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Batch growth kinetic studies for elimination of phenol and cyanide using mixed microbial culture

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ABSTRACT

The efficiency of a mixed microbial culture to eliminate phenol and cyanide from aqueous solution was estimated in a batch process. The influence of initial concentration of phenol and cyanide on the microbial growth was calculated for the range of initial concentration between 50 and 1500 mg/L of phenol and 5–150 mg/L of cyanide. A maximum specific growth rate of 0.0663 h^{-1} was observed at 250 mg/L of phenol and 0.143 h^{-1} at 25 mg/L of cyanide concentration in the growth medium. The specific growth rate of the culture followed substrate inhibition kinetics. Monod, Haldane, Edward, Yano and Koga, Luong, Han-Levenspiel, and Powell growth kinetic models were used to determine the growth kinetics of mixed culture. Edward, Luong and Han and Levenspiel models were established as best fit to the specific growth rate data for phenol. However, for cyanide Haldane and Edward models were found to be best with lowest Marquardt's percent standard deviation (MPSD) values. The degradation kinetics of phenol and cyanide was found to follow a three-half order model at all initial concentrations with lowest MPSD value.

1. Introduction

Phenol and cyanide are considerably significant pollutants, according to the United States Environmental Protection Agency (USEPA) [1,2]. Industries like oil refinery (10–100 mg/L of phenol and 0–2.25 mg/L of cyanide), petrochemicals (6.42–88.03 mg/L of phenol and 0–15 mg/L of cyanide), coke oven plant (485 mg/L of phenol and 50 mg/L of cyanide) and steel manufacturing (342–487 mg/L of phenol, 4–15 mg/L of cyanide) etc., are generally responsible for discharge of phenol and cyanide in their effluents [3–5]. There are indications of serious environmental pollution owing to its poisonous nature towards human as well as marine life [6]. Owing to these causes elimination of phenol and cyanide from wastewater prior to final discharge into water bodies is mandatory. Degradation or removal of such toxicants from the wastewater completely has therefore turned out to be a stimulating assignment for the researchers working in the associated arena. In contrast to with conservative treatment methods like adsorption on activated carbon, coagulation and oxidation process; biological treatment techniques have revealed a better presentation for handling such pollutants for removal from wastewater [1,6]. Biological decontamination of phenol and cyanide by microbes has numerous advantages over chemical and physical treatment methods comprising of the capacity to convert toxic products into non-toxic by-products and the low cost of treatment methods [3,7].

A large amount of information is available in literature on the biodegradation of phenol and cyanide from mono substrate system by pure bacteriological cultures [3,7–22]. However, biodegradation of phenol and cyanide from binary substrate system using an indigenous mixed bacterial consortium, is a comparatively less reported aspect. In mono substrate system, during biodegradation of pollutant, the growth inhibition of biomass takes place owing to the higher substrate toxicity. This inhibition by substrate reduces the biomass growth rate and biomass yield.

Generally the effluents from industries comprise a mixture of pollutants posing difficulties in their degradation. This is due to the fact that degradation of different compounds require different values of operating conditions. Additionally, one substrate may inhibit the degradation of other substrate(s) existing in the same effluent. This interaction is due to toxicity of compounds and antagonism effect for microbial enzymes [23].

For this reason, it is essential to study the effect of substrates on their biodegradation as well as their interaction with one another. In binary substrate system the rate of biodegradation remains slow, generally owing to competitive inhibition, and increased toxicity of substrate(s) [20,24]. Several kinds of substrate interaction arrangements together with non-competitive inhibition and competitive inhibition have been evaluated in a binary substrate system [25].

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Phenol and cyanide interferes with the metabolism of growing microbial cells thereby inhibiting their further growth. Microbial cultures of *Pseudomonas putida* MTCC 1194 and *Serratia odorifera* MTCC 5700 have reported to be very effective for treatment of phenol and cyanide bearing wastewaters [3,10,26]. However, the literature on simultaneous biodegradation of phenol and cyanide, using a mixed bacterial consortium is rare. Thus, a study was considered to observe the removal of phenol and cyanide from wastewaters by mixed consortium cultures of bacterium *P. putida* MTCC 1194 and *S. odorifera* MTCC 5700. The objective of the current study was to evaluate the growth kinetics of mixed culture and simultaneous degradation of phenol and cyanide in batch process.

2. Materials and methods

2.1. Chemicals and reagents

Phenol and cyanide used in the experimentation were of analytical grade, inorganic salts and glucose used in microbial growth media were of reagent grade with more than 99% purity. These chemicals and other reagents were procured from Himedia Laboratories Pvt., Ltd., Mumbai, India. The pH of the solutions was regulated using dilute HCl and NaOH.

2.2. Growth medium and microorganism

An indigenous mixed microbial culture of *P. putida* MTCC 1194 and *S. odorifera* MTCC 5700, effective in degradation of phenol and cyanide was used. *P. putida* MTCC 1194, was supplied by Microbial Type Culture Collection, Chandigarh, India. The strain *S. odoriferra* MTCC 5700 was isolated from coke waste water in laboratory by Agarwal and Balomajumder [10]. The mixed culture was firstly grown in 250 ml flask comprising 100 mL of mineral salt medium, containing the composition (mg/L): Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl, $MgSO_4{\cdot}7H_2O$ and Glucose at pH 8. The batch treatment of phenol and cyanide was achieved under aerobic condition. Prior to culture growth, the medium was steam sterilized in an autoclave at 121 °C for 20 min at 15 psi pressure. For biodegradation studies, same medium accompanied with phenol and cyanide was used.

2.3. Estimation of biomass and substrate concentrations in the sample

To evaluate biomass growth, optical density (OD) measurement method was used. The concentration of biomass in the sample was observed by evaluating the absorbance of turbid medium at 600 nm wavelength by using a UV–vis spectrophotometer (HACH DR 5000). Microbial cells were grown overnight and the flasks were removed at pre-determined time intervals, to study the biomass growth. 2 ml sample volume was taken from flasks and centrifuge at 10000*g* for 10 min at ambient conditions. The pellet thus obtained was washed with the double distilled water two times and completely dried in an oven. After vaporization of water, dry biomass was weighed and appropriate dilutions were obtained. OD_{600} of the biomass was then transformed to dry cell weight through a calibration curve, achieved by plotting absorbance at OD_{600} versus dry cell weight of biomass. Each experiment was repeated three times and the average values were used to get true experimental values. Phenol and cyanide concentrations in biomass free supernatant were analyzed quantitatively at 510 nm for phenol and 520 nm for cyanide using the colorimetric method using 4-aminoantipyrine and picric acid, respectively using a UV–vis spectrophotometer methods [27].

2.4. Batch biodegradation experiments

All batch biodegradation experiments were accomplished in 250 ml flask with working volume of 100 ml containing mixed culture in mineral salt medium and phenol and cyanide as sole source of carbon and nitrogen, respectively. Initial concentration of phenol and cyanide was varied from 50 to 1500 mg/L and 5 to 150 mg/L, respectively. Phenol and cyanide biodegradation was carried out in an orbital shaker (Metrex, MO-250, India) at 30 °C and 120 rpm 2 mL inoculum was added to every flask initially. The freshly acclimatized mixed culture was directly transferred from liquid growth medium broth to mineral salt medium comprising phenol and cyanide at various concentrations. Treated samples were collected at consistent time intervals, and centrifuged (10,000*g* for 10 min) for measuring biomass concentration. The supernatant was analyzed for remaining phenol and cyanide concentration. The triplicate set of experiment was used to obtain average results.

The biodegradation efficiency was calculated according to following equation:

$$\text{Biodegradation efficiency}(\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where, C_i and C_f are the initial and the instantaneous concentrations of the phenol and cyanide.

2.5. Modeling of the growth kinetics and biodegradation kinetics

In this study, the growth and biodegradation kinetic models were solved by using nonlinear least squares methods using MS Excel 2012 software. Following section summarizes various models used, the details of which are well described by Costa et al. [28].

3. Kinetic models

3.1. Microbial growth kinetics

The kinetic substrate inhibition models have been used to explain the growth of microbial culture in the presence of phenol and cyanide in this study.

The specific growth rate of microbial culture at different initial concentration of phenol and cyanide were designed by the subsequent equation:

$$\mu_s = \frac{\ln(x_2 - x_1)}{(t_2 - t_1)} \quad (2)$$

where, x is the biomass concentration (mg/L), at time t (h), and μ_s is the specific growth rate (h^{-1}) [29].

Further, the specific substrate degradation rate (q, h^{-1}) was determined by the subsequent equation

$$q = -\frac{\ln(x_2 - x_1)}{(S_1 - S_1)} \quad (3)$$

where, x and S are the biomass and substrate concentrations, respectively in mg/L at time t (h) [30].

The Monod and Haldane models have been extensively used to describe the growth kinetics of micro-organisms [31,32]. These model equations are based on the specific growth rate, but may also be correlated to the specific substrate degradation rate [32–34].

The Monod's model relates the concentration of substrate to the specific growth rate of microorganism by the subsequent equation [35]:

$$\mu_s = \frac{\mu_{max} S}{K_s + S} \quad (4)$$