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Study of the initial glycerol concentration effects upon bacterial cells adaptation and biodegradation kinetics on a submerged aerated fixed bed reactor using biocell® packing

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- ✓ Synthetic solution.

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Abstract

The present work provides an experimental study of the effect of the glycerol concentration on removal performances in a Submerged Aerobic Fixed-Film (SAF) Reactor performing in batch mode. This study consisted in adding various initial substrate concentrations of synthetic solutions (glycerol) in order to assess their effects on biodegradation kinetics and then on bacterial cells adaptation. For this purpose, three series of tests were made. The first two series were carried out with initial Chemical Oxygen Demand concentrations respectively of 330 mgO_2/L (SL) and 1120 mgO_2/L (SH). The third series (SS) was carried out with gradually increasing concentrations, in which initial COD values were 480, 590 and 760 mgO_2/L. It has been found that the adaptation time decreased when the initial concentration increased. But, when synthetic solutions were renewed for a new adaptation, better performances were observed. Increasing initial COD solutions (SS test) showed excellent performances; in fact, it seems combine both, the advantage of low substrate concentration (better adaptability, interesting time for adaptation – case of SL test) and of a high concentrated synthetic solutions (better treatment performance and high biodegradation kinetics constant – case of SH test).

1. Introduction

Submerged Aerobic Fixed-Film Reactors (SAF) are fixed biomass reactors. They are mainly used for carbonaceous and ammonia removal in the aerobic treatment of urban wastewaters. They are also used in secondary or in some cases in tertiary treatment. Today, these reactors have several applications in industry [1]–[5]. They combine compactness and high removal efficiencies in a large range of hydraulic and organic load.
In SAF, biological degradation of organic matters is made according to the following reactions:

Several organic substrates can be used as a source of carbon and energy for purifying micro-organisms[6]–[8], both in aerobic or anaerobic wastewater treatment systems. In this work, the substrate used is glycerol as a proper external carbon source. Its biological degradation has been studied by some authors either in aerobic or anaerobic treatment [8]–[12].

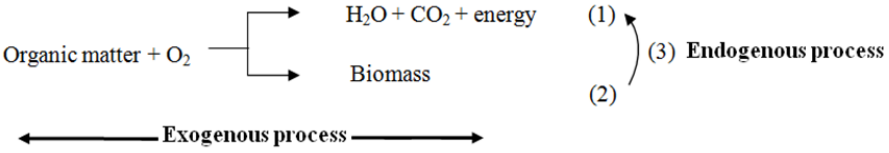

Figure 1: Removal biological process of organic matter

The SAF performances can be assessed by the yield (Y). Its expression uses the chemical oxygen demand (COD) as follows [2]:

$$\boldsymbol{Y}\ (\%) = \frac{\boldsymbol{L_0 - L}}{\boldsymbol{L_0}} \qquad \text{Equation 1}$$

Where:
Y: Yield (%) removal of the substrate (in terms of COD)
L_0: Initial organic Load of the substrate, expressed in COD (mgO_2/L)
L: Final organic Load of the substrate at time t, expressed in COD (mgO_2/L)

The purification performances of Submerged Aerobic Fixed-Film Reactors can also be assessed by calculating biodegradation kinetic constants of organic pollution. In batch reactor, the most significant period in the growth cycle is the exponential growth phase, when the population of biomass is perfectly adapted to the substrate. The first-order model, neglecting endogenous respiration, provides accurate simulations of biodegradation kinetics. It can be written as follows [2], [13]:

$$\frac{\boldsymbol{dL}}{\boldsymbol{dt}} = -\boldsymbol{k_T}\ .L \qquad \text{Equation 2}$$

Where:
t: Time (d)
k_T: First-order biological oxygen demand(BOD)biodegradation kinetics constant expressed as (d^{-1}) depending on the temperature according to the following expression:

$$\boldsymbol{k_T} = \boldsymbol{k_t}.\boldsymbol{\theta}^{(\boldsymbol{T}-\boldsymbol{T_0})} \qquad \text{Equation 3}$$

Where:
k_t: First-order BOD biodegradation kinetics constant at Standard temperature T_0= 20°C, expressed as (d^{-1})
θ: Temperature coefficient equal to 1.03.Typical range of the temperature activity coefficient for aerobic attached bacteria process is from 1.02 to 1.08[14].
T: Temperature of the synthetic solution expressed in °C close or below to 20ºC.

The integration of Equation 2, taking into account Equation 3, provides the expression of substrate degradation in time [4]:

$$\boldsymbol{L} = \boldsymbol{L_0}.\boldsymbol{10}^{-\boldsymbol{k_t}.\boldsymbol{\theta}^{(\boldsymbol{T}-\boldsymbol{T_0})}.\boldsymbol{t}} \qquad \text{Equation 4}$$

The linearization of this equation allows determining the constant k_t using semi-logarithmic coordinates according to Equation 5.

$$\boldsymbol{log}\frac{\boldsymbol{L}}{\boldsymbol{L_0}} = -\boldsymbol{k_t}.\boldsymbol{\theta}^{(\boldsymbol{T}-\boldsymbol{T_0})}.\boldsymbol{t} \qquad \text{Equation 5}$$

Other physicochemical parameters such as conductivity, pH, Dissolved oxygen and Turbidity can be linked to the assessment of SAF performances [3], [15], and could give some explanations about the efficiency of this bioreactor in wastewater treatment.

In this paper, tests were made to evaluate the evolution of the adaptation with increasing synthetic solution concentrations. Three tests series were realized: tests with 330 mgO_2/L (SL), tests with 1120 mgO_2/L (SH) and tests with increasing concentrations (SS) (480, 590 and 760 mgO_2/L). Synthetic solutions were added successively after each substrate removal in the bioreactor.

2. Experimental

Experiments were carried out in a reactor operating in batch mode. It was constituted by a cylindrical column, height of 1 m and a diameter of 12.5 cm, made of opaque PVC. The air was introduced at the bottom of the column with an air flow equal to 10 L/h.

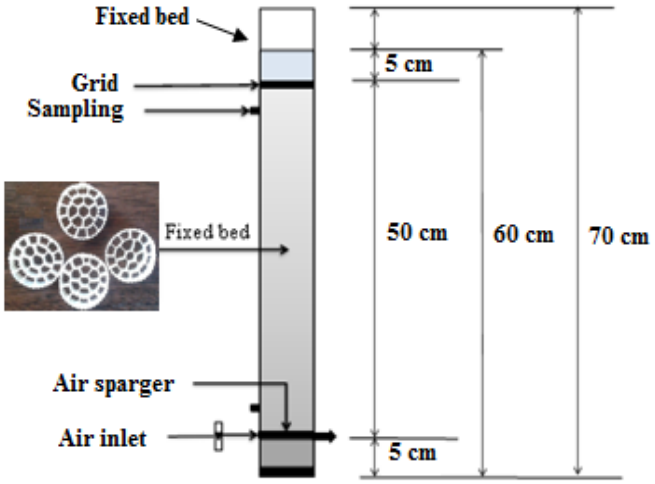

Figure 2: Experimental setup

A Biocell® packing (Figure 2) filled in the reactor, was supported by a grid placed above the air sparger at the bottom of the column for maintaining the packing fixed. Another grid was used to fix this packing to avoid its flotation by the effect of crossing air bubbles. The physical properties of the packing are illustrated in Table I.

Table I**:** Physical properties of packing

Name	Biocell®
Color	White
Material	PET
Average particle diameter (mm)	12
Average particle height (mm)	10
Specific surface area (m^2/m^3)	~ 700
Density (kg/m^3)	130
Number of particles ($.10^5$ / m^3)	6.66
Bed porosity (%)	92.5

Synthetic solutions were prepared in COD/N/P ratio of 100/4/1 [2], [16] (Table II), using distilled water. A high quality Glycerol was used as the organic substrate. Salts of sodium nitrate (Nitrogen source) and potassium phosphate (Phosphorus source) and some oligo elements ($FeSO_4$, $MgSO_4$, $MnSO_4$ and $CaSO_4$) with high quality were also added to synthetic solutions. Doses of oligo elements were low[2], [17], [18]. To initiate the adaptation and biodegradation of substrate, inoculation was made by bacterial flora was extracted from soil [19], [20]. The physicochemical characteristics of synthetic solutions are presented in Table II.

Table II: Physicochemical characteristics of synthetic solutions

			Low			High			Increasing		
			SL1	SL2	SL3	SH1	SH2	SH3	SS1	SS2	SS3
Characteristics of synthetic solutions	COD	(mgO_2/L)	330	330	330	1120	1120	1120	480	590	760
	T°	(°C)	25	27.1	25.8	14.3	15.7	16.5	25.5	26.4	26.1
	Cond	(µS/cm)	1185	1185	1185	1955	1922	1917	1320	1540	1620
	Turb	(NTU)	10	15	10	10	18	20	28	14	18
	pH	-	7.92	7.92	7.92	8.23	8.14	8.45	7.63	7.87	8.01
	DO	(mg/L)	-	-	-	9.35	9.75	9.32	10.3	10.3	10.4

The composition of synthetic solutions is presented on Table III. Concentrations are in mg/L

Table III: Synthetic solutions compositions

	Low			High			Increasing		
	SL1	SL2	SL3	SH1	SH2	SH3	SS1	SS2	SS3
$C_3H_8O_3$	300	300	300	1000	1000	1000	400	500	650
$NaNO_3$	12	12	12	40	40	40	16	23.6	30.4
KH_2PO_4	3	3	3	10	10	10	4	5.9	7.6
$ZnSO_4, 7H_2O$	0.3	0.3	0.3	1	1	1	0.4	0.5	0.7
$FeSO_4, 7H_2O$	0.3	0.3	0.3	2	2	2	0.4	0.5	0.7
$MnCl_2$	0.3	0.3	0.3	3	3	3	0.4	0.5	0.7
$CaSO_4$	0.07	0.07	0.07	0.25	0.25	0.25	0.1	0.14	0.2
$MgSO_4, 7H_2O$	0.03	0.03	0.03	0.1	0.1	0.1	0.04	0.05	0.07

Three series of adaptation tests were performed. In the first tests, two series of synthetic solutions concentrations 330 mgO_2/L (SL) and 1120 mgO_2/L (SH)) were added; synthetic solutions were renewed for each new adaptation. In the third series, increasing concentrations were performed by adding successively: 480 mgO_2/L, 590 mgO_2/L and 760 mgO_2/L (SS).

The procedure followed in these experiments was:

- The first test: consisted in successive additions of synthetic solutions SL1, SL2 and SL3 with Low concentration equal to 330 mgO_2/L of glycerol;
- The second test: consisted in successive additions of synthetic solutions SH1, SH2 and SH3 with high concentration equal to 1120 mgO_2/L of glycerol;
- The third test: consisted in adding successively increasing concentrations of glycerol of SS1 = 480 mgO_2/L, SS2 = 590 mgO_2/Land 760 mgO_2/L.

In each of these tests, the first synthetic solution was added to the Submerged Aerobic Fixed-Film Reactor, filled with a clean packing. After total glycerol removal, residual solutions were evacuated and drained out for 15 minutes, and the second synthetic solution was added without washing the bioreactor. This procedure has been used for all the tests cited above.

Methods:

Reactor performances monitoring were made by testing some parameters such as Chemical Oxygen Demand (COD), this parameter was measured in accordance with the French standard NF T90-101 (February 2001),. This latter was monitored using a photometer type Palintest 7000, which allowed also the measure of turbidity. pH and dissolved oxygen were measured using a Hach sensor, 40d-HQ-Multi parameters which can also measure the temperatures of water and air. The conductivity was measured by an Orion model 125. Samples from the bioreactor were filtered through filters having a pore size of 0.45 µm for measuring the soluble COD concentrations. For the other measurement parameters, they were realized directly *insitu*.

3. Results and discussion

The representation of COD results are illustrated on Figures 3-a, 3-b and 3-c. Figures 3-a and 3-b show that the adaptation duration increased with the initial concentration of substrate, and decreased during adaptation series tests of SL and SH. According to the study of Shimp and Pfaender,[21], the non-adapted bacterial cells takes more time to grow and consume the substrate. In the same way, Amrouche and Hellal, [22] tested the biodegradation of phenol in a batch reactor, they found that the duration of adaptation phase increased with substrate concentration.

The evolution of adaptation phase was mentioned by some authors [11], [23]. They treated a synthetic petrochemical wastewater containing benzoic acid in a laboratory scale using a sequential batch reactor (SBR). They tested concentrations of synthetic solutions of 50, 100, 150 and 200 mg/L, which were inoculated by an activated sludge collected from municipal sewage treatment plant and aerated for acclimatization in synthetic solution of 200 mg/L of benzoic acid. This study showed that the purification yield curve versus time presents at the beginning, best performances for low concentrations. Concerning the SS tests (Figure 3-c), high performances has been shown. Bacteria cells adapted from a low concentration (480 mgO_2/L) react efficiently when an increase of Glycerol concentration was made. Bacteria cells which were already adapted to low concentrations of substrate couldgrow easily in new rich-substrate solutions.

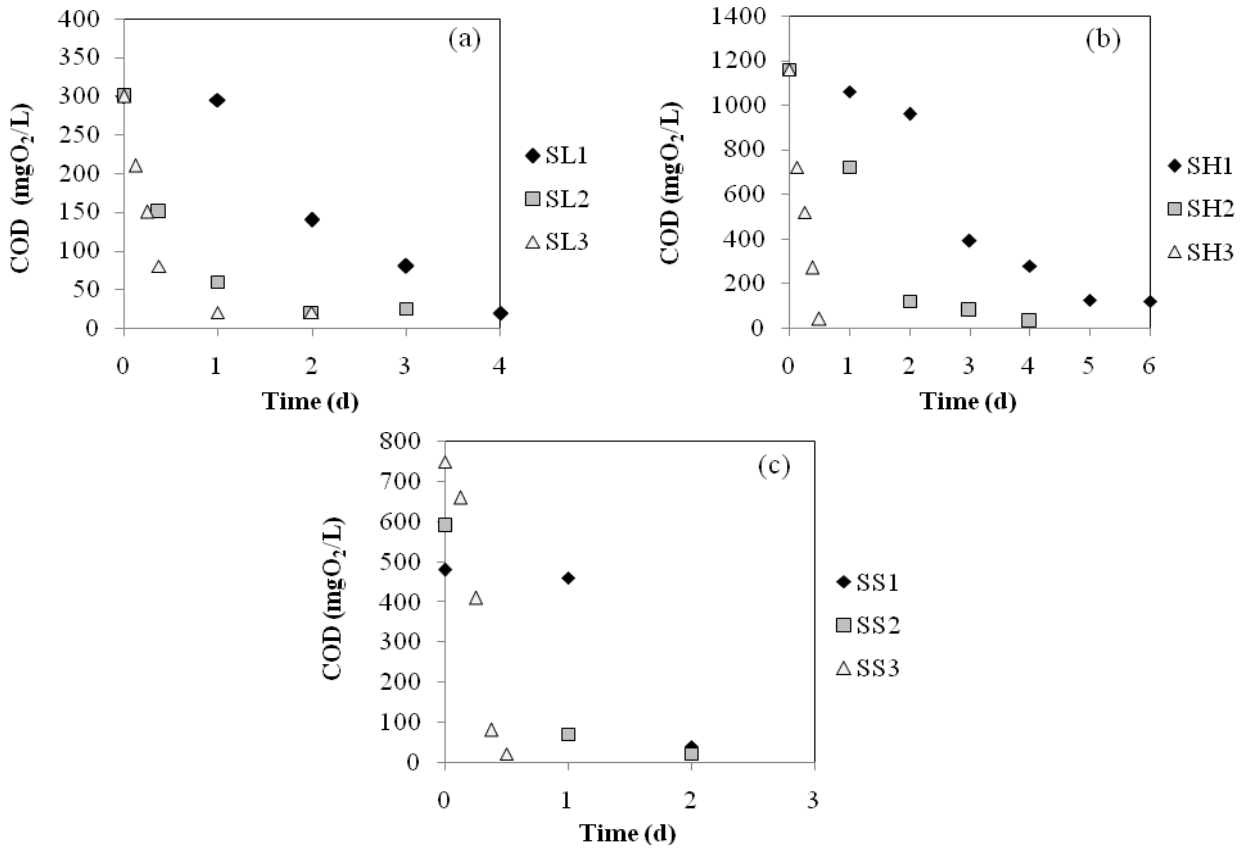

Figure 3: Evolution of Chemical Oxygen Demand COD of SL (a), SH (b) and SS (c)

Figure 4 represents the biodegradation kinetics constants of the adaptation of SL, SH and SS at T°=20°C.

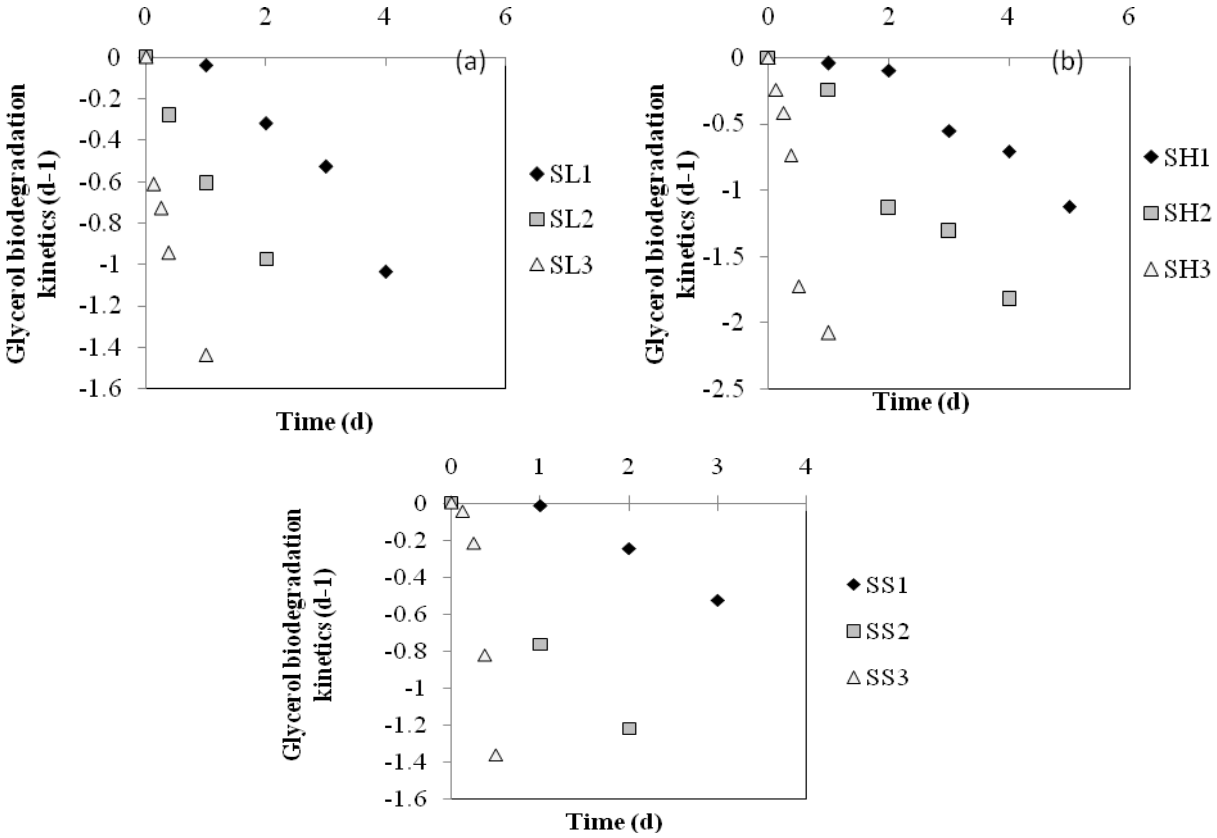

Figure 4: Semi-logarithmic glycerol biodegradation kinetics constants of SL (a), SH (b) and SS (c) versus time

The duration of the adaptation phase was 1 day for SL1 and SS1 and 2 days for SH1. Otherwise, the biodegradation kinetics improves with adaptation for each test series. A difference has been noted between biodegradation kinetics constants of SL1 and SH1 during the first adaptation (Tab. IV). It showed that the biodegradation kinetics constant of glycerol is better at low concentrations[24].

Table IV: Adaptation duration and biodegradation kinetic constants in SL, SH and SS

		Addition 1	Addition 2	Addition 3
SL	Adaptation duration (h)	24	12	3
	Biodegradation kinetic (d^{-1})	0.41	0.53	1.00
	Purification yields	17 %	80 %	93.4 %
SH	Adaptation duration (h)	48	24	6
	Biodegradation kinetic (d^{-1})	0.23	0.46	1.67
	Purification yields	8.7 %	37.9 %	98.3 %
SS	Adaptation duration (h)	24	8	3
	Biodegradation kinetic (d^{-1})	0.33	0.64	2.90
	Purification yields	16.7 %	87.9 %	97.4 %

Moreover, the analysis of Table IV and Figure 5 showed that, despite their low adaptability for high concentrations of glycerol during the first adaptation (k_t = 0.23 d^{-1} for SH1 against 0.41 d^{-1} for the low concentration SL1), which confirms the results of Lekhlif et *al.*[11] study, the bacteria cells reached a relatively high biodegradation kinetic constants at the third adaptation (1.67 d^{-1} for SH3 against 1.00 d^{-1} for SL3) (Table IV) ; however, this degradation required a longer adaptation time (twice compared to low concentration).

In presence of SS solutions, bacteria cells presented excellent performances (Table IV and Figure 5). The obtained biodegradation kinetics were 0.33 d^{-1}, 0.64 d^{-1} and 2.90 d^{-1}. In presence of non-toxic substrate (glycerol) bacteria cells combined both the advantage of a low concentration synthetic solution (better adaptability, good adaptation time similar to SL1) and the advantage of a high concentrated synthetic solution (better treatment performance and high biodegradation kinetics constant).

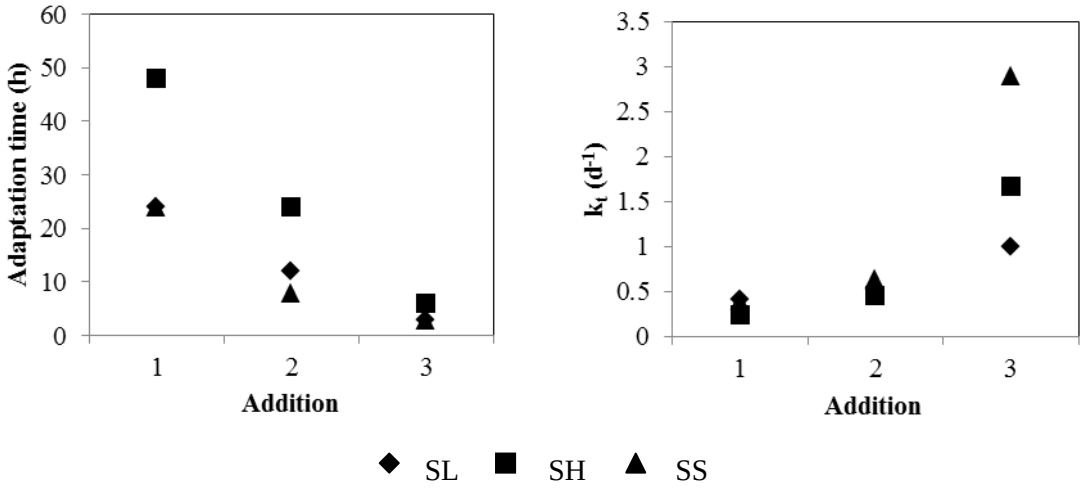

Figure 5: Evolution of adaptation times and biodegradation kinetics constants of Glycerol

The representation of turbidity results is illustrated on Figure 6. It has been shown that turbidity variation is slightly random at the beginning of each test. It could be explained by the inoculation of soil bacteria, which contains a suspension of solid particles (<15µm). It could be also due to the detached biofilm which have not been drained after each test. So, during each adaptation phase, turbidity increases and follows in the same time the decrease of COD. This phenomenon could be also explained by the EPS formation by bacteria cells [25]. After the degradation phase (decrease of COD), turbidity measured after this phase consisted, mainly, on biomass. A decrease of turbidity was also observed for SL3 and SH3 corresponding to suspended solid matters reduction after one day of aeration. The endogenous respiration may begin after the substrate depletion. For different tests, the turbidity reached a maximum (Figure 7). Corresponding times for maximum turbidity are illustrated in Table V.

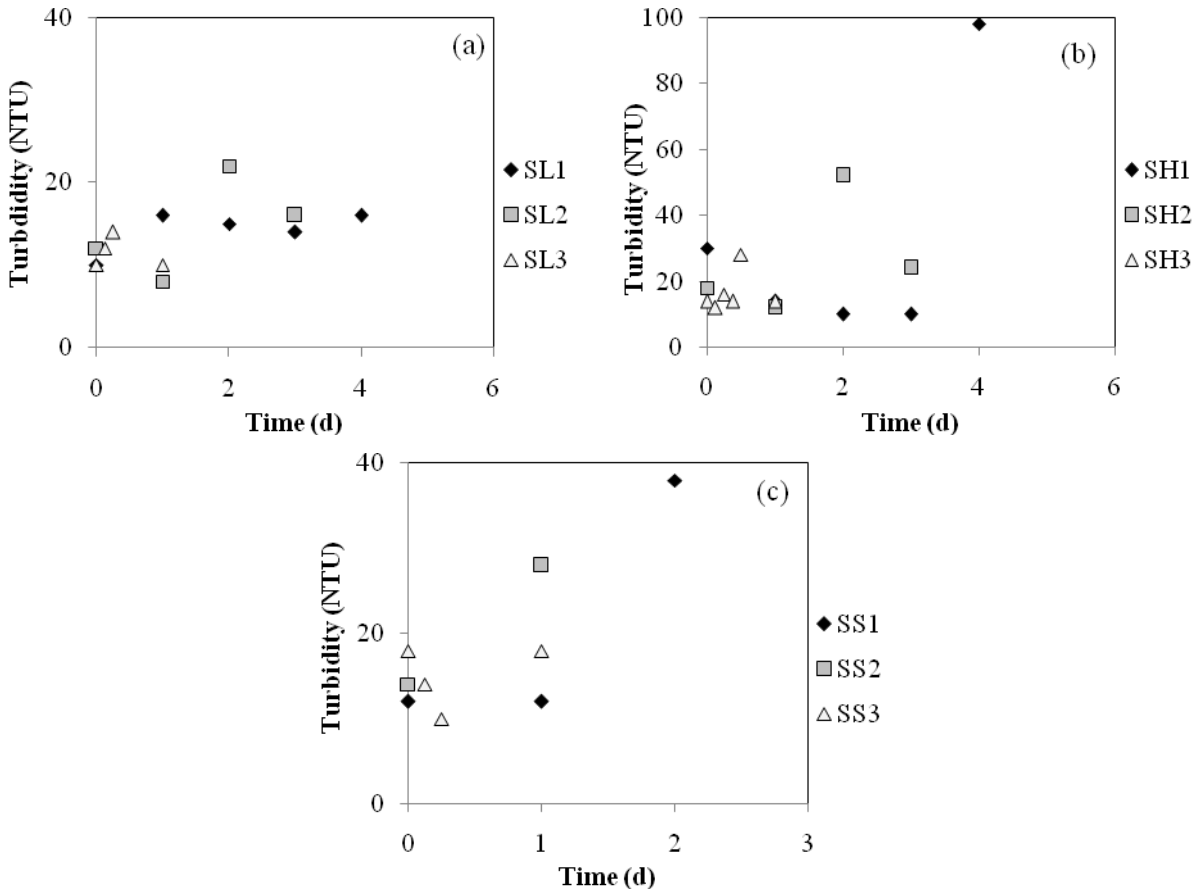

Figure 6: Turbidity evolution for SL, SH and SS

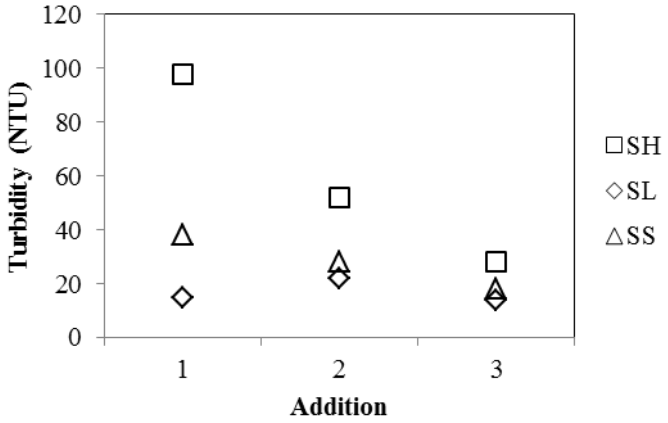

Figure 7: Maximum turbidity evolution for SL, SH and SS

Table V: Adaptation duration and biodegradation kinetics constants in SL, SH and SS

		Addition 1	Addition 2	Addition 3
SL	Maximum turbidity (NTU)	32	22	14
	Time (h)	48	24	12
SH	Maximum turbidity (NTU)	108	52	28
	Time (h)	72	24	12
SS	Maximum turbidity (NTU)	38	28	18
	Time (h)	24	12	9

This Figure shows that in the first phase adaptation, turbidity is high for SH, due probably to the multiplication of bacterial cells (Eq. 1) which was not yet adapted to attach to the supports bioreactor. However, the turbidity of SS and SH were low, this could be explained by the easy adaptation at low concentrations and after each addition of new synthetic solutions as notified above; during adaptation, the bacterial cells should be acclimated against the substrate and bioreactor hydrodynamic.

The big decrease of the turbidity of SH tests was the result of adapted bacteria cells increase and the high colonization of the biocell packing surface. Some physical interception mechanisms are the main reason of the decrease of turbidity values. The adsorption and flocculation of biofilm decreases the quantity of suspended solids on the bioreactor, those suspended elements are adsorbed by Extracellular polymeric substances (EPS) secreted by bacteria [25,26].

As it was illustrated on Figure 8, and during biodegradation, a very slight variation was observed, the pH measured in synthetic solutions is resulted from an equilibrium between bacterial cells catabolism process (CO_2 formation from eq.1) and from the denitrification process (increase of pH), as reported by some researchers [19]. Et-taleb et al., [27] has mentioned that the decomposition of organic matters in anaerobic conditions allows an increase of pH. It could also be affected by the stripping of CO_2 [28]. In all experiments, the pH increase coincided with the increase of turbidity observed in all tests.

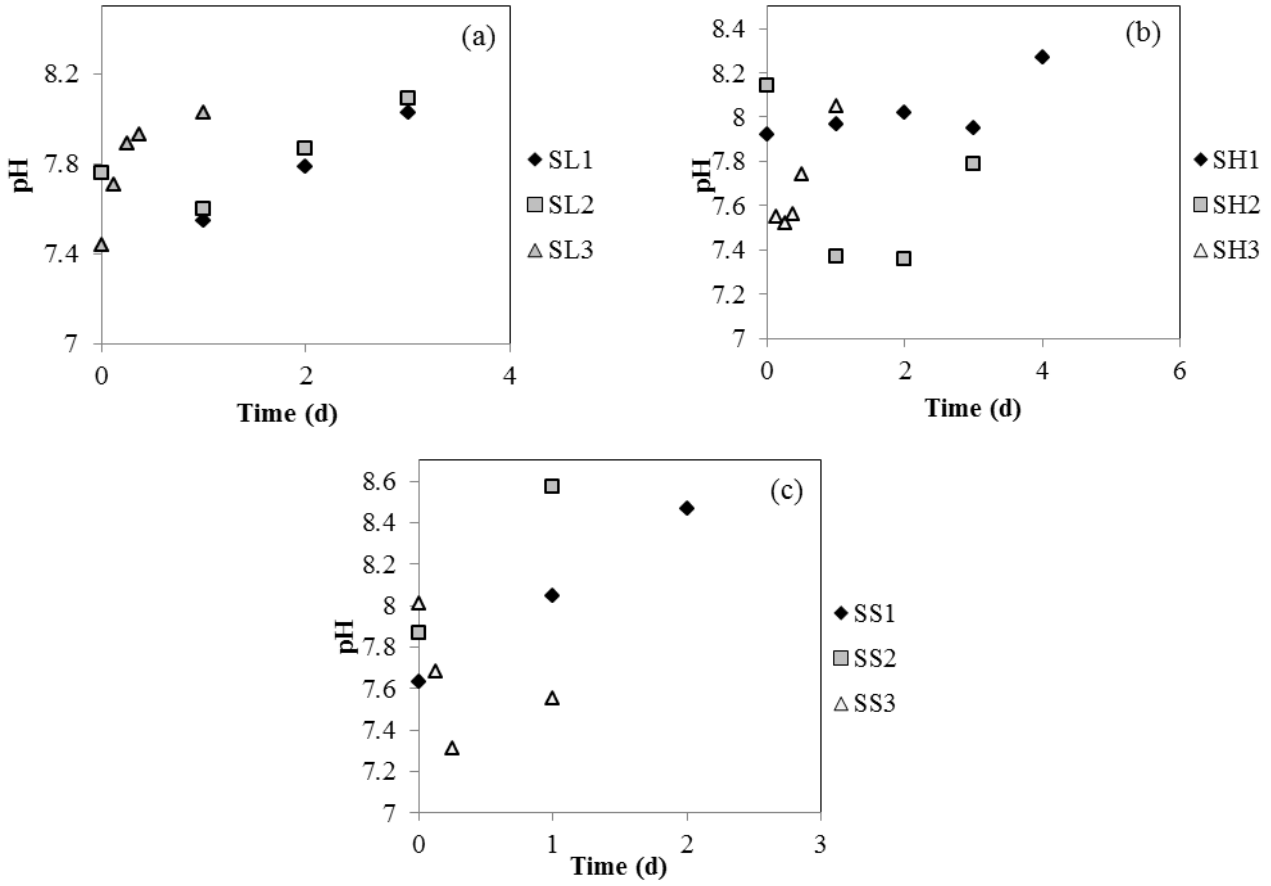

Figure 8: pH evolution of SL, SH and SS

Figure 9 shows the evolution of dissolved oxygen versus time of SH and SS wastewaters. It has been shown that DO varies in the same way with adaptability tests [24]. It decreased because of the biological degradation and the biological suspended matter [2], [29].

Concerning the third addition, DO concentration decreased to 8.42 mg/L for SH3 and 4.82 mg/L for SS3 and then increased probably because of the substrate concentration depletion. The difference in these oxygen concentrations was probably due to the quantity of biomass colonizing the bioreactor packing. This result confirmed the biodegradation kinetic constants founded previously k_t: 1.67 d^{-1} for SH3 and k_t: 2.90 d^{-1} for SS3.

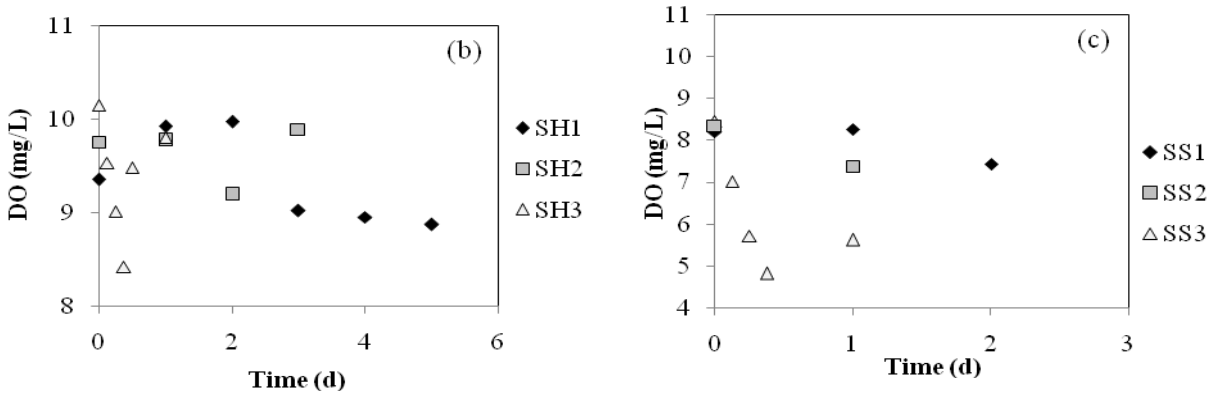

Figure 9 : Dissolved oxygen evolution SH and SS

Conclusions

The tests carried out in this study yielded promising results and help us to draw the following conclusions. In all the tests, the COD decreased due to a decrease of the dissolved organic matter. The substrate was consumed by bacterial cells, depending on the adaptation state of bacteria cells and the concentration of the substrate. The monitoring of glycerol biodegradation kinetics k_t, relating to a first-order kinetic showed that k_t decreased with the initial concentration of the substrate (0.41 d^{-1} for 330 mgO_2/L against 0.23 d^{-1} for 1120 mgO_2/L). But, when synthetic solutions were renewed for a new adaptation, we have observed that the highest were CODs, the better were performances. The biodegradation kinetic constants were respectively 0.23, 0.46 and 1.67 d^{-1} for 1120 mgO_2/L against 0.41, 0.53 and 1.00 d^{-1} for 330 mgO_2/L.SS tests showed excellent performances. The biodegradation kinetics constants were 0.33, 0.64 and 2.90 d^{-1}. Increasing concentrations during adaptation seems combine both, the advantage of a low concentration synthetic solution (better adaptability, interesting time for adaptation relatively similar to SL) and of a high concentrated synthetic solution (better treatment performance and high biodegradation kinetic constant of 2.9 d^{-1}).The turbidity varied at the beginning for the first addition and after each refilling of the bioreactor by the synthetic solution. It was affected by the soil solid suspension used to inoculate the synthetic solution and also by particles of detached biofilm which have not been drained. It decreased quickly because of EPS formation by bacteria. The pH presented some slightly variations, differentiated early-stage curves at the beginning, some increased values were observed due to the denitrification which is occurred in biological suspended matter. The DO decreased and increased then when the substrate concentration becomes low, especially in the third adaptation when the substrate was eliminated. DO increases when COD decreases.

The physicochemical parameters monitored during these tests showed consistent variations, which explained the phenomena encountered during the biological process taking place in the SAF Reactor. The different parameters of monitoring (Turbidity, COD, pH, dissolved Oxygen) can be associated between them as follows:

- The turbidity increased when COD decreased, the increased turbidity coincided with the beginning of the degradation phase;
- The pH increased when the turbidity increased, it was due to the denitrification;
- The decrease of the DO was linked to the COD decrease.

Abbreviations

COD	Chemical Oxygen Demand (mgO2/L)	***pH***	Potential of Hydrogen
DO	Dissolved Oxygen (mg/L)	***PVC***	Polyvenyl chloride
EPS	Extracellular polymeric substance	***SH***	Synthetic wastewater with high intial COD of 1120 mgO2/L
k_t	Biodegradation kinetics constant at T=20°C	***SAF***	Submerged Aerated Fixed-Film Reactor
k_T	Biodegradation kinetics constant at ambient temperature	***SL***	Synthetic wastewater with low initial COD of 330 mgO2/L
L	Final organic load (mgO2/L)	***SM***	Suspended matters
L_0	Initial organic load (mgO2/L)	***SS***	Synthetic wastewater with increasing initial COD of 480, 590, 760 mgO2/L
N	Nitrogen	***SWW***	Synthetic wastewater
P	Phosphorous	***T***	Temperature (°C)
PET	Polyethylene terephthalate	T_0	Temperature of 20°C
WWTP	Wastewater Treatment Plant		

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