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Phenol removal from industrial wastewater using chitosan-immobilized Pseudomonas putida

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Abstract

The present study deals with degradation of phenol in industrial wastewater using Pseudomonas putida. Biodegradation process at various initial phenol concentrations ranging from 50 to 200 mg/l was evaluated at different conditions. Phenol removal as single source of carbon at initial phenol concentration of 200 mg/l took place within 22 days. Phenol/ glucose mixture used as dual system to improve phenol degradation. The presence of glucose as supplementary substrate degraded phenol at initial concentration of 200 mg/l within 19 days. Acclimated Pseudomonas putida was able to degrade phenol at initial phenol concentration of 200 mg/l within 15 days. It was also revealed that phenol degradation using acclimated Pseudomonas putida immobilized on chitosan was carried out at the shortest period of time in contrast to the other conditions. The obtained results represented that microorganism was able to consume phenol as a substrate.

Keywords: Acclimation, Chitosan, Immobilization, Phenol Biodegradation, Pseudomonas putida

1. Introduction

Rapid accumulation of chemical contaminants in biosphere harshly endangers human health and environment [1-2]. Among these recalcitrant chemicals, phenolic compounds are identified as substances with high stability and toxicity. They are known as aromatics in which a hydroxyl group is attached to a benzene ring [3-4]. Phenolic compounds are commonly present in various industrial waste streams such as oil refining, resin and plastic, coal gasification plants, coke oven, pulp and paper plants, pharmaceutics and agro-industrial units [5]. The ingestion of phenol in the human body resulted in serious health risk such as protein degeneration, paralysis of the central nervous system and liver and kidney damage [6]. The presence of phenol in

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water leads to formation of noxious ploychlorinated phenols. These compounds impart taste and odour to water, even at low concentration of 2.0 µg/l [7-9]. It is also lethal to aquatic life in concentration in the range of 5–25 mg/l [10]. Due to high toxicity, phenol is regarded as high priority water pollutant [11] and is the most effective organic pollutants listed by U. S. EPA [1, 12]. Therefore, it is considered necessary to degrade phenol before discharging into the water stream. Conventional treatment methods of wastewater containing phenol and their compounds can be classified as physical and chemical treatments [13]. In recent years, biological phenol removal emerges as an efficient alternative because these processes produce no toxic end products and results to mineralize organic pollutants in an environmental and economical way [14]. There are many reported in literature on aerobic degradation of phenol by wide variety of fungi and bacteria cultures [15, 16]. However, *Pseudomonas putida* has been attracted much interest because of biodegradation of phenols [17, 18]. Immobilization of microbial cells is one of the approaches which enhance microbial cell stability, increase the possibility of the microbes to deal with the stock load [19] and prevent the biomass-liquid separation requirement. Various matrices with the aim of bacterial biomass entrapment have been also reported in the literature for immobilization of microbes [20]. Chitosan is an alkaline deacetylated of chitin which attracted much notice on removal of phenolic compounds because of its high bulk density, hydrophilicity, biocompatibility and significant biodegradability. The chitosan matrix show porous enough to allow phenol ions to be freely transported through the matrix which may be due to the presence of active sites such as amino ($-NH_2$) groups in the chitosan. In the present paper, olive mill wastewater (OMW) produced during olive oil production was selected as a case study. Different conditions were employed to phenol biodegradation. First, cell growth and phenol degradation as single source of carbon were monitored. Then, biodegradation of phenol in the presence of glucose as co- substrate was examined. Phenol biodegradation using acclimated bacteria was also evaluated. Finally, acclimated *Pseudomonas putida* immobilized on chitosan were used to degrade phenol. In all experiments, initial phenol concentration varied from 50 to 200 mg/l with interval of 50.

2. Materials and methods

2.1. Microorganism and media

Pseudomonos putida (PTCC 1694) species which degrades phenol was obtained from Persian Type Culture Collection (PTCC), Tehran, Iran. The stock culture of the bacteria was stored in nutrient broth agar at 4 °C. The culture was cultivated and enriched in 1000 ml of a growth medium contained: 1.5 g K_2HPO_4, 0.4 g KH_2PO_4, 2.0 g yeast extract, 0.3 g glucose, 0.0015 g $CaCl_2$, 0.4 g $(NH_4)_2SO_4$. The OMW as a source of phenol was provided from Olive Oil factory from north of Iran. Chitosan (degree of deacetylation ≥75%, pH 7), calcium chloride, sodium hydroxide, and acetic acid were supplied by Sigma-Aldrich (Germany). All other chemicals used for the experiments were analytical graded and provided by Merck (Darmstadt, Germany).

2.2. Experimental System and Analytical Techniques

To recover the activity of the stock culture, one loop of bacteria from the culture-contained agar was transferred to 20 mL of the nutrient broth in a flask. The flask was then incubated in an incubator-shaker (Stuart, S1500, UK) at 26℃ and 180 rpm. All experiments were carried out in a 250 ml Erlenmeyer flask that contained 100 ml of the media. The media were autoclaved and inoculated with a fresh culture where the size of inoculums was 5% (v/v) [21]. For the acclimatization the bacteria with phenol, 5mL of the bacteria-laden growing medium was transferred into a 100 mL serum bottle containing 50 mL of growing medium with 50 mg/L of phenol. The serum bottle was then maintained at 26℃ in an incubator-shaker at 180 rpm. To culture the acclimatized bacteria, 5 mL of the medium was then transferred into a 250 ml serum bottle which contained 100

ml growing medium with 50 mg/l of phenol. Again, the serum bottles were incubated at pervious mentioned conditions. Finally, the medium was centrifuged at 2000×*g* for 15 min, and the centrifuged pellets were harvested for further use [21]. Phenol concentration was determined by a direct photometric method using 4-amino antipyrine as the color reagent according to standard methods of analysis [22]. Before quantifying phenol, samples were centrifuged (Hitachi Universal 320R, UK) at 5000 rpm for 10 min. The calibration curves were prepared and the incorporated data were found linearly fitted with regression value of 0.98. Biomass concentration in the samples was determined by measuring its absorbance at 600 nm wavelength using a UV–Visible Spectrophotometer (Unico, 2100 series, USA) (data are not shown). For immobilization of *Pseudomonas putida*; first, the culture at exponential growth phase mixed with sodium alginate 3% (w/v) solution. Then, the mixture solution (sodium alginate–culture) was dripped into a bath of chitosan–$CaCl_2$ solution at room temperature using a syringe. Once the slurry was added to the bath, beads of chitosan/alginate with entrapped cells were formed (4mm) [23].

3. Results and discussion

The experiments were done at different conditions. First, Cell growth and phenol biodegradation as single substrate system was evaluated. Then, Biomass concentration and phenol biodegradation were monitored in the presence of glucose as Supplementary Substrate. The effect of using acclimated bacteria to phenol on phenol degradation was also investigated. Acclimation process save microbial cells from inhibitory and toxicity effects [24]. Finally, acclimated bacteria were immobilized on chitosan to improve phenol biodegradation. In all experiments, initial phenol concentration varied from 50 to 200 mg/l with interval of 50.

3.1. Culture growth and biodegradation of phenol as single substrate

Figures 1 and 2 show cell growth and concentration profiles based on phenol as single source of carbon, respectively. Initial phenol concentration varied from 50 to 200 mg/L with interval of 50. After inoculated the media with *Psudomonas putida*, phenol removal was tracked for 22 days. The samples were taken at time interval of 24 hours. As the Figure 1 depicts, the culture required at least 22 days for complete degradation of phenol. The required time duration for full phenol removal with initial concentration of 50, 100, 150 and 200 mg/L was 11, 16, 19 and 22 days, respectively. The trend of phenol concentration variation is depicted in Figure 2. Generally, phenol is highly toxic and recalcitrant compound; therefore, it resists to biodegradation showing a prolonging degradation period. However, it is biodegradable in general.

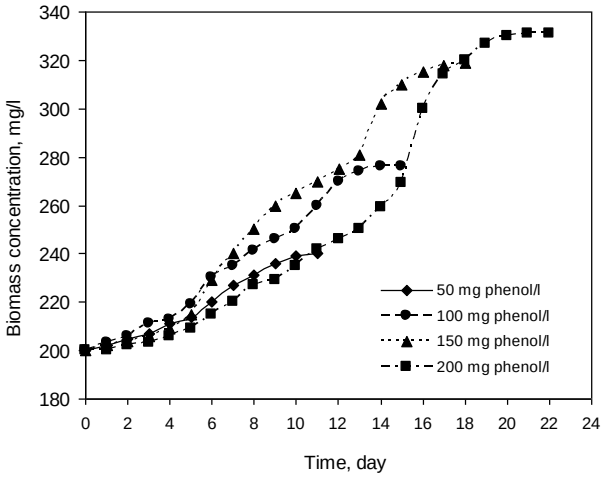

Figure 1. Cell growth with defined initial phenol concentrations while phenol is a single substrate.

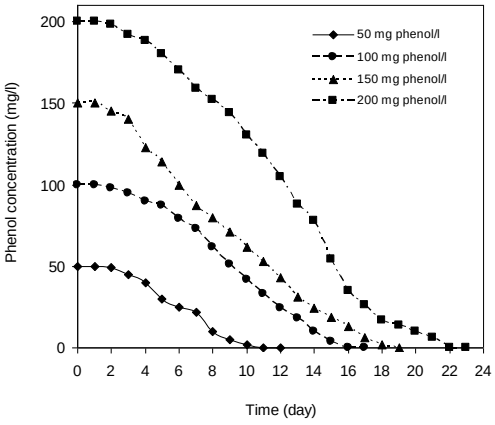

Figure 2. Phenol degradation as single substrate with defined initial phenol concentrations.

3.2. Culture growth and biodegradation of phenol on dual substrates system

Cell growth and phenol biodegradation were monitored at various phenol concentrations ranging from 50 to 200 mg/l in the presence of glucose at a constant concentration of 2000 mg/l. Glucose is a co- substrate for bioaugmentation [25]. The samples were also taken at time interval of 24 hours. It can be monitored (Figure 3) that complete degradation of phenol in the presence of glucose as an easy biodegradable substrate took place in shorter period of time rather than the single substrate system. This is in agreement with Tay et al. [26] which found that adding 2 g/L glucose improve the phenol removal. The employed mixed culture was able to degrade phenol at concentration of 200 mg/l within 19 days.

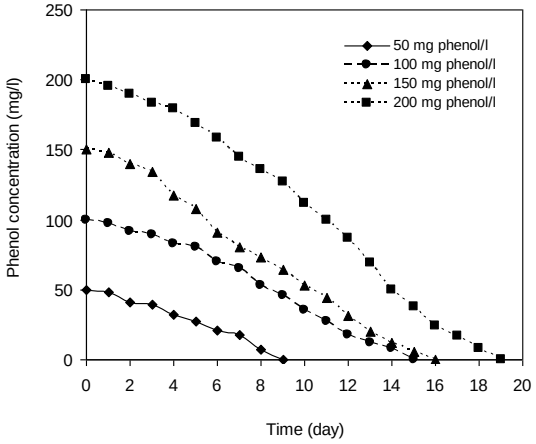

Figure 3. Phenol degradation with respect to incubation time for initial phenol concentrations in presence of 2000 mg/ l glucose in a dual substrate system**.**

Biomass concentration was yielded by utilization of glucose and phenol as dual substrates. It emerges that cell growth was related to consumption of glucose at initial days of degradation process since a significant growth primarily occurred (Figure 4). During exponential phase, microorganism attained maximum growth (168 mg/l) at phenol concentration of 200 mg/l within 19 days.

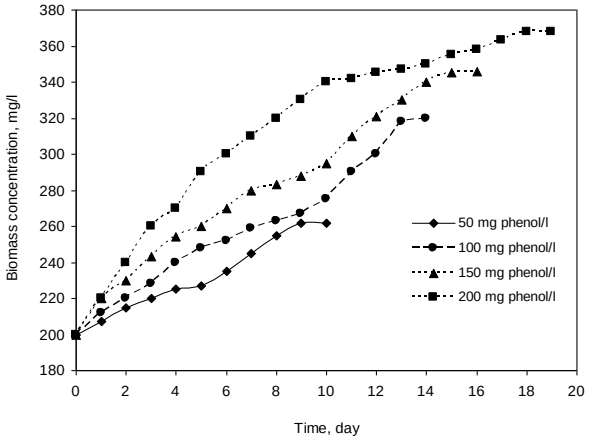

Figure 4. Biomass concentration profiles for initial phenol concentrations in presence of 2000 mg/ l glucose in dual substrates system**.**

3.3. Culture growth and biodegradation of phenol using acclimated microorganism on single substrates system

Figures 5 and 6 show phenol biodegradation and biomass concentration using acclimated bacteria, respectively at various phenol concentrations ranging from 50 to 200 mg/l. Adoption period can improve the system stability [27] and can also increase phenol biodegradation. Therefore, the media first acclimated with phenol as mentioned in section 2.2. After acclimatization the bacteria with phenol, experiments have been done for evaluating phenol biodegradation. The amount of phenol in the media was estimated at time interval of 24 hours. Time duration of phenol removal is reduced duo to adoption process. Phenol was degraded at time period of 6, 9, 13 and 15 days for initial phenol concentration of 50, 100, 150 and 200 mg/L, respectively. Because of adoption period, the bacteria growth was begun immediately and no lag phase was observed. With phenol degradation, the microorganism simultaneously reaches to the stationary phase in a short period of time. The maximum microbial growth (170 mg/L) was obtained at phenol concentration of 200 mg/L at the 14^{th} day of operation.

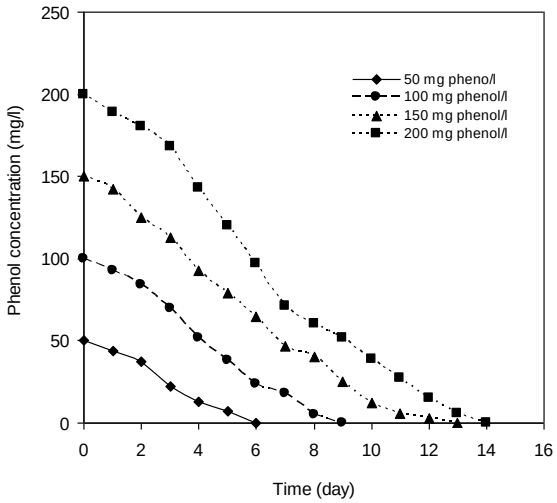

Figure 5. Phenol degradation with respect to incubation time for initial phenol concentrations using acclimated bacteria.

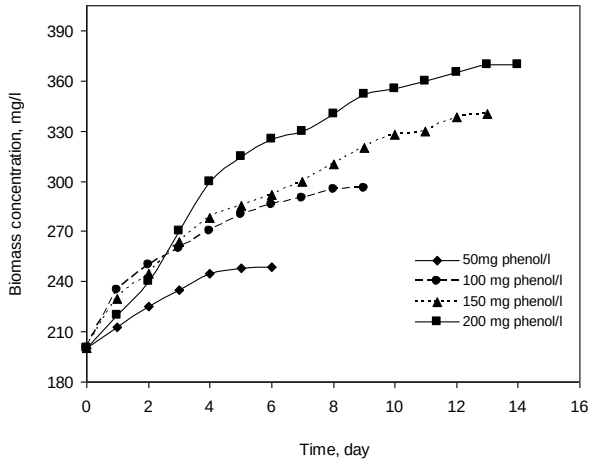

Figure 6. Biomass concentration profiles for initial phenol concentrations using acclimated bacteria**.**

3.4. Culture growth and biodegradation of phenol using acclimated Pseudomonas putida immobilized on chitosan

Taking into consideration the importance of immobilization on conquest of inhibitory effect of phenol [28], the acclimated microorganism was immobilized on chitosan. Figure 7 depicts the phenol biodegradation at initial phenol concentration ranging from 50 to 200 mg/L using acclimated bacteria immobilized on chitosan. The obtained results reveal that immobilization has great potential to improve the phenol degradation. Phenol degradation at initial concentration of 50, 100, 150 and 200 mg/l were achieved within 2, 4, 5, and 7 days, respectively.

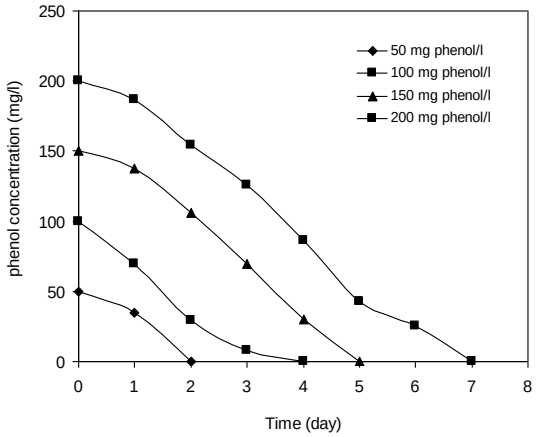

Figure 7. Phenol degradation with respect to incubation time for initial phenol concentrations using acclimated bacteria immobilized on chitosan.

4. Conclusions

The experiments have been done at different conditions. Phenol biodegradation as single source of carbon at initial phenol concentration of 200 mg/l took place within 22 days. It was observed that the existence of glucose as an easy biodegradable substrate has positively affected on phenol biodegradation process. Acclimated *Pseudomonas putida* was able to degrade phenol at initial phenol concentration of 200 mg/l within 15 days. The shortest period of time for phenol degradation was achieved using acclimated bacteria immobilized on chitosan. The Cells were able to grow by consuming phenol. It is also concluded that biological treatment can be a remarkable alternative instead of traditional processes such as chemical and physical treatments.

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