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Salicylate biodegradation by various algal-bacterial consortia under photosynthetic oxygenation

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Abstract

Four green microalgae (*Chlorella sorokiniana*, *Chlorella vulgaris*, *Scenedesmus obliquus* and *Selenastrum capricornutum*), a wild Bolivian microalga strain and two cyanobacteria (*Anabaena catenula* and *Microcystis aeruginosa*) were compared for tolerance to salicylate, O_2 production capacity and ability to support salicylate degradation by a *Ralstonia basilensis* strain in symbiotic microcosms with the microalgae. *Microcystis aeruginosa* had the highest tolerance to salicylate at 500 mg l^{-1} and 1500 mg l^{-1} but only produced 0.7 mg O_2 l^{-1} h^{-1} in the absence of pollutant. *Chlorella sorokiniana* resisted salicylate at 1500 mg l^{-1} with the highest O_2 production in the absence of salicylate (26 mg l^{-1} h^{-1}) closely followed by the Bolivian microalga (23 mg l^{-1} h^{-1}) and *Chlorella vulgaris* (21 mg l^{-1} h^{-1}). *Selenastrum capricornutum* and *Anabaena catenula* were completely inhibited by salicylate at 500 mg l^{-1}. When inoculated with *Ralstonia* sp. and supplied with salicylate, *Chlorella sorokiniana* had the highest removal rate (19 mg l^{-1} h^{-1}), followed by the wild Bolivian strain (18 mg l^{-1} h^{-1}) and *Chlorella vulgaris* (14 mg l^{-1} h^{-1}).

Introduction

The biodegradation of many toxic and recalcitrant organic contaminants is traditionally carried out under aerobic conditions, which requires intensive aeration and mixing to achieve high degradation rates. Unfortunately, this aeration is costly and can cause the hazardous stripping and dispersion of volatile and even non-volatile toxic compounds (Bell *et al.* 1993, Guieysse *et al.* 2001).

An alternative mode of oxygenation is based on the use of photosynthetic microorganisms (Oswald 1988). When light is present, the microalgae furnish the O_2 required by the aerobic bacteria to mineralize organic pollutants. The CO_2 released by the bacteria is then taken-up by microalgae, mitigating in this way the release of this greenhouse gas in the atmosphere (Guieysse *et al.* 2002). Thus, organic pollutants could be converted into only biomass and O_2. Hence, the use of microalgae as O_2 suppliers potentially offers a safer degradation alternative when volatile organic compounds (VOCs), or phenolic compounds are to be aerobically degraded since no external aeration is needed. However a potential limitation is that microalgae can be inhibited by pollutant toxicity, which could result in process failure (Borde 2001). In addition, the algal growth can also inhibit bacterial activity by releasing toxic metabolites, increasing the temperature and keeping high O_2 levels (Skulberg 2000, Oswald 1988). Yet, algae can also enhance bacterial activity by producing biosurfactants that increase the pollutant bioavailability or by producing extracellular matter that helps in the co-metabolic degradation (Muñoz *et al.* 2003, Wolfaardt *et al.* 1994). Likewise, bacterial growth can either enhance microalgal metabolism by releasing growth-promoting factors (De-Bashan *et al.* 2002) or inhibit it by production of algicidal extracellular metabolites (Baker *et al.* 1978, Dakhama *et al.* 1993). Hence, due to the complex interaction between

algae and bacteria, the selection of the O_2 supplier is fundamental to process design.

Four species of green microalgae, *Chlorella sorokiniana*, *Chlorella vulgaris*, *Scenedesmus obliquus* and *Selenastrum capricornutum*, two cyanobacteria, *Anabaena catenula* and *Microcystis aeruginosa*, and a newly isolated Bolivian microalga were tested in symbiosis with a *Ralstonia basilensis* strain for biodegradation of salicylate (chosen as model contaminant). Emphasis was given to pollutant toxicity, O_2 production and salicylate removal rates.

Materials and methods

Culture conditions and microorganisms

The four green microalgae and the wild Bolivian microalga were cultivated using a mineral salt medium (MSM) previously described by Guieysse *et al.* (2002) and composed of (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. pH was adjusted at 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).

A modification of the BG 11 medium previously described by Rippka *et al.* (1979) was used for cyanobacteria cultivation and was composed of (g l^{-1} in distillated water): $NaNO_3$, 1.5; $K_2HPO_4 \cdot 3H_2O$, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; EDTA, 0.001; Na_2CO_3, 0.02; H_3BO_3, $6.1 \cdot 10^{-5}$; $MnSO_4 \cdot H_2O$, $1.69 \cdot 10^{-4}$; $ZnSO_4 \cdot 7H_2O$, $2.87 \cdot 10^{-4}$; $CuSO_4 \cdot 5H_2O$, $2.5 \cdot 10^{-6}$; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $1.25 \cdot 10^{-5}$.

Chlorella sorokiniana strain 211/8k (Sorokin & Krauss 1958) was from the Culture Center of Algae and Protozoa (Cambridge, UK). *Chlorella vulgaris* strain H 1987 was from the Collection of Algae of Charles University of Prague (Czech Republic). *Scenedesmus obliquus*, *Selenastrum capricornutum* and *Microcystis aeruginosa* strains were from the Ecology Department of Lund University (Sweden). *Anabaena catenula* was from the Culture Collection of Göttingen (Germany). A wild type Bolivian microalga strain from the Pampa Aullagas River (Oruro, Bolivia) was enriched by repeated transfers into MSM supplied with 1.25 g $NaHCO_3$ l^{-1}. Isolation of the strain was performed by cultivating diluted samples of the microalgae in agar plates (using MSM enriched with glucose, peptone and yeast extract at 3.125, 0.0625 and 0.0625 g l^{-1}, respectively).

Ralstonia basilensis (Genbank accession number AY047217, Borde *et al.* 2003) was used for salicylate biodegradation. Inoculum preparation was performed according to Borde *et al.* (2003).

All tests were performed in duplicate and incubated at 26 °C in a rotary shaker (150 rpm) under continuous illumination (Philips TLD 36 W/840 fluorescent lamps) at 7000 lux.

Salicylate inhibition test

Salicylate inhibition tests were performed in 155 ml glass flasks closed with butyl septa and sealed with aluminum caps to avoid any gas exchange with the surrounding atmosphere. The flasks were filled with 50 ml MSM and sodium salicylate was supplied at either 500 or 1500 mg l^{-1}. Control tests were run under similar conditions but were not supplied with salicylate. Two ml green microalgae culture (or the Bolivian strain) was inoculated into the systems. To remove most of the atmospheric O_2 and provide CO_2 for algal autotrophic growth, the headspace of the flasks was filled with N_2/CO_2 (70/30 v/v), except for *Selenastrum capricornutum* for which N_2/CO_2 (80/20 v/v) was used. For *Microcystis aeruginosa*, the flasks were filled with BG 11 medium enriched with 300 mg $NaHCO_3$ l^{-1} and 5 ml of pure culture was then added as inoculum to reach a total liquid volume of 100 ml. An inert N_2 gas phase was used in this particular test. The levels of O_2 and CO_2 in the flask headspaces were recorded by GC analysis. Liquid samples of 0.5 ml were withdrawn to monitor microbial growth.

Anabaena catenula inhibition tests were performed in 250 ml conical flasks closed with cotton plugs. The flasks were supplied with 195 ml of BG 11 medium and 5 ml of pure culture of the cyanobacteria. Sodium salicylate was supplied at the concentrations referred to above. Microbial growth was monitored by periodically measuring the dry weight of the cultures because this cyanobacterium tended to form large and heterogeneous flocs, not allowing the withdrawal of heterogeneous samples.

Degradation tests

Salicylate degradation tests were performed in 155 ml glass flasks. Sodium salicylate was added from a stock solution to reach an initial concentration of 800 mg l^{-1} in the flasks. The flasks were then filled with MSM and inoculated with *Ralstonia basilensis* (1% v/v) and the microalgae to reach an initial OD_{550} of 0.05 in all the flasks. Degradation tests with *Microcystis aeruginosa* (performed in BG 11 medium) were inoculated with *Ralstonia basilensis* (1% v/v) and 5 ml cyanobacteria.

The final volume was 125 ml in all the flasks and the flask headspaces were filled with an inert N_2 atmosphere to remove the atmospheric O_2. The flasks were finally sealed with rubber septa. New amendments to reach the same initial concentration were performed when salicylate was completely degraded. After each amendment the flasks were refilled with fresh MSM to restore the initial liquid volume (125 ml). Liquid samples of 1.5 ml were periodically withdrawn to monitor microbial growth, salicylate concentration, and nutrient uptake (PO_4^- and NO_3^-). The gas composition in the flasks headspaces was also daily recorded.

Influence of photosynthetic extracellular metabolites on Ralstonia basilensis *growth*

Each photosynthetic microorganism was autotrophically grown without adding sodium salicylate as described above for the inhibition control tests. Then, 25 ml culture broth was removed, filtered through a 0.2 μm cellulose acetate filter to remove the microalgae and transferred into new 155 ml cultivation flasks. These new flasks were then amended with fresh sodium salicylate (800 mg l^{-1}) and inoculated with *Ralstonia basilensis* to give 53 mg dry wt l^{-1}. The flasks were finally sealed with butyl septa, allowing the diffusion of the atmospheric O_2 contained in the headspace to support salicylate biodegradation. The bacterial activity was followed by measuring the salicylate degradation and the gas phase composition. Controls were made using fresh medium (MSM or BG 11 medium depending on the photosynthetic microorganism tested) instead of photosynthetic broth.

Influence of bacterial extracellular metabolites on microalgal or cyanobacterial growth

Ralstonia basilensis was grown in conical shake-flasks on sodium salicylate (800 mg l^{-1}) for 48 h (to ensure complete salicylate degradation) in MSM or BG 11 medium. Then, 50 ml liquid culture was withdrawn, filtered through a 0.2 μm cellulose acetate filter and transferred into 155 ml cultivation flasks. The flasks were then inoculated with the corresponding microalga or cyanobacterium and cultivated autotrophically as described above. Controls were made using fresh medium (MSM or BG 11 medium depending on the photosynthetic microorganism tested) instead of the *Ralstonia basilensis* broth. The levels of O_2 and CO_2 in the flask headspaces were monitored by GC analysis.

Analytical procedures

Optical density at 550 nm was used as indicator for microbial growth except for the tests performed with *Anabaena catenula,* where dry weight was determined instead.

Dry weight of the cultures of *Anabaena catenula* was monitored by withdrawing and filtering 5 ml samples through 0.2 μm pre-dried and pre-weighed membrane filters. The filters were weighed again after 24 h of drying at 105 °C.

For salicylate analysis, 1 ml samples were centrifuged for 10 min at 13 800 $\times$ g. Portions of supernatant were then transferred to HPLC vials for analysis. Samples were eluted isocratically using a mobile phase composed of methanol, water and acetic acid (60/39/1, by vol.) at 0.5 ml min^{-1}. Detection was performed at 280 nm. External standards were used to enable quantitative determination and the detection limit achieved was less than 1 mg l^{-1}.

Gas phase composition was analyzed by gas chromatography using a HayeSep Q 80–100 mesh column and a thermal conductivity detector. The column was at 70 °C, whilst the injector and detector were maintained at 110 °C and 150 °C, respectively. Helium was used as the carrier gas at 10 ml min^{-1}.

Nitrate and orthophosphate concentrations were analyzed colorimetrically using a FIAStar 5000 analyzer (FOSS TECATOR AB).

Results and discussion

In the following, all the rates were calculated from the slope of the salicylate/O_2/CO_2/NO_3^- concentration versus time curves. The rates are given as the average of duplicates with the corresponding 95% confidence interval calculated with at least 3 points.

Table 1. Maximum O_2 production and CO_2 removal rates for the tested photosynthetic microorganisms in the presence of salicylate.

Microorganisms	Salicylate (mg l^{-1})	r_{O2} (mg l^{-1} h^{-1})	r_{O2} (%)	$-r_{CO2}$ (mg l^{-1} h^{-1})	$r_{O2}/-r_{CO2}$
C. vulgaris[a]	0	20.9 ± 0.6	100	12.4 ± 0	1.6 ± 0
	500	15.5 ± 0.8	74	9.6 ± 0	1.6 ± 0
	1500	0	0	0	–
C. sorokiniana[a]	0	26 ± 3.7	100	15 ± 3.6	1.7 ± 0.3
	500	19.9 ± 0.8	77	11.3 ± 3.2	1.8 ± 0.3
	1500	13.9 ± 2.8	53	9.3 ± 1.7	1.5 ± 0.3
Sc. obliquus[a]	0	15.9 ± 1.9	100	15 ± 3.6	1.4 ± 0.2
	500	7.7 ± 0.3	48	5.9 ± 0.4	1.3 ± 0.1
	1500	0	0	0	–
Bolivian microalgae[a]	0	22.8 ± 1.6	100	15.5 ± 0.6	1.5 ± 0.1
	500	19.3 ± 0.9	85	13.2 ± 1.1	1.5 ± 0.1
	1500	0	0	0	–
S. capricornutum[a]	0	18.9 ± 2.3	100	10.8 ± 1.4	1.5 ± 0.1
	500	0	0	0	–
M. aeruginosa[b]	0	0.7 ± 0.1	78	–	–
	500	0.9 ± 0	100	–	–
	1500	0.7 ± 0	78	–	–

[a]Microalgae were grown on MSM under a N_2/CO_2 atmosphere.
[b]Cyanobacteria grown on BG 11 medium enriched with 300 mg $NaHCO_3$ l^{-1}.

Salicylate inhibition test

Chlorella sorokiniana showed the highest O_2 production rate in the absence of salicylate, with a maximum rate of 26 ± 3.7 mg O_2 l^{-1} h^{-1} (Table 1). *Chlorella vulgaris*, *Selenastrum capricornutum* and the Bolivian microalga produced O_2 at similar rates (20 mg O_2 l^{-1} h^{-1} approx.). *Scenedesmus obliquus* showed a maximum O_2 production rate 39% lower than that of *Chlorella sorokiniana* (15.9 ± 1.9 mg O_2 l^{-1} h^{-1}) and *Microcystis aeruginosa* production rates were not higher than 1 mg l^{-1} h^{-1}, which confirms the importance of the microalgae selection in the O_2 supply design. Hence, when salicylate is not present, the microalgae tested can be classified according to their O_2 producing capacity as follows: *Chlorella sorokiniana* > Bolivian microalgae ≈ *Chlorella vulgaris* ≈ *Selenastrum capricornutum* > *Scenedesmus obliquus* > *Microcystis aeruginosa*. [O_2 production rates for *Microcystis aeruginosa* were obtained using $NaHCO_3$ as carbon source since high concentrations of CO_2 (given by atmospheres of CO_2/N_2 20/80 v/v) inhibited its growth.] *Anabaena catenula* could not be cultivated either under N_2/CO_2 atmosphere or with enriched BG 11 medium.

O_2 production by the cyanobacterium, *Microcystis aeruginosa*, was not inhibited by salicylate (EC_{50} > 1500 mg l^{-1}, where EC_{50} is defined as the concentration of salicylate that inhibits O_2 production by 50%). *Chlorella vulgaris*, *Chlorella sorokiniana* and the Bolivian microalga showed a similar tolerance towards the pollutant, when present at 500 mg l^{-1}. EC_{50} values of approx. 500 and 1500 mg l^{-10} were observed for *Scenedesmus obliquus* and *Chlorella sorokiniana*, respectively. *Selenastrum capricornutum* and *Anabaena catenula* were completely inhibited in the presence of 500 mg salicylate l^{-1} and, consequently, were unsuitable for further work.

Salicylate toxicity varies greatly among species: in the presence of 500 mg salicylate l^{-1}, *Chlorella sorokiniana* and the wild type strain gave the highest O_2 production rates and had the highest oxygenation capacity in the presence of 1500 mg pollutant l^{-1}. Algal selection is, thus, important under continuous treatment since a large increase in the inlet pollutant concentration could dramatically decrease the photosynthetic activity and cause a process failure.

A $O_{2production}/CO_{2removal}$ final ratio of approx. 1.6 was found for all the tested green microalgae. This ratio was independent from salicylate concentration, showing that inhibition did not affect the

Table 2. Maximum salicylate degradation and N-NO_3 uptake rates in the biodegradation tests with the different algal-bacterial microcosms tested.

Algae species	*Chlorella sorokiniana*	*Chlorella vulgaris*	*Scenedesmus obliquus*	*Microcystis aeruginosa*[a]	Bolivian strain
Salicylate removal ($mg\ l^{-1}\ h^{-1}$)	18.5 ± 2.4	14.1 ± 0.6	5.2 ± 0.2	1.3 ± 0.3	17.5 ± 1.2
NO_3-N uptake ($mg\ l^{-1}\ h^{-1}$)	0.78 ± 0.1	0.82 ± 0.03	0.41 ± 0.03	0.08 ± 0.05	0.87 ± 0.12
NO_3-N/salicylate ($g\ g^{-1}$)	0.04 ± 0.01	0.06 ± 0	0.08 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

[a]BG 11 medium was used for salicylate biodegradation.

Table 3. Influence of photosynthetic extracellular metabolites on *Ralstonia basilensis* growth.

r ($mg\ l^{-1}\ h^{-1}$)	*Chlorella sorokiniana*	*Chlorella vulgaris*	Bolivian strain	*Sc. obliquus*	Control (MSM)	*Microcystis aeruginosa*[a]	Control BG 11
$-\mathbf{r}_{O2}$	66 ± 4	75 ± 11	69 ± 6	63 ± 7	70 ± 9	64 ± 15	71 ± 5
$\mathbf{r}_{CO2}$	74 ± 8	69 ± 1	73 ± 5	65 ± 8	74 ± 9	55 ± 4	54 ± 6
$-\mathbf{r}_s$	108 ± 3	102 ± 13	106 ± 17	103 ± 4	100 ± 10	104 ± 4	110 ± 10

[a]BG 11 medium enriched with 300 mg $NaHCO_3\ l^{-1}$ was used for salicylate biodegradation and cyanobacterial cultivation.
$-\mathbf{r}_{O2}$ – Maximum O_2 depletion rate.
$\mathbf{r}_{CO2}$ – Maximum CO_2 production rate.
$-\mathbf{r}_s$ – Maximum salicylate removal rate.

stoichiometry of the process. For *Microcystis aeruginosa* tests, the experimental conditions made it impossible to determine this ratio. Microalgae growth always paralleled O_2 production throughout the entire experimentation.

Salicylate was not significantly used as carbon source by the green microalgae or the cyanobacteria since this would have led to higher growth (data not shown).

Degradation tests

Salicylate removal was totally supported by photosynthetic oxygenation, since salicylate was neither photodegraded nor assimilated by the microalgae and since the *Ralstonia basilensis* strain is not able to use salicylate in the absence of O_2 (Guieysse *et al.* 2002). Thus, *Chlorella sorokiniana* and the Bolivian strain supported the highest degradation rates (18.5 ± 2.4 and 17.5 ± 1.2 $mg\ l^{-1}\ h^{-1}$ respectively), followed by *Chlorella vulgaris* (14.1 ± 0.6 $mg\ l^{-1}\ h^{-1}$), *Scenedesmus obliquus* (5.2 ± 0.2 $mg\ l^{-1}\ h^{-1}$) and *Microcystis aeruginosa* (1.3 ± 0.3 $mg\ l^{-1}\ h^{-1}$) (Table 2). These results are in agreement with the inhibition tests since the microalgae with the highest O_2 production rates also supported the fastest degradation.

When *Microcystis aeruginosa* was used as O_2 supplier, salicylate removal stopped after 260 h and 44% of the initial salicylate remained in the system (Figure 1a). This was due to a lack of phosphate (data not shown) because the BG 11 medium only contains 0.031 g $K_2HPO_4\ l^{-1}$. This limitation was described by Aiba *et al.* (1982) who reported a dramatic decrease in the photosynthetic activity of *Chlorella ellipsoidea* in phosphorate-limited environments.

Microbial growth was well correlated with salicylate disappearance for all the microalgae tested (Figure 1b). However absorbance is not a robust parameter because it strongly depends on the pigment content (Martinez *et al.* 1995) and the metabolites excreted. Consequently no solid statement can be made based solely on these measurements.

For all the microalgae tested, the CO_2 concentration progressively increased whilst O_2 remained at a low concentration during salicylate biodegradation. When the pollutant was completely depleted, O_2 increased rapidly as previously described (Guieysse *et al.* 2002), confirming that dissolved O_2 measurements could be used to monitor the biodegradation processes.

Nitrate uptake correlated and was proportional to salicylate removal with all the microalgae tested. $NO_3\text{-}N_{consumption}$/$salicylate_{consumption}$ ratios ranging

Table 4. Influence of bacterial extracellular metabolites on photosynthetic organisms growth.

$\mathbf{r}_{O2}$ (mg $l^{-1}h^{-1}$)	*Chlorella sorokiniana*	*Chlorella vulgaris*	Bolivian strain	*Scenedesmus obliquus*	*Microcystis aeruginosa*[a]
Control	23 ± 1	22 ± 2	21 ± 1	16 ± 2	0.5 ± 0.1
Bacterial broth	22 ± 1	21 ± 1	21 ± 2	17 ± 1	0.6 ± 0.2

[a]BG 11 medium enriched with 300 mg $NaHCO_3$ l^{-1} was used for salicylate biodegradation and microalgae cultivation.
$\mathbf{r}_{O2}$ – Maximum O_2 production rate.

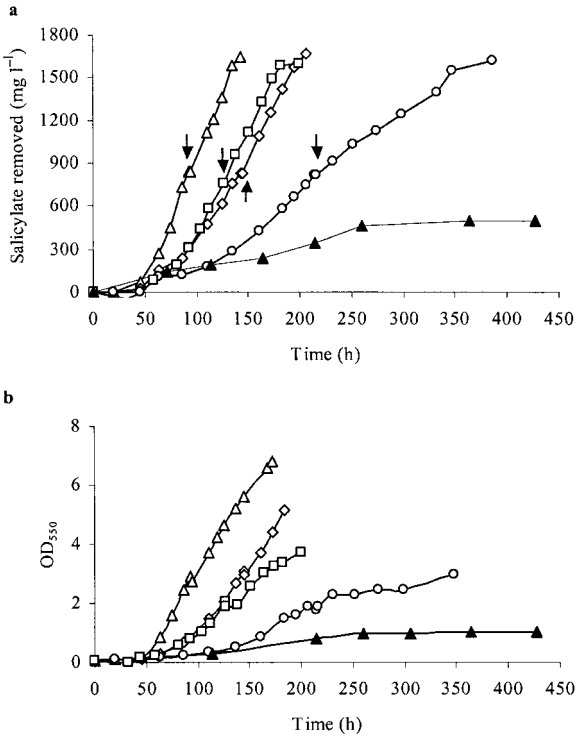

Fig. 1. Time course of salicylate biodegradation (a) and microbial growth (b) in the biodegradation tests supplied with 800 mg l^{-1} of sodium salicylate and inoculated with *Ralstonia basilensis* and (△) *Chlorella sorokiniana*, (◊) *Chlorella vulgaris*, (□) Bolivian microalga, (○) *Scenedesmus obliquus*, (▲) *Microcystis aeruginosa*. Salicylate was amended at time 0 h for all the tests and at 94 h, 127 h, 144.5 h, 226 h, for *Chlorella sorokiniana*, Bolivian microalga, *Chlorella vulgaris and Scenedesmus obliquus*, respectively. Arrows show the time when sodium salicylate was amended again to 800 mg l^{-1} into the systems.

from 0.04 to 0.08 were found for the microalgae tested meaning that *N*-removal capacity greatly varies among species (Table 2). Except for *Microcystis aeruginosa*, the percentage of phosphate taken up was always lower than 16% of the initial phosphate concentration.

Influence of photosynthetic extracellular metabolites on Ralstonia *growth*

Microalgae growth can inhibit bacterial activity by increasing the pH or temperature of the cultivation broth or by excreting toxic metabolites (Oswald 1988, Skulberg 2002). On the other hand, beneficial effects of microalgae metabolism on bacterial activity are also present in nature (Woolfaardt *et al.* 1994). None of these effects were found in the present study, as follows from the maximum O_2 and salicylate depletion rates (Table 3).

Influence of bacterial extracellular metabolites on microalgal or cyanobacterial growth

Bacteria can be either detrimental (Baker *et al.* 1978, Dakhama *et al.* 1993) or beneficial (Mouget *et al.* 1995, Haines *et al.* 1974, De-Bashan *et al.* 2002) to microalgae. In the present study, none of these effects were observed (Table 4).

Conclusions

Hazardous volatilization of toxic compounds, such as VOCs or phenolic compounds, is likely to occur when intensive bubbling or mechanical aeration is required to support the biodegradation process. Photosynthetic oxygenation could overcome this problem since O_2 is produced and consumed '*in-situ*' without any need of external O_2 transfer.

Under the experimental conditions tested, *Chlorella sorokiniana* was the most suitable microalgae both in terms of O_2 production rate and salicylate tolerance followed by the Bolivian microalga and *Chlorella vulgaris*. Although *Microcystis aeruginosa* had the highest tolerance to salicylate, it had only very low degradation rates, which confirms that a proper balance between oxygenation capacity and pollutant tolerance

should be considered in the design process. *Scenedesmus obliquus* has the highest nitrate uptake per salicylate degraded (0.08 g g^{-1}). No significant enhancement or inhibition effects due to extracellular metabolites on the microalgae activity by the bacterium tested or vice versa were observed in the symbiotic systems. Microalgae selection determines the process efficiency (in terms of removal rates) and stability towards the process variables (pollutant load, temperature). A high specific O_2 production rate, high CO_2 tolerance (since CO_2 has been shown to accumulate throughout biodegradation process) and high tolerance towards the target pollutant are crucial. In addition, the algae should not release toxic compounds or inhibit bacterial activity and vice versa.

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