Comparative study of suspended and attached activated sludge processes in the treatment of wastewater containing toxic substances

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***Abstract* – Although activated sludge communities are considered stable, the presence of toxic substances, such as Cr(VI), 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(VI) effects on the performance and on 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. The protozoan species were identified, quantified, and correlated to the efficiency of each system through microscopic observations. High removal rates of the organic loading, ammonia–nitrogen, and total phosphorus were observed after the acclimatization period of the activated sludge, even at high Cr(VI) dosages. The reactors with the PVA biofilm carriers exhibited increased removal of Cr(VI) from the synthetic sewage. Differences in the abundance and diversity of the protistans were observed at various Cr(VI) influent concentrations. Sludge Biotic Index (SBI) values decreased at the start of the operation, indicating the effects of Cr(VI) influent on the activated sludge microfauna. However, the acclimatization of the activated sludge microorganisms to Cr(VI) concentrations was indicated by the increase of the SBI index after 45 days of operation. This fact indicates that a healthy activated sludge microfauna, performing adequate biodegradation processes, can be retained after gradual acclimatization.**

Keywords: Wastewater Treatment, Activated sludge, Hexavalent chromium (Cr(VI)), protozoa, biocarriers

I. INTRODUCTION

Activated sludge is a widely used process, based on the development of appropriate bacterial aggregates and other associated organisms in an aeration tank; these organisms are easily separated from the aqueous phase during the subsequent sedimentation. The microscopic observations have shown that these flocs are multi-layered porous structures, in which macroflocs are formed by smaller aggregates or microcolonies, resulting from the reproduction of microorganisms. The binding between the different levels may be devoted to the internal hydrophobic properties (Adav et al., 2008) to extracellular polymeric substances (EPS) composition (Henriques et al., 2007) or even to multivalent cation bridging (Henriques et al., 2007). EPS are large molecular weight compounds that result from bacteria secretion, cell lysis, hydrolysis, and from the absorption of wastewater organic matter onto the bacteria (Adav et al., 2007, Weber et al., 2007). Bacteria can also produce EPS to protect themselves against the presence of xenobiotic substances. Ciliated protozoa play an essential role in the whole process by removing dispersed bacteria through grazing, which otherwise may result in high turbidity effluents (Wilen et al., 2007). 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; Cr salts are extensively used in several industrial processes such as tanneries, electroplating, textile, dyeing, and metal finishing industries. Cr may exist in the trivalent [Cr(III)] and hexavalent [Cr(VI)] state. Cr(VI) compounds (chromates and dichromates) are highly toxic and are considered as mutagens and carcinogens. Various studies (Costerton et al., 1995) exhibited differences in the treatment process efficiency, due to shock loads from a wide range of toxic substances. The morphology, composition, and sensitivity of the activated sludge in the presence of various toxic substances change and result in different floc structures and operational parameters that may determine the efficiency of the process. 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. 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 (Stewart, 2002). However, to the best of our knowledge, limited studies were conducted on the effects of Cr(VI) 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 biofilm carriers. The objectives of this study were: a) the monitoring of the composition and response of the activated sludge protistan community to the exposure to various Cr(VI) concentrations, b) the investigation of the potential effect of Cr(VI) on the efficiency of an activated sludge process, c) the correlation of these responses and effects to the effluent quality, and d) the comparison of the ability of the activated sludge microfauna to remove Cr(VI) in the presence of PVA biofilm carriers and in a conventional activated sludge setup.

II. Methodology

Six 2L glass beakers were used as the bench scale activated sludge reactors (reactors R and RB) at room temperature (Figure 1). Continuous aeration was provided by three air pumps using two air diffusers in each system. Start up of the reactor was conducted by the addition of 250 mL of an activated sludge sample 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 beads. The characteristics of each bench scale reactor are shown in Table 1. The pH of each system was about 7.5 – 8.5. The reactors were initially fed with synthetic wastewater containing Cr(VI) in order for the activated sludge microorganisms to be acclimatized to the corresponding experiment conditions. After the acclimatization period, Cr(VI) in the desired concentrations was added to the synthetic wastewater and the operation of the reactors was monitored as a function of time.

Figure 1. Reactors supplied with PVA beads

TABLE I: REACTOR CHARACTERISTICS

Reactor	PVA beads	Influent Cr(VI) concentration, mg/L
R1		1
R2		5
R3		10
RB1	+	1
RB2	+	5
RB3	+	10

Each system was operated in subsequent cycles of four days. At the end of each cycle, sludge sedimentation was taking place by turning off the air pumps. 400 mL of the supernatant water was withdrawn from each system for further analysis, and were replaced by fresh synthetic wastewater containing the desired Cr(VI) concentration (Kargi et al., 2006). 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±50 mg/L, TN = 60±3 mg/L and P = 18±2 mg/L. The Cr(VI) source was potassium dichromate that was prepared as a stock solution at a concentration of 10 g/L. Samples of each reactor were analysed for MLSS content, COD, ammonia nitrogen, phosphates, and Cr(VI) concentrations; all parameters measured according to standard methods of analysis (APHA, 1989). 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. 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, which is able to indicate the "health" of the activated sludge (Madoni, 1994).

III. Selected Results

The bench scale activated sludge systems were operated for a total period of 60 days, in order to evaluate the effect of Cr(VI) on the operation performance. The MLSS content in each system as a function of time is presented in Figure 2. The highest MLSS values, 3500 mg/L, were observed in the reactors R without the addition of PVA beads at the highest Cr(VI) influent concentration (10 mg/L). The increased MLSS concentration in activated sludge systems 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.

Additionally, due to the continuous aeration of the aerobic activated sludge reactors, chromium 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 (Yetis et al., 1999).

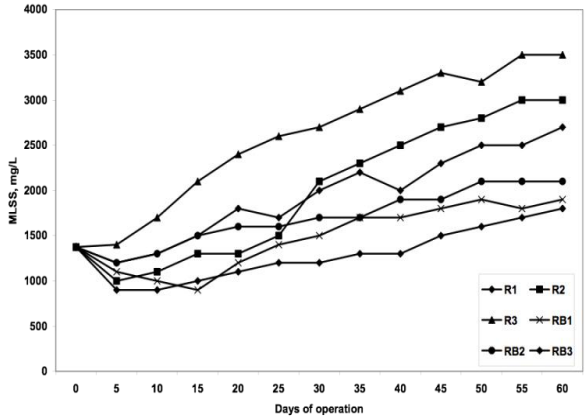

Figure 2. MLSS concentration in all reactors as a function of operation time

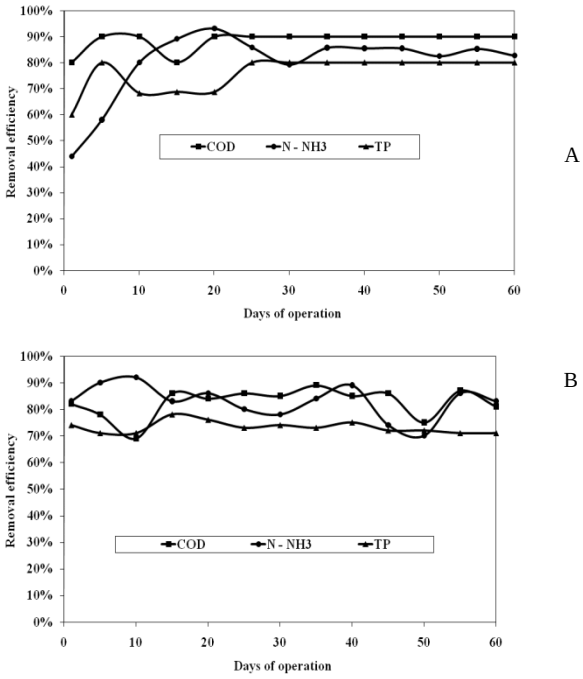

Figure 3. Removal efficiencies of COD, ammonia – nitrogen (N-NH3) and total phosphorus (TP) with time are shown as average values for the reactors A) without (R) and B) with (RB) the presence of PVA biofilms.

The removal efficiencies of COD, total phosphorus, and ammonia – nitrogen with time are shown as average values for the reactors without (R) and with (RB) the presence of PVA biofilms in Figures 3A and 3B, 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 Cr(VI) concentration, and the efficient utilization of the carbon source by the sludge microfauna.

Nitrification was more efficient after the 10th day of operation in all systems supplied with PVA gel beads (Figure 3B); however fluctuations in the effluent concentration of nitrites were observed the cases of the reactors operating only with activated sludge (Figure 3A). The nitrification process was not affected by the addition of high Cr(VI) concentrations. However, other researchers noticed that the addition of 1 mg/L 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, although the microbial Cr(VI) removal was not affected.

The efficiency of the systems in removing phosphates was generally low in all reactors but after the acclimatization period of the activated sludge, the phosphate effluent values decreased significantly reaching up to 0.7 and 1.2 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 (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 (Kargi et al.,2005).

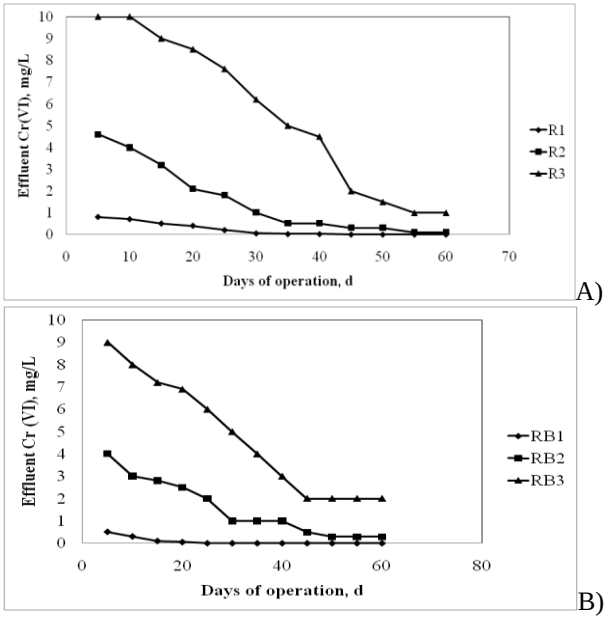

Figure 4. Effluent Cr(VI) concentrations for A) activated sludge reactors (R) and B) biofilm reactors (RB)

In general, Cr(VI) removal during the initial acclimatization period was low, at longer operation time Cr(VI) removal was enhanced (Figure 4). As a result Cr(VI) removal rates that were measured exceeded in all cases 30% at extended operation times; even in the system treating the highest Cr(VI) concentration (10 mg/L), low effluent Cr(VI) concentration was observed at prolonged operation time. The corresponding acclimatization time depended upon the influent Cr(VI): the highest the influent Cr(VI) content the longest the acclimatization

period. However, it should be noted that lower Cr(VI) values were observed in the reactors supplied with PVA biofilms (RB).

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 (Papadimitriou et al., 2007). The increase of MLSS in the systems (Figure 2) 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 slight variable between the different Cr(VI) influent concentrations.

In the reactors supplied with PVA biofilm carriers, 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 biofilm carriers 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 (Figure 5):

- Dominance of crawling ciliates on the sludge flocs (Weber et al., 2007)
- Proliferation of stalked ciliates serving as platform for attachment of bacteria.
- Complete dominance of the stalked ciliates (mostly *Opercularia* (Figure 6) and *Vorticella* in the initial periods and the carnivorous species *Podophrya* (Figure 7) and *Tokophrya* (Figure 8) after the introduction of 10 mg/L influent Cr(VI)).
- Overgrowth of the stalked ciliates having as a result their consequent death or escape of the biofilm and overtake of the free swimming ciliates.
- 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 condense flocs.

Moreover, protozoa excrete growth stimulating compounds that enhance bacterial activity and enhance the nutrient and organic carbon flux through cilia beating (Ratsak et al., 1996). Madoni et al., 1996, identified three distinct characteristics for an efficient activated sludge performance: a) high microfauna density, at least 106 cells/L, 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.

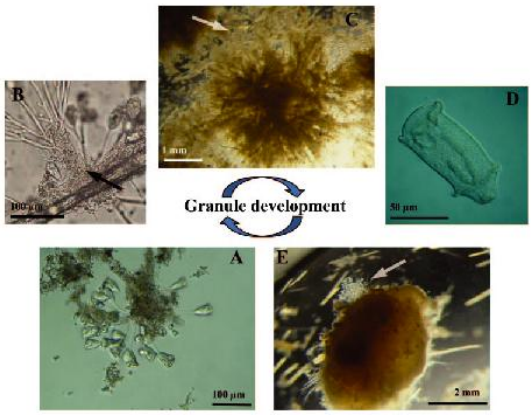

Figure 5. Stages of PVA beads colonization by protistans (modified from Weber et al., 2007)

Figure 6. *Opercularia sp*

Figure 7. *Podophrya sp*

Figure 8. *Tokophrya sp*

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 should 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 – 9 (Figures 9 & 10). 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 sludge, respectively (Madoni, 1994). The estimated SBI values as a function of operation time in the reactors treating Cr(VI) containing influents are shown in Figures 9 & 10. SBI in all cases decreased after the introduction of Cr(VI) in the influent. Increased Cr(VI) 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 60th day of operation, related to the improved removal efficiency of organic load, nutrients, and Cr(VI) in the reactors, indicating a well colonized and stable sludge associated to an excellent biological activity (Papadimitriou et al., 2007).

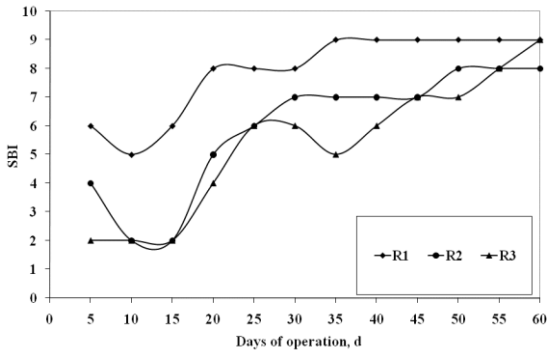

Figure 9. SBI values as a function of operation time for activated sludge reactors.

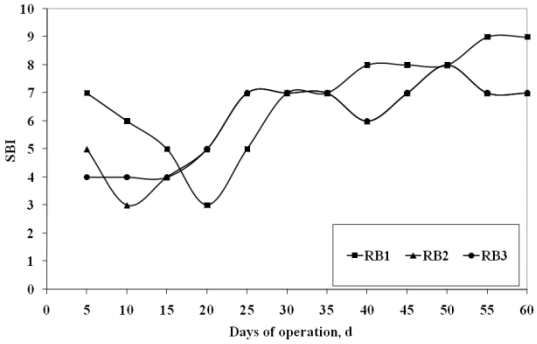

Figure 10. SBI values as a function of operation time for biofilm reactors (RB)

IV. CONCLUSIONS

The addition of Cr(VI) to the influent, even at high dosages, did not affect 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 Cr(VI) removal rates were observed at prolonged operation times, possibly due to its adsorption/precipitation on the activated sludge organisms. However, the Cr(VI) addition affected the relative protistan community by shifting from sessile to carnivorous species with the increase of influent Cr(VI) concentration. In all cases, the community SBI value was stabilized after a certain period of time (45 days).

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REFERENCES

Adav S.S, Lee D.J., Tay J.H., (2008). Extracellular polymeric substances and structural stability of aerobic granule Wat. Res. 42, 1644-1650.

Henriques I. D.S., Love, N.G., (2007). The role of extracellular polymeric substances in the toxicity response of activated sludge bacteria to chemical toxins Wat. Res., 41, 4177-4185.

Adav S.S, Lee D.J, Lai J.Y., (2007). Intergeneric coaggregation of strains isolated from phenol degrading aerobic granules. Appl Microbiol Biotechnol., 79, 657–661.

Weber S. L., Schleifer D.W., Fried, J., (2007). Microbial Composition and Structure of Aerobic Granular Sewage Biofilms App. Environm. Microb., 73, 6233-6240.

Wilen B.M., Onuki M., Hermansson M., Lumley D., Mino T., (2007). Microbial community structure in activated sludge floc analysed by fluorescence in situ hybridization and its relation to floc stability. Wat. Res., 42, 2300 – 2308.

Costerton J.W., Lewandowski Z., Calwell D.E., Korber D.R., Lappinscott H.M., (1995). Microbial biofilms, Annu, Rev. Microbiol. 49, 711 – 745.

Stewart P.S., 2002. Mechanisms of antibiotic resistance in bacterial biofilms. Int. J. Med. Microbiol. 292, 107–113.

Kargi F., Gikla, Sinem, 2006. Biosorption of zinc (II) ions onto powdered waste sludge (PWS): kinetic and isotherms. Enzyme Microb. Technol. 38, 705 – 710.

APHA, (1989). Standard Methods for the Examination of Water and Wastewater, 17th Edition. American Publication Health Association, Washington DC.

Madoni P., (1994). A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. Wat.Res. 28, 67 – 75.

Yetis U., Demirer G. N., Gokcay C. F., (1999). Effect of chromium (VI) on the biomass yield of activated sludge. Enzyme and Microbial Technology 25, 48 – 54.

Kargi F., Uygur A., Baskaya H. S., (2005). Phosphate uptake rates with different carbon sources in biological nutrient removal using a SBR. Journal of Environmental Management 76, 71 – 75

Madoni P., Davoli D., Gorbi G., Vescovi L., (1996). Toxic effect of heavy metals on the activated sludge protozoan community. Wat. Res. 30, 135 – 141.

Ratsak, C. H., Maarsen, K. A., Kooijman, S. A. L. M., (1996). Effects of protozoa on carbon mineralization in activated sludge, Wat. Res., 30, 1 – 12.

Papadimitriou Ch., Palaska G., Lazaridou M., Samaras P., Sakellaropoulos G.P., (2007). The effects of toxic substances on the activated sludge microfauna. Desalination 211, 177–191.