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Influence of Biofilm on Activated Carbon on the Adsorption and Biodegradation of Salicylic Acid in Wastewater

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Abstract This paper presents a study of the combined process of adsorption and biodegradation in solid biologically activated carbon (AC) for the removal of salicylic acid aimed at determining the influence of the presence of biofilm on the process. Adsorption on AC and biodegradation of free cell cultures were studied separately so as to compare their performance with that of the combined biosorption system. The formation of bacterial biofilm on the surface of the carbon was investigated. The study was carried out using a range of synthetic solutions containing between 15 and 500 mg/L salicylic acid simulating an industrial effluent from the pharmaceutical industry. An individual bacterium, *Pseudomonas putida* (DSM 4478), was used to study the differentiated effects. Filtrasorb 400 and GAC 830 ACs were used in the adsorption processes and Filtrasorb 400 in the biofilm formation and combined biosorption processes. As regards, combined adsorption/biodegradation results indicated that the bioactivated carbon system outperformed the combination of conventional AC and biological water treatment processes when working with high pollutant concentrations.

Keywords Biofilm · Salicylic acid · Activated carbon · Biodegradation · *Pseudomonas putida*

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1 Introduction

Salicylic acid (o-hydroxylbenzoic acid) is a typical pollutant in industrial wastewater (Khenniche and Aissani 2010). Salicylic acid (SA) is a common derivative of phenol. It presents a serious environmental hazard due to its high toxicity and tendency to accumulate in the environment. As a result, efficient removal and recycling of SA from aqueous solutions have received a great deal of attention in recent years (Wang et al. 2012a, b).

Different methods designed to remove phenol compounds have been put forward, including the application of biodegradation processes using pure cultures of microorganisms specially adapted to metabolize pollutants (González et al. 2001). Besides, the use of pure cultures contributes to our understanding of biodegradation processes under well-defined conditions. The use of *Pseudomonas putida* cultures appears to be an interesting alternative for this purpose, not only due to its importance as a bacterium capable of carrying out the degradation of phenolic compounds but also because it is commonly found in the mixed cultures present in activated sludge, where it plays an important role in removing toxic compounds for the environment (Loh and Bin 2008).

The treatment of wastes using activated carbon (AC) is considered an effective method for the removal of phenol compounds from waste solutions due to its large surface area, microporous nature, high adsorption capacity, high purity, and easy availability (Qadeer and Rehan 2002; Özkaya 2006).

AC adsorption systems may therefore be applied to produce a high quality effluent from sewage treatment

plants which can be reused for various purposes. However, despite its high adsorption capacity, AC can only maintain this adsorption capacity for a short time once its available adsorption sites become exhausted by adsorbed organic pollutants. It is well known that AC is also a good support media for microbial growth. Thus, biologically AC with an attached biomass can effectively remove organic pollutants both by adsorption and biodegradation (Xing et al. 2008).

In wastewater treatment, a reactor with immobilized microbial cells may offer several advantages over processes with a suspended biomass. These advantages include (1) retention of a high concentration of microorganisms in the reactor, (2) protection of cells from toxic substances, (3) prevention of suspended particles from the effluent, and (4) lower capital costs for single reactor systems than that for individual processes and to the fact that less frequent regeneration of the carbon will result in lower energy requirements and operating costs (Walker and Weatherley 1999; Olmstead and Weber 1991). Although the aforementioned reports highlight obvious advantages of bioactivated carbon (BAC) columns over conventional granular activated carbon (GAC) systems in laboratory and pilot plant tests, it should be noted that few specifically designed BAC systems have been employed for industrial wastewater treatment. This may be due to physical disadvantages such as the increased pressure drop due to clogging by microbial growth (Characklis 1981). This problem may be alleviated, however, by frequent backwashing and air scouring to remove excess biomass (Ying and Weber 1979). The advantage provided by BAC systems of shielding bacteria from toxic effects could also serve as a drawback should pathogenic bacteria attached to the carbon be shielded from disinfection (LeChevalier et al. 1984).

There are numerous studies on the advantages of BAC processes over conventional biodegradation systems using free cells to remove organic matter. Some authors have reported an apparent synergism in the combined BAC process (Orshansky and Narkis 1996). However, there are reports that organic matter removal in these systems is a simple addition of effects from carbon adsorption and biological removal (Tsuneda et al. 2002), (2003). These contradictory findings indicate that the mechanisms involved in the combined BAC process have not been fully elucidated. In order to evaluate the degree of synergism of the BAC process to remove a given hazardous/toxic compound, many researchers have often studied its removal using biodegradation alone, then the efficiency of AC to remove such a compound, and finally the performance of the combined BAC process (Ferro Orozco et al. 2010).

The aim of the present study was to investigate SA removal efficiency using a combined process of adsorption and biodegradation, testing the influence of the presence of biofilm on the individual processes, i.e., AC adsorption and biodegradation by *P. putida*. The efficiency of this system was compared with AC adsorption and biological treatment via *P. putida* biodegradation. Biofilm formation on AC by *P. putida* was analyzed in order to obtain the optimal conditions.

2 Materials and Methods

2.1 Solutions

A pharmaceutical wastewater was simulated for the initial tests. Synthetic solutions with concentrations between 15 and 500 mg/L SA were prepared with SA (Panreac Qca. S.A.) and NaOH to increase pH to a suitable value for use in the mineral medium of *P. putida*. The solutions were used to test adsorption with AC, biodegradation with *P. putida*, the formation of attached biomass, and finally biosorption. The pH of these solutions was approximately 11–12.

The following chemical reagents were used in this study: meat extract, peptone, and agar, manufactured by Cultimed, KH_2PO_4, K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4$ 7 H_2O, and $C_6H_{11}NO_7Fe$ manufactured by Panreac Qca S.A., NaCl, NaOH, CaC_{12} 12·H_2O, and $Fe(NO_3)_3$ 9·H_2O manufactured by Sigma-Aldrich and tryptone manufactured by Biokar Diagnostics.

2.2 Activated Carbon

Filtrasorb 400 (Chemviron carbon Ltd., UK) and GAC 830 (Cabot Norit Americas Inc, USA) carbons were used in this study. The former, F400, has a surface area of 1,050 m^2/g, an iodine number of 1,050 mg/(g min), density of 861 g/L, and an effective size and moisture content of 0.6–0.7 mm and 2 %, respectively. GAC 830 has a surface area of 950–1,050 m^2/g, an iodine number of 920 mg/(g min), density of 907 g/L, and an effective size and moisture content of 1 mm and 2 %, respectively.

2.3 Bacteria

A specific bacterium, *P. putida* [Leibniz Institute DSMZ-German Collection of Microorganism and Cells Cultures, Germany (DSM 4478)], was chosen to degrade SA. This bacterium was selected because it is one of the main microorganisms present in sludges, is harmless, and metabolizes under aerobic conditions, at 30 °C and neutral pH, making it suitable for use in the treatment of industrial effluents.

2.4 Experimental Methods

2.4.1 Batch Adsorption Experiments

Prior to use in experiments, both ACs were rinse with distilled water to remove fines and dried at 105 °C in an oven. The carbon was then cooled in desiccators, where it remained until use.

Batch adsorption experiments were conducted at room temperature (20 °C) using synthetic solutions, varying the AC dosage from 0.12 to 0.5 g in the SA solution (250 mL) in order to determine a suitable L/S ratio [volume of liquid (mL)/mass of AC (g)] for subsequent experiments. 250 mL of each SA solution (15–115 mg/L) was respectively placed in different 500 mL stirred tanks adding a specific amount of AC to each tank. The solution was equilibrated for 90 min in a mechanical shaker at 250 rpm monitoring the SA concentration over time. This time was considered sufficient to reach operative equilibrium. The volume of the samples extracted from the tank each time was 2 mL, which did not substantially change the solution volume.

The adsorbed amount (q) from equilibrium experimental data was calculated via the following equation (Xing et al. 2008):

$$q = \frac{V(C_i - C_e)}{M} \quad (1)$$

where q is the adsorbed amount (mg/g), V is the volume (L) of solution, C_i is the initial SA concentration in the wastewater (mg/L), C_e is the equilibrium SA concentration (mg/L), and M is the amount of adsorbent (g).

2.4.2 Salicylic Acid Biodegradation

Nutrient medium was prepared by dissolving the following reagents in 1 L of distilled water: peptone (5 g), meat extract (3 g), KH_2PO_4 (0.422 g), K_2HPO_4 (0.375 g), $(NH_4)_2SO_4$ (0.244 g), $MgSO_4\ 7{\cdot}H_2O$ (0.05 g), $C_6H_{11}NO_7Fe$ (0.054 g), $CaCl_2\ 12{\cdot}H_2O$ (0.015 g), and NaCl (0.015 g) (Juang and Tsai 2006). The mineral salt medium was composed of the following reagents (g/L): KH_2PO_4 0.422, K_2HPO_4 0.375, $(NH_4)_2SO_4$ 0.244, $MgSO_4\ 7\ H_2O$ 0.05, $C_6H_{11}NO_7Fe$ 0.054, $CaCl_2\ 12\ H_2O$ 0.015, NaCl 0.015, and tryptone 0.05 (Juang and Tsai 2006). SA was added after sterilization by filtration through a Millipore filter of 0.22 μm. The final pH of these solutions should be 7 since this is the optimum value for *P. putida*; NaOH may be added, if necessary, to control the pH. The SA concentrations used ranged between 50 and 500 mg/L.

A biokinetic test of SA biodegradation was performed in a batch system to evaluate the biokinetic parameters. These experiments employed 100 mL of mineral medium in 250 mL Erlenmeyer flasks (Monteiro et al. 2000), 30 °C, 200 rpm, in which SA plus mineral salt medium was seeded with the acclimated suspended biomass (Juang and Tsai 2006; Silva et al. 2007). The biomass was previously introduced into the nutrient medium for 16 h at 150 rpm (Loh and Yu 2000; Silva et al. 2007) and 30 °C (Ploux et al. 2007), the time the bacterium takes to reach the exponential growth phase.

Samples were extracted at the onset of the batch kinetic test and subsequently at regular intervals to measure SA and the concentration of suspended biomass. The batch kinetic test provided data on the variation in SA and the concentration of suspended biomass over time.

2.4.3 Biofilm Formation

Live cells of a salicylic-degrading bacterium, *P. putida* DSM 4478, were immobilized on granular AC by adsorption. The AC was sterilized before use in the biofilm formation process.

The formation of biofilm on AC was carried out in a batch experiment by contacting carbon with *P. putida* bacteria for 24 h in a nutrient medium (Walker and Weatherley 1999). The L/S ratio [volume of liquid (mL)/mass of AC (g)] used in these series of experiments was 1,000. The experimental conditions were 30 °C, two different volumes, 100 mL in 250 mL Erlenmeyer and 1,000 mL in 2,000 mL Erlenmeyer, were used, and an initial concentration of SA of 100 mg/L. Experiments were performed employing different agitation speeds, 50

and 150 rpm, and inoculums sizes, 0.05 and 0.1 optical density units (OD, 600 nm).

The number of adsorbed cells was estimated by the difference in dry weight before and after the adsorption process and colony count determination.

In order to test whether the presence of SA influences the formation of biofilm on AC and subsequent SA biosorption, the same procedure was performed for biofilm formation as explained in the previous paragraph. In this case, however, 100 mg/L of SA was not added, the biological AC being subsequently subjected to a process of biosorption.

2.4.4 Combined Adsorption–Biodegradation

In almost all wastewater applications, AC and BAC have been used in continuous processes. However, analysis of batch processes can provide useful design data that enable continuous processes to be developed (Walker and Weatherley 1999). In this study, three batch systems were studied using the aerated stirred tank setup (Ong et al. 2008; Orshansky and Narkis 1996; Walker and Weatherley 1999).

1. Bacteria immobilized on F400 AC (Living biofilm-GAC).
2. Dead bacteria immobilized on F400 (Dead biofilm-GAC).
3. F400 granular AC with no biological activity (GAC).

The AC was sterilized prior to contact to minimize bacterial growth by heat, submitting it to 100 °C for 12 h in a sterile container (Walker and Weatherley 1999).

For the immobilized cell systems, the bacterial culture was contacted with 0.1 g F400 carbon in nutrient broth for 24 h before addition to the reaction vessel. To achieve a dead immobilized cells system, 0.1 g of F400 with biofilm was introduced in an oven at 100 °C for 30 min before the biosorption process (Ong et al. 2008). Finally, adsorption using the granular carbon system meant the addition of 0.1 g F400 to the reactor. Each of these systems was contacted with 100 mL of an autoclaved synthetic mineral salt medium in 250 mL Erlenmeyer flasks. These operated at a pH of 7, a temperature of 30 °C, and 200 rpm agitation speed, the conditions being controlled by a New Brunswick Scientific incubator.

Samples were taken from the Erlenmeyer flasks every 2 h and centrifuged (5 min at 13,200 rpm) to separate the biomass from the supernatant, thus enabling analysis of cell density and the concentration of SA.

2.5 Analytical Methods

The standard iron complex (Fe^{3+}) method (Snell and Snell 1953) was used to determine the concentration of SA in water. SA reacts with ferric ions to form a violet-colored complex, which was determined by colorimetric analysis at 530 nm on a UV/Vis (Thermo Scientific) spectrophotometer.

OD was measured at 600 nm on a UV/Vis (Shimadzu) spectrophotometer to monitor outgrowth of microorganisms during fermentation.

All experiments were performed in duplicate, and the process was repeated on subsequent days under identical conditions. Furthermore, the samples were analyzed in triplicate; the reproducibility of the concentration measurements was mostly within 5 %.

3 Results and Discussion

In the first stage of this research study, each process (adsorption and biodegradation) was studied separately.

3.1 Batch Equilibrium

As previous results show that the minimum L/S ratio which yields the highest capacity in both cases is equal to 1,000, this ratio was selected as the value for all the tests.

Several runs were carried out to obtain the capacities of the F400 and GAC 830 ACs. These were contacted with different SA solution concentrations (15–115 mg/L) with an L/S ratio=1,000 mL/g. The results show that equilibrium is reached in less than 90 min in all cases.

Two different models, the Langmuir and Separation Factor isotherms (CFS), were used to define the relationship between the loading capacity and the solution concentration. The equations of the isotherms are

$$\text{Langmuir}: q_i = \frac{K_{eq} \cdot q_t \cdot C_i}{1 + K_{eq} \cdot C_i} \tag{2}$$

where K_{eq} is the equilibrium constant, q_t is the maximum capacity of the AC, and C_i is the solution concentration of species i.

$$\text{Separation Factor}: q_i = \frac{K_{eq} \cdot q_t \cdot C_i}{C_T + (K_{eq} - 1) \cdot C_i} \quad (3)$$

where C_T is the minimum salicylic equilibrium concentration in the solution with a saturated AC.

The equilibrium constants and the maximum adsorption capacity were obtained. Experimental results were fitted to the Langmuir and CFS isotherms and are shown in Fig. 1a, b. The different isotherm parameters obtained are summarized in Table 1.

In view of the results, the SA adsorption capacity of Filtrasorb F400 is higher than that of GAC 830. Accordingly, F400 was used in subsequent experiments. Adsorption equilibrium experimental data showed a better fit to the Langmuir isotherm model. Otero et al. (2004) obtained a maximum capacity of 351 mg/g from fitting equilibrium data on the adsorption of SA (pH around 5) with F400. Significant differences between the values obtained in the present study and those reported by Otero et al. (2004) can be explained by the pH of the solutions. In our case, the solutions of SA are dissolutions with a basic pH (11.5), which produces a decrease in the adsorption of the pollutant by AC (Karimi-Jashni and Narbaitz 1997).

3.2 Batch Kinetics

The process was monitored until equilibrium was reached with all solutions. After 45 min, the solution concentration of SA does not change substantially, so operational equilibrium may be assumed. This value was taken from the loading experiments conducted in stirred tanks using synthetic solutions with initial concentrations of between 15 and 115 mg/L SA. The concentration profiles can be studied using the film mass transfer and pore diffusion kinetics models to determine the mass transfer mechanisms.

- Film mass transfer model

In this model, the retention of SA is assumed to be limited by the external surface of the particle. The behavior of this model can be described by the following equation (Torre et al. 2006):

$$q_i(t) = C_0 (V_L/V_S) \left(1 - \frac{C^*}{C_0}\right) \left(1 - \exp\left(\frac{-K \cdot t}{V_L/V_S}\right)\right) \quad (4)$$

where q_i is the concentration of species i in the AC at time t, K is the apparent constant of mass transfer, C^* is the equilibrium concentration of SA in the solution, V_L is the volume of liquid, V_s is the volume of AC, and C_0 is the initial SA concentration in solution.

The apparent constant of mass transfer, K, can be determined by linear regression using the experimental data, fitting these to Eq. (4). The AC beads are of constant size and have a spherical geometry; therefore, $K_f = K/a_p$. Hence, a comparison of the experimental results for SA concentration and the theoretical values obtained with Eq. (3) will give the goodness of the obtained value for all the experiments. Experimental kinetic results were fitted to the film mass transfer model and are shown in Fig. 2a.

- Pore diffusion model

This model considers carbon to be a porous matrix. In this study, we used the system described for Torre

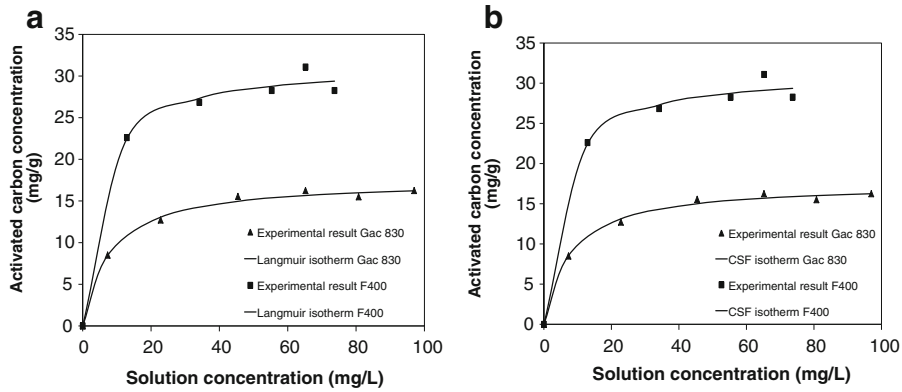

Fig. 1 Fitting of experimental data to the Langmuir isotherm (**a**) and to the FSC isotherm (**b**), employing a synthetic solution of salicylic acid (15–115 mg/L), 20 °C, 250 rpm, L/S: 1,000, pH:11.5

Table 1 Isotherms parameters obtained for both activated carbons

	Filtrasorb 400		GAC 830	
	Langmuir isotherm	CFS isotherm	Langmuir isotherm	CFS isotherm
K_{eq}	0.194 (L/mg carbon)	46.99	0.128 (L/mg carbon)	21.9
q_t (mg SA/g carbon)	31.4	30.7	17.5	16.7
C_T (mg SA/L solution)	–	240	–	160

et al. (2006) and a FORTRAN subroutine, PDECOL, to solve these equations. The subroutine uses the method of orthogonal collocation on finite elements to solve the system of nonlinear differential equations.

Figure 2b shows the fit of the experimental results to this model. The good agreement between experimental data and the theoretical prediction shows the goodness of the model.

The apparent constants of mass transfer and diffusion coefficient values were obtained from the fit of the experimental data shown in Table 2 for the ACs used in this study.

The results suggest that the kinetics is slightly faster for the GAC 830 carbon than for F400. However, as the adsorption of SA by Filtrasorb 400 is 40 % higher compared to GAC 830, F400 was chosen for subsequent biofilm formation and biosorption tests.

Other examples of values for the pore diffusion coefficient would be 1.85×10^{-10} m^2/s for the F400 carbon with SA (Otero et al. 2004), 7×10^{-12} m^2/s for orthochlorophenol and an AC particle diameter equal to 1.015 mm (Chourio et al. 1997), and 9.5×10^{-12} m^2/s for F400, 2-nitrophenol and pH 13 (Karimi-Jashni and Narbaitz 1997). The apparent constant of mass transfer values of around $(0.4–2)\times10^{-4}$ m/s was obtained for phenolic compounds (Navia et al. 2003), 8.02×10^{-4} m/s for F400, 2-nitrophenol and pH 13 (Karimi-Jashni and Narbaitz 1997), and 1.36×10^{-8} m/s for F400, SA and pH 5 (Otero et al. 2004).

3.3 Salicylic Acid Biodegradation

The suspended biomass data show a typical growth curve with a well-defined growth phase followed by a constant growth phase (Fig. 3a). The SA concentration and suspended biomass data provide an a priori estimation of the biokinetic parameters for evaluating the growth rate of the suspended biomass and the utilization rate of SA.

Batch cultures of SA-utilizing bacteria were conducted in media containing initial SA concentrations ranging between 50 and 500 mg/L. Cellular growth of *P. putida* at different initial concentrations of SA and the consumption of SA depending on the initial concentration are shown in Fig. 3a, b, respectively.

Figure 3a shows a steady increase in biomass over time for all initial concentrations of SA. Employing

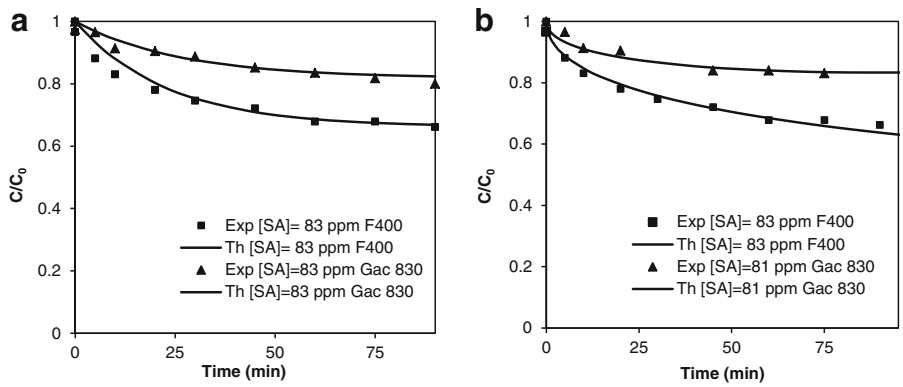

Fig. 2 Fitting of experimental theoretical kinetic data to the film mass transfer model (**a**) and to the pore diffusion model (**b**), employing a synthetic solution of salicylic acid (15–115 mg/L), 20 °C, 250 rpm, L/S: 1,000, pH:11.5

Table 2 Mass transfer constant and pore diffusivities calculated by film mass transfer and pore diffusion model respectively for activated carbon F400 and GAC 830

Experiments (F400)	D_p(m^2/s)	K_f(m/s)	Experiments (GAC 830)	D_p(m^2/s)	K_f(m/s)
Synthetic solution, $C_{0\ AS}$=35 mg/L	8.6×10^{-11}	7.7×10^{-5}	Synthetic solution, $C_{0\ AS}$=35 mg/L	6.95×10^{-11}	9.95×10^{-5}
Synthetic solution, $C_{0\ AS}$=60 mg/L	2.4×10^{-11}	5.1×10^{-5}	Synthetic solution, $C_{0\ AS}$=60 mg/L	6.95×10^{-11}	1.00×10^{-4}
Synthetic solution, $C_{0\ AS}$=85 mg/L	2.8×10^{-11}	7.3×10^{-5}	Synthetic solution, $C_{0\ AS}$=80 mg/L	6.95×10^{-11}	9.90×10^{-5}
Synthetic solution, $C_{0\ AS}$=95 mg/L	2.8×10^{-11}	8.8×10^{-5}	Synthetic solution, $C_{0\ AS}$=95 mg/L	6.95×10^{-11}	1.1×10^{-4}
Synthetic solution, $C_{0\ AS}$=100 mg/L	2.8×10^{-11}	8.3×10^{-5}	Synthetic solution, $C_{0\ AS}$=115 mg/L	6.95×10^{-11}	2.7×10^{-4}

initial SA concentrations of 423 and 529 mg/L, the biomass remains constant from 10 h until 24 h.

Figure 3b shows that 100 % degradation was obtained in 6 h for initial concentrations of 60 and 110 mg/L and in 10 h for 225 mg/L SA. When the initial SA concentrations were 423 and 529 mg/L, however, only 81 and 72 % of the initial concentration were degraded in 10 h, respectively, obtaining 100 % removal in both cases after 24 h.

Exact comparisons of degradation efficiency with literature results are not direct because there are differences in the cell density and medium compositions. Without considering these factors, it has been reported by Juang and Tsai (2006) that the range between 138 and 587 mg/L of SA was degraded within 15–63 h, respectively. In the researches of Loh and Yu (2000), SA concentrations between 20 and 600 mg/L were biodegraded with times removal of the contaminant between 9 and 23 h, respectively.

The specific growth rate was calculated by means of the following equation (Juang and Tsai 2006):

$$r_X = \frac{dX}{dt} = \mu\ X \tag{5}$$

where r_x is the rate of biomass growth, X is the cell number or mean cell concentration, μ is the specific biomass growth rate, and t is time. The variation in specific growth rate (μ) versus SA concentration (S) obtained from batch tests is shown in Fig. 4. The Haldane equation for substrate-inhibited growth was fitted to the specific biomass growth rate data (μ) versus SA concentration (S) (Juang and Tsai 2006). The following parameter values were obtained: μ_{max}=0.25 h^{-1}; substrate-affinity constant, K_s=61.25 mg SA/L; substrate-inhibition constant, K_i=704.93 mg SA/L; and a correlation coefficient, r^2, of 0.991.

Literature survey on salicylic degradation shows that the K_i, μ_{max}, and K_s values ranges from 719.6 mg/L, 0.137 h^{-1}, and 15.33 mg/L using *P. putida* CCRC 14365 (Juang and Tsai 2006) to 1,201.7 mg/L, 0.52 h^{-1}, and 36.2 mg/L using *P. putida* ATCC 17484 (Loh and Yu 2000). The K_i and μ_{max} values obtained in the present work were (704.93 mg/L and 0.25 h^{-1}) which fall within the literature ranges. On the other hand, the K_s value (61.26 mg/L) determined in this work is

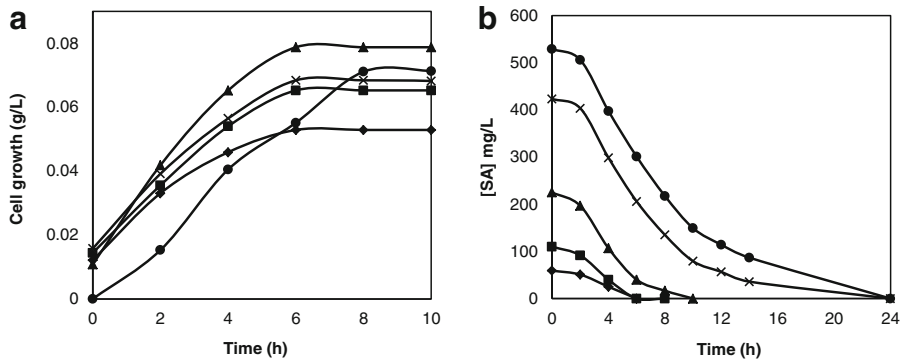

Fig. 3 Growth and salicylic acid degradation of *Pseudomonas putida* in a mineral salt medium containing 50–500 mg SA/L, 30 °C, 200 rpm, pH:7 (*black diamond* 59 mg/L, *black square* 110 mg/L, *black up-pointing triangle* 225 mg/L, × 423 mg/L, and *black circle* 529 mg/L) **a** Growth of *Pseudomonas putida* and **b** salicylic acid degradation

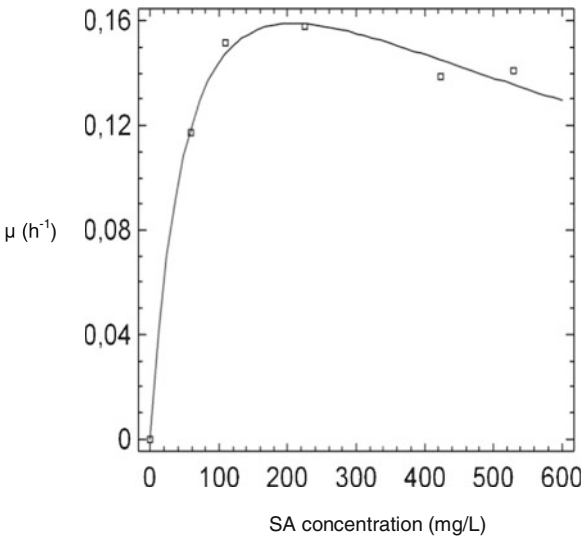

Fig. 4 Specific growth rate of suspended biomass in a salicylic acid solution (50–500 mg/L), 30 °C, 200 rpm pH: 7 (*white square* experimental data – model)

slightly higher than that found in other works indicating that *P. putida* (DSM 4478) cells have a higher SA affinity.

3.4 Biofilm Formation

Biofilm formation on AC was analyzed by varying the inoculated concentration of initial biomass and the agitation speed of the medium. The agitation speeds and inoculum sizes used in these experiments were 50 and 150 rpm and 0.05 and 0.1 OD units, respectively. Experiments were performed employing two different volumes of medium: 100 and 1,000 mL.

Table 3 shows the results obtained in the experiments. No significant differences were found in terms of biomass formation for the different process operating conditions. However, the results show that the number of colony forming units (cfu) is slightly higher at 50 rpm and 0.05 OD units than that obtained under other conditions.

Table 3 Results of the dry weight of biomass and colony forming units for the formation of biofilm on activated carbon

Inoculum size	*N* (rpm)	V_m (mL)	mg bio/g AC	cfu/mL	cfu/g AC
0.05	150	100	256.9	3.6×10^{8}	3.6×10^{10}
0.05	150	1,000	320.5	5.9×10^{8}	5.9×10^{10}
0.05	50	100	335.7	7.5×10^{8}	7.5×10^{10}
0.1	150	100	243.8	2.9×10^{8}	2.8×10^{10}
0.1	150	1,000	309.2	5.1×10^{8}	5.1×10^{10}

For the combined adsorption–biodegradation process, biofilm attachment on AC occurred under operating conditions of 50 rpm and 0.05 OD units.

In this work, the value of attached biomass on AC in optimal conditions found was 2.9×10^{5} cfu/cm^2. Data of attached biomass on AC in batch biofilm formation processes have not been found in the literature; however, some data are available in several works regarding column biofilm formation. For example, Gibert et al. (2013) estimate the amount of attached biomass on active carbon in column experiments, being 1×10^{6} cfu/cm^2 in the top of the column. In other work about biofilm formation on surfaces exposed to treated water, Van der Kooij et al. (1995) indicate biomass values adhered between 8.7×10^{3} and 2.5×10^{4} cfu/cm^2 on glass and values between 1.1×10^{4} and 2.9×10^{4} cfu/cm^2 on Teflon. In batch operations, Ploux et al. (2007) determined the amount of biomass attached on two different surface areas. These surfaces were constituted by terminated self-assembled monolayers (SAMs) on silicon wafers. For CH_3- and NH_2-surfaces, values around $(3–3.5)\times10^{7}$ cfu/cm^2, respectively, were reached in 24 h. So the values of attached biomass found in this work are in the same range that the values obtained in other systems for other authors.

Furthermore, the absence or presence of SA in the process of biofilm formation did not produce significant differences in the performance of the subsequent biosorption in either the pollutant removal rate or the rate of biomass formation in the medium.

3.5 Biofilm Influence on Adsorption and Biodegradation

Simultaneous adsorption and biodegradation of SA were studied in a series of batch experiments.

Figure 5a, b shows the SA uptake over time using two different systems, AC and dead biofilm. Duplicates biosorption process with dead biomass are shown in the figure (indicated as Dead biofilm-Gac 1 and Dead biofilm-Gac 2). This process was carried out at initial salicylic concentrations of 100 and 500 mg/L.

AC can reduce the concentration of SA from 85 to 38 and from 458 to 374 mg/L in 25 h, so it presents an adsorption capacity of 40.5 and 72.3 g/L, respectively. In this case, the AC adsorption capacity was higher than that obtained in Section 3.1. This is explained by the

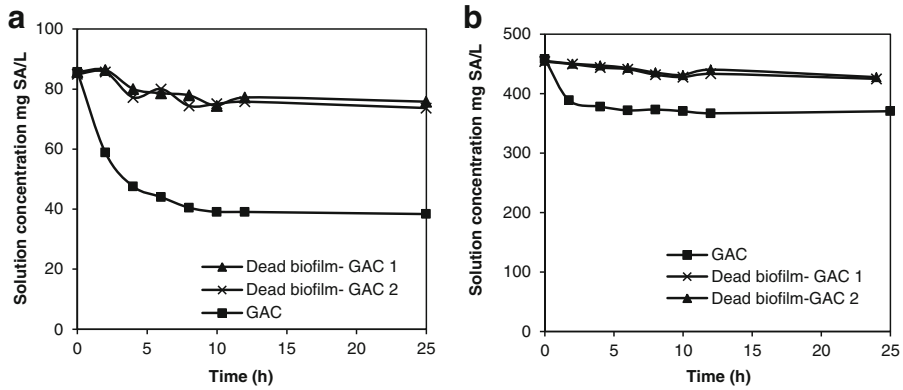

Fig. 5 Variation in the concentration of salicylic acid over time using dead biofilm and activated carbon systems. Initial concentration of acid: 100 mg/L (**a**) and 500 mg/L (**b**), 30 °C, L/S:1,000 and 200 rpm, pH:7

difference in pH between the experiments: in Section 3.1, the solution had a basic pH (11.5), while in this case, the pH of the solution was neutral, around 7. Karimi-Jashni and Narbaitz (1997) reported that increasing the pH of the solution hinders pollutant adsorption by the AC. In view of the results, the adsorption of the pollutant by the AC was adversely affected by the basicity of the medium.

In the case of the dead bacteria immobilized on AC system, only 12 and 6 % of SA was removed. The AC of the system is not able to adsorb SA because the biomass plugs its active sites or the AC is saturated during the process of biofilm formation with the acid and other components present in the medium (Ong et al. 2008; Walker and Weatherley 1999).

Figure 6a, b compares SA removal over time in the case of the biodegradation with free cells system and for biosorption with biofilm attached on the AC. Duplicates biosorption process with Living biofilm are shown in the figure (referred as Living biofilm-Gac 1 and Living biofilm-Gac 2).

Bioadsorption performed significantly better than AC adsorption. The results indicate that the same amount of AC achieves almost 100 % pollutant removal in the biosorption process, whereas the removal of SA in the case of adsorption is equal to 55 and 20 % for initial SA concentrations of 100 and 500 mg/L, respectively.

Comparing the biofilm system versus the free cells system, a difference in performance is observed depending on the initial concentration of SA. When the initial concentration is low (100 mg/L), the free cells system is more effective, as it removes the pollutant in a shorter period of time. When the initial concentration of acid is higher (500 mg/L), however, the immobilized biomass system is more effective even though biosorption is slightly lower in the first hours of the experiment and the system has a greater lag period.

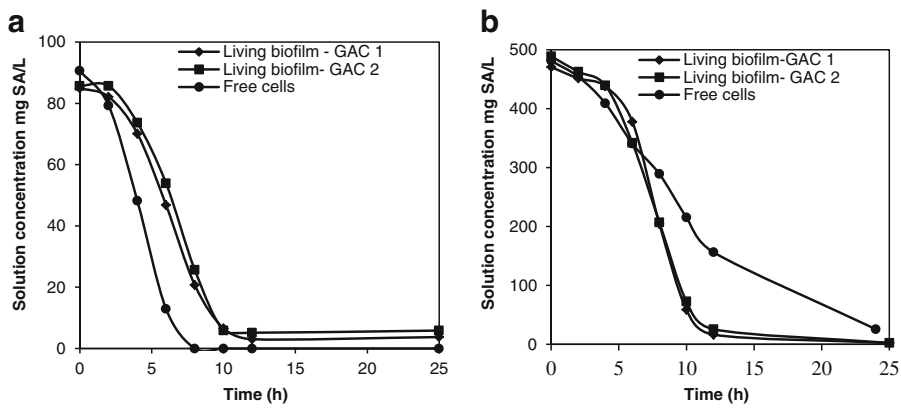

Fig. 6 Variation in the concentration of salicylic acid over time using biofilm and free cells systems. Initial concentration of acid: 100 mg/L (**a**) and 500 mg/L (**b**) 30 °C, L/S:1,000 and 200 rpm, pH:7

Table 4 Kinetics parameters for the system with biomass immobilized and free cells system

		$Y_{X/S}$	μ (h^{-1})	r_{max} (mg/L h)	Bioadsorption time (h)	Maximum concentration of biomass (mg/L)
500 mg/L	Bacteria immobilized	0.364	0.301	77.2	12	169
	Free cells	0.142	0.033	36.7	26	42.8
100 mg/L	Bacteria immobilized	0.746	0.293	13.1	25	58.9
	Free cells	0.691	0.276	17.7	8	42.2

In this case, AC bioadsorption using synthetic wastewater was found to require a longer time to reach equilibrium. Dissolved organic matter was removed from the wastewater quickly within the first 45 min, after which the removal rates increased gradually over the next 12 h. The explanation for this is that the adsorption of AC was more predominant at the beginning, while after the available sites were occupied, the organic matter was biodegraded by the activity of microorganisms which colonized the external surface and macropores of the carbon (Xing et al. 2008).

These conclusions are confirmed by the specific growth rate, maximum speed of degradation, cell yield, degradation time, and maximum concentration of biomass values shown in Table 4.

The consumption associated with the growth substrate, cell yield, and their relationship is represented as follows:

$$(X - X_0) = Y_{X/S}(S_0 - S) \quad (6)$$

where $Y_{X/S}$ is the cell yield coefficient, which is a measure of the amount of biomass produced per unit of substrate consumed, and the subscript 0 represents the initial conditions.

The observed difference in performance between the free cells and immobilized biomass systems is due to the fact that the biomass is much more protected from the toxicity of the medium in the biofilm attached to AC setup. In addition, Prober et al. (1975) showed that AC is able to enrich dissolved oxygen. This oxygen is probably also utilized by the microorganisms adsorbed on the AC.

Pai et al. (1994), Orshansky and Narkis (1996), Xing et al. (2008), and Walker and Weatherley (1999) investigated biosorption processes with different contaminants. Although different contaminants and bacteria were used, these authors found an improved contaminant removal due to the use of the biofilms. For example, Pai et al. (1994) observed that the presence of biofilm improved the rate of degradation of phenol by *Rhodococcus sp.*, increasing the value of the degradation rate from 0.432 g/Ld when operating with free cells to 2.91 g/Ld when biological AC was used. Moreover, Walker and Weatherley (1999) found that the removal of Acid Blue Color (TB4R) with *P. putida* (NICMB 9776) increased from 30 % using free cells to 90 % using biofilm on AC after 24 h.

4 Conclusions

In this paper, the influence of biofilm on individual processes of adsorption of SA on AC and its biodegradation by *P. putida* were studied. First, the individual processes were tested separately, finding that Filtrasorb 400 AC had a higher capacity than the other carbon studied. The equilibrium constant and the maximum capacity of F400 were 46.99 and 26.6 g/L AC (30.74 mg/g), respectively. The biodegradation of SA by *P. putida* follows a substrate-inhibition kinetics that can be fitted to the Haldane equation, with the following parameters: μ_{max}=0.25 h^{-1}, K_s=61.25 mg SA/L, Ki=704.93 mg SA/L, and a correlation coefficient, r^2, of 0.991. Furthermore, the system of dead biomass attached to the AC did not show biosorption capacity. Thus, the presence of biofilm on AC has a negative effect on the adsorption process.

The free cells system employing an initial concentration of SA of 100 mg/L achieved a pollutant removal efficiency of 85.7 and 100 % at 6 and 8 h, respectively. However, when the initial concentration was 500 mg/L SA, the respective removal efficiencies were 67.5 and 94.7 % at 12 and 24 h. The bioadsorption system employing an initial concentration of 100 mg/L needed 10 h to obtain a removal efficiency of 93.16 % SA. When employing an initial pollutant concentration of

500 mg/L, however, the removal efficiency was 94.7 % at 12 h. As regards the combined adsorption/biodegradation process, the results showed that the biofilm outperformed the combination of conventional AC and biological water treatment processes in the case of working with high pollutant concentrations, as it is able to remove SA in a shorter period of time. This effect occurs in this case because the biological system is protected from adverse environmental conditions.

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