Hydraulic retention time effects on wastewater nutrient removal and bioproduct production via rotating algal biofilm reactor

Sahand Iman Shayan [a,b,*], Foster A. Agblevor [b], Lorenzo Bertin [a], Ronald C. Sims [b]

[a] *Department of Civil, Chemical, Environmental and Materials Engineering (DICAM), University of Bologna, via Terracini 28, 40131 Bologna, Italy*

[b] *Department of Biological Engineering, Utah State University, Logan, UT 84322, United States*

** Corresponding author at: Department of Civil, Chemical, Environmental and Materials Engineering (DICAM), University of Bologna, via Terracini 28, 40131 Bologna, Italy.*

E-mail address: sahand.imanshayan@studio.unibo.it (S. Iman Shayan).

HIGHLIGHTS

- Rotating algal biofilm reactors were designed for wastewater nutrient removal.
- Municipal wastewater in secondary stage was fed to the algal–bacterial culture.
- Hydraulic retention time was main variable factor in fed-batch mode operation.
- Competitive environment was applied in the bioprocess of mixed culture.
- Nutrient removal and carbon storage were affected by hydraulic retention time.

ABSTRACT

Rotating algal biofilm reactor (RABR) technology was successfully employed in an effective strategy to couple the removal of wastewater nutrients with accumulation of valuable bioproducts by grown algae. A secondary stage municipal wastewater was fed to the developed system and the effects of the hydraulic retention time (HRT) parameter on both nutrient removal and bioproduct production were evaluated under fed-batch operation mode. Two sets of bench scale RABRs were designed and operated with HRTs of 2 and 6 days in order to provide competitive environment for algal growth. The HRT significantly affected nitrogen and phosphorus uptakes along with lipid and starch accumulations by microalgae in harvested biofilms. Domination of nitrogen removal in 2-day HRT with higher lipid accumulation (20% on dried weight basis) and phosphorus removal in 6-day HRT with higher starch production (27% on dried weight basis) was observed by comparing the performances of the RABRs in duplicate runs.

1. Introduction

Conventional wastewater treatment methods need to be imple- mented by post-treatment approaches if they cannot fulfill the effluent standards. Algal systems associated with conventional methods have the potential of improving quality in effluent of wastewater treatment plant by reducing nutrient and metal loads into freshwater ecosystems (Hoffmann, 1998).

Several efforts into nutrient removal via conventional mechan- ical or biological and also chemical technologies along with their drawbacks in costs, energy consumptions, maintenance and vol- ume of produced waste sludge, have been reported (Lin et al., 2002; Tchobanoglous and Burton, 1991). Among them, development of processes based on the exploitation and recovery of microalgae under the physiological condition of immobilized cells was proposed. In particular, nutrient removal from municipal wastewater by immobilized *Chlorella vulgaris* and *Scenedesmus obliquus* was studied (Ruiz-Marin et al., 2010). Furthermore, an algal-based immobilization process in secondary wastewater treatment (He and Xue, 2010) and application of different coagu- lants to harvest microalgae uptaking nitrogen and phosphorus from municipal wastewater (Udom et al., 2013) were developed. These studies demonstrated the feasibility of nutrient removal by employment of different microalgae cultures, but challenges related to process scaling up and costs have still to be faced up. Thus, incorporation of biofilm photobioreactors in wastewater treatment processes to reduce the harvesting costs and improve the removal efficiency can play a significant role in perspective of developing economically feasible systems. In fact, removal of dissolved nitrogen and phosphorus is improved by maximizing algae production in a biofilm system, which allows higher concentrations and easier harvesting of valuable biomass (Christenson and Sims, 2012). Further studies in this field were car- ried out with (a) polymeric sheets for biofilm generation in a flow cell biofilm reactor dedicated to nutrient removal in a post treat- ment process (Boelee et al., 2011), (b) horizontal flat panels in large scale photobioreactors for phosphorus removal from tertiary step using microalgal biofilm (Sukač ová et al., 2015), (c) algal–bacterial biofilm photobioreactor to eliminate carbon, nitrogen and phos- phorus from concentrate and domestic wastewater (Posadas et al., 2013), (d) submerged membrane module for algal biofilm growth and nutrient removal using *C. vulgaris* strain (Gao et al., 2015), and (e) Twin-Layer photobioreactor for separation of immo- bilized microalgae (Shi et al., 2014). Defects of these studies might be found in culture medium and use of specific strain, installation of air compressor, ecological and economic implications. On the other hand, concerns about global energy demand, as well as neg- ative impact of petroleum based energy on environment, entice the attentions to the algal research as viable energy source (Quinn and Davis, 2015).

Sustainable treatment process could be achieved by the exploitation of algae, which can couple nutrient removal with pro- duction of biomass accumulating bioproducts, such as lipids, pro- teins, carbohydrates and residual biomass for anaerobic digestion. In this frame, this study covers lipid and starch produc- tion that can be converted to biodiesel and bioethanol by trans- esterification and fermentation processes, respectively. Various strategies have focused on high lipid and starch production from algae, both employing single strains or mixed cultures, through the regulation and mutation of strains, gravitation enrichment technique and inhibition of starch synthesis pathway (Goodson et al., 2011; Hassanpour et al., 2015 and Li et al., 2010); however, those approaches were carried out with genetically modified organisms or single strain of microalgae fed with laboratory pre- pared water solutions which only simulated actual wastewaters. Conversely, this study focused on the exploitation of a photoau- totrophic mixed culture made up of algae and bacteria capable of accumulating lipid and starch while removing nutrients from sec- ondary stage municipal wastewater. In particular, RABR system was employed as algal photobioreactor. Needful substrate and nutrients for biofilm formation were available from wastewater nitrogen and phosphorus sources, without external nutrient addition.

Development of algal biofilm technology in wastewater treatment process faces limitations of information about algal growth needs, biofilm formation, nutrient removal efficiencies and stan- dard operating conditions. In fact, investigation on performances and processes related to algal biofilm technologies are needed, despite the occurrence of established heterotrophic attached growth systems for wastewater treatment (Kesaano and Sims, 2014). The aim of this study was finding out the role of HRT on nutrient removal and bioproduct production in RABR systems, as a crucial parameter to distinguish target systems depending on main process aim (nutrient removal or carbon storage). To this aim, two sets of RABRs operated under fed-batch mode with HRTs of 2 and 6 days on the mixed culture medium in order to obtain the dominant photobioreactor beside effective operation of nutrient removal incorporation with bioproduct production.

2. Materials and methods

2.1 Experimental setup and operation

All experiments were performed in 8 L bench scale RABRs, oper- ated at 4.8 rpm and temperature of 21|25 °C (dark|light). Each reactor was composed of a plastic cylindrical wheel with 10 cm diameter and installed cotton cords onto wheel as the biofilm sub- stratum with average length of 28 inch (71 cm) and 0.25 (0.63 cm) inch diameter. Cylinder rotated inside the 50% of reac- tor volume and light irradiance was on the top led biofilm forma- tion on cotton ropes. Also, reactors were illuminated under 8 h dark and 16 h light period with sodium vaporizer lamp (1000 W/ SWBL) at light intensity of 200 ± 20 lmol m^{-2} s^{-1} with Apogee Quantum Meter (MQ-200) at level of biofilm surface. Also, UV filter sheets were installed over the RABRs in order to prevent critical distribution of algae strains by UV irradiations from light (Holzinger and Lütz, 2006). Biomass harvesting was applied via scraper after taking out the rope on cylinder. The pH was measured every 2 days with transmitter (Mettler Toledo, Switzerland). The RABRs were operated under fed-batch mode in two cases of, 2-day HRT and 6-day HRT. Prior to fed-batch operation, the same conditions described above were applied on the same reactors in batch mode of operation in order to specify the biomass growth parameters, growth phases and process time period. Duplicate system was considered to the whole reactors except microscopic qualification of growth media.

2.2 Biomass growth analysis

Municipal wastewater from the pond C, a secondary stage, in the city of Logan's treatment facility, UT, USA, was prepared and preserved at 4 °C before feeding into the reactors. In order to find out the biomass seeding period and growth curves, duplicate RABRs in batch operation was designed including biomass inocula- tion through adding 10% (v/v) ratio of sludge from the same pond. Before adding, sludge properties of total, fixed and volatile solid analysis were performed by means of oven at 103 °C and muffle furnace at 550 °C for 12 h and 1 h, respectively (USEPA Method 1684). The inoculation samples involved mixture of various algae strains that microscopic analysis reported based on the algal key and previous works (Bellinger and Sigee, 2015; Griffiths, 2009).

Biofilms harvesting were regulated for every 2 days and 4 h after light period's beginning. Removed biofilm from rope was col- lected into weighted aluminum bowl and dried for 12 h at 103 °C. Then after, dried biomass was cooled in desiccator and weighted as biomass cell dried weight (CDW, g m^{-2}). Fed-batch operation was accomplished according to the results from batch mode to find out the growth phase startup day. In order to perform 2-day HRT and 6-day HRT, liquid mediums were drained and refilled as 1/2 and 1/6 of the reactor

volume, respectively, every 24 h with light per- iod initiation. Sampling from reactor content for biomass growth was just focused for optical density (OD) by UV spectrophotometry (Shimadzu, UV 1800).

2.3 Nutrient removal analysis

Wastewater characterizations were analyzed before feeding to the RABR system. TSS and VSS both were measured based on stan- dard gravimetric method (USEPA Methods 8158 and 8164). During the experiment, samples were taken every 2 days from liquid med- ium except total nitrogen (TN) and total phosphorus (TP) as daily inlet–outlet of the cycle. Concentrations of TN, TP and ammonium (NH^+-N) were determined spectrophotometrically (Hach, DR 2700) using the persulfate digestion method (Hach methods 10071-72), molybdovanadate with acid persulfate digestion (Hach method 10127) and salicylate method (Hach Method 10031), respectively. Nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate (PO^{3-}) and sulfate (SO^{2-}) concentrations in the broth medium were monitored and measured by ion chromatography (IC) using a Dionex IonPac AS12A anion-exchange column set at a flow rate of 1.5 ml min^{-1}. IC data also, were analyzed via Chromeleon 7 Chromatography Data System (CDS) software.

2.4 Bioproduct analysis

Bioproduct analysis focused on carbon storages as lipid and starch. Harvested biofilms in fed-batch mode as same as biomass growth analysis, were dried at 103 °C then, prepared for lipid deter- mination procedure via colorimetric method the same as described by Mishra et al. (2014) and starch determination using modified methods (Asada et al., 2012; Mooij et al., 2014). A known amount of dried biomass was sonicated inside 2 M HCl for about 30 min and the solution was heated for 3 h in boiling water as hydrolyzation process. Afterward, neutralized by 2 M NaOH and centrifuged for 15 min at 4000 rpm. Finally, after filtration by 0.2-lm pore size filter, dissolved glucose monomer concentration was determined by high-performance liquid chromatography (Shimadzu Scientific) using BP- $100H^+$ column from Benson Polymeric (USA). Starch content was cal- culated by multiplying factor 0.9 to the glucose according to starch determination method (McCready et al., 1950).

3. Results and discussions

3.1 Biomass growth in batch operation of RABR

In order to determine the whole batch process duration, a con- ventional biomass growth curve as a function of the time was defined. This allowed preliminary considerations about the HRT parameter, which was applied in the development of subsequent fed-batch processes. Duplicate RABRs were fed with characterized municipal wastewater and sludge for inoculation and seeding (Table 1). During 21 days of experiment, the lag, growth and stationary phase were achieved through logarithmic areal density versus time, (Fig. 1A). A 3-days long lag phase was observed and consecutive exponential growth phase for microalgal biofilms occurred from day 3 to 11, after which the linear part of natural log transformed growth data and stationary phase followed until day 21. The maximum specific growth rate measured during the exponential phase was 0.149|006 day^{-1} (mean|range) for algal bio- films. The maximum areal biomass density measured through the stationary phases was 16.44 g m^{-2} on average. Moreover, the bio- film production rate as total accumulated biofilm's areal density over the time taken to reach stationary phase, reached 0.96 g m^{-2} d^{-1} in batch mode. Growth of microalgae in the bulk medium was analyzed via optical density at 750 nm wavelength (Fig. 1B). Results from OD analysis demonstrate biofilms formation and biomass tendency to grow on the cotton cord. The pH variations actuated the bulk medium to values over 8.5. High ranges of pH, indicates possibility of domination in bicarbonate uptake via speci- fic algae strain (Dubinsky and Rotem, 1974) and this can affect nutrient removal during the experiment.

3.2 Biomass growth and nutrient removal in fed-batch operation

Fed-batch operation was accomplished as 2 sets of duplicate RABRs in 2 days and 6 days of HRT for 21 days of experiment. Draining and refilling were performed after 3 days of batch mode operation, according to results about lag phase of seeding period. The CDW analysis of biofilm provided growth curve as natural log- arithmic data versus time for fed-batch operation to make the same diagram in batch operation. Maximum growth rate in this operation mode was reached 0.2124|0.022 day^{-1} (mean|range) for 2-day HRT and 0.1924|0.018 day^{-1} (mean|range) for 6-day HRT. Also maximum biofilm production per area was obtained in 2-day HRT with 30.1 g m^{-2} which was 28 g m^{-2} for 6-day HRT. Furthermore, the biofilm production rates, calculated as the total biofilm areal density accumulated divided by the time taken to reach stationary phase, were 1.74 and 1.64 g m^{-2} day^{-1} for biofilms in 2-day HRT and 6-day HRT, respectively. Growth curves for the algal biofilms and also in suspension for hydraulic retention times of 2 and 6 days are shown in Fig. 2. Prominent algal genera in 21 days of experiment according to microscopic analysis

of both HRTs with 40x magnification included *Scenedesmus, Chlorella, Cyanobacteria, Oocystis, Ankistrodesmus and Synura*. Removal of the nutrients, which are mostly nitrogen and phosphorus in algal research, is a basic requirement of wastewater post-treatment to gain acceptable standards of effluents. Using algal-based reactors improves nutrient uptake through thebiomass which could be harvested from the effluent. Initial total nitrogen and phosphorus concentrations of the feed were 49.5 mg-N L^{-1} (HRT 2)–46.5 mg-N L^{-1} (HRT 6) and 14.95 mg-P L^{-1} (HRT 2)–15.15 mg-P L^{-1} (HRT 6), respectively. After 21 days of treatment process beside of pH raising up to 9, broth medium reached 1.6 and 1.15 mg L^{-1} of TN and TP, respectively in 2-day HRT, which were 1.85 and 0 mg L^{-1} of TN and TP, respectively in 6-day HRT. Significant amounts of sulfate (SO^{2-}), lead to consider removal potentials in both HRTs. The reactor feed for 2-day HRT had 58.7 mg-S L^{-1} of sulfate and RABR decreased it by 30.6 mg-S L^{-1} with average rate of 1.62 mg-S L^{-1} d^{-1}, which was 60.3–29.24 mg-S L^{-1} in 6-day HRT with average rate of 2.16 mg-S L^{-1} d^{-1}.

Nitrogen as ammonium (NH^{+}), nitrate (NO^{-}) and nitrite (NO^{-}) were analyzed during the experiment (Fig. 3), with initial concentra- tions of 5.2 mg L^{-1}, 0.08 mg L^{-1} and 0.24 mg L^{-1}, respectively for 2-day HRT and subsequently 5.05 mg L^{-1}, 0.08 mg L^{-1} and 0.25 mg L^{-1}, respectively for 6-day HRT. The removal efficiencies in overall process were obtained 92% (NH^{+}), 44% (NO^{-}) and 57% (NO_2^{-}) for 2-day HRT and subsequently, 100% (NH_4^{+}), 47% (NO_3^{-}) and 58% (NO_2^{-}) for 6-day HRT. Phosphorus as orthophosphate (PO^{3-}) was also measured during the experiment with initial con- centrations of 3.52 mg L^{-1} (HRT 2) and 3.33 mg L^{-1} (HRT 6). Overall phosphate removal efficiencies were reached to 97% and 79% for the HRT of 2 and 6 days, respectively. Nutrient concentrations in each two days were collected as soon as day 4 began with draining- refilling step. It is obvious that ammonium uptake is almost complete in both HRTs despite of other nitrogen sources and this indicates preferential utilization of ammonium by algae over nitrate (Eustance et al., 2013).

The pH variations in both HRTs were compatible with possibility of bicarbonate domination chiefly, in the range of 8.5–9. On the other hand, nitrification process in the reactor contains chemoau- totrophic bacteria which need a carbon source such as CO_2 or HCO_3^{-}, favorable initial conditions, lead bacteria to grow. However, pH ranges out of 8–8.5 effect on reaction rate and provide incomplete nitrification of ammonia (Sawyer et al., 1974). Thus; refilling the reactor with fresh feed, accumulates nitrite (NO_2^{-}) ions. This can be mentioned in both HRTs after day 9 that pH values had raising trend.

In order to find out the domination of nutrient removal in treat- ment process, the inlet and outlet concentrations of total nitrogen, total phosphorus, were depicted for each batch (Fig. 4). High amount of nitrogen uptake than phosphorus could be extracted through the curves in 2-day HRT, unlike significant phosphorus removal rate than nitrogen in 6-day HRT (Table. 2). This can be demonstrated better, through the uptake rate's ratio as phosphorus

over nitrogen (Fig. 5). During the fed-batch operation since day 4, it is clear that phosphorus consumption rate in 6-day HRT is over than 2-day HRT which means that phosphorus is finished prior than nitrogen. Likewise; nitrogen consumption rate in 2-day HRT is more than 6-day HRT. Using the removal potentials for both HRTs in each cycle (Table. 2), the maximum removal potential in 2-day HRT is owned for nitrogen as 94%, whereas in 6-day HRT is 99.5% for phosphorus.

Both HRTs have increasing trend in nutrient removal potentials in conjunction with biofilm growth but, RABR system with 2-day HRT prefers to remove nitrogen and with 6-day HRT prefers to use phosphorus according to results. With respect to the analysis of TN and TP along with different nitrogen types in the bulk medium and also orthophosphate concentrations, most of the nitrogen and phosphorus include the organic types. On the other hand, long retention time gave higher removal capacity than short one for NH^{+}, NO^{-} and NO^{-} unlike better PO_3^{-} removal in short HRT. This means that there is sufficient time to nitrogen conversion and utilization in 6-day HRT and organic nitrogen is assimilated with biofilm growth in 2-day HRT. Moreover, long retention time led organic phosphorus conversion to the orthophosphate and assimi- lation, probably due to the high activity of the phosphatase at the cell surface. In a mixed culture of different strains, these can effect on carbon cycle to produce lipid or starch with respect to the com- petitions to uptake nitrogen or phosphorus.

According to nitrogen and phosphorus removal efficiencies in this study, they both are superior to most of previous reports as about 80% of TN and TP removal by twin layer biofilm reactor (Shi et al., 2014), immobilized process for secondary stage munic- ipal wastewater that represents 52% of TN and 92% of TP removal efficiency (He and Xue, 2010) and also, 96% of TN and 99% of TP removal efficiency by cultivating *Chlorella kessleri*, *C. vulgaris* and marine algae species (Caporgno et al., 2015). Therefore, it is a com- parable strategy of treatment to reach optimal stage with no spec- ified strain, nutrient addition and costly harvesting method.

3.3 Lipid and starch production

In order to distinguish carbon flow into accumulation of mole- cules, bioproducts' results for both HRTs were described versus time in case of lipid and starch (Fig. 6). The maximum bioproduct production was obtained at the end of experiment as lipid in 2-day of HRT and starch in 6-day HRT which are more than 20% and 27% of dried biofilm, respectively. This shows the preferability of 2-day and 6-day HRT in lipid and starch production, respectively. Regard- ing nutrient removal results, mixed culture's biology and impacts of nutrient starvation and phosphorus ratio over nitrogen that can change carbon flow's direction in

lipid or starch production (Zhu et al., 2015), domination of lipid production in short HRT than long, probably could be consequence of inhibition in AGPase enzyme's activity to reduce starch accumulation. As a case study about synthetic pathway of lipid and starch's accumulation, (Li et al., 2015) priority of lipid production through a specific strain, *Chlorella sorokiniana*, over starch in the N-depleted condition have been discussed. Using nutrient removal capacities and domination of organic nitrogen and organic phosphorus uptake in short and long retention times, respectively, daily improvement of removal capacities for both N and P along with biofilm growth in both HRTs may give the possibility of specific genera for both algae and bac- teria in the mixed culture to provide nutrient starvation through the rough removal process. Therefore; in the mix autotrophic envi- ronment several parameters can influence on lipid or starch synthetic.

Similar to the nutrient removal, bioproducts analysis repre- sented comparable results considering lipid and hydrocarbon pro- duction by the rotating algal biofilm system and flat panel photobioreactor as 7–22% for lipid and 38–48% for hydrocarbon using *C. Vulgaris* (UTEX #265) (Gross et al., 2013). In another study, Hassanpour et al. investigated on gravitation enrichment of mixed culture to screen lipid and starch producing specious separately in a sequencing batch reactor using specific medium. Maximum lipid and starch production reached by lipid producing photobioreactor with values of 30% and 36%, respectively on the basis of VSS.

In comparison with other investigations to improve the nutrient removal or carbon storages, ecology-based environment which has been used in this study by HRT variation and competitive condi- tions can represent advantages of biodiversity in the mixed culture, compatibility with other variables and optimization possibility in process design (Mooij et al., 2015).

Effective algal process that uses RABR to provide selective con- ditions by designing suitable HRT for both nutrient removal and bioproduct production, offers a simple, inexpensive and sustain- able method. This can be applied on various wastewaters to find out dominant biodiversity, and large scale optimization with aims of above.

4. Conclusions

Removal and utilization of municipal wastewater nutrients were established through biofilm technology using microalgae to uptake them. Designed RABRs were operated in fed-batch opera- tion for two HRTs of 2 days and 6 days. Experiments during 21 days demonstrated superiority of RABR with 2-day HRT in nitrogen removal and 6-day HRT in phosphorus uptake. Notable results described advantage of 2-day HRT in lipid and 6-day

HRT in starch accumulation at the end of experiment. The HRT based sifting may represent new strategy to combine nutrient removal from wastew- ater with dominant lipid and starch synthesis to produce increased biodiesel and bioethanol, respectively.

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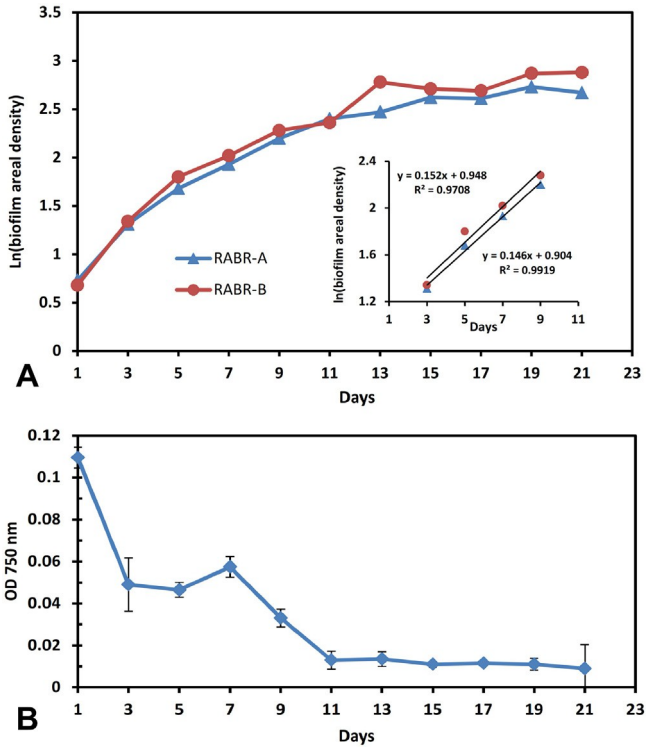

Fig. 1. Algal biomass growth profile as natural log transformed data for attached biofilm (A) showing the exponential phase (days 3–10) with equations and R^2 values describing exponentially phase of biofilms and also, optical density @ 750 nm for suspension (B) in batch mode during the experiment. Error bars represent standard deviation ($n = 2$).

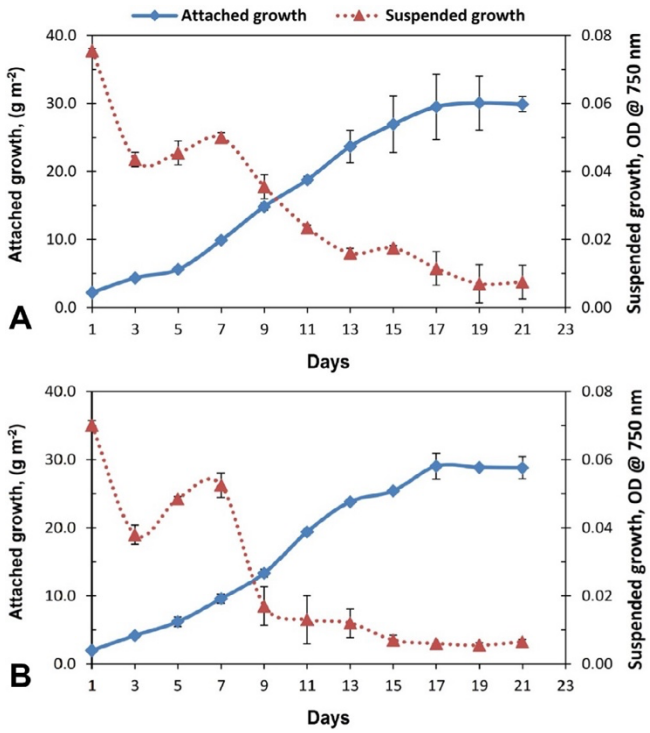

Fig. 2. Algal biomass growth profile in attached biofilm and broth medium for 2-day HRT (A) and 6-day HRT (B) in cases of attached growth (solid lines) and suspended growth (dotted lines). Error bars represent standard deviation ($n = 2$).

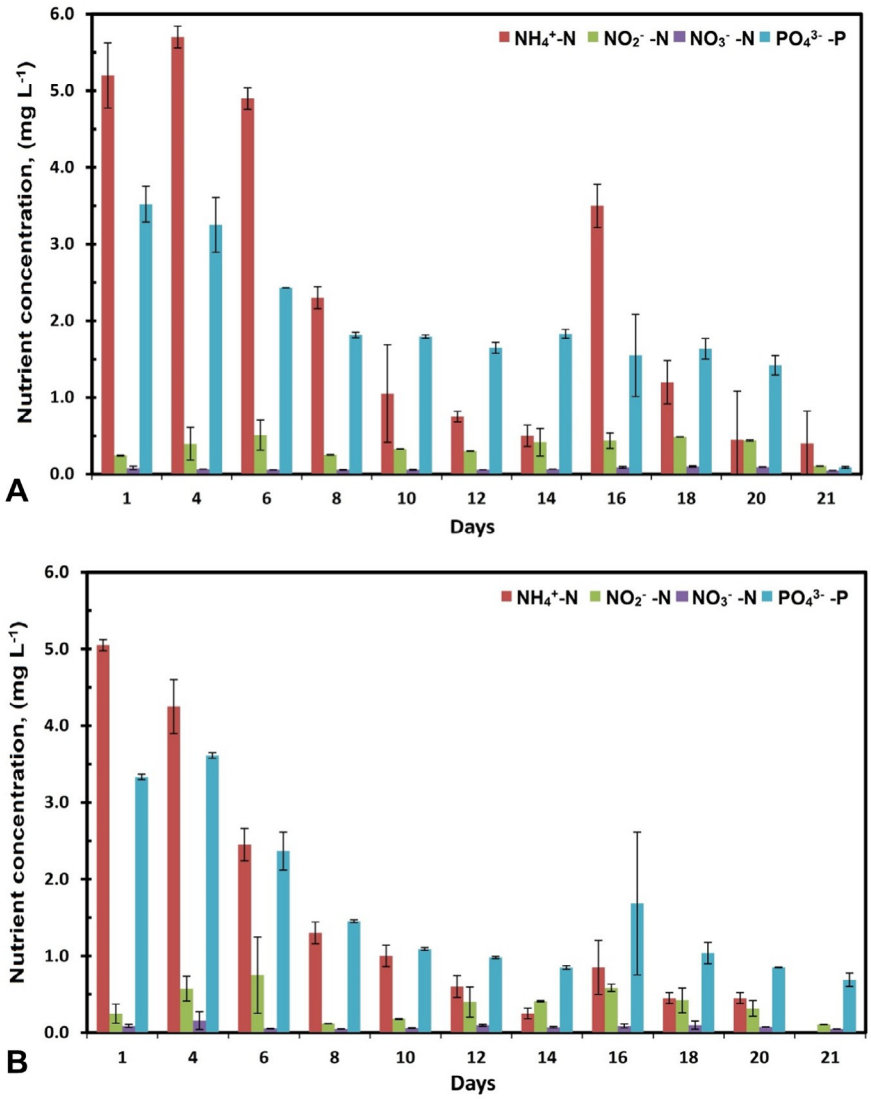

Fig. 3. Nutrient concentration profile as ammonium (NH^{+}-N), nitrite ($NO^{—}$-N), nitrate ($NO^{—}$-N), phosphate ($PO^{3—}$-P) in fed-batch mode during the experiment for 2-day HRT (A) and 6-day HRT (B). Error bars represent standard deviation ($n = 2$).

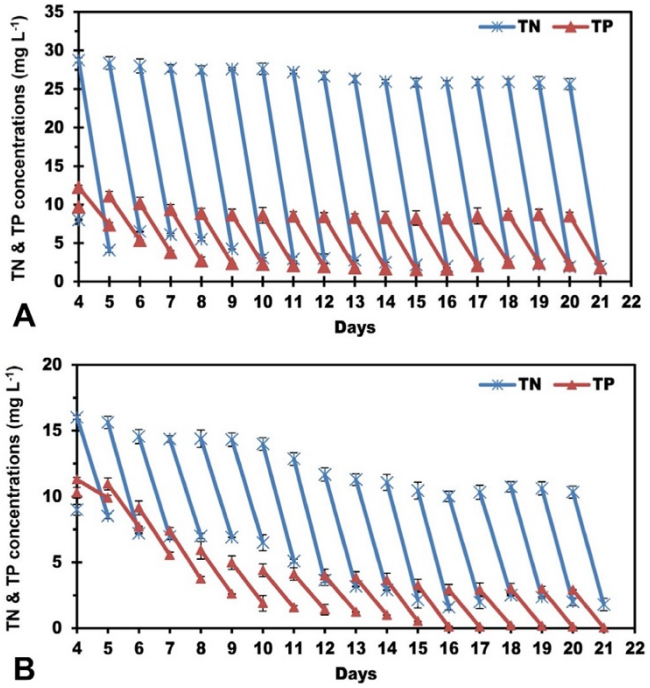

Fig. 4. Inlet and outlet concentrations of TN and TP in each batch of 2-day HRT (A) and 6-day HRT (B); Error bars represent standard deviation (n = 2).

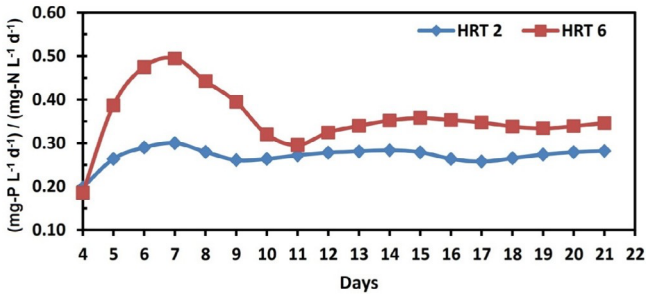

Fig. 5. Uptake rates of phosphorus over nitrogen, during the experiment in 2-day HRT and 6-day HRT.

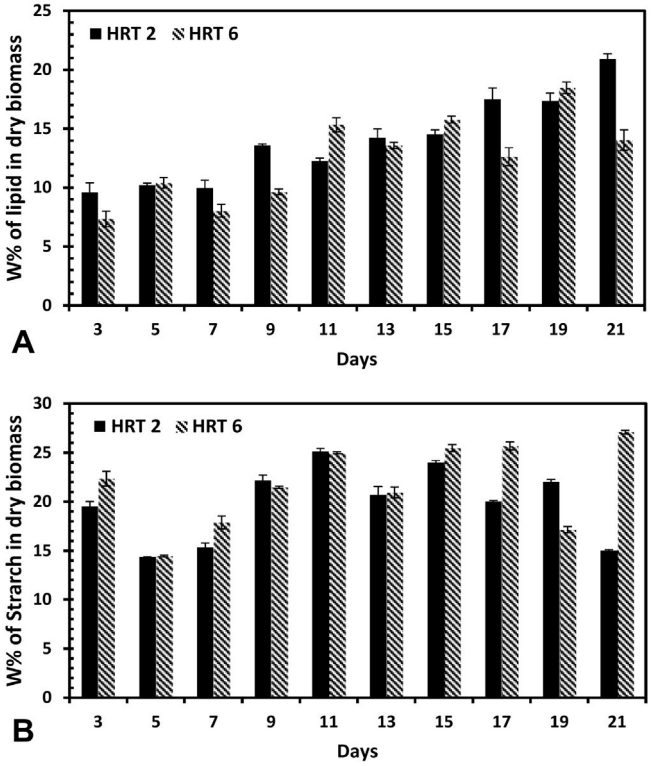

Fig. 6. Accumulated lipid (A) and starch (B) profile during the experiment in 2-day HRT and 6-day HRT. Error bars represent standard deviation ($n = 2$).

Table 1. Specificaion of the wastewater and sludge used for inoculation and seeding.

Wastewater characteristics		Sludge characteristics	
TSS (mg/L)	53.3	Total solid (%WB)	7.7
VSS (mg/L)	46.6	Fixed solid (%WB)	70
COD (mg/L)	63.1	Volatile solid (%WB)	30
Nitrite as N (mg/L)	0.33		
Nitrate as N (mg/L)	0.45		
Ammonium as N (mg/L)	9.9		
Phosphate as P (mg/L)	3.49		
Sulfate (mg/L)	58.3		
TKN (mg/L)	49.2		
Total nitrogen (mg/L)	50		
Total phosphorus (mg/L)	15		

Table 2. Removal capacities and removal rates of TN and TP in each batch for 2-day HRT and 6-day HRT.

Days	2-day HRT				6-day HRT			
	N		P		N		P	
	% Removal	mg L^{-1} d^{-1}	% Removal	mg L^{-1} d^{-1}	% Removal	mg L^{-1} d^{-1}	% Removal	mg L^{-1} d^{-1}
4	85.8	24.66	39.9	4.89	46.7	7.48	12.3	1.39
5	77.1	21.88	51.9	5.77	53.9	8.43	29.7	3.26
6	78.2	21.91	62.6	6.35	52.1	7.58	39.4	3.60
7	80.1	22.19	71.0	6.66	51.3	7.36	49.3	3.64
8	84.6	23.27	73.5	6.51	52.0	7.48	55.9	3.31
9	88.8	24.51	73.9	6.40	54.6	7.81	61.8	3.08
10	89.4	24.72	75.7	6.52	63.5	8.88	64.6	2.84
11	89.0	24.23	77.4	6.59	71.6	9.20	66.0	2.72
12	89.6	23.92	78.8	6.65	72.8	8.51	69.1	2.76
13	90.5	23.80	79.9	6.69	74.3	8.39	74.0	2.85
14	91.6	23.82	81.1	6.75	80.6	8.92	85.8	3.14
15	92.3	23.84	80.5	6.65	84.7	8.86	96.9	3.17
16	91.1	23.50	74.9	6.20	80.1	8.02	96.7	2.83
17	90.0	23.28	70.5	6.00	75.8	7.83	93.2	2.72
18	91.4	23.72	72.1	6.29	78.1	8.40	94.4	2.84
19	92.6	23.93	75.3	6.55	81.2	8.62	96.6	2.88
20	93.7	24.03	78.5	6.71	82.5	8.53	98.8	2.89
21	93.7	23.95	80.6	6.75	81.3	8.26	99.5	2.84