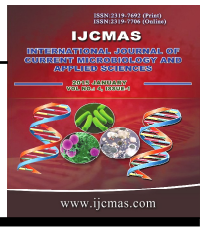
Int.J.Curr.Microbiol.App.Sci (2015) 4(1): 625-634

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 1 (2015) pp. 625-634**
http://www.ijcmas.com



Original Research Article

Bioflocks structure from enriched lab-scale stabilization ponds used to remove high chromium concentrations

L.F.Prieto-Contreras[1,2], F.J.Avelar-González[1],V.M.Loera-Muro[1], C.Quiñones-Valles[1], A.Loera-Muro[1], E.M.Ramírez-López[1], F. Esparza-García[3] and A. L. Guerrero-Barrera[1]*

[1]Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Mexico
[2]Doctorado en Ciencias Biológicas, Universidad Autónoma de Aguascalientes, Mexico
[3]Departamento de Biotecnología e Ingeniería, CINVESTAV-IPN, Mexico
**Corresponding author*

Keywords

Bioflocks, Enriched stabilization ponds, Chromium removal, Bacterial composition.

A B S T R A C T

Architecture of bioflocks from labscale anoxic stabilization ponds enriched with activated sludge, used to remove efficiently high concentrations of chromium VI, until 153 mg/l with near 99% of removal efficiency, was determined by scanning electronic microscopy. This flocculated biomass showed a biofilm-like architecture formed by cylindrical towers and scattered structures on the surface showing liquid channels inside the bioflock, characteristic of biofilm architecture, but they were not attached to any inert surface. Before chromium treatment bioflocks had a gram negative: gram positive bacteria proportion of 50: 50%. After chromium treatment these proportion changes to 70: 30%, respectively. The viability of flocculated biomass before and after chromium treatment was almost 100%, determined by Live/Dead Bactlight Kit (Molecular Probes). Before chromium treatment, bioflocks were characterized and the bacteria present were isolated, biochemically characterized and sequenced using rRNA, these bacteria were *Aeromonas hydrophila* AKR1, *Curtobacterium sp* Fek 20, *Staphylococcus saprophyticus* OTUC3, *Bacillus sp* By130 (A) Ydz-ds, *Citrobacter sp* I101-10, *Pseudomonas aeruginosa*, *Escherichia coli* UMN 026, *Morganella morganii* BH-16, *Klebsiella pneumoniae* subsp. *pneumoniae* MGH78578, *Aeromonas salmonicida* subsp. *salmonicida* and *Enterobacter sp* YRL01. After chromium treatment only were found *Escherichia coli* UMN 026, *Acinetobacter sp* TPR 15 and *Morganella morganii* BH-16. These results show a selection of chromium resistant species.

Introduction

Stabilization ponds are a cost-efficient method for the treatment of municipal and non-toxic industrial effluents. Enrichment procedure using activated sludge from a municipal wastewater treatment plant has been developed to increase the biomass concentration and activity in stabilization ponds, which produces better efficiency and capacity to remove high organic and toxic phenol loads. The enriched ponds removed

organic matter twice faster and phenol seven times faster than traditional ponds, and the flocculated biomass resulted from the enrichment procedure was the main responsible of the removal of both contaminants (Avelar *et al.,* 2001; Avelar *et al.,* 2003; Ramos *et al.,* 2005, 2007). However, the architecture and the structure of this flocculated biomass are unknown.

Microorganisms in nature have been development survival strategies against adverse environment, so they could survive as microbial consortia like biomats or biofilms (Boles and Horswill, 2008; Chandra and Ghannouum, 2004; Hu *et al.,* 2005). Biofilms are bacterial communities that include one or several species, which are adhered to inert surfaces or tissue alive. Biofilms produces extracellular matrix that usually are compound by exopolysaccharides (EPS), proteins and sometimes nucleic acids (Chandra *et al.,* 2001; Donlan, 2002; Hu, *et al.,* 2005; Palmer and Stoodley, 2007; Parsek and Fuqua, 2004; Post *et al.,* 2004; Rice *et al.,* 2005; Singh *et al.,* 2003; Yi *et al.,* 2004).

Biofilms promotes metabolic interactions as antagonism, mutualism, interspecific competition and commensally relationships. These communities increase the antibiotic and antimicrobial resistance (Boles and Horswill, 2008; Kirisits *et al.,* 2005; Lindsay and Von Holy, 2006; Mangallappalli-Illathu and Korber, 2006; Parsek and Fuqua, 2004; Spoering and Lewis, 2001; Suthereland *et al.,* 2007).

Priester *et al.* (2006) found that relationship between metals and bacteria involve EPS, which promotes nutrient retention, adhesion cell-substrate, signaling cell to cell and protection of individual cells from depredators and chemical degradation. EPS also could stabilize heavy metals (Priester *et al.,* 2006).

In a biofilm could be found photosynthetic, chemosynthetic, chemolithotrophic, motile and non-motile bacteria, which live in symbiosis. Biofilm could have Gram negative and Gram positive mixture of bacteria or only one type of Gram bacteria. In this work are explored the biomass flocculated structure by scanning electron microscopy, the bacterial composition and the changes produces in both blioflocks before and after treatment with chromium (153 mg/l).

Materials and Methods

Bacterial isolation

Biomass flocculated (30 ml) without or with chromium treatment (153 mg/l) was homogenized and washed three times with 0.1X PBS. 100 µl of a 1:20 dilution of this homogenized biomass were grown on agar BHI or BHI with 100 mg/l of K_2Cr_2O7 pH 5 and incubated at 30°C all night long (Leung *et al.,* 2000). Bacterial colonies were selected according with their morphology. Every colony was re-cultured individually. These colonies were incubated at 30°C, 24 hrs and after Gram staining was done to check their purity. After isolation, biochemical characterization was done using Api NE or Api E galleries (Biomériéux).

Bacterial nucleic acid extraction, reverse transcription polymerase chain reaction and molecular identification of bacterial strains.

After biochemical characterization of all isolated bacterial strains, each was grown in brain heart infusion media (Beckton-Dickinson) at 30°C for 24 hrs. After, the extraction of nucleic acids was done according to Sambrook and Russell method (2001), PCR was performed by using universal rRNA primers 16s (27f)

AGAGTTTGATCMTGGCTCASG and 16s (1492R) TACGGYTACCTTGTTACGACT T. For amplification reaction 200 ng of nucleic acids was used, with 40 pM of each primer and 25 µl of 1X PCR Master Mix (Fermentas), used for final volume (50 µl) of reaction mixture. PCR conditions were 30 cycles at 94°C for 30 min, 48°C 1 min and 72°C for 2 min. The purification and sequentiation were done by Macrogen Corp. Every sequence was compared with GenBank data (NCBI) for the identification of the bacterial strains.

Viability-test

Duplicated random samples were taken from flocculated biomass with or without chromium treatment (153 mg/l). As negative control, flocculated biomass from labscale stabilization ponds without chromium treatment was used. Flocculated biomass was homogenized and washed three times with 0.1X PBS. Dead/Live BacLight kit (Molecular Probes) was used in order to test viability. Images were taken with microscope, Zeiss Axioscope 40, and captured using the Image Pro Plus System (Cybernetics).

Flocculated biomass scanning electron microscopy

Flocculated biomass was homogenized, washed and diluted as mentioned in isolation section. Three microliter of 1:20 dilution was taken and air dried. After this, samples were dehydrated in alcohol with increased dilution series (60–100%). The excess humidity was removed with liquid CO_2, in critic point chamber (Tousimis). Dehydrated samples were covered using chamber Desk II. Samples were observed with scanning electron microscope Jeol LV-5900. The images were captured with microscopy software.

Chromium removal

Samples were taken every third day from effluents of stabilization ponds enriched with bioflocks treated with chromium (153 mg/l), as well as control ponds. Using atomic adsorption the chromium concentration was determined as Avelar et al. (2003), described.

Results and Discussion

Microorganism identification

From bioflocks from random sampling were isolated and identified by biochemical characterization and molecular sequentiation of several species. They were characterized using 16S rRNA partial sequence analysis. From no treated system were isolated: *Aeromonas hydrophila* AKR1, *Curtobacterium sp* Fek 20, *Staphylococcus saprophyticus* OTUC3, *Bacillus sp* By130 (A) Ydz-ds, *Citrobacter* spI101-10, *Pseudomonas aeruginosa*, *Escherichia coli* UMN 026, *Morganella morganii* strain BH-16, *Klebsiella pneumoniae* subsp. *pneumoniae* MGH78578, *Aeromonas salmonicida* subsp. *salmonicida* and *Enterobacter sp* YRL01. While from chromium treated systems the following were isolated: *Escherichia coli* UMN 026, *Acinetobacter* spTPR 15, *Morganella morganii* BH-16.

Viability test

Bioflocks from stabilization ponds control or chromium treated are shown in figure 1. Their viability was explored by Dead/Live BacLight Kit (Molecular Probes). Panels A and C shows the optic views of bioflocks while panels B and D shows the results of viability test observed by epifluorescence. Green microorganisms were alive, while red microorganisms were dead. For both bioflocks without or with chromium

treatment the viability found was almost 90%. Figure 2 showed the Gram type proportion in the bioflocks before and after chromium treatment. Before chromium treatment proportion between Gram positive and Gram negative bacteria was 50:50 (Fig. 2A); whilst after chromium treatment the Gram positive proportion was reduced to 30% while Gram negative bacteria increased to 70% (Fig. 2B). In Figure 2B also showed crystals that are surrounded by biomass.

Bioflocks structure observed by scanning electron microscopy

The stabilization ponds flocculated biomass from both systems with or without chromium treatment showed a biofilms like structure. They show bacteria in attachment phase, also growth structures and detaching structures with water channels as it is shown in Fig. 3 and Fig. 4, but these biofilms are not attached to a solid surface as normal biofilms are. In Fig. 4 it is shown that at least diversity, apparently the bioflock structure is less tight.

The pollution caused by heavy metals in wastewater is a serious problem because these elements are not biodegradable and can accumulate in living tissues (Deng *et al.*, 2006; Quintelas *et al.*, 2009). The conventional methods for heavy metal removal from industrial effluents are precipitation, coagulation, ion exchange, cementation, electro-dialysis, electro-winning, electro-coagulation and reverse osmosis (Ahluwalia and Goyal, 2007; Quintelas *et al.*, 2009). These technologies are expensive, mainly when applied to dilute solutions they not have a good efficiency and usually generating huge volumes of sludge containing high levels of heavy metals, which have to be disposed. Due to these, new technologies are necessary (Cossich *et al.*, 2004; Quintelas *et al.*, 2009). In these sense, stabilization ponds enriched with bioflocks represent a good alternative, because they have a high removal capacity and resistance to high chromium concentration due to a biomass increasing, microbial population selection with acclimatization and with high organic levels as well as with the treatment with other xenobiotics (Avelar *et al.*, 2003; Chambless *et al.*, 2006; Ramos *et al.*, 2005).

Acclimatized systems showed chromium removal capacities of 99%, instead of the no enriched system that have only 5% of this removal capacity (data not shown). Here, the results show that chromium treatment produces a microorganism selection in bioflocks. Thus, control bioflocks showed eleven different bacterial species, instead of chromium treated systems that only showed three different species, this microbial selection caused by a xenobiotic in high level has been found also for other authors in activated sludge (Leung *et al.*, 2000; Ye *et al.*, 2005; Vilchez, 2007; Stewartt, 2008). As results showed, all the experiment cells still alive, but there was a specific selection.

The selected species in the stabilization ponds treated with chromium, *Escherichia coli*, *Acinetobacter* sp and *Morganella morganii*, showed adaptation to chromium stress conditions. These species as other authors have been proposed could have synergic metabolism to remove chromium (Burmolle *et al.*, 2006; Teitzel and Parsek, 2003; Stewartt and Franklin, 2008).

Figure.1 Bioflocks from control stabilization ponds. (A) Structure observed by light microscopy (400X). (B) Viability test using Dead/Live BactLight kit (Molecular Probes) in comparison withbioflocks from stabilization ponds treated with 153 mg/L chromium. (C) Structure observed by light microscopy (400X). (D) Viability test using Dead/Live BactLight kit. The observation was made by epifluorescence using FITC filter (400X)

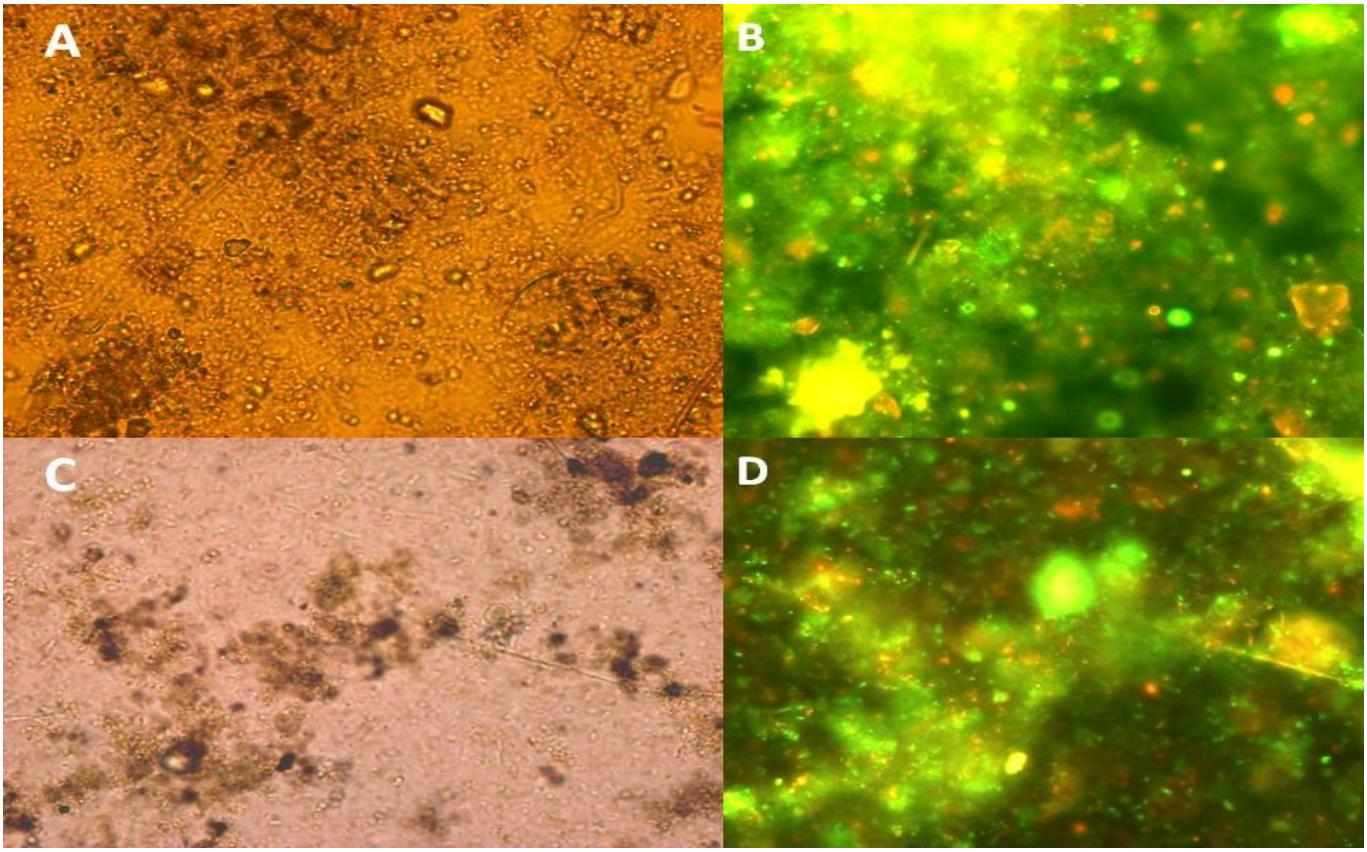


Figure.2 Gram staining of stabilization ponds bioflocks. (A) Control bioflock, Gram positive: Gram negative proportion 50: 50%. (B) Bioflock treated with 153 mg/L of chromium. Gram positive: Gram negative proportion 30:70% (400X)

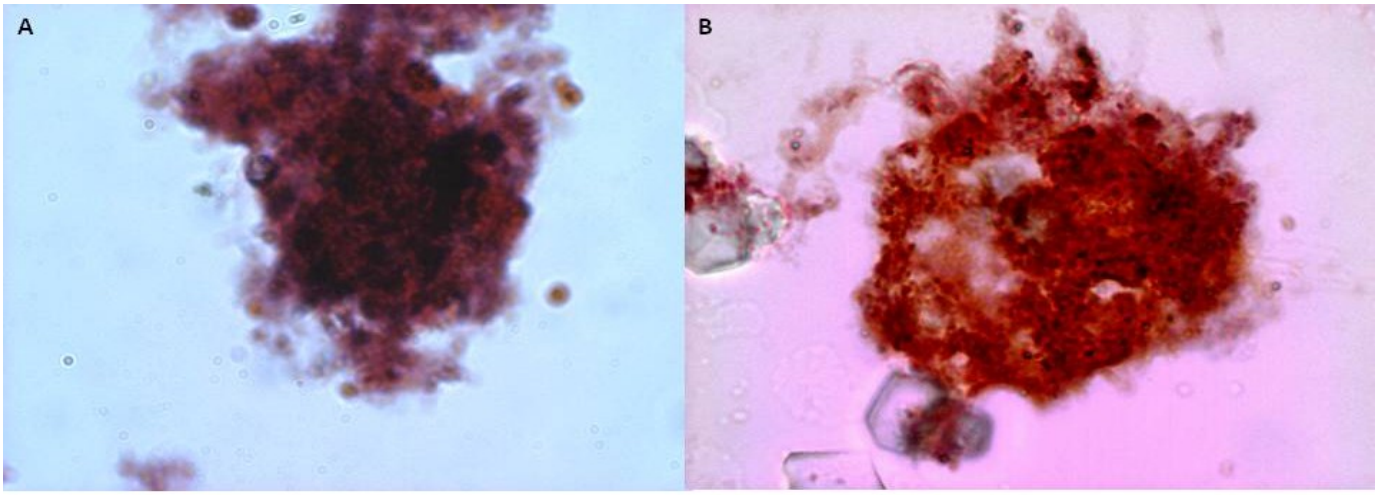


Figure.3 Scanning electron micrographies of control bioflock without chromium treatment. (A) Water channel structure. (B) Biofilm structure (C and D) Mixed-species heterotrophic biofilm grown shown different kind of bacteria and some water channel between them

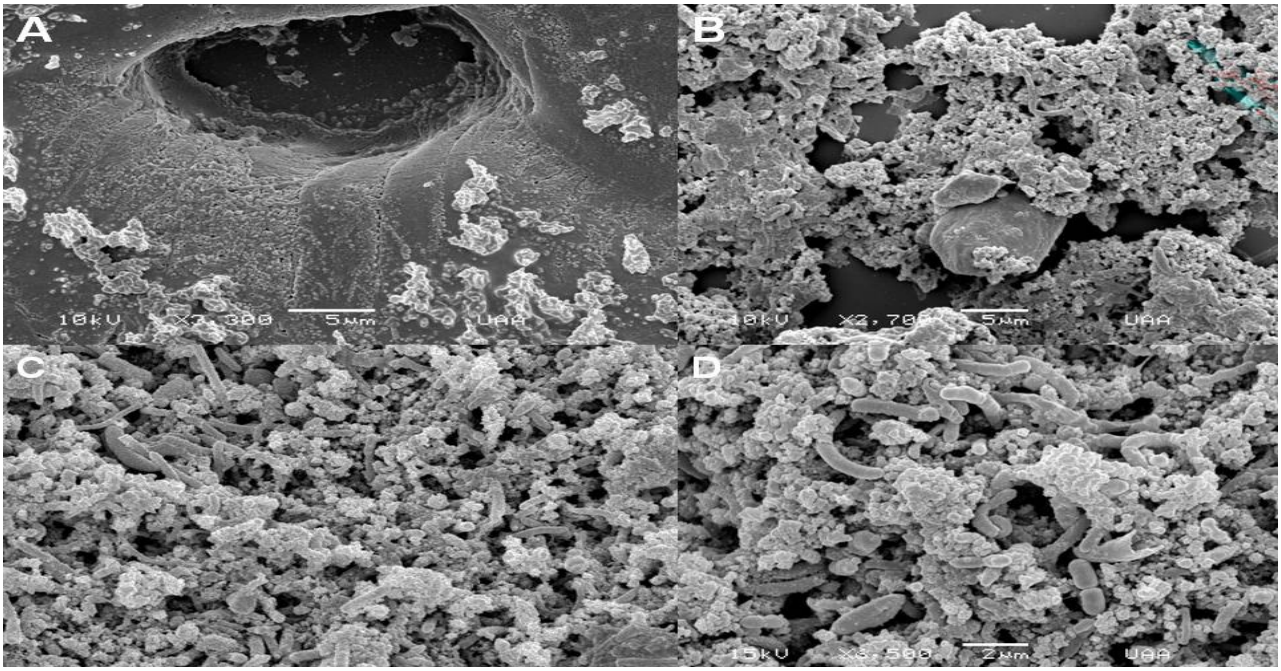
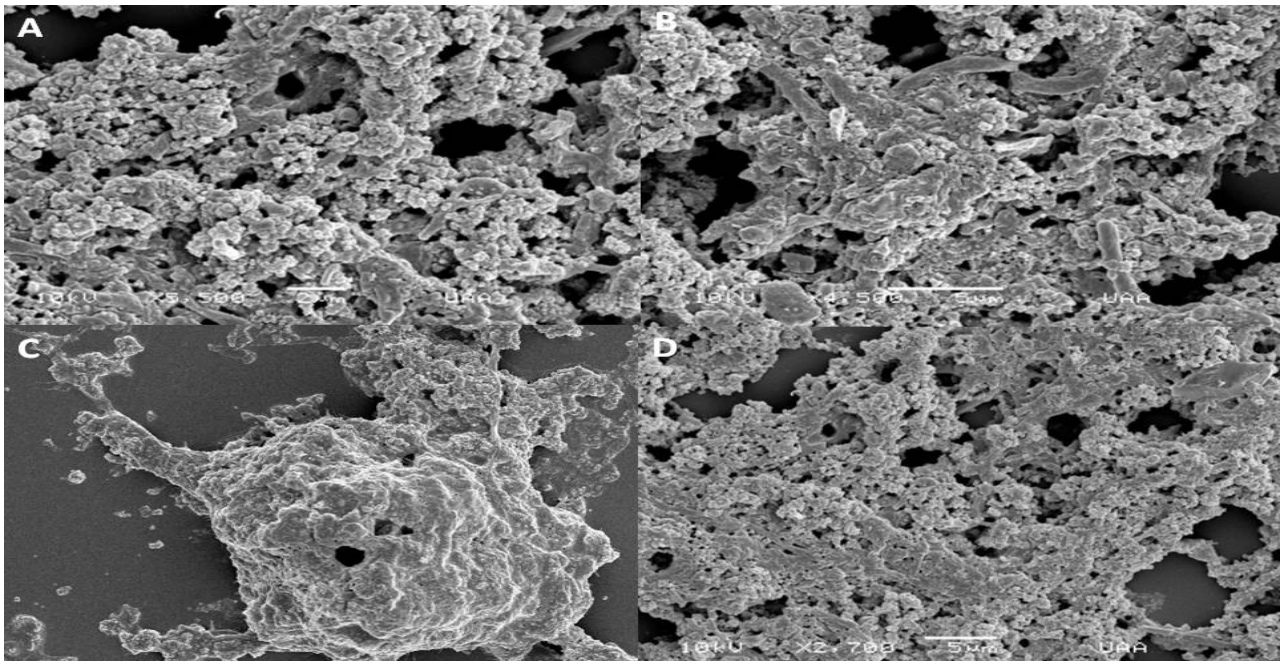


Figure.4 Scanning electron micrographies of chromium treated bioflock (153 mg/L). (A) and (B) show microorganism growth. (C) shows a close up of water channel in biofilm structure. (D) Mixed-species heterotrophic biofilm grown shown different kind of bacteria and some water channel between them



Molokwane et al. (2008) have been found that bacteria consortia collected from a waste water treatment plant in South Africa in aerobic conditions completely remove chromium VI (150 mg/L), after 150 h and in that conditions *Bacillus* sp and *Microbacterium* sp are selected. Their results are similar to the results obtained in the present work.

E. coli and *Acinetobacter* sp have been reported as good microorganisms to remove chromium VI from tanneries or from industrial waste water (Srivastava and Thakur, 2007; Quintelas *et al.*, 2008), and *Morganella morganii* has been found multiplied during the transit of the organic residues through the gut of worms, but no removal of chromium capability has been found before of the present work (Parthasarathi *et al.*, 2007), however its capability could be related with phosphatase mediated chromium accumulation that is observed in *M. morganii* with other heavy metals (Macaskie *et al.*, 1994).

One of the resistance mechanisms reported in several bacterial species are biofilms. A bacterial biofilm is a community of microorganisms attached to a solid surface using extracellular polymeric substances (Branda *et al.*. 2005; Wood *et al.*, 2006). In nature, biofilm formation has several steps, attachment, growth and detaching (Langmark *et al.*, 2005). During the process, water channels formation is the main characteristic. *Pseudomonas aeruginosa* during biofilm development exhibits mushroom-like structures which have been attributed to stress forces conditions (Stoodley *et al.*, 1999) and substrate gradients (Chang *et al.*, 2003) that could be involved differential gene expression (Espinoza-Urgel, 2003).

In our systems are found mushroom like structures (Figs. 4 and 5), these mushroom structures form channel that is reported are used for nutrient and waste flow. The bioflocks seems to be biofilms that not are attached to any inert surface (Drudge and Warren, 2014; Ofițeru *et al.*, 2014). They are attached between them probably by exo-polysaccharides which are high expressed when high chromium levels are present in *Acinetobacter* sp (data not shown). More studies are necessary to show what kind of exo- polysaccharide this specie is producing.

References

Ahluwalia, S. S., and Goyal, D. 2007. Microbial and plant derived biomass for removal ofheavy metals from wastewater. *Bioresour. Technol.* 98: 2243–2257.

Avelar, F. J., Martínez-Pereda P., Thalasso, F., Rodríguez-Vazquez, R., Esparza-García, F.J. 2001. Upgrading of Facultative Waste Stabilisation Ponds Ander High Organic Load. *Biotechnol. Lett.*, 23(14): 1115–1118.

Avelar, F. J., Martínez-Pereda, P., Thalasso, F., Rodríguez-Vázquez, R., Alba, J. and Esparza-García, F. J. 2003. Phenol Removal in Upgraded Facultative Waste Stabilisation Ponds. *Environ. Technol.*, 24(4): 465–470.

Boles, B. R., and Horswill, A. R. 2008. agr-Mediated Dispersal of *Staphylococcus aureus* Biofilms. *PLoS Pathog.*, 4(4): e1000052. doi:10.1371/journal.ppat.1000052.

Branda, S. S, Vik Å Friedman, L., Kolter, R. 2005. Biofilms: thematrix revisited. *Trends. Microbiol.*, 13: 20–26.

Burmolle, M., Webb, J. S., Rao, D., Hansen, L.H. Sorensen, S.J., and Kjelleberg, S. 2006. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by sinergistic interactions in multispecies biofilms. *Appl. Environ. Microbiol.,* 72(6): 3916–3923.

Chambless, J.D., Hunt, S.M., and Stewart, P.S. 2006. A Thre-Dimensional Computer Model of Four Hypothetical Mechanisms Protecting Biofilms From Antimicrobials. . *Appl. Environ. Microbiol.,* 72 (3): 2005–2013.

Chandra, J., and Ghannouum, M. A. 2004. Fungal Biofilms. Microbial biofilms. ASM Press. Washington, D.C. Pp. 30–42.

Chandra, J., Kuhn, D.M., Mukhererjee, P.K., Hoyer, L.L., McCormick T., and Ghannoum, M. A. 2001. Biofilm Formation by the Fungal Pathogen *Candida albicans* Development, Architecture, and Drug Resistance. *J. Bacteriol.,* 183(18): 5385–5394.

Chang, I, Gilbert, E. S, Eliashberg, N, Keasling, J. D. 2003. A three dimensionalstochastic simulation of biofilm growth andtransport-related factors that affect structure. *Microbiol.,* 149: 2859–2871.

Cossich, E. S., Da Silva, E. D., Tavares, C. R. G., Filho, L. C. and Ravagnani, T. M. K., 2004.Biosorption of chromium (III) by biomass of seaweed *Sargassum sp*. in a fixedbedcolumn. *Adsorption,* 10: 129–138.

Costerton, J. W., Cheng, G., Gersey, T. I., Ladd, J. G., and Nickel, M. 1987. Bacterial biofilm in nature and disease. *Annu. Rev. Microbiol.,* 41: 435–464.

Deng, L., Su, Y., Su, H., Wang, X., Zhu, X., 2006. Biosorption of copper (II) and lead (II)from aqueous solutions by nonliving green algae *Cladophora fasciculares:* equilibrium, kinetics and environmental effects. *Adsorption,* 12: 267–277.

Donlan, R. M. 2002. Biofilms: Microbial life on surfaces. *Emerg. Infect. Dis.,* 8: 881–890.

Drudge C.N., and L.A. Warren 2012. Prokaryotic horizontal gene transfer in freshwater lakes: implications of dynamic biogeochemical zonation. *J. Environ. Protect.,* 3: 1634–1654

Espinosa-Urgel, M. 2003. Resident parking only: rhamnolipidsmaintain fluid channels in biofilms. *J. Bacteriol.,* 185: 699–700.

Häussler, S., and Becker, T. 2008. The Pseudomonas Quinolone Signals (PQS) Balances Life and Death in *Pseudomonas aeruginosa* Populations. *PLoS Pathog.,* 4(9): e1000166. doi:10.1371/journal.ppat.1000166.

Hu, Z., Hidalgo G., Houston, P.L., Hay, A.G., Shuler, M.L., Abruña, H.D., Ghiorse, W.C., and Lion, L.W. 2005. Determination of Spatial Distributions of Zinc Active Biomass in microbal Biofilms by Two-Photon Lase Scanning Microscopy. *Appl. Environ. Microbiol.,* 71(7): 4014–4021.

Kirisits, M.J., Prost, L., Starkey, M., Parsek, M.R. 2005. Characterization of Colony Morphology Variants Isolated from *Pseudomonas aeruginosa* Biofilms. *Appl. Environ. Microbiol.,* 71(8): 4809–4821.

Langmark, J., Storey, M. V., Ashbolt, N. J., and Stenstro, T. H.2005. Accumulation and Fate of Microorganisms and Microspheres in Biofilms Formed in a Pilot-Scale Water Distribution System. *Appl.*

Environ. Microbiol., 71: 706–712.

Leung, W.C., Wong, M.F., Chua, H., Lo W., Yu, P.H., and Leung, C.K. 2000. Removal and Recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal wastewater. *Water. Sci. Technol.*, 41(12): 233–240.

Lindsay, D., and Von Holy, A. 2006. Bacterial biofilms within the clinical setting: what healthcare professionals should know? *J. Hosp. Infect.*, 64: 313–325.

Macaskie, L. E., Bonthrone, K. M. and Rouch, D. A. 1994. Phosphatase-mediated heavy metal accumulation by a *Citrobacter sp*. and related enterobacteria.*FEMS. Microbiol. Lett.*, 121: 141–146.

Mangallappalli-Illathu, A.K. and Korber, D.R. 2006. Adaptative Resistance and Differential Protein Expression of*Salmonella enteric* Serovar Enteriditis Biofilms Exposed to Benzalkonium Chloride.*Antimicrob. Agents. Chemother.*, 50(11): 3588–3596.

Molokwane, P. E, Meli, C. K, Chirwa, E. M. 2008. Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading.*Water. Sci. Technol.*, 58(2): 399–405.

Ofițeru ID, Bellucci M, Picioreanu C, Lavric V, Curtis TP (2014) Multi-scale modelling of bioreactor-separator system for wastewater treatment with two-dimensional activated sludge floc dynamics. *Water Res.*, 50: 382–395.

Ophir, T., and Guntnick, D. L. 1994. A papele for exopolysaccharide in the protection of microorganism from dessication. *Appl. Envirom. Microbiol.*, 60: 740–745.

Palmer, R. J., and Stoodley P. 2007. Biofilms 2007: Broadened Horizons and New Emphases. *J. Bacteriol.*, 189(22): 787–807.

Parsek, M. R., and Fuqua, C. 2004. Biofilms 2003: Emerging Themes and Challenges in Studies of Surface- Associated Microbial Life. *J. Bacteriol.*, 186(14): 4427–4440.

Parthasarathi, K., Ranganathan, L. S, Anandi, V., Zeyer, J. 2007. Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. *J. Environ. Biol.*, 28(1): 87–97.

Post, J. C., Stoodley, P. Hall- Stoodley, L., and Ehrlich, G. D. 2004. The role of biofilms in otolaryngologic infections. *Curr. Opin. Otolaryngol. Head. Neck. Surg.*, 12: 185–190.

Priester, J. H., Olson, S. G., Webb, S.M., Neu, M.P., Hersman. L.E., and Patricia, A.H. 2006. Enhanced Exopolymer Production and Chromium Stabilizatión in *Pseudomonas putida* Unsaturated Biofilms. *App. Environ. Microbiol.*, 72(3): 1988–1996.

Quintelas, C., Fernandes, B., Castro, J., Figueiredo, H., and Tavares, T. 2008. Biosorption of Cr(VI) by three different bacterial species supported on granular activated carbon: a comparative study. *J. Hazard. Mater.*, 153(1–2): 799–809.

Quintelas, C., Fonseca, B., Silva, B., Figueiredo, H., and Tavares, T. 2009. Treatment of chromium(VI) solutions in a pilot-scale bioreactor through a biofilmof *Arthrobacter viscosus* supported on GAC. Bioresour. *Technol.*, 100: 220–226.

Ramos, M. S., Dávila, J. L., Esparza, F., Thalasso, F., Alba, J., Guerrero, A. L., and Avelar, F. J. 2005. Treatment of wastewater containing high phenol concentrations using

stabilisation ponds enriched with activated sludge. *Water Sci. Technol.,* 51(12): 257–260.

Ramos, M. S., Dávila, J. L., Thalasso F., Guerrero A. L., Ramírez-López E. M., Esparza-García, F., and Avelar, F. J. 2007. Labscale Anoxic Stabilization Ponds Operated Under High Organic Loads: Long Term Effect of Enrichment with Activated Sludge. *Environ. Technol.,* (28): 693–699.

Rice, S.A., Koh, K.S., Queck, S.Y., Labbate, M., Lam K.W., and Kjelleberg, S. 2005. Biofilm Formation and Sloughing in *Serratia marcescens*Are Controlled by Quorum Sensing and Nutrient Cues. *J. Bacteriol.,* 187(10): 3477–3485

Sambrook J, Russel D 2001. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York. 1–3, 372 pp.

Singh, R., Stine, O. C., Smith, D. L., Spitznagel, J. K., Labib, M. E., and Williams, H. N. 2003. Microbial Diversity of Biofilms in Dental Unit Water Systems. *App. Environ. Microbiol.,* 69(6): 3412–3420.

Spoering, A.L., and Lewis, K. 2001. Biofilms and Planktonic Cells of *Pseudomonas aeruginosa* Have a Similar Resistance to killing by Antimicrobials. *J. Bacteriol.,* 183(23): 6746–6751.

Srivastava, S., and Thakur, I. S. 2007. Evaluation of biosorption potency of *Acinetobacter sp*. for removal of hexavalent chromium from tannery effluent. *Biodegradation,* 18(5): 637–46.

Stewartt, P.S., and Franklin M.J. 2008. Physiological Heterogeneity in biofilms, *Nat. Rev. Microbiol.,* 6: 199–210.

Stoodley, P., Lewandowski, Z., Boyle, J. D., Lappin-Scott, H. M. 1999. Theformation of migratory ripples in a mixed species bacterialbiofilm growing in turbulent flow. *Environ. Microbiol.,* 1: 447–455.

Suthereland, I. W., Hughes, K. A., Skillman, L. C., and Tait, K. 2007. Interaction of phage and biofilms. *Water. Sci. Technol*., 55: 377–385.

Teitzel, G.M., and Parsek, M.R. 2003. Heavy Metal Resistance of Biofilm and Planktonic *Pseudomonas aeruginosa*. *App. Environ. Microbiol.,* 69(4): 2313–2320.

Vilchez, R., Pozo, C., Gómez, M.A., Rodelas, B., González-López, J. 2007. Dominance of sphingomonads in a copper exposed biofilm community for groundwater treatment. *Microbiology,* 153: 325–337.

Wood, T. K., Gonzalez Barrios, A. F., Herzberg, M. and Lee, J. 2006. Motility influences biofilm architecture in *Escherichia coli*. *Appl Microbiol. Biotechnol.,*72: 361–367.

Ye, J., Kandegedara, A. Martin, P., Rosen, B.P. 2005 Crystal Structure of the *Staphylococcus aureus* pI258 cadC Cd(II)/Pb(II)/Zn(II)-Responsive Represor. *J. Bacteriol.,* 187(12): 4214–4221.

Yi, K., Rasmussen, A.W., Gudlavalleti, S.K., Stephens, D.S. and Stojiljkovic, I. 2004. Biofilm Formation by Neisseria meningitides. *Infect. Immun.,* 72(10): 6132–6138.