Bioresource Technology 218 (2016) 969–979

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Technical potential of microalgal bacterial floc raceway ponds treating food-industry effluents while producing microalgal bacterial biomass: An outdoor pilot-scale study

Sofie Van Den Hende[a,b,*], Veerle Beelen[a], Lucie Julien[a,c], Alexandra Lefoulon[a,c], Thomas Vanhoucke[d], Carlos Coolsaet[d], Stanislaus Sonnenholzner[b], Han Vervaeren[a,e,1], Diederik P.L. Rousseau[a,1]

[a] *Laboratory of Industrial Water and Ecotechnology (LIWET),*[2] *Department of Industrial Biological Sciences, Ghent University, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium*

[b] *Centro Nacional de Acuicultura e Investigaciones Marinas, CENAIM,*[3] *Facultad de Ciencias de la Vida, Escuela Superior Politécnica del Litoral, ESPOL, Campus Gustavo Galindo, Km 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador*

[c] *Ecole des Métiers de l'Environnement (EME),*[4] *Campus de Ker Lann, Avenue Robert Schuman, F-35170 Bruz, France*

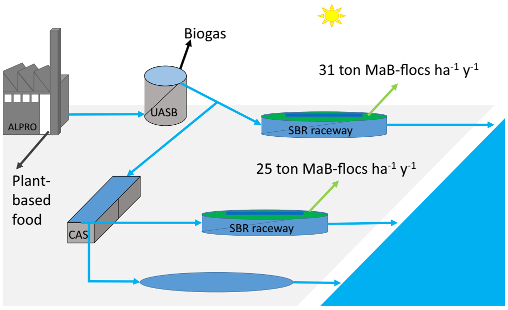
[d] *Alpro,*[5] *Vlamingstraat 28, B-8560 Wevelgem, Belgium*

[e] *Ghent University, Centre for Advanced Process Technology for Urban Resources (CAPTURE), B-9000 Ghent, Belgium*

HIGHLIGHTS

- Outdoor pilot-scale MaB-floc SBR raceway pond treating two food-industry effluents.
- Upflow anaerobic sludge blanket (UASB), conventional activated sludge (CAS).
- Photosynthetic aeration was sufficient for nitrification of UASB effluent.
- Low phosphorus removal in pond treating CAS effluent.
- High biomass productivities of 24.9–31.3 ton TSS ha_{pond}^{-1} $year^{-1}$.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 May 2016
Received in revised form 11 July 2016
Accepted 13 July 2016
Available online 17 July 2016

ABSTRACT

To replace costly mechanical aeration by photosynthetical aeration, upflow anaerobic sludge blanket (UASB) effluent of food-industry was treated in an outdoor MaB-floc raceway pond. Photosynthetic aeration was sufficient for nitrification, but the raceway effluent quality was below current discharge limits, despite the high hydraulic retention time (HRT) of 35 days. Hereafter, conventional activated sludge (CAS) effluent of food-industry was treated in this pond to recover phosphorus. The two-day HRT results in a more realistic pond area, but the phosphorus removal efficiency was low (20%). High biomass

---

Abbreviations: BOD_5, 5-day biological oxygen demand; CAS, conventional activated sludge; COD, chemical oxygen demand; dSVI, diluted sludge volume index; EC, electrical conductivity; HRT, hydraulic retention time; MaB-floc, microalgal bacterial floc; RP, reactive phosphate; SBR, sequencing batch reactor; TIC, total inorganic carbon; TIN, total inorganic nitrogen; TON, total organic nitrogen; TN, total nitrogen; TOC, total organic carbon; TP, total phosphorus; TS, total solids; TSS, total suspended solids; UASB, upflow anaerobic sludge blanket; VS, volatile solids; VSS, volatile suspended solids.

[*] Corresponding author at: Laboratory of Industrial Water and Ecotechnology (LIWET), Department of Industrial Biological Sciences, Ghent University, Graaf Karel de Goedelaan 5, B-8500 Kortrijk, Belgium.

E-mail addresses: shende@espol.edu.ec, sofie_vdhende@yahoo.com (S. Van Den Hende).

[1] Shared last author.

[2] www.ibw.ugent.be.

[3] www.cenaim.espol.edu.ec.

[4] www.ecole-eme.fr.

[5] www.alpro.com.

http://dx.doi.org/10.1016/j.biortech.2016.07.065

productivities were obtained, i.e. 31.3 and 24.9 ton total suspended solids ha_{pond}^{-1} $year^{-1}$ for UASB and CAS effluent, respectively. Bioflocculation enabled successful harvesting of CAS effluent-fed MaB-flocs by settling and filtering at 150–250 μm to 22.7% total solids. To conclude, MaB-floc raceway ponds cannot be recommended as the sole treatment for these food-industry effluents, but huge potential lies in added-value biomass production.

Keywords:
MaB-floc
Algae
Wastewater
Bioflocculation
Nitrification
Phosphorus

1. Introduction

In food-industry, water plays a vital role, and this industry produces a large amount of wastewater, i.e. 2–73 m^3 wastewater ton^{-1} product (Tchobanoglous et al., 2003). In Belgium, anno 2014, food-industry is the largest industry, accounting for 27% of all industrial enterprise (Preillon and Decoster, 2015). The 4532 companies of the Belgian food and drink sector represent a turnover of € 48 billion, and 81.8% of that turnover is realised in Flanders, the northern region of Belgium (Preillon and Decoster, 2015). In this region, high-loaded food-industry wastewater treatment is currently mainly performed in biological systems consisting of an upflow anaerobic sludge blanket system (UASB) for conversion of organic matter to biogas, followed by a mechanically aerated conventional activated sludge (CAS) system for removal of nitrogen and phosphorus. Two major challenges in these wastewater treatment systems are: (1) the high cost of mechanical aeration of CAS, and (2) phosphorus recovery and conversion into valuable biomass. Firstly, in mechanically aerated CAS systems, 45–75% of the total energy consumption of 1.1–2.4 MJ per m^3 wastewater is spent to convert organic carbon into CO_2, while nitrogen is 'wasted' by nitrification/denitrification aeration (Henze et al., 2008; Tchobanoglous et al., 2003). The latter is a significant cost for the food-industry, and, therefore, the food-industry is in search for cheaper alternatives. Secondly, enhanced phosphorus recovery from wastewater by microalgae is of strong interest nowadays, especially as agriculture is looking out to a phosphorus-scarce future (Solovchenko et al., 2016). Also because of this phosphorus scarcity, discharge limits for phosphorus are becoming more stringent in Northwestern Europe. For example, the current discharge limit is 2 mg total phosphorus (TP) L^{-1} for food-industry effluents in Flanders (Vlarem II, 1995), but is expected to decrease to below 1 mg TP L^{-1}. Therefore, phosphorus removal and recuperation technologies are of strong interest to the food-industry and other wastewater-producing industries in this region.

Microalgae can contribute in the needed redesign of wastewater treatment systems. Being photosynthetic microorganisms, microalgae lower the need for mechanical aeration by providing oxygen via photosynthesis, reduce the CO_2 emission by using CO_2 for growth, scavenge resources (C, N, P) from the wastewater, and convert solar energy in valuable biomass (Richmond, 2006). The past decade, there has been a renewed interest in dual-purpose microalgal technology which couples wastewater treatment based on photosynthetic aeration with the production of microalgal biomass (Solovchenko et al., 2016; Wang et al., 2015; Park et al., 2013; Santiago et al., 2013; Udom et al., 2013; Park and Craggs, 2010; Richmond, 2006). Nevertheless, the implementation of this technology has been hampered by various challenges. The relatively high cost of conventional systems for the separation of the microalgal biomass from the treated wastewater is still a challenging matter (Udom et al., 2013). To overcome this harvesting challenge, the innovative concept of microalgal bacterial floc sequencing batch reactors (MaB-floc SBRs) was developed (Van Den Hende et al., 2011). MaB-flocs contain, next to bacteria, microalgae and/or cyanobacteria (further referred to as microalgae, unless otherwise stated, to ease reading). These consortia of bacteria and microalgae aggregate to flocs with a size of around 300 μm to 1000 μm via bioflocculation by growing them in an SBR (Van Den Hende et al., 2011; Van Den Hende et al., 2014a, 2014b). In this SBR, MaB-flocs are fed with wastewater during the day phase. During the night phase, MaB-flocs settle in the reactor. In this way, MaB-floc biomass and treated wastewater are separated in the reactor. After this settling phase, the supernatant of the reactor, which is MaB-floc-free effluent, can be discharged. This separation via bioflocculation avoids expensive microbial biomass removal techniques for effluent discharge, such as the addition of flocculants followed by centrifugation (Udom et al., 2013). This bioflocculation is a large advantage for the implementation on industrial scale of algae-based wastewater treatment.

On lab scale, MaB-floc SBRs showed promising results for both the treatment of upflow anaerobic sludge blanket (UASB) effluent from an industrial company processing plant-based food in Flanders as for the concomitant microbial biomass production (Van Den Hende et al., 2014b). On industrial scale, MaB-floc SBRs should be operated in outdoor raceway ponds, because raceway ponds tend to be the easiest to install and to operate, the cheapest, and the most durable microalgae culture systems (Richmond, 2006). To determine the technical potential of sunlight and algae-based systems for wastewater treatment and biomass production, experiments should be performed outdoors and at pilot scale, as the conversion of lab-scale data to pilot-scale data is not straightforward (Van Den Hende et al., 2014a; Park and Craggs, 2010). Till date, to the best of the authors' knowledge, no scientific data is available on wastewater treatment and biomass production in MaB-flocs SBR raceways receiving food-industry effluents.

To assess the technical potential of MaB-floc technology for treatment of food-industry effluents and concomitant biomass production in Northwestern Europe, experiments were performed in an outdoor pilot-scale raceway pond (28 m^2) in Belgium. Two different food-industry effluents were compared. Firstly, UASB effluent was treated from winter till summer to screen its potential for treatment by photosynthetically aerated MaB-flocs as alternative for conventional activated sludge systems (CAS) with mechanical aeration. Secondly, CAS effluent was treated from summer to winter to screen its potential for further phosphorus polishing. In this paper, the following key parameters evaluating the technical feasibility of MaB-floc SBR raceway ponds for CAS and UASB effluent are presented: (1) wastewater treatment (effluent quality, nutrient removal, and need for flue gas sparging), and (2) MaB-floc biomass characteristics (floc settling, chlorophyll, productivity, and harvesting).

2. Materials and methods

2.1. Wastewater and inoculum

UASB effluent and CAS effluent originated from the company Alpro which produces plant-based food (Wevelgem, Belgium) (Fig. 1; Table 1). UASB effluent was pretreated in a settling tank, and subsequently collected in a buffer tank before feeding to the MaB-floc raceway pond, as described earlier (Van Den Hende et al., 2014a), and further referred to as 'UASB raceway influent'. Twice a week, settled sludge was removed from this settling tank. CAS effluent was collected in the pilot facility buffer tank without prior pretreatment before feeding to the MaB-floc raceway pond, and further referred to as 'CAS raceway influent'. MaB-floc inoculum for the UASB experiments originated from treatment of pikeperch aquaculture wastewater and consisted of MaB-flocs which were dominated by *Klebsormidium* and/or *Ulothrix* sp. (Van Den Hende et al., 2014a). This MaB-floc inoculum was stored at 4 °C prior to its use. MaB-floc inoculum for the CAS experiment originated from the UASB experiment of this study.

2.2. Reactor setup and operation

The pilot-scale MaB-floc facility was operated outdoors (Company Alpro, Wevelgem, Province West-Flanders, Belgium) as an SBR, and consisted of an influent pretreatment settling and buffer tank with influent pump (EUS EVAK Taichung, Taiwan), MaB-floc SBR raceway pond stirred with 2 propeller pumps (Dreno, Italy) and one effluent pump (Industrial pump system bvba, Belgium), effluent buffer tank, harvesting tanks, PLC for steering and data logging, gas boiler for heating (Bulex, Belgium), heating system under the raceway tank, flue gas injection system and tubing, as described earlier (Van Den Hende et al., 2014a) (Fig. 1). The effective pond depth was 0.35 m, resulting in an effective reactor volume of 10.464 m^3. To enhance start-up during winter and to avoid too cool temperatures during spring and autumn, the raceway was heated to a minimum temperature of 12 °C. The SBR cycles consisted of (1) a settling phase during night, (2) an effluent discharge phase in the morning, (3) an influent feeding phase in the morning, and (4) a stirring phase during day time (Table A.1; supplementary data).

The raceway was fed with UASB raceway influent from 24 February 2014 until 13 July 2014 (UASB days 0–140), and with CAS raceway influent from 14 July 2014 until 13 November 2014 (CAS days 0–122). The hydraulic retention time (HRT) was 35 days during the UASB experiment. This HRT decreased from 11.7 to 2.06 days during the adaptation period (CAS days 1–45), and was kept at 2.06 days during the rest of the CAS experiment (CAS days 46–122). To adapt the MaB-floc inoculum from nutrient-poor pikeperch aquaculture wastewater to nutrient-rich UASB raceway influent, the MaB-floc raceway was started up with diluted UASB raceway influent (1 part UASB effluent and 34 parts drinking water), but fed with undiluted UASB raceway influent from day 2 on. In the CAS experiment, synthetic flue gas containing 89 ± 2 g CO_2 Nm^{-3} (Lindegas, Belgium; balance N_2 and no O_2) was sparged in the MaB-floc raceway at 5–8 L min^{-1} when the reactor pH was above 9.00.

MaB-flocs, raceway influent and effluent were sampled twice a week. MaB-floc samples were analyzed for total suspended solids (TSS), volatile suspended solids (VSS), chlorophyll *a*, physiological condition of photosynthetic microorganisms (assessed by the A_{664b}:A_{665a} ratio of MaB-floc extracts), and diluted sludge volume index (dSVI). Influent and effluent samples were analyzed for pH, electrical conductivity (EC), total inorganic carbon (TIC), total organic carbon (TOC), chemical oxygen demand (COD), 5-day biological oxygen demand (BOD_5), turbidity, total nitrogen (TN), total ammoniacal nitrogen (TAN), nitrite (NO_2^-), nitrate (NO_3^-), total inorganic nitrogen (TIN; sum of TAN, N-NO_2^- and N-NO_3^-), TON (total organic nitrogen; difference between TON and TIN), TP, and reactive phosphate (RP).

2.3. Harvesting of MaB-flocs

MaB-flocs were harvested once a week directly from the raceway pond to maintain a MaB-floc density in the raceway of around 0.500 g TSS L^{-1}, for UASB and CAS raceway influent, respectively. Harvesting of MaB-flocs consisted of two steps: (1) pumping from the MaB-floc raceway to a settling tank to allow the MaB-flocs to settle for 1 h, and pumping the supernatant back into the raceway pond, in order to obtain a MaB-floc slurry; and (2) pumping this MaB-floc slurry in a filter bag with pore size of 150–250 μm (Lampe, Belgium), followed by dewatering this MaB-floc slurry in this bag, firstly by natural gravity filtering, and secondly by putting this bag in a hydropress (4 bar; Enotecnica Pillan, Italy) in order to obtain a MaB-floc cake, as described earlier in detail (Van Den Hende et al., 2014a). MaB-floc reactor liquor of the settling tank before settling, supernatant of the settling tank after settling, gravity filtrate and press filtrate were analyzed for VSS and TSS. Dewatered MaB-floc cakes were analyzed for total solids (TS) and volatile solids (VS).

2.4. Analytical methods

All parameters for wastewater and MaB-flocs were analyzed as given by Van Den Hende et al. (2014a). Removal rates and efficiencies were calculated from daily raceway influent and effluent values of the period when MaB-flocs were adapted to each wastewater type (days 64–133 for UASB effluent; days 50–122 for CAS effluent; 2 samples a week). All values given are averages and standard deviations of single points repeated in time of the latter operation periods, unless otherwise stated.

2.5. Statistics

Statistical analyses were carried out using PASW Statistics 22.0 software (SPSS Inc., USA). Normal distribution of data was screened with a Shapiro-Wilk test and homogeneity of variances with a Levene's test. When normal data distribution and homogeneity of variances was observed, significant differences in means of influent and effluent characteristics were analyzed with parametric one-way ANOVA and a Tukey's posthoc test; otherwise, non-parametric Kruskal Wallis including a Bonferroni correction was used ($p < 0.05$). Correlations were quantified with a parametric Pearson's R test (two-tailed significance, $p < 0.05$) or a non-parametric Spearman's r_s test (two-tailed significance, $p < 0.05$).

3. Results and discussion

3.1. UASB effluent

3.1.1. Wastewater treatment

The studied effluent of the UASB system of a company producing plant-based food was rich in carbon, nitrogen and phosphorus (Table 1). This UASB effluent, termed 'UASB raceway influent', was treated in an outdoor MaB-floc raceway pond operated as a SBR. Every morning, effluent of this raceway, termed 'UASB raceway effluent' was discharged. The UASB raceway influents and effluents were compared for pH, EC, turbidity, carbon, nitrogen, and phosphorus species (Table 1).

MaB-floc systems are based on photosynthetic aeration as a sunlight-powered alternative for conventional treatment systems based on mechanical aeration of CAS. Dissolved oxygen concentrations increased typically from 0 mg L^{-1} to 2–16 mg L^{-1} during day, and decreased from 2 to 16 mg L^{-1} to 0 mg L^{-1} during night. It is well-known that photosynthetic growth of microalgae increases the pH (Richmond, 2006). MaB-flocs, a consortium of microalgae and bacteria, increased the UASB raceway influent pH during most of the reactor operation period. This suggests that the daily pH increase due to TIC removal via photosynthesis and/or precipitation was higher than the daily pH decrease due to TIC production

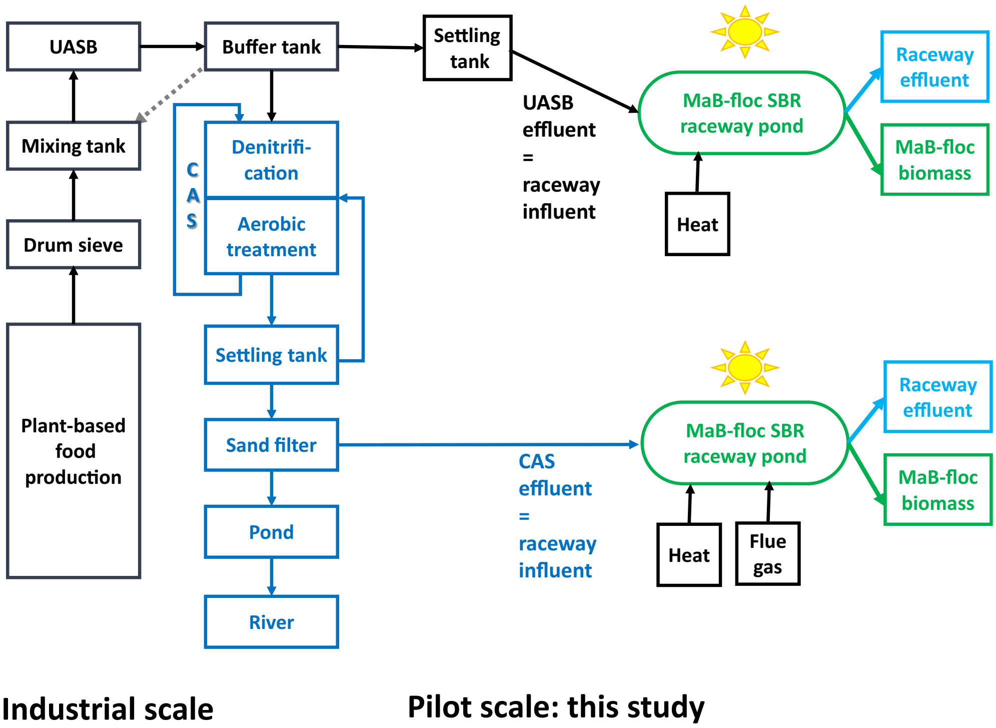


Fig. 1. Origin of wastewaters treated in pilot-scale MaB-floc SBR raceway ponds: UASB and CAS effluents from industrial food-producing company Alpro in Belgium UASB: upflow anaerobic sludge blanket; CAS: conventional activated sludge.

Table 1
Composition of influent and effluent of a MaB-floc SBR raceway treating UASB and CAS effluent from food-industry.

Parameter	Unit	UASB[1]		CAS[1]	
		Influent	Effluent	Influent	Effluent
pH		8.15 ± 0.22[b]	8.65 ± 0.38[a]	8.25 ± 0.13[b]	8.86 ± 0.19[a]
EC	mS cm^{-1}	2.81 ± 0.11[a]	2.30 ± 0.16[b]	2.23 ± 0.24	2.19 ± 0.16
TIC	mg C L^{-1}	377 ± 29[a]	278 ± 51[b]	259 ± 25	243 ± 29
TOC	mg C L^{-1}	199 ± 22[a]	96 ± 25[b]	79 ± 26	55 ± 21
TC	mg C L^{-1}	587 ± 39[a]	382 ± 23[b]	338 ± 17[a]	298 ± 30[b]
COD	mg O_2 L^{-1}	635 ± 86[a]	208 ± 54[b]	57 ± 36	29 ± 18
BOD_5	mg O_2 L^{-1}	318 ± 70[a]	47 ± 22[b]	6 ± 6	2 ± 3
Turbidity	NTU	215.0 ± 44.9[a]	44.1 ± 11.6[b]	13.8 ± 11.2	7.7. ± 3.1
TN	mg N L^{-1}	116.4 ± 26.5[a]	53.4 ± 17.9[b]	8.03 ± 2.59[a]	4.76 ± 2.47[b]
TON	mg N L^{-1}	35.5 ± 24.0	14.6 ± 13.2	3.30 ± 1.97	2.10 ± 1.50
TIN	mg N L^{-1}	84.5 ± 9.7[a]	38.9 ± 10.0[b]	4.65 ± 1.46[a]	2.99 ± 1.52[b]
TAN	mg N L^{-1}	84.3 ± 9.6[a]	1.43 ± 3.22[b]	3.94 ± 1.74[a]	0.35 ± 0.30[b]
NO_2^--N	mg N L^{-1}	0.01 ± 0.01[b]	16.1 ± 18.0[a]	0.47 ± 0.49	0.22 ± 0.26
NO_3^--N	mg N L^{-1}	0.19 ± 0.05[b]	21.2 ± 11.8[a]	0.24 ± 0.10[b]	2.42 ± 1.40[a]
TP	mg P L^{-1}	14.79 ± 3.37[a]	9.81 ± 0.97[b]	2.81 ± 1.08	2.24 ± 0.93
RP	mg P L^{-1}	9.97 ± 2.13	8.48 ± 1.03	2.37 ± 0.84	2.17 ± 0.87

[1] Averages and standard deviations of data points of the time period of days 64–133 for UASB effluent; of days 50–122 for CAS effluent; 2 samples a week.

[a] Significant ($p < 0.05$) differences between influent and effluent of UASB experiments, or between influent and effluent of CAS experiments are indicated with a different letter according to a parametric one-way ANOVA and Tukey's posthoc test, or a non-parametric Kruskal Wallis including a Bonferroni correction.

[b] Significant ($p < 0.05$) differences between influent and effluent of UASB experiments, or between influent and effluent of CAS experiments are indicated with a different letter according to a parametric one-way ANOVA and Tukey's posthoc test, or a non-parametric Kruskal Wallis including a Bonferroni correction.

via microbial respiration. The UASB raceway effluent which was discharged in the morning stayed below the currently allowed effluent discharge level of 9.5 (Table 1). Therefore, injection of flue gas to lower the pH, as demonstrated for microalgae-based raceway systems treating nutrient-poor wastewater, such as for sewage (Park and Craggs, 2010) and aquaculture wastewater (Van Den Hende et al., 2014a), was not needed.

A significant decrease of the studied organic carbon parameters were observed (Table 1). Over 50% of COD, BOD_5, and TOC were removed from the UASB raceway influent without mechanical aeration. This removal remained stable in time. Next to TOC, also a significant decrease in the TIC concentration of UASB raceway influent was observed during the MaB-floc treatment (Table 1). This removal of TIC and the increase of the pH (Table 1) are typical effects of photosynthesis (Richmond, 2006). This shows that at least a part of the microalgae which were present in the MaB-floc raceway pond were performing photosynthesis and producing oxygen for aerobic processes such as nitrification.

For 1 mol of TOC removed from the UASB raceway influent, 0.50 ± 0.94 mol of TIC was removed from this influent. This demonstrates that the *in situ* TIC production from TOC oxidation was lower than the TIC removal by CO_2 uptake for autotrophic microbial growth (e.g. photosynthesis), CO_2 emission to the air and/or *in situ* (bi-)carbonate precipitation. The simultaneous decrease in TIC and decrease in EC (Table 1), an indicator of the total amount of dissolved ions in water, shows that ions and inorganic carbon were removed from the effluent. This means that at least a part of the TIC could have been precipitated with these ions to (bi-)carbonate salts.

Next to carbon, removal of nitrogen is of importance in wastewater treatment. During the total operation period of the MaB-floc raceway treating UASB wastewater, the TIN, TN and TON removal remained relatively stable (Fig. 2d–f), but the TAN, NO_2^-, and NO_3^- concentrations of the effluent changed sharply in time (Fig. 2a–c). The TAN removal steadily increased during the first 60 days of reactor operation (Fig. 2a). Hereafter, the TAN removal remained stable until the end of the operation period, resulting in an average removal efficiency of 99.2 ± 0.6% (day 70–133). After around 30 days, NO_2^- was produced in the MaB-floc raceway, where after it decreased (Fig. 2b), but still resulted in an average concentration in the effluent of 2.56 ± 2.30 mg NO_2^--N L^{-1} (day 99–133). From day 60 on, also the NO_3^- concentration increased (Fig. 2c). These results demonstrate an increased nitrifying activity in the MaB-floc raceway. This increased nitrifying activity of the microbial community in time might have been related to the increasing reactor temperature from winter to summer. Indeed, nitrifying bacteria have an optimal growth temperature of between 25 °C and 30 °C (Tchobanoglous et al., 2003). Moreover, certain microalgae species inhibit the presence of ammonium oxidizing bacteria (Risgaard-Petersen et al., 2004). During the reactor operation period the dominant microalgal species in MaB-flocs shifted from *Ulothrix* sp. or *Klebsormidium* sp. present in the inoculum (Van Den Hende et al., 2014a) to most probably *Desmodesmus* sp. (based on morphological characteristics observed via light microscopy) which was the dominant microalgae species, and unidentified coccal green microalgae species. It remains to be confirmed whether this shift effected the enhanced nitrification.

When at day 73, the NO_2^- concentrations of the raceway effluent were at their highest, TAN, NO_3^- and NO_2^- concentrations in the MaB-floc raceway were determined during a 24 h SBR cycle (Fig. 3). During daytime, NO_2^- increased, while TAN and NO_3^- decreased (Fig. 3). During nighttime, NO_2^- decreased, while TAN remained stable, and NO_3^- first increased to later on steeply decrease (Fig. 3). The growth of ammonium oxidizing and nitrite oxidizing micro-organisms is inhibited by light (Olson, 1981), and by a pH below or above their optimum of around 8.2 and 7.9, respectively (Park et al., 2007). Therefore, a possible explanation could be that during daytime oxidation of NO_2^- to NO_3^- was inhibited by an increased light intensity and pH, and thus resulted in an increase of NO_2^-. Whereas during nighttime, light intensity and pH lowered and NO_2^- was converted into NO_3^- while the settled layer of MaB-flocs was still aerobic; and later on NO_3^- was converted into N_2 via denitrification while the settled layer of MaB-flocs had become anoxic. Also Wang et al. (2015) observed a decrease of NO_2^- during night in a photo-sequencing batch reactor (PSBR) treating anaerobically digested swine manure centrate with addition of organic carbon during night. The latter authors attributed this decrease of NO_2^- to denitritation and to inhibition of nitrite oxidizing micro-organisms due to high concentrations of ammonia and nitrite and low dissolved oxygen concentration in the PSBR. In this study, the TIN only slightly decreased during daytime, but decreased more steeply during nighttime (Fig. 3), which might be a result of denitritation and/or denitrification. It remains to be confirmed whether denitritation and/or denitrification took place in the MaB-floc raceway.

Overall, these results show that MaB-floc raceway ponds can provide adequate levels of oxygen needed for nitrifying bacteria without mechanical aeration. More research is needed to elucidate diurnal fluctuations in nitrogen removal mechanisms in MaB-flocs to further optimize the nitrogen removal and conversion into biomass.

As for TP, a significant removal was obtained, but the TP removal efficiency was much lower compared to nitrogen (Table 2). Moreover, relatively strong oscillations in the RP concentration were observed, and no significant net decrease in the RP concentration was obtained (Table 1). As microalgal growth was observed in this MaB-floc raceway (Section 3.1.2 ‘MaB-floc characteristics’) and phosphorus is essential for microalgal growth (Richmond, 2006), at least some of the phosphorus must have been removed by microalgae present in MaB-flocs. Further research is needed to elucidate whether these microalgae produced extracellular phosphatase which converted organic phosphorus into dissolved orthophosphate, took up organic phosphorus in the form of phytates, and/or associated with bacteria which released RP from organic phosphorus (Solovchenko et al., 2016).

The average ratio of the molar TC removal rate: molar TN removal rate was low (Table 2). Indeed, on average, for every mole of TN removed, 4.06 mol of TC was removed. This means that not all TN could have been removed by photosynthetic biomass production. Also, the ratio of molar TN removal rate: molar TP removal rate was high. On average 38.1 mol TN was removed for each mole TP removed. These ratios are in line with those of treatment of this UASB effluent in a lab scale MaB-floc SBR (4.62 ± 1.49 and 22.9 ± 6.3, respectively for the TC:TN and TN:TP molar removal rate ratios) (Van Den Hende et al., 2014b). These values are also much higher than the typical ‘Redfield’ ratios reported for microalgal biomass, i.e. TC:TN range from 4 to 17, and TN:TP range from 5 to 19 (Geider and La Roche, 2002). Moreover, the MaB-floc productivities showed no significant positive correlation with the removal rates of C, N and P parameters ($r_s = 0.200$, $p < 0.800$; $R = -0.508$, $p < 0.111$; $r_s = 0.105$, $p < 0.687$, respectively). These data demonstrate that microalgal biomass productivity was not the only mechanism for nutrient removal from wastewater in a MaB-floc SBR treating this UASB effluent. Other nitrogen and phosphorus removal mechanisms may have played a role such as settling, predation, volatilization of wastewater compounds, adsorption of particulate matter and dissolved compounds, denitrification, luxury uptake and precipitation.

Overall, the quality of the effluent of the MaB-floc raceway pond was still too poor to be discharged to the river. Indeed, the values of COD, BOD_5, TN and TP of the MaB-floc raceway effluent were still above the current discharge limits of 6.5–9.5, 120, 25, 15 and 2, respectively (Table 1), despite the high HRT of 35 days. Further research is needed to improve the effluent quality of this MaB-floc system, but an increase in HRT is not recommended, as the applied HRT already results in 14.6 ha_{pond} per daily 1500 m^3 effluent of this food company. In comparison, the company Alpro currently applies a HRT of 43 h for its denitrification and nitrification system of this UASB effluent. A HRT of 2–3 days is not realistic for a MaB-floc raceway pond when treating this UASB effluent in a temperate climate. Therefore, in Northwestern Europe, where cheap land area in the vicinity of industry is rather scarce, the implementation of MaB-floc SBR technology on industrial scale cannot be recommended as the sole treatment for highly polluted wastewater such as UASB effluent from food-industry. Further increase in nutrient removal rates are hence needed before turning MaB-floc technology into an industrial reality for treatment of UASB effluent of food-producing industries in this region. Analysing the microbial community structure of MaB-floc raceway ponds will be an important tool to elucidate the nutrient removal mechanisms and improve the nutrient removal rates.

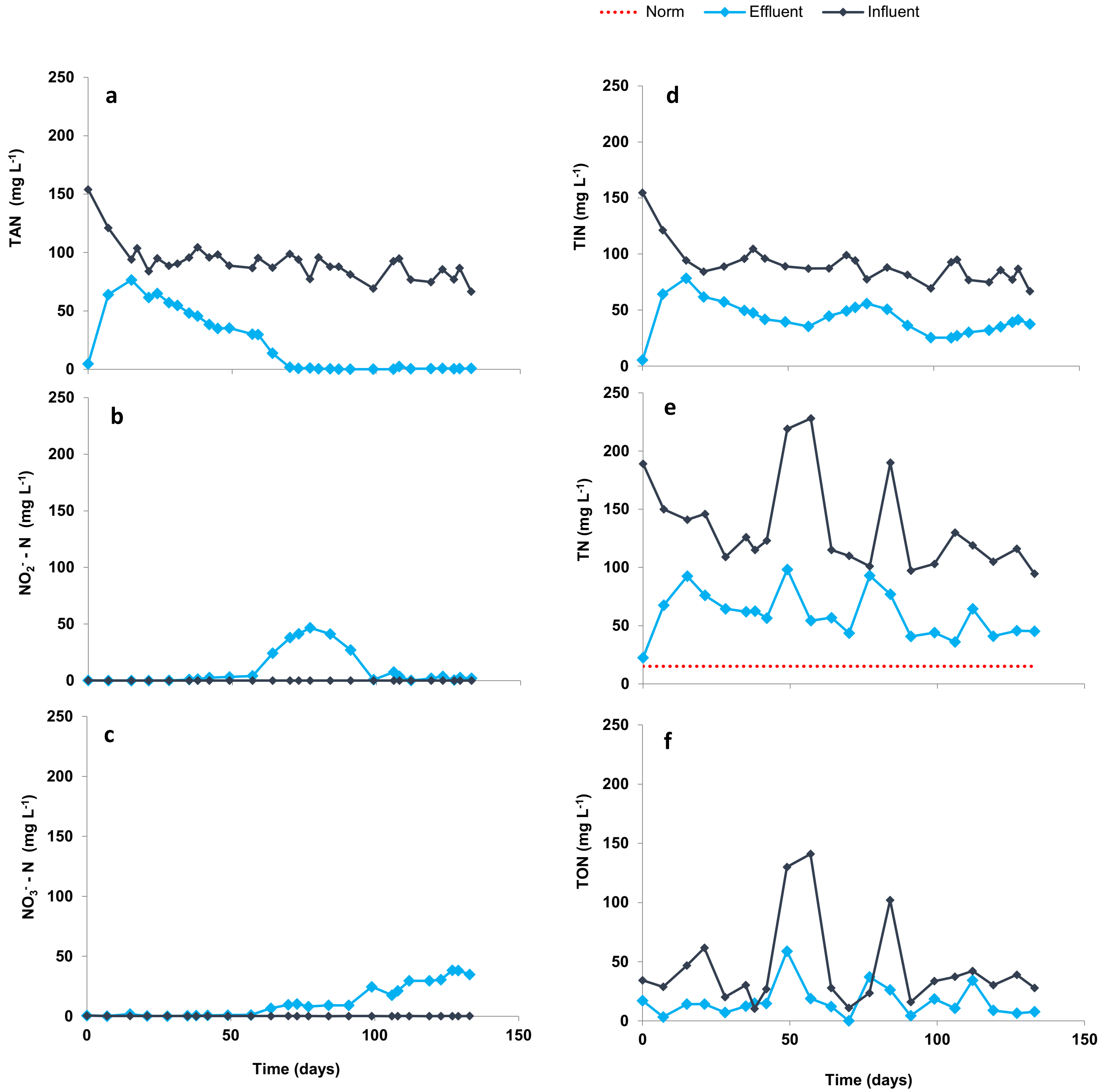


Fig. 2. Evolution in time of nutrient species of influent and effluent of the MaB-floc SBR raceway treating UASB effluent from food-industry: TAN (a), NO_2^- (b), NO_3^- (c), TIN (d), TN (e), TON (f).

3.1.2. MaB-floc characteristics

A first important requirement of MaB-flocs is adequate settling, because it is crucial for safeguarding the discharge of biomass-free effluent and for obtaining a high biomass recovery. The average dSVI value for MaB-flocs treating the UASB effluent (Table 3), that is the volume of 1 g of MaB-flocs after 30 min of settling, was in line with the values obtained earlier for this wastewater in lab-scale experiments, i.e. 69–81 mL g^{-1} TSS (Van Den Hende et al., 2014b). Compared with the dSVI of aquaculture wastewater-fed MaB-flocs obtained at pilot scale (Van Den Hende et al., 2014a), this dSVI is four times higher. As the HRT was 35 days and the reactor depth was 35 cm, only the upper 1 cm (from 35 cm to 34 cm water height) of treated wastewater was daily withdrawn from the raceway pond. According to the dSVI values, the sludge blanket of settled MaB-flocs was lower than 4 cm, and thus a sufficient MaB-floc settling was still obtained to avoid discharge of MaB-flocs.

The density of settled MaB-flocs of 9.1 ± 1.5 g TSS L^{-1} is close to the range obtained on lab scale, i.e. 12–15 g TSS L^{-1} (Van Den Hende et al., 2014b). This demonstrates that up-scaling from lab to pilot scale did not strongly effect the MaB-floc settling properties, and that settling by gravity provides a first harvesting step without addition of expensive and water-contaminating flocculants (Udom et al., 2013).

A second important set of characteristics are the physiological condition and the content of microalgae in MaB-flocs, because microalgae are crucial for photosynthetic aeration and of importance for biomass valorization. The A_{664b}:A_{665a} ratio is an indicator for the physiological condition of the microalgae (Van Den Hende et al., 2011). A ratio of 1.7 indicates pure chlorophyll *a*, and a good

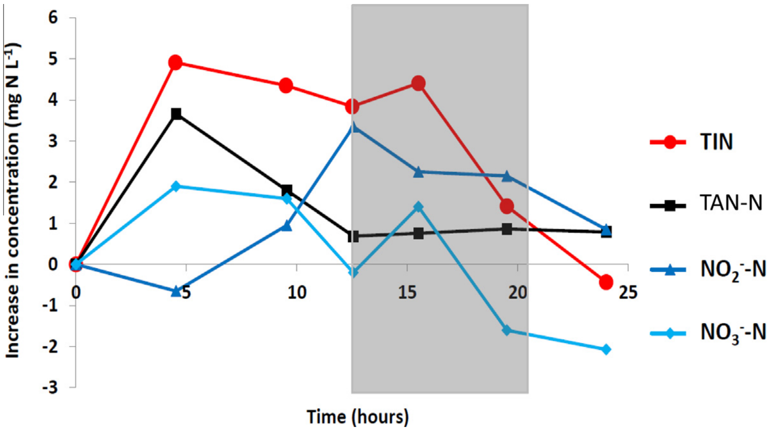


Fig. 3. Diurnal fluctuations of the increase in concentrations of nitrogen species in the MaB-floc SBR raceway pond treating UASB raceway influent at day 73 during daytime (white zone) and nighttime (grey zone).

Table 2
Removal efficiencies and rates of MaB-floc SBR raceways treating UASB effluent and CAS effluent from food-industry and drum filter effluent from aquaculture.

Para- meter	Removal efficiencies (%)			Removal rates (mg $L_{reactor}^{-1}$ day^{-1})		
	Food		Aquaculture[2]	Food		Aquaculture[2]
	UASB[1]	CAS[1]		UASB[1]	CAS[1]	
TIC	26 ± 13	6 ± 7	45 ± 19	2.8 ± 1.5	7.6 ± 8.9	9.8 ± 5.8
TOC	52 ± 9	26 ± 27	33 ± 51	2.9 ± 0.5	11.7 ± 12.9	3.0 ± 5.4
COD	67 ± 8	33 ± 60	28 ± 48	12 ± 3	13 ± 18	4 ± 7
BOD_5	85 ± 6	45 ± 63	53 ± 56	8 ± 2	2 ± 2	1 ± 2
TN	53 ± 17	37 ± 35	31 ± 17	1.80 ± 0.75	1.59 ± 1.56	3.6 ± 3.0
TON	60 ± 51	34 ± 38	n.d.[3]	0.60 ± 0.62	0.75 ± 0.86	n.d.[3]
TIN	53 ± 12	32 ± 39	n.d.	1.28 ± 0.34	0.58 ± 0.83	n.d.
TAN	98 ± 4	89 ± 12	n.d.	2.37 ± 0.28	1.74 ± 0.87	n.d.
NO_2^--N	–[4]	–[4]	n.d.	−0.46 ± 0.52	−1.06 ± 0.70	n.d.
NO_3^--N	–[4]	–[4]	n.d.	−0.60 ± 0.34	0.14 ± 0.28	n.d.
TP	31 ± 14	20 ± 18	64 ± 22	0.14 ± 0.10	0.28 ± 0.25	0.29 ± 0.13
RP	12 ± 19	7 ± 32	n.d.	0.04 ± 0.07	0.10 ± 0.34	n.d.

[1] Averages and standard deviations of data points of the time period of days 64–133 for UASB effluent; of days 50–122 for CAS effluent; 2 samples a week.
[2] Treatment of pikeperch aquaculture wastewater in the same outdoor MaB-floc SBR raceway pond (Van Den Hende et al., 2014a).
[3] No data.
[4] Values not presented, because very low concentrations present in the influents.

Table 3
Characteristics of MaB-floc biomass in raceway ponds treating UASB effluent and CAS effluent from food-industry.

Parameter	Unit	UASB[1]	CAS[1]
Density			
TSS before harvest	mg TSS L^{-1}	0.680 ± 0.093	0.668 ± 0.074
VSS before harvest	mg VSS L^{-1}	0.591 ± 0.093	0.428 ± 0.057
TSS after harvest	mg TSS L^{-1}	0.549 ± 0.077	0.585 ± 0.060
VSS after harvest	mg VSS L^{-1}	0.489 ± 0.074	0.368 ± 0.053
Composition			
VSS:TSS	%	86.7 ± 4.7	64.1 ± 3.8
Chlorophyll *a* content	mg Chla g^{-1} TSS	3.20 ± 1.49	7.52 ± 0.96
Chlorophyll *a* content	mg Chla g^{-1} VSS	3.65 ± 1.62	11.8 ± 1.6
$A664_a$:$A665_b$	–	1.44 ± 0.10	1.60 ± 0.06
Settling			
dSVI	mL g^{-1} TSS	113 ± 18	38 ± 15
Productivity			
Volumetric TSS productivity	mg TSS $L_{reactor}^{-1}$ d^{-1}	24.5 ± 27.7	17.9 ± 20.9
Volumetric VSS productivity	mg VSS $L_{reactor}^{-1}$ d^{-1}	21.5 ± 23.1	10.5 ± 19.1
Areal TSS productivity	g TSS m_{pond}^{2} d^{-1}	8.56 ± 9.70	5.98 ± 7.30
Areal VSS productivity	g VSS m_{pond}^{2} d^{-1}	7.53 ± 8.10	3.66 ± 6.69

[1] Averages and standard deviations of data points of the time period of days 64–133 for UASB effluent; of days 50–122 for CAS effluent; 2 samples a week.

physiological condition of microalgae; while a ratio of 1.0 indicates pure pheophytin *a*, and no living microalgae. The average A_{664b}:A_{665a} ratio of around 1.4 (Table 3) demonstrates the presence of pheophytin *a* in MaB-flocs, and suggests a moderate toxicity of the photosynthetic microorganisms to the UASB effluent, or a lack of nutrients. The chlorophyll *a* content of MaB-flocs was 0.365 ± 0.162% of VSS. This is in the lower range of those of pure microalgae or cyanobacteria cultures of 0.17–4.36% (Piorreck et al., 1994), and suggests a low content of photosynthetic microorganisms in MaB-flocs; a hypothesis confirmed by microscopy. A high VSS:TSS ratio of MaB-flocs was obtained (Table 3). This ratio is similar to the result obtained for this wastewater in lab scale experiments, i.e. 87.0 ± 7.8% (Van Den Hende et al., 2014b). A high VSS:TSS ratio, and thus low ash content, is important for biomass valorization.

A third important characteristic of MaB-flocs is their biomass productivity. To date, high microalgal biomass productivities are still regarded as priority in wastewater-treating high rate algal ponds in order to make resource recovery from these ponds economically feasible (Solovchenko et al., 2016; Richmond, 2006). Without improvements, MaB-floc productivities were expected to be lower than lab-scale results because of the lower temperature, lower photon flux, and lower irradiated surface-to-volume ratio. Indeed, the volumetric TSS and VSS productivities obtained at pilot scale (Table 3) were 10 times less compared with previous results on lab scale, i.e. 257 ± 116 mg TSS $L^{-1}_{reactor}$ day^{-1} and 223 ± 90 mg VSS $L^{-1}_{reactor}$ day^{-1} (Van Den Hende et al., 2014b). The volumetric biomass productivities were higher than those obtained with the MaB-floc SBR treating aquaculture wastewater on pilot scale (Van Den Hende et al., 2014a), especially for the VSS productivity which almost tripled. However, they are twice lower compared to the results of Park and Craggs (16.7 g TSS m^{-2} day^{-1}) (2010), obtained in a HRAP with sewage and CO_2 addition and a HRT of 4 days; and 1.5 times lower than 11.4 g VSS m^{-2} -day^{-1} obtained by Santiago et al. (2013), in a HRAP with UASB effluent. One of the reasons for these relatively lower biomass productivities obtained in this study might be biomass loss due to predators. The fact that around 20% of the measured biomass productivities were negative, and that MaB-floc predators were observed (mainly mosquito larvae), confirm this assumption.

Based on these pilot-scale results (Table 3), an average productivity of 31.3 ton TSS $ha^{-1}_{pond\ area}$ year^{-1} or 27.5 ton VSS $ha^{-1}_{pond\ area}$ -year$_{-1}$ is calculated. Assuming that per ha MaB-floc pond 1.5 ha land is needed, a 20.3 ton TSS $ha^{-1}_{land\ area}$ year^{-1} and 18.3 ton VSS $ha^{-1}_{land\ area}$ year^{-1} would be obtained in case of UASB effluent. These values are higher than productivities of conventional crops in Belgium yielding, after several decades of engineering, maximum 12–16 ton dry matter ha^{-1} year^{-1} (Peeters, 2010). Since for MaB-flocs, these high productivities are already obtained without any research being done to improve their biomass yield, these results show the large potential for microalgal biomass production in MaB-floc raceway ponds on UASB effluent.

Overall, MaB-flocs grown on UASB effluent showed an adequate settling and relatively high biomass productivity, but a rather low microalgae content. Further research is needed on the valorisation of this biomass, in, for example, a biorefinery concept.

3.2. CAS effluent

3.2.1. Wastewater treatment

To screen whether MaB-floc technology can be used as an additional phosphorus polishing step, CAS effluent of food-industry was treated in an outdoor MaB-floc raceway pond. The CAS effluent which was fed to the MaB-floc raceway is termed 'CAS raceway influent'; the resulting effluent after treatment in the MaB-floc raceway is termed 'CAS raceway effluent' (Table 1, Table 2). Due to the low concentrations of nutrients (N, P) in this CAS raceway influent (Table 1), the HRT was changed from 35 days for UASB raceway influent to 2.06 days for CAS raceway influent. This leads to a more realistic scenario of 0.9 ha_{pond} per daily 1500 m^3 wastewater produced by the food company of this case study, compared to the UASB effluent scenario. The TP concentrations of both the CAS raceway influent and CAS raceway effluent were above the current discharge limit of 2 mg TP L^{-1} (Table 1). At the industrial site, all this CAS effluent is stored in a buffer pond and mixed with other nutrient-free effluents of this company, so that the final effluent which is discharged to the river Leie meets current discharge limits including for P. Although during the CAS experiment the average TP removal rate in the MaB-floc reactor doubled compared to UASB experiment (Table 2), the TP removal efficiency of around 20% (Table 2) is still too low compared to other phosphorus removal technologies (Henze et al., 2008; Tchobanoglous et al., 2003).

During treatment of CAS raceway influent in a MaB-floc raceway, the pH increased during the light phase and decreased during the dark phase. The pH decrease at dark was not sufficient to reach the effluent discharge limit of 9.5. Moreover, an elevated pH can negatively impact on photosynthesis and microalgal growth through altered uptake of trace metals, and changes in membrane transport processes and metabolic functions (Clark and Flynn, 2000). A pH above 9 leads to shifts in inorganic carbon species, with the concentration of HCO_3^- and CO_3^{2-} increasing, while CO_2 decreasing (Richmond, 2006). Most microalgae preferentially uptake CO_2 through passive diffusion over HCO_3^- or CO_3^{2-}, because the uptake of the latter two carbon species require metabolically expensive carbon concentrating mechanisms (Richmond, 2006). Nitrifying bacterial communities have been reported to have an optimal growth at a pH around 8 in wastewater-treating ponds (Antoniou et al., 1990). Therefore, flue gas was sparged in the raceway during day time. In this way, the average pH of CAS raceway effluent was below the limit of 9.5 (Table 1). Only low volumetric flue gas flow rates were applied of maximum 0.00096 vvm (volume gas per volume reactor per minute). This is of importance because a decreased flue gas flow rate means (1) less gas pumping and thus lower energy and maintenance cost, and (2) a smaller number of flue gas blower systems in the raceway pond, and thus decreased capital and maintenance costs. Moreover, by applying a low flue gas flow rate, an increase in the TIC of the raceway effluent was avoided, and MaB-floc treatment still resulted in a significant decrease of the TC concentration (Table 1).

Similar to the UASB case study, TAN was removed (Fig. 4a), NO_2^- remained stable (Fig. 4b) and NO_3^- was produced (Fig. 4c) in the MaB-floc raceway receiving CAS influent (Table 1). This demonstrates nitrification by MaB-flocs. There was a significant difference between the TIN and TN of the influent and the effluent, but not for TON (Table 1; Fig. 4d–f). On average, for each mole of nitrogen removed, 16.4 mol carbon was removed, and suggests that not all carbon removal was due to biomass production. The relatively high ash content of MaB-flocs of 35.9 ± 3.8% of TSS (Table 3) suggests that carbon could have been removed as (bi-)carbonate precipitates. Light and fluorescence microscopy confirmed the presence of crystals in the MaB-flocs grown on CAS raceway influent. The average ratio of TN molar removal rate: TP molar removal rate (Table 3) was in line with that for microalgal biomass, i.e. TN:TP of 5–19 (Geider and La Roche, 2002), and no additional nitrogen loss via denitrification is expected.

Overall, sunlight-based MaB-floc treatment of CAS effluent cannot (yet) be recommended as an efficient phosphorus-scavenging technology for Northwestern Europe. Further research is needed to improve the phosphorus removal, by, for example, adding limiting micronutrients which may enhance both chemical phosphorus precipitation and microalgal biomass growth.

Furthermore, future research should include detailed analyses of the microbial community structure of MaB-floc raceway ponds to elucidate the major nutrient removal mechanisms and increase the nutrient removal rates.

3.2.2. MaB-floc characteristics

Compared to MaB-flocs grown on UASB raceway influent, MaB-flocs grown on CAS raceway influent showed a decreased dSVI (Table 3). This means an improved settling in the UASB raceway

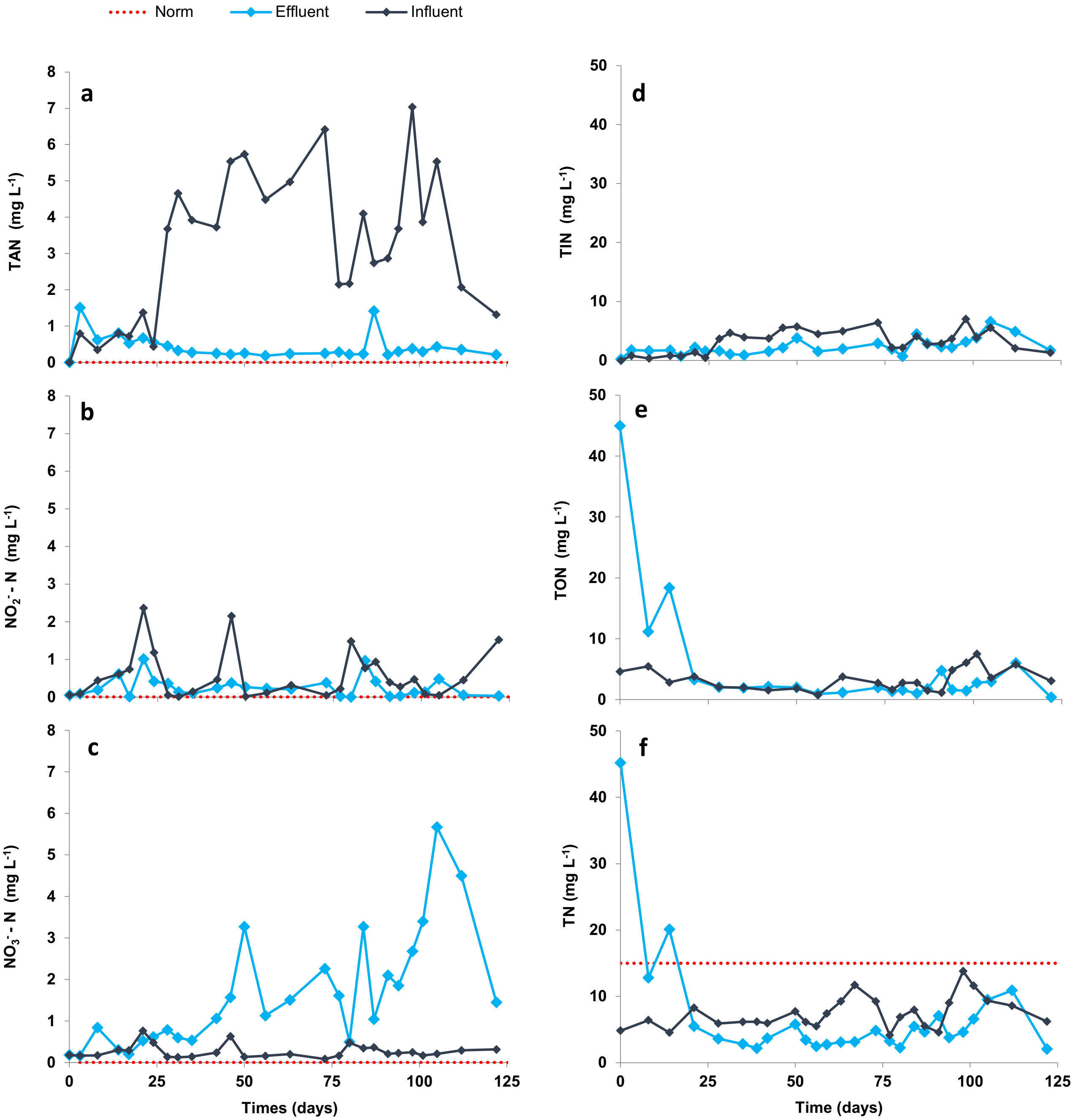


Fig. 4. Evolution in time of nutrient species of influent and effluent of the MaB-floc SBR raceway treating CAS effluent from food-industry: TAN (a), NO_2^- (b), NO_3^- (c), TIN (d), TN (e), TON (f).

influent compared to the UASB raceway influent scenario. Likewise, the VSS:TSS of the latter MaB-flocs decreased (Table 3). This is in line with a previous study, where it was observed that a decreased VSS:TSS ratio was positively correlated with a decreased dSVI, and thus improved settling (Van Den Hende et al., 2014a). This improved settling could be explained by the presence of crystals in the MaB-flocs (light and fluorescence microscopy). The obtained dSVI of the MaB-flocs of this CAS case study was low enough to avoid MaB-floc loss via daily withdrawal of half of the raceway volume.

Changing the wastewater type from white-coloured UASB raceway influent rich in TAN to transparent CAS raceway influent poor in TAN (Table 1), strongly improved the physiological condition of the microalgae and the chlorophyll *a* content of MaB-flocs. Indeed, both the A_{664b}:A_{665a} ratio and the chlorophyll *a* content of MaB-flocs increased during the adaptation period (data not shown), and remained stable from day 50 till day 122 (Table 3). This chlorophyll *a* result suggests a high content of photosynthetic microorganisms in MaB-flocs as it is in the mid-range of those of pure microalgae or cyanobacteria cultures of 0.17–4.36% (Piorreck et al., 1994). Microscopic observations confirmed the dominance of large colonies of up to 1000 μm of coccal cyanobacteria in MaB-flocs grown on CAS raceway influent, with a morphology similar to *Aphanothece* sp. and *Aphanocapsa* sp. Based on DNA fingerprinting, cyanobacterial species present in these MaB-flocs were identified as closely related to *Geminocystis* sp. strain NIES-3708 (97.3% similarity), and closely related to an uncultured cyanobacteria species (97.7% similarity) (Van Den Hende et al., 2016).

Cyanobacteria such as *Aphanothece* sp. and *Aphanocapsa* sp. are of interest for production of pigments (e.g. phycocyanine). Recently, it was shown that these MaB-flocs contain a relatively high content of the high-value phycochemicals C-phycocyanin, C-phycoerythrin and neophytadiene (Van Den Hende et al., 2016). This demonstrates the stronger potential of CAS raceway influent for microalgal biomass production in an outdoor raceway in Northwestern Europe based on bioflocculation compared to UASB raceway influent.

The volumetric TSS and VSS productivities of MaB-flocs grown on CAS raceway influent were lower compared to the results obtained for UASB raceway influent (Table 3). Based on these pilot-scale results, 24.9 ton TSS $\text{ha}^{-1}_{\text{pond area}}$ year^{-1} and 15.3 ton VSS $\text{ha}^{-1}_{\text{pond area}}$ year^{-1} is calculated. These biomass productivities are similar to those obtained with the MaB-floc SBR treating aquaculture wastewater on pilot scale (Van Den Hende et al., 2014a), and to results obtained in autumn-spring-winter in a HRAP with recycling microalgal bacterial biomass in a HRAP with settling cone in New Zealand (3.3–12.7 g VSS m^{-2} day^{-1}) (Park et al., 2013). However, they are twice lower compared to the results of Park and Craggs (2010) who obtained 16.7 g TSS m^{-2} day^{-1} in a HRAP receiving sewage and CO_2 and had a HRT of 4 days; and those of Santiago et al. (2013) who obtained 11.4 g VSS m^{-2} day^{-1}in a HRAP with UASB effluent. Assuming that per ha MaB-floc pond 1.5 ha land is needed, a 16.6 ton TSS $\text{ha}^{-1}_{\text{land area}}$ year^{-1} and 10.2 ton VSS $\text{ha}^{-1}_{\text{land area}}$ year^{-1} would be obtained in case of CAS effluent. These values are in the higher range of conventional crops in Belgium yielding maximum 12–16 ton dry matter ha^{-1} year^{-1} (Peeters, 2010). Since for MaB-flocs, a high productivity is obtained without any research being done to improve this productivity, this study shows the huge potential of MaB-floc raceways for microalgal biomass production.

A key factor for sustainable wastewater treatment and concomitant biomass production by microalgae is an efficient and cost-effective biomass harvesting (Udom et al., 2013). Microalgae harvesting represents 20–60% of the total microalgae production costs (Udom et al., 2013). In this study, harvesting consisted of two steps: (1) concentration by 1 h gravity settling in a settling tank, and (2) dewatering of the settled biomass by gravity filtering followed by press filtering. Gravity settling increased the MaB-floc density with a factor 45 to 30.3 ± 11.1 g TSS L^{-1} or 19.4 ± 7.1 g VSS L^{-1}. These results are in the same range as for microalgae which were grown on sewage in an outdoor raceway pond and harvested via gravity settling, i.e. 18–35 g VSS L^{-1} (Park et al., 2013). Settling resulted in a MaB-floc loss of 10.3 ± 6.0% of TSS. The MaB-floc loss due to settling obtained in this study was lower compared to the one obtained in a gravity algal settler (33% after 1–3 days) (García et al., 2006), higher compared to the MaB-floc TSS loss of aquaculture wastewater-fed MaB-flocs of 7.9 ± 5.9% (Van Den Hende et al., 2014a), but still in the same range as compared to microalgae settling by flocculants addition (2–15%, after 1 h settling) (Udom et al., 2013), and in the same range as by bioflocculation in a biomass-recycling raceway treating sewage (14–75% yearly average after 1 h of settling) (Park et al., 2013). Moreover, in the present study, the MaB-floc biomass present in the supernatant was not lost, as it was pumped back into the raceway to increase biomass recovery during the 10 times longer settling period at dark, and to enable raceway stirring without decreasing the HRT (i.e. adding extra influent).

The second harvesting step resulted in a total loss of 10.7 ± 6.4% of TSS, with 5.0 ± 3.6% of TSS lost during gravity filtering, and 5.8 ± 5.3% of TSS lost during press filtering. This is slightly better compared to previous lab-scale results where a TSS loss of 12.0 ± 5.6% was obtained during filtering of MaB-flocs grown on food-industry wastewater (Van Den Hende et al., 2014b). A relatively large pore size was used (150–250 μm) for filtering. Further optimization by finding the optimal pore size is needed. Dewatering increased the TSS densities of settled MaB-floc slurry to a MaB-floc cake of 22.7 ± 3.7% TS and 14.1 ± 1.8% VS. This value is double as high compared to a lab-scale study with food-industry wastewater with 11.8 ± 1.5% TS (Van Den Hende et al., 2014b). The average harvestable biomass productivity, calculated based on harvesting efficiencies in accordance to Park et al. (2013), was 5.24 g TSS m^{-2} d^{-1} or 3.36 g VSS m^{-2} d^{-1}.

Recently, it was shown that MaB-flocs grown on CAS effluent contain high contents of phycobiliproteins (Van Den Hende et al., 2016). These results demonstrate the huge potential of MaB-floc technology to convert CAS raceway influent into phycobiliprotein-rich MaB-flocs in Northwestern Europe where land area is available. Moreover, research is warranted (and is part of current research) to optimize this technology in regions where light and temperature conditions are less fluctuating, such as in countries in tropical climates.

4. Conclusions

Outdoor pilot-scale MaB-floc SBR raceway ponds converted food-industry effluents into biomass. In case of UASB effluent, photosynthetic aeration was sufficient for nitrification, but the effluent quality was below current limits, despite the high HRT of 35 days. In case of CAS effluent, the 2-day HRT is more realistic, but phosphorus removal efficiency was low. For both effluents, high biomass productivities were obtained. Huge potential lies in added-value biomass production, especially for phycobiliprotein-rich MaB-flocs grown on CAS effluent. Further research should include optimization of the wastewater treatment and implementation in tropical countries where land area is available.

Acknowledgements

This work was supported by the INTERREG IVB NWE Program, the Flemish Government, and Province West-Flanders within the EnAlgae project. The authors greatly acknowledge Alpro n.v. for the pilot reactor location, electricity, wastewaters and technical support; Bebouwen and Bewaren n.v. and CATAEL b.v.b.a. for the construction of the pilot-scale MaB-floc raceway pond facility; Rita Beyaert for making all MaB-floc harvesting filter bags; Jeroen Van Wichelen of UGent for the algae species determination; Jorge Sonnenholzner of ESPOL-CENAIM for his valuable comments and proofreading.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.07.065.

References

Antoniou, P., Hamilton, J., Koopman, B., Jain, R., Holloway, B., Lyberatos, G., Svoronos, S.A., 1990. Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. Water Res. 24 (1), 97–101.

Clark, D.R., Flynn, K.J., 2000. The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton. In: Proc. R. Soc. Lond. B 267 (1447), 953–959.

García, J., Green, B.F., Lundquist, T., Mujeriego, R., Hernández-Mariné, M., Oswald, W.J., 2006. Long-term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresour. Technol. 97, 1709–1715.

Geider, R.J., La Roche, J., 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur. J. Phycol. 37 (1), 1–17.

Henze, M., van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment: Principles. IWA Publishing, London, Modelling and Design, p. p511.

Olson, R.J., 1981. Differential photoinhibition of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum. J. Mar. Res. 39, 227–238.

Park, J.B.K., Craggs, R.J., 2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. Water Sci. Technol. 61 (3), 633–639.

Park, S., Bae, W., Chung, J., Baek, S.-C., 2007. Empirical model of the pH dependence of the maximum nitrification rate. Process Biochem. 42 (12), 1671–1676.

Park, J.B.K., Craggs, R.J., Shilton, A.N., 2013. Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. Water Res. 47, 4422–4432.

Peeters, A., 2010. Country Pasture/Forage Resource Profile for Belgium. FAO AGPC, Rome, p. 61, <http://www.fao.org/ag/AGP/ AGPC/doc/Counprof/Belgium/belgium.htm>.

Piorreck, M., Baasch, K.H., Pohl, P., 1994. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochem. 23 (2), 207–216.

Preillon, N., Decoster, W., 2015. Innovative Food Solutions. Belgian Foreign Trade Agency, p. 64, <http://www.abh-ace.be/sites/default/files/Economic_studies/toral_publication/aa048_sectorale_studie_food_full.pdf>.

Richmond, A., 2006. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwell Sciences Ltd., Oxford, UK, p. p588.

Risgaard-Petersen, N., Nicolaisen, M.H., Revsbech, N.P., Lomnstein, B.A., 2004. Competition between ammonia-oxidizing Bacteria and benthic microalgae. Appl. Environ. Microbiol. 70 (9), 5528–5537.

Santiago, A.F., Calijuri, M.L., Assemany, P.P., Calijuri, M.C., Dos Reis, A.J., 2013. Algal biomass production and wastewater treatment in high rate algal ponds receiving disinfected effluent. In: Environ. Technol. 34 (13–16), 1877–1885.

Solovchenko, A., Verschoor, A.M., Jablonowski, N.D., Nedball, L., 2016. Phosphorus from wastewaters to crops: an alternative path involving microalgae. Biotechnol. Adv. 34 (5), 550–564.

Tchobanoglous, G., Burton, F.L., Stensel, H.D. (Eds.), 2003. Wastewater Engineering Treatment and Reuse, fourth ed. McGraw-Hill Higher Education, Metcalf & Eddy Inc., Boston, p. 823.

Udom, I., Zaribaf, B.H., Halfhide, T., Gillie, B., Dalrymple, O., Zhang, Q., Ergas, S.J., 2013. Harvesting of microalgae grown on wastewater. Bioresour. Technol. 19, 101–106.

Van Den Hende, S., Vervaeren, H., Saveyn, H., Maes, G., Boon, N., 2011. Microalgal bacterial floc properties are improved by a balanced inorganic/organic carbon ratio. Biotechnol. Bioeng. 108 (3), 549–558.

Van Den Hende, S., Beelen, V., Bore, G., Boon, N., Vervaeren, H., 2014a. Up-scaling aquaculture wastewater treatment by microalgal bacterial flocs: from lab reactors to an outdoor raceway pond. Bioresour. Technol. 159, 342–354.

Van Den Hende, S., Carré, E., Cocaud, E., Beelen, V., Boon, N., Vervaeren, H., 2014b. Treatment of industrial wastewaters by microalgal bacterial flocs in sequencing batch reactors. Bioresour. Technol. 161, 245–254.

Van Den Hende, S., Beyls, J., De Buyck, P.-J., Rousseau, D.P.L., 2016. Food-industry effluent-grown microalgal bacterial flocs as a bioresource for high-value phycochemicals and biogas. In: Algal Res. 18, 25–32.

Vlarem, I.l., 1995. Besluit van de Vlaamse Regering houdende algemene en sectorale bepalingen inzake milieuhygiëne, Vlarem II (1 juni 1995). Available from <http://navigator.emis.vito.be/milnav-consult/>.

Wang, M., Yang, H., Ergas, S.J., van der Steen, P., 2015. A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR). Water Res. 87, 38–48.