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Treatment efficiency and sludge characteristics in conventional and suspended PVA gel beads activated sludge treating Cr (VI) containing wastewater

C.A. Papadimitriou[a,*], H.K. Karapanagioti[b], P. Samaras[c], G.P. Sakellaropoulos[a]

[a]*Chemical Process Engineering Research Institute and Department of Chemical Engineering, Aristotle University of Thessaloniki, P.O. Box 1520, 54006 Thessaloniki, Greece*
Tel. +30 2310 498 254; email: papadim@cperi.certh.gr
[b]*Department of Chemistry, University of Patras, 26500 Patra, Greece*
[c]*Department of Pollution Control Technologies, Technological Educational Institute of W. Macedonia, 50100 Kozani, Greece*

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ABSTRACT

Although activated sludge microbial communities are considered stable, the presence of toxic substances, such as Cr, in the influent may induce changes in the activity and the performance of a wastewater treatment plant. The main objective of this study was the determination of Cr effects on the performance and the protistan community of an activated sludge. Six laboratory scale activated sludge reactors, three conventional and three with PVA gel beads, were supplied with synthetic sewage containing Cr (VI), at three concentrations: 1, 5, and 10 mg l^{-1}. The protozoan species were identified, quantified, and correlated to each system's efficiency through microscopic observations. Additionally, the extracellular polymeric substances (EPS) in the form of proteins and polysaccharides were determined. High removal rates of organic compounds (90%), ammonia–nitrogen (95%), total phosphorus (80%), and chromium (90%) were observed even at high Cr dosages. The abundance and diversity of the protistans were observed under all Cr loadings. Based on the composition of the protistan community, sludge biotic index (SBI) values decreased at the start of the operation but increased gradually with operation time. Chromium addition affected the relative protistan community by shifting from sessile to carnivorous species with an increase of influent chromium concentration.

Keywords: Activated sludge; Biofilm; Chromium; Extracellular polymeric substances (EPS); Protistan; Polyvinyl alcohol (PVA) gel bead; Biocarrier; Sludge biotic index (SBI)

1. Introduction

The activated sludge process is based on the enrichment of bacteria and other microorganisms in an aeration tank; these organisms form aggregates and are easily separated from the aqueous phase during a subsequent sedimentation phase. These flocs have been shown to have multi–layered, porous structures that form around smaller aggregates or micro-colonies. The binding between the different levels may be due to the hydrophobic properties of extracellular polymeric substances (EPS) [1,2,3] or multivalent cation bridging [1]. EPS are large molecular weight compounds produced by active bacteria, cell lysis, hydrolysis or from other factors in the wastewater source [1,2,4]. Bacteria can also produce EPS to protect themselves from the presence of xenobiotic substances. Additionally, aerobic aggregation of the suspended flocs is a phenomenon that is frequently observed in sequential batch reactors (SBR) [5]. Ciliated protozoa play an essential role in the overall process by removing dispersed bacteria through grazing, which otherwise may result in high turbidity in

*Corresponding author.

the effluent [6,7]. The presence of toxic substances in the influent may induce changes in the whole food web of the activated sludge ecosystems affecting their activity and the performance of the wastewater treatment plant.

Chromium is a common pollutant found in industrial effluents; chromium salts are used in several industries such as tanneries, electroplating, textile, dyeing, and metal finishing industries. Chromium may exist in the trivalent [Cr (III)] and hexavalent [Cr (VI)] state. Hexavalent chromium compounds (chromates and dichromates) are highly toxic and are considered as mutagens and carcinogens. Due to the severe toxicity of Cr (VI), the agency for toxic substances and diseases registry (ATSDR) classifies it as the sixteenth most hazardous substance [8].

Various studies [9] exhibited differences in treatment efficiency of activated sludge following shock loads from a wide range of toxic substances. The morphology, composition, and sensitivity of activated sludge can change in the presence of various toxic substances, which can inhibit floc formation and other operational parameters.

The use of various types of supporting materials in conjunction to activated sludge processes such as gels, activated carbon, or plastic carriers, may enable the formation of biological aggregates containing several levels of organization acting as porous substrates where cells are embedded on the matrix [10]. Such structures are defined as biofilms [10]. One of the most well known properties of the biofilm structure is its increased resistance to xenobiotic substances in comparison to free bacterial cells in suspension [11]. This property is of particular importance since the EPS barrier appears to function through sorption and/or reaction of matrix components with the toxic substances, thus protecting the 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 their reduced toxicity potential as the metal becomes less bioavailable in comparison to systems without significant quantities of EPS [12,13].

Despite extensive research on microbial reduction of chromium (VI) by pure cultures [14,15], data on chromium reduction by activated sludge are limited. Some studies reported the use of activated sludge microorganisms or dried waste sludge for metal removal from aqueous solutions [16]. Imai et al. [17] reported the reduction of chromium (VI) to chromium (III) in the dissolved phase of a batch activated sludge system. Stasinakis reported the ability of activated sludge to reduce chromium (VI) to chromium (III) in a range of concentrations between 0.5 and 10 mg l^{-1} [18]. In addition, they investigated the reduction of chromium (VI) in a continuous activated sludge system. For chromium (VI) concentrations between 0.5 and 5 mg l^{-1}, activated sludge could be used for bioremediation of chromium containing wastewaters. However, to the best of our knowledge, no other studies were conducted on the effects of hexavalent chromium on the treatment efficiency of the activated sludge and its effects on sludge structure and composition, comparatively with and without the addition of polyvinyl alcohol (PVA) gel bead biocarriers. The objectives of this work were to: (a) study the composition of an activated sludge protistan community at various chromium concentrations, (b) investigate the effect of chromium on the efficiency of an activated sludge process, (c) correlate the responses and effects to the effluent quality, and (d) compare the ability of the microfauna to remove chromium in an integrated activated sludge process containing a PVA biocarrier with that of 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 with a mixed liquor suspended solids (MLSS) content of 5.5 g/l. 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 moving bed biocarrier reactor systems [19]. The characteristics of each bench scale reactor are shown in Table 1. The pH of each system was 7.5–8.5. The reactors were initially fed with synthetic wastewater containing chromium in order for the activated sludge microorganisms to be acclimatized to the corresponding experimental conditions. Prior to the addition of chromium 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 to continuous feed conditions and to different carbon source than that of the synthetic wastewater. 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 the phosphorus and nitrogen content of the synthetic wastewater. After the acclimatization period, chromium in the desired concentrations was added to the

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

Reactor	Activated sludge 250 ml with Mixed Liquor Suspended Solids (MLSS) content of 5.5 g/l	PVA beads (100 ml in each reactor)	Influent Cr (VI) concentration, $mg\,l^{-1}$
R1	+		1
R2	+		5
R3	+		10
RB1	+	+	1
RB2	+	+	5
RB3	+	+	10

synthetic wastewater and the operation of the reactors was monitored as a function of time (up to 60 d).

Each system was operated in cycles of four days. 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 desired chromium concentration was added and the operation was restored.

2.2. Composition of the synthetic wastewater

The synthetic wastewater used was prepared by the addition of sodium acetate CH_3COONa (PANREAC) as the carbon and energy source; NH_4Cl (PANREAC), Na_2HPO_4 (PANREAC), $MgSO_4{\cdot}7H_2O$ (MERCK) and $NaHCO_3$ (PANREAC) as nutrient sources. Trace minerals such as NaCl (PANREAC), ($BAKERCaCL_2{\cdot}2H_2O$ (MERCK) and $FeCL_3{\cdot}6H_2O$ (MERCK) were added in the feeding solution [16]. 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 an initial COD content of $1200 \pm 50\ mg\,l^{-1}$, TN = $60 \pm 3\ mg\,l^{-1}$ and P = $18 \pm 2\ mg\,l^{-1}$. The chromium source was potassium dichromate that was prepared as a stock solution at a concentration of $10\ g\,l^{-1}$ and was kept in the refrigerator at 4°C.

2.3. Physicochemical analysis and microfauna observations

Samples of each reactor were analysed for MLSS, COD, ammonia nitrogen, phosphates, and chromium concentrations; all parameters were measured in accordance with Standard Methods [20]. For the analysis of protozoan community, aliquots of 200 μl were collected from each reactor at different time periods. Analysis was conducted for the identification of species in vivo according to standard methods using an optical microscope (OPTIKA) at 10x 40x and 100x magnification [21]. Small flagellates were counted by placing the sample on a Fuchs–Rosenthal 3.2 μl chamber. Identification and quantification of the protistan community was performed in order to calculate the sludge biotic index (SBI) value using a two-way table [22].

2.4. Analysis of extracellular polymeric substances

EPS were extracted from activated sludge obtained from both reactors. All samples were analyzed in triplicates. The process of extraction was performed in four steps, using a modification of the respective methods presented in the literature [23]:

Step 1: Washing (recovery of slime material). About 0.05 g of the activated sludge was placed into centrifuge tubes along with 10 ml of distilled water and gently shaken prior to centrifugation at 3500 rpm for 10 min.

Step 2: Striping (recovered capsule—bound material). The liquid was decanted from the centrifuge tubes and collected as slime material. Solids were resuspended in 0.85% NaCl solution and then centrifuged again. Liquids from the two washing steps were combined.

Step 3: EDTA extraction. A 10 ml dose of 10% EDTA was added to 10 ml of the combined sample and left for 3 h at 4°C and then centrifuged at 6000 rpm for 30 min.

Step 4: Filtration and collection of EPS. The supernatant was filtered in order to remove free cells.

The EPS quantified in the form of proteins and carbohydrates. Proteins were analyzed by the modified Lowry method. Quantification was performed at 650 nm using bovine serum albumin as a standard [24]. Carbohydrates were analyzed by the anthrone method as total carbohydrates. Quantification was performed at 625 nm using glucose as a standard [24].

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 days, in order to evaluate the effect of chromium on the operation performance. The MLSS content in each system as a function of time is presented in Fig. 1. The highest MLSS values, $3500\ mg\,l^{-1}$, were observed in the reactors R without the addition of PVA beads at the highest chromium influent concentration ($10\ mg\,l^{-1}$). All three reactors RB with the addition of PVA seem to level of at lower values than all reactors R (1500–3500 mg/l). The increased MLSS concentration in activated sludge systems could be attributed to the increased ATP synthesis, which resulted in increased

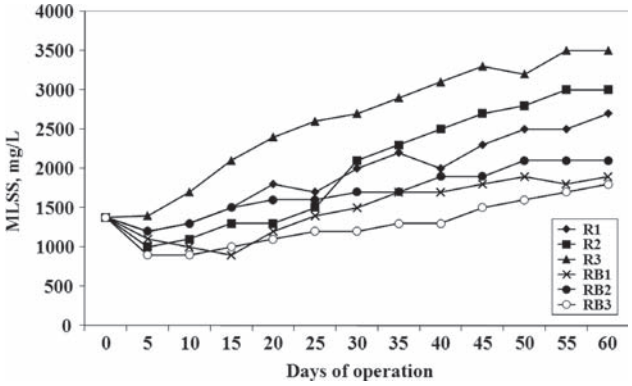

Fig. 1. MLSS concentration in all reactors as a function of operation time.

amounts of ATP that could be used as an additional energy source by activated sludge microorganisms stimulating their growth.

Additionally, due to the continuous aeration of the aerobic activated sludge reactors, chromium ions may catalyze extended oxidation of the synthetic wastewater substrate, producing energy of combustion in excess of the energy available at normal conditions resulting in increased MLSS content [25].

The removal efficiencies of COD, ammonia–nitrogen, and total phosphorus with time are shown as average values for the reactors without (R) and with (RB) the presence of PVA biofilms in Figs. 2A and 2B, respectively. COD values were measured in the supernatants from each system during the initial operation stages. However, effluent COD values decreased with time possibly due to metabolic adaptation of the microorganisms to the corresponding chromium concentration, and the efficient utilization of the carbon source by the sludge microfauna.

Nitrification was more efficient after the 10th d of operation in all systems supplied with PVA gel beads (Fig. 2B); however fluctuations in the effluent concentration of $N–NH_3$ were observed in the cases of the reactors operating only with activated sludge (Fig. 2A). Generally, in this study, the nitrification process was not affected by the addition of high chromium concentrations. However, other researchers [18] noticed that the addition of 1 mgl^{-1} Cr (VI) in an activated sludge system slightly decreased the growth of heterotrophic nitrifiers, while significant inhibition was observed at Cr (VI) concentrations exceeding 5 $mg\,l^{-1}$, although the microbial Cr (VI) removal was not affected.

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 reaching up to 0.7 $mg\,l^{-1}$ and 1.2 $mg\,l^{-1}$ for

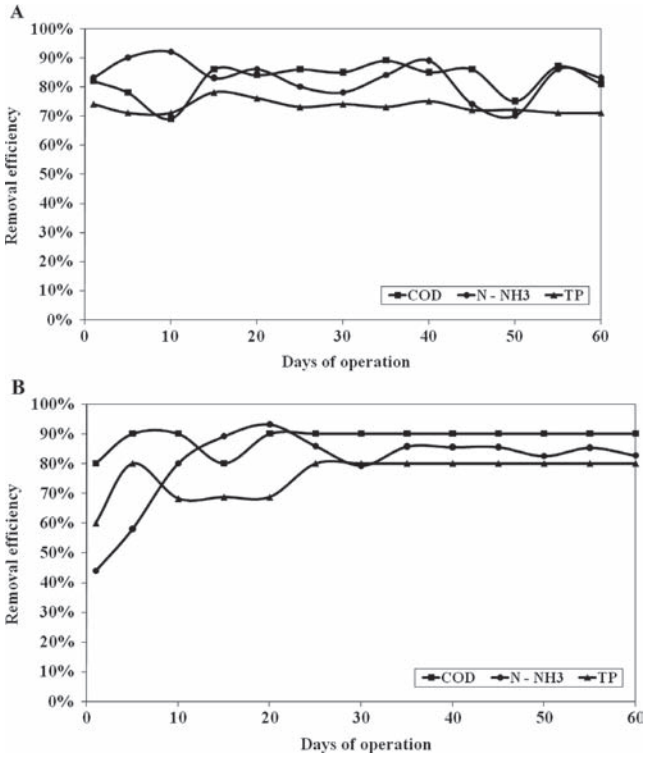

Fig. 2. Removal efficiencies of COD, ammonia—nitrogen ($N-NH_3$) and total phosphorus (TP) with time are shown as average values for the (A) activated sludge reactors (R) and (B) biofilm reactors (RB).

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-hydroxybutyrate synthesis during the anoxic (aerobic) 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 synthesis during the oxic phase [26].

In general, chromium removal during the initial operation time was low but then, at longer operation time chromium removal was enhanced (Fig. 3). As a result chromium removal rates that were measured exceeded in all cases 30% at extended operation times; even in the system treating the highest chromium concentration (10 $mg\,l^{-1}$), low effluent chromium concentration was observed at prolonged operation time. The corresponding acclimatization time depended upon the influent chromium: the highest the influent chromium content the longest the acclimatization period. Moreover from our results it is indicated that RB reactors exhibited lower Cr (VI) effluent concentrations at the initial stages of operation. Although some studies exhibited the ability of PVA attached biofilms to perform copper biosorption [28], the ability of the biofilms to perform

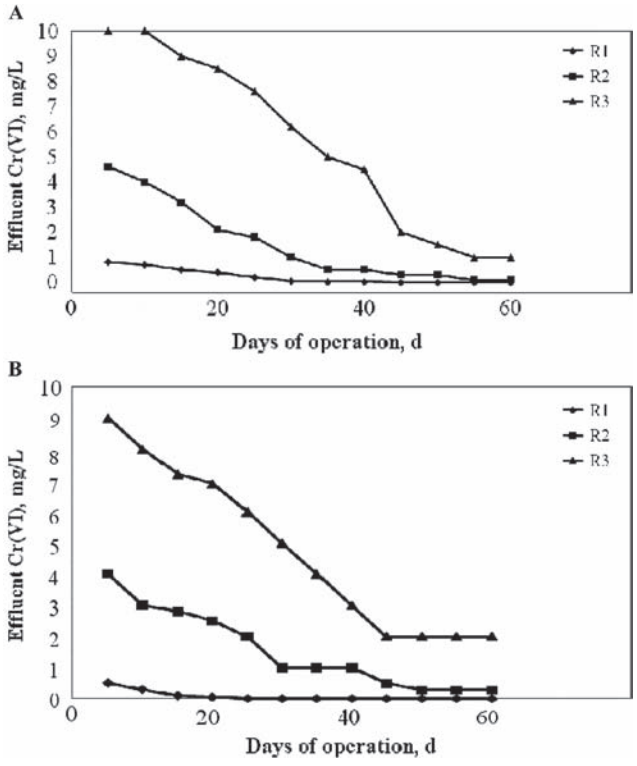

Fig. 3. Effluent Cr (VI) concentrations for (A) activated sludge reactors (R) and (B) biofilm reactors (BR).

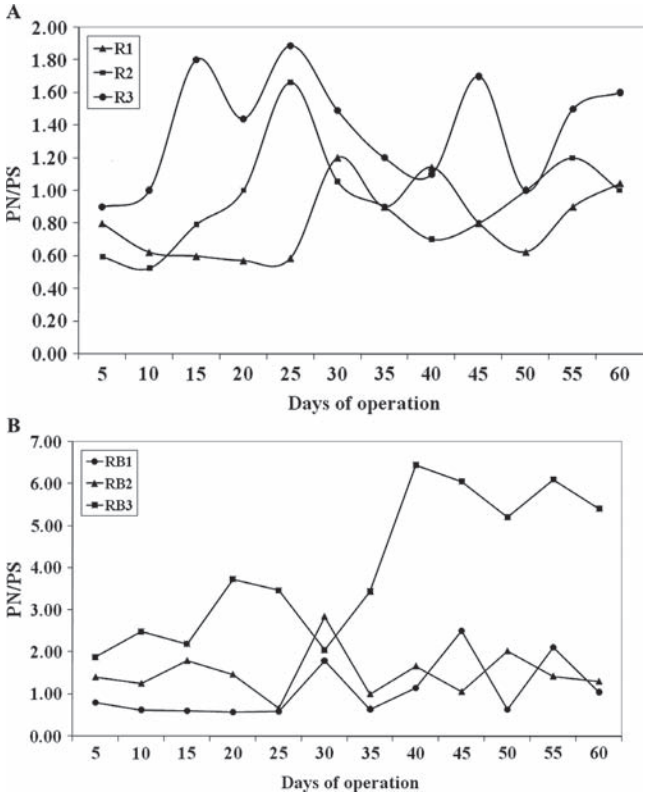

Fig. 4. PN:PS ratio for (A) activated sludge reactors (R) and (B) biofilm reactors (BR).

biosorption of chromium removal is still under study. Another possible mechanism of chromium removal is by sorption to the extracellular polymeric substances.

3.2. Extracellular polymeric substances

A natural response of the microorganisms upon exposure to unfavourable/toxic conditions is to stimulate production of EPS, which are mainly carbohydrates (acetyl, succinyl, pyruvyl and sulfonates) and proteins (glycoproteins, lipoproteins). EPS are the main elements for the formation of biofilms. EPS can serve as nutrient reserves to ensure survival under famine conditions [32] or as a protective shield against the toxic substances [12]. They delay or prevent the toxicants to enter the inner cell structures by diffusion limitation and/or by chemical reactions. Various results concerning the relationship between proteins and polysaccharides and stability of the activated sludge aerobic aggregates have been reported in previous studies. According to these results the ratio of proteins to polysaccharides (PN:PS) might indicate the hydrophobicity of the organisms. The higher the PN:PS ratio the higher the hydrophobicity and consequently, the greater the aerobic granulation [3,28]. On the other hand, other studies showed that the high polysaccharide content facilitated the cell to cell adhesion [28–30]. As shown in Figs. 4A and 4B, PN:PS ratio is higher for the RB reactors compared to the R reactors indicating higher hydrophobicity of the granules in biofilm reactors. The higher hydrophobicity in the present study, resulted in better activated sludge granulation, as it was indicated through microscopic observations and consequently to the enhanced sedimentation ability of the aerobic granules.

3.3. Microfauna observations and SBI

The activated sludge flocs examined microscopically showed dominance of the various ciliated groups in the reactors varied throughout the experimental period. At the beginning of the operation in the reactors with high Cr (VI) influent concentrations free swimming bacterivorous species along with flagellates were dominant, while significant decrease of sessile species was observed. This phase can be considered as a transitory phase, where a non-easily biodegradable substance, Cr (VI) introduced and thus, the sessile species—indicators of well stabilized sludge—decreased [31]. The increase of MLSS in the systems (Fig. 1) initiated the second phase of protozoan succession where dominance of crawling and sessile species occurs. The increase of these

species was particularly profound in the reactors operating with high Cr (VI) influent concentrations, where the most significant increase of MLSS was observed. In the stable phase of the reactors, sessile species were dominant in all cases, with their presence being slightly variable between the different chromium influent concentrations.

In the reactors supplied with PVA biocarriers, changes were observed at the species dominating the fringes of the biofilm aggregates, having as a consequence the alteration of biofilm structure and its degree of attachment on the biocarriers and the alteration of the EPS profiles due to the dominance of different bacterial species through time. Previous studies have reported changes of the biofilm structure during its succession. The succession process of the biofilm can be divided into 4 distinct phases:

1. Dominance of crawling ciliates on the sludge flocs [5,29].
2. Proliferation of stalked ciliates serving as platform for attachment of bacteria [32].
3. Complete dominance of the stalked ciliates (mostly *Opercularia* and *Vorticella* in the initial periods and the carnivorous species *Podophrya* and *Tokophrya* after the introduction of 10 mgl^{-1} influent chromium) [33].
4. Overgrowth of the stalked ciliates having as a result their consequent death or escape of the biofilm and overtake of the free swimming ciliates [5,32].

The last step of the succession process results to the detachment of the activated sludge aggregates from the plastic carriers and the formation of less dense and thus lighter flocs. Moreover, protozoa excrete growth stimulating compounds that enhance bacterial activity and enhance the nutrient and organic carbon flux through cilia beating [32]. Madoni [22] identified three distinct characteristics for an efficient activated sludge performance: (a) high microfauna density, at least 10^6 cellsl^{-1}, (b) specific composition based on attached and crawling ciliates, with the absence of flagellates, which along with the free swimming ones are typical at the colonization stage, (c) diversified community, where no group dominates numerically by a factor greater than 10. All the above inter and intra species relationships have been categorized and quantified in order to comprise an index, the sludge biotic index (SBI) that could be used to evaluate the sludge quality and consequently the potential efficiency of the activated sludge in degrading influent pollutants. sludge biotic index (SBI) values ranged from 2 to 9 (Fig. 5). The prevalence of the certain microfauna groups is dependent on the physicochemical and operational parameters of the wastewater parameters. SBI may range from 0 to 10 indicating the worst and best "health" quality of activated

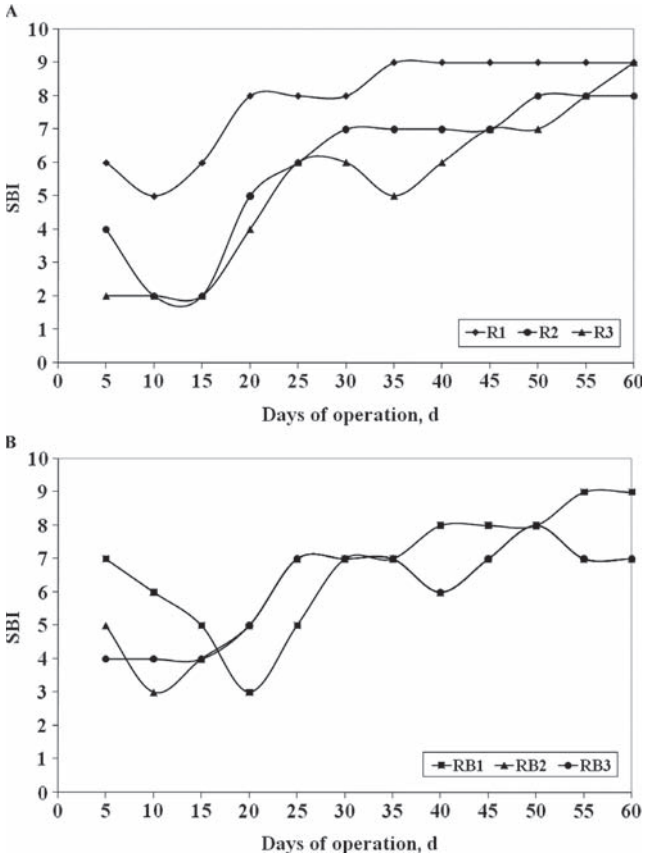

Fig. 5. SBI values as a function of operation time for (A) activated sludge reactors (R) and (B) biofilm reactors (BR).

sludge, respectively [22]. The estimated SBI values as a function of operation time in the reactors treating chromium containing influents are shown in Fig. 5. SBI in all cases decreased after the introduction of chromium in the influent. Increased chromium concentrations stimulated severe effects on the activated sludge protists and thus, a sharp decrease of the SBI values was observed. A significant increase of SBI occurred after the 45th d of operation along with the improved removal efficiency of organic load, nutrients, and Cr in the reactors, indicates a well colonized and stable sludge associated to an excellent biological activity [33].

4. Conclusions

The addition of chromium to the influent, even at high dosages, did not effect the organic matter removal and the nitrification process, resulting in high quality effluents. However, enhanced organic load and nutrient removal was observed in the case of biofilm reactors (RB) compared to conventional activated sludge reactors (R). In addition high chromium removal rates were observed at prolonged operation times, possibly due

to its adsorption/precipitation on the activated sludge organisms and on the EPS. The PN:PS ratio indicated higher hydrophobicity of the granules in biofilm reactors. The chromium addition affected the relative protistan community by shifting from sessile to carnivorous species with the increase of influent chromium concentration. In all cases, the activated sludge was slowly acclimatized to chromium inflow. PVA gel beads may be used as complementary to activated sludge processes for the treatment of wastewaters with relatively low concentrations of Cr (VI), since they are able to support greater abundance of microorganisms in order to perform enhanced biodegradation processes. Moreover the EPS form granules with greater hydrophobicity suggesting enhanced protection of the microorganisms from Cr (VI) and better sedimentation ability of the sludge granules.

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