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# THE PERFORMANCE OF A THREE-PHASE FLUIDIZED BED REACTOR IN TREATMENT OF WASTEWATER WITH HIGH ORGANIC LOAD

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**Abstract -** An experimental study was carried out aiming to evaluate the performance of a three-phase fluidized bed bioreactor (FBBR) used to treat milk wastewater. In this study three different concentrations of milk wastewater substrate (462, 825 and 1473 mg $O_2$/L) were tested. Using the same number of support particles, the results demonstrate that the average efficiency of COD removal decreased as the concentration of organic load in the substrate was increased. The growth of microorganism in the FBBR was followed by a count of viable cells in both liquid phase and the biofilms attached to the support. An increased number of viable cells were observed inside the reactor when it was used to degrade higher organic loads, with most of the cells on the support. The higher concentration of active biomass was responsible for achieving a relatively high absolute degradation of the wastewater containing the high organic load.
Keywords: wastewater treatment, fluidized bed bioreactor, viable COD, biofilm

## INTRODUCTION

The final disposal or destination of residues, specially the wastewater generated by industrial processes (such as the milk industry, slaughter houses, tanneries, breweries, food-processing industries, etc.) and that from domestic sewage, has become a serious problem to be tackled by big cities (Di Bernardo et al., 1989). The wide variety and large quantity of chemical compounds found in these wastewaters are responsible for most of the environmental pollution of aquatic bodies (Metcalf and Eddy, 1991).

Biological treatment of wastewater, using either aerobic or anaerobic microorganisms, has been used to degrade organic loads. According to Bergamasco (1996), cell respiration is the main mechanism involved in the biodegradation carried out by microorganisms in aerobic processes. This process uses oxygen as the main electrons acceptor, there by causing the oxidation of organic compounds. It transforms complex molecules into simpler and more stable ones (Lehninger, 1976). This respiratory metabolism liberates the necessary energy for maintenance and growth of bacterial cells. While total oxidation of organic compounds can be achieved in the aerobic process, oxidation in the anaerobic process is only partial and occurs in the absence of oxygen. In the latter process microorganisms interact in order to cause a stable

and self-regulating fermentation of organic compounds producing $CH_4$ and $CO_2$.

In the 30's the fluidized bed bioreactor (FBBR) appeared as a new alternative for biological treatment of wastewaters. In this type of reactor a high concentration of biomass is maintained inside because, microorganisms are attached to support particles. In order to achieve aerobic degradation in this reactor, support particles are fluidized by the flowing wastewater, which must have been previously oxygenated or flow co-currently together with an air stream.

Applications of FBBRs to treat wastewater appeared only towards the end of the 70's and the beginning of the 80's with several advantages over the conventional treatment used up to that time. These advantages have been discussed extensively in the literature (Distler, 1995; Kroumov et al, 1999; Cohen, 2001; Souza, 2002). The main advantages are the lower hydraulic retention time (HRT) and the small size of equipment required.

In FBBRs it is possible to achieve a high concentration of biomass depending on the operational conditions used in the process and the type of support used to immobilize the microorganisms, which are found within a complex structure of cells and their extracellular products, referred to as a biofilm. The cell volume inside the biofilm is only a small part of its total volume. Polysaccharides represent around 65%, while proteins represent 10 to 15% of the extracellular products within the biofilm (Lazarova and Manem, 1995).

Dense particles were traditionally used in FBBRs as a support for the biofilm. Entrainment of support particles from the reactor was problem that appeared as the biofilm grew, decreasing particle density. Lertpocasombut et al. (1988) suggested the use of lower density support particles to avoid entrainment of particles from the reactor. Several authors then suggested (Bergamasco, 1996; Tavares, 1992) use of polymeric material particles, which allows prior treatment to improve biofilm adhesion and growth on particle surfaces.

In spite of the advantages of three-phase FBBRs in degrading wastewater, most of the works in the literature is related to the treatment of wastewater with low organic load. In general, these works deal with wastewater containing inlet chemical oxygen demand (COD) not higher than 350 mg/L (Souza, 2002).

In order to degrade higher concentrations of COD, it is necessary to have enough viable biomass available for degradation to occur within the reactor. Viable biomass can be determined by means of classic methods of microbiology (spread and pour plate methods) that determine the number of viable cells. In relation to this, Münch and Pollard (1997) presented a methodology to determine the COD of viable biomass in different types of reactors used for wastewater treatment.

Using advantages offered by the FBBR to achieve aerobic degradation of wastewater, this work aims to study the performance of a three-phase FBBR with low-density support particles in treating milk industry wastewater containing high organic loads by quantifying the amount of viable biomass within the reactor.

## MATERIALS AND METHODS

### Experimental Apparatus

A sketch of the three-phase FBBR used in this work to treat wastewater from the milk industry is shown in Figure 1. The reactor consists of a perspex (acrylic) column with a height of 2m and an internal diameter of 0.11 m where small support particles are fluidized. Above this column there is a cylindrical disaggregating zone (0.5 m high with a 0.24 m ID) to separate contacting phases. A cylindrical PVC settler with a height of 0.8 m and ID of 0.24 m was coupled to the disaggregating zone. The liquid affluent, air stream and liquid recycling stream are fed at the bottom of the column with co-current contact.

The fluidizing support particles are small PVC disks that had been previously treated with concentrated nitric acid solution ($HNO_3$ 95% w/w) to improve microbial adhesion. The density, average diameter and average thickness of these support particles are 1.37 g/cm$^3$, 3.45mm and 1.12 mm, respectively.

### FBBR Operation

A mixed culture of aerobic and facultative anaerobic microorganisms, obtained from the sludge of the municipal sewage treatment, (SANEPAR), had been previously acclimated with a synthetic milk wastewater during 11 days before its inoculation in the reactor. The synthetic wastewater used in this acclimatization had a COD of 300 mg $O_2$/L and its composition is shown in Table 1.

The FBBR was inoculated carried out by feeding the previously acclimated mixed culture into the reactor under fluidisation conditions during 24 hours, using a batch of 100L of a synthetic wastewater with a COD of 450 mg/L. Then wastewater was gradually

fed in during a period of three days until reaching 18 L/h. Two different recycling and aeration flow rates were used in the experiments. The number of PVC support particles used comprised 0.5 m. of the column height (static height). The reactor was operated during 348 days, divided into four experimental phases 1, 2A, 2B and 3, according to the inlet feed concentration or organic loads of synthetic milk wastewater and hydrodynamic operational conditions as described in Table 2.

In all experiments the aeration flow rate used plus an extra aeration in the feeding of wastewater maintained the oxygen concentration inside the FBBR above 2 mg/L. The oxygen concentration was monitored using a Digimed DM4 oxymeter.

Reactor operation in all experimental phases was monitored measuring several parameters: pH, temperature, COD and protein and polysaccharide contents. COD analyses were carried out using the closed reflux colourimetric method described in APHA (1980). Protein content was measured by the Löwry method (Löwry et al., 1951) and the polysaccharide content was measured by the Dubois method (Dubois et al., 1956) in triplicate samples.

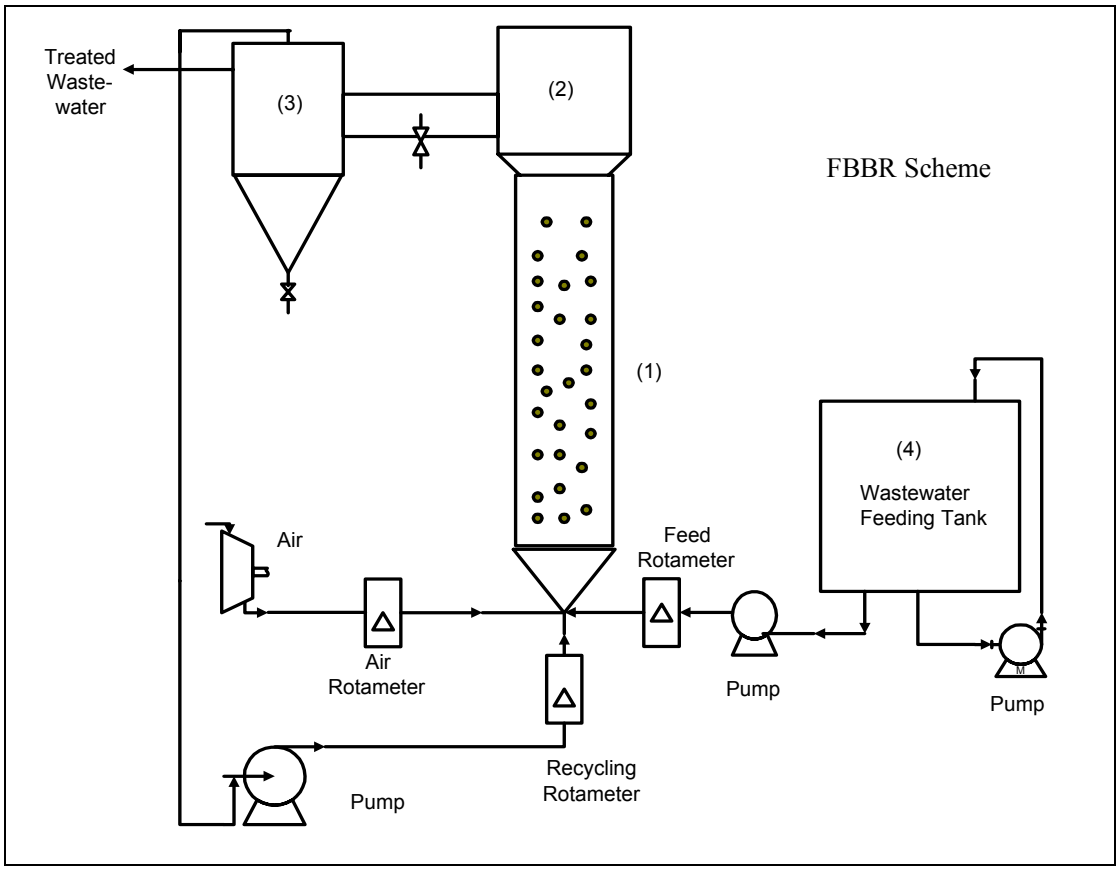


**Figure 1:** Experimental rig

**Table 1: Composition of synthetic milk wastewater for a COD of 300 mg $O_2$/L**

| Compound | Concentration (mg/L) |
|---|---|
| Whole powdered milk | 150.0 |
| Anhydrous D-glucose p.a. | 150.0 |
| Urea p.a. | 14.4 |
| Monobasic potassium phosphate | 7.2 |

**Table 2: Operational conditions used in three-phase FBBR**

| | Experimental Phase | | | |
|---|---|---|---|---|
| **Parameters** | **1** | **2A** | **2B** | **3** |
| Operation time (days) | 256 | 36 | 35 | 21 |
| Inlet feed concentration (mg $O_2$/L) | 460 | 825 | 825 | 1475 |
| Hydraulic retention time (min)* | 25 | 25 | 41 | 41 |
| Bed expansion (%) | 50 | 50 | 150 | 150 |
| Feed flow rate (L/h) | 18 | 18 | 18 | 18 |
| Aeration flow rate (L/h) | 13.21 | 13.21 | 34.31 | 34.31 |
| Recycle flow rate (L/h) | 1317 | 1317 | 2457 | 2457 |
| Liquid velocity – $U_L$ (m/h) | 141 | 141 | 261 | 261 |

* Based on expanded bed volume

## Determination of Viable Biomass

The viable biomass was obtained from the number of cells present in liquid phase, which was determined by using the spread plate method on nutrient agar for counting live cells (Baker et al., 1983). According to Münch and Pollard (1997) the viable biomass COD of a biological degradation process can be obtained from the equation

$$C_{\text{Biomass COD}} = C_{\text{cells}} \cdot m_{\text{cell}} \cdot i_{\text{COD X}} \quad (1)$$

where $C_{\text{cells}}$ is the number of cells per liter (CFU/L), $m_{\text{cell}}$ is the dry weight of a bacterial cell (mg/cell) and $i_{\text{COD X}}$ is the COD equivalent of biomass (mg COD/mg of biomass).

In this work the values of $14\times10^{-11}$ mg/cell and 1.416 mg COD/mg of biomass were used for $m_{\text{cell}}$ and $i_{\text{COD X}}$, respectively. These values were also used by Münch and Pollard (1997) for bacterial cells found in wastewater treatment. The value of $C_{\text{cells}}$ was determined by the viable cells counting method. In this method liquid samples containing cells were treated twice with ultrasound (0.2 A during 2 min) to disaggregate cells. After that they were filtered with isopore polycarbonate filters with 5-μm diameter pores (Millipore). The filter was then aseptically torn into four pieces to allow adequate coverage of the filter by sterile 0.1M phosphate buffer at pH 6.5. The filter was sonicated twice for 2 min in 10 ml of sterile buffer. This procedure was repeated in order to assure that all cells present in the sample passed through the membrane. The bacterial concentration in these suspensions was determined by plating on nutrient agar (Biobrás).

In determining the number of viable cells, rod-shaped bacteria were found to be the main representatives of the dense population. None of the predominant colonies belonged to groups of fungi. In addition, microscopic examination of biomass samples showed that among the numerous bacteria, protozoa were occasionally observed.

The viable biomass found in the biofilm attached to support particles was analyzed after removal of the biofilm from the support by two sequential ultrasound treatments. The respective number of viable cells was determined by the same procedure as that used for the liquid phase, but instead this number was expressed as number of cells per gram of support particles.

## RESULTS AND DISCUSSION

Phase 1 was the longest experimental phase. It was necessary to extend it to ensure stable conditions in the reactor, such as biofilm formation. In this phase the reactor was fed with the lowest concentration of substrate (COD ≈ 460 mg $O_2$/L).

The average COD removal in this phase was 67.6%, according to results presented in Figure 2. In this figure removal is expressed in a control chart, which includes, in addition to the average removal percentage achieved after regime establishment, the upper and lower control limits with a 3σ amplitude within a confidence interval of 99.7%.

COD removal results for phases 2A, 2B and 3 are presented in a similar way (control chart) in Figure 3. A comparison between phases 1 and 2A shows that as the inlet concentration of substrate in the wastewater was increased from 460 to 825 mg $O_2$/L,

the percentage of COD removal decreased to 44.1%. The same operational condition of an HRT (hydraulic retention time) of 25 min was maintained in these two phases. In order to increase COD removal, the HRT in phase 2B was increased to 41 min maintaining the same substrate inlet concentration (825 mg $O_2$/L). The results presented in Figure 3, in which average COD removal increased 5.2%, reaching 49.3%, illustrate this fact.

Maintaining the HRT at 41 min, but increasing the substrate inlet concentration from 825 to 1475 mg $O_2$/L, average COD removal decreased only marginally to 48.7%, as shown by results for phases 2B and 3 presented in Figure 3.

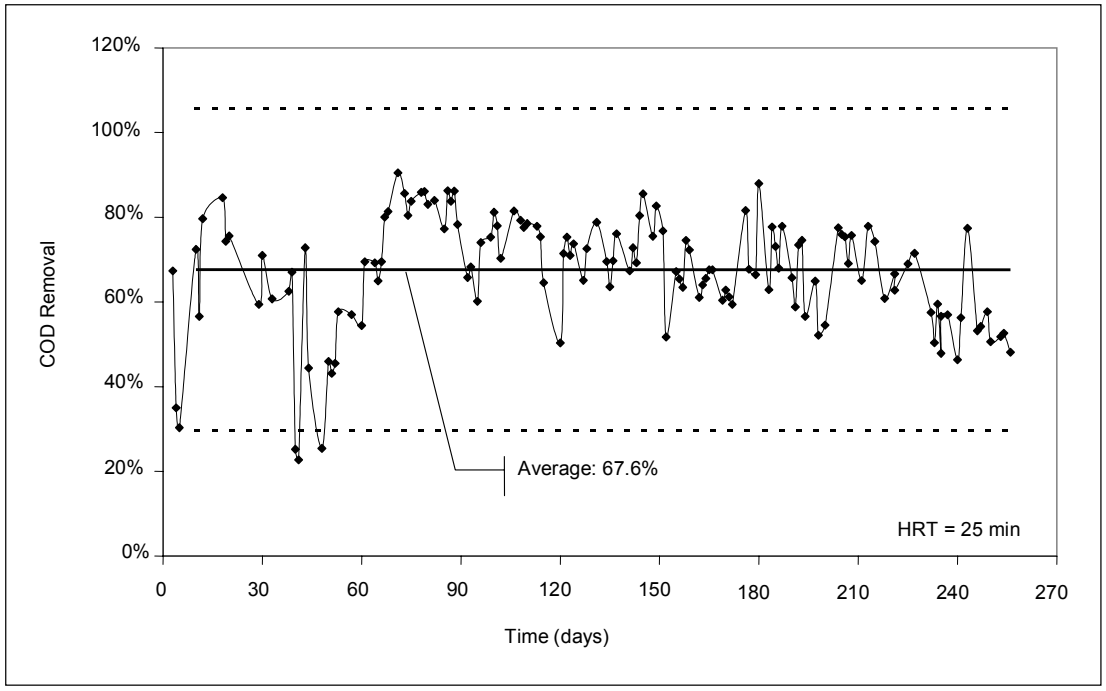


**Figure 2:** Efficiency of COD removal in FBBR (phase 1)

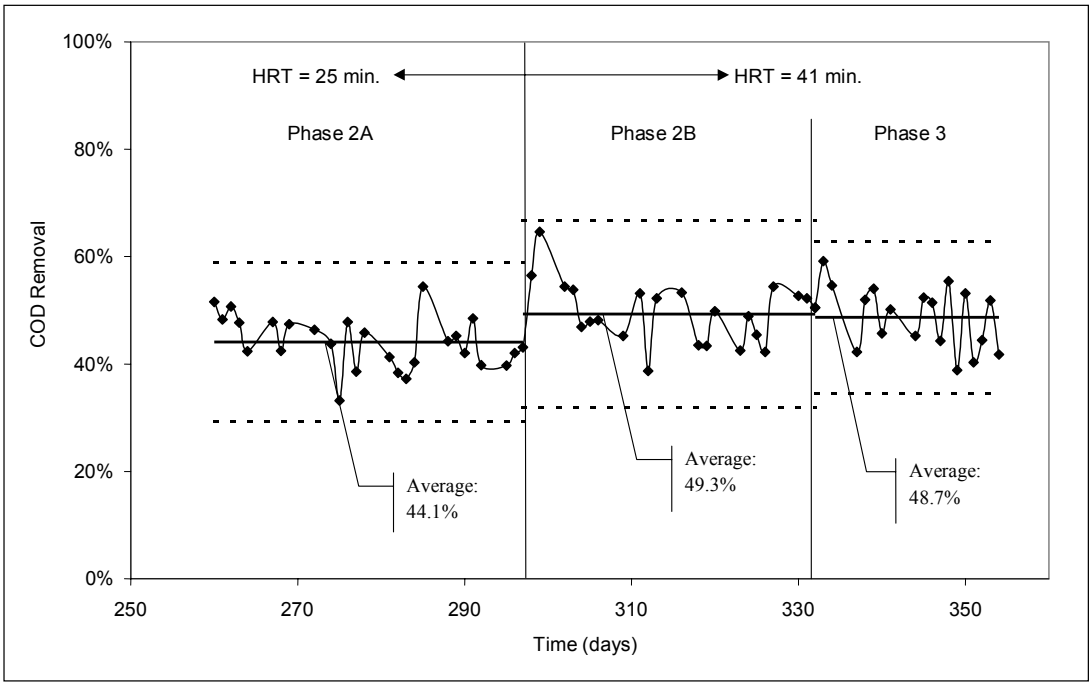


**Figure 3:** Efficiency of COD removal in FBBR (phases 2A, 2B and 3)

Table 3 illustrates the variation in measured values of pH and temperature inside the FBBR for all experimental phases. According to Pelczar et al. (1996) conditions under which microorganisms grow better are in temperatures ranging from 25 to 40 °C and values of pH between 4 and 9. The conditions presented show that this temperature range was not maintained within this interval on only one day during wintertime. Therefore these conditions were enough to ensure good degradation inside the FBBR. Data presented show that the increase in substrate inlet concentration resulted in a reduction in the relative removal of COD; however, if this removal is compared to absolute removal, as shown in Table 3, a higher removal is observed for higher substrate inlet concentrations.

The values of polysaccharide content in both inlet and outlet reactor streams demonstrated that the FBBR is able to remove around 90% of the polysaccharides, as the average removal for phases 1, 2A, 2B and 3 were 88.1%, 90.4%, 89.0% and 89.4%, respectively.

The Löwry method (Löwry et al., 1951) was used to quantify total proteins both in the feed and outlet reactor streams. It was verified that the protein content in the outlet stream was higher than that in the feed stream, due to the cells in suspension, that had possibly detached from the biofilms on particles.

The Löwry and Dubois methods were adapted to quantify proteins and polysaccharides in biofilms attached to support particles (Bergamasco, 1996). This was done using samples consisting of ten particles inserted in a 1 ml volume of distilled water. The of protein or polysaccharide contents of the samples were obtained in the same manner as for the liquid samples. Using this volume and the weight of the ten particles, protein or polysaccharide contents were converted into mg of proteins or mg of polysaccharides per gram of support particles. Table 4 shows the average values for proteins and polysaccharides present on support particles for all experimental phases.

Data presented in Table 4 show an increase in both the average contents of protein and of polysaccharide in the biofilms when the substrate concentration in the wastewater was increased. Polysaccharides have been reported in the literature (Bergamasco, 1996) to be the cementing agent responsible for microorganism adhesion to support particles. Figure 4 shows the gradual increase in average contents of both proteins and polysaccharides in biofilms supported on fluidizing particles. Therefore, the higher polysaccharide content together with the higher protein contents observed can indicate a more intense colonization of the biofilms. This allowed degradation of higher organic loads, as shown by the data for absolute COD removal presented in Table 3.

**Table 3: Operational conditions in the FBBR and COD removal in all experimental phases**

| Phase | Temp. (°C) | pH | HRT (min) | $COD_{in}$* (mg/L) | Relative COD Removal (%) | Absolute COD Removal (mg $O_2$/L) |
|---|---|---|---|---|---|---|
| 1 | 19 - 41 | 5.2 - 7.3 | 25 | 462.4 | 67.6 | 313.4 |
| 2A | 33 - 41 | 4.2 - 6.3 | 25 | 825.1 | 44.1 | 365.0 |
| 2B | 33 - 41 | 4.2 - 6.3 | 41 | 823.8 | 49.3 | 406.8 |
| 3 | 28 - 39 | 4.3 - 5.1 | 41 | 1472.9 | 48.7 | 754.7 |

* average values

**Table 4: Average proteins and polysaccharides on biofilm attached to support particles**

| Phase | Proteins (mg/g support) | Polysaccharides (mg/g support) |
|---|---|---|
| 1 | 3.91 | 8.07 |
| 2A | 4.88 | 8.50 |
| 2B | 5.89 | 8.39 |
| 3 | 6.67 | 10.95 |

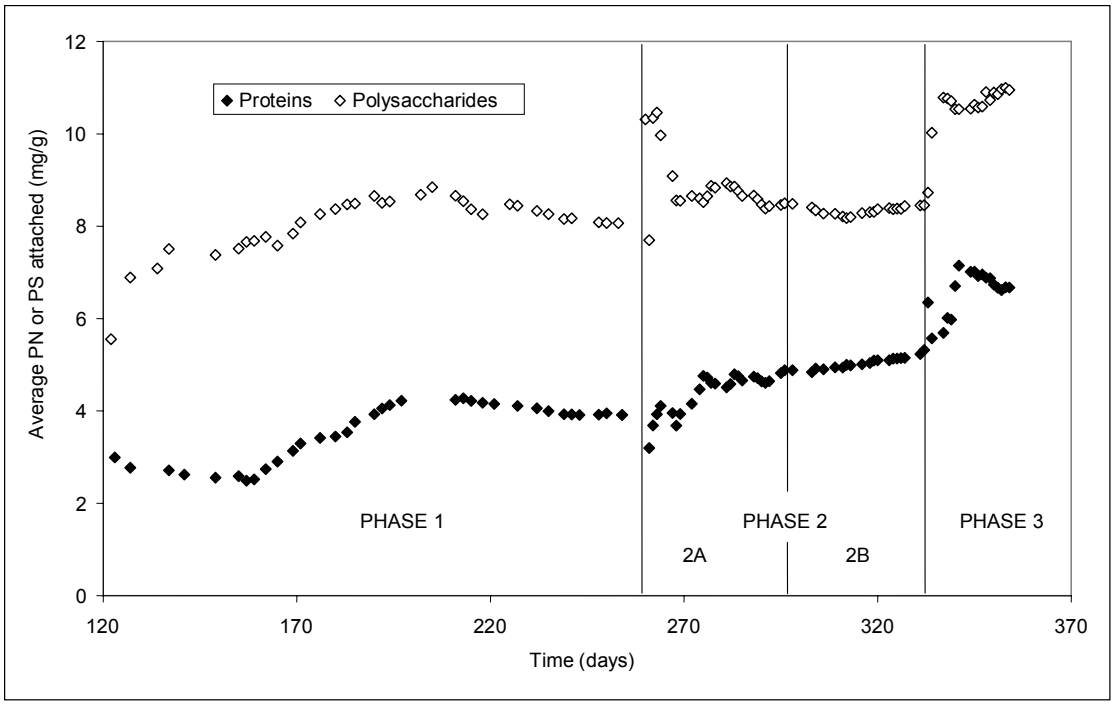


**Figure 4:** Cumulative average protein or polysaccharide content attached to support particles during all experimental phases

## Viable Bacterial Biomass

The numbers of viable cells in the biofilms attached to the support particles are shown in Table 5 for experimental phases 1, 2B and 3. In this table the amount of biofilm attached to support particles, obtained gravimetrically based on 10 particles but expressed as mg of biofilm per g of support, is also presented. As the biofilm is not evenly distributed on all particles, a smaller amount of biofilm on support particles sampled was observed for experiments of phase 3 than that for phase 2B. Although a larger amount of biofilm was to be expected, the results still demonstrate an increase in the percentage of viable cells in biofilms as the FBBR treated wastewater containing higher organic loads.

Results for the liquid phase inside the FBBR are presented in Table 6. These includes the number of viable cells and the corresponding COD and total COD inside the reactor. Similar to results presented for attached biofilms (Table 5), a significant increase in number of viable cells is observed as the concentration of substrate increases.

These results can be compared to those for biofilms attached to the support. After transforming the latter into a volume base using particle density, the values $6.7\times10^{11}$, $24.5\times10^{11}$ and $38.9\times10^{11}$ colony forming units per liter of support were obtained for phases 1, 2B and 3, respectively. These larger values, although roughly estimated, do confirm the higher level of activity of the biomass on the fluidizing support particles. Results in Table 6 also demonstrate that the COD of the viable biomass is only a small portion (around 10%) of the total COD in the reactor.

In order to remove a higher amount of organic load, it is necessary to increase the biomass in the FBBR. The results obtained in this work clearly show that degradation of higher organic loads was obtained at the expense of an increase in the active biomass. The results demonstrate an increase in the viable biomass attached to the support as the concentration of substrate was increased, but it seems that there is a limit to this growth of viable biomass. A factor that has limited this growth could be the lack of surface area provided by the number of support particles, as the same number of support particles was used in all experiments. The hydrodynamic condition is another limiting factor that may not have allowed development of thicker and denser biofilms to an extent that resulted in effective mass transfer rates. In addition to these factors, inhibition by products or substrate concentration may also play an important role in the rates of COD removal.

**Table 5: Biofilm and viable cells attached to support particles**

| | Experimental Phases | | |
|---|---|---|---|
| **Parameter** | **1** | **2B** | **3** |
| Number of viable cells ($\times 10^9$ CFU/g support) | 0.49 | 1.79 | 2.84 |
| Biofilm attached to support (mg/g of support) | 4.31 | 6.44 | 6.35 |
| % Viable cells in the biofilm | 1.58 | 3.89 | 6.27 |

**Table 6: Results of suspended microorganisms in the liquid phase**

| | Experimental Phases | | |
|---|---|---|---|
| **Parameter** | **1** | **2B** | **3** |
| Number of viable cells ($\times 10^{10}$ CFU/L) | 6.9 | 22.5 | 57.5 |
| COD of viable biomass(mg $O_2$/L) | 13.7 | 44.6 | 113.9 |
| COD inside FBBR(mg $O_2$/L) | 150 | 440 | 755 |

## CONCLUSIONS

Results presented in this paper have clearly shown that it is possible to degrade wastewaters containing high organic loads in a three-phase fluidised bed bioreactor, since hydrodynamics and operational conditions, such as surface area of support particles, are assured in order to maintain enough viable biomass inside the reactor.

Analysis of viable biomass demonstrated that the main factor responsible for degradation performed is the biomass that becme attached to support particles, although some degradation may occur by suspended active cells as the reactor was operated in a mixing regime (high recycle rates).

## ACKNOWLEDGEMENTS

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## NOMENCLATURE

| | |
|---|---|
| COD | chemical oxygen demand |
| CFU | colony forming units |
| $C_{cells}$ | number of cells per liter (CFU/L) |
| $m_{cell}$ | dry weight of a bacterial cell (mg/cell) |
| $i_{COD\ X}$ | COD equivalent of biomass (mg COD/mg of biomass). |

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