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Treatment of wastewater by mixed bacterial consortium in continuous reactors packed with solid waste

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ABSTRACT

Phenol is one of the priority pollutants as declared by EPA (USA). Very few comparative studies have been carried out with continuous packed bed reactors (PBR) for bacterial degradation of phenol and applying the result in real time situation. The present study compared the performance of a co-current and a counter-current continuous PBR in degrading phenol by a mixed consortium of bacteria isolated from the East Calcutta Wetlands based on both rate of phenol degradation and phenol removal efficiency. Different process parameters like air flow rate, hydraulic retention time and temperature were varied for both the systems. Performance of the co-current continuous PBR was found to be more efficient. To validate the performance of this system in actual application in industry a real time phenolic industrial effluent was fed to this system. Phenol degradation rate was 1.43 mg/l min and the phenol removal efficiency of this co-current continuous PBR was 72.85%.

1. Introduction

In developing countries like India urbanization and industrialization go hand in hand. The necessity for prolific industrialization is undeniable. Setting up of industry in a region leads to an overall development of the region primarily by establishment of townships in and around the industrial belt. The bulk methods for treatment fail to reduce the levels of specific organic and inorganic pollutants to meet the environmental regulations. As a result, the surface water gets contaminated with organic and inorganic pollutants discharged by industrial effluents and due to seepage the ground water is also contaminated. Industries like petrochemicals, coal coking, coal gasification, tanneries etc discharge phenolic effluent into the environment [1,2]. Ground water contamination may occur due to leakage in the gaskets and bearings of the pipelines carrying phenolic wastewater [3]. Phenolic compounds are aromatic pollutants which are toxic to human health. Exposure to phenol above the safety limit leads to central nervous system disorder, myocardial depression, hypothermia, renal failure, hepatitis damage, gastrointestinal problem and even blindness. Phenol is carcinogenic too [4]. Phenol is the representative of all phenolic compounds and the safety limit of phenol concentration in effluent is set as 0.168 mg/l by EPA (USA) [5].

To reduce the phenol concentration present in industrial effluent within safety limit degradation of phenol by both chemical and biological treatments are prevalent. The chemical treatments like adsorption, stripping are though fast are expensive. Moreover, degradation of phenol chemically leads to formation of toxic intermediates. On the other hand, biological treatments are economic, could process a varied range of phenol concentration and more importantly lead to complete mineralization of phenol [2,6–8].

Aerobic and anaerobic bacteria, fungi had been acclimatized to degrade hydrocarbons and it had been reported that a mixed bacterial consortium was more efficient in degrading phenol as compared to its constituent bacteria degrading individually [9–11]. Majority of the work done in phenol degradation by biological method considered batch process [12,10,11] and concentrated primarily on kinetic parameter evaluation. Very few works [13,3] studied the continuous mode of operation in phenol degradation by mixed culture. In this work both co-current and counter-current continuous phenol biodegradation processes were studied and compared.

Micro-organisms for biodegradation of phenol were mostly sourced from industrial sewage water in previous works [14]. The present study had used soil microbes from a novel site for this purpose. The site was known as East Calcutta Wetlands (ECW, 22°24′N-22°36′N; 88°32′E, 12,500 ha in area) and was declared as a Ramsar site on 19th August 2002 by Ramsar Convention Bureau. Since 1868 ECW acted as the most important urban and industrial waste treatment and recycling site of Kolkata, a major metropolitan city of India. It was the dumping ground of domestic waste of the city as well as the industrial sewage effluent from the industries at the outskirts. The soil bacteria of ECW were expected to have a resistance against organic pollutants as this area was receiving a mixed waste for a long time and was thus rich in

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biodiversity.

The degradation rates and removal efficiencies of phenol by these soil microbes under various mean hydraulic retention times, air flow rates and temperature conditions were observed and process optimizations were done for both co-current and counter current processes. Moreover, the performance characteristics under the optimized conditions were determined against real time industrial effluent.

2. Materials and methods

2.1. Chemicals and reagents

Glucose and all the inorganic salts used in preparation of MS media were reagent grade. All other chemicals used were of analytical grade. All these chemicals and other reagents for analytical study were purchased from Merck, India.

2.2. Collection and maintenance of samples

An indigenous mixed microbial culture expected to have potential to degrade phenol and phenolic compounds were collected from a sewage-based agricultural field of the East Calcutta Wetlands, India. 10gm of collected soil sample was suspended in 0.8% saline water and incubated at 32 °C overnight in a shaker at 100 rpm. Then the soil suspension was centrifuged at 3000 rpm for 3 min. The supernatant containing the mixed microbial cells was added to nutrient broth and cultured overnight for enhanced growth in nutrient rich media at 32 °C. The mixed culture was maintained on nutrient agar slant and stored at 4 °C for future use.

2.3. Micro-organisms and culture conditions

Inoculum was prepared in nutrient broth with the microorganisms preserved in nutrient agar slant. 24-h old culture was inoculated into MS media (10%, v/v) in an Erlenmeyer flask. The composition of MS media was (mg/L): KH_2PO_4:680, K_2HPO_4: 1730, $FeSO_4$:30, NH_4NO_3:100, $CaCl_2$: 20, $MnSO_4$:30 supplemented with glucose. Glucose in the MS media was then gradually replaced by increasing concentration of phenol till phenol served as the sole source of carbon. By 16 s rDNA sequence data analysis it was found that *Psychrobacter* sp., *Stenotrophomonas maltophilia, Bacillus subtilis* and *Escherichia coli* were present in the mixed culture [15].

2.4. Reactor, packing and immobilization procedure

Two 1000 ml bench scale reactors with aspect ratio (H:D) of 10:1 made of Borosil glass packed with claychips and designed to function in co-current and counter-current modes respectively were used as packed bed reactors (PBR). One reactor had an inlet port 110 mm from the base of the reactor and the other had an inlet port 15 mm from the base of the PBR. There were two outlet ports at 25 mm and 50 mm from the base of each PBR respectively. Oxygen was supplied from the lower end of the reactors from a compressed air source at varying flow rates from 0 to 3 Lpm. The air flow rate was controlled and measured by a rotameter. The rate of aeration was sufficient to maintain saturated DO (dissolved oxygen) level (4.5 mg O_2/L) in the reactor medium so as to assume growth not to be limited by DO level. Schematic diagrams of the reactors are given in Fig. 1a and b.

The clay chips used here as the packing materials were broken pieces of earthen tea cups used in Kolkata(India) by tea vendors to serve tea. The cups were non-biodegradable and caused environmental pollution as bulk garbage. The used tea cups were collected from the local tea vendors, washed and dried under the sun. Then they were broken into smaller pieces and similar sized clay chips were selected for packing the reactor. The void volume (ε) was 0.81707 and the equivalent diameter, D_p, was calculated from the following formula:

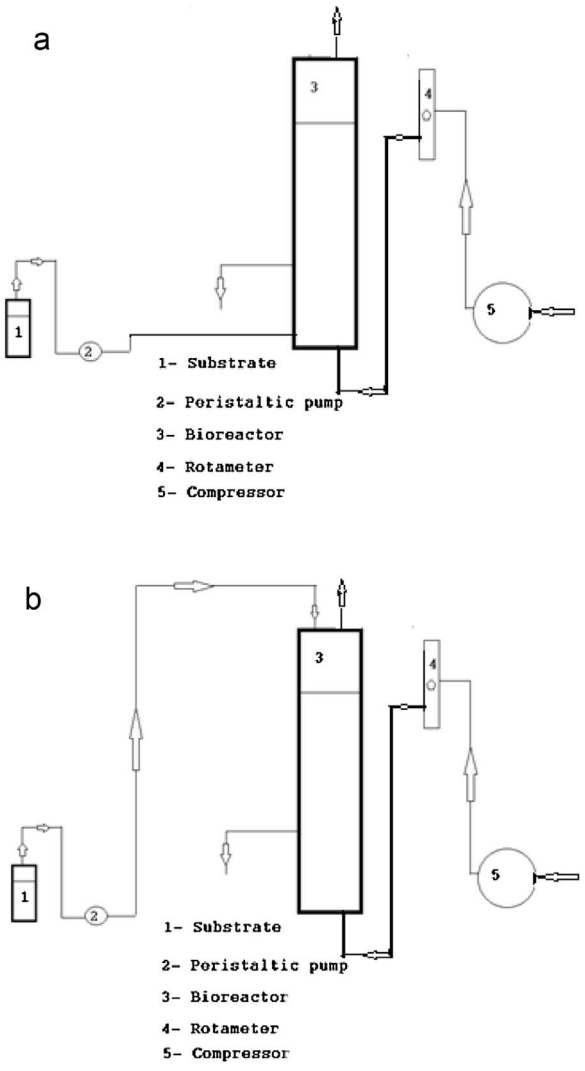

Fig. 1. (a) Schematic diagram of continuous co-current reactor system.(b) Schematic diagram of continuous counter current reactor system.

$D_p = \sqrt[3]{(6\,V_p/\pi)}$, where V_p = mean volume of a chip in cubic centimeter. The value of D_p was found to be 1.08 cm. The bed volume was 80% of the total reactor volume.

2.5. Scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS) study

SEM and EDS were done with clay chips, bacteria immobilized clay chips to obtain their topographical characterization and mineral composition. SEM photographs were taken after coating the samples with palladium by JFC-1600 Autofine Coater with a scanning electron microscope (JSM 6360) using 17 kV. EDS was done to ascertain the in carbon percentage using instrument INCA-MICS, 01736-03-04.

2.6. Process description for removal of phenol from synthetic media and real time industrial effluent

The phenol acclimatized mixed bacterial culture was suspended in 800 mg/l of phenol in MS media. The phenol was the sole source of carbon for the mixed culture. The mixed culture was allowed to grow in phenol media for 24 h. The optical density of this 24h-old cell suspension was measured at 600 nm by UV-VIS spectrophotometer (Shimadzu). This mixed bacterial culture was circulated through the PBR with the help of peristaltic pump at the rate of 6 ml/min for 12 h