Biodegradation Kinetic Studies for the Removal of Iso Propyl Alcohol (IPA)

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Abstract

Iso propyl alcohol (IPA) is a toxic volatile organic compound (VOC) that is emitted from various paint, pharmaceutical, lithography, and rubber manufacture industries. These emissions may lead to an adverse effect on air quality and thus endanger public health and welfare. The most serious requirements for removing VOCs from waste gases in recent years necessitate the development of innovative and cost-effective treatment alternatives. The aerobic biodegradation of IPA by an acclimated mixed culture obtained by sewage treatment plant is carried out in this study for the range of 200 – 700 mg/L of initial IPA concentration. Substrate inhibition occurred for more than 300 mg/L of IPA concentration. The results obtained in present study are fitted with the Haldane model with coefficient of determination (R^2 = 0.989). The biodegradation rate kinetic parameters are obtained for zero order and three and half order kinetic models.

1. INTRODUCTION

Isopropyl Alcohal (IPA) is one such VOC having a boiling point of 83^0 C, which vaporizes at normal atmospheric conditions. It is flammable, clear, colorless liquid and is slightly odorous. IPA has wide applications as a solvent in paint, pharmaceutical, lithography, and rubber manufacture industries. It is used as a cleaning agent in semiconductor and printing industries. It is one of the components of many antiseptic and cosmetic products [1]. The workspace exposure limit (WEL) and OSHA permissible exposure limit (PEL) is 400 mg/L for 8 h exposure [2,].

Inhalation of IPA vapors can cause gastrointestinal pain, cramps, nausea, vomiting, and diarrhea. IPA vapors can also cause eye irritation, possible corneal burns and eye damage [3].

The biological treatment of VOCs has been attracting more attention these days than physical and chemical methods as they are cost effective and create almost negligible secondary pollutant. Biological methods include variety of microorganism's community which is known to utilize VOCs as a carbon source for their growth [4]. The studies were reported for biodegradation of IPA using mixed culture as it can be predominantly used for large scale bio based treatment plants [6].

In the present study, biodegradation of IPA was studied. The objective of this study was to understand the biodegradation of IPA with better quantitative experimental data. Thus the work involved the development of acclimated mixed culture for the biodegradation of IPA. The effect of time on initial concentration of IPA and biomass concentration ranging from 200-700 mg/L was also studied. The biodegradation data was then tested with some growth kinetic models.

2. MATERIALS AND METHODS

2.1 Preparation of media

All the materials used (IPA and various nutrient media) were of analytical grade of Merck (India) brand. Stock glucose solution was prepared by dissolving 10 g of glucose in 100 ml distilled water. The media, Minimal Salt Medium (MSM), was prepared having the following composition (in g/l): K_2HPO_4 – 0.8, KH_2PO_4 – 0.2, $CaSO_4.2H_2O$ – 0.05, $MgSO_4.7H_2O$ – 0.5, $(NH_4)_2SO_4$ – 1.0, $FeSO_4$ – 0.01 in distilled water and was autoclaved.

2.2 Microorganism culture conditions

An aerobic mixed microbial culture was obtained from the Municipal Sewage Treatment Plant of Birla Institute of Technology & Science (BITS) Pilani. The sludge was kept for settling for almost 3-4 hours in a room at around 25^0 C (away from sunlight). 10 gm of settled sludge was taken and thoroughly mixed with 100 ml of distilled water. The shaking was carried out gently and then sludge was allowed to settle. 50 ml of supernatant was taken and centrifugation was carried out for 2 minutes at 10,000 rpm in a Centrifuge. The pellet obtained after the centrifugation was further used for the microbial growth and the supernatant was discarded.

2.3 Inoculation procedure

The inoculation was carried out in 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 37^0 C for 48 hours. The sufficient microbial culture (measured by optical density value of more than 0.1 at 540 nm) was obtained. This obtained microbial culture was then used for obtaining the microbial culture acclimated with IPA. For this, 100ml of MSM was added with 1 ml of glucose and 20 µl of IPA to start with and was kept in rotary shaker for almost 48 hours. This procedure was followed for 3 weeks by decreasing the amount of glucose from 1 to 0 ml with a decrement of 200 µl every time and simultaneously increasing IPA amount from 20 to 60 µl with an increment of 10 µl. Hence the acclimated mixed culture was obtained after 3 weeks cycle.

2.4 Biodegradation study

Batch biodegradation experiments were conducted in the concentration range of 200 - 700 mg/L with the values of 200, 300, 400, 500, 600 and 700 mg/L individually in 250 ml Erlenmeyer flasks sealed with cotton plugs. 100 ml of MSM was autoclaved and 2 ml of pre-cultured suspension (acclimated mixed culture) and known amount of IPA were added to it to maintain the required concentration. The amount of IPA added was 27, 40, 54, 68, 82 and 95 µl (based on density and boiling point of IPA) to maintain 200, 300, 400, 500, 600 and 700 mg/L of IPA concentration respectively. All experiments were carried out in an orbital shaking incubator set at 150 rpm and 37^0 C. The samples were collected at different intervals for different concentrations ranging from 200 to 700 mg/L based on visual observation (turbidity). The time interval was different for different samples.

2.5 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). Samples were then centrifuged at 10000 rpm for 2 minutes to separate biomass and supernatant (aqueous IPA solution) [7]. Dry weight of biomass was obtained and concentrations of IPA in aqueous samples were measured using a gas chromatograph (Model 5700 series, Nucon Engineers, India). The temperatures of injection port, detector and oven were maintained at 150^0 C, 150^0 C and 200^0 C, (based on boiling points of the compound and specified ranges of gas chromatograph) 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.

3. RESULTS AND DISCUSSION

In the present study, biodegradation of IPA was studied for the initial concentration range of 200-700 mg/L using acclimated mixed culture.

3.1 Effect of time on initial IPA concentration

Initial concentration of any VOC has a significant influence on the growth of microbial culture [7]. Fig. 1 shows the time profile of IPA biodegradation for concentration ranging from 200 – 700 mg/L using the acclimated mixed culture. It was found that the acclimated mixed culture degraded IPA in 14 h, 18 h, 21 h, 24 h, 26 h and 28 h for 200, 300, 400, 500, 600 and 700 mg/L of initial IPA concentration respectively. The IPA concentration was found decreasing with time showing the utilization of IPA by the microorganisms as it acts as a carbon source for their growth [7].

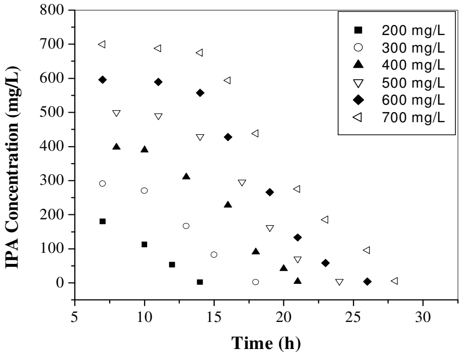

Fig. 1 Residual IPA concentration Vs time for different initial IPA concentration

3.2 Effect of time on biomass concentration

Fig.2 shows the change in biomass concentration of the mixed culture (as OD540) at different times for initial IPA concentration values ranging from 200 – 700 mg/L. Biomass concentration increases with increase in time until all IPA was utilized by microorganisms. The maximum biomass concentrations were obtained as 0.104, 0.137, 0.155, 0.151, 0.143 and 0.139 g/l for initial IPA concentrations of 200, 300, 400, 500, 600 and 700 mg/L, respectively. The biomass concentration increased with increase in initial IPA concentration from 200 to 300 mg/L. This indicated that IPA has less inhibition effect on growth of microorganisms as shown by small lag phase. This indicated that above 300 mg/L of initial IPA concentration, substrate inhibition effect was predominant.

3.3 Biodegradation growth kinetics

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 [8], Powell kinetic model [9], Haldane model [10] are reported in the literature. These models are helpful in the understanding of the behavior of the biological processes and predicting the IPA concentrations in the treatment systems.

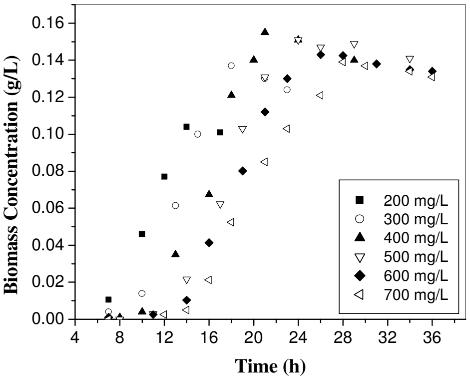

Fig. 2 Change in biomass Concentration with respect to time for different initial IPA concentration

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 at various initial values of IPA concentration are used to calculate the specific growth rate using Eq. 1.

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

where, μ is the specific growth rate (1/h); x is the biomass concentration (g/l) at time t (h); S is the substrate (IPA) concentration (mg/L).

Fig.3 shows the specific growth rate values at different values of initial IPA concentration. The values of specific growth rate were obtained as 0.329, 0.337, 0.309, 0.295, 0.269 and 0.246 1/h for the initial IPA concentration values of 200, 300, 400, 500, 600 and 700 mg/L respectively. The specific growth rate increases up to 300 mg/L of initial IPA concentration and then decreases with increase in concentration (see Fig. 3). In microbial processes, some higher concentrations of the substrate inhibit the growth of the microorganisms due to which the growth rate falls. In the present study, it is observed that the inhibition effect on microorganisms starts at low initial concentration values of IPA as compared to earlier studies [11]. Hence in this study, basic growth models such as Monod and Powell were not considered. 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. Also, the Monod and Powell models do not consider the self inhibition effect which was exhibited during the (IPA) biodegradation process. The present work includes the application of substrate inhibition model such as Haldane model.

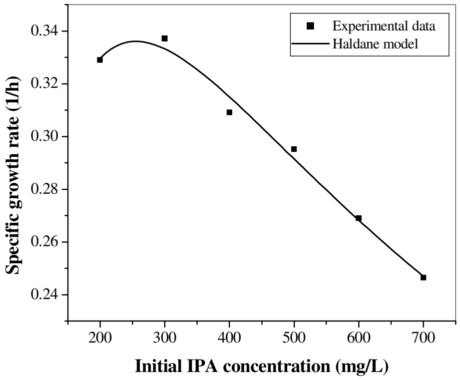

Fig. 3 Experimental and theoretically obtained specific growth rate for different growth kinetics models at different initial IPA concentrations

3.3.1 Haldane's Model

IPA biodegradaton was clearly subjected to the self-inhibition as shown by the decrease in specific growth rate at the start of the biodegradation of IPA with increasing initial IPA concentration as discussed in the earlier section (Biodegradation kinetics). In such cases, substrate inhibition is considered by incorporating the substrate inhibition constant (K_I) in Monod's Model. Among the various substrate inhibition models, Haldane's model has been widely used [12, 13]. Haldane model was originally proposed for substrate inhibition in 1968. According to Haldane model, the specific growth rate can be represented by Eq. (2).

$$\mu = \frac{\mu_m S}{K_s + S + \left(S^2 / K_I \right)} \qquad (2)$$

The model equation is non linear and it was solved using a professional graphics software package ORIGIN (version 6) to obtain the kinetic constants and were listed in Table 1. Fig. 3 shows the fit of Haldane model with the experimental results. The value of coefficient of determination (R^2 = 0.989) showed that the present data confirmed well to the Haldane model. The obtained value of affinity constant, K_s, and substrate inhibition constant, K_I, were 241.6 and 270.75 mg/L respectively. For self-inhibitory compounds, there is a critical substrate concentration, C_{crt}, which is defined by Eq. (3), above which the substrate removal rate falls due to self-inhibitory effect [18].

$$C_{crt} = \sqrt{K_s K_I} \qquad (3)$$

Critical IPA concentration was obtained as 255 mg/L. The corresponding specific growth rate was 0.3361/h. The obtained value of K_s (241.6) was less than the critical IPA concentration which also confirms the self inhibition effect. Observed K_I value was also low, which means that IPA is toxic to the cells even at low concentration ranges. In Haldane model, only the substrate utilization term was considered and the biomass production from the utilization of substrate was neglected.

3.4 Biodegradation rate kinetics

Several kinetic approaches for describing the transformation of organic compounds such as IPA into biomass by suspended microorganisms were being evaluated for biodegradation processes [14, 15]. The rate of disappearance of substrate is 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 [22]. The first-order and second-order kinetics were not widely preferred as they suffered from the limitation that they do not take into account of the biomass growth. Therefore in biodegradation processes, generally three-half-order kinetics is used [22]. 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 IPA degradation is given by Eq. 4:

$$\frac{dS}{dt} = -k_1 S - aES \qquad (4)$$

where k_1 is the proportionality constant (1/time), E is the cell concentration (g/l) and a is the proportionality constant (1/(biomass concentration)(time)). After integration and simplification, Eq. 4 reduces to Eq. 5 [22].

$$Y = -k_1 - \frac{k_2 t}{2} \qquad (5)$$

$$P = S_0\left(1 - e^{-k_1 t - \left(k_2 t^2\right)/2}\right) + k_0 t \qquad (6)$$

k_1 and k_2 were found by plotting Y against t which gave a straight line. k_0 and S_0 are zero-order rate constant and substrate concentration at zero time, respectively.

Eqn.8 contains four unknown parameters and is highly nonlinear. It also requires the initial estimates of S_0 and k_0 for the calculation of kinetic parameters (k_1 and k_2). 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 Eq. 7 and 8 respectively:

$$\frac{dS}{dt} = -k_0 \qquad (7)$$

$$S = S_0 - k_0 t \qquad (8)$$

The zero-order constants were evaluated used in finding the three-half-order kinetic constants and three-half-order kinetics are also shown in Fig. 4.The obtained value of coefficient of determination (R^2 = 0.959 – 0.995) indicated that the three-half-order kinetic model is suitable to explain the IPA biodegradation rate kinetics using acclimated mixed culture over a wide range of operating conditions.

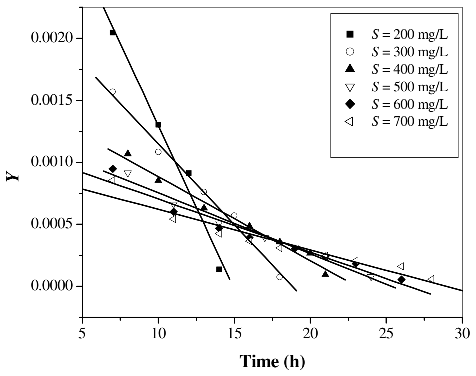

Fig. 4 Three-half-order kinetics for biodegradation of IPA at different initial IPA concentrations

4. CONCLUSIONS

The present study was focused on the microbial degradation of IPA. It showed that the time required for utilizing IPA using acclimated mixed culture increased by increasing the initial concentration of IPA. The specific growth rate was found at various initial concentrations of IPA. The maximum specific growth rate was obtained at 300 mg/L of initial IPA concentration. Substrate inhibition occurred for more than 300 mg/L of IPA concentration. The obtained experimental data was fitted with Haldane model. 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 IPA.

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