Simultaneous nutrient recovery and algal biomass production from anaerobically digested sludge centrate using a membrane photobioreactor

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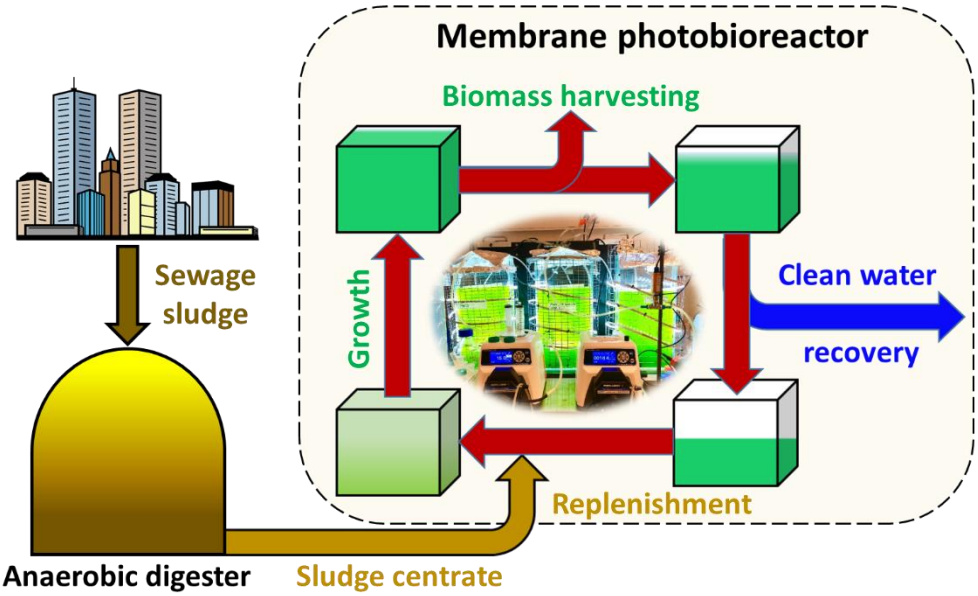
Graphical abstract



Highlight

- Continuous algae growth could be achieved by MPR using sludge centrate.
- Nutrient loading had indiscernible impact on biomass growth.
- Nutrient removal efficiency increased as nutrient loading rate decreased.
- Nutrient removal efficiency increased as HRT increased.
- Backwashing completely restored water flux decline caused by microalgae deposition.

Abstract

This study aims to evaluate the performance of *C. vulgaris* microalgae to simultaneously recover nutrients from sludge centrate and produce biomass in a membrane photobioreactor (MPR). Microalgae growth and nutrient removal were evaluated at two different nutrient loading rates (sludge centrate). The results show that *C. vulgaris* microalgae could thrive in sludge centrate. Nutrient loading has an indiscernible impact on biomass growth and a notable impact on nutrient removal efficiency. Nutrient removal increased as the nutrient loading rate decreased and hydraulic retention time increased. There was no membrane fouling observed in the MPR and the membrane water flux was fully restored by backwashing using only water. However, the membrane permeability varies with the hydraulic retention time (HRT) and biomass concentration in the reactor. Longer HRT offers higher permeability. Therefore, it is recommended to operate the MPR system in lower HRT to improve the membrane resistance and energy consumption.

Keywords: Biomass production; *C. vulgaris*; membrane photobioreactor; nutrient recovery; sludge centrate.

1. Introduction

Municipal wastewater is a valuable resource in a circular economy because it can be used to recover and reuse energy, nutrients, and clean water (Ansari et al., 2016; Nguyen et al., 2021; Vu et al., 2021b). In wastewater treatment plants (WWTPs), most of the organic input from wastewater is anaerobically digested to produce biogas which is a source of clean energy and digestate (a mixture of solid and liquid residue from anaerobic digestion) (Vutai et al., 2016). Digested sludge centrate is the liquid fraction after digestate dewatering that has been reported as the concentrated source of nutrients (i.e. nitrogen and phosphorus). The ammonia and phosphate contents in sludge centrate can reach up to 1 and 0.5 g/L, respectively (Li et al., 2020; Liu et al., 2021; Vu et al., 2021b; Wang and Lee, 2021). The high nitrogen and phosphorus content in a small volume of sludge centrate offers an excellent opportunity for nutrient recovery.

Nutrient recovery from sludge centrate is a win-win solution for nutrient management in WWTPs. Even if only 30% of nutrients in sewage end up in sludge centrate, the standard practice of returning this stream to the headwork for further treatment can have a negative impact on WWTPs (Abeysiriwardana-Arachchige et al., 2020). Examples include nutrient organic carbon imbalance, struvite blockage, and failure to meet stringent effluent discharge standards (Ansari et al., 2016; Vu et al., 2019). Thus, nutrient recovery from sludge centrate can simultaneously improve compliance with effluent discharge standards while also lowering maintenance costs due to the significant reduction in struvite blockage. At the same time, valuable fertilizers can be made from the recovered nutrients.

To date, several techniques have been developed and applied to recover nutrients from wastewater, such as sludge centrate. Examples include direct stripping (Ye et al., 2020), ion exchange (Wirthensohn et al., 2009), electrodialysis (Ward et al., 2018), chemical precipitation (Ansari et al., 2016; Daneshgar et al., 2018), membrane filtration (Ansari et al., 2016; Shin et al., 2021), and microbial electrochemical processes (Barua et al., 2019; Nancharaiah et al., 2016). They have proven their efficacy and potential in recovering nutrients from wastewater. However, majority of these processes are primarily focused on phosphorus recovery rather than a combination of both nitrogen or phosphorus (Barua et al., 2019). Furthermore, high chemical and energy consumptions continue to be major barriers to commercialisation of these technologies (Ansari et al., 2016; Cong Nguyen et al., 2020; Ward et al., 2018)

Microalgae-based treatment has recently emerged as a cost-effective and environmentally-friendly method of removing and recovering nutrients from wastewater (Abeysiriwardana-Arachchige et al., 2020; Ahmed et al., 2022). Microalgae use sun light as the energy source, carbon dioxide from the atmosphere as the carbon source, and nitrogen and phosphorus from wastewater to grow. Microalgae based wastewater treatment has numerous advantages including low operating costs (Ahmed et al., 2022), carbon capture (Deprá et al., 2020; Nagarajan et al., 2019), the production of biochemical feedstock (Khoo et al., 2019), and biofuel from algal biomass (Vo et al., 2018).

Microalgae-based treatments for removing nutrients from wastewater and producing biomass in photobioreactors have been demonstrated in several studies (Sayedin et al., 2020; Zhou et al., 2017). Zhou et al. (2017) reported that *Spirulina platensis* in saline wastewater could remove 80% of total nitrogen and 93% of total phosphorus, and achieve 0.76 g/L in biomass content. In a more recent study, Sayedin et al. (2020) showed nitrogen and phosphorus removal efficiencies of 95% and 78% from anaerobic digestate, respectively, by *Chlorella sorokiniana*. However, microalgae-based technology has a high space requirement, thus, it has been rarely commercially applied for removing nutrients from wastewater (Gao et al., 2016). A major technical challenge is to increase the microalgae content in the reactor for process intensification and reduction in space requirement (Gao et al., 2016; Nguyen et al., 2020).

The aforementioned challenge can be addressed by incorporating a submerged ultrafiltration membrane with the bioreactor to form a membrane photobioreactor (MPR). In the MPR, a high algal biomass concentration can be achieved at a low hydraulic retention time allowing for process intensification. Furthermore, this method can be easily scaled up.

Therefore, this study aims to evaluate the effectiveness of an MPR following an ingenious operation cycle in simultaneously recovering nutrients and producing microalgal biomass from sludge centrate. The feasibility of continuous operation of the microalgae system is demonstrated via monitoring its stable performance. Additionally, the effects of hydraulic retention duration and the rate of sludge centrate loading on nutrient removal and biomass generation are investigated. The results from this study are expected to be a stepping-stone to valorise resources from high strength wastewater.

2. Materials and methods

2.1. Microalgae inoculum and sludge centrate

The freshwater green microalgae strain *C. vulgaris* (CS-41) from the Australian National Algae Culture Collection, CSIRO Microalgae Research (Hobart, TAS, Australia) was used in this study. The microalgae were incubated in MLA medium at the University of Technology Sydney culture collection (Vu et al., 2021a). A concentrated microalgae solution was prepared from the culture collection and used as inoculum. This was accomplished by removing the supernatant from the culture and centrifuging the remainder at 3,000 rpm for 5 minutes.

Sludge centrate from a high speed centrifuge at a full scale wastewater treatment plant (located in Sydney, Australia) was used as the nutrient source to cultivate the microalgae. Large particles were removed from the sludge centrate using a 75 μm stainless steel filter mesh. The raw sludge centrate is at pH 6.95 and had 253 mg/L COD, 998 mg/L NH_3-N, and 312 mg/L PO_4^{3-}. The total nitrogen (TN) and total phosphorus (TP) were 1012 and 318 mg/L, respectively.

2.2. Experimental systems

Three identical 3.5 L glass reactors were used to cultivate microalgae (Supplementary Data). The internal dimensions of each reactor were 20 cm in length, 4 cm in width, and 45 cm in height. In order to ensure adequate mixing, each reactor's microalgae culture was aerated at a rate of 1 L/min using a stone diffuser positioned at the bottom of the reactor. The air was cleaned using a 0.45 μm cartridge filter. The reactor was illuminated with a surrounding LED strip at a light intensity of approximately 100 μmol/m^2/s in a 16:8 -hour light:dark cycle. This light/dark cycle condition has been established in our previous work as a favourable condition for *C. vulgaris* growth (Nguyen et al., 2020). These operational conditions were consistent throughout the experiments regardless of the operation modes of the microalgae reactor.

In the MPR, a polyvinylidene difluoride ultrafiltration (UF) hollow fiber membrane module (Mitsubishi Rayon Co., Ltd) was used to withdraw the treated water (Figure 1A). The nominal pore size and total surface area of the module were of 0.04 μm and 0.073 m^2, respectively. A Masterflex Peristaltic pump (Cole-Parmer, USA) connected to the membrane module was used to extract clean water from the MPR. A pressure transducer (PT30 model, Extech Instruments, United States) was inserted in the suction line of the pump to monitor the changes in transmembrane pressure during operation.

2.3. Experimental protocols

Microalgae growth and nutrient removal were evaluated at two nutrient (sludge centrate) loading rates. The feed solutions to the MPR were prepared by diluting raw sludge centrate 12.5 and 25 times using clean water corresponding to high and low nutrient loading rates, respectively. This work aims to demonstrate the effectiveness of the MPR in maintaining stable performance in terms of microalgae growth and nutrient removal. Therefore, the pretreated sludge centrate (section 2.1) was further filtered through 1 μm filter paper prior to the dilution step in order to minimise any impacts caused by the presence of bacteria and turbidity in the medium.

Microalgae cultures in three reactors were inoculated simultaneously using diluted sludge centrate corresponding to each nutrient loading rate presented ealier. Each reactor were inoculated by dosing 50 mL of the concentrated microalgae culture (section 2.1) into 2950 mL of diluted sludge centrate in order to achieve a biomass content of approximately 145 mg/L. Each reactor had a working volume of 3 L. During the stationary phase, one reactor remained in batch mode. The other two reactors were switched to the MPRs at HRT of 3 and 5 days, respectively.

Algal biomass extraction and sludge centrate feeding were conducted once a day in four steps (Figure 1B). First, 100 mL of the microalgae culture was collected from each reactor, which was subsequently used for the measurement of biomass content and nutrient removal. Second, 900 and 500 mL of treated water were extracted through the membrane from each reactor over 1 hour corresponding to HRT of 3 and 5 days, respectively. In practice, the treated water from a microalgae system would be mixed with the raw feed solution for the next cultivation cycle. In this study, the treated water was not reused for cultivation so that a constant nutrient loading can be achieved for systematic comparison. Instead, the above described fresh culture media were used for daily feeding the system. Third, after the filtration process, fresh diluted sludge centrate solution was fed to the reactor to maintain the HRT of 3 and 5 days, respectively. Finally, the microalgae reactor was operated under steady conditions for the remaining duration of the day.

[FIGURE 1]

At the end of the MPR experiment, membrane permeability was measured at the final microalgae content in the reactor. The initial membrane flux was adjusted to 20 $L/m^2.h$ and the transmembrane pressure during filtration was recorded for 150 min for permeability calculation. During the permeability tests, the permeate was returned to the reactor to

maintain constant liquid volume and microalgae concentration. The MPR was continuously aerated with air at 1.5 L/min through a diffuser placed in the bottom of the reactor. The permeability test was conducted in replication. At the end of each filtration cycle, the membrane module was backwashed at 40 L/m^2.h using clean water and aerated at 1.5 L/min for 5 min.

2.4. Analytical methods

Chemical oxygen demand (COD) was measured by the US-EPA Standard Method 5220 using a HACH DRB200 COD reactor and HACH DR3900 spectrophotometer. Ammonium (NH_3-N), total nitrogen (TN) and total phosphorus (TP) were determined by HACH standard kits using the HACH DR3900. Orthophosphate (PO_4^{3-}) was measured using ion chromatography (IC) (Thermo Fisher, Australia). The system was equipped with a Dionex AS-AP auto-sampler and a Dionex AS19 IC column (7.5 μm pore size, 4 mm diameter and 250 mm length). The sample injection volume was 10 μL. The analysis conducted using potassium hydroxide eluent with the following gradient (time [min]: concentration [mM]) (0-10: 10; 10-25: 45; 25-27: 45; 27-30: 10; 31: stop run).

The optical density and dry weight of microalgae culture were determined daily using a UV spectrophotometer (UV 6000 Shimadzu; Australia) at a wavelength of 680 nm and by gravimetric analysis, respectively to assess microalgae growth. For the optical density measurement, 3 mL of homogeneous microalgae cell suspension was transferred into a cuvette to measure the optical density. For gravimetric analysis, 50 mL of microalgae cell suspension was filtered through a 1.1 μm pre-weighed glass filter paper. The filter paper was then dried at 60 °C for 4 hours to a constant mass. A linear regression coefficient (R^2) of 0.96 was confirmed between the optical density and dry weight biomass.

3. Results and discussions

3.1. Biomass production

Results in Figure 2A confirm that microalgae can thrive in sludge centrate. At both nutrient loading rates, there was no observable lag phase, which indicates good adaption of *C. vulgaris* to sludge centrate as the growth medium (Figure 2A). In batch mode, the microalgae grew rapidly and reached a stationary phase with a biomass concentration of 1,100 mg/L at day 6 at both loading rates. The specific growth rates under both nutrient loadings were similar at 0.34 day^{-1} in batch mode. The biomass content and specific growth rate in this study were similar or higher than those reported in previous studies using nutrient rich effluent or aquaculture wastewater as culturing media (Boonchai and Seo, 2015; Gao et al., 2016). Results in this study confirm that sludge centrate was sufficient to maintain high microalgal biomass productivity. Another reason is that biomass production could be promoted by the heterotrophic growth of *C. vulgaris* with the presence of organic carbon in sludge centrate (Gim et al., 2016).

In batch mode, the microalgae population collapsed after 12-14 days of continuous operation (Figure 2A). This ecological collapse is expected and mainly due to the limited illumination and depletion of limiting nutrients, especially nitrogen, as evidenced by the complete removal of ammonia in the effluent in batch mode at the stationary phase (see supplementary material). In addition, beyond the stationary phase, the microalgae cultures were highly alkaline at pH 9.35 (data not shown), which was unfavourable for *C. vulgaris* growth (Sakarika and Kornaros, 2016). The observed phenomenon is consistent with the growth stages of microalgae (i.e. lag, exponential growth, stationary, and death stages) in previous photobioreactor studies (Vo et al., 2018; Vu et al., 2020).

By contrast to batch mode, the MPR could achieve stable biomass production (Figure 2B&C). In the MPR, regular extraction of microalgal biomass and treated water as well as the replenishment of fresh feed improved the biomass production at both nutrient loading rates. Biomass content in the MPR was 40% higher than that in batch mode (at HRT of 3 days). The observed improved biomass content in the MPR was due to the retention of microalgal biomass by the membrane. The sufficient supply of nutrients from daily fresh feed replenishment to the MPR could also promote the growth of microalgae, thus increasing biomass content. While the main focus of this study was on microalgae growth in the MPR,

additional work is also recommended to examine any long term changes in cell morphology and content caused by sludge centrate.

In the MPR, nutrient loading did not show any discernible impact on biomass growth (Figure 2). The microalgal biomass contents at low and high nutrient loading rates were similar in the MPR (Figure 2B and 2C). This is because in the MPR, the system is not limited by nutrients. Microalgal biomass content in the MPR of approximately 1.6 g/L in this study is much higher than that (i.e. approximately 0.9 – 1.1 g/L) reported in previous works in the literature (Gao et al., 2016; Nguyen et al., 2020). Thus, illumination for photosynthesis has probably become the limiting factor in this study. Furthermore, feeding the reactor with high ammonium content (approximately 80 mg/L) on a daily basis may cause toxicity to *C. vulgaris* microalgae, reduce cell viability, and retard the biomass production (Collos and Harrison, 2014; Zheng et al., 2019).

[FIGURE 2]

In MPR mode, microalgal biomass production is regulated by HRT. A low HRT resulted in higher microalgal biomass production (Figure 2B and 2C). The impact of HRT on microalgal biomass production was more profound at the low nutrient loading rate. This is because the larger volume of withdrawal effluent and the replenishment of fresh feed could result in better control of the culture pH and improved illumination for microalgae growth. The obtained pH values of the microalgae culture using the low rate of sludge centrate at HRT of 3 and 5 days after stabilisation of biomass growth were approximately 7.6 and 8.3, respectively.

3.2. Organic matter and nutrient removal from sludge centrate

The removal of COD by *C. vulgaris* microalgae was minimal. This outcome is expected because microalgae are autotrophs, meaning they can obtain energy from light and grow using CO_2 rather than organic carbon to grow. There is an increase in COD residue from sludge centrate addition in the effluent (Figure 3). Nevertheless, the COD residue reached an equilibrium after about four days in the MPR. The observed increase in COD residue is due to the dilution effect at the beginning of the MPR operation and initial chemoautotrophic microalgae growth. In batch mode, COD was removed completely by the microalgae culture, which could be attributed to *C. vulgaris*'s chemoautotrophic growth and enhanced organic carbon metabolism under nitrogen-starved conditions (Su, 2021).

[FIGURE 3]

At the beginning of the MPR operation, nutrient content in the treated water remained at a low level as evidenced by the high removal efficiency over the first few days (Figure 4). This initial increase in nutrient removal can also be attributed to the dilution effect discussed above in relation to COD removal. Nutrient removal eventually reached a stable value in all experiments as the equilibrium of nutrient input and output was reached.

Nutrient loading rate has a significant impact on nutrient removal efficiency (Figure 4). Nutrient removal at the high loading rate was only half of that at the low loading rate. As discussed in section 3.1, increased nutrient loading did not affect biomass growth. The main mechanism of nutrient removal is mentioned to be biomass production combined with nutrient consumption for microalgae assimilation. Thus, higher nutrient input and low utilisation for biomass growth could result in higher nutrient content in the effluent and decreased removal efficiency.

[FIGURE 4]

HRT also has a significant impact on nutrient removal efficiency (Figure 4). On average, 80% ammonia and 72% phosphate could be removed from sludge centrate at low nutrient loading rate with HRT of 5 days. Under low nutrient loading rate, 5 days HRT showed better nutrient removal with approximately 30% increase compared to 3 days HRT. This result could be attributed to more adequate contact time for the nutrient assimilation by microalgae at longer HRT. Furthermore, the elevated pH (i.e. pH 8.3) of the culture after 5 days HRT could promote ammonia volatilisation, thus increasing nitrogen removal efficiency.

3.3. Membrane permeability

Backwashing completely reversed the membrane water flux. This was demonstrated by insignificant differences in the initial membrane permeability between duplicate experiments regardless of HRTs (Figure 5). The change in the membrane permeability followed a similar pattern throughout the specific filtration process. The membrane permeability decreased significantly during the first 60 minutes and then remained stable (Figure 5). The rapid deposition of microalgae cells on the membrane surface caused by high hydrodynamic drag force could explain the significant reduction in permeability during the early stages of filtration. The constant permeability after reaching a steady-state value could be attributed to the equilibrium of deposition phenomenon, which occurred as a large number of microalgae cells were swept away from the membrane surface by the shear force generated by the aeration in the reactor.

[FIGURE 5]

The permeability of the membrane was determined by the amount of biomass in the reactor (Figure 5). Longer HRT (i.e. 5 days) resulted in higher permeability (Figure 5). The longer HRT with lower biomass concentration, as shown in section 3.1, could reduce the severity of microalgae deposition on the membrane, thus improving the permeability. A higher permeability value indicates that the membrane resistance is low and that a larger volume of the medium can be filtered in the same amount of time. These findings imply that operating the MPR at short HRT is recommended due to the low membrane resistance and consequently lower energy consumption.

4. Conclusion

The feasibility of using an MPR for simultaneous nutrient recovery and algal biomass production from anaerobic sludge centrate was demonstrated. In this study, it can be concluded that in comparison to the batch mode reactor, the MPR allows for continuous cultivation of microalgae with 40% higher biomass content. The effects of nutrient loading on biomass growth were negligible. Reduced nutrient loading rate and increased HRT resulted in improved nutrient removal efficiency. The permeability of the membrane was determined by the amount of biomass in the reactor. After backwashing using only water, the water flux could be fully recovered.

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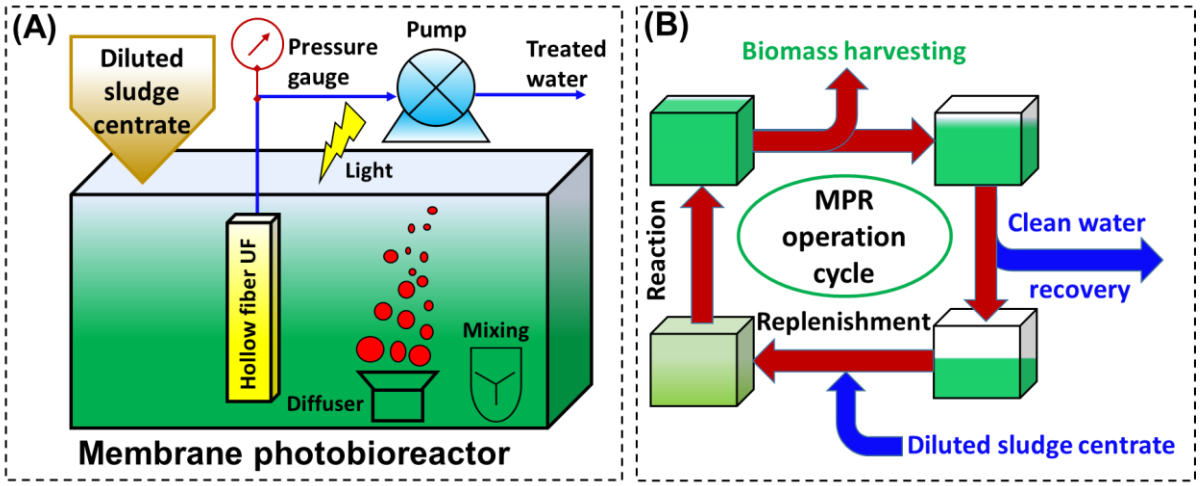


Figure 1. Schematic diagram of experimental systems in this study, which presents (A) membrane photobioreactor and (B) MPR operation cycle.

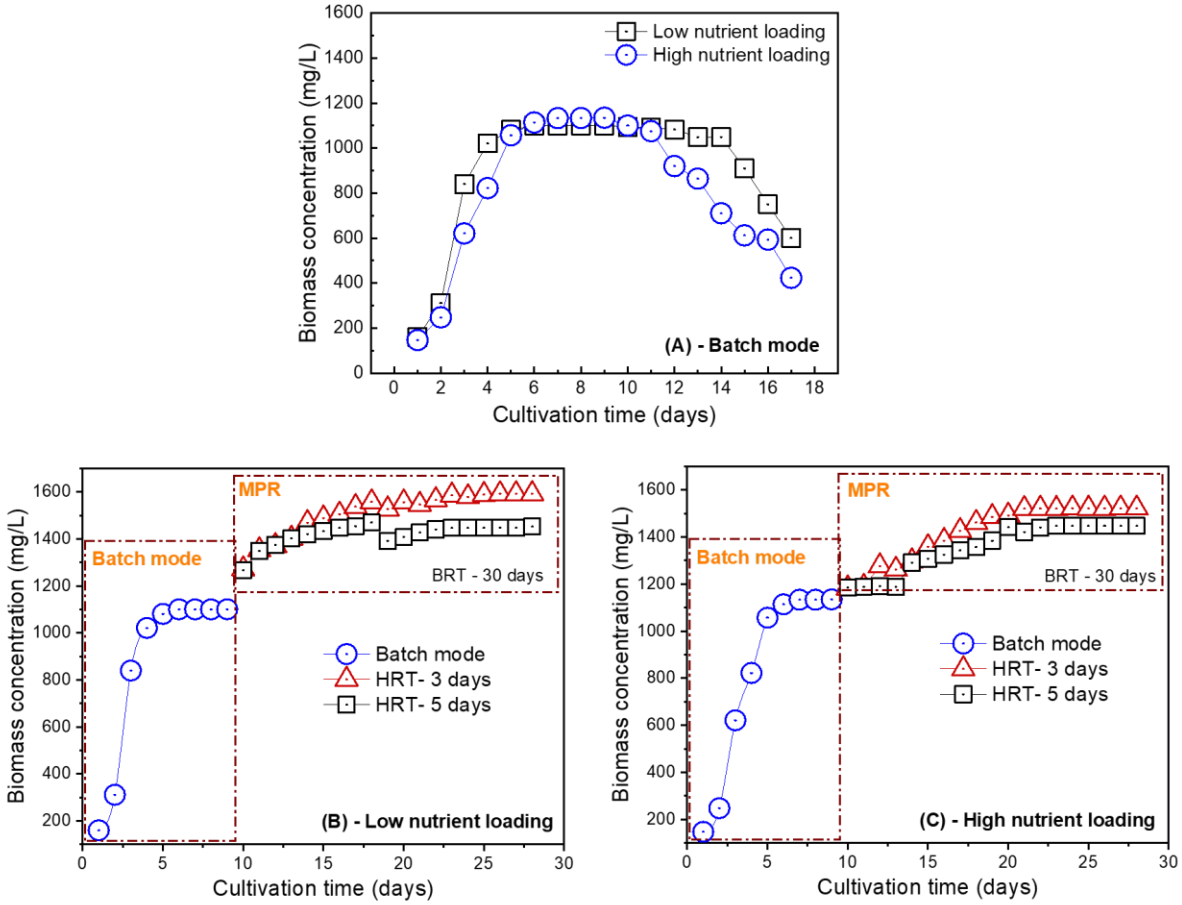
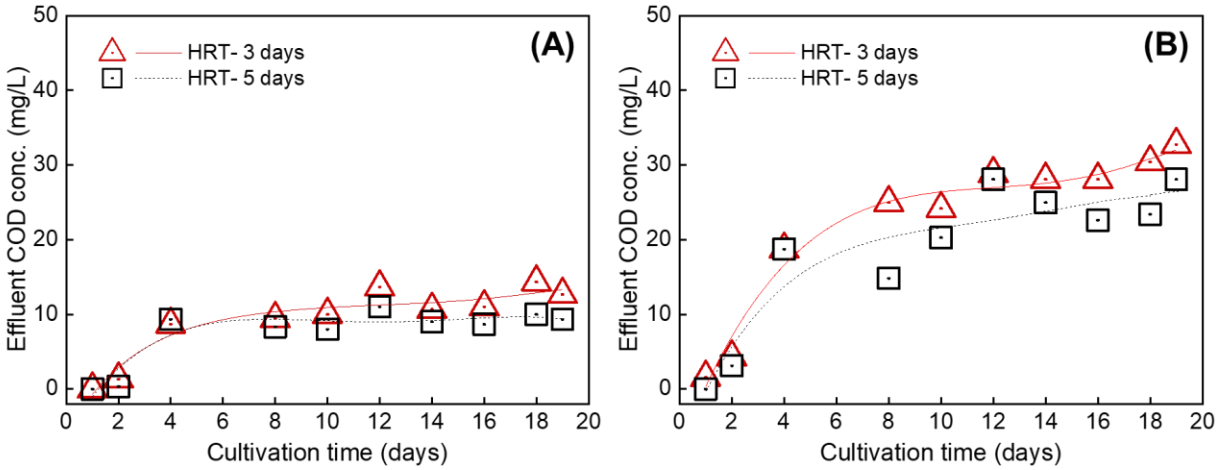


Figure 2. Changes in biomass production of (A) batch mode microalgae reactor and the MPR at (B) low nutrient loading and (C) high nutrient loading.



448

449 **Figure 3.** Changes in COD concentration in the MPR effluent (permeate) over time at (A)

450 low and (B) high rate of sludge centrate and different HRTs.

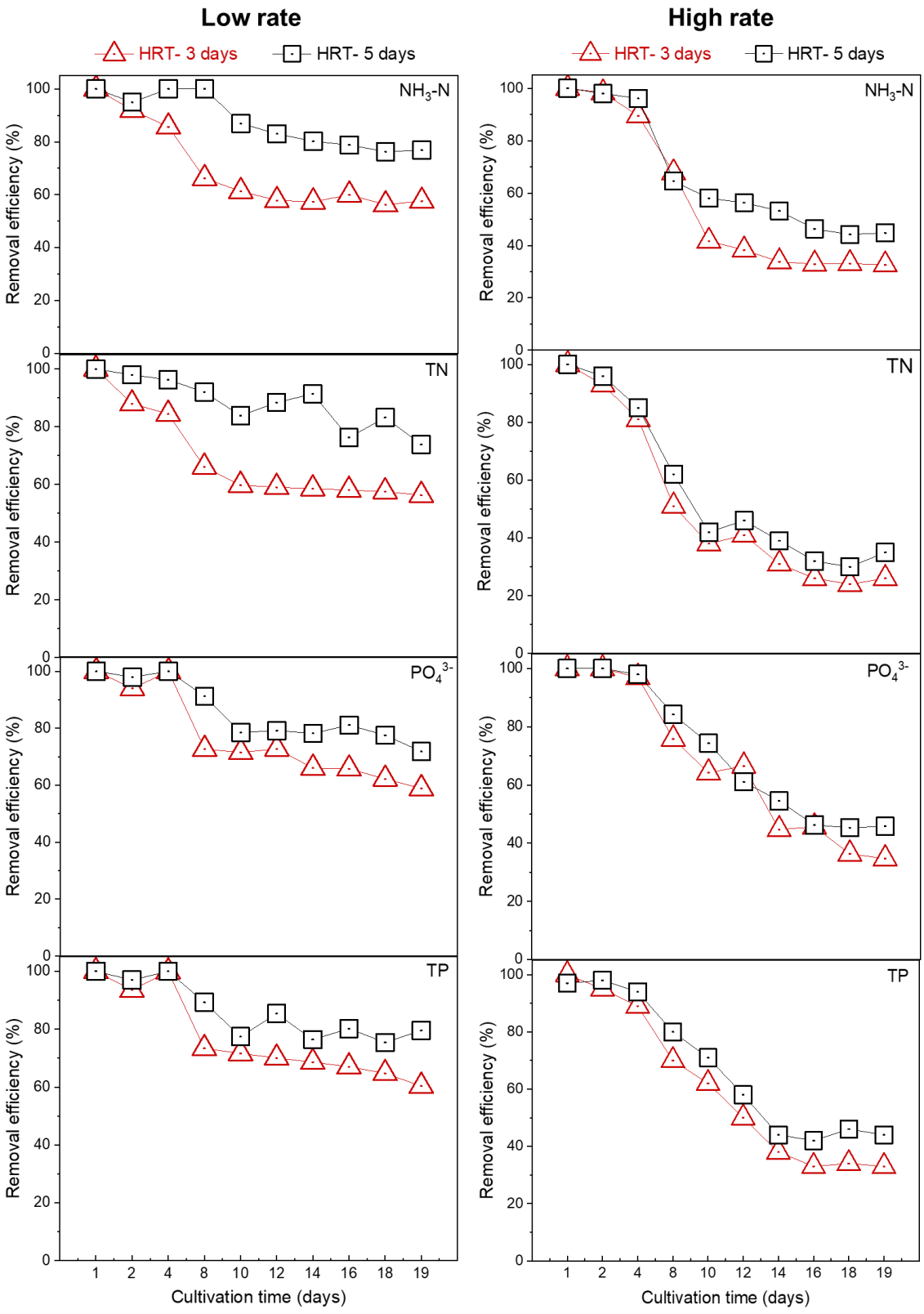
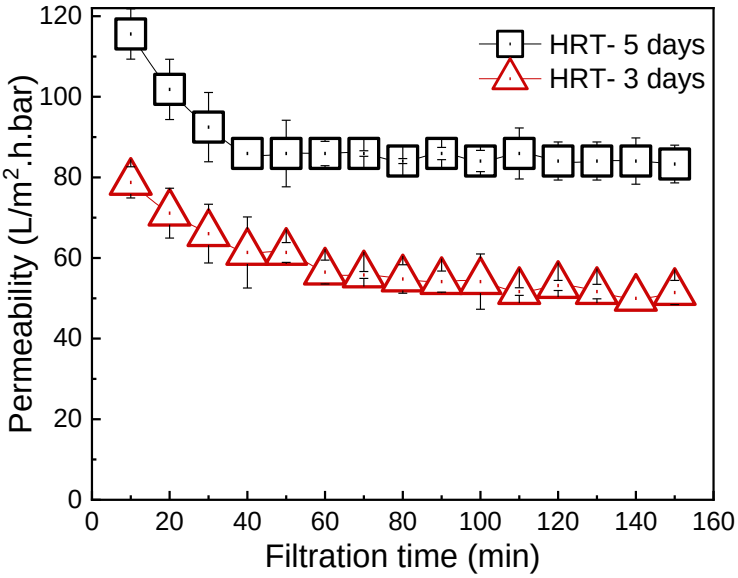


Figure 4. Nutrient removal from sludge centrate in the MPRs at different rate of sludge centrate and different HRTs.



454

455 **Figure 5.** Comparison in membrane permeability of microalgae culture at low loading rate of
456 sludge centrate and different HRTs. Values and error bars are the mean and standard
457 deviation of two replicate experiments.

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