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Treatment efficiency and biomass characteristics in conventional activated sludge and suspended PVA-gel biocarrier systems treating phenol-containing wastewater

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Abstract

Protozoa play a significant role in activated sludge systems, grazing on dispersed bacteria while supporting a healthy food web in biological wastewater treatment ecosystems. Operational parameters and influent properties are the most important factors affecting the composition of activated sludge microfauna. The objective of this study was to assess treatment efficiency and protozoan populations in activated sludge systems with and without the addition of polyvinyl alcohol (PVA)-gel beads treating synthetic wastewater containing phenol. Six reactors - three supplemented with PVA-gel beads – were used for the treatment of phenol at concentrations up to 10 mg/L. During the course of this study, dominance of species with known attachment characteristics was observed mainly in the reactors with PVA-gel beads. In addition, enhanced removal efficiencies of nutrients and organic loading were evident in the reactors with PVA-gel beads. Introduction of phenol at a concentration of 5 mg/L did not significantly affect the operation of the reactors and the composition and abundance of the microfauna, while a high phenol removal rate was maintained. However, an increase of influent phenol to 10 mg/L resulted in the formation of foam along with the appearance of *Zooglea* sp in the activated sludge reactor, while an increase in the abundance of crawling species was observed in the reactors with PVA-gel beads

Keywords: phenol, protozoa, microfauna, polyvinyl alcohol (PVA), wastewater treatment

1. Introduction

Activated sludge is a widely used process, based on the development of appropriate bacterial aggregates and other associated organisms in an aeration process. The use of ciliated protozoa found in activated sludge biocenosis, as indicators of operational efficiency, has been widely advocated (Madoni, 1994; Puigagut *et al.*, 2005; 2007; Salvado *et al.*, 2004; Nicolau *et al.*, 2005). Microfauna play an essential role in the whole process by removing dispersed bacteria, through grazing, which otherwise may result in highly turbid effluents. Moreover, microfauna reported to reduce sludge production (Rensink and Rulkens, 1997), affect the floc size distribution through predation activity (Puigagut *et al.*, 2007), and increase settleability of the mixed liquor in secondary sedimentation (Ratsak *et al.*, 1996). Protozoa have been used for the assessment of operational problems in wastewater treatment plants (Al-Shahwani and Horan, 1991; Fried *et al.*, 2000). Several studies have been reported addressing the correlation of protozoan populations to treatment parameters and effluent quality; these studies were based on monitoring the protozoan micro-fauna in municipal activated sludge plants (Al-Shahwani and Horan, 1991; Fried *et al.*, 2000; Esteban *et al.*, 1991; Chen *et al.*, 2004). In recent decades, Madoni (Madoni, 1994; Madoni *et al.*, 2000) probed the potential use of protozoa as indicators of wastewater treatment efficiency. The inter- and intra-species relationships have been categorized and quantified in order to comprise an index, the Sludge Biotic Index (SBI), which can be used to evaluate the quality and potential efficiency of the activated sludge in degrading influent pollutants. However, influent composition has also been considered as having an important role in the formation of microfauna.

Recent studies (Clauss *et al.*, 1998) have indicated that seeding a conventional activated sludge wastewater treatment reactor with fine mineral particles may enhance solid/liquid separation and nitrogen removal; successful settling has been reported in good colonized activated sludge (Puigagut *et al.*, 2005). These characteristics are dependent on factors such as retention time, oxygen concentration, predation/grazing effects, levels of filamentous bacteria and floc size (Liao *et al.*, 2006). The use of various types of supporting materials in conjunction to activated sludge processes, such as gels, activated carbon or plastic carriers, may enhance the retention of effective biomass by the formation of biological aggregates, containing several levels of organization, acting as porous or protective matrixes where the colonized cells are defined as biofilms (Costerton *et al.*, 1995). Increased resistance to xenobiotic substances in comparison to free bacterial cells in suspension is a well-known characteristic of the biofilm structure (Steward, 2002; Sfaelou *et al.*, 2015a). This property is of particular importance since the extracellular polymeric substances (EPS) barrier appears to function through sorption and/or reaction of matrix components with the toxic substances, resulting in the protection of bacteria in activated sludge flocs and biofilms. The interaction between the toxic compounds and the negatively charged residues in bound EPS protein may also bind cationic species of heavy metals, leading to a reduced toxicity potential, due to the metal becoming less bioavailable (Guibaud *et al.*, 2005; Comte *et al.*, 2008). Another advantage of biocarriers is the formation of an anoxic zone that facilitates nutrient removal (Sfaelou *et al.*, 2015b).

The objectives of this research were to conduct a study addressing: a) the composition and response of the activated sludge protistan community to the exposure of various phenol concentrations, b) the potential effects of phenol on the efficiency of an activated sludge process, c) the correlation of these responses and effects on the effluent quality, and d) the ability of the activated sludge microfauna to remove phenol using polyvinyl alcohol (PVA) biofilm carriers, versus a conventional activated sludge setup.

2. Materials and methods

2.1. Operation of the reactors

Six 2–L glass beakers were used as the bench-scale activated sludge reactors (reactors R and RB) at room temperature. Continuous aeration was provided by three air pumps using two air diffusers in each reactor. Start-up of the reactor was conducted by the addition of 250 mL of activated sludge collected from the aeration tank of a full-scale activated sludge unit. Three reactors (reactors RB) were supplied with 100 mL of PVA gel beads (4 mm diameter spheres with a solids content of about 10%, porosity of 90% and specific gravity of 1.025; Kuraray Co., Tokyo, Japan). The effective specific surface area of PVA was shown to be 2500 m^2/m^3, using a calibrated mathematical model to simulate data from a moving-bed biocarrier reactor systems (Levstek *et al.*, 2010). The characteristics of each bench scale reactor are shownin Table 1. The pH of each system was 7.5–8.5. The synthetic wastewater used in the study was composed of sodium acetate CH_3COONa 2.472 g/L (PANREAC) as carbon and energy source, while NH_4Cl 60 mg/L (PANREAC), Na_2HPO_4 18.7 mg/L (PANREAC), $MgSO_4 \bullet 7H_2O$ 100 mg/L (MERCK) and $NaHCO_3$ 1.3393 gr/L (PANREAC) were used as the required nutrients. In order to obtain a nutritionally balanced wastewater, the composition of the synthetic wastewater was adjusted to yield a COD/N/P ratio of 100/5/1.5 with initial content of COD of 1200±50 mg/L, TN = 60±3 mg/L and P = 18±2 mg/L (Kargi *et al.*, 2005).

The reactors were initially fed with synthetic wastewater containing phenol in order for the activated sludge microorganisms to be acclimatized to the corresponding experimental conditions. Prior to the addition of phenol in the synthetic wastewater, acclimatization of the activated sludge microorganisms to the batch conditions and to the new influent composition was achieved. The activated sludge microorganisms obtained from the municipal wastewater treatment plant were used under continuous-feed conditions with a different carbon source than that of the synthetic wastewater; thus, acclimatization was necessary.

Table 1. Reactor characteristics (R reactors operated under activated sludge, RB reactors operated under activated sludge and PVA-gel beads)

Reactor	Activated sludge 250 mL	PVA-gel beads (100 mL in each reactor)	Influent phenol concentration, mg/L
R1	+		1
R2	+		5
R3	+		10
RB1	+	+	1
RB2	+	+	5
RB3	+	+	10

The duration of the acclimatization period was about 10 d, by the end of that period microorganisms were able to perform about 90% removal of the organic load and over 85% removal of phosphorus and nitrogen content of the synthetic wastewater. After the acclimatization period, phenol at the planned concentrations was added to the synthetic wastewater and operation of the reactors was monitored as a function of time (up to 60 d).

Each system was operated in cycles of 5 d. At the end of each cycle, the sludge was settled by turning off the air pumps and 400 mL of the supernatant was withdrawn for quantitative analyses. Subsequently, 400 mL of fresh synthetic wastewater containing the planned phenol concentration was added and operation was restored.

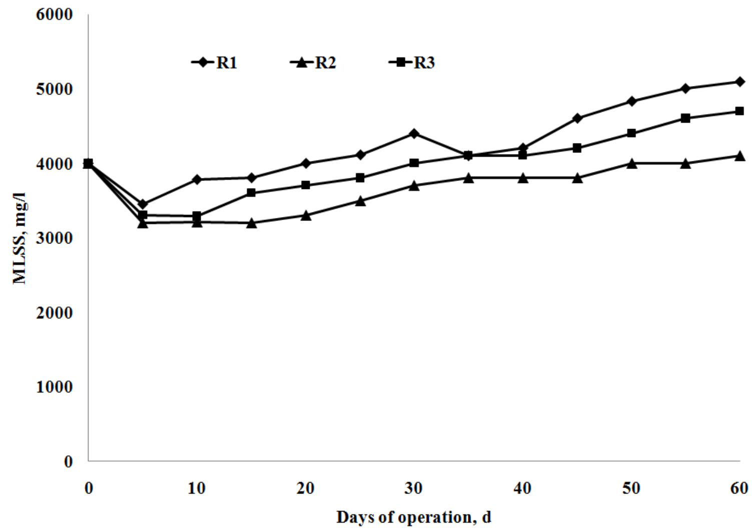
2.2. Physicochemical analysis and microfauna observations

Samples of each reactor were analysed for MLSS, COD, ammonia nitrogen, phosphates, and phenol concentrations in accordance with Standard Methods (APHA, 1997). For analysis of the protozoan community, aliquots of 200 μL were collected from each reactor at different time periods. Analyses were conducted for the identification of species *invivo* according to standard methods using an optical microscope (OPTIKA) at 10x, 40x, and 100x magnification (Lee *et al.,* 1972). Small flagellates were counted by placing the samples on a Fuchs–Rosenthal 3.2-μL chamber. Identification and quantification of the protistan community was conducted in order to calculate the Sludge Biotic Index (SBI) using a two-way table (Madoni, 1994).

3. Results and discussion

3.1. Physicochemical characteristics of the reactors

The bench-scale activated sludge systems were operated for a total period of 60 d, in order to evaluate the effect of phenol on operation performance. The MLSS content in each system as a function of time is presented in Figure 1. It is considered that the initial more pronounced drop in MLSS concentrations in the reactors with the PVA-gel beads (Figure 1.B) was due to the suspended biomass (or some part thereof) attaching to the PVA-gel biocarrier matrix. With that sorption demand being met, the suspended biomass, having first access to applied substrates, began to grow, gradually catching up with the activated sludge levels in the reactors without biocarrier addition (Figure 1.B).



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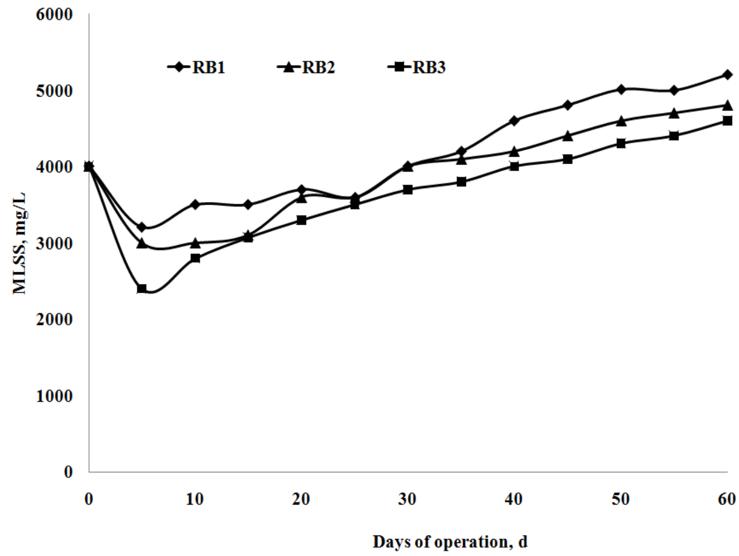
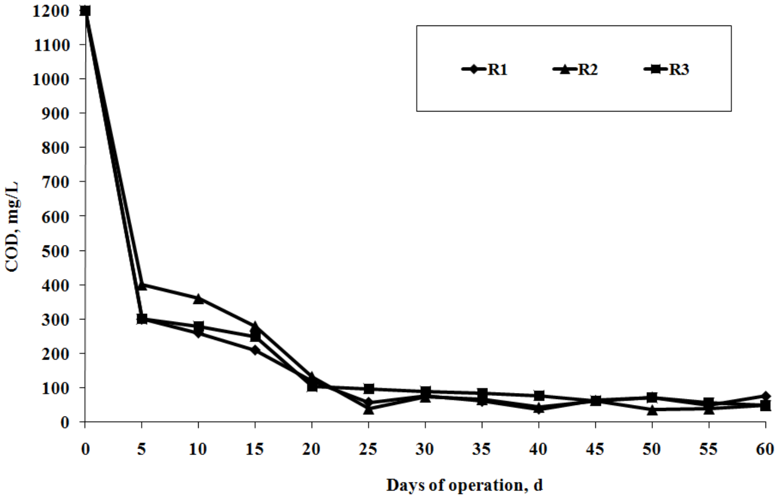


(b)

Figure 1. MLSS concentrations in reactors as a function of operation time: A) activated sludge B) activated sludge with PVA-gel beads

The highest MLSS values of 5200 mg/L was observed in reactor R1 operating with activated sludge, while in all reactors the MLSS was increasing with increase of operation time. The increase of MLSS concentration in both types of reactors with time could be attributed to the increased ATP synthesis, which resulted in increased amounts of ATP that could be used as an additional energy source by activated sludge microorganisms stimulating their growth (Papadimitriou *et al.*, 2010). COD values (Figure 2) were measured in the supernatants from each system during the initial stages of operation. However, effluent COD values decreased with time possibly due to metabolic adaptation of the microorganisms to the corresponding phenol concentration, and the efficient utilization of the carbon source by the sludge microfauna.



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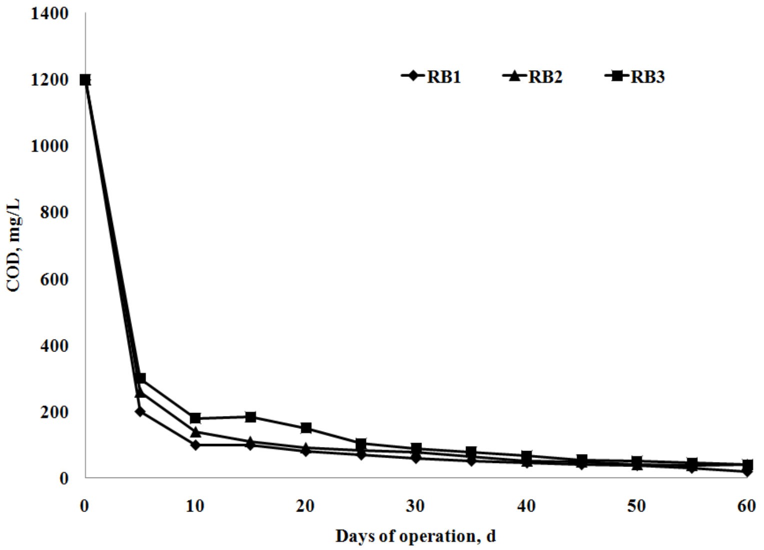
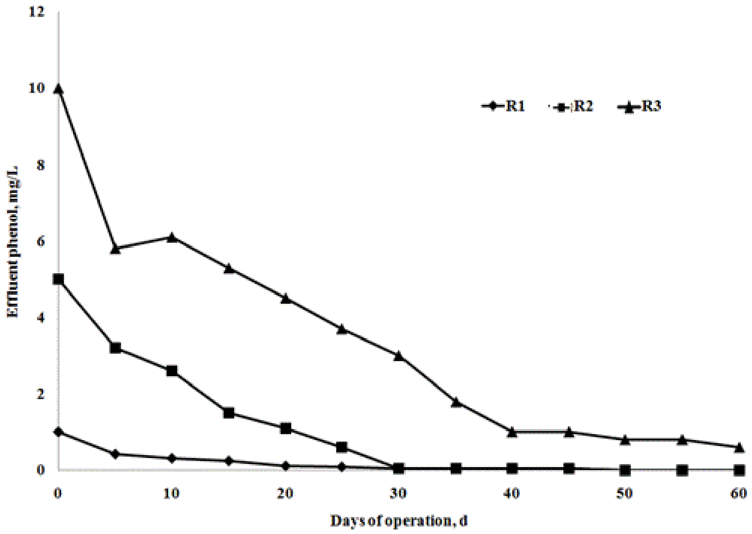


(b)

Figure 2. COD effluent concentration in reactors as a function of operation time A) activated sludge B) activated sludge with PVA-gel beads

Ammonia was removed in percentages over 80% in both types of reactors without pronounced differences between the reactors, while an inhibition of the nitrification process was observed with the higher influent phenol concentrations (data not shown). Initially, the efficiency of the systems in removing phosphates was generally low in all reactors but after some time, the phosphate effluent values decreased significantly to 1.9 mg/L and 0.5 mg/L for the R3 and RB3, respectively. The efficient removal of phosphates could be attributed to sodium acetate that was the sole carbon source in the synthetic wastewater. Sodium acetate may take part in the poly-hydroxy-butyrate synthesis during the anoxic (anaerobic) phase of a wastewater treatment plant for generation of large amounts of ATP, associated to enhanced phosphate uptake, which in turn is used in polyphosphate formation during the oxic phase (Kargi *et al.*, 2005).



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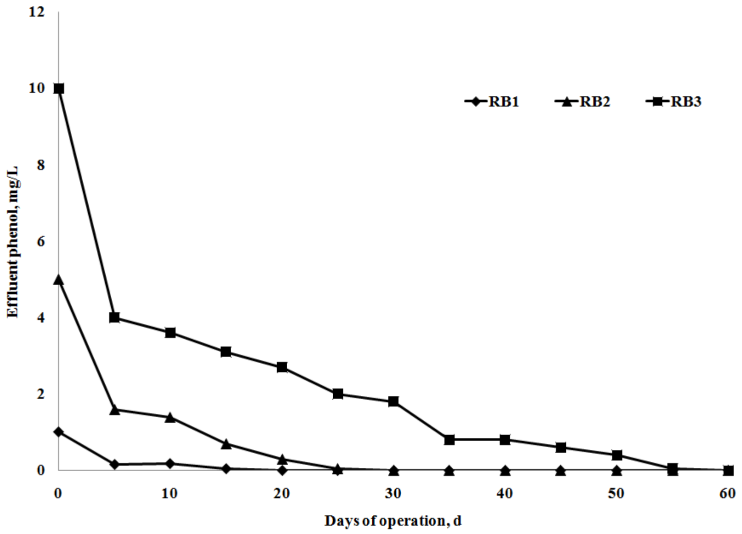

(b)

Figure 3. Effluent phenol concentrations in reactors as a function of operation time: A) activated sludge B) activated sludge with PVA-gel beads

Phenol effluent concentrations were proportional to the influent concentrations as in all cases the reactors operating with 10 mg/L phenol influent exhibited higher effluent concentrations (Figure 3). Additionally, both type of reactors presented negligible effluent phenol concentrations after 30 d of operation. However, the reactors supplied with PVA-gel beads presented significantly lower effluent concentrations since the early stages of operation.

3.2. Microfauna observations

Sessile species were more abundant in the reactors supplied with PVA-gel beads (RB) than the reactors with only activated sludge (R), while species such as *Vorticella* and *Opercularia* were present in both systems occupying 30% and 42% of the total sessile species, respectively. Carnivorous species and free-swimming ciliates were more abundant in the case of R reactors than in the RB reactors. *Aspidisca sp* showed increased abundance in the RB system. In the activated sludge reactors (R), *Podophrya* occupied a significant part of the protistan microfauna, while in PVA-gel bead supplied reactors (RB) *Tokophrya sp* co-dominated in comparable abundances with *Opercularia sp*, with *Litonotus sp* to occupy greater part of the activated sludge protistan community. The addition of 5 mg/L phenol in the influent did not affect the removal efficiencies of the reactors and no effects were observed in the composition of microfauna. *Arcella sp* was present in enhanced populations in all reactors. *Carchesium sp* was particularly profound in reactors supplied with PVA-gel beads. The presence of certain species of the microfaunal community in the aeration tanks of activated sludge plants reflected the diversity of the influent composition. *Arcella* sp presented negative correlation to nitrate nitrogen and MLSS content, but positive factor for SS and BOD_5 removal capacity. The concentration of suspended solids in the mixed liquor presented a poor correlation to all species, with values varying between – 0.184 to 0.155 for bench scale units (Madoni *et al.*, 1994). Nitrate – nitrogen content and nitrification capacity appeared to be best correlated to protozoan composition with correlation factors ranging from -0.755 up to 0.110, while an efficient correlation was also found for effluent suspended solids concentration with values from – 0.421 up to 0.355 (Papadimitriou et al 2011). Most of the sessile (*Carchesium polypinum*, -0.317) and swimming ciliates (*Colpidium campylum*, -0.377) in the bench reactors correlated to BOD_5 removal capacities (Papadimitriou *et al.*, 2011). Indicating that these protozoa may be considered as indicators of good performance of an activated sludge plant treating high strength wastewater. In all reactors, the addition of 10mg/L influent phenol resulted in sludge foaming and increase of *Zooglea sp*.

4. Conclusions

The operation of the activated sludge systems and the corresponding microfauna development was examined in this study during treatment of synthetic wastewater with the addition of phenol. The concentrations of phenol added in the influent had a direct effect on the activated sludge microfauna in both cases of reactors. In all reactors, the addition of 10 mg/L influent phenol resulted in sludge foaming and increase of Zooglea sp. In the reactors supplied with PVA-gel beads (RB), the activated sludge microfauna was dominated mainly by sessile species which were favored by the surface areas on the PVA-gel beads. Sessile species tend to form more stable community food webs within the community of activated sludge microorganisms, thus enhancing the biodegradation process. Removals of organic compounds and nutrients were not significantly affected by the addition of low phenol concentrations, however the increase of phenol to 10 mg/L decreased these removal efficiencies. The reactors supplied with PVA-gel beads presented significantly lower effluent phenol concentrations since the early stages of operation compared to the conventional activated sludge setup.

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