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Biofilm Characteristics and Compositions in Fluidized Porcelanite Bioreactors: An Experimental Work

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Abstract: Two systems of Fluidized Porcelanite Bioreactors (FPBR) were designed and constructed at Al-Rustamiyah Sewage Treatment Plant, South of Baghdad. The first system consists of Upflow Expanded Bed Reactor (UEBR) which is connected in sequence with aerated fluidized bed reactor (AFBR), while the other system is composed of two identical upflow expanded reactors operated in parallel. In order to evaluate the biofilm compositions and its characteristics on Porcelanite aggregates or fragment surfaces, an experimental measurements of the biofilm formation using the light microscopy in fixed media have been undertaken. Systems operation consists of various distinct phases, where influent substrate concentrations were taken from primary sewage and clarifier effluent of Al-Rustamiyah sewage.

Three effective parameters, that could influence the rate of biofilm growth such as superficial velocity, substrate, and biomass concentration, were investigated. The rate of biofilm was tested in two types of aeration; the first one was aerated directly compared with second one, which was operated by indirect aeration. Also the rate of biofilm losses for different influent flow rates, substrate concentrations, locations of packing bed in the reactor, and the periods of operation was evaluated. This work showed the main following conclusions:

1. The physical, chemical and biological analyses depend on the environmental conditions to which the attachment surface is exposed. The large portion of biofilm composition was water with 90.4%, and with a small value of volatile fraction ranged to 9.2%, while the fixed fraction is composed of 0.4% of total biofilm by mass.
2. Organic and chemical composition of the fixed fraction of biofilm was determined, where the primary constituents' carbon (C) was to be 58%, and nitrogen (N) to be 15%, and phosphorus (P) to be 2%, while the fixed solid composition was 25%.
3. The inorganic composition of fixed fraction of biofilm varies with chemical and organic properties of bulk water, chemical and physical properties of media, which composes mainly of silica with (45%), and with Fe^{+2} (20%), Mn^{+2} (14%), Al^{+3} (10%), Ca^{+2} (6%) and Mg^{+2} (5%).

Key words: Biofilm, fluidized bed, bioreactor, porcelanite.

Introduction

Generally, biofilms are layers like aggregations of microorganisms and their extra cellular polymers are attached to a solid surface (Characklis, 1973; Trulear & Characklis, 1979; Costerton, 2004). Biofilms, which are naturally immobilized cells, occur ubiquitously in nature and increasingly important in engineered processes used in pollution controls (Cunningham et al., 1991; Purevdorj & Stoodley, 2004). Biofilms development brings about changes in particle size, density and hydraulic drag coefficient (Costerton, 1999; Lewandowski, 2000). The growth, detachment, density and thickness of biofilm depend on parameters like velocity of water, properties

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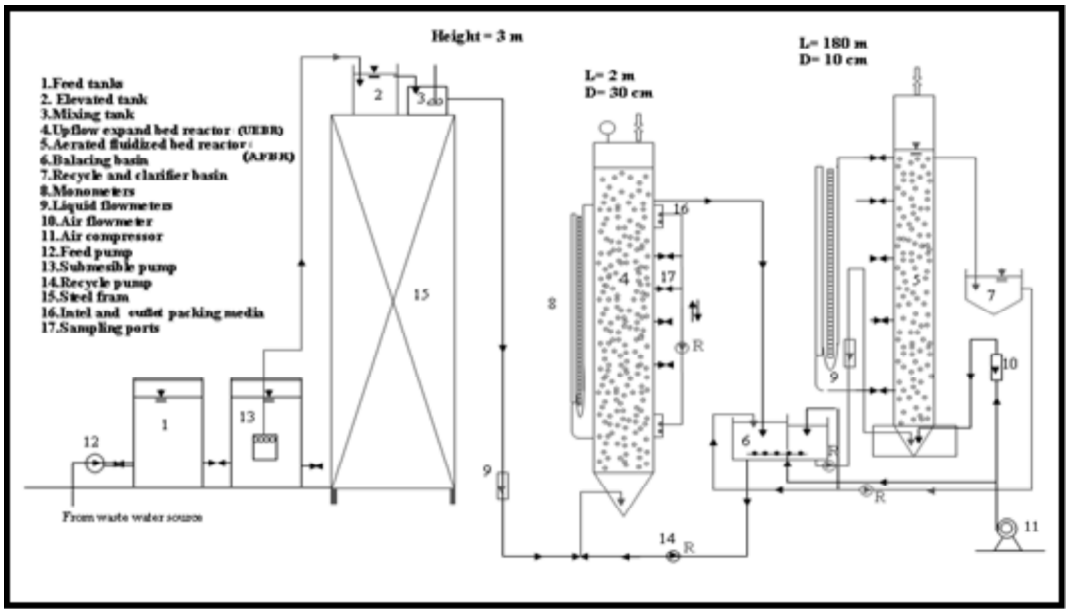


Figure 1: Schematic diagram of the first pilot scale (field study).

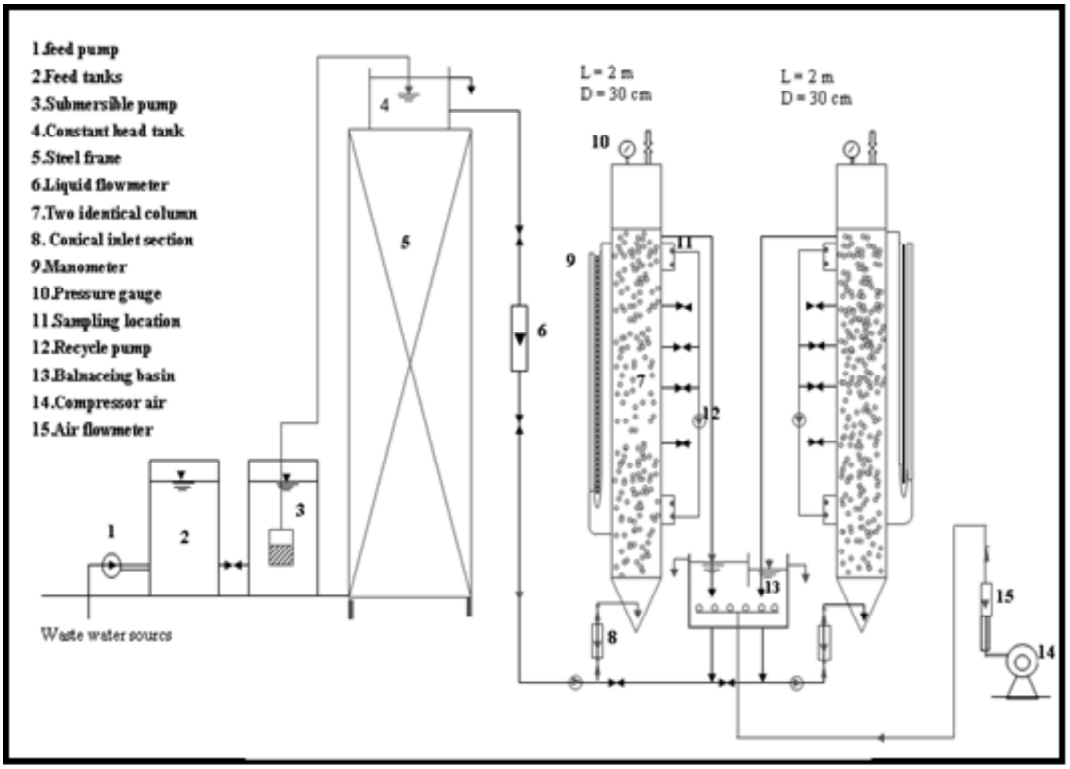


Figure 2: Experimental setup of the pilot scale (field study).

of packing material, load of suspended solids and organic nutrient compounds (Geesey et al., 1992; Scheuerman et al., 1998). Biofilm density is a function of upflow velocity which decreases with increase in the upflow velocity. Biofilm thickness, however, is independent of upflow velocity achieving a steady state thickness which is unaffected by upflow velocity (Huang et al., 1992).

Biofilm is affected by substrate flux in FBRs. Rittmann and McCarty (1980a) found that when flux is large, biofilms become deep and biomass at the attachment surface approaches zero activity. When flux is small, the biofilm on carrier material is thin and approaches

Photo (1): Field pilot scale (First system).

complete substrate penetration (Rittmann, 1982). An advantage of expanded fluidized bed reactors over other biological treatment methods is the high loading capacity made possible by the adhesion and growth of microbiological organisms upon the suspended medium (Atkinson et al., 1981; Cooper & Wheeldon, 1981).

Materials and Methods

The field experimental setup used in this study is presented in (Figures 1 and 2). The first system consists of upflow expanded bed reactor (UEBR) which is connected in sequence with aerated fluidized bed reactor (AFBR), Photo (1), while the other system is composed of two identical upflow expanded reactors which are operated in parallel, Photo (2).

Experimental Design and Operation

The bed pressure drop and bed expansion are monitored at different superficial liquid velocities ranging from 7.5 m/hr to 72 m/hr. The design and the operating parameters that are important to maintain high biomass concentrations in the reactor include volume of media, biofilm density and biofilm thickness. However, biofilm thickness and expanded bed height become important operating parameters, which affect expansion and biomass concentration. The temperature and pH values in the laboratory model scale are maintained in the range of 24-26°C and 7.5-8.6°C, respectively. Also, the dissolved oxygen is kept 2-4 mg/l during the experimental run.

First Pilot-scale System

Design parameters and operation of the first pilot-scale system are shown in Tables 1 and 2, respectively. The operation of the first pilot system has a duration of approximately 12 months. The operation of system

Photo (2): Field Pilot Scale (Second System).

consists of various distinct phases, as shown in Tables 1 and 2. The influent substrate concentrations are taken from primary sewage and clarifier effluent of Al-Rustamiyah Sewage Treatment plant. Minimum hydraulic retention time (HRT) is chosen according to the maximum of the feed and recycle pumps which are used in the pilot-scale systems, while the other variables are chosen according to the reasonable expected range (Cooper and Wheeldon, 1981).

Second Pilot Scale System

The identical reactors are designed and operated continuously for period of approximately (six) months to test the performance of the expanded and fluidized bed system and to determine the biokinetic and biofilm parameters. This system is operated with effluent recycles to ensure sufficient and constant fluidization of the beds. The feeds are introduced into the reactor on the suction side of the recycle pumps. Thus, the feed to recycle ratio with varying feed flow rates while the total flow rate to both reactors remained constant throughout the experimental work.

A pseudo-steady state is achieved in both reactors during the last two months, after start up phase from the viewpoints of BOD_5 and COD effluent and biomass accumulation (XfLf). After about three months of operation, the system appeared to have reached a steady state condition. There is no appreciable differences between the two reactors and both reactors performed well. However, the removal efficiency of BOD_5 and COD

Table 1: Operational Conditions of the First Field Pilot-Scale system

Parameters	*Experimental Phases*				
	Phase I	*Phase II*	*Phase III*	*Phase IV*	*Phase V*
1. Upflow Expanded Bed Reactor (UEBR)					
Feed flow (l/hr)	120	180	240	300	360
Recycle flow (l/hr)	300	420	600	720	900
Upflow velocity (m/hr)*	7	10.5	15	18	22
Hydraulic retention time (HRT) (min)**	45	30	23	18	15
Settled bed high (cm)	105	105	110	110	110
Settled bed porosity	0.52	0.52	0.52	0.58	0.54
Weight of settled bed (kg)***	22.5	21	23.5	20	22
Interstitial velocity (m/hr)	13.5	20	29	30	40
2. Aerated Fluidized Bed Reactor (AFBR)					
Feed flow (l/hr)	12	24	36	48	60
Recycle flow (l/hr)	72	72	108	108	150
Upflow velocity (m/hr)*	10.7	12.2	18.3	20	26.7
Hydraulic retention time (HRT) (min)**	70	35	23	18	15
Settled bed high (cm)	100	100	102	102	105
Settled bed porosity	0.54	0.54	0.52	0.53	0.53
Weight of settled bed (kg)***	2.75	2.8	3	2.85	2.8
Interstitial velocity (m/hr)	20	22.5	35	38	50

* Upflow velocity based on feed and recycles flow: $HLR = \frac{Q + Q_r}{A}$

** HRT based on feed flow only and volume of empty bed: $HRT = \frac{V}{Q}$

Table 2: Parameters operation condition of the First Field Pilot-Scale systems under Pseudo-Steady State

Parameters	*Experimental Phases*				
	Phase I	*Phase II*	*Phase III*	*Phase IV*	*Phase V*
1. Upflow Expanded Bed Reactor (UEBR)					
Operation time (days)	65	60	52	44	40
Number of measurements	35	30	30	29	28
Feed $TBOD_5$ concentration (mg/l)	(64-181)*	(85-189)	(98-210)	(105-296)	(86-428)
	93**	133	139	196	261
Feed TCOD concentration (mg/l)	(82-243)*	(123-299)	(143-375)	(188-342)	(128-617)
	129**	209	255	262	370
Hydraulic retention time (HRT) (min)	45	30	23	18	15
Feed flow rate (l/hr)	120	180	240	300	360
Recycle flow rate (l/hr)	300	420	600	720	900
2. Aerated Fluidized Bed Reactor (AFBR)					
FOperation time (days)	65	60	52	44	40
Number of measurements	35	30	30	29	28
Feed $TBOD_5$ concentration (mg/l)	(32-77)*	(44-92)	(63-122)	(71-179)	(78-211)
	55**	908	7	118	135
Feed TCOD concentration (mg/l)	(63-122)*	(68-131)	(78-197)	(98-248)	(88-288)
	81**	96	120	169	
Hydraulic retention time (HRT) (min)	70	35	23	18	15
Feed flow rate (l/hr)	12	24	36	48	60
Recycle flow rate (l/hr)	72	72	108	108	150

* Range value

** Average value

are more relatively than 70% of each reactor, then, the steady state data collected which is used to determine the biokinetic constant (Rmax and Kmax) (Rittmann & McCarty, 1980b).

The porcelanite rock was prepared for the sieving to obtain a relatively medium range of size 2.5-4.75 mm and then washed with a distilled water and dried in oven at 105 °C for at least 24 hrs. The periods of the experiment vary and depend each phase more particularly on the time needed to achieve stability and to reach a steady state. Between each phase to achieve a steady-state condition, each system is left to equilibrate over a period of 15 to 20 days. Daily measurements of the effluent were taken over a period of 10 days and samples collected and analyzed to check the stability of the systems. Major parameters include COD, BOD_5, SS, VSS, NH_4^+ and NO_3.

Wastewater Quality Analysis

The field experiments are monitored for a period of approximately 12 months. The actual wastewater quality sampling programme has lasted for a period of approximately eight months through the evaluation of physical, chemical and biological parameters. Table 3 summarizes all measurements obtained by a sampling procedure which are conducted according to the standard methods for the examination of water and wastewater (Murga et al., 1998; Huang et al., 1998). A comprehensive sampling programme is carried out on the basis of both 24 hour flow proportional samples and discrete sampling every few hours for a 24-hour period. Different wastewater quality analyses are conducted at Al-Rustamiyah Laboratory and at Environmental Research Center of Hazard Office (Ministry of Sciences and Technology).

Biological Tests

Since the primary concern of this study is to establish a pseudo steady-state condition for the laboratory model and field pilot-scale systems, biofilm thickness has to be measured frequently. Biomass is determined on the basis of the average volatile solids (AVS) attached to the support materials in a given volume of the reactor.

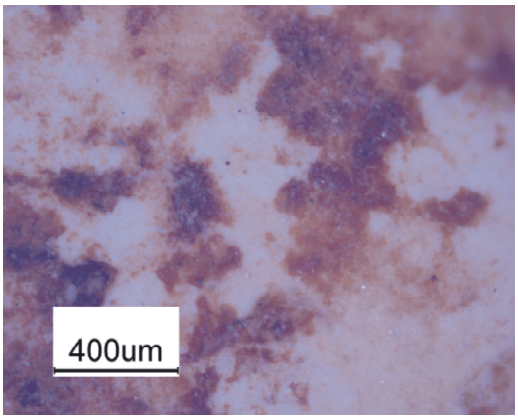
To estimate the attached volatile solids, packing medium is taken from the sampling ports along each reactor, the procedure for biomass analysis follows the steps outlined in Table 4. Characklis (1981) found that water comprises about 99% of the biomass mass and assumed that 1 ml of organism is equivalent to 1 mg wet weight and that dry weight is equal to one tenth of wet weight. The biofilm dry mass density (ρ_{bd}) can be also determined from the weight of biofilm thickness if the

Table 3: Description of analytical methods used for frequency of sampling

Parameters	*Frequency*	*Description*	*Method*
pH	Daily	pH-meter pH probe	
Dissolved oxygen (DO)	Daily	DO-meter oxygen probe	
TBOD * SBOD **	Twice per week	DO-meter and incubator (Incubation bottle)	
TCOD SCOD	Twice per week	Colorimetric Hatch, model 35 (COD) reactor	Colorimetrically method which is a modification of dichromate reflux method
Suspended solids (SS)	Twice per week	Whatman Glass Microfiber Filter Papers (0.45 μm)	Gravimetric method
Volatile suspended Solids (VSS)	Twice per week	Whatman Glass Microfiber Filter Papers (0.45 μm)	Gravimetric method
Ammonium-Nitrogen (NH_4^+-N)	Weekly	Colorimeter, Model DR/IA, Hatch	Nessler method
Nitrite-Nitrogen (NO_3^--N)	Weekly	Colorimeter, Model DR/IA, Hatch	Cadmium reduction method
Nitrate-Nitrogen (NO_2^--N)	Weekly	Colorimeter, Model DR/IA, Hatch	Diazotization method

* Represented total BOD_5 and COD; All parameters measured in mg/l except pH.

** Represented soluble BOD_5 and COD which are initially filtering samples through a 45 μm glass fiber filter before measured.



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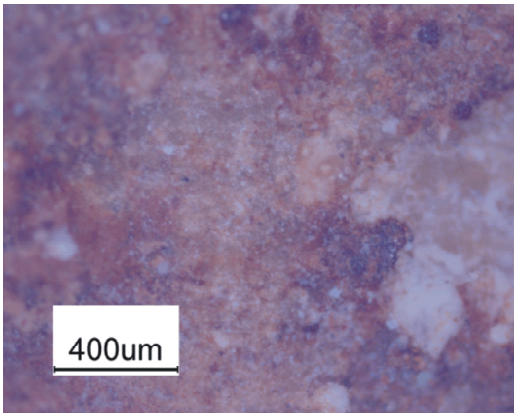

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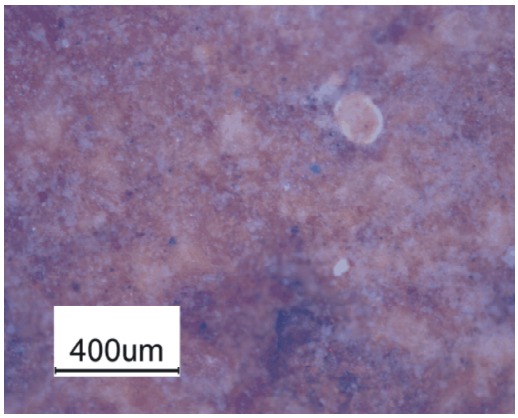

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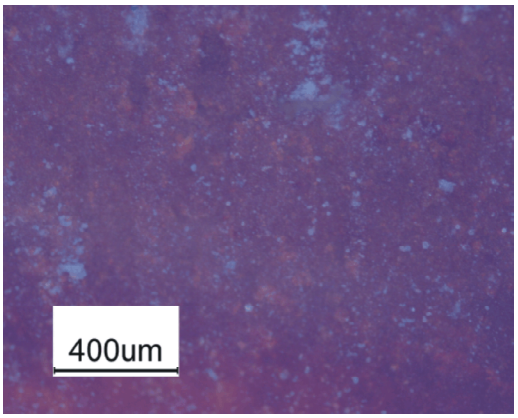
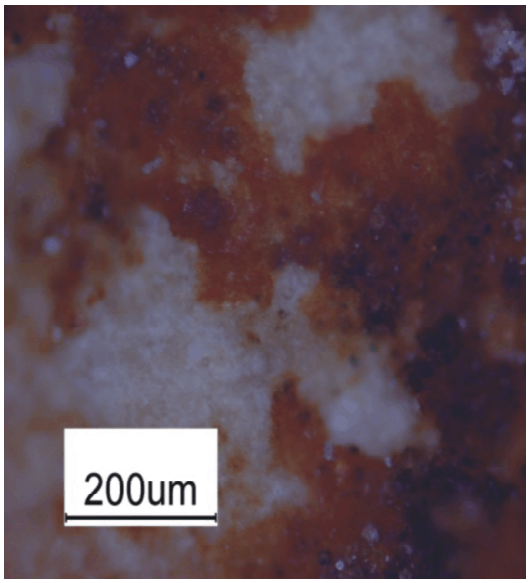


Photo (6)

Biofilm growth on porcelanite surface after 1, 3, 6 and 9 weeks respectively, for operation conditions: 2-4 mg/l dissolved oxygen concentration, direct aeration, high concentration TCOD 186 mg/l and superficial velocity = 0.0146 m/s (for water mixed with Carmosine E-122).



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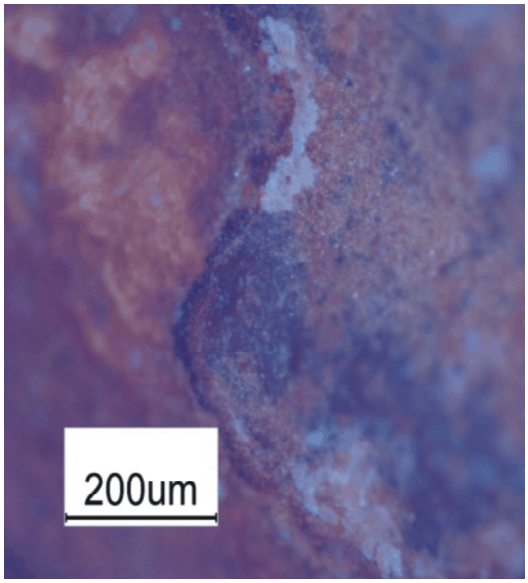

Photo (8)

Photos (7) and (8) show the biofilm growth on porcelanite surface after 1 and 9 weeks respectively, for operation conditions: 2-4 mg/l dissolved oxygen concentration, for TCOD 297 mg/l and superficial velocity = 0.006 m/s (for water mixed with Carmosine E-122).

Table 4: Biomass analysis procedure (Bryers and Characklis, 1992)

Step	*Procedure*
1	Weigh dry "dish".
2	Drain water from sample; place in dish.
3	Over dry at 106ºC "over night".
4	Weigh cooled desiccated sample and dish.
5	Ignite sample and dish at 600 ºC for 1 hour.
6	Cool in desiccators and weigh.
7	Acid wash sample with % HCl.
8	Rinse with distilled water.
9	Over dry at 106 ºC "over night".
10	Cool in desiccators and weigh.
	Calculation
11	Step 4 - step 1 = Weight of media and solids.
12	Step 10 - step 1 = Weight of media.
13	Step 4 - step 6 = Weight of volatile solids.
14	Step 4 - step 10 = Weight of total solids.
15	gm TSS/gm media = step 14/step 12 gm VSS/gm media = step 13/step 12

biofilm mass is known. However, the biofilm dry mass density reflects the attached dry mass per unit wet biofilm volume. On the other hand, the wet biofilm mass can be converted to volume by assuming a density of 1 g/cm^3. If this volume is divided by the total surface area of the bed reactor, an average biofilm thickness is obtained.

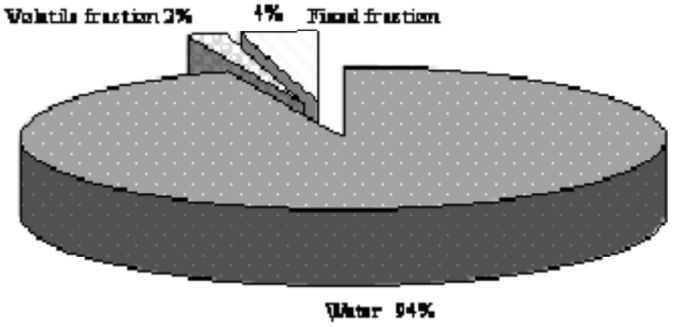

Figure 3: Chemical properties of biofilms obtained from the porcelanite surfaces

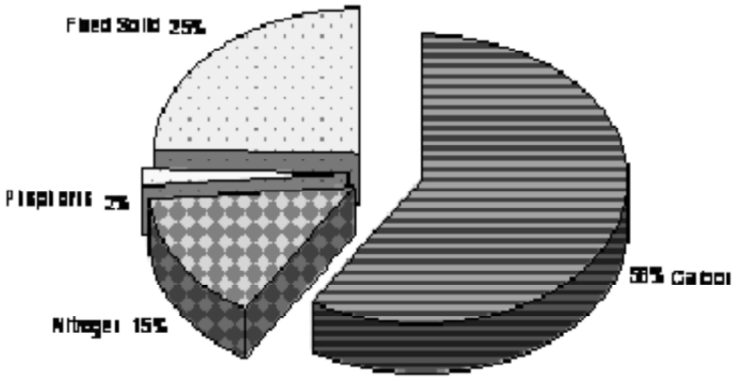

Figure 4: Chemical composition of biofilms obtained in BFBR emphasizing the primary constituents (C, N, P).

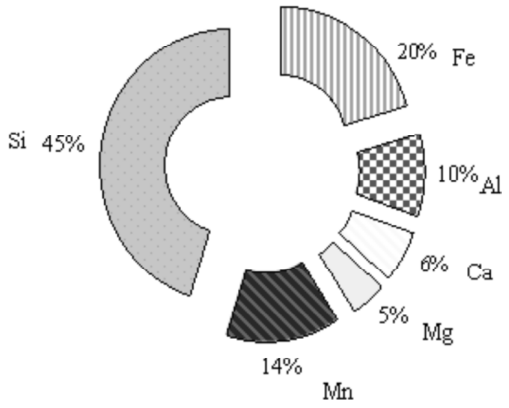

Figure 5: Chemical composition of fixed fraction of biofilms claimed from the porcelanite surfaces.

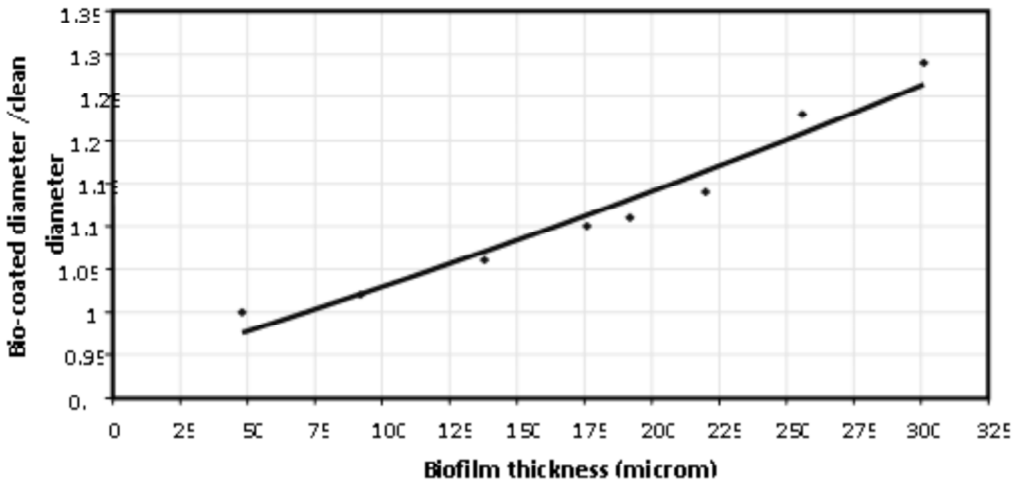

Figure 6: The effect of biofilm thickness on biocoated diameter in BFBR.

Results

Biofilm Formation and Composition

Observations of the biofilm growth formation phases attached to the surface packing media in the fluidized bioreactors system are the major important objectives in this study. However, the biofilm growth is responsible for removal, through microbial metabolism of the biodegradable components from the wastewater passing up the bed.

Photos (3) to (6) show the surface of a virgin porcelanite surface with different magnification powers of light microscope with various operation systems (TCOD = 186 mg/l and superficial velocity 0.0146 m/sec) with various systems operation. The surface of porcelanite media is covered with biomass and very few macro pores are visible. Because biofilm shears the media by the expanding and fluidizing effect under direct aeration, most of the biofilms are less thin than 220 μm.

Photos (3) and (4) show that the porcelanite surface is partially covered with biofilm, where some voids within the media matrix seem to be filled completely with biological solids and many of the media have a thin biofilm around the media solids less than 186 μm, possibly because of the scouring action of rising air bubbles in the reactor. However, the biomass penetrated inside the structure of the particles leads to a decrease in the area available for adsorption.

Physical, chemical and biological properties of biofilm are experimentally determined depending on the environmental condition to which the attachment surface is exposed. The organic and chemical composition of biofilm obtained in the laboratory experiments emphasizing the primary constituents carbon (C), nitrogen (N), and phosphorus (P) is graphed in Figures (3) and (4). The organic composition of the biofilm is strongly related to the energy and carbon sources available for metabolism. Inorganic composition of biofilm undoubtedly varies with the chemical composition of the bulk water, and probably affects the physical and biological structure of the film. The inorganic composition observed in selected biofilm in the laboratory scale system is presented in Figure 5.

The growth of biofilm on the media surface increases the size of the porcelanite particles and laterally increases the biocoated particles and their bioparticle diameter ratio (dbr), as shown in Figure 6. Accumulative volume rate changes upon the biofilm growth and porosity and the rate of variation of vbr is correlated linearly with porosity with

R^2 = 0.9, as shown in Figure 7. Experimental measurement of porcelanite bioparticles density remarks low values (1050 kg/m^3) in the medium biological section, when biofilm thickness is less than 150 μm for continuous operation of laboratory model scale. However, the packing media density 10 decreases from 1230 kg/m^3 to 1000 kg/m^3 with the growth of biofilm thickness from 50 μm to 300 μm at constant superficial velocity approach to 0.01 m/sec (Figure 8).

Biofilm Loss Rate Estimation

As the biofilm grows thicker on the packing media, the fluid shear stress of the biofilm interface generally increases. Also, as thick biofilm becomes thicker, the potential for substrate, oxygen, or nutrient limitation in the deeper portions is great. These limitations may weaken the biofilm matrix and cause biofilm loss rate.

Figure 9 indicates that the biofilm loss rate increases with the increase in biomass accumulation (XfLf) at a constant flow velocity 0.01 m/sec under steady state

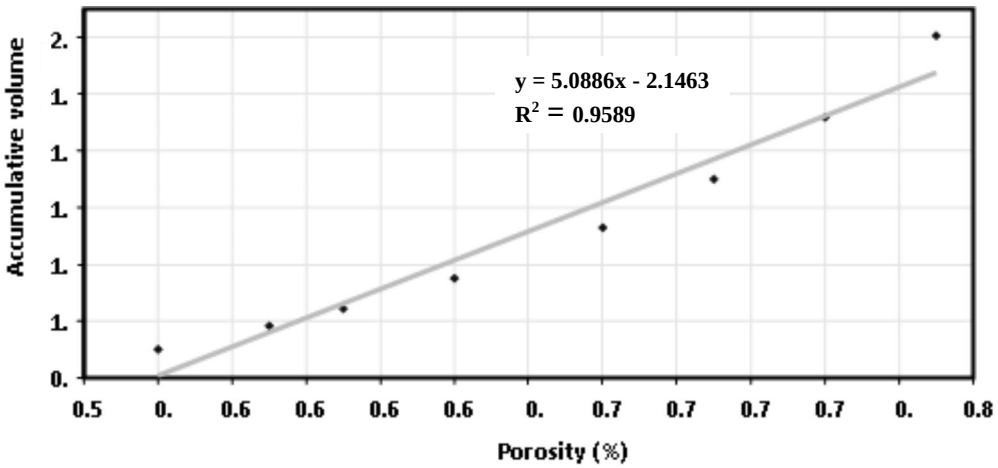

Figure 7: Effect of bed porosity on accumulative volume ratio in BFBR.

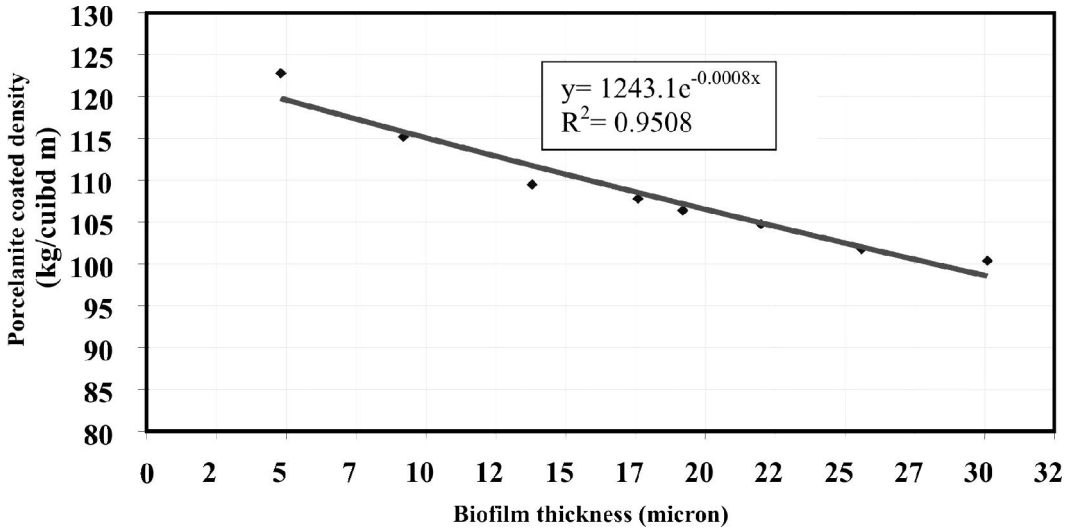

Figure 8: The effect of biofilm thickness on the density of porcelanite with attached microbial growth in BFBR.

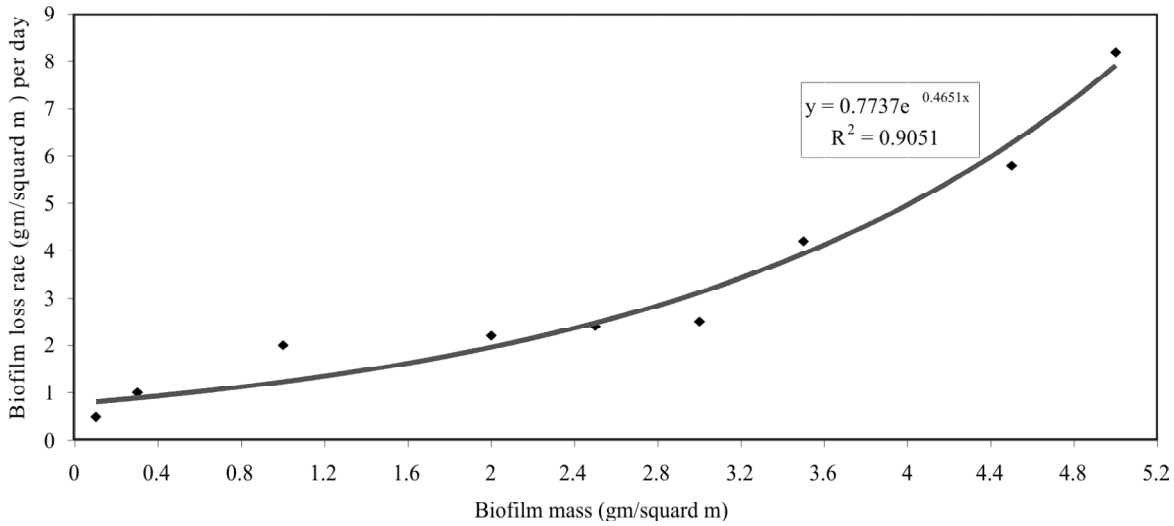

Figure 9: Biofilm loss rate as a function of biofilm mass per area in BFBR.

operation of fluidized bed reactor. The correlation is an exponential approximation in the range of (XfLf) from 0.1 g BVS/m^2 to 5 g BVS/m^2. Characklis (1981) found a linear relationship between biofilm loss rate and XfLf in the range of 0.78 g BVS/m^2 to 4.8 g BVS/m^2 and biofilm loss rate between 0.25 and 8.3 g/m^2.day. However, the significance of detachment or sloughing appears to increase at higher flow velocities.

Conclusions

1. Three effective parameters, which are found experimentally and influence the rate of biofilm growth, are superficial velocity, substrate and biomass concentration.

2. The rate of biofilm rapidly grows in system aerated directly compared with other systems operated by indirect aeration (aerated by the stream of influent).

3. The biomass accumulation in high concentration 297 mg TCOD/l is higher than the same operation condition treated at low substrate concentration influent 127 mg TCOD/l.

4. The physical, chemical and biological analyses depend on the environmental conditions to which the attachment surface is exposed. The large portion of composition is water 90.4% and the small value of volatile fraction 9.2%, while the fixed fraction is composed of 0.4% of total biofilm (by mass).

5. The organic and chemical composition of fixed fraction of biofilm is obtained from the laboratory

experiments emphasizing the primary constituents carbon C (58%), nitrogen N (15%), and phosphorus P (2%), while the fixed solid composition is 25%.

6. The inorganic composition of fixed fraction of biofilm varies with chemical and organic properties of bulk water, chemical and physical properties of media and its structure, silica composes 45%, while Fe composes 20%, Mn 14%, Al 10%, Ca 6% and Mg 5%.

7. The upflow expanded bed reactor (UEBR) followed by aerated fluidized bed reactor AFBR showed more improvement of BOD_5 and COD removal efficiencies from 60% to 80% $TBOD_5$ and from 59% to 79% TCOD, respectively. Then, this system could be a very promising alternative for the treatment of the domestic sewage, since the system can be designed at HRT less than one hour in both reactors resulting in a very compact and low cost treatment unit.

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