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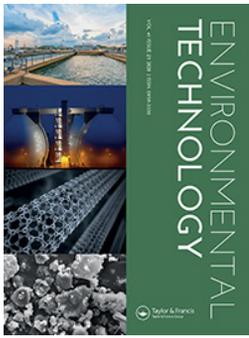
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Effect of slow biodegradable substrate addition on biofilm structure and reactor performance in two MBBRs filled with different support media

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ABSTRACT

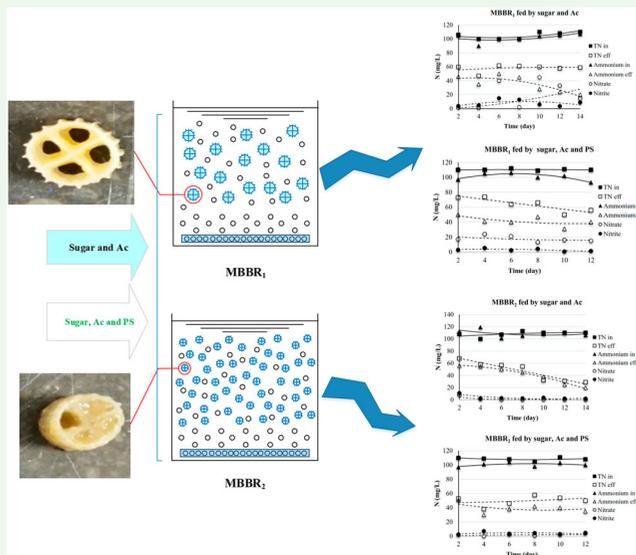
In this study, two moving-bed biofilm reactors (MBBR₁ and MBBR₂) filled with different size of carrier media (Kaldnes K₁ and Kaldnes K₁ micro, respectively) were subjected to soluble (sugar and sodium acetate (Ac)) substrate and mixture of soluble and particulate (particulate potato starch (PS)) substrate in a very high organic loading rate (12 kgCOD/m³·d) at different temperatures (26 and 15°C, in MBBR₁ and MBBR₂, respectively). The effects of carrier type and substrate on biofilm structure and reactor performance have been studied. Starch was removed by adsorption at the biofilm surface and hydrolyzed which caused substrate gradient in MBBR₁, however, hydrolyzed uniformly within biofilm in MBBR₂. The biofilm of MBBR₁ was irregular due to filamentous structure growth due to the substrate gradient, while, it was regular in MBBR₂ due to uniform distribution of substrate. The performance of both MBBRs in ammonium, COD and TN removal decreased significantly when the amount of small particles in the reactor increased owing to feeding by starch, which led to biomass density decline. The type of media affected the quantity and distribution of attached biomass, which in turn influenced the activity of specific microbial functional groups in the biofilm. The biofilm in MBBR₂ was thicker and consequently nitrogen removal by denitrification was much higher. The lower temperature did not affect negatively the reactor performance in MBBR₂.

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MBBR; slowly biodegradable substrate; potato starch; simultaneous nitrification-denitrification (SND); particle size distribution



1. Introduction

Compared to the suspended biomass process, Moving Bed Biofilm Reactor (MBBR) has definite advantages such as higher biomass concentration, higher chemical oxygen demand loading, sturdy tolerance to loading

impact, longer sludge age, lower hydraulic retention time (HRT), higher volumetric removal rates, no sludge recirculation, relatively small area requirements and no sludge bulking issues [1,2]. The MBBR process has proved to be a very simple and efficient technology in

municipal and industrial wastewater treatment strategies. In 2009 there were more than 600 MBBRs operating in 50 countries [3]. The utilization of attached instead of suspended biomass benefits a very compact reactors and easier separation of the bio-solids from the treated effluent [4]. The moving bed biofilm reactor (MBBR) is a growing biofilm technology which has acquire considerable attention in the wastewater treatment in the last 20 years [5]. It is based on the use of generously moving plastic carrier elements with density a little lighter than that of water in which microorganisms form biofilms [6]. The MBBR technology promotes biofilm attachment and growth on engineered carriers that are maintained in constant suspension. The attached biofilms are preserved and protected from abrasion with other carriers in the interior spaces of the MBBR carriers [7]. Consequently, the biofilm carriers in the MBBR play a significant role in microbial attachment control, as well as the type of reactor operation and process effectiveness. To date, various carriers have been introduced in the MBBR process, including polyethylene plastics, polyurethane sponge, polyvinyl alcohol gel, biodegradable polymer, granular activated carbon, polymer foam pads, nonwoven media, etc., [8–13]. This biofilm process has been extensively used for the treatment of synthetic [14,15], domestic [16,17] and industrial wastewaters [16,18]. Environmental conditions such as substrate availability and hydrodynamics might lead to various physical structures of biofilms, rough or smooth, porous or compact biofilms [19–22]. Given the benefits presented by the MBBR process such as compactness, flexibility and high quality effluent production, a rapidly growing market for this technology has been established worldwide [4]. Since nitrogen compounds are a significant threat to natural aquatic ecosystems, mainly because they play an important role in the eutrophication process, the environmental legislation has become increasingly stringent concerning nitrogen concentration in the effluents from wastewater treatment plants (WWTPs), which are major sources of nitrogen compounds [23]. Traditionally, biological nitrogen removal from wastewater has been accomplished using nitrification and heterotrophic de-nitrification [24]. Since the carrier material employed in MBBR processes effect the attachment and distribution characteristics of the biofilm, it is interesting to understand how the type of support media will influence the activity of nitrifiers in situations where overgrowth of heterotrophs takes place, such information may potentially be used in the selection of appropriate biofilm carriers for MBBRs treating high loaded wastewaters [4]. Most of the studies on MBBR up to now, focused on optimizing its performance, such as the optimal filling degree [25], the effect of carrier geometry [26] or microbial community structure [27]. There are

Table 1. Characteristics of Kaldnes K_1 bio-carrier.

Characteristics	Kaldnes K_1 [4]	Kaldnes K_1 micro [28]
Material	High-density polyethylene cylinder	High-density polyethylene cylinder
Shape		
Nominal diameter (mm)	9.1	6
Nominal length/thickness (mm)	7.2	6
Apparent density (kg/m^3)	150	413
Specific surface area (m^2/m^3)	500	900

lacks of studies, which investigate the biofilm structure development on the carriers in MBBR system, which is fed by particulate substrate (e.g. potato starch) and effect of the carriers' size, temperature and substrate on MBBRs' performance, in COD and nitrogen removal. In the light of this background, this work attempted to evaluate the effect of soluble and particulate substrates on COD and nitrogen conversions in two lab scale MBBRs. In order to perceive the influence of the temperature and support media on MBBR's overall performance as well as on the dynamics of floc and biofilm throughout the experiment, each reactor was filled with different sizes of carriers (same in material and shape) which were operated at different temperatures.

2. Material and methods

2.1. Experimental equipment and carriers

Two aerobic MBBR systems consisting of an influent tank and reactor were used in this study. The reactors were made of transparent Plexiglas in a rectangular shape (the volume of MBBR₁ and MBBR₂ were 5.25 and 3L, respectively) which were aerated by porous stone diffusers located at the bottom of the systems and compressed air was supplied to them. The pH and DO were constant at 7 and 2 mg/L, the temperature for MBBR₁ and MBBR₂ were 26 and 15°C, respectively. The flow rate for both of MBBRs was 31.5 L/d. HRT (Hydraulic Retention Time) for MBBR₁ and MBBR₂ was 4 and 2.3 hr, respectively. Kaldnes K_1 and Kaldnes K_1 micro carriers were used for MBBR₁ and MBBR₂, which their characteristics are demonstrated in Table 1. In order to have the same surface area for biofilm development in both reactors, the filling fraction was chosen to 40% and 22% of the operational volume in the reactors for MBBR₁ and MBBR₂, respectively.

2.2. Biomass inoculum

MBBRs were inoculated with activated sludge taken from Harnaspolder wastewater treatment plant (South Holland, Netherlands). Total suspended solids (TSS) and

volatile suspended solids (VSS) were 3 and 2 g/L respectively. At first, two MBBRs were started- up with a mixture of sugar and Sodium Acetate (Ac). Once the sugar and Ac were fully converted in the reactors, we added PS in the influent.

2.3. Influent medium

The applied synthetic wastewater for both reactors consisted of two media. The media were separated in carbon source (Medium A: C-source with a final concentration of 2 gCOD/L; 3.6 mM $MgSO_4 \cdot 7H_2O$; 4.7 mM KCl) and medium B (69 mM NH_4Cl ; 4.2 mM K_2HPO_4 ; 2.1 mM KH_2PO_4 ; 15 mL milk; 10 mL/L trace element solution according to [29]). Every time, 150 mL of both media was dosed to the reactor together with 1.2 L of tap water. The final dosage of COD-load and N-load during the experiments were 12 and 21 kgCOD/m³-day and; 0.6 and 1 kgN/m³-day for MBBR₁ and MBBR₂, respectively. During the first period, a mixture of sugar and sodium acetate (Ac) (ratio 1:1) was used as carbon source for both of reactors, while, in the second period, particulate potato starch (PS) was added as carbon source as well as sugar and sodium acetate (ratio 1:1:1). The starch vessel was continuously stirred [30].

2.4. Analytical methods

The analyses are divided in continuous measurements (online), daily to weekly measurements, some other less frequent measurements and the cycle measurements. Total suspended solids (TSS) and volatile suspended solids (VSS) were measured for floc, as described in [31] and for biofilm as described in [4]. Biofilm thickness was determined by microscopy.

COD, $NH_4^+ - N$, $NO_2^- - N$, $NO_3^- - N$ and TN concentrations in the bulk liquid were determined

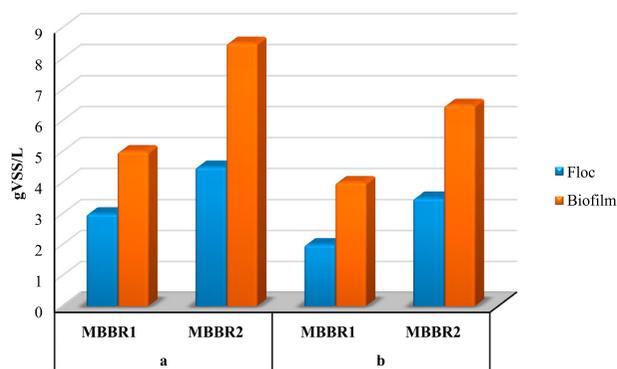


Figure 1. Biomass (floc and biofilm) concentration in MBBR₁ and MBBR₂ in soluble substrate fed period (a) and soluble with particulate substrate fed period (b).

spectrophotometrically by use of standard test kits (Dr. Lange type LCK; manufacturer: Hach Lange, Dusseldorf, Germany). Particulate COD could be visualized by colouring the starch particles with an Iodine solution prior to microscopy (according to [30]).

3. Results

3.1. Biofilm formation

Biomass concentration increased from 1 gVSS/L at the beginning of the operation to 8 (floc = 3 gVSS/L, biofilm = 5 gVSS/L, Figure 1a) and 13 gVSS/L (floc = 4.5 gVSS/L, biofilm = 8.5 gVSS/L, Figure 1a) on day 45 for MBBR₁ and MBBR₂, respectively (30 days for startup). Within 45 days, almost all of the carriers got 80% and 100% covered in MBBR₁ and MBBR₂, respectively (determined by microscopy, Figure 2). On day 45, with sugar and Ac as substrate, the estimated averaged thicknesses of the biofilm were 0.47 ± 0.1 and 1.2 ± 0.1 mm in MBBR₁ and MBBR₂, respectively (Figure 2) and the biofilm density were 13 and 32 gVSS/lbiofilm in MBBR₁ and MBBR₂, respectively (Figure 3). These parameters indicated that the biofilm was dense, quite round and smooth in both MBBRs, as observed previously with glucose as substrate [32,19], that being said, the biofilm in the MBBR₂, was even much denser. From day 45 (start of starch addition along with sugar and Ac) to 65, the biomass concentration and density started to decrease due to the biomass washout and biofilm detachment, until 6 gVSS/L (floc = 2 gVSS/L, biofilm = 4 gVSS/L, Figure 1b) and 6.5 gVSS/lbiofilm in MBBR₁ and 10 gVSS/L (floc = 3.5 gVSS/L, biofilm = 6.5 gVSS/L, Figure 1b) and 29 gVSS/lbiofilm in MBBR₂ (Figure 3). Almost 100% of the carriers were partially covered (50% covered) in MBBR₁, on the other hand, all of carriers in MBBR₂ were still 90% covered (based on the microscopy, Figure 4). The biofilm became lighter, softer, less smooth and irregular in both reactors by adding starch (Figure 4). Visual observations indicated that the biofilms, which formed during the starch degradation, were mainly detached by sloughing/abrasion (after day 45). The developed biofilm in glucose (sugar) and Ac mixture, was smooth, while, the biofilm obtained in starch was fluffy and rough. SVI_{30} increased from 120 to 185 mL/gTSS for both MBBRs, after adding PS in the influent. Feeding by PS led to the presence of many small particles in the bulk liquid (Figure 5). Presence of those small particles accelerated biomass wash out increase, since, the settling rate of these small particles was lower than the applied settling rate of 12 m/h [30]. This phenomenon brought about biomass concentration decline in PS fed period.

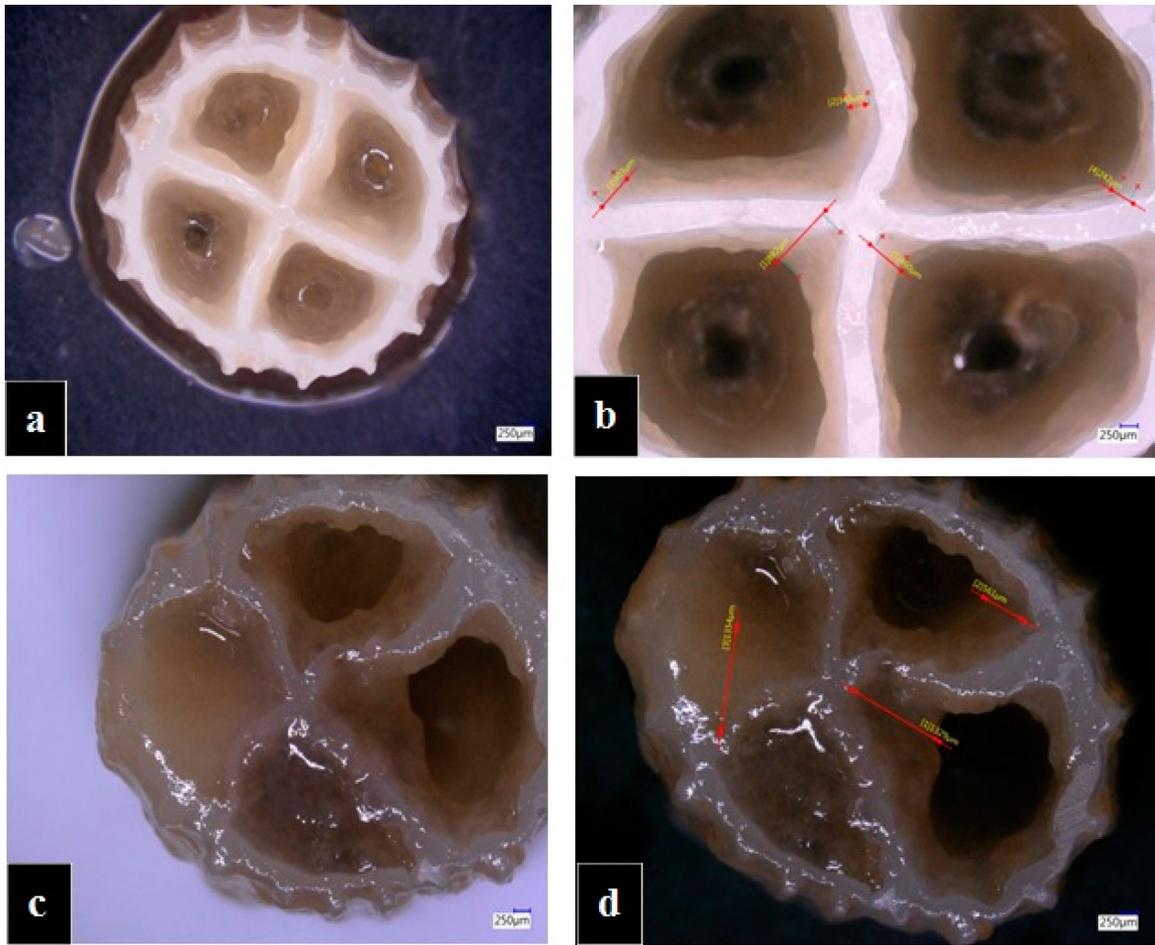


Figure 2. Covered carriers and biofilm thickness in Sugar and sodium acetate feed MBBR (MBBR₁ a, b and MBBR₂ c, d).

3.2. Reactor performance

Results of two MBBRs' performance (in 2 periods) are shown in Figure 6. Nitrogen removal efficiency during soluble substrate feeding, was 55% and 75% in MBBR₁ and MBBR₂, respectively. In fact according to what

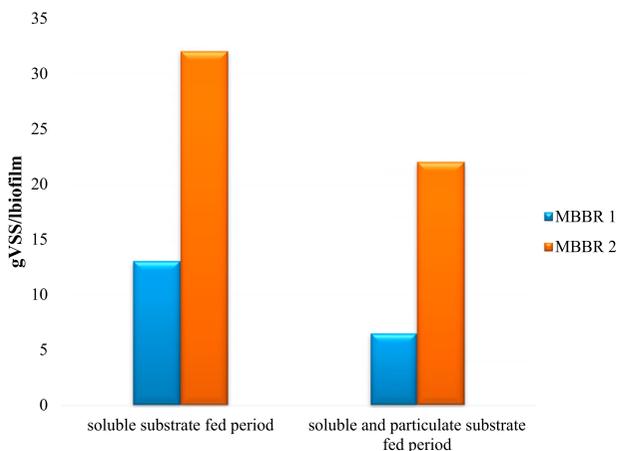


Figure 3. Biofilm density in MBBR₁ and MBBR₂.

Bassin et al. [4] indicated at high organic loading rate; the amount of the utilized nitrogen in order to biomass synthesis became more relevant, consequently, less ammonium was available for nitrification, therefore, the amount of the generated oxidized nitrogen (nitrate/nitrite) decreased and overall nitrogen conservation declined in the bulk. Furthermore, by considering the amount of soluble nitrogen in effluent (as ammonium, nitrite and nitrate) and the expected amount for fully aerated nitrifying reactors with no anoxic zones (calculated by subtracting the utilized nitrogen in biomass growth from the total nitrogen in the influent), it could be realized that 2% and 25% of nitrogen in MBBR₁ and MBBR₂, respectively, were still missing. Possibly, this amount of nitrogen was lost in to the atmosphere in the form of nitrogen gas (N₂) resulted from de-nitrification, which Bassin et al. [4] acquired the same results as well. In MBBR₁ with partially covered carriers, which could not get anoxic condition for de-nitrification, it was low (2%), on the other hand, in MBBR₂, with completely full and saturated carriers, the anoxic condition was applied in order to denitrification, thus, 25% of total

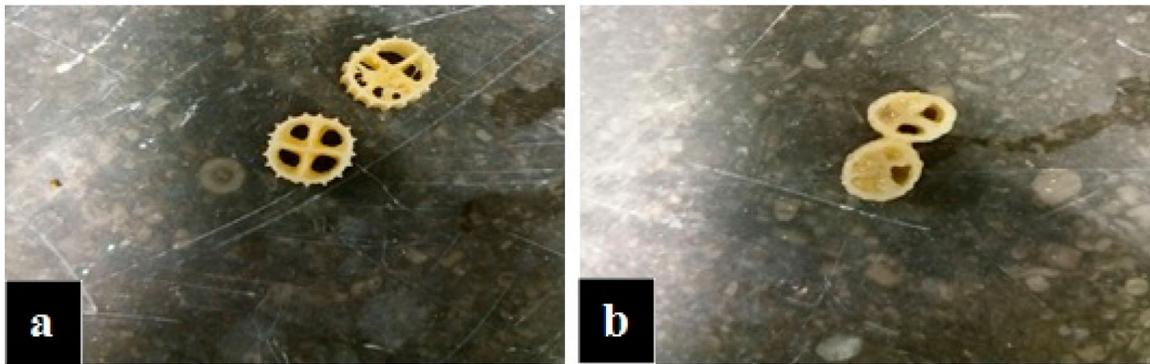


Figure 4. Less dense and irregular biofilm after adding PS, in MBBR₁, carriers are 50% covered (a) and in MBBR₂, carriers are still 90% full (b).

nitrogen was disappeared. Total nitrogen removal decreased to 42% and 58% in sugar, Ac and PS fed MBBR₁ and MBBR₂, respectively, owing to biofilm concentration and thickness deterioration. Nitrification efficiencies were around 70% and 85% for sugar and Ac fed MBBR₁ and MBBR₂, respectively. Ammonium was not completely removed in both MBBRs fed by

sugar and Ac and even decreased to 57% and 62% in MBBR₁ and MBBR₂ fed by sugar, Ac and PS, respectively. Over 96% and 99% of filterable COD removal (based on the filterable COD in the effluent) achieved in MBBR₁ and MBBR₂ fed by mixture of sugar and Ac as substrate, respectively. Total COD in effluent were 75% and 85% in MBBR₁ and MBBR₂ fed by sugar, Ac and PS,

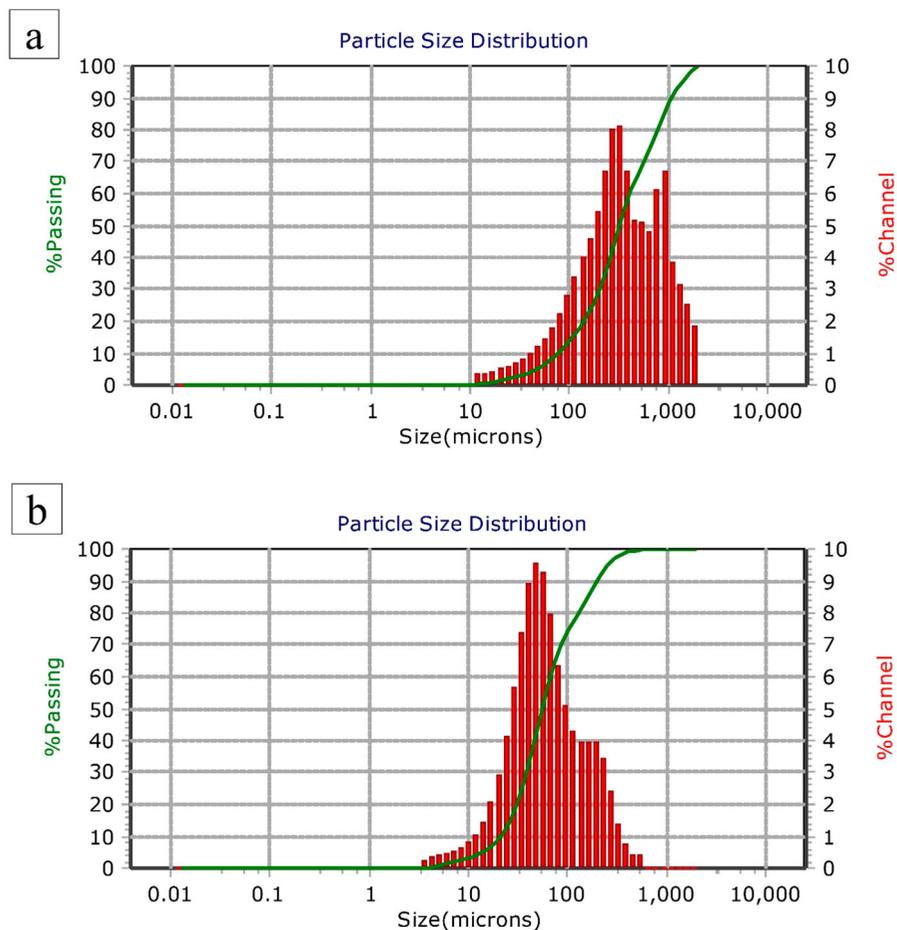


Figure 5. Particle size distribution of sugar and Ac fed (a) and PS fed (b) both MBBRs; in soluble fed system (a), particles are mostly between 100–1000 micron but in PS fed system, particles are mostly between 20–80 micron (b).

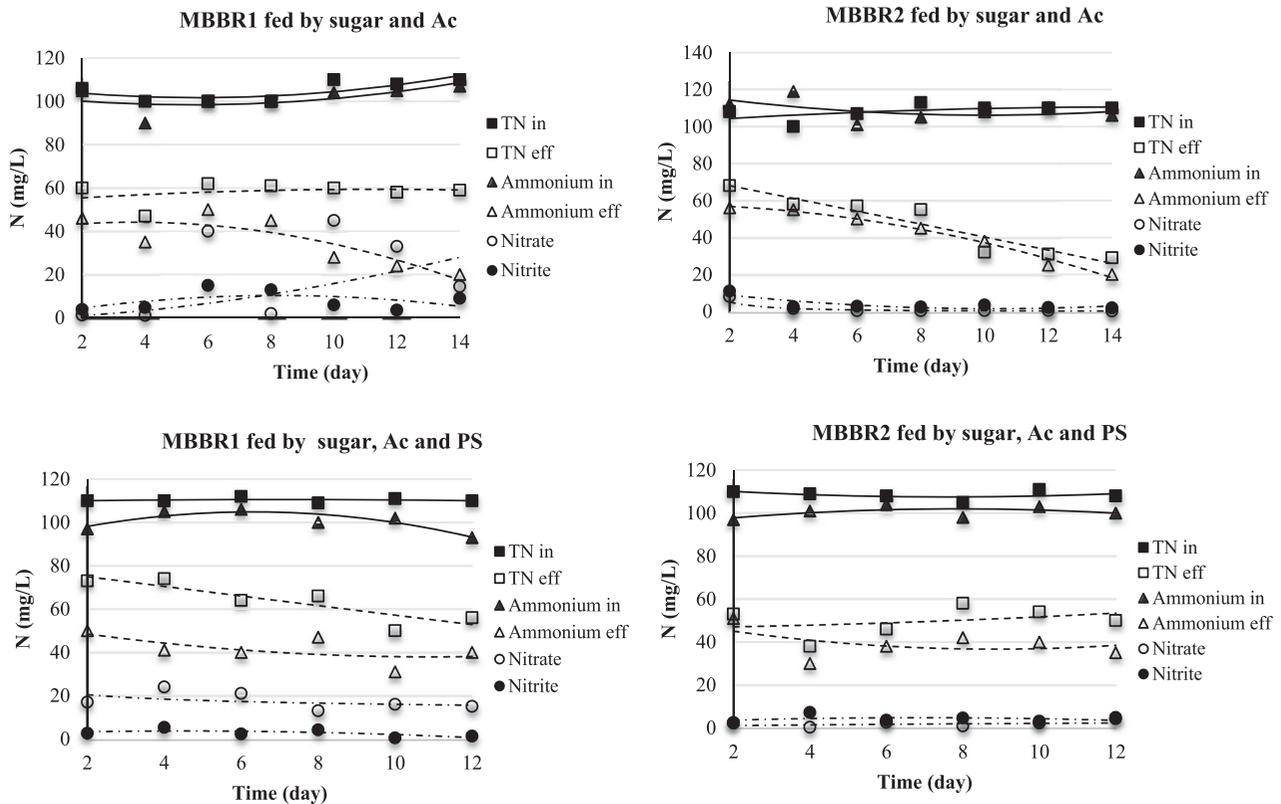


Figure 6. Nitrogen concentrations in influent and effluent of MBBRs at 2 different patterns of feeding.

respectively. The sludge growth yields which were evaluated from effluent suspended solids and variations in biomass dry weight in the reactor, were 0.4 and 0.55 gCOD/gCOD in MBBR₁ and MBBR₂ fed by sugar and Ac and 0.39 and 0.3 gCOD/gCOD in MBBR₁ and MBBR₂ fed by sugar, Ac and PS, respectively.

3.3. Starch degradation

Adsorption of starch onto biofilm could be visualized by submerging a carrier in an iodine solution [30]. The adsorbed starch particles could be seen by bright-field microscopy on the biofilm as black dots after one day feeding (Figure 7a, b). After one day aeration, iodine coloured particulate starch was not visible on the surface of the biofilm (Figure 7c, d), thus, we cut the carriers with a razorblade to see if starch was spread over the surface and inside the biofilm. Black dots were visible inside the biofilm in MBBR₁ (Figure 7e, f), while, they were not visible inside the biofilm in MBBR₂ (Figure 7g, h). Observations of coloured starch demonstrated that one day feeding was sufficient to hydrolyze all the particulate starch on the biofilm surface and inside biofilm in MBBR₂, though, it was sufficient to hydrolyze starch only on the surface of the biofilm (not inside biofilm) in MBBR₁. Whereas De Kreuk et al. [30] indicated that, one hour anaerobic feeding was insufficient to

hydrolyze all particulate starch on the granule surface. Even after more than two hours aeration, iodine coloured particulate starch was still visible on the surface of the granules. Furthermore, soluble substrate (e.g. sugar or Ac), adsorbed mostly onto the surface of the biofilm which caused a slow release and consumption of hydrolyzed substrate near the surface, while PS might be able to penetrate more into the biofilm, thus, it might be hydrolyzed and consumed more at the inside of the biofilm.

4. Discussion

4.1. Effect of carrier type on attached biomass accumulation and biofilm sloughing

According to the literatures, the key factor in MBBR design is the available effective surface area for biomass growth [33,34]. In this regard, when the specific surface area is defined for a given carrier, its size and shape are not usually notable in design purposes [33]. In this study, the media filling ratio was chosen in order to get the same specific area for biofilm growth in both reactors. Due to this fact, since both MBBRs were subjected to the same feeding pattern, similar attached biomass concentrations were expected in both systems, while, it was observed that

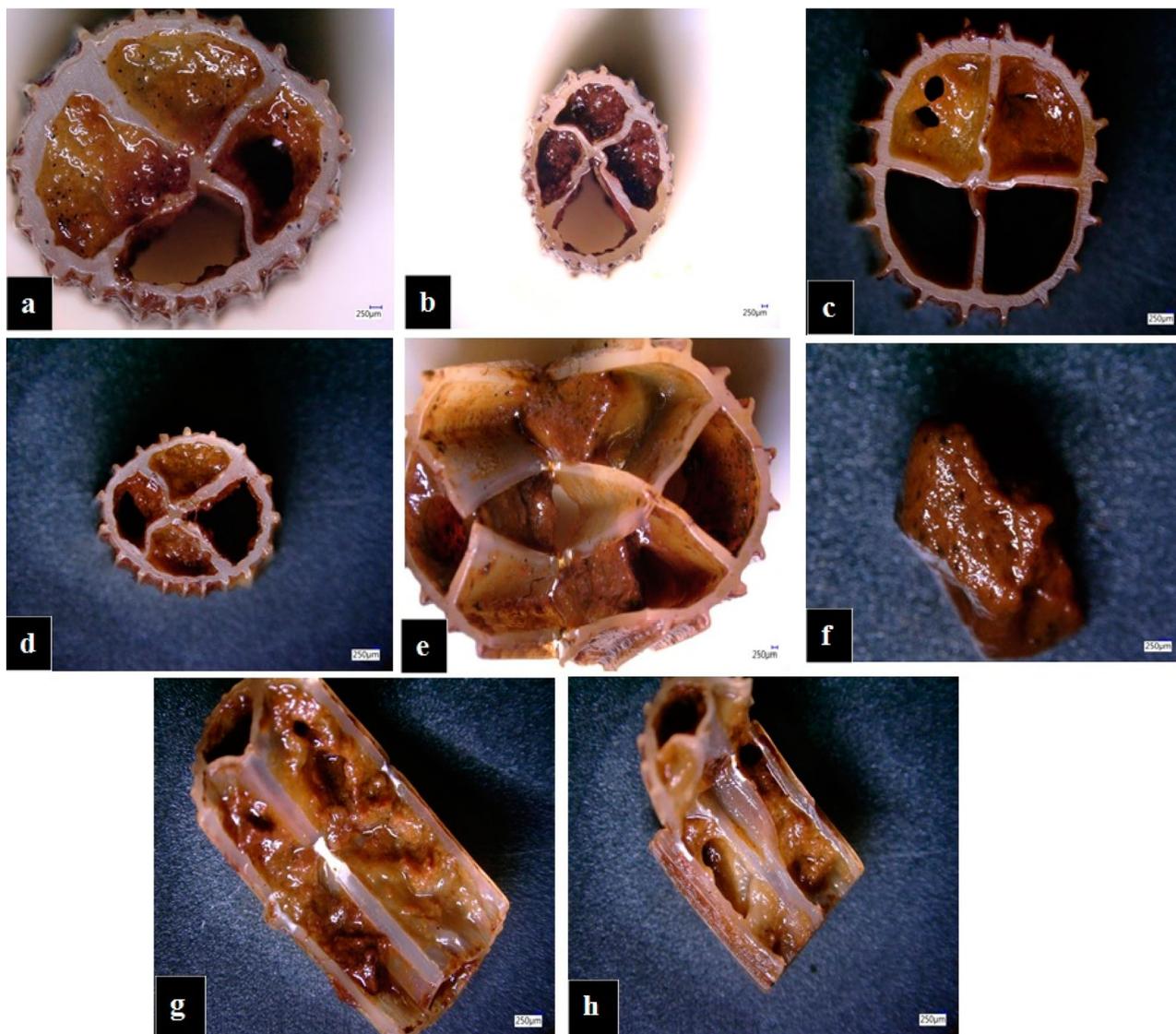


Figure 7. Starch adsorption and slow degradation, illustrated by iodine coloured biofilm (starch is coloured purple) after the feeding period in MBBR₁ (a) and MBBR₂ (b) after overnight aeration when purple dots are not visible on the surface of biofilm in MBBR₁ (c) and in MBBR₂ (d), after overnight aeration and cutting carrier when purple dots are visible inside the biofilm in MBBR₁ (e,f), but not visible inside the biofilm in MBBR₂ (g,h).

the Kaldnes K₁ micro carriers (media used in MBBR₂) allowed obtaining higher attached biomass concentrations compared to those reached in MBBR₁, where the Kaldnes K₁ were employed as support media. These findings suggested that the amount of the attached biomass, which could be achieved in a MBBR system, did not only depend on the theoretical biofilm surface area shown by the support material, but also on the carrier configuration and size. The Kaldnes K₁ micro carriers in MBBR₂ were much smaller than the conventional Kaldnes K₁ and the grown biofilm inside them were more packed and dense. This condition favoured the attachment of biofilm and the amount of the detached solids tended to decrease.

4.2. Influence of starch and carrier type on biofilm morphology

As De Kreuk et al. [30] described in their study, in systems without substrate gradients (suspended biomass in flocs), regular and compact structures are anticipated, whereas in systems with substrate gradients, filamentous organisms will proliferate. Martins et al. [35,36] have indicated that in activated sludge systems, suspended solids are incorporated in the open sludge flocs, where the hydrolysis within the floc triggers a constant release of substrate. More or less the same (low) substrate concentration is available throughout the entire floc, consequently micro-gradient would be missing, extreme outgrowth of



Figure 8. Kaldnes K1 micro carriers still almost full of biomass after PS feeding and Kaldnes K1 carriers which have very thin layer of biofilm after PS feeding.

filamentous organisms would be diminished and sludge bulking would be prohibited. Contrary, in aerobic granules [30] and MBBR₁ system in this study (Figure 7e, f), starch was mainly hydrolyzed at the surface of the granules and biofilm. Produced substrate was consumed locally, boosted substrate gradients inside the granules or biofilm and incited the outgrowth of filamentous structures. This was in line with other biofilm and fully aerated aerobic granular sludge researches, which low substrate concentrations in the reactor jointly with the presence of oxygen (or nitrate) led to irregular growth of aggregates [30,37–39]. On the other hand, in biofilm system in MBBR₂, PS was adsorbed at the biofilm surface and mostly hydrolyzed inside the biofilm (Figure 7g, h) which commenced the persistent release of substrate, thus, there was no substrate gradient inside biofilm in MBBR₂ and regular biofilm without any filamentous structure were grown inside MBBR₂. It can be included that Kaldnes K₁ micro carriers preserved regular biofilm without any filamentous structure and resistant against the slowly biodegradable substrate. Kaldnes K₁ carriers, which were inside MBBR₁ have had less dense biofilm which were washed out in large amount by adding PS (Figure 8).

4.3. Influence of substrate and carrier type on nitrogen removal

Starch removal mechanism deteriorated total nitrogen removal, which remained lower than those reported in studies using acetate as substrate. Nitrogen removal was 55% and 42% in MBBR₁ fed by sugar, Ac and fed by sugar, Ac, PS, respectively, and 75% and 58% for the MBBR₂ fed by sugar, Ac and fed by sugar, Ac and

PS respectively. This decline could be explained by biomass wash out due to slow hydrolyze of PS. Same results were perceived for nitrification and ammonium removal. Ammonium removal was 70% and 57% in MBBR₁ fed by sugar, Ac and fed by sugar, Ac, PS, respectively, and 85% and 62% in MBBR₂ fed by sugar, Ac and sugar, Ac and PS, respectively. Deficiency of simultaneous nitrification and de-nitrification (SND) could be elucidated by partially covered (not saturated by biofilm) Kaldnes K₁ carriers which caused low nitrogen removal in MBBR₁. A quota of nitrogen in the influent, which was not back as oxidized nitrogen (nitrite and nitrate) or residual non nitrified ammonium, was feasibly removed via de-nitrification as Bassin et al. [4] mentioned in their work as well. Despite the fact that aerobic condition was predominated in the bulk liquid, the thick biomass layer on the carrier media in MBBR₂ (Kaldnes K₁ micro) resulted in oxygen mass transfer limitation and enabled anoxic condition establishment in the inner zone of the biofilm, where de-nitrification could take place. Therefore nitrogen removal in MBBR₂ was superior compared to MBBR₁.

Taking into account that the amount of utilized nitrogen for biomass growth was similar in both systems, the observed higher nitrogen loss in MBBR₂ was possibly due to the higher thickness of the biofilm in protected surface area of the carrier, which favoured the development of anoxic environment in MBBR₂. Derived results from this study suggested that simultaneous nitrification and de-nitrification (SND) could be accomplished in MBBR reactors which are preserved under high bulk oxygen concentration with no deliberate anoxic phase, provided that the biofilm is sufficiently thick ($1.2 \text{ mm} \pm 0.1 \text{ mm}$). Furthermore, the magnitude of SND and the associated nitrogen removal were dependent on the specific carrier type. These observations emphasized the requirement for considering not only the effective surface area, but also the configuration of the media (e.g. size and shape) and the biofilm characteristics (thickness and biomass content) for better description of the biological conversions.

4.4. Influence of temperature on two reactors' performance

The relationship between nitrifying kinetics and temperature has been adequately modelled in previous studies between temperatures of 10°C to 28°C using the Arrhenius temperature correction coefficient (θ); with θ values being reported between 1.086 and 1.109 and an average θ value of 1.09 being suggested for MBBR systems [40]. The relation between nitrification

rate and temperature can be found as Equation (1) [7]:

$$K_2 = K_1 \theta (T_2 - T_1) \quad (1)$$

where θ is the Arrhenius correction coefficient, K is the removal rate ($\text{gN}/\text{m}^2\cdot\text{d}$) and T is temperature ($^{\circ}\text{C}$). As the influent nitrogen surface loading rate were 1.2 and $1.1 \text{ gN}/\text{m}^2\cdot\text{d}$ in MBBR_1 and MBBR_2 , respectively and the effluent ammonium surface rate were 0.24 and $0.23 \text{ gN}/\text{m}^2\cdot\text{d}$ (Figure 6a,b; the effluent concentration of ammonium were 20 mg/L in both reactors), it can be inferred that the nitrogen removal rate were 0.96 and $0.88 \text{ gN}/\text{m}^2\cdot\text{d}$ in MBBR_1 and MBBR_2 , respectively. The nitrogen removal rate in MBBR_2 (K_{MBBR_2}), calculated according to Equation (1), was $0.37 \text{ gN}/\text{m}^2\cdot\text{d}$.

$$\begin{aligned} (0.96 = K_{\text{MBBR}_2}(1.09)(26 - 15)) &\rightarrow K_{\text{MBBR}_2} \\ &= 0.37 \text{ gN}/\text{m}^2\cdot\text{d} \end{aligned}$$

The achieved removal rate in MBBR_2 from experiments (Figure 6b), indicated that the removal rate was 2.38 times higher than what Equation (1) says, thus, the temperature effect on nitrogen removal in MBBR_2 was not considerable.

5. Conclusions

The particulate substrate (e.g. Starch) in the influent of MBBR_1 (filled by Kaldnes K_1 media) was removed by adsorption at the biofilm surface, after which it was hydrolyzed. The substrate gradients that came into existence due to the hydrolysis caused irregular filamentous outgrowth on the biofilm. While, starch in the MBBR_2 (filled by Kaldnes K_1 micro) was hydrolyzed inside the biofilm and uniformly within biofilm which there was no substrate gradient and no filamentous structure; the biofilm in MBBR_2 was regular and much denser. The size of the carrier directly influenced the distribution and amount of attached biomass. Total nitrogen removal was achieved as a result of nitrogen assimilation by heterotrophs and denitrifying activity in the inner (anoxic) layer of the biofilm, despite the maintenance of high bulk oxygen concentrations in both reactors (especially in MBBR_2 which there was thicker biofilm, total nitrogen removal efficiency was pretty higher), which even lower temperature of MBBR_2 (15°C) could not affect the reactor performance negatively in COD and nitrogen removal.

Disclosure statement

No potential conflict of interest was reported by the authors.

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