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Batch biodegradation of toluene by mixed microbial consortia and its kinetics

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ABSTRACT

Biodegradation of toluene in a batch system by mixed microbial culture isolated from cow dung compost was investigated. Batch biodegradation of toluene was carried out at various initial concentrations ranging from 50 to 500 mg l^{-1} at room temperature (28 °C) with initial a pH value of 6.85 maximum observed specific growth rate was 0.062 h^{-1} and maximum removal efficiency of toluene was 84% at 100 mg l^{-1}. Biochemical characterizations were carried out by different sugar fermentation tests to identify the microorganisms responsible for biodegradation of toluene. *Pseudomonas species, Bacillus species* and *Escherichia coli* were found to be dominant strains among the mixed culture. The effect of pH and inoculum size on biodegradation of toluene at initial concentration of 100 mg l^{-1} was also studied. Different substrate inhibition models (Haldane, Edwards and Levenspiel) were fitted with the experimental data.

1. Introduction

There is a widespread contamination of surface water due to the presence of aromatic compound, which is released to the environment by various industries. Aromatic compounds have a wide range of applications in the form of solvents and raw materials for paints, resins, varnishes, organic dye, etc. They are also used as solvent for rubber and plastics (Arinjay et al., 2005; Sung et al., 2001).

Among these, toluene is a highly toxic (50 mg l^{-1}) and carcinogenic compound. Owing to high water solubility (0.174–0.187%), higher volatility and high mobility, toluene is a universal environmental contaminant (Slominska et al., 2012; Durmusoglu et al., 2010). Toluene is used in the manufacture of polycols, antioxidants, corrosion inhibitors, polyurethane, adhesives and polyethylene terephthalate (PET) solid state resins. It is also widely used as a process solvent (Mathur and Majumder, 2008).

Physical and chemical methods used for the treatment of VOCs incur high operational costs; emits secondary pollutants or convert pollutants from one phase to another. Biological treatment appears to be cost effective and environmental friendly technique for treating toluene as it can be easily degraded under anaerobic or aerobic conditions in the presence of microorganism. Further, literature survey reveals that a few researchers (Singh and Celin, 2010; Mathur and Majumder, 2010; Li et al., 2006; Tazdait et al., 2013; Choi and Oh, 2001; Weimin et al., 2014) attempted to study the biodegradation of benzene, toluene and other aromatic compound using microbial free cells as well as immobilized cells. Through the literature review on biodegradation kinetics, the capacities of microorganism to degrade the aromatic compounds were well understood by means of various inhibition models such as Haldane model (1965), Levenspiel model (1988) and Edwards model (1970).

The main focus of this research work is to degrade toluene using mixed culture. Usually, pure culture is used in most of the studies, whereas in this study, an attempt was made to biodegrade toluene using mixed culture derived from cow dung compost. Cow dung compost consists of microorganisms and macroorganisms which are used to take advantage of changing temperature, moisture, oxygen content and pH. It also contains nutrients such as nitrogen, phosphorus and potassium, which are essential for the microbial growth. Toluene commonly used as a solvent in process industry is taken as a model pollutant and tested for removal in a batch shake

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flask by using mixed microbial culture isolated from the compost. The effect of pH and inoculum dosage on the biodegradation of toluene was also studied. Batch biodegradation kinetics was performed and the results were correlated with various substrate inhibition models. Biochemical characteristics of mixed culture were done by morphology and sugar fermentation test.

2. Materials and methods

2.1. Microorganisms and culture media

The microbial mixed culture was obtained from cow dung compost collected from nearby farmyard. The culture was initially grown in 250 ml Erlenmeyer flask containing 100 ml of mineral salt medium (MSM) containing the following composition (g/L): Na_2HPO_4 – 5, K_2HPO_4 – 4.0, KH_2PO_4 – 4.0, $(NH_4)_2$ PO_4 – 1.0, $MgSO_4{\cdot}7H_2O$ – 0.25, $CaSO_4$ – 0.25, $FeSO_4{\cdot}H_2O$ – 0.08, Dextrose – 2.0 in distilled water at pH of 6.85 under ambient condition.

2.2. Batch degradation studies

The mixed microbial culture was pre-cultured in 100 ml of the MSM containing 25 mg l^{-1} of toluene for about 48 h. Biodegradation of toluene was carried out over a concentration range of 50–500 mg l^{-1} individually in 250 ml Erlenmeyer flasks. Flasks were closed with cork and sealed with aluminum foil to minimize the loss of toluene by evaporation. Samples were collected at different period of time intervals and analyzed for residual toluene concentration and biomass.

2.3. Analytical method

Toluene in the liquid phase was analyzed by HPLC chromatograph equipped with UV detector (model UV -1700, SHIMADSU) at 254 nm. The column used was C_{18} bond pack 3 μm (25 cm × 4.6 mm) with a mobile phase consisting of methanol-water (70–30). The flow rate was set at 1 ml min^{-1} with a residence time of 2.4 min.

The biomass concentration was estimated using wet weight method. A 1 ml of sample was taken in Eppendorf tube. Then it was centrifuged at 2000 revolution per minute (rpm) for 20 min. The supernatant liquid was separated from the biomass. The eppendorf tube containing biomass was weighed. The difference between the weight of eppendorf tube with biomass and the empty eppendorf tube was calculated and taken as the biomass concentration (Ravi et al., 2013).

3. Results and discussion

3.1. Biodegradation of toluene

Batch study experiments were carried out in Erlenmeyer flask (250 ml) with working volume of 150 ml to understand the potential of a mixed culture to degrade toluene and used to estimate the micro kinetics of the elimination process.

To study the biomass growth profile of mixed culture using toluene as the substrate, the initial concentration was varied from 50 to 500 mg l^{-1} in the mineral salt medium. Experiments were carried out in duplicate and the average of the value is taken for plotting graph. The biomass growth pattern was observed and shown in Fig. 1. It was observed that the biomass growth profile, particularly at low concentrations (<100 mg l^{-1}) was found to be a typical conventional biodegradation process with lag, exponential and stationary phase. However, at higher toluene concentrations (>100 mg l^{-1}) the growth rate was significantly reduced due to toxicity of the substance. A marginal reduction in the slope of growth curve was observed after a concentration of 100 mg l^{-1}. Similarly, growth inhibition was found in toluene biodegradation at 100 mg l^{-1}.

At different concentrations, specific growth rate (μ) was calculated using the formula

$$\mu = \frac{\ln \frac{x_2}{x_1}}{(t_2 - t_1)} \quad (1)$$

where μ is the specific growth rate, $h.^{-1}x_1$, g/l is the biomass concentration at time t_1, $h.x_2$, g/l is the biomass concentration at time t_2, h.

The specific growth rate profile was shown in Fig. 2. The specific growth rate increased with toluene concentration increases until reaching a maximum of 0.062 (1 h^{-1}). For a further increase the toluene concentration, there is a decrease in specific growth rate, indicating substrate inhibition above 100 mg l^{-1}.

The removal efficiency profile for toluene at different

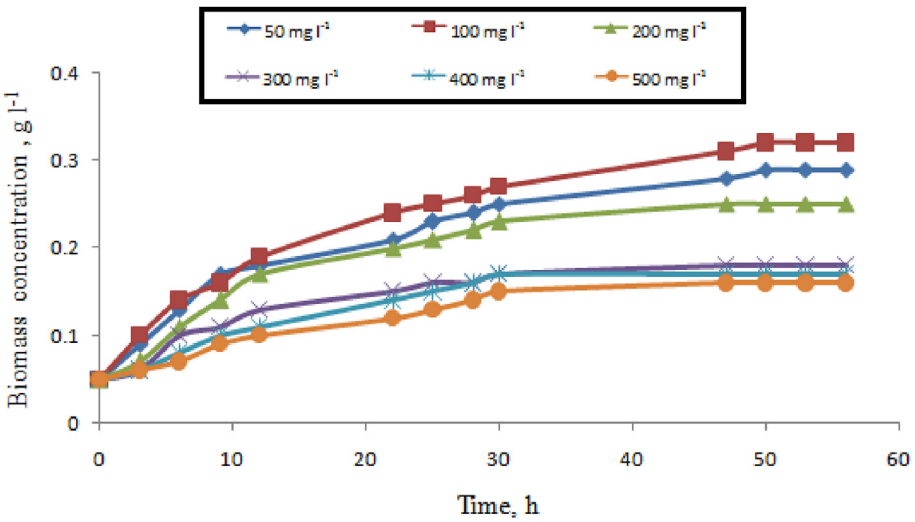

Fig. 1. Biomass concentration profile for different toluene concentration.

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