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

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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: $T = K \cdot \dot{\gamma}^n$. Where: (T) was the shear stress, (K) indicated the sludge viscosity, ($\dot{\gamma}$) 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 leaks, 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 °T, 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 biosynthesis 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. Viscosity 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 determined 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% H₂SO₄ solutions), 27-28 °C, 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: $T = K \cdot \dot{\gamma}^n$; where: (T) was the shear stress or force required to move a given area of the fluid activated sludges (Newton / m² = 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 / m² or Pa x seconds or Poise = dyne.s /cm²); ($\dot{\gamma}$) shear rates defined as the movement rate of 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 \pm standard error; the Student's two-tailed t-test was used to evaluate the differences between controls (0 phenanthrene levels 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 biodegradation 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 (10⁵ cells/ m³), most of the flocs were around 3 μ m, and the cellular yield was 0.3-0.6 mg dry wt/ ml. In contrast, with higher inoculums (10⁹ cells/ m³), flocs of 2.0 μ m 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 10⁹ cells/m³ in shake flasks and 10¹² cells/ m³ in non-shaked flasks, small unstable flocs formed at the 5th 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 $\geq 2.5 \mu\text{m}$ or small to $< 0.6 \mu\text{m}$; 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**).

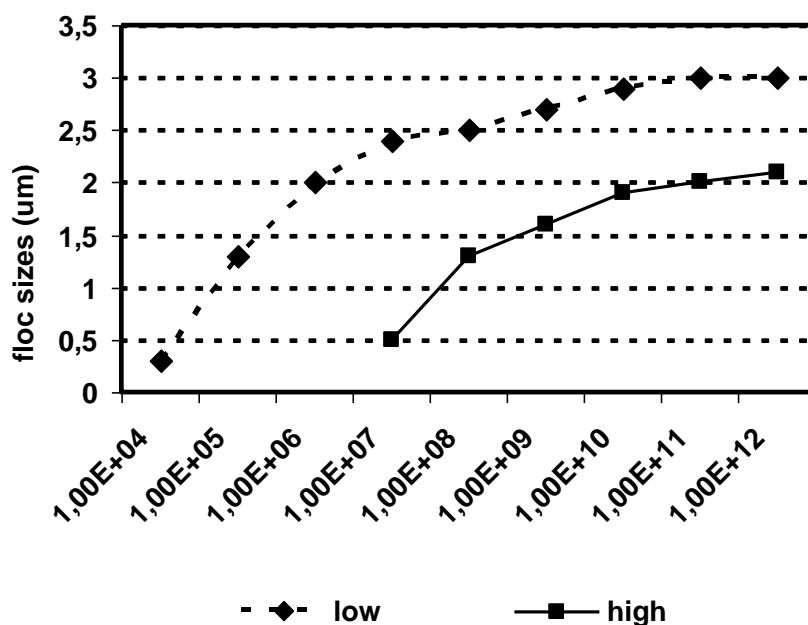


Figure 1: *L. ochracea* floc sizes in relation to the inoculum sizes in the polluted sludges

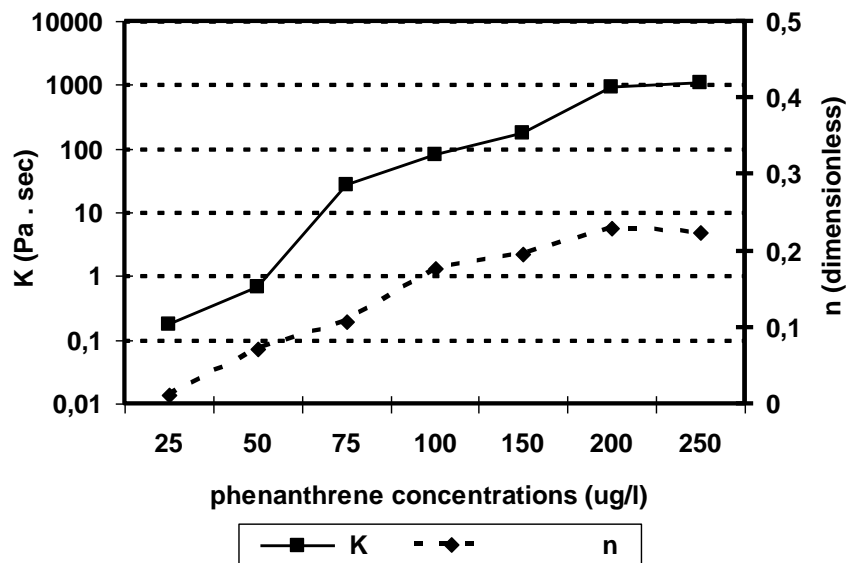


Figure 2: Power law parameters K (consistency index, Pa . seconds) and η (dimensionless) obtained from the activated sludge

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

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

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, pollutants 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.

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