Research Article

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Phenol and nitrogen removal in microalgal–bacterial granular sequential batch reactors

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Abstract

BACKGROUND: Microalgal–bacterial systems work on the principle of the symbiotic relationship between algae and bacteria. The ability of algal–bacterial photobioreactors for the treatment of wastewater containing ammonia and phenol has been poorly addressed. In this work a self-sustaining synergetic microalgal–bacterial granular sludge process was thus developed to treatment of industrial wastewater based upon the low cost of photosynthetic oxygenation and the simultaneous phenol and nitrogen removal. The performance of a conventional sequential batch reactor (SBR) based on aerobic bacterial communities (SBR_B) and a microalgal–bacterial granular SBR (SBR_{MB}) were comparatively assessed. The major challenges associated with microalgal–bacterial systems were discussed.

RESULTS: A complete removal of phenol (100 mg L^{-1}) was achieved in both reactors. The reactors SBR_B and SBR_{MB} showed similar performance in term of removal of inorganic nitrogen. Nitrogen mass balances estimated nitrogen assimilation, nitrification and denitrification. Higher simultaneous nitrification and denitrification (70% SND) occurred in SBR_B as determined by mass balances. The higher nitrogen assimilation (17.9%) by the microalgal–bacterial biomass compensated the lower denitrifying activity in SBR_{MB} (54% SND), resulting in a removal of inorganic nitrogen (61%) similar to that obtained in SBR_B (66%). N_2O was not detected in the headspace of any system.

CONCLUSION: Granular microalgal–bacterial consortia implemented in SBR constitute an efficient method for industrial wastewater treatment achieving complete removal of ammonia and phenol. The application of SBR_{MB} would be more cost effective than SBR_B mainly due to the significant energy savings in SBR_{MB} resulting in a sustainable system that contributes to the circular bioeconomy.

Keywords: bacterial granules; *Chlorella sorokiniana*; nitrogen; phenol; photobioreactor; simultaneous nitrification and denitrification

INTRODUCTION

Wastewater from chemical industries commonly contains high concentrations of ammonia, sulfur, phenols and other hydrocarbons.[1,2] The uncontrolled discharge of such hazardous contaminants can cause adverse effects on natural ecosystems.[3] The implementation of pollutant emission standards in the oil refining industry in 2017 has brought a renewed attention to phenol and nitrogen removal.[4]

Phenol has been identified as a priority contaminant by United States Environmental Protection Agency (USEPA). USEPA has set a water quality concentration limit for phenol <1 ppb in surface water,[5] while toxic concentrations range between 9 and 25 mg L^{-1} for humans and aquatic life.[6] In addition, exposure to nitrate, nitrite and ammonia above the maximum allowed limits are able to cause multiple disorders in humans and natural ecosystems. Thus, nitrogen discharge limits of 30 mg L^{-1} for ammonia and 10 mg L^{-1} for the sum of nitrite and nitrate concentrations are required by the USEPA and Health Canada.[7]

Multiple techniques are nowadays commercially available for the removal of nitrogenous and phenolic compounds from chemical industry wastewater, which include chemical, physical as well as biological methods.[8] Biological treatment methods exhibit

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lower operating costs, are more environmentally friendly, and can support a complete mineralization of pollutants (such as the conversion of phenol to CO_2 and H_2O, and of nitrogenous compounds to N_2) compared to their physical/chemical counterparts.[9] Traditionally, aerobic bioprocesses are preferred over their anaerobic counterparts based on their higher biodegradation kinetics and tolerance to pollutant effects.[9] However, phenolic contaminants are inhibitors of nitrifying microorganisms (even at low concentrations).[10] Phenolic compounds such as phenol have potential inhibitory or toxic effects over conventional biological treatment systems, specifically over the nitrification process. Nitrification does not typically occur at phenol concentrations above 5.0 mg L^{-1} for pure culture of nitrifying bacteria or activated sludge.[2] Therefore, the use of two or three stages to separate the biological processes of phenol removal, nitrification and anaerobic denitrification has been proposed.[9,10] However, multistage process configurations for biological treatment of toxic wastewaters involve large facilities[2] and higher operational costs. For instance, methanol as external carbon source for denitrification was added in a three-stage process for coke wastewater treatment.[11] In pre-denitrification activated sludge systems for the treatment of toxic effluents, instability or failure of the nitrification process, probably caused by the presence of toxic compounds (phenols, cyanides), is commonly reported.[12] In this context, the addition of activated carbon allows the reduction of phenol inhibition on nitrification at the expense of increased treatment costs.[12]

Simultaneous removal of nitrogen and phenolic compounds would reduce costs and areal footprint.[13] Several studies have been conducted to remove phenol and ammonia simultaneously using a single system. Nitrification could be achieved in artificial wastewater containing both phenol and ammonia when phenol was completely removed or its concentration reduced to a level non-inhibitory to nitrification.

Inhibition caused by high-strength phenolic wastewaters can be overcome by cell immobilization.[2] A gel-immobilized and phenol-acclimated bacterial consortium showed nitrification at phenol concentrations above the inhibitory levels.[2] Sequential batch reactors (SBRs) based on granulated aerobic biomass have emerged as a promising technology to effectively treat chemical wastewater in a single-stage process.[14]

Aerobic granules possess a higher tolerance to dangerous pollutants, broader pollutant degradation portfolio and higher settling rates than conventional activated sludge flocs.[15-18] The presence of different redox conditions inside the granules allows simultaneous nitrification–denitrification (SND) in granular SBRs.[16] Therefore, this technology represents a promising alternative to conventional methods for the simultaneous removal of phenolic and nitrogenous compounds from chemical industries wastewater.[18] Unfortunately, bacterial granular SBRs require a high energy demand for oxygen supply and mixing, and generate a continuous stream of CO_2, and also aerosol and phenol volatilization.[19,20] Aeration represents 35–50% of the total energy consumption in conventional wastewater treatment plants.[21]

Microalgal–bacterial systems can overcome the above-mentioned limitations of granular bacterial SBRs. Indeed, algal–bacterial consortia represent an ecological and sustainable alternative for wastewater treatment, with an outstanding potential to remove nutrients and phenolic compounds at a low oxygenation cost and in the absence of hazardous pollutant stripping.[22] In addition, the biomass produced during wastewater treatment can serve as feedstock for the generation of biofuels and third generation of biofertilizers.[22,23] In algal–bacterial photobioreactors, microalgae produce O_2, which can be used by microorganisms to degrade inorganic and organic pollutants, and assimilate the CO_2 released during mineralization of organic pollutants by bacterial activity. In general, this synergistic association is typical of natural ecosystems and constitutes the basis for organic matter degradation in aerobic stabilization ponds, simultaneously allowing a very cost-effective aeration and CO_2 consumption.[24]

Algal–bacterial systems are efficient for the treatment of toxic compounds such as phenol.[25] In addition, the potential of an anoxic–aerobic algal–bacterial system, based on an enclosed anoxic bioreactor followed by an open photobioreactor and a settler, to carry out nitrification–denitrification has been recently demonstrated.[26] However, difficulty of separation of the biomass, the high land requirement of open photobioreactors or the high construction costs of enclosed systems limit the application of this photosynthetic platform.[25] Algal–bacterial granular sludge is a new low-carbon technology, with merits in municipal wastewater treatment, utilizing typically photobioreactors operated in SBR mode.[27,28]

However, despite the potential of granular algal–bacterial SBRs, the use and application of this innovative photobioreactor configuration in the treatment of chemical industry wastewater containing ammonia and phenol have been poorly addressed to date.[29]

Accordingly, this work aims to compare the efficiency of a conventional bacterial granular SBR and an algal–bacterial granular SBR for the simultaneous removal of phenol and inorganic nitrogen from synthetic chemical industry wastewater. In the present work, the contribution of the different biotic and abiotic processes for nitrogen removal was quantified using mass balances.

Table 1. Composition of synthetic wastewater

Composition	SWW_1	SWW_2
$(NH_4)_2SO_4$ (g L^{-1})	0.6060	0.6060
KH_2PO_4 (g L^{-1})	0.0489	0.0489
K_2HPO_4 (g L^{-1})	0.0395	0.0395
$C_2H_3NaO_2$ (g L^{-1})	1.566	0.944
C_6H_5OH (g L^{-1})	0.000	0.200
Trace element solutions (mL L^{-1})	1	1
COD:N	100:15	100:15

MATERIALS AND METHODS

Microorganisms

Chlorella sorokiniana strain 211/8k was obtained from the Culture Centre of Algae and Protozoa (Cambridge, UK). Inocula were prepared according to Borde *et al.*[30] Aerobic granules were taken from a lab-scale SBR fed with a synthetic wastewater containing sodium acetate and ammonia.[31]

Wastewater

Synthetic wastewater 1 (SWW_1) contained (g L^{-1}): $(NH_4)_2SO_4$ (0.6060), KH_2PO_4 (0.0489), K_2HPO_4 (0.0395) and $C_2H_3NaO_2$ (1.566). SWW_2 contained (g L^{-1}): $(NH_4)_2SO_4$ (0.6060), K_2PO_4 (0.0489), K_2HPO_4 (0.0395), $C_2H_3NaO_2$ (0.944) and C_6H_5OH (0.2). Both SWW_1 and SWW_2, with the same chemical oxygen demand (COD):N ratio of 100:15, were supplemented with two trace element solutions, 1 mL of each solution per 1 L of synthetic wastewater[31] (Table 1).

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Experimental set-up

Two different SBR configurations were used for comparative purposes. An SBR that operated only with bacterial granules (henceforth referred to as SBR_B), and an SBR containing microalgal–bacterial granules (henceforth referred to as SBR_{MB}) (Fig. 1). Both SBRs consisted of bubble acrylic columns with an internal diameter of 9.5 cm, height/diameter ratio of 3.5, and total and working volumes of 2.5 and 2 L, respectively. SBR_B had three stone diffusers at the bottom of the reactor to generate an upward culture broth flow favorable to granulation. A superficial upflow air velocity of 0.9 cm s^{-1} (corresponding to 2.4 L min^{-1}) was set. On the other hand, SBR_{MB} was constantly stirred at 200 rpm via magnetic agitation. The microalgal–bacterial system was constantly illuminated using light-emitting diode (LED) lamps at a photosynthetic active radiation of 200 µmol m^{-2} s^{-1} at the external reactor walls. Both SBR systems were operated at 25 °C and constant pH of 7.5 ± 0.1 with H_2SO_4 (1 mol L^{-1}) and NaOH (0.5 mol L^{-1}) using an automatic pH control system (EVopH-P5, BSV Electronic SL, Barcelona, Spain).

SBR operation

An abiotic test to assess the stripping of NH_3-N in the mechanically aerated SBR was performed. The stripping experiment was carried out with 2 L SWW_2 at pH 7 and an aeration rate of 0.9 cm s^{-1}. The abiotic SBR was operated in the absence of biomass and sampled at 0, 2, 4, 6 and 24 h to measure NH_3 concentration.

SBR_B was inoculated with 1000 mg total suspended solid (TSS) L^{-1} of granular sludge stored at 4 °C. On the other hand, SBR_{MB} was inoculated with a mixture of *C. sorokiniana* (0.2 g L^{-1}) and granular sludge (1.8 g TSS L^{-1}) obtained from SBR_B under steady state. Both SBRs were operated with volumetric organic loading rate (OLR), ammonia nitrogen loading rate (NLR) and phosphorous loading rate (PLR) of 600 mg COD (L d)$^{-1}$, 90 mg N (L d)$^{-1}$ and 12 mg P (L d)$^{-1}$, respectively (COD:N:P ratio = 100:15:2). Both SBR_B and SBR_{MB} were operated with consecutive cycles (24 h): feeding period (1 min), aerobic period (1432 min), settling phase (5 min), supernatant extraction (1 min) and idle time (1 min).[32] In both SBRs, the cellular retention time (CRT) and hydraulic retention time (HRT) were set at 20 and 2 days, respectively. SBR_B was operated for 180 days until steady state was achieved, while SBR_{MB} was operated for 40 days.

The reactors were operated with alternating phases of organic carbon availability (feast period) and starvation (famine period), known as feast/famine regime. The feast phase extended from the start of feeding to the first hours of the operating cycle, with consumption of more than 80% of the carbon and energy source. The famine phase lasted from when the feast phase had finished to the end of the cycle (24 h). During the feast period all nutrients were available. Organic carbon was consumed (>80%) in both studies for the bacteria and the microalgae in this period. On the other hand, during the famine period, the main component available was ammonia and remaining organic carbon was completely removed.

The performance of the SBRs was evaluated by periodically measuring the concentrations of ammonia nitrogen (NH_3-N), nitrate (NO_3^--N), nitrite (NO_2^--N), total soluble nitrogen (TN), nitrous oxide in the reactor headspace (N_2O), total solids (TS), TSS, phenol (C_6H_6O) and soluble chemical oxygen demand (COD_S). Settling properties and granule size of the biomass were periodically determined.

Nitrogen mass balance

A nitrogen mass balance was performed in order to estimate nitrogen assimilation, nitrification, as well as denitrification. The biomass composition of the bacterial and microalgal–bacterial granules was estimated using the elemental formula of bacterial ($CH_{1.8}O_{0.5}N_{0.2}$) and microalgal ($CH_{1.7}O_{0.4}N_{0.15}P_{0.0094}$) biomass.[33,34]

N_2 (N_G, mg N L^{-1}) produced by denitrification and stripping was estimated according to Eqn (1),[35] as follows:

$$N_G = \Delta(NH_3\text{–}N) - \Delta(NO_X\text{–}N) - \Delta(NH_3\text{–}N)_X \quad (1)$$

where $\Delta(NH_3\text{-}N)$ is the concentration of ammonia nitrogen removed (mg N L^{-1}), $\Delta(NO_X\text{-}N)$ is the oxidized nitrogen (NO_3^- and NO_2^-) concentration accumulated via nitrification in the culture broth (mg N L^{-1}), and $\Delta(NH_3\text{-}N)_X$ involves the ammonia nitrogen concentration utilized for the growth of bacteria and microalgae during an operational cycle. Thus, $\Delta(NH_3\text{-}N)_X$ was estimated, at steady state, according to Bucci *et al.*,[35] as follows:

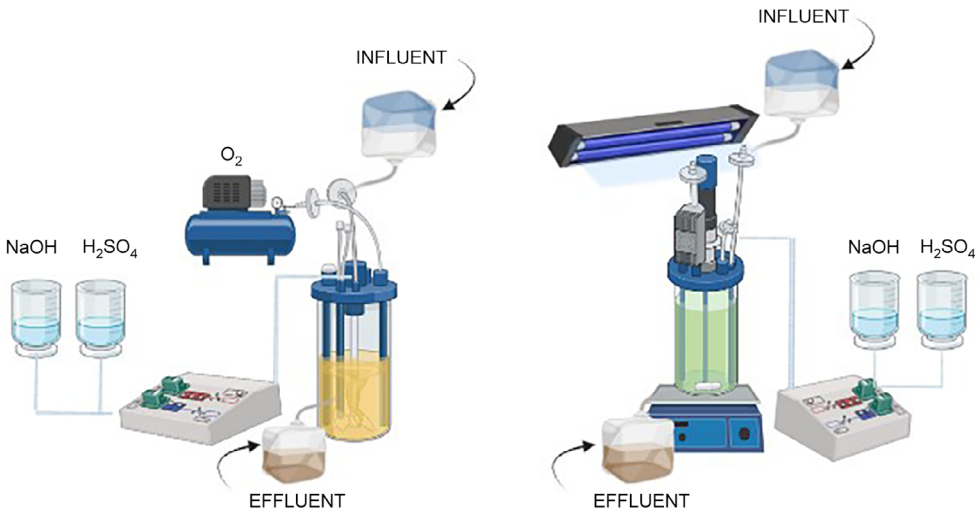

Figure 1. (a) Scheme and photograph of the bacterial granular SBR (left) and the microalgal–bacterial granular SBR (right).

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$$\Delta(NH_3{-}N)_X = \left(\frac{1}{a} y_{N/X}\, bX_A PV\right)/V \quad (2)$$

where X_A is the concentration of active biomass (mg X_A L^{-1}), $y_{N/X}$ is the stoichiometric coefficient that relates the nitrogen and carbon contents in the biomass (0.2 mmol N C mmol X_A^{-1}), *a* corresponds to 24.6 mg X_A C $mmol^{-1}$ assuming the elemental formula of biomass ($CH_{1.8}O_{0.5}N_{0.2}$), *b* corresponds to the atomic mass of nitrogen (14 mg N mmol N^{-1}), PV is the volume of biomass wasted (0.1 L) per operational cycle of the SBR and *V* is the working volume (L) of each reactor. In addition, $\Delta(NH_3\text{-}N)$ is the sum of $\Delta(NH_3\text{-}N)_X$ and ammonia nitrogen consumed by the nitrification process ($\Delta(NH_3\text{-}N)_N$). Therefore, $\Delta(NH_3\text{-}N)_N$ was estimated as shown in Eqn 3:

$$\Delta(NH_3{-}N)_N = \Delta(NH_3{-}N) - \Delta(NH_3{-}N)_X \quad (3)$$

Nitrogen removal by the denitrification process (%SND) was estimated as follows:

$$\%SND = \left(\frac{\Delta(NH_3{-}N)_N - \Delta(NO_X{-}N)}{\Delta(NH_3{-}N)_N}\right) 100 \quad (4)$$

Overall, a generic microalgal–bacterial population preferentially consumes nitrogen as ammonia (NH_3) under aerobic conditions during carbon assimilation, and nitrate (NO_3) under anoxic conditions as electron acceptor.[36] In addition, some strains of microalgae have the ability to use toxic compounds, including phenols, as carbon and energy sources.[37]

Denitrification assays

Gas-tight 120 mL glass serum bottles were used to confirm the denitrification capacity of the bacterial granular sludge. The flasks were filled with 80 mL SWW_2 (without ammonia), 1.8 g L^{-1} granular biomass and 43 mg L^{-1} NO_3-N as electron acceptor at a neutral pH. The serum bottles were closed with rubber septa and aluminum caps, and flushed with helium for 10 min to provide anaerobic conditions. The cultivation broths were continuously stirred under magnetic agitation (120 rpm) at 25 °C to foster the gas–liquid equilibrium and maintain the granular sludge in suspension. The O_2, N_2 and CO_2 headspace composition was monitored periodically by gas chromatography–thermal conductivity detection. The denitrification assay was conducted in duplicate.

Analytical procedures

Liquid samples were filtered by means of cellulose acetate filters (0.45 μm) prior to determination of soluble parameters. NH_4^+-N concentration was measured in a spectrophotometer (U-200, Hitachi, Tokyo, Japan) using the Nessler method at 425 nm. NO_2^--N and NO_3^--N concentrations were determined by high-performance liquid chromatography–ion conductivity (HPLC-IC) using a 515 HPLC pump (Waters, Milford, MA. USA) coupled with a Waters 432 202 IC detector and equipped with an IC-Pak Anion HC column (150 mm × 4.6 mm). TSS and TS concentrations were quantified by Standard Methods.[38] TN concentrations were determined using a TOC-V CSH analyzer equipped with a TNM module (Shimadzu, Kyoto, Japan). For granular biomass, the particle size distribution was determined using a Mastersizer E 20003.14. Settling properties of the granular biomass were determined by means of the sludge volume index (SVI) after 30 min sedimentation (SVI_{30}, mL g TS^{-1}). Temperature and dissolved oxygen concentrations in the bioreactor cultivation broths were monitored using a ProfiLine 3320 m coupled with a sensor CellOx 325 (WTW, Frankfurt am Main, Germany).

Nitrous oxide (N_2O) concentration was measured using a gas chromatography with electron capture detection (GC-ECD; Scion 436, Bruker, Palo Alto, CA, USA), equipped with an HS-Q packed column (1 m × 2 mm ID × 3.18 mm OD) (GC–ECD, SCION 436–GC. Bruker, USA). Injector, detector and oven temperatures were set at 100, 300 and 40 °C respectively. Helium was used as carrier gas (20 mL min^{-1} flow rate). External standards of N_2O in N_2 prepared in volumetric bulbs (Sigma Aldrich, Saint Louis, MO, USA) were used for N_2O quantification.

Phenol concentration was measured colorimetrically by the 4-amino-antipyrine method.[39] Meta, *ortho*- and some *para*-substituted phenolic compounds react, under alkaline conditions and the presence of ferricyanide, with 4-amino-antipyrine, producing a reddish product, which is measured at 510 nm. Absorbance was linearly correlated with phenol concentration (<10 mg L^{-1}). The calibration curve was periodically prepared using phenol standards (Sigma Aldrich, 98% purity) of known concentrations in a range of 1–8 mg L^{-1}. A sample (5 mL) of the mixed liquor was taken from the SBRs and centrifuged (2000 rpm, 10 min). The pellet was discarded, and then 1 mL 4-amino-antipyrine solution (20.8 mmol L^{-1}) in sodium carbonate (0.25 mol L^{-1}), and 1 mL ferricyanide solution (83.4 mmol L^{-1}) in sodium carbonate (1 mol L^{-1}) were added to the supernatant previously filtered by membranes of 0.45 μm. After an incubation time of 10 min, the absorbance was measured with a UV-2550 spectrophotometer (Shimadzu) at 510 nm. The blank was prepared by replacing the sample volume with distilled water. Finally, the anthrone method, a modification of the procedure proposed by Osborne and Voogt,[40] was used to determine intracellular glycogen concentration.

Analysis of SBR stability

At different CRTs, the specific COD_S uptake rate (q_{CODS}, mg COD_S g^{-1} TSS h^{-1}), specific removal rates of ammonia ($q_{NH3\text{-}N}$, mg NH_3-N g^{-1} TSS h^{-1}), phenol (q_{Phenol}, mg phenol g^{-1} TSS h^{-1}), and total soluble nitrogen (q_{TNs}, mg TN g^{-1} TSS h^{-1}) were determined. Both the SBR_B and SBR_{MB} were considered under stable operation when constant values (standard deviation <15%) of mean granular size, qNH_3-N, $qCOD_S$, q_{Phenol}, q_{TNs} and SVI were achieved. Phenol removal efficiency ($\%Phenol_R$) was determined from Eqn 5:

$$\%\ Phenol_R = 100 \times \frac{(Phenol)_O - (Phenol)_F}{(Phenol)_O} \quad (5)$$

where $(Phenol)_O$ and $(Phenol)_F$ correspond to the concentrations of phenol at the beginning and at the end of the SBR cycle, respectively.

Statistical analysis

The indicators of the process performance were statistically analyzed by means of an ANOVA at 95% confidence level and Tukey's honest significance test to compare the performance of the bioreactors.

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RESULTS AND DISCUSSION

Phenol and nitrogen removal in SBR_B

After 40 days of operation, the SBR_B operated at a phenol loading rate of 100 mg L^{-1} d^{-1} achieved almost complete removal of phenol and acetate within the first 2 h of the 24 h cycle (Fig. 2). However, the stability in SBR_B based on granule size and settling properties was reached after 60 days of operation (corresponding to three CRTs). Under steady state, a mean granule size of 0.7 mm, SVI of 40 mL g^{1} TSS and biomass concentration of 1.8 ± 0.2 g TSS L^{-1} were recorded. A high DO concentration (>7.0 mg O_2 L^{-1}), as a result of the high aeration rate to maintain the granules in suspension, avoided oxygen limitation during carbon and nitrogen oxidation.

The fast depletion of phenol and acetate shown in Fig. 2 allowed dividing the cycle into a short initial feast period (first 2 h), accounting for 80% of the removal of CODs (phenol + acetate), followed by a famine period which lasted until the end of the 24 h cycle. In the feast period (Fig. 3(a)), the intracellular carbon and energy reserves (glycogen) increased from 70 to 300 mg glycogen L^{-1} as the concentration of CODs decreased from 610 to 100 mg L^{-1}. In addition, a phenol removal of 79% (Table 2) was obtained during the feast period. The specific removal rate and removal efficiency of CODs averaged 138 ± 4 mg COD_S g^{-1} TSS h^{-1} and 83.3%, respectively (Table 2). During the famine period, glycogen concentration gradually decreased (Fig. 3(a)) as a result of microbial growth. During this period, phenol was completely degraded (Fig. 2).

The mean q_{Phenol}, estimated from the beginning of the cycle until the complete removal of phenol, was 13.8 mg phenol g^{-1} TSS h^{-1}. Such a specific phenol removal rate was significantly higher than the value estimated from data reported by Xu *et al.*[41] in an activated sludge reactor (1.1 mg phenol g^{-1} TSS h^{-1}) with aerobic and anoxic stages, operated at an OLR of 160 mg phenol L^{-1} d^{-1} and 40 mg phenol L^{-1}. On the other hand, Tay *et al.*[42] reported a q_{Phenol} of 14.4 mg phenol g^{-1} TSS h^{-1} for aerobic granules fed with concentrations of acetate and phenol of 100 mg L^{-1}. However, it should be taken into account that Tay *et al.*[42] operated the SBR at a volumetric OLR ~7.5 times greater than that used in the present study and a lower HRT of 8 h. Higher phenol removal rates, 55 mg phenol g^{-1} TSS h^{-1} was observed in aerobic granules exposed to higher initial phenol concentrations of 250 mg phenol L^{-1} and 1000 mg phenol L^{-1} respectively.[43,44]

On the other hand, NH_3-N was also gradually removed during the feast and famine period due to an active nitrification process and assimilation via microbial growth (Fig. 3(b)). The removal of ammonia via nitrification was correlated with the continuous generation of nitrate until the end of the cycle, which amounted to <30% of the initial NH_3-N. Interestingly, NO_2^--N accumulated during the feast period, and gradually disappeared after 6 h of SBR_B operation. The removal of NH_3-N during a 24 h cycle averaged 93% (Table 2).

Nitrification involves the oxidation of ammoniacal nitrogen up to nitrate, through several intermediates such as nitrite. The oxidation of ammoniacal nitrogen during the feast period competes with the oxidation of the carbon source. Nitrifiers compete with heterotrophic bacteria for the oxygen available in the mixed liquor mainly during the feast period, where the high metabolic activity of heterotrophs can induce low oxygen concentrations. Among nitrifiers, nitrite-oxidizing bacteria (NOB) have lower affinity for oxygen and lower growth rates than ammonia-oxidizing bacteria (AOB). Therefore, under conditions of strong microbial competition for oxygen, the ammonia oxidation rate is higher than the nitrite oxidation rate, leading to nitrite accumulation. At the end of the feast phase, NOB activity increases under a less severe oxygen competition, gradually oxidizing the accumulated nitrite into nitrate. Thus, NOB activity is likely higher during the famine period, where there is no available external carbon.

Nitrification extended from the beginning of the feast phase to the end of the famine period, which entailed a gradual ammonium removal throughout the operating cycle (Fig. 3(b)). However, the specific ammonia removal rate decreased by ~45% in the famine phase (Table 2). Most soluble COD from phenol and acetate was removed during the feast period, likely causing a decrease in the ammonium uptake rate by heterotrophic growth in the famine phase. In this period, a low heterotrophic bacterial

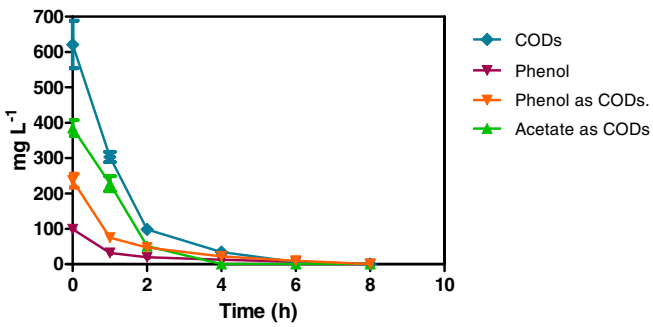

Figure 2. Effect of time on phenol, acetate and COD_S concentration in SBR_B. Assays were carried out at an initial phenol concentration of 100 mg L^{-1}.

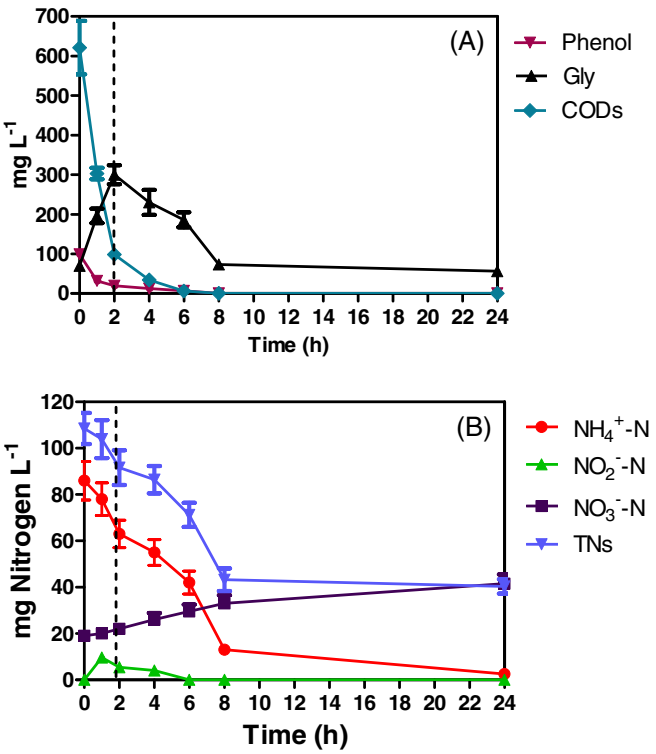

Figure 3. Variation in the concentrations of: (a) phenol (▼), COD_S (♦) and glycogen (▲); and (b) NH_4^+-N (●), NO_3^--N (■), NO_2^--N (▲) and TNs (▼) during wastewater treatment in SBR_B.

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Table 2. Key performance indicators of phenol biodegradation and nitrogen removal in SBR_B and SBR_{MB}

Operating cycle	Parameter	SBR_B (COD:N:P 100:15:2)	SBR_{MB} (COD:N:P 100:15:2)
24 h cycle	NH_4^+-N removal (%)	93 ± 2.3*	100*
	N inorganic removal (%)	66 ± 4.0**	61 ± 4.1**
	Nitrification (%)	86 ± 3.2**	82 ± 3.0**
	Phenol removal (%)	100	100
	SND (%)	70 ± 4.2*	54 ± 4.1*
	N_G (mg L^{-1})	48 ± 2.5*	42 ± 2.7*
	N_G (%)	53.3 ± 3.1*	47 ± 3.4*
Feast phase	q_{CODs} (mg COD_S (g^{-1} TSS h^{-1})	138 ± 4.1*	113 ± 2.5*
	NH_4^+-N removal (%)	30 ± 2.8*	42 ± 3.3*
	Phenol removal (%)	79 ± 4.1*	85 ± 0.7*
	$q_{NH3\text{-}N}$ (mg NH_3-N (g^{-1} TSS h^{-1})	9.2 ± 0.6**	9.1 ± 0.8**
	q_{TNs} (mg TN (g^{-1} TSS h^{-1})	6.3 ± 0.5**	6.8 ± 0.8**
Famine phase	NH_4^+-N removal (%)	65 ± 5.2**	58 ± 4.2**
	Phenol removal (%)	21 ± 1.6**	15 ± 1.5**
	$q_{NH3\text{-}N}$ (mg NH_3-N (g^{-1} TSS h^{-1})	5.1 ± 0.1*	11 ± 1.8*
	q_{TNs} (mg TN (g^{-1} TSS h^{-1})	6.8 ± 0.7*	4.3 ± 2.1*

Note: Statistical analysis: (Shapiro–Wilk $P < 0.05$).
*$P < 0.05$;
** $P > 0.05$.

growth rate, mainly sustained by intracellular glycogen, was expected. This phenomenon could explain the decrease in $q_{NH3\text{-}N}$ observed in the famine period. The assimilated NH_3-N estimated from Eqn 2 represented 12.6 mg N L^{-1}, which accounted for 14% of the ammonium removed throughout the cycle. The remaining ammonia (86%) was removed via nitrification. The generation of nitrogen gas (N_G) in the wastewater treatment cycle accounted for approximately 53% of the removed ammonium, which confirmed that denitrification played a relevant role in SBR_B (Table 2). The occurrence of N_2O gas during the SBR_B operation was not detected by GC-ECD. At the end of the cycle, TN concentrations matched the sum of the inorganic nitrogenous species (NH_3-N, NO_3-N), which ruled out the presence of soluble organic nitrogen. The removal efficiency of inorganic nitrogen at the end of the 24 h cycle corresponded to 66%, with a contribution of SND of 70% (Table 2). Similar efficiencies of nitrification (86.6%), denitrification (59.5%) and total nitrogen removal (60.5%) were reported during the domestic wastewater treatment in an aerobic granular SBR operated with a short period of anaerobic feeding followed by an extended aerobic period.[45]

Nitrogen and phenol removal in industrial wastewater is typically achieved alternating anoxic and aerobic conditions. For instance, a complete removal of phenol (1000 mg L^{-1}) was reported via nitrite-based denitrification using phenol as carbon source, followed by aerobic phenol oxidation and partial nitrification in an activated sludge SBR operated at a dilution rate of 0.25 d^{-1}. However, while 75% of the ammonia nitrogen from the influent was transformed to N_2, the remaining 25% of nitrogen present as nitrite required further treatment.[46] Likewise, Xu *et al.*[41] reported a maximum SND value of 30.5% at an OLR of 160 mg phenol L^{-1} d^{-1} in an activated sludge reactor operated with intermittent aeration (aerobic and anoxic stages) using phenol as organic carbon and electron donor in the denitrification stage. In the present study, higher SND and removal of soluble TN (~70%) were achieved in a single-stage aerobic granular sludge SBR at 100 mg phenol L^{-1} d^{-1}.

Removal of phenol and nitrogen in SBR_{MB}

The microalgal–bacterial granular biomass achieved steady-state concentrations of 2.3 ± 0.5 g L^{-1} after 20 days of operation. The average size of the granules increased from 0.7 to 1.2 mm and the SVI decreased from 40 ± 3 to 20 ± 3 mL g^{-1} TSS ($P < 0.05$), which suggested that microalgae enhanced the sedimentation properties of the granular biomass, which allowed generation of microalgal–bacterial flocs and granules.

The feast period in SBR_{MB} lasted 2 h, when the COD_S was removed by >85%. Likewise, the famine period lasted from hour 2 until the cycle ends (Fig. 4(a)). During the feast period, COD_S was consumed at a specific rate of 113 mg COD_S g^{-1} TSS h^{-1} and phenol at 18.5 mg g^{-1} TSS h^{-1}. Such a symbiotic growth of algae and bacteria would avoid the mass transfer limitations of CO_2 and O_2. The remaining external carbon source was completely depleted within the first 2 h of the famine period (Fig. 4(a)). Although the specific removal rate of CODs was faster in SBR_B during the feast period, the removal of phenol was better in SBR_{MB} during the feast period.

Nitrification was active at the beginning of the SBR_{MB} cycle, with a NH_3-N removal of 42% during the feast period, concomitant with the generation of NO_3^--N and NO_2^--N (Fig. 4(b) and Table 2). During the famine period, nitrification supported a steady accumulation of nitrate (Fig. 4(b)). On the other hand, the nitrogen assimilated throughout the operating cycle was ~16.1 mg N L^{-1} according to Eqn 2. N_G production, which represented about 47% of the ammonia removed, had a relevant role in the removal of inorganic nitrogen in SBR_{MB} (61%).

The removal efficiency of ammonia in both SBRs was high, although SBR_{MB} supported higher NH_4^+ removal than SBR_B. In SBR_{MB}, ammonia nitrogen was completely removed within 8 h (90 mg NH_3-N L^{-1}), while only 80 mg NH_3-N L^{-1} was removed in

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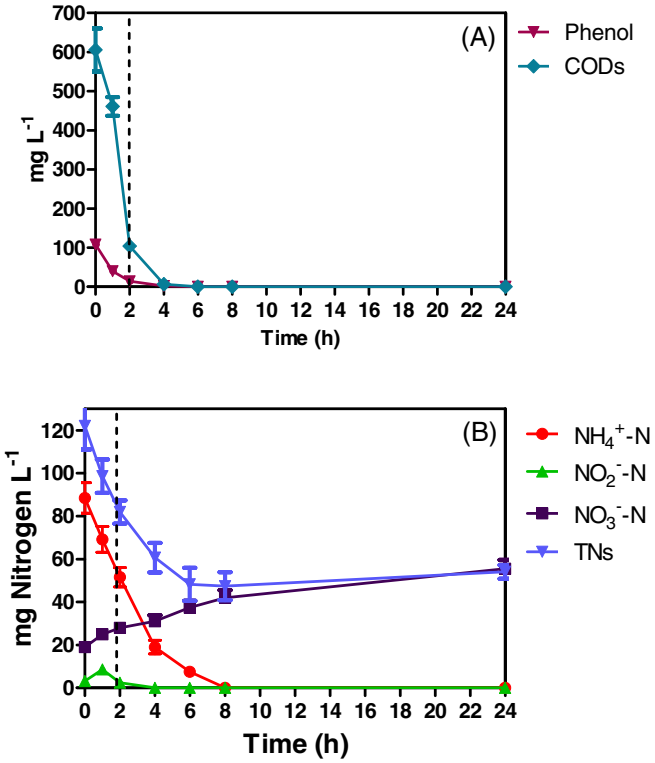

Figure 4. Variation in the concentrations of: (a) phenol (▼) and COD_S (♦); and (b) NH_4^+-N (●), NO_3^--N (■), NO_2^--N (▲) and TNs (▼) during wastewater treatment in SBR_{MB} (microalgal–bacterial granular biomass).

a similar period of time in SBR_B (Figs 3(b) and 4(b)). The soluble effluent TN after a settling time of 5 min in SBR_B and SBR_{MB} accounted for 40 ± 3 and 55 ± 5 mg L^{-1}, respectively. The removal of inorganic nitrogen was similar in both SBRs (SBR_B and SBR_{MB}). However, N_G and SND were lower in SBR_{MB} compared to SBR_B. Indeed, higher nitrogen assimilation as microalgal–bacterial biomass likely compensated for the lower denitrifying activity in SBR_{MB}, resulting in a removal of inorganic nitrogen similar to that obtained in SBR_B.

In addition, $q_{NH3\text{-}N}$ was higher in SBR_{MB} compared to SBR_B, particularly during the famine period, which was likely attributed to the synergistic relationship between bacteria and algae. This finding has been consistently reported in the literature.[47] N_2O was not detected in any reactor.

The highest value of the free ammonia (FA) in both SBR_B and SBR_{MB} was 1.85 mg L^{-1}. At this point, it should be stressed that FA values of 1.0–10 mg of NH_3 L^{-1} are considered inhibitory for NOB and might induce nitrite accumulation in the culture broth. It should be noted that in both systems NH_3-N concentration was high at the beginning of the cycle, which could explain the temporary accumulation of nitrite observed in Figs 3(a) and 4(a).

Interestingly, stripping of phenol did not occur under the experimental conditions tested in either reactor SBR_B (operated with a superficial upflow air velocity of 0.9 cm s^{-1}) or SBR_{MB} (stirred at 200 rpm). Similarly, the removal of phenol by air stripping was negligible during the first 24 h of the experiment in a fluidized bed reactor containing initial phenol concentrations ranging between 20 and 1000 mg L^{-1}, and operated at air velocities ranging between 1.3 and 3.9 cm s^{-1}.[48] In an internal-loop airlift reactor operated at an air flow rate of 3–12 L min^{-1}, corresponding to a superficial upflow air velocity of 1.0–4.0 cm s^{-1}, phenol removal by stripping accounted for 5–11%.[49] Therefore, air stripping is only relevant at very high gas velocities and high phenol concentrations. In this context, phenol loss by air stripping must be monitored particularly in aerobic bacterial SBRs operated at higher superficial upflow air velocities and phenol loading rates.

Microalgae provide photosynthetic oxygenation required for the oxidation of phenol and acetate by bacteria without the need for an external source of oxygen. This symbiotic association of microalgae–bacteria could reduce operating costs and improve process safety compared to conventional aerobic wastewater treatment processes.

Microalgal-based wastewater treatment serves many purposes, such as safe environmental release and the recovery of nutrients in the form of biomass, reinforcing the principles of circular economy via waste valorization. Recent technological developments have shown the feasible integration of microalgal cultivation in wastewater treatment plants.[50]

The environmental impacts of microalgal-based wastewater treatment are lower in seven categories compared to conventional bacterial processes: ozone depletion, cumulative energy demand, freshwater eutrophication, climate change, water consumption, freshwater ecotoxicity and photochemical ozone formation.[51] The major environmental concerns of this green technology involve ammonia stripping at alkaline pH and the end-use applications of the wastewater-derived microalgal biomass.[50]

Menger-Krug *et al.*[47] analyzed the energy and emission balances of integrating microalgal cultivation into wastewater treatment plants (WWTP) with biogas production from anaerobic digestion of biomass followed by electricity generation from biogas. Co-digestion of biomass, from microalgal systems integrated into WWTPs, significantly increased biogas production in comparison to WWTPs without microalgae systems. On-site microalgal cultivation with only resources present in the wastewater treatment process can significantly improve the energy balance of the wastewater plant by 41–71% when 60–80% of the CO_2 generated from biological processes and from the biogas combustion is used for algae cultivation. In the optimistic scenario of a complete utilization of the CO_2 produced during wastewater treatment for algal growth, WWTP with microalgal systems can even support a net energy production. Similarly, Tua *et al.*[51] evaluated the environmental improvements related to the integration of a microalgal side-stream process within a municipal WWTP by means of a life cycle assessment, which allowed estimation of a reduction of the cumulative energy demand of about 50–80% for optimized scenarios. The lower demand for electricity compared to conventional activated sludge WWTPs was mediated by the additional production of electricity from the biogas generated by the anaerobic digestion of the microalgal biomass, and the lower energy consumption in the main biological processes as a result of the absence of mechanical aeration.

In this work, the microalgal–bacterial aggregates of the SBR_{MB} showed a specific phenol removal rate 2–100 times higher than those reported for microalgal systems, which agrees with results of previous studies.[52] Thus, an axenic culture of the microalga *Chlorella pyrenoidosa*, exposed to an initial phenol concentration of 100 mg L^{-1} in an orbital shaker (140 rpm), supported a specific phenol removal rate of ~10.4 mg phenol g^{-1} TSS h^{-1}.[53] Likewise, *Chlorella* sp. and the filamentous microalga *Tribonema minus*, cultivated in column photobioreactors, supported a specific removal rate of ~0.3 and 0.6 mg phenol g^{-1} TSS h^{-1}, respectively, using

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flasks shaken three to five times per day, at an initial phenol concentration of 250 mg L^{-1} and continuous irradiance of 50 µmol photons m^{-2} s^{-1}.[54] Microalgal cultures typically require the addition of carbonate salts[54] or CO_2[52] as a carbon source for autotrophic growth. In the present study, the microalgal–bacterial system SBR_{MB} achieved similar removal efficiencies for phenol and inorganic nitrogen to those of the aerobic granular bacterial SBR in the absence of both aeration and addition of external inorganic carbon.

CONCLUSIONS

Successful development of algal–bacterial granules for treating synthetic wastewater was realized and indicated by good COD removal, biomass retention, settleability and average granule size. This work systematically compared granular SBR operated with bacterial biomass and mechanical aeration, and irradiated algal–bacterial biomass. The reactors SBR_B and SBR_{MB} showed similar performance in terms of removal of inorganic nitrogen, as well as a complete removal of phenol. Denitrification was more active in SBRB; however, higher nitrogen assimilation by the microalgal–bacterial biomass led to faster and complete removal of ammonia in SBRMB with respect to SBRB. No nitrous oxide production was observed regardless of the prevailing microbial community.

In the algal–bacterial granular system, ammonia and phenol showed complete and better removal efficiencies and higher phenol biodegradation rates than stand-alone microalgal cultures reported in the bibliography. Also, algal–bacterial granules would protect microalgae against toxicity inhibition effects.

The application of SBR_{MB} would be more efficient than SBR_B for its lower energy demand, cost effectiveness and potential resource recovery. The highly synergetic reaction between microalgae and bacteria in microalgal–bacterial granules was essential for achieving stable removal of organics, phenol and nitrogen under non-aerated conditions.

It appears that SBR_{MB} can be reasonably considered as an alternative for environmentally sustainable wastewater treatment.

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AUTHOR CONTRIBUTIONS

Paula Bucci: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft; Octavio García-Depraect: methodology, supervision; Enrique José Marcos Montero: methodology; Noemí Zaritzky: reviewing; Alejandro Caravelli: formal analysis, review and editing; Raúl Muñoz: funding acquisition, project administration, resources, supervision, review and editing.

CONFLICT OF INTEREST

The authors declare that they do not have any conflicts of interest.

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