of zero-order and three-half-order kinetic models at different initial MIBK concentrations

S. no.	Initial MIBK concentration, S_0 (mg l^{-1})	Zero order kinetics			Three half order kinetics		
		k_0	S_0	R^2	$k_1 \times 10^4$	$k_2 \times 10^3$	R^2
1	200	13.79	179.07	0.991	5.53	7.55	0.995
2	300	21.77	368.43	0.983	1.81	3.26	0.997
3	400	45.92	917.98	0.988	0.460	1.09	0.990
4	500	40.97	1021.91	0.989	0.327	0.898	0.968
5	600	44.14	1194.12	0.995	0.229	0.697	0.983
6	700	49.49	1368.49	0.983	0.165	0.552	0.993

using acclimated mixed culture over a wide range of operating conditions.

Conclusions

The present study focused on the growth and biodegradation kinetics of acclimated mixed culture for MIBK. The obtained results showed that the time required for utilizing MIBK using mixed culture increased by increasing the initial concentration of MIBK. The maximum specific growth rate was obtained at 600 mg l^{-1} of initial MIBK concentration. Substrate inhibition occurred for more than 600 mg l^{-1} of MIBK concentration. The different growth kinetic models such as Monod, Powell, Haldane, Luong and Edward model were tested. The obtained values of coefficient of determination obtained for Luong model suggested that this model suits the biodegradation kinetics of MIBK better than the other inhibition models of Haldane and Edwards. The higher value obtained for critical inhibitor concentration indicated that the higher efficiency of the microbial culture to grow in the presence of MIBK. Biodegradation rate kinetics using zero-order and three-half-order kinetic models were tested and three-half-order kinetic model was found suitable for the biodegradation of MIBK.

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