IJBPAS, February, 2015, 4(2): 239-249

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

POWER RHEOLOGY MODEL TO ASSESS BACTERIA GROWTH IN ACTIVATED SLUDGE POLLUTED WITH PHENANTHRENE

M. CRISTINA ROMERO[1, 4*], JUAN C. CHIARAVALLI[2], M. INÉS URRUTIA[3] & M. MORENO KIERNAN[5]

Fac.Cs.Veterinarias, [1]Cát. Micología Médica e Industrial, [2] Cát. Zootecnia General; [3]Fac. Cs. Agrarias y Forestales, calle 60 y 119, UNLP; [4]CONICET; [5]Minist. de Salud, Prov. de BA. Argentina.

(cmriar@yahoo.com.ar, proganaderia21@yahoo.com.ar; urrutia@cespi.unlp.edu.ar)

(*[1]Correponding author: cmriar@yahoo.com.ar, TE: 0054-221-425 0577, Calle 528 bis e/ 11 and 12 nº 1632, 1900, La Plata, Argentina).

ABSTRACT

Environmental biotechnology is a scientific and engineering system that use microorganisms to detoxificate solid, liquid and gaseous wastes. Activated sludge is a biotechnology that clean up sewage and industrial wastewaters using air and biological flocs. Different mathematical models expressed the bacteria growth in relation to the sludge parameters. The Power model described the relationship between the shear rates of the sludges with hydrocarbon and *Leptothrix* spp. flocs sizes; and was expressed as: $T = K \cdot \gamma^n$. Where: (T) was the shear stress, (K) indicated the sludge viscosity, (ɣ) was the shear rates and η was the flow behavior index. The assays were performed to evaluate the thick wooly flocs formation with and without phenanthrene. At low inoculum sizes (10^5 cells/ m^3), most of the flocs were larger than 3 µm, and the cellular yield was 0.3-0.6 mg dry wt/ ml. In contrast, with higher inoculums (10^9 cells/m^3), flocs of 2 µm were observed, and the bacterial yield was around a constant value c.a. 1.6 g dry wt/m. The relation of the inoculum with the flocs sizes responded to an hyperbolic curve. This phenomenon was related to poor growth due to oxygen limitation and hydrocarbon presence inside the large flocs. The experimental data were tested by the Power model, and the R^2 obtained indicated the goodness of the fit to the bioassays. The optimization of bioremediation

strategies like the bacterial bioaugmentation in activated sludge, confirmed that the Power model provided the best prediction of viscosity that determined the aeration with the suspended solids and phenanthrene present in the sludges.

Keywords: Activated Sludge - flocs -- *Leptothrix* spp. - Phenanthrene - Power Law - Viscosity

INTRODUCTION

Environmental contamination by petroleum and its derivatives is a world wide complex problem. Considerable hydrocarbons amounts had been released into water, soils due to pipeline lacks, transport accidents, storage tank ruptures **[1, 2]**. Hydrocarbon sludge are generated in oil-water separation systems, waste-oil accumulation, crude-oil storage tanks, maintenance and reparation of oil-tanks ships, floating platforms, harbor basin and cleaning of fuel service station **[3, 4, 5]**. Environmental biotechnology is a scientific and engineering system that use microorganisms and their products to detoxificate solid, liquid and gaseous wastes, by monitoring the detoxification processes **[6, 7]**. Wide spectrum of hazardous organic and inorganic materials could be detoxified by wild microorganisms **[8, 9, 10]**, and numerous methods break toxicants under diverse conditions (aerobic/anaerobic habitats, Δ pH, Δ ºT, organic/inorganic presence, soil texture; sorption/desorption process) **[11, 12, 13]**.

Activated sludge had been used to clean up sewage and industrial wastewaters using air and biological flocs composed by bacteria, protozoa, rotifers and fungi **[14, 15, 16]**. The microbial morphology is a prerequisite for this treatment and cellular aggregation depended on inoculum sizes, growth rates, age, metabolites, polymers, surfactants biosysthesis and chelators presence **[17, 18]**. Moreover, it was difficult to define the mechanisms by which flocs took form, as they depended on many factors like temperature, composition medium, viscosity, dissolved oxygen level and mix intensity. They affected the culture rheology, which was responsible for biological mass, heat and pollutant detoxification during *in-vitro* culture **[19, 20]**.

Different mathematical models expressed the filamentous bacteria growth in relation to the variables that dominated the activated sludges, this system had been considered as non-Newtonian fluid **[21, 22]**. Others relationships, like Power, Herschel-Bulkley, Bingham, Sisko, Carreau or Casson have been applied to perform the flow properties and specific functions of the sludges **[23, 24]**. However, there is a lack of reliable literature for viscosity in wastewater treatments, activated sludge due to its biological nature is a non-Newtonian system and time-dependent behavior, inducing important space-time variations of the sludge samples **[25]**. Therefore, the purposes of this article were to determine the flocs size developed by a filamentous bacteria in aromatic hydrocarbons presence, to evaluate the Power model to predict the flow behavior index in polluted sludge treatments with phenanthrene and to improve the bioassay factors necessary for the bioremediation strategies.

MATERIALS AND METHODS

Filamentous bacteria *Leptothrix* spp was isolated from sediments of the petroleum refinery effluents, La Plata, Argentina, in agar-mineral medium with phenanthrene (0, 25, 50 75, 100, 150, 200 and 250 µg phe/l) as sole carbon and energy source. The mineral and aromatic hydrocarbon medium had been already described **[8]**. The assays were performed in the same liquid medium to evaluate the thick wooly flocs formation with and without (control flask) aromatic hydrocarbon.

Floc sizes were analyzed by sieving bacterial filamentous aggregates, bore sizes 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 µm obtained by a diffraction sensor (Mastersizer 2000, Malvern), using spheres of the same volumes, by triplicate. Viscocity determinations were carried out with a high resolution C-VOR viscosimeter with a computer, the system was a plane-cone with a 60 mm ø. Measures were obtained by increasing the shear stress from 0.01 to 8.00 Pa, by duplicate.

The bacteria biomass from each bioassays with the diverse phenanthrene levels, were determinated by dry weights, by filtering 20 ml sludge through pre-weighed glass fiber filters (grade GF/C, 4.25 cm, Whatman), then they were washed and dried in a microwave oven (15 min at low power) and left in a dessicator for 24 hours before reweighing, until constant weight.

The stirred bioreactor with a single turbine, pH and temperature sensors was filled with 4 l of the sludge, at pH 6.0 controlled at 1.0 by automatic addition of titrants (2 M NaOH or 20% H_2SO_4 solutions), 27-28 ºC, air-flow rate at 1 vol/vol/min air/medium, the agitation system operated at 400 rpm and polyethylene glycol **(MW 2000, Sigma)** as antifoam. Other details of the equipments and the bioassays had been already described **[26]**.

The Power model described the relationship between the shear rates of the polluted sludge treatments with hydrocarbon and *Leptothrix* spp. aggregate sizes. The model was expressed as: $T = K \cdot \gamma^n$; where: (T) was the shear stress or force required to move a given area of the fluid activated sludges (Newton / m^2 = Pa); (K) termed as consistency index indicated the sludge viscosity, the higher the value is, the higher the apparent viscosity; it's defined as the ratio of shear stress over shear rate (Newton seconds / m^2 or Pa x seconds or Poise = dyne.s /cm^2); (ɣ) shear rates defined as the movement ratepf the fluid (1 /s) and η was the flow behavior index which specified the fluid sludge tendency to shear thin (dimensionless); the shear rate η of Newtonian fluids equals to 1 **[27, 28]**.

Statistics. All the experiments, inoculum cultures and phenanthrene assays were done by triplicate. The results are expressed as the arithmetic mean ± standard error; the Student´s two-tailed t-test was used to evaluate the differences between controls (0 phenanthrene leves or the initial data of the bioassays) and experimental means, with $P < 0.5$ being considered significant.

RESULTS AND DISCUSSION

Slurry-phase biological treatment is a relatively new development for the remediation of hazardous wastes, offering significant advantages over other biodetoxification technologies currently in use. It is highly effective for a variety of wastes, and its degradation rates is up to ten times faster than land treatments.

Leptothrix spp., an aerobic and chemoheterotrophic genus, that grew forming thick wooly flocs with range in color from orange to dark brown in relation to the Fe and Mn oxidation levels. It was worthy to highlight that while there are four recognized species, *L. ochracea* was the dominant one in iron-rich freshwater environments, and this specie was selected as it was able to growth with three fused benzene rings-aromatic hydrocarbon, and formed spherical flocs of different sizes in phenanthrene presence.

At low inoculum sizes (10^5 cells/ m^3), most of the flocs were around 3 µm, and the cellular yield was 0.3-0.6 mg dry wt/ ml. In contrast, with higher inoculums (10^9 cells/ m^3), flocs of 2.0 µm were observed, and the bacterial yield was around a constant value c.a. 1.6 g dry wt/m. The relation of the inoculum sizes with the flocs sizes responded to an hyperbolic curve **(Figure 1)**.

This phenomenon was related to poor growth rate due to oxygen limitation and hydrocarbon presence inside the large flocs. The relation with the agitation state of the flasks with 10^9 cells/m^3 in shake flasks and 10^{12} cells/ m^3 in non-shaked flasks, small unstable flocs formed at the 5^{th} culture day, that disappeared a week later. With the increase of the inoculum level, wooly growth was the prevalent development. The average floc size was inversely proportional to the inoculum sizes; furthermore, it appeared that within large inoculum ranges the mean aggregate value reached a plateau, remaining close to 1.8 µm.

The aggregate size distributions showed a bimodal relation, with two flocs sub-populations, one either large to ≥ 2.5 µm or small to < 0.6 µm; in the first case the cells grew around the hydrocarbon particle, in the second one no-phenanthrene fragment was observed inside. On the contrary, in the control sludge without phenanthrene only one population was observed with average floc of 0.8 µm.

Some models showed deficiencies to describe the overall profile of activated sludge systems. The presence of upper and lower Newtonian regions coupled with the Power law made the interpretation and application of rheological data a challenging task. Other relationships involved more parameters to describe the activated sludge flow. The Herschel-Bulkley model corrected this deficiency by replacing the plastic viscosity term in the Bingham equation with the Power expression.

The experimental data obtained from the polluted sludge treatments with phenanthrene and *Leptothrix* spp. were tested by the Power model; the correlation coefficient (R^2) obtained by multiple non-linear regressions and the least square technique indicated the goodness of the fit to the bioassays **(Figure 2)**. The numbers and sizes of the bacteria flocs and the phenanthrene particles determined T, that was the shear stress or force required to move a given area of the fluid sludges **(Table I)**.

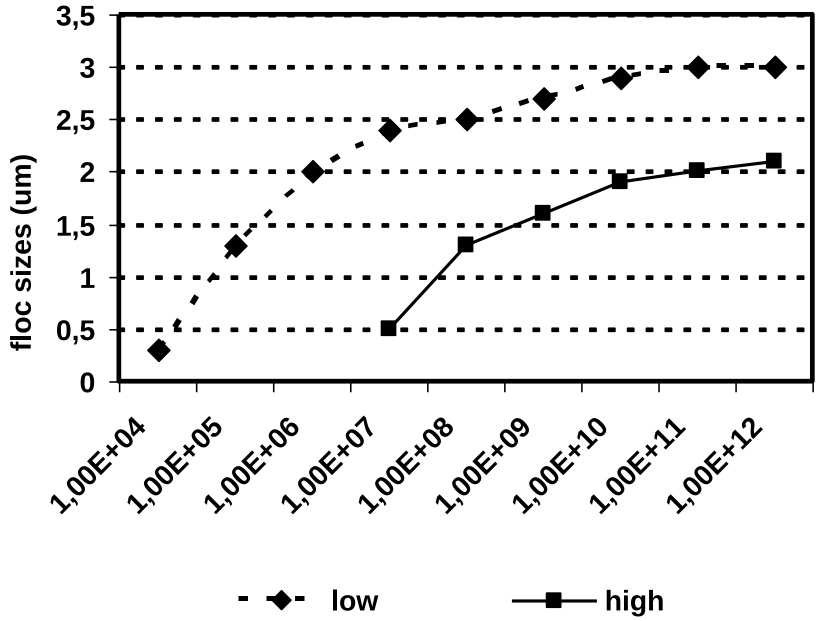

Figure 1: *L. ochracea* floc sizes in relation to the inoculum sizes in the polluted sludges

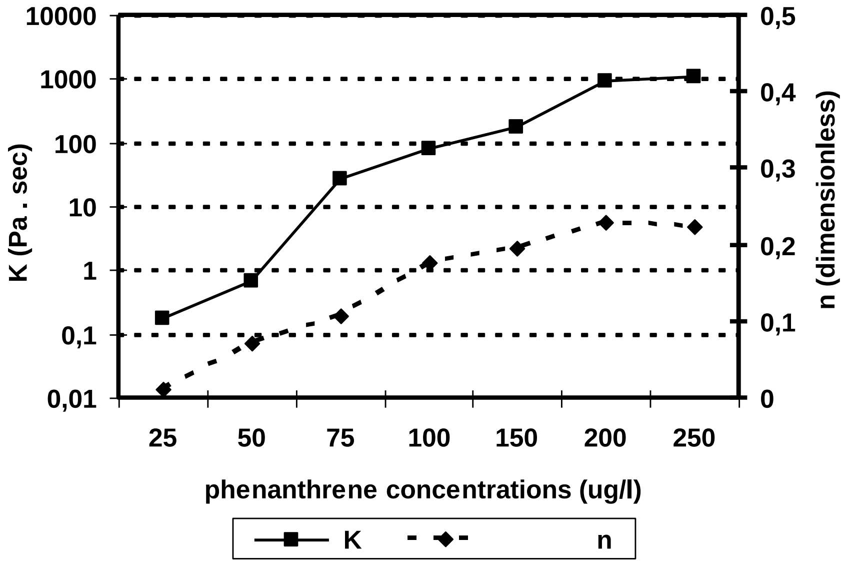

Figure 2: Power law parameters K (consistency index, Pa . seconds) and η (dimensionless) obtained from the activated sludge

Table I: Experimental parameters K (consistency index, Pa . seconds), η (dimensionless) and correlation coefficient (R^2) obtained in the polluted sludge

phenanthrene		parameters	
	K	η	R^2
µg/l	1.71E-01	7.06E-02	0.983
25	6.87E-01	1.13E-02	0.989
50	6.58E-01	8.73E-02	0.968
75	9.37E-01	2.06E-01	0.979
100	1.75E+00	9.56E-02	0.997
150	9.21E+02	2.29E-01	0.991
200	1.08E+03	3.44E-01	0.989
250			

DISCUSSION

To evaluate the phenanthrene sludge structure and flow characteristics, seven models for viscosity of non-Newtonian fluids had been considered by different authors, like Bingham plastic, Herschel-Bulkley, Power, Casson, Sisko, Carreau and Cross equation. These models were especially useful to assess the hydrodynamic and computational dynamic of the fluids. Polluted activated sludge processes, where viscosity plays a major role on the hydrodynamic regime, oxygen transfer and mass transport, influenced the system performance **[29, 30]**.

Several mathematical relationships have been developed to describe the relationship between shear stress and shear rate of non-Newtonian fluids. These equations characterized the flow properties and determine the ability of a fluid to perform specific functions. The most frequently applied models had been the Power law and Bingham plastic ones, each of them has two adjustable parameters. Moreover, others relationships involved three or four adjustable parameters. It is necessary to include a third parameter to describe the flow of the fluids in the upper or lower Newtonian region as well as the Power law region **[31, 32]**. The Herschel-Bulkley equation corrected some deficiency, but assumes that the flow is homogeneous, situation that was not suitable for activated sludge. The Casson and Sisko equations had been used to describe the flow in the Power law and upper Newtonian regions. The four parameter models, like the Carreau one was used over the entire range of shear rates; but the four parameter relations had been difficult to apply because there is seldom enough data to allow good model fitting **[33, 34]**.

Some researchers studied the link between the rheological properties and the sludges parameters. The Herschel-Bulkley relation was proper to describe the viscosity of activated sludge at high concentrations **[21, 30**]; besides, the Bingham model was better to characterized weakly concentrated sludge. Others presented that the Power law model was the best for representing the viscosity of activated sludge for a low shear rate range **[22, 32]**.

By other hand, *Leptothrix* spp. had been isolated from diverse metal rich aquatic environments, including lake bottom sediments, wells and groundwaters **[35, 36]**. The OD limitation determined that cells formed smaller flocs, resulting greater surface area per unit cell-volume, and increased oxygen transfer to bacteria **[7]**. Moreover, an increment in growth rate was correlated not only with the decreased in flocs size, but also with the hydrophobicity of cell-walls, as expressed in contact angles of cells with water. Different conditions yielded smaller flocs reducing the cell wall hydrophobicity, like temperature, shear rates, pollutans presence, microbial biomass **[8, 9, 37]**.

CONCLUSION

The phenanthrene levels, the bacterial floc sizes and the shear rates determined the activated sludge flow. The viscosity increased with the hydrocarbon levels and decreased exponentially with higher shear rates. Power law showed good agreement with the experimental data and provided better prediction of the cultural factors. The optimization of diverse bioremediation strategies like the bacterial bioaugmentation in activated sludge processes, where viscosity determined the hydrodynamic regime and the oxygen transfer influenced the system performance; and could be applied to select the most suitable bio-detoxification process.

ACKNOWLEDGMENTS

This work was supported by grants from the National Council of Scientific and Technological Research - CONICET; and from the National University of La Plata, UNLP, La Plata, Argentina.

REFERENCES

[1] Rittmann B and McCarty PL, Environmental biotechnology: principals and applications. 2001. McGraw-Hill, New York, 768 pp.

[2] Evans GG and Furlong J, Environmental biotechnology: theory and application. 2011. John Wiley & Sons (eds.), 2nd. ed., pp 29 - ISBN 0470975385 / 9780470975381

[3] Christou M and Konstantinidou M, Safety of offshore oil and gas operations: lessons from past accident analysis. Ensuring EU hydrocarbon supply through better control of major hazards. 2012. Europ. Com. Joint Res. European Union, Luxembourg (eds). pp 58.

[4] Westenhaus B, Trucks, trains, or pipelines. The best way to transport petroleum. 2013. MIT Press Energy Initiative, US.

[5] Leveson NG, Risk management in the oil and gas industry. 2011. MIT Press Energy Initiative, US.

[6] Coccia AM, Gucci PMB, Lacchetti I, Beccaloni E, Paradiso R, Beccaloni M and Musmeci L, Hydrocarbon contaminated soil treated by bioremediation technology: microbiological and toxicological preliminary findings. Environ. Biotech. 5, 2009, 61-72.

[7] Das N and Chandran P, Microbial degradation of petroleum, hydrocarbon contaminants: an overview. Biotech. Research Internat, Review Article, 2011, 1-13.

[8] Romero MC, Hammer E, Hanschke R, Arambarri AM and Schauer F, Biotransformation of biphenyl by filamentous fungi *Talaromyces helicus*. World J. Microb. Biotech. 21, 2005, 101-106.

[9] Romero MC, Urrutia MI, Reinoso EH and Moreno Kiernan M, Benzo[*a*]pyrene degradation by soil filamentous fungi. J. Yeast Fungi Research, http://www.academicjournals.org/JYFR. 1, 2010, 025-029.

[10] Romero MC, Urrutia MI, Reinoso EH, Della Vedova R and Reynaldi FJ, Atrazine degradation by wild filamentous fungi. Global Res. J. Microb. 4 (1), 2014, 10 –16.

[11] Romero MC, Reinoso EH, Urrutia MI and Reynaldi FJ, Impact of metals exposure and resiliency values in wild filamentous fungi. Inter. J. Biol. Phar. Allied Sciences 2 (10), 2013, 1814-1824. ISSN: 2277-499. http//www.ijbpas.com.2013.Romero.et.al

[12] Akratosa CS and Tsihrintzis VA, Effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Ecolog. Engineer. 29, 2007, 173-191.

[13] Romero MC, Urrutia MI, Reinoso EH and Moreno Kiernan A, Effects of the sorption/desorption process on the fluoranthene degradation by wild strains of *Hansenula angusta* and *Rhodotorula minuta*. Inter. Res. J. Microbiol. 2 (7), 2011, 230-236. http://interesjournals.org/IRJM/Content/2011%20content/August.htm - ISSN 2141-5463.

[14] Singh H, Mycoremediation. 2006. John Wiley & Sons, Inc. (eds.) 592 pp.

[15] Caia Q-Y, Mo C-H, Wu Q-T, Zeng Q-Y, Katsoyiannisc A and Férard J-F, Bioremediation of polycyclic aromatic hydrocarbons (PAHs) contaminated sewage sludge by different composting processes. J. Hazar. Mat. 142 (1-2), 2007, 535-542.

[16] Cecen F and Aktas O, Activated carbon for water and wastewater treatment: integration of adsorption and biological treatment. 2011. John Wiley & Sons (Eds.). 406 pp. ISSN 3527639462 / 9783527639465.

[17] Carrère H, Dumas C, Battimelli A, Batstone DJ, Delgenès JP, Steyer JP and Ferrer I, Pretreatment methods to improve sludge anaerobic degradability: a revie. J. Hazardous Materials 246, 2010, 577–581.

[18] Romero MC, Moreno Kiernan M, Moreno Kiernan A and Urrutia, MI, Fluoranthene degradation induced morphological and funtional changes in *Dipodascus ingens* biotypes. Rev. Mex. Micol. 22, 2006, 7-12. ISSN 0187-3180.

[19] Bräuer SL, Increased abundance of *Gallionella* spp., *Leptothrix* spp. and total bacteria in response to enhanced Mn and Fe concentrations in a disturbed Southern Appalachian High Elevation Wetland. Geomicrob. J. 29 (2), 2012, 124-138.

[20] Forster CF, The rheological and physico-chemical characteristics of sewage sludges. Enzyme Microb. Technol. 30, 2002, 340-345.

[21] Guibaud G, Dollet P, Tixier N, Dagot C and Baudu M, Characterisation of the evolution of activated sludges using rheological measurements. Process Biochem, 39, 2004, 1803–1810.

[22] Hasar H, Kinaci C, Unlu A, Togru, H and Ipek U, Rheological properties of activated sludge in a sMBR. Biochem. Eng. J. 20, 2004, 1-6.

[23] Mikkelsen LH, The shear sensitivity of activated sludge Relations to filterability, rheology and surface chemistry. Colloids Surf. A. 182, 2001, 1–14.

[24] Mori M, Seyssiecq I and Roche N, Rheological measurements of sewage sludge for various solids concentrations and geometry. Process Biochem. 41, 2006, 1656–1662.

[25] Seyssiecq I, Ferrasse JH and Roche N, State-of-the-art: rheological characterization of wastewater treatment sludge, Biochem. Eng. J 16, 2003, 41-56.

[26] Romero MC, Urrutia MI and Moreno Kiernan A, Bioreactor treatment of aromatic hydrocarbons by indigenous micoflora and *Gliocladium viride*. Global Res. J. Microbiol. 2 (2), 2012, 118-123. http://www.globalresearchjournals, org/?a=journal&id=grjm.

[27] Rosenberger S, Kubin K and Kraume M, Rheology of activated sludge in membrane bioreactors. Eng. Life Sci., 2 (9), 2002, 269-275.

[28] Khalili Garakani AH, Mostoufi N, Sadeghi F, Hosseinzadeh M, Fatourechi H, Sarrafzadeh MH and Mehrnia MR, Comparison between different models for rheological characterization of activated sludge. Iran. J. Environ. Health. Sci. Eng. 8 (3), 2011, 255-264.

[29] Mardani S, Mirbagheri A, Amin MM and Ghasemian M, Determination of biokinetic coefficients for activated sludge processes on municipal wastewater. Iran. J. Environ. Health. Sci. Eng., 8 (1), 2011, 25-34.

[30] Baudez JC, Ayol A and Coussot P, Practical determination of the rheological behavior of pasty biosolids. J. Environ. Manage. 72, 2004, 181–188.

[31] Forster CF, The rheological and physico-chemical characteristics of sewage sludges. Enzyme Microb.

Technol. 30, 2002, 340-345.

[32] Pollice A, Giordano C, Laera, G, Saturno D and Mininni G, Physical characteristics of the sludge in a complete retention membrane bioreactor. Water Res. 41, 2007, 1832–1840.

[33] Lotito V, Spinosa L, Mininni G and Antonnaci R, The rheology of sewage sludge at different steps of treatment, Water Sci. Technol. 36 (11), 1997, 79–85.

[34] Bougrier C, Carr`ere H and Delgenes JP, Solubilisaiton of waste activated sludge by ultrasonic treatment, Chem. Eng. J. 106 (2), 2005, 163–169.

[35] Johnson KW, Carmichael MJ, McDonald W, Rose N, Pitchford J, Windelspecht M, Karatan E and Bräuer SL, Increased Abundance of Gallionella spp., Leptothrix spp. and Total Bacteria in Response to Enhanced Mn and Fe Concentrations in a Disturbed Southern Appalachian High Elevation Wetland, Geomicrob. J. 29 (2), 2012, 124-138.

[36] Stein LY, Jones G, Alexander B, Elmund K, Wright-Jones C and Nealson KH, Intriguing microbial diversity associated with metal-rich particles from a freshwater lake. FEMS Microbiol Ecol 42, 2002, 431–440.

[37] Park JH, Feng Y, Ji P, Voice TC, Megharaj M, Ramakrishnan B, Venkateswarluk K, Sethunathan N and Naidu R, Bioremediation approaches for organic pollutants: a critical perspective. Environ Int. 37, 2011, 1362–1375.