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Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture

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ABSTRACT

A wastewater-born and settleable algal-bacterial culture, cultivated in a stirred tank photobioreactor under lab conditions, was used to remove the carbon and nutrients in municipal wastewater and accumulate biomass simultaneously. The algal-bacterial culture showed good settleable property and could totally settle down over 20 min, resulting in a reduction of total suspended solids from an initial 1.84 to 0.016 g/l. The average removal efficiencies of chemical oxygen demand, total kjeldahl nitrogen and phosphate were 98.2 ± 1.3%, 88.3 ± 1.6% and 64.8 ± 1.0% within 8 days, respectively, while the average biomass productivity was 10.9 ± 1.1 g/m^2·d. Accumulation into biomass, identified as the main nitrogen and phosphorus removal mechanism, accounted for 44.9 ± 0.4% and 61.6 ± 0.5% of total inlet nitrogen and phosphorus, respectively. Microscopic analysis showed the main algae species in the bioreactor were filamentous bluegreen algae. Furthermore, denaturing gradient gel electrophoresis and 16S rDNA gene sequencing revealed that the main bacteria present in the photobioreactor were consortia with sequences similar to those of *Flavobacteria, Gammaproteobacteria, Bacteroidia and Betaproteobacteria*. This study explores a better understanding of an algae-bacteria system and offers new information on further usage of biomass accumulated during treatment.

1. Introduction

The concept of algal-bacterial culture as an engineered system in domestic and industrial wastewater treatment has experienced increased momentum over the past few years (Bordel et al., 2009; de-Bashan et al., 2002; Garcia et al., 2000; Gutzeit et al., 2005; Medina and Neis, 2007; Munoz et al., 2005). It is especially favorable in regions with year-round high solar radiation and temperature as the removal is an entirely natural process. When illuminated, algae produce oxygen that can be used by aerobic bacteria to biodegrade pollutants whilst, in return, they consume the carbon dioxide released from bacterial respiration (Oswald, 1988), which provides a cheaper and safer alternative to mechanical aeration and contributes to CO_2 mitigation (Guieysse et al., 2002; Munoz and Guieysse, 2006).

Another advantage of this technology is that more nitrogen and phosphorus could accumulate into the algal and bacterial biomass during the removal process. When using a bacterial system to treat acetonitrile (1 g/l), only 26% N–NH_4^+ was assimilated into biomass. Under the same conditions using algal-bacterial culture, 53% N–NH_4^+ was assimilated into algae

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and bacteria biomass (Munoz, 2005). Oswald also inferred that, under optimal operating conditions (e.g., sufficient light intensity and a proper bioreactor configuration), almost all the available ammonia nitrogen appeared in the form of algal cell material (Oswald and Gotass, 1957). All the above gives algal-bacterial biomass, accumulated during wastewater treatment, the potential to be used as a fertilizer in agriculture (Benemann et al., 1977; Mulbry et al., 2005).

The major limitation of the exploitation of this technology is the requirement for cost-effective biomass harvesting techniques. A technical separation unit consisting of filtration or centrifugation has to be applied (Mohn, 1988), but this will raise the operation cost. Adding chemicals such as Ca^{2+} or slaked lime will result in secondary pollutants (Imase et al., 2008; Nurdogan and Oswald, 1995). Using an immobilization system is another possible solution (Mallick, 2002; Moreno-Garrido, 2008), but all media are costly and inefficient over a long operation time. Therefore, a more effective biomass harvesting strategy such as a settleable algae-bacteria system is required.

The identification and biometry of the dominant algal species in the algal-bacterial culture were well-studied in previous works (Godos et al., 2009; Garcia et al., 2000; Oswald, 2003), but little information was available about the bacterial community involved in this symbiotic system. Investigation of the microbial composition and their functionalities could provide some insights into the biological catalytic and symbiotic mechanisms. Moreover, the purification capacity could potentially be improved by addressing microbial constraints.

In this study, for the first time, a settleable algal-bacterial culture was cultivated from domestic wastewater, and its treatment efficiencies, biomass generation, N and P accumulation processes and microbial diversity were investigated.

2. Material and methods

2.1. Settleable algal-bacterial culture enrichment

The algae inoculum was obtained from the second clarifier wall of the Suderburg municipal wastewater treatment plant (County of Uelzen, Lower Saxony, Germany). The collected algae solution (exposure to bacteria was unavoidable) was firstly settled down for 1 h, and then 30 g (wet weight) of settled solid was used as algae inoculum for algal-bacterial culture enrichment. The wastewater collected from the second clarifier at the same site was used as medium, and 600 ml pretreated wastewater collected from the same plant (after preliminary screening, grit removal and primary sedimentation process) was used as bacterial inoculum and nutrient supply. The settleable algal-bacterial culture was cultivated under laboratory conditions at around 19 °C. The stirred tank photobioreactor (for culture enrichment) was made of transparent PVC 40 cm in depth and 29 cm in diameter. The total volume of the medium in the reactor was 14 l (approx. 25 cm in depth). Constant mixing was maintained using a magnetic stirring bar (100 rpm) to avoid algae sedimentation. Two compact fluorescent lamps (Sylvania, F20W/860/E27) were used to irradiate the tank with about 360 $\mu E\,s^{-1}\,m^{-2}$ (measured at the top of the liquid surface) for a period of 12 h per day. In order to cultivate the settleable algal-bacterial culture, the mixing procedure was stopped every 23 h for 1 h and the floating biomass was discarded with a screen (0.5 mm). 600 ml pretreated wastewater was exchanged after sedimentation every 3 days to maintain a nutrient supply. After one month of cultivation, dark green and pea green microalgae were visible and distributed evenly in the reactor.

2.2. Experimental operation

The same laboratory-scale reactor system as in the cultivation process was used for the batch mode. The pretreated wastewater was used as feed for the reactor in the following experiments, unless otherwise stated. The characterization of the pretreated wastewater used in the different batches is shown in Table 1. Before starting the batch experiment, the algal-bacterial biomass was allowed to settle by stopping the stirrer for half an hour. At the end of each 8-days cycle, 12.5 l of suspension was removed and replaced by fresh wastewater as above. The irradiation device was the same as that for the cultivation process. The photoperiod was a 12 h light-12 h dark cycle. 150 ml samples for further analysis (see below) were collected near the midway of the reactor with a pipe every day, 4 h after starting the irradiation period.

2.3. Analytical procedures

The temperature and dissolved oxygen (DO) were measured near the midway of the reactor by using a microprocessor oximeter (Oxi 320/SET. WTW, Germany) coupled with an O_2 sensor (CellOx 325, WTW, Germany). pH was determined using a Crison pH electrode (pH 197-S). Chemical Oxygen Demand (COD), Total Kjeldahl Nitrogen (TKN) and Total Suspended Solid (TSS) were analyzed according to DIN 38409-H 41(44), DIN EN 25663-H11 and DIN ISO 11465 (DEV., 2002). NH_4^+, total phosphorus and dissolved phosphorus (PO_4^{3-}) were determined according to DIN 38406-E5-1 and DIN EN ISO 6878-D11 (DEV., 2002) using a UV/Vis Spectrometer (Perkin Elmer, Lambda 40, USA). NO_3^- and NO_2^-

Table 1 – Characterization of wastewater.

Parameter	Unit	First batch	Second batch	Third batch	Fourth batch
Chemical oxygen demand (COD)	mg O_2/l	132.7 ± ±3.0	103.0 ± ±5.0	190.9 ± ±3.0	140.8 ± ±4.0
Total organic carbon (TOC)	mg C/l	49.8 ± 1.2	36.9 ± 1.5	63.6 ± 1.0	52.1 ± 0.8
TKN	mg N/l	25.7 ± 0.3	25.3 ± 0.2	23.1 ± 0.6	35.4 ± 0.3
Ammonium	mg N/l	14.6 ± 1.0	18.4 ± 0.8	17.0 ± 1.2	18.9 ± 1.0
Total phosphorus	mg P/l	4.9 ± 0.1	3.9 ± 0.1	4.7 ± 0.1	3.8 ± 0.1

were determined using an Ion Chromatograph (Dionex DX-100, USA) according to DIN EN ISO 10304-1 (DEV., 2002). Total Organic Carbon (TOC) and Inorganic Carbon (IC) were determined using a TOC analyzer (Elementar liqui TOC II, Germany) according to DIN EN 1484-H3 (DEV., 2002). Before analysis of the above parameters in liquid, samples were membrane filtered (0.45 μm). To measure nitrogen or phosphorus in biomass, the samples were first divided into two identical parts. One part was homogenized, while another part was filtered to remove the solids. Nitrogen or phosphorus in biomass was calculated as the total nitrogen or phosphorus difference between the homogenized samples and the filtered samples. All the experiments were performed in duplicate. An optical microscope (OLYMPUS CHT, Japan) was used for morphological characterisation of microalgae.

2.4. *Community analysis*

Bacteria of the algae-bacteria system were collected at the end of each batch test by centrifugation at 10,000 rev min^{-1} for 10 min at 4 °C. Each sample was washed twice with phosphate buffer (pH 7.0). Genomic DNA was isolated using the QIAamp™ DNA Stool Mini Kit (QIAGEN, 51504) according to the manufacturer's instructions. The polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE) and 16S rDNA analysis were done as described previously (Su et al., 2009; Schauer et al., 2000). Dominant bands were sequenced (Macrogen, the Netherlands). Sequences were subjected to Basic Local Alignment Search Tool (BLAST) and Ribosomal Database Project (RDP) analysis (Zhang et al., 2009). Phylogeny was determined with the RDP classifier and Sequmatch. The sequences used here have been deposited in the GenBank under the accession numbers HQ327478–HQ327485.

3. Results and discussion

3.1. *Settleability of the algal-bacterial culture*

The settleability of the cultivated algal-bacterial culture was investigated and is shown in Fig. 1. It showed good settleability, as nearly all the algal-bacterial biomass settled to the bottom of glass cylinder within 20 min, resulting in a reduction of TSS from an initial 1.84 to 0.016 g/l. The corresponding sludge settling ratio (SV %) was 12%, which also implied its good settleability (Sekine et al., 1984). Compared with an uncultivated algal-bacterial culture, the good settleability of the system might be due to the special cultivation strategy used in this study. The alternate mixing and non-mixing operation in the cultivation period promoted the selection of settleable algae and bacteria, which provided an effective way to harvest algal-bacterial biomass. The harvest technology of this settleable system has three advantages over other algal-bacterial harvesting technologies (Mohn, 1988; Imase et al., 2008; Nurdogan and Oswald, 1995). First, the operating cost was greatly reduced, as no extra energy (or equipment) was required. Second, secondary pollutants were eliminated during operation and biomass harvest, as no extra chemicals were added. Third, the settleability could be guaranteed during long-term operation. The sedimentation was the characteristic of the cultivated algal-bacterial culture and not dependent on any immobilization medium which was inefficient over long time operation (Mallick, 2002; Moreno-Garrido, 2008). A microscopic photograph of the developed algal-bacterial flocs is shown in Fig. 2. From Fig. 2A, it can be seen that the main species in the bioreactor were filamentous blue-green algae. Fig. 2B shows the wastewater bacteria attached to the algae filaments, which forms the cooperative system. Obviously, binding mechanisms supporting the bio-flocculation process led to the formation of settleable biomass. There may be some factors responsible for the settling process, such as the algae cell surface properties, extracellular polymeric substances (EPS) and the content of cations, which will influence the formation and the stability of the settleable algal-bacterial biomass (Gutzeit et al., 2005).

3.2. *Carbon and nutrients removal in municipal wastewater with algal-bacterial culture*

3.2.1. *Temperature, dissolved oxygen and pH*

Changes in temperature, pH and dissolved oxygen during operation were monitored to determine their effects on the treatment process. As shown in Fig. 3A, the culture temperature

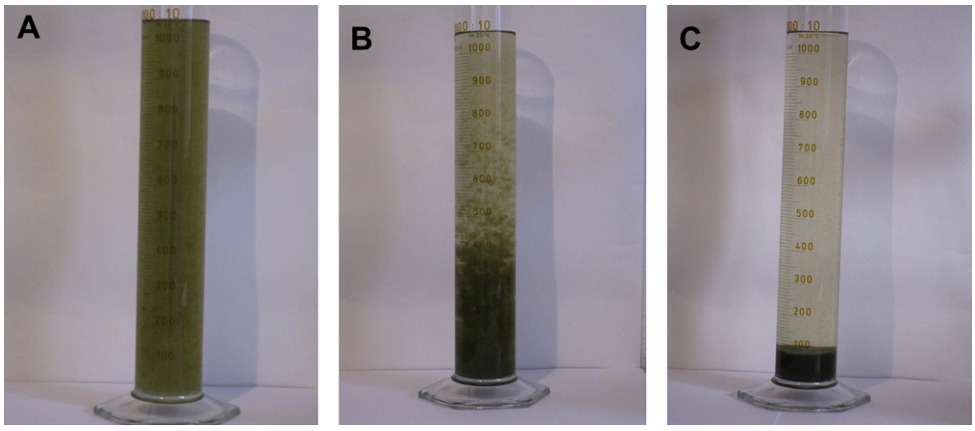

Fig. 1 – Settleability of algal-bacterial biomass. (A) Initial completely mixed sample. (B) After 5 min of sedimentation. (C) After 20 min of sedimentation.

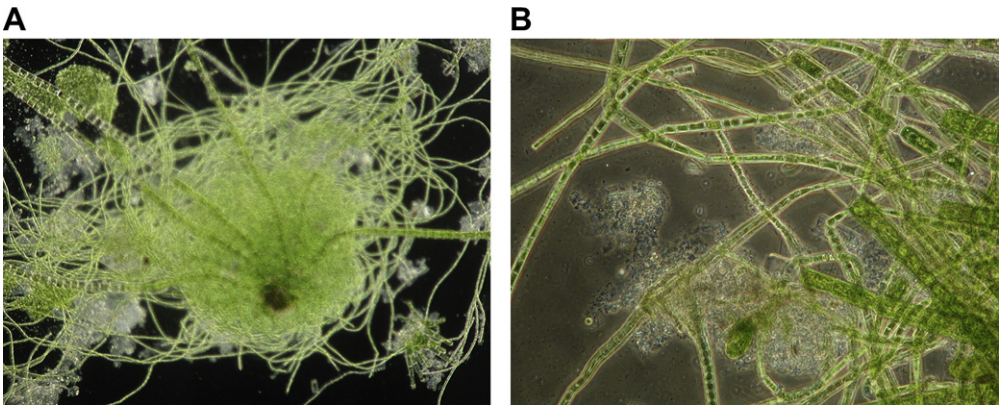

Fig. 2 – Microscopic photographs of algal-bacterial flocs. (A) Light microscope × 100. (B) Light microscope × 400.

was around 12 °C (the same as outdoor temperature) at the beginning of each batch test. It might be due to the fact that the wastewater was added into the bioreactor immediately after collection from the municipal wastewater plant. After that, the temperature increased to room temperature and remained stable until the end of each batch test.

Similarly, at the beginning of each batch test, the dissolved oxygen concentration (DO) was the same as that of the pre-treated wastewater. When starting the batch run, the DO values dropped significantly to around zero, which indicated that after initial consumption of the DO in the wastewater, the O_2 released from the algal photosynthesis was almost consumed by processes such as heterotrophic carbon oxidation and nitrification. After three days, the oxygen concentration increased gradually to around 2 mg/l and continued to increase until it was around 5.5 mg/l (70% saturation) at the end of each batch run (Fig. 3A).

No significant variation in culture pH during the four batches was detected in the system. Only a slight pH decrease occurred due to the intensive nitrification over the first five days. After that, pH increased gradually to 8.4 (see Fig. 3B). There are several factors which may influence the culture pH, such as micro-algal growth (pH increase as a result of CO_2 uptake), NH_4^+ nitrification (pH decrease due to the release of H^+) and the excretion of acidic or basic metabolites from organic matter biodegradation (Gonzalez et al., 2008b).

3.2.2. *Elimination of organic carbon*

As shown in Fig. 4, the COD decreased with time and was lower than 3 mg/l at the end of each batch test. The COD and TOC removal efficiencies were around 98% and 75.2% for the four batches, respectively (Fig. 4). Both algae and bacteria were able to use organic carbon through mixotrophic or heterotrophic metabolism (Abeliovich and Weisman, 1978). It was obvious that carbon sources were eliminated significantly

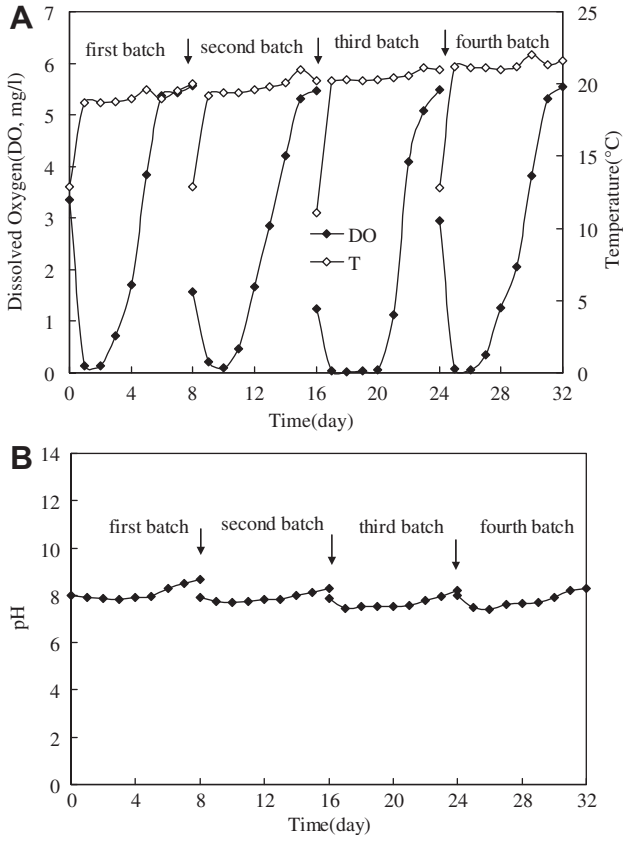

Fig. 3 – Changes in temperature, dissolved oxygen and pH in the algal-bacterial system. (A) Temperature and dissolved oxygen. (B) pH. Arrows indicate the start of a new batch test.

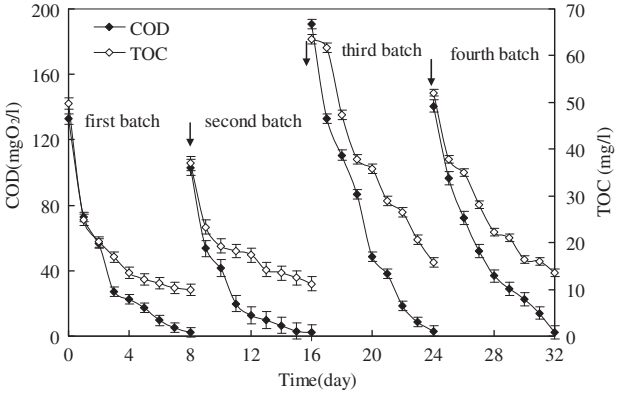

Fig. 4 – The concentration of COD and TOC in the algal-bacterial system over four batch runs. Arrows indicate the start of a new batch test.

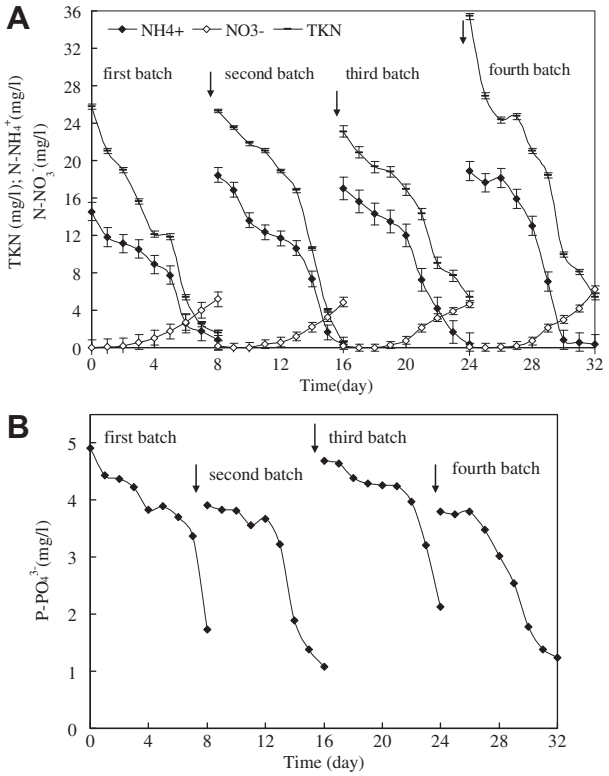

Fig. 5 – The concentration of TKN, NH_4^+, NO_3^- and PO_4^{3-} over four batch runs. (A) TKN, NH_4^+ and NO_3^-. (B) PO_4^{3-}. Arrows indicate the start of a new batch test.

within the first four days (Fig. 4). This result was in agreement with that of previous studies (Gutzeit et al., 2005). The COD and TOC removal were slow after the fourth day. There were two possible reasons for this. First, the carbon source left in the system was small after the fourth day. Second, the remaining carbon may be some colloidal, slowly biodegradable material. Usually, carbon was the limiting factor when algae were cultured in sewage. However, algae may also obtain CO_2 from the air in an open system, when the amount of carbon (inorganic and organic) in wastewater is inadequate for algal photosynthesis (Gonzalez et al., 2008a; Oswald, 1988).

3.2.3. *Elimination of nitrogen*

The influent nitrogen was mainly in the form of $N–NH_4^+$ (70–90%), followed by total organic nitrogen (10–30%). Fig. 5A shows the TKN, $N–NH_4^+$ removal and $N–O_3^-$ generation process. $N–NH_4^+$ removal efficiencies for all the four batches were nearly 100%, while the TKN removal efficiency were slightly different, ranging from 76.6% to 97.8% (Fig. 5A). The TKN removal efficiency in the last two batches was slightly lower compared with that in the first two batches. Obviously, there was still some organic nitrogen (ON, the difference of TKN and $N–NH_4^+$) in the effluent. The ON might be made of a small amount of inseparable organic matter produced during algae growth and wastewater treatment processes Oswald and Gotass, 1957. Additionally, $N–NO_3^-$ was always detected in the effluent due to incipient nitrification (around 5.2 mg/l). Nitrite was never detected at the end of each batch run.

The nitrogen balance was also investigated for a better understanding of nitrogen removal mechanisms. Based on total nitrogen removal and biomass concentration (discussed later), nitrogen assimilation into biomass accounted for approx. 52.9 ± 0.3%, 43.1 ± 0.4%, 43.0 ± 0.5% and 40.7 ± 0.4% of the total inlet nitrogen in the four batches, respectively (Table 2). The contribution of ammonia volatilization to total nitrogen removal in this system could be negligible due to the low NH_4^+ concentrations and relatively low pH (<8.5). Conversion into nitrate only accounted for 20.0 ± 0.2%, 18.3 ± 0.3%, 19.0 ± 0.1% and 17.4 ± 0.1% of total inlet nitrogen in the four batches, respectively (Table 2). The remaining missing nitrogen might be due to denitrification processes, which could occur at DO below 2 mg/l (Godos et al., 2009).

3.2.4. *Elimination of phosphate*

The time course of $P–PO_4^{3-}$ is shown in Fig. 5B. The removal of phosphate was a much slower process compared to that observed for nitrogen, but the same general pattern was apparent (Fig. 5B). The removal efficiencies of phosphate ranging from 54.5% to 72.6% were observed. The relatively slower and lower $P–PO_4^{3-}$ removal efficiencies, compared with $N–NH_4^+$, were probably due to the fact that nitrogen was the limiting nutrient, not phosphate, in this system. Previous studies have shown that the optimal ratio for maximum nitrogen and phosphorus uptake by algal-bacterial culture is N:P = 30:1 (Chevalier and de la Noue, 1985). However, the ratio of nitrogen to phosphorus was lower than 3 in this study.

Similarly, the balance of phosphorus was also investigated. Phosphorus accumulation into the biomass was still the main mechanism in this system, which accounted for 62.6 ± 0.5%, 66.6 ± 0.4%, 50.1 ± 0.6% and 67.1 ± 0.5% of the total inlet phosphorus for the four batches, respectively (Table 2). Phosphorus can be eliminated through both biotic phosphorous assimilation into the biomass and abiotic phosphorous precipitation (Godos et al., 2009). Nurdogan and Oswald (1995) reported on abiotic P removal which took place mainly in the

Table 2 – Nitrogen and phosphorus balance over the four batch tests.

Batch	Inlet TN (mg N/l)	Outlet TN (mg N/l)	Inlet nitrogen oxidized in NO_3^- (%)	Inlet nitrogen accumulated in biomass (%)	Inlet Phosphorus (mg P/l)	Outlet Phosphorus (mg P/l)	Inlet phosphorus accumulated in biomass (%)
1	25.8 ± 1.3	6.8 ± 1.5	20.0 ± 0.2	52.9 ± 0.3	4.9 ± 0.1	1.7 ± 0.1	62.6 ± 0.5
2	25.5 ± 1.0	5.4 ± 0.9	18.3 ± 0.3	43.1 ± 0.4	3.9 ± 0.1	1.1 ± 0.1	66.6 ± 0.4
3	23.3 ± 1.8	10.1 ± 1.0	19.0 ± 0.1	43.0 ± 0.5	4.7 ± 0.1	2.1 ± 0.1	50.1 ± 0.6
4	35.5 ± 1.3	11.7 ± 0.7	17.4 ± 0.1	40.7 ± 0.4	3.8 ± 0.1	1.2 ± 0.1	67.1 ± 0.5

form of orthophosphate precipitation at high pH (9–11). In our system, the pH below 9 was not sufficient to promote this removal mechanism.

3.3. Biomass generation and nutrients accumulation processes

3.3.1. Biomass generation during the operation

As shown in Fig. 6A, the TSS increased during operation, from 0.6 g/l at the beginning to 1.84 g/l at the end. The mean biomass generation rate was 10.9 ± 1.1 g/m^2 d. These values were lower than those reported by previous studies, in which maximum biomass productivities of 27.7 g/m^2 d were observed with 10 times diluted swine manure (2417 ± 481 mg COD/l; 214 ± 53 mg NH_4^+/l) during June and August in high rate algae ponds (HRAPs) in Valladolid, Spain (average irradiations 7062 ± 81 Wh/m^2 d) (de Godos et al., 2009). The lower availability of carbon and nitrogen in municipal wastewater compared with diluted swine manure, together with the lower irradiation, may be possible reasons for this.

Although there was continuous algal and bacterial growth in the system during the treatment process, the removal efficiencies of both carbon and nutrients and their general patterns were quite similar for the four batches. One possible reason for this might be that the further increased algae concentration has less effect on the uptake rate, since the nutrient uptake by algae was also determined by other factors (e.g., illumination conditions and the irradiated water surface area of the reactor) (Chevalier and de la Noue, 1985).

3.3.2. Nutrient accumulation processes

The accumulation of nitrogen and phosphorus in biomass during the treatment process is shown in Fig. 6B. The nitrogen concentration in biomass increased from 27.8 to 32.6 mg/g, at a rate of nearly 0.15 mg/g d. Meanwhile, the phosphorus concentration in the biomass increased from 0.72 to 4.74 mg/g, at a rate of 0.12 mg/g d. The most rapid accumulation period of nitrogen and phosphorus occurred in the first batch. After that, the accumulation rates were much lower and the amount of nitrogen and phosphorus in biomass was stable. It has been reported that the biomass generated from a swine manure removal process, ranging from 22.9 to 76.6 mg N/g biomass and from 4.3 to 25.2 mg P/g biomass, had fertilizer value to promote plant growth (Mulbry et al., 2005; Mulbry et al., 2006). The concentrations of nitrogen and phosphorus in biomass in this study were comparable to those of the swine manure treatment system. However, it is worth noting that the nutrient concentration in municipal wastewater is much lower than that of swine manure, so it leaves room to improve the accumulation of nitrogen and phosphorus in the settleable algal-bacterial culture.

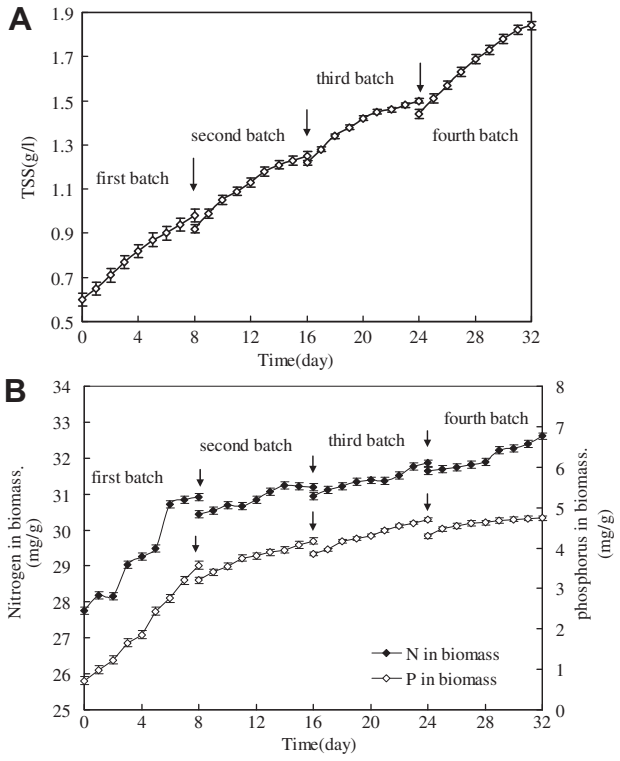

Fig. 6 – Changes in total suspended solids, nitrogen and phosphorus accumulation in biomass over four batch runs. (A) Total suspended solids. (B) Nitrogen and phosphorus concentration in biomass. Arrows indicate the start of a new batch test.

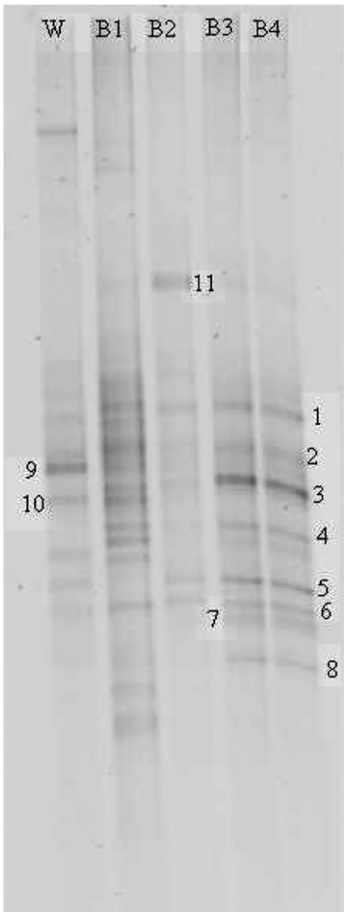

Fig. 7 – DGGE bands of bacteria communities. W: wastewater; B1 to B4: batch 1 to 4; 1 to 11: the name of each band.

Table 3 – DGGE 16S rDNA band identifications.

Band	Run					Genbank accession no.	Closest relatives (%Sequence similarity[d])	Class[c]
	W[a]	B1	B2	B3	B4			
1		•[b]	•	•	•	HQ327478	Uncultured *Flavobacteria* bacterium clone ATB-LH-7295 (96%)	*Flavobacteria*
2		•		•	•	HQ327479	Uncultured bacterium isolates DGGE gel band a3 (81%)	*Gammaproteobacteria*
3				•	•	HQ327480	Uncultured bacterium clone sls1360 (97%)	*Flavobacteria*
4		•		•	•	HQ327481	Uncultured bacterium clone LL141-8H16 (92%)	*Bacteroidia*
5	•		•	•	•	HQ327482	*Dysgonomonas* sp. enrichment culture clone YFZ1 (100%)	*Bacteroidia*
6	•	•	•	•	•	HQ327483	Uncultured bacterium 6week9 (94%)	*Bacteroidia*
7				•	•	HQ327484	Uncultured *Bacteroidetes* bacterium clone 298 (83%)	*Bacteroidia*
8				•	•	HQ327485	Uncultured bacterium clone RW6944 (86%)	*Betaproteobacteria*

a Inoculum.
b Existence under the condition.
c The phylotypes were assigned to phyla based on Ribosomal Database Project II (RDP II) taxonomy classifications.
d Percent values represent similarities between the associated DGGE band sequence and the closest match sequence from GenBank.

3.4. *Community analysis*

The DGGE profiles of the bacteria community sampled from the reactor at the end of each batch run as shown in Fig. 4 are summarized in Fig. 7. It was clear that the bacterial populations changed with time and became stable after operation through three batches (Fig. 7). At the same time, bacteria related to nutrient removal might have been enriched and stable, as indicated by the high removal efficiency observed along the successive tests, as shown in Fig. 4 and Fig. 5. Some bacteria in the acclimatized bacteria consortium were not present in the inoculum, suggesting that some new communities were enriched after operation. The above results also indicate that the bacteria community in algal-bacterial culture required time to acclimate to this commensalism system.

In order to provide greater insight into microbial ecology and diversity, eight predominant species extracted from DGGE bands were sequenced. Based on the 16S rDNA gene library results (Table 3), the acclimatized bacteria consortium was predominated by *Bacteroidia* (50% of clones), followed by *Flavobacteria* (25% of clones), *Betaproteobacteria* (12.5% of clones) and *Gammaproteobacteria* (12.5% of clones). It has been observed that *Flavobacteria* and *Bacteroidetes* phylotypes were present in high numbers in ammonia-oxidizing processes (Ducey et al., 2010; Nakano et al., 2008; Zang et al., 2008). Bafana et al. (2007) also observed that a *Gammaproteobacteria* (54%) phylotype was dominant in the acclimatized sludge of wastewater treatment plants. It was noticed that the band 1, 3 and 5 were stronger than other bands during operation (Fig. 7), thus their respective microorganisms might play a very important role in wastewater nutrient removal.

4. Conclusion

A settleable algal-bacterial culture, cultivated from wastewater, was successfully used to treat municipal wastewater in a stirred tank photobioreactor. The algal-bacterial culture showed good settleability, while the total suspended solid could be reduced to 0.016 g/l within 20 min sedimentation. The average removal efficiencies of COD, TKN and phosphate were 98.2 ± 1.3%, 88.3 ± 1.6% and 64.8 ± 1.0% within 8 days, respectively, while the average biomass productivity was 10.9 ± 1.1 g/m^2·d. Biomass uptake was the main mechanism for nutrient removal. The main algae species in the bioreactor were filamentous blue-green algae, while the main bacteria present in the photobioreactor were a consortium with sequences similar to *Flavobacteria, Gammaproteobacteria, Bacteroidia and Betaproteobacteria*. This study provides new insights into rapidly settleable algal-bacterial culture enrichment strategies and supplements the information on microbial ecology and diversity in algal-bacterial culture.

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