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Biofilm photobioreactors for the treatment of industrial wastewaters

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ABSTRACT

A flat plate and a tubular packed-bed photobioreactor with an algal–bacterial biofilm attached onto Poraver® beads carriers, a flat plate and a tubular photobioreactor with the biofilm attached onto the reactor walls, and an algal-turf reactor were compared in terms of BOD removal efficiencies, elimination capacities, and stability. A control column photobioreactor with suspended algal–bacterial biomass was also tested to compare the performance of biofilm photobioreactors with conventional algal-based processes. When the algal–bacterial biomass was immobilized onto Poraver® the process never reached a steady state due to a poor homogenization in the bioreactor. When the biofilm was formed onto the reactor wall (or reactor base) the process was stable. A maximum degradation rate of 295 mg BOD l^{-1} h^{-1} was achieved in the algal-turf reactor although control experiments performed in the dark showed atmospheric O_2 diffusion represented 55% of the oxygenation capacity in this system. BOD removal rates of 108, and 92 mg BOD l^{-1} h^{-1} were achieved in the tubular and flat plate biofilm reactors, respectively, compared to 77 mg BOD l^{-1} h^{-1} in the control suspended bioreactor. In addition, all biofilm photobioreactors produced an easily settleable biomass. Evidence was found that biomass attachment to the reactor's wall improved stability.

1. Introduction

External oxygen supply is often required during the treatment of hazardous effluents which are poorly suitable for anaerobic treatment in order to supply the bacterial community with enough O_2 to carry out pollutant mineralization. This is costly and can cause the hazardous release of aerosols and toxic volatile contaminants such as phenolic compounds or toxic organic solvents [1–4]. Photosynthetic oxygenation overcomes these limitations by *in situ* O_2 generation, which is safer and cheaper as sunlight is the main energy source [5]. More precisely, the microalgae produce the oxygen required by the aerobic bacteria to mineralize organic pollutants, which in turn use the carbon dioxide released by the bacteria [6]. Unfortunately, photosynthetically oxygenated processes are often limited by the slow growth of microalgae, the risk of microalgal inhibition and by the difficulty of removing microalgae from the treated effluent [7,8]. Poor algae harvesting also reduces the possibility of biomass recirculation in order to achieve high removal rates in small reactors [6]. This means continuous processes must be operated at rather high hydraulic retention times to avoid cell washout (2–6 d; [9]). None of the common industrial approaches (filtration, centrifugation, microstraining, etc.) have been proven to be economical and suitable to large-scale microalgal removal [7]. In fact, the high harvesting costs can even jeopardize the economic viability of high-quality algal biomass production processes [10].

Biomass immobilization can overcome these limitations by maintaining a high microbial activity at all operating conditions, protecting cells from pollutant toxicity and producing an effluent containing easily settleable microbial flocks [11]. Immobilization into biopolymers such as carrageenan, chitosan, or alginate has been successfully tested in the lab but the weakness of these matrices during long term operation and their high cost have restricted their large scale application [7]. New methods for biomass immobilization are therefore required to support photosynthetically oxygenated processes. This study was conducted to evaluate the potential of several biofilm photobioreactor configurations using a *Chlorella sorokiniana–Ralstonia basilensis* consortium immobilized

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Table 1
Design parameters of the photobioreactors tested

	Flat plate-Poraver®	Tubular Poraver®	Flat plate	Tubular	Algal-turf	Column
Illumination	Side	Side	Side	Top	Top	Side
Material	Glass	Glass	Plexiglas	PVC	PVC	Glass
Reactor volume (cm^3)[a]	105	134	709	251	313	600
Illuminated surface (cm^2)	250	812	1290	500	487	600
Surface[b]/volume (cm^{-1})	2.38	6.06	1.82	1.99[c]	1.55	1

[a] In the following, all volumetric rates are expressed per unit of total reactor volume.
[b] Outer illuminated surface.
[c] In this particular case the total surface to volume ratio does not correspond to the illuminated surface/volume ratio (3.98 *vs* 1.99).

onto foamed-glass beads carriers (Poraver®) and onto the reactor walls. A suspended column photobioreactor with the same consortium was also tested as control. Salicylate was chosen as model contaminant for being moderately toxic towards microalgae and for not being biodegraded by *C. sorokiniana* [5]. Hence, in this study, the algae only served to produce the oxygen necessary by *R. basilensis* to mineralize the pollutant. This work constitutes, to the best of our knowledge, the first application of enclosed biofilm photobioreactors for the treatment of industrial wastewaters.

2. Material and methods

2.1. Microorganisms and culture conditions

All experiments were performed using the following mineral salt medium (MSM) ($g\,l^{-1}$): KNO_3, 1.25; $MgSO_4{\cdot}7H_2O$, 0.625; $CaCl_2{\cdot}2H_2O$, 0.1105; H_3BO_3, 0.1142; $FeSO_4{\cdot}7H_2O$, 0.0498; $ZnSO_4{\cdot}7H_2O$, 0.0882; $MnCl_2{\cdot}4H_2O$, 0.0144; MoO_3, 0.0071; $CuSO_4{\cdot}5H_2O$, 0.0157; $Co(NO_3)_2{\cdot}6H_2O$, 0.0049; EDTA, 0.5; KH_2PO_4, 0.6247; K_2HPO_4, 1.3251. The pH was adjusted to 6.8 with KOH and the medium was autoclaved before use ($MgSO_4{\cdot}7H_2O$ was autoclaved separately and added to the sterile culture medium afterwards to avoid salt precipitation). The flat plate reactor was however operated with MSM supplied with 0.4 g $(NH_4)_2SO_4\,l^{-1}$ instead of KNO_3 in order to investigate the effect of pH on process performance. Sodium salicylate (Merck, min. 99.5%) was used as model contaminant.

C. sorokiniana strain 211/8k was purchased from the Culture Centre of Algae and Protozoa (Cambridge, UK). Culture conservation and inoculum preparation were carried out according to Guieysse et al. [12]. The MSM used for algal cultures was enriched with a sterile solution of glucose, peptone and yeast extract to reach the final concentration of 3.125, 0.0625 and 0.0625 g/l, respectively. The algae was maintained on agar plates (enriched MSM plus agar, 10 g/l) at room temperature (23 °C) under continuous illumination (4000 lux) for 10 days and stored at 4 °C afterwards. The algae were transferred to new agar plates every month. Algal inocula were mixotrophically grown in 250-ml flasks at room temperature with enriched MSM, under continuous illumination (Phillips TLD 36W/840 fluorescent lamps) at 9000 lux (Lutron LX-101 Lux Meter) and shaken on a rotary-shaker at 150 rpm.

A *Ralstonia basilensis* strain (Genbank accession number AY047217) was used for salicylate biodegradation [5]. To furnish fresh inocula *R. basilensis* was periodically transferred to 250-ml flasks supplied with 800 mg/l of salicylate (using a stock solution of 20 g/l) in MSM, and incubated at room temperature on a rotary shaker (IKA KS 501 shaker, Staufen, Germany) at 150 rpm.

2.2. Experimental

Six photobioreactor configurations were tested (Fig. 1): a flat plate and a tubular photobioreactor with the algal–bacterial biofilm attached onto 4–8 mm Poraver® foamed-glass beads [13], a flat plate and a tubular photobioreactor with the biofilm attached onto the bioreactor walls, an algal-turf reactor shallow open pond [14] with the biofilm attached to the reactor base, and a column photobioreactor with suspended algal–bacterial biomass and no biomass retention (chemostat operation). The main characteristics of each photobioreactors are shown in Table 1. The reactors were operated under continuous illumination at 180 $\mu E\,m^{-2}\,s^{-1}$ (impinging irradiance at the outer photobioreactor surface) and at 25 °C. No temperature control was carried out in any of the experiments. The temperature of the bioreactors was thus the result of the balance between the energy input (impinging heat from the fluorescent lamps and microalgal conversion of light into heat) and the heat dissipated via photobioreactor's surface. Sodium salicylate at 2 g/l in MSM (concentration periodically analyzed in order to check the correct preparation of the feed) was continuously added to the reactors using peristaltic pumps (Alitea C4-MIDI).

The performance of these photobioreactor configurations (evaluated based on salicylate removal efficiency (RE) and elimination capacity (EC)) under several hydraulic retention time (HRT) was investigated by gradually increasing the effluent flow rate (3.3–0.2 d of HRT) at constant salicylate concentration ($2\,g\,l^{-1}$ in MSM-periodically checked in order to check the correct preparation of the feed). In this regard, photobioreactor configuration constituted the core parameter evaluated considering that both the illuminated surface to volume ratio and culture pH (influenced by microalgal activity) are intrinsically linked to the tested configuration and the fact that the same illumination was provided at photobioreactors' surface. EC and RE were calculated according to the following expressions:

$$EC = \frac{Q}{V_{reactor}}(S_{in} - S_{out}) \quad (1)$$

$$RE = \frac{(S_{in} - S_{out})}{S_{in}}100 \quad (2)$$

where $V_{reactor}$ is the reactor volume (l); Q corresponds to the feed flow rate ($l\,h^{-1}$); S_{in} and S_{out} are the inlet and outlet sodium salicylate concentrations, respectively ($mg\,l^{-1}$).

In this regard, photobioreactor configuration constituted the core parameter evaluated considering that both illuminated surface to volume ratio and culture pH (determined by microalgal activity) are intrinsically linked to the tested configuration and the fact that the same illumination was provided at the outer photobioreactors' surface. After each change in the HRT, each reactor was allowed to equilibrate, and once salicylate concentration was stable, the reactor was monitored for a period of at least two HRTs. During that period, between three and six samples were withdrawn, and each sample was considered to be a replicate for this specific steady state. Early studies showed *R. basilensis* consumed 0.88 g of $O_2{\cdot}g$ sodium salicylate^{-1} [5] and this value was used to convert salicylate removal rates into BOD removal rates in the following. No dissolved oxygen concentration was herein measured due techni-

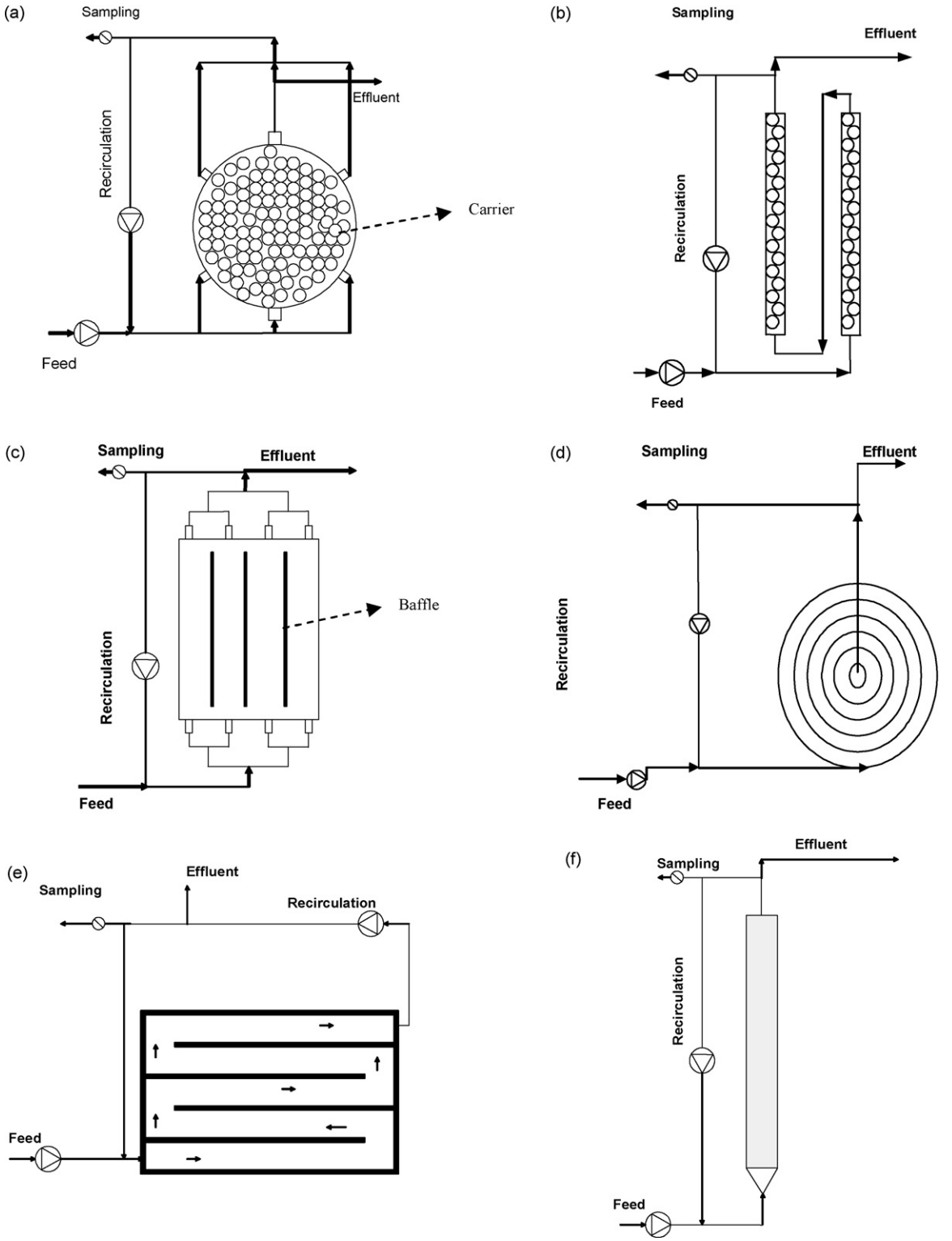

Fig. 1. Schematic representation of the flat plate photobioreactor with biomass immobilized on Poraver® carriers (a); tubular photobioreactor with biomass immobilized on Poraver® carriers (b); flat plate photobioreactor with biomass immobilized as biofilm onto the reactor wall (c); tubular photobioreactor with biomass immobilized as biofilm onto the reactor wall (d); algal-turf reactor (e); and column photobioreactor with suspended biomass (f).

cal complexity imposed by the particular configuration and size of the photobioreactors tested (packed-bed reactors, tube diameters of 1 cm or plate width <1 cm). The attachment and confinement of the algal–bacterial population inside the biofilm photobioreactors hindered the estimation or direct measurement of algal–bacterial growth rates in the enclosed bioreactors. In the column reactor the algal–bacterial biomass concentration was estimated by daily measurement of the absorbance of the suspended culture. There exits moreover an inherent challenge on the quantification of the individual populations of microalgae and bacteria separately, even in

suspended growth bioreactors, due to the comparable size of both microorganisms. Estimations of microalgae concentration based on chlorophyll measurement are also rather inaccurate due to the ability of microalgae to modify its chlorophyll content depending on light availability.

2.3. Analytical procedures

Light input was measured as photosynthetic active radiation (PAR) using a LI-170 sensor (LI-COR, NE, USA). A Schott pH meter with a 3 M/KCL Schott electrode (Schott glas, Germany) was used for pH determination.

For salicylate analysis, 1-ml samples were centrifuged in microcentrifuge tubes for 10 min at 13000 × *g* using a centrifuge Biofuge 13 (Heraeus Instruments, Germany). Portions of supernatant were then transferred to HPLC vials for analysis. HPLC-UV analysis was performed using a Varian 9010 liquid chromatograph (Varian, Walnut Creek, USA) with a Varian ProStar 420 autosampler and a Varian 9050 variable wavelength monitor. The column used was a Supelcosil LC-8, 5 μm (Supelco, Bellefonte, USA) [8]. Samples were eluted isocratically using a mobile phase composed of methanol, water and acetic acid (60:39:1, v:v:v) at a flow rate of 0.5 ml min^{-1}. UV detection was performed at 280 nm.

3. Results and discussion

The packed-bed bioreactors never reached a steady state (Fig. 2). Salicylate removal efficiencies ranged from 100% to 23% (corresponding to EC of 28–123 mg BOD l^{-1} h^{-1} at 0.6 d HRT) in the flat plate and 100–74% (34–54 mg BOD l^{-1} h^{-1} at 1.6 d of HRT) in the tubular photobioreactor. Supplying the medium with NH_4^+ as nitrogen source in the flat bed reactor stabilized the pH value of the culture broth to 6.6–6.9 compared to 6.8–8.5 in the tubular packed-bed reactor. Thus, while microalgal activity increased culture pH in the tubular packed-bed reactor due to the photosynthetic CO_2 consumption, NH_4^+ assimilation in the flat plate brought about the release of H^+ with the subsequent stabilization in culture pH. However, this did not improve salicylate removal. Consequently, a pH-mediated inhibition unlikely caused the observed periodic process collapses. In addition, considering the EC achieved in this study, the empirical N content of algal–bacterial biofilms in algal-turf scrubbers (typically 4–5%; [15]), the N content of the MSM supplemented with $(NH_4)_2SO_4$ l^{-1}, and the low affinity constant of microalgae for N ($k_s \approx 0.01$ mg l^{-1} [16]) nitrogen limitation was unlikely to occur. Large gas bubbles (2–10 mm ID) accumulated inside both packed-bed photobioreactors after episodes of complete salicylate removal as a result of the poor homogenization inherent to these photobioreactor configurations. Thus, when the photosynthetic O_2 production exceeded the inlet BOD load (due to an excess in irradiance, dissolved CO_2, and nutrients), O_2 concentrations in the cultivation medium rapidly increased as observed by Muñoz et al. [6], which likely caused the accumulation of gas bubbles as proper medium homogenization was not provided. This increase in O_2 concentration (up to 400% saturation values) is likely to cause a severe inhibition of both microalgal and bacterial activity, which might explain the temporary declines observed in the performance of the packed-bed photobioreactors. Zones of biomass decay were also observed in the flat plate bioreactor, which was also linked to homogenization problems. Hence, the observed decrease in process efficiency could be due to temporary reductions in the active biomass fraction (O_2-mediated microbial inhibition or biomass decay) and/or in the real HRT as the formation of gas bubbles reduced the working volume of the reactor.

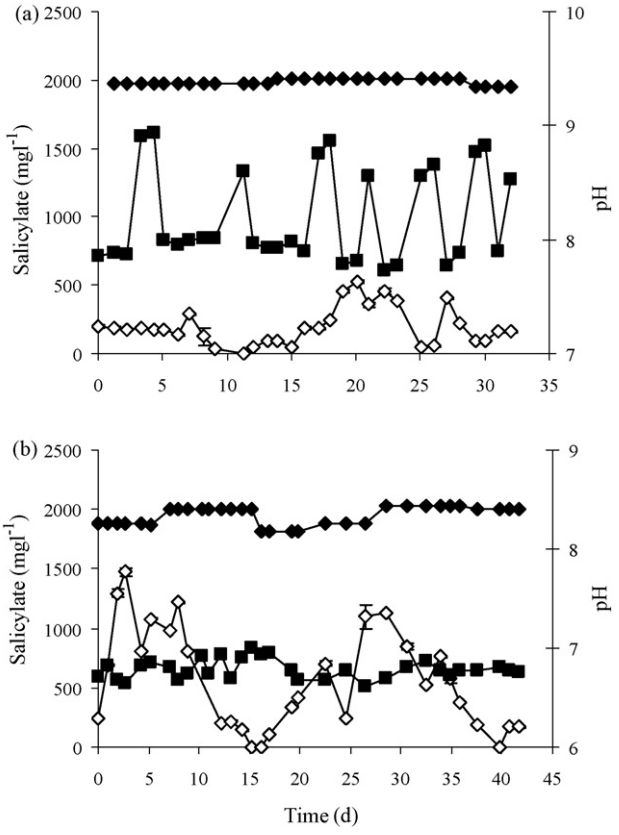

Fig. 2. Time course of salicylate inlet (♦) and effluent (◊) concentration, and pH (■) in the tubular (a) and flat plate (b) photobioreactors with biomass immobilized on Poraver® carriers and operated at 1.6 and 0.6 d HRT, respectively. Vertical bars represent standard deviation from three to six replicates.

When the biomass was attached onto the bioreactor walls or onto the reactor base (alga-turf reactor), the process remained stable (constant RE, pH and EC) and could be operated at 100% RE. The HRT influenced salicylate removal efficiency but a decrease on the HRT was always followed by an increase in the EC, suggesting that O_2 production, and therefore microalgae activity did not limit the process. In fact, pollutant diffusion often limits microbial activity in biofilm systems [11]. The highest performance at 100% RE was recorded in the algal-turf reactor at 1.3 d HRT (136 ± 2 mg BOD l^{-1} h^{-1}, Fig. 3), while the tubular and flat bed photobioreactors exhibited similar performances with maximum EC of 32 ± 0 and 27 ± 1 mg BOD l^{-1} h^{-1} at 100% RE, at 2.3 and 3 d HRT, respectively (Fig. 3). RE decreased in all biofilm reactors when HRT was decreased further below these critical values (corresponding to 100% RE). Maximum EC of 295, 108, and 92 mg BOD l^{-1} h^{-1} were achieved at 76, 34, and 45% RE in the algal-turf, tubular, and flat bed photobioreactors, respectively. However, control experiments performed in dark conditions in the algal-turf reactor showed atmospheric O_2 diffusion provided 55% of oxygenation capacity in this open system. By comparison, salicylate biodegradation completely stopped when both tubular and flat plate reactors were deprived from light, indicating that photosynthetic oxygen exclusively supported salicylate biodegradation. The performance of photosynthetically oxygenated processes is often limited by microalgal activity, and therefore light availability at high biomass concentration [6]. Hence, similar efficiencies in the tubular and flat bed bioreactors were expected since both photobioreactors offered the same illuminated surface to volume ratio, a parameter that determines the volumetric oxygenation capacity in photobioreactors [17].

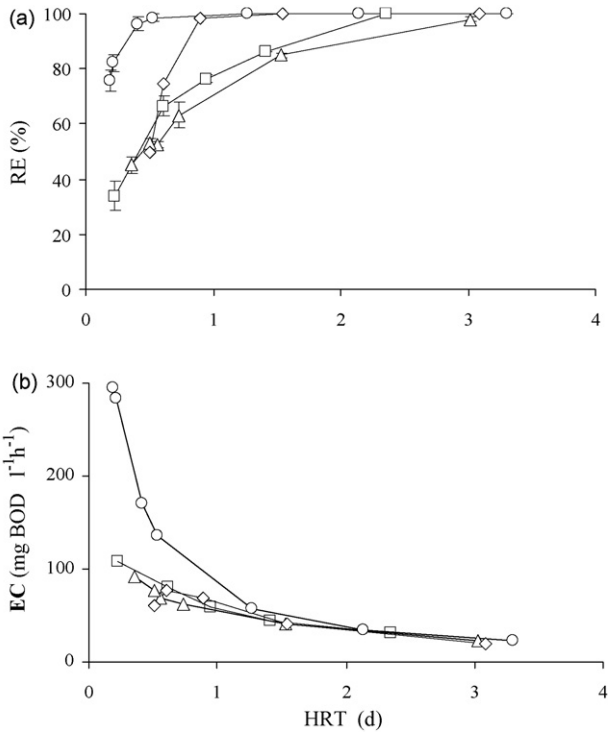

Fig. 3. Influence of the HRT on the salicylate removal efficiency (a) and elimination capacity (b) in an algal-turf reactor (○), a tubular (□) and flat plate (△) reactor with biomass immobilized onto the photobioreactor's wall and a column photobioreactor with suspended biomass (◊). All reactors were operated at 180 μE m^{-2} s^{-1}, and 25 °C. Vertical bars represent the standard deviations from three to six replicates.

The control column reactor achieved a maximum EC of 77 ± 3 mg BOD l^{-1} h^{-1} at 0.6 d HRT and 80% RE. However, despite this lower performance (likely due to the lower surface to volume ratio, 1 *vs* 1.99–1.82) the column photobioreactor exhibited a higher EC at 100% (69 mg BOD l^{-1} h^{-1}) compared to the tubular and flat bed reactors, where the highest removal rates were achieved at lower RE. This is especially important during the treatment of toxic organic pollutants, where process EC should be compared at high RE. Thus, without taking into account potential scaling effects, reactors of approximately 67 and 40 m^3 would be required to remove 150 kg BOD d^{-1} (i.e. 300 m^3 wastewater d^{-1} loaded with 0.5 g BOD l^{-1}) by 99% in suspended (0.9 d HRT) and algal-turf (0.53 d HRT) systems, respectively.

RE dropped by 24% and 25% when the HRT decreased from 0.5 to 0.2 d, and from 0.9 to 0.6 d in the algal-turf and suspended reactor, respectively. By comparison, RE only dropped by 37%, and 26% in the flat and tubular when the HRT decrease from 3 to 0.7 d and from 2.3 to 0.9 d, respectively. In the former photobioreactor configuration, cells were exposed to increasing salicylate concentration, which could have inhibited microalgal metabolism as Muñoz et al. [8] reported that 500 mg salicylate l^{-1} inhibited *C. sorokiniana* photosynthetic O_2 production by approximately 23%. It is however very difficult to distinguish the direct effects derived from decreasing HRT from that of salicylate concentration as the HRT also influences the suspended biomass concentration, and thereby the light utilization efficiency and microbial activity [6]. The sharp drop in RE is however characteristic of systems where microalgae are directly exposed to the toxic inhibitory pollutant [6] because the actively growing microalgae (exposed to light) in the algal-turf and in suspended photobioreactors were directly exposed to salicylate from the reactor broth. As salicylate concentration in the bioreactor increased when the HRT was lowered, the active microalgae were likely inhibited, which reduced the specific O_2 production rates and the overall RE. By comparison, salicylate concentration was likely lower at the reactor wall than in the bulk liquid phase where microalgae were growing actively in the flat and tubular reactors due to the pollutant diffusion and degradation throughout the biofilm. Indeed, the performance of these processes is always intrinsically linked to microalgal activity, which itself is a function of pollutant toxicity. Consequently, the higher the toxicity of the pollutant is, the lower the photosynthetic oxygen production and therefore the lower the performance of the photobioreactor. For instance, phenol is known to exhibit a much higher toxicity towards *C. sorokiniana* growth than salicylate, and therefore much lower efficiencies are expected when treating this compound in algal–bacterial systems [5]. However, the degradation of phenol entirely driven by photosynthetic oxygen supply was still feasible under similar conditions than those herein reported [18].

The effluents produced from all the biofilm photobioreactors remained clear except for the periodical occurrence of large biomass aggregates of detached biofilm (visual observation). By comparison the effluent from the control column photobioreactor was characterized by the presence of freely suspended biomass. This is in agreement with the observations of Meiring and Oellermann [19] who successfully reduced the chlorophyll content of a wastewater effluent from an algal pond using a biotrickling filter fed with the effluent and less than 10% of the raw wastewater in order to render the filter partly heterotrophic and increase the elimination of suspended microalgae. This observation confirmed the ability of biofilm-based bioreactors (operated under heterotrophic conditions) to retain algal–bacterial biomass.

4. Conclusions

The natural ability of microalgae to attach to photobioreactors' walls, which in mass algal production constitutes a severe operational problem, was herein applied to enhance the performance of algal–bacterial biodegradation processes for the treatment of toxic industrial wastewaters. This study indicated that biofilm photobioreactors allow to attain RE and EC as high as the one achieved with suspended cultures, while favoring the production of easily settleable biomass. Biomass retention showed thus a remarkable potential in algal-based processes to maintain an optimum microbial activity and to improve the final quality of the effluent. However, biofilm-based photobioreactors might present two major limitations that could restrict their widespread application: first, photoinhibition in outdoors cultures could occur due to the fact that immobilized microalgae at the reactor surface are constantly exposed to a high photon flux density; and secondly there is a potential risk of clogging due to biomass overgrowth.

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