Biodegradation of phenol in a draft-tube spouted bed bioreactor with biomass attached to hydrogel particles

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Abstract

The performance of a draft-tube spouted bed bioreactor (DT-SBB) packed with hydrogel particles for biomass immobilization has been used to treat a phenolic wastewater in continuous mode. The biomass support particles were made of a cyclodextrin-based polymer and then seeded with an acclimated mixed culture. Due to the low density of such particles and also the bioreactor design, the pumping energy required to maintain a moving bed of the resulting bioparticles was very low and a recirculation flow was not necessary. The inlet phenol concentration and the phenol inlet loading rate were the operating variables studied. Phenol removal efficiency was used to monitor the process. The DT-SBB showed a high removal capacity (up to 2.8 kg-phenol/m^3d) with a high efficiency (>99%). The removal capacity of the DT-SBB was found to be limited by the availability of dissolved oxygen in periods with high phenol elimination rate. The removal efficiency decreased significantly for higher loading rates (>3.1 kg/m^3d) because of phenol inhibition. The evolution of the distribution of microbial populations was also investigated. A predominance of gram-negative bacteria (especially the genera *Pseudomonas* and *Acinetobacter*) was observed during the periods of maximum degradation.

Keywords: spouted bed bioreactor, draft-tube, cyclodextrin polymer, hydrogel biomass support, phenol aerobic degradation.

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1 Introduction

Phenol, as a basic chemical used worldwide, is a major pollutant present in effluents from chemical process industries. Low concentrations of aqueous phenol are harmful to the environment and human health (Saha *et al.* [1]). Conventional methods for treating phenolic wastewaters include physical-chemical processes which lead to secondary pollution. Contrarily, biological treatments remove phenol yielding innocuous end-products. In particular, biological techniques using microorganisms attached to inert solid particles usually provide higher biomass concentrations (and therefore higher removal rates) than those achieved with suspended microorganisms. Moreover, moving bed biofilm systems such as fluidized bed bioreactors promote good heat and mass transfer characteristics. This type of reactor generally requires the recirculation of part of the effluent in order to permit the movement of bed bioparticles. On the other hand, the lighter the support material the lower the energy required for fluidization.

The main aim of this work is to study the operation of a draft-tube spouted bed bioreactor (DT-SBB) with hydrogel beads as biomass carrier particles for wastewater treatment. Phenol was chosen as the model pollutant.

The spouted bed is a fluid-solid contacting technique somehow derived from fluidized beds, but with its own characteristics. The fluid (in this case air and wastewater) enters the vessel through a spouting nozzle at the base, and flows through the bed of solid particles (Fan *et al.* [2]). The resulting hydrodynamic pattern shows three distinct regions: an upward jet (spout), a downward moving-packed bed (annulus) and a characteristic fountain above the bed surface (Çeçen-Erbil [3], Cui and Grace [4]). Furthermore, in bioreactors, the shear forces exerted on the surface of the solids by the recirculation between the spout and annulus provides a good control of the growth of biofilm thickness (Livingston and Chase [5]). However, the use of spouted bed reactors is often limited by the existence of a maximum spoutable height or the largest height of particle bed which is possible to move in this contact regime. The inclusion of an internal draft tube solves this drawback and enhances the circulation pattern (Zhao *et al*. [6]).

Beads of a hydrogel material composed of β-cyclodextrin polymer (CDP) were used as biomass support particles. Since its swollen density is slightly higher than that of water, CDP beads can be fluidized with a very low energy requirement. The use of CDP as support for microorganisms has been previously reported in fluidized bed reactors (Sevillano *et al.* [7, 8]).

2 Material and methods

2.1 Experimental setup

A schematic of the experimental system is depicted in Figure 1. A conical-base spouted bed bioreactor (diameter 51 mm, height 0.70 m) made of glass (Afora, Spain) was equipped with a PVC draft-tube (diameter 16 mm, height 0.23 m).

The inlet spouting flow (for air, nutrients and phenol solution) was entered through a nozzle (diameter 8 mm) at the conical-base of the column. A clarifier was coupled to the bioreactor outlet in order to retain the detached biomass. The aqueous phenol and nutrient solutions were separately supplied by two peristaltic pumps (Masterflex L/S, USA). No effluent recirculation flow was used. The air flow rate was regulated by a flowmeter (Aalborg, USA). The DT-SBB was operated in continuous mode at room temperature. The air flow was kept enough to assure a proper oxygen saturation level in the liquid phase. The inlet concentration and flow rate of phenol (i.e., the inlet phenol loading rate) were the operating variables considered. The substrate loading rate is defined as the phenol mass flow per unit reactor volume. The biodegradation efficiency was calculated as the relative difference between the inlet and outlet phenol loading rates.

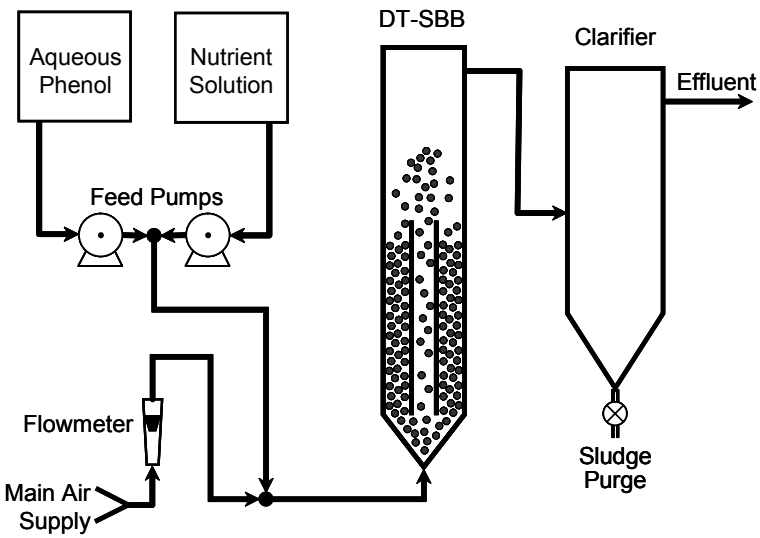

Figure 1: Schematic diagram of the experimental DT-SBB.

2.2 Moving media

Hydrogel CDP particles (nearly spherical in shape) were prepared by crosslinking β-cyclodextrin with epichlorohydrin. A detailed description can be found in Romo *et al.* [9]. A batch of CDP beads (bed height 19 cm) with a diameter interval of 1.25–1.60 mm and a swollen density of 1.06 g/mL was initially loaded into the reactor and then inoculated with an acclimated microbial mixed culture.

A synthetic wastewater with a variable phenol concentration (2.0–2.5 g/L in the feed tank) and equilibrated with a modified Brunner mineral medium (DSMZ Medium 457, Germany) was applied. In this case, the concentration of

ammonium sulphate was doubled compared to the standard recipe in order to minimize nitrogen limitation conditions.

2.3 Analytical methods

Phenol concentration was determined by spectrophotometry (Hewlett Packard 8452, USA) at 270 nm. Effluent samples were centrifuged (Heraeus 200, Germany) and filtered (0.45 μm), according to Goswami *et al.* [10], just before measuring the outlet phenol concentration. Dissolved oxygen was also measured in the effluent (Hanna Instruments HI91410, Italy).

2.4 Microbiological analysis

Bacteria within the microbial consortium before inoculation and from the bioreactor (attached to CDP beads and suspended in broth) were isolated by plating in general and selective agar media. In order to recover the maximum number and type of microorganisms, different general agar plates have been used: yeast agar, nutrient agar and plate count agar (Biolife, Italy). Selective media used were *Pseudomonas* agar (cetrimide) (Oxoid, UK) and *Burkholderia cepacia* agar (Microkit, Spain). In addition, selective plates of yeast agar supplemented with different quantities of phenol were used to identify the most resistant strains to pollutant (Puhakka *et al.* [11]). To determine quantitatively the viable cells in the samples, several decimal dilutions were placed on the surface of agar plates. After incubation at 30ºC for 24-72 h, plates containing between 30 and 300 colonies were selected for enumeration with a colony counter (Suntex 560, Taiwan). The characterization of microbial communities was carried out by biochemical assays. Individual colonies isolated from the phenol-degrading consortium were streaked on agar plates and incubated at 30ºC for 48 h. The identification of microbial isolates was performed by gram staining, enzyme assays (catalase and oxidase) and API test strips (BioMerieux API 20 NE, API 50 CH, France) (Prpich and Daugulis [12]).

3 Results and discussion

3.1 Biofilm growth

The biomass growth on the hydrogel particles in the DT-SB reactor fed with aqueous phenol can be observed in Figure 2. Although not well appreciated in the B/W photograph, the support beads were clear and transparent before microbial inoculation (day 0). Progressively, the appearance of the particles was getting darker as the solid surface was being colonized. Since the stagnant bed height scarcely increased after the whole colonization of the support media, the thickness of the growing biofilm remained quite stable throughout the study. This is a significant difference with fluidized bed bioreactors in which biofilm thickness can increase appreciably (Sevillano *et al.* [8]).

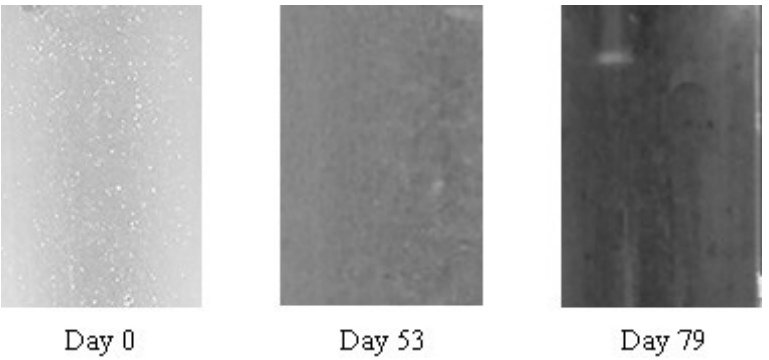

Figure 2: Biofilm colonization of bed particles in the DT-SBB.

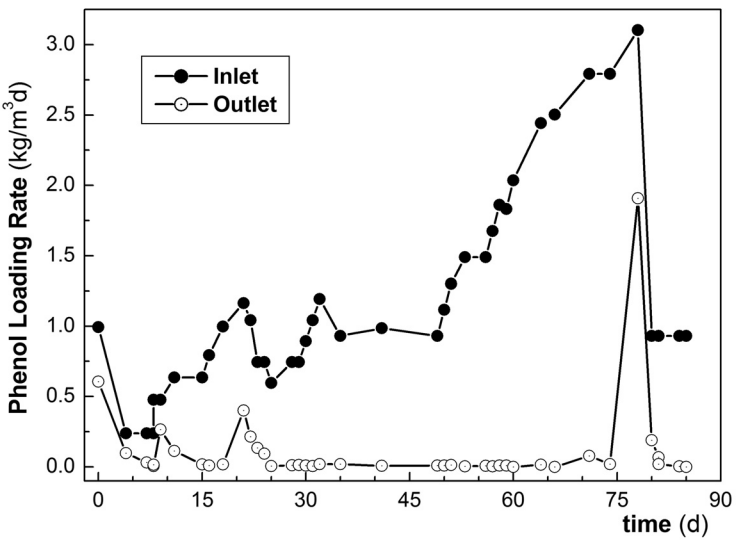

Figure 3: Phenol loading rates in the DT-SBB operation with CDP bioparticles.

3.2 Bioreactor performance

The reactor was operated continuously for three months. The inlet concentrations of phenol and nutrient solutions were deliberately kept high, and low the respective flow rates, in order to extend the feed supply capacity. Thereby, the values of hydraulic residence time were notably high (8–32 h) compared to those of real effluents, although the inlet substrate loading rate was as high as 3.1 kg-phenol/m^3d. The organic loading rates in the influent and effluent streams are

shown in Figure 3, whereas the outlet phenol concentration and the removal efficiency of the DT-SBB are plotted in Figure 4.

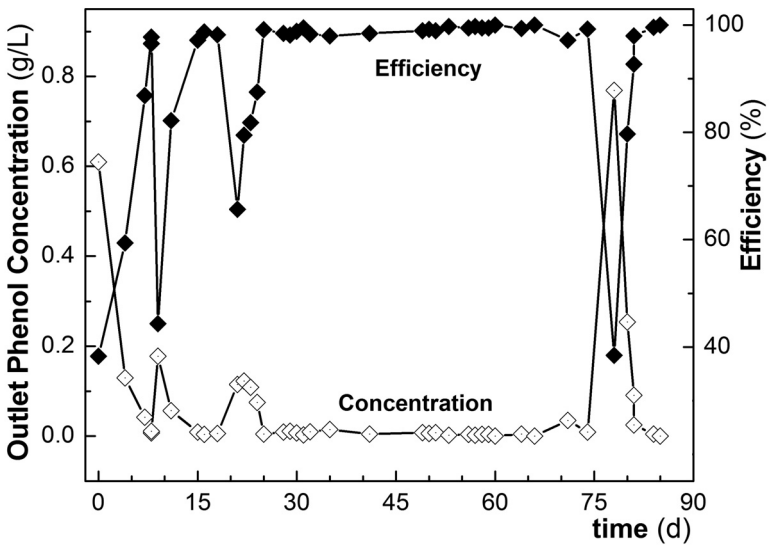

Figure 4: Outlet phenol concentration and elimination efficiency in the DT-SBB.

As can be observed, the DT-SBB worked with low inlet loading rate of substrate at the starting-up in order to promote the colonization of the support particles by microorganisms. At the end of the initial acclimation period, the system was treating about 1.0 kg-phenol/m^3d with a high removal efficiency (>95%). However, at the beginning of this acclimation period, the inlet load was so high that the system became oversaturated and high values of dissolved oxygen were reached.

From day 50 and on, the pollutant loading rate entering the bioreactor was progressively increased up to very high values while keeping the conversion efficiency high. This good bioreactor performance was maintained for nearly one month (until day 75). The maximum elimination capacity was achieved in the last week of this period (from day 70 to day 75) with 2.79 kg-phenol/m^3d and a removal efficiency higher than 99% (i.e., a phenol concentration of 19 mg/L in effluent).

By day 80, when the inlet loading rate had been raised up to 3.10 kg-phenol/m^3d, the removal capacity of the system was saturated and the removal efficiency decreased significantly (below 40%) with the outlet phenol concentration increasing up to 0.77 g/L. As expected, the stronger the substrate inhibition the higher the phenol concentration in the effluent. This behavior is characteristic of the metabolism of toxic compounds, such as was observed by Watanabe *et al.* [13] in an activated sludge process when the inlet phenol

concentration exceeded the upper limit of 2.0 kg/m^{3}d. Several factors could explain the difference in the maximum biodegradable phenol load between that reported and that determined in the present work. First, the DT-SBB is a fixed biofilm reactor and the other was a suspended biomass system, and it is well-known the larger resistance of the former compared to the latter (Sá and Boaventura [14]). Furthermore, the use of a hydrogel support with proven sorption ability towards phenol (Romo *et al.* [15]) would enhance the protection of the biofilm from shock-loading (Sevillano *et al*. [8]). After the saturation point was reached, as the phenol removal capacity dropped abruptly, the inlet load was reduced to 0.93 kg/m^{3}d in order to recover the process efficiency.

On the one hand, dissolved oxygen (DO) could have been a limiting factor in the DT-SBB performance. The DO concentration as measured in the DT-SBB effluent is given in Figure 5. An air flow rate range of 5.4–13.8 L/h was applied. The maximum air flow entering the bioreactor was limited because of the risk of elutriation of bed particles. The fluctuations in the DO concentration during the first three weeks were due to the decoupling between aeration and biological activity during the acclimation period. High values of DO concentration would be related to bad working conditions with poor degradation activity, whereas low DO values would be associated with high elimination capacity. Nevertheless, very low DO levels (close to zero) with high substrate removal rates would be showing a problem of oxygen mass transfer. Therefore, the improvement of the aeration system can play an important role in the enhancement of the DT-SBB performance.

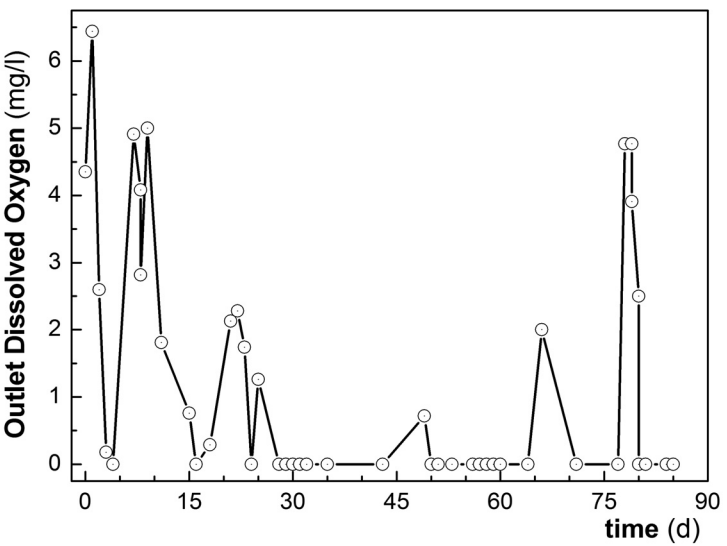

Figure 5: Dissolved oxygen profile in the DT-SBB effluent.

3.3 Microbiological studies

Table 1 shows the isolated microorganisms during the phenol adaptation phase before being inoculated into the DT-SBB. The predominant species present in the microbial consortium included both gram-positive and gram-negative bacteria, being the genus *Bacillus* the most abundant (about 80% of total).

Table 1: Identified microorganisms in the inoculum during the phenol acclimation period.

Isolate	Concentration (CFU/mL)	Characteristics
Bacillus circulans 1	$4.6x10^5$	Gram-positive, catalase-positive
Burkholderia cepacia	$6.7x10^4$	Gram-negative, oxidase-positive
Bacillus thuringiensis	$5.0x10^4$	Gram-positive, catalase-positive
Ochrobactrum anthropi	$2.3x10^3$	Gram-negative, oxidase-positive
Alcaligenes xilosoxidans	$1.1x10^3$	Gram-negative, oxidase-positive

The identification and enumeration of the microbial consortium within operational phase of the DT-SBB showed a strong selection of gram-negative bacteria, where genera *Comamonas* and *Acinetobacter* appeared to be the predominant (Table 2). As shown in Figure 3, the increase of the inlet loading rate of phenol, together with the hydrodynamic conditions of the DT-SBB, has a marked selective effect on the microbial community. None of the gram-positive bacteria identified in the acclimation period were isolated during the bioreactor operation.

Table 2: Identified microorganisms in DT-SBB samples.

Isolate	Concentration (CFU/mL)	Characteristics
Comamonas acidovorans	$2.1x10^7$	Gram-negative, oxidase-positive
Acinetobacter baummanni	$1.2x10^7$	Gram-negative, oxidase-positive
Ochrobactrum anthropi	$8.0x10^6$	Gram-negative, oxidase-positive
Burkholderia cepacia	$6.0x10^6$	Gram-negative, oxidase-positive

4 Conclusions

In conclusion, hydrogel particles of a cyclodextrin-based polymer have been shown to be an effective support medium for attached biomass in a draft-tube spouted bed bioreactor treating a phenolic wastewater. The shear forces caused by the spouting hydrodynamics on the bed particles exert a good control of biofilm growth while maintaining an efficient phenol biodegradation. In addition, since no water recirculation flow was necessary to move the bed due to the low support density, the DT-SBB operational costs should be smaller than

those of conventional fluidized bed bioreactors. Experimental results showed that this system could be used to satisfactorily treat effluents containing high phenol inlet loading rates (up to 2.8 kg/m^{3}d) with high removal efficiency (>99%). *Pseudomonas* and *Acinetobacter* were the predominant genera in optimal conditions of DT-SBB performance. Despite the high phenol removal efficiency achieved, more research is needed to improve the aeration efficiency in order to avoid oxygen-limiting conditions as well as any changes in microbial community by substrate inhibition.

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