Journal of Hazardous Materials 162 (2009) 476–481

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Batch growth kinetics of an indigenous mixed microbial culture utilizing *m*-cresol as the sole carbon source

Pichiah Saravanan[a], K. Pakshirajan[b], Prabirkumar Saha[a,*]

[a] Department of Chemical Engineering, Indian Institute of Technology Guwahati, Assam 781039, India
[b] Department of Biotechnology, Indian Institute of Technology Guwahati, Assam 781039, India

ARTICLE INFO

Article history:
Received 2 January 2007
Received in revised form 8 March 2008
Accepted 16 May 2008
Available online 21 May 2008

Keywords:
m-Cresol
Biodegradation
Kinetics
Substrate inhibition
Indigenous mixed microbial culture

ABSTRACT

An indigenous mixed microbial culture, isolated from a sewage treatment plant located in Guwahati was used to study biodegradation of *m*-cresol in batch shake flasks. *m*-Cresol concentration in the growth media was varied from 100 mg/L to 900 mg/L. The degradation kinetics was found to follow a three-half-order model at all initial *m*-cresol concentrations with regression values greater than 0.97. A maximum observed specific degradation rate of 0.585 h^{-1} was observed at 200 mg/L *m*-cresol concentration in the medium. In the range of *m*-cresol concentrations used in the study, specific growth rate of the culture and specific degradation rates were observed to follow substrate inhibition kinetics. These two rates were fitted to kinetic models of Edward, Haldane, Luong, Han-Levenspiel, and Yano-Koga that are used to explain substrate inhibition on growth of microbial culture. Out of these models Luong and Han-Levenspiel models fitted the experimental data best with lowest root mean square error values. Biokinetic constants estimated from these two models showed good potential of the indigenous mixed culture in degrading *m*-cresol in wastewaters.

1. Introduction

m-Cresol is a derivative of phenol and is a compound regarded as a priority pollutant by the United States Environmental Protection Agency (USEPA) [1–3]. Industries such as pulp and paper mills, textile mills, coal gasification units, herbicides and fungicides industry, etc., are mainly responsible for discharging *m*-cresol in their aqueous effluents [2,4,5]. *m*-Cresol, a methylated derivative of phenol, leads to serious environmental contamination due to its toxicity towards aquatic biota [1,2]. Mainly due to these reasons, its removal from wastewater before final discharge is required. Among the available treatment methods for degrading *m*-cresol, microbe-based processes seem more promising and economical [1–6]. Although reports on biodegradation of phenolic compounds by pure microbial cultures are known, biodegradation of *m*-cresol using indigenous mixed microbial community is relatively less reported. Aerobic degradation using an indigenous mixed microbial community may be advantageous in complete assimilation (CO_2 and H_2O) of *m*-cresol without producing any toxic residues in the process [2].

Gallego et al. evaluated the biodegradation of 2-chlorophenol, phenol and *m*-cresol as mixed components by using pure and mixed indigenous cultures in aerobic reactors. They observed the biodegradation of *m*-cresol with an initial concentration of 50 mg/L in 27 h by pure bacterial cultures [7]. Recently Yan et al. showed the biodegradation of *m*-cresol in batch shake flask with pure culture of *Candida tropicalis*. They observed that culture could degrade *m*-cresol with a maximum initial concentration of only 280 mg/L within 66 h. In addition, they also studied the intrinsic kinetics of cell growth and substrate degradation with *m*-cresol [1].

However, the reports on biodegradation of high concentration *m*-cresol, employing mixed microbial cultures are limited. Hence the objective of the present work was therefore to study the kinetics of growth and *m*-cresol degradation in batch shake flasks using an indigenous mixed microbial culture. Also, suitable substrate inhibition models, found in the literature were applied to the experimental data to estimate the biokinetic constants with an aim to scale-up the process.

The adopted substrate inhibition models along with their mathematical form have been described below: the earliest model on microbial growth kinetics, the Monods model (1949), relates growth rate of micro-organism to the concentration of a single growth controlling substrate represented by the following

* Corresponding author. Tel.: +91 361 2582257; fax: +91 361 2582257.
E-mail addresses: prabirkumar.saha@gmail.com, p.saha@iitg.ernet.in (P. Saha).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.05.069

equation[8]:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (1)$$

where μ is the specific growth rate (h^{-1}), μ_{max} the maximum specific growth rate (h^{-1}), S the substrate concentration (mg/L) at time t, and K_s is the half saturation coefficient (mg/L). Haldane (1968) proposed the first and most popular model for substrate inhibition kinetics. This model form is given in the following equation [9]:

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

where K_i is the substrate inhibition constant (mg/L). Due to its significance it was widely adopted by most of the researchers. Yano and Koga proposed an model (1969), based on a theoretical study on the dynamic behavior of single vessel continuous fermentation subject to the growth inhibition at high concentration of rate limiting substrate, e.g., the acetic acid fermentation from ethanol, the gluconic acid fermentation from glucose, the tannase fermentation with tannic acid as the sole carbon source, a bacterium production from pentane, etc. The model form is given in the following equation [10]:

$$\mu = \frac{\mu_{max}}{(K_s/S) + 1 + \sum_{j=1}^{n} (S/K_j)^j} \quad (3)$$

where K is the positive constant. Similarly, Edward (1970) proposed a kinetic model (Eq. (4)), which was the modified form of Haldane model. But he found that his model did not show better result as compared to Haldane model [11].

$$\mu_i = \mu_{max} \frac{S}{S + K_s + (S^2/K_{si})(1 + S/K)} \quad (4)$$

where K_{si} is the substrate inhibition constant (mg/L) and K is the constant. The model proposed by Luong (1987) as represented in Eq. (5), appeared to be useful for representing the kinetics of substrate inhibition. Though the proposed model is of generalized Monod type, but accounts for substrate stimulation at its both, low and high, concentrations. The model has the capability to predict the values of S_m, the maximum substrate concentration, above which the growth is completely inhibited [12].

$$\mu = \frac{\mu_{max} S}{K_s + S} \left[\frac{1 - S}{S_m}\right]^n \quad (5)$$

Han and Levenspiel (1988) proposed a model (Eq. (6)) to express substrate degradation rate. This model involves a delay function, which has an exponential form and incorporate the critical product or substrate concentration corresponding to the inflection point on the growth [13,14].

$$q = \frac{q_{max} S[1 - (S/S_m)]^n}{K_s + S - [1 - (S/S_m)]^m} \quad (6)$$

where q is the specific substrate degradation rate (h^{-1}), q_{max} the maximum specific substrate degradation rate (h^{-1}), S_m the critical inhibitor concentration (mg/L) above which the reactions stops, and m and n are the empirical constants.

In addition to the substrate inhibition model, the kinetics of *m*-cresol degradation by the mixed culture, were applied to growth associated, non-growth-associated kinetic models and three-half-order model which are used to describe degradation of organics by micro-organisms [15–17]. The form of these different types of models and their validity, in relation to initial substrate concentration, S_0, and half-saturation constant, K_s, is given in the following equation:

- Non-growth associated:

$$\text{Zero order}: \quad S = S_0 - k_0 t, \quad S_0 \gg K_s \quad (7)$$

$$\text{First order}: \quad S = S_0 \exp(-k_1 t), \quad S_0 \ll K_s \quad (8)$$

$$\text{Monod with no growth}: \quad K_s \ln \frac{S}{S_0} + S - S_0 = -k_2 t, \quad k_2 = \mu_{max} X_0, \quad S_0 \sim K_s \quad (9)$$

- Growth associated:

$$\text{Logarithmic}: \quad S = S_0 + X_0[1 - \exp(\mu_{max} t)], \quad S_0 \gg K_s \quad (10)$$

$$\text{Logistic}: \quad S = \frac{S_0 + X_0}{1 + (X_0/S_0)[\exp(K(S_0 + X_0)t)]}, \quad K = \frac{\mu_{max}}{K_s}, \quad S_0 \ll K_s \quad (11)$$

$$\text{Monod with growth}: \quad K_s \ln \frac{S}{S_0} = (S_0 + X_0 + K_s) \ln \frac{X}{X_0} - (S_0 + X_0)\mu_{max} t, \quad S_0 \sim K_s \quad (12)$$

- Three-half-order kinetic model:

$$y = \frac{1}{t} \ln \frac{S_0 - (S_0 - S + k_0 t) + k_0 t}{S_0} = -k_1 - \frac{k_2 t}{2}, \quad \text{where } P = S_0 - S + K_0 t \quad (13)$$

where k_0, k_1, k_2 = zero-, first-order and second-order rate constants, S_0 is the substrate concentration (mg/L) at time $t = 0$, and X_0 is the biomass concentration (mg/L) at time $t = 0$.

2. Materials and methods

2.1. Chemicals and reagents

m-Cresol used in the study was of an analytical grade, glucose and inorganic salts used in preparing microbial growth media were of reagent grade. All the chemicals and other reagents were purchased from Merck®, India.

2.2. Micro-organism and culture conditions

An indigenous mixed microbial culture, potent to degrade phenolic compounds including *m*-cresol, was isolated and enriched from a sewage treatment plant located in Guwahati, India. The isolation procedure as reported by Nuhoglu and Yalcin was adopted in this study [18]. The culture was initially grown in 250 mL Erlenmeyer flask containing 100 mL of mineral salt medium (MSM) having the composition (mg/L): $(NH_4)_2SO_4$ 230 g/L, $CaCl_2$ 8.0 g/L, $FeCl_3$ 1.0 g/L, $MnSO_4 \cdot H_2O$ 100 g/L, $MgSO_4 \cdot 7H_2O$ 100 g/L, K_2HPO_4 500 g/L, KH_2PO_4 250 g/L and glucose 2 g/L and pH 7.0 under agitation condition (150 rpm). The culture was then acclimatized, over a period of 1 month, to grow in MSM containing *m*-cresol as the sole carbon source up to a concentration of 1000 mg/L. The detailed acclimatization phase of the culture to degrade *m*-cresol is presented in Fig. 1.

2.3. Batch biodegradation study

All biodegradation experiments using the indigenous mixed microbial culture, acclimatized to grow using *m*-cresol as the sole