A hybrid anaerobic and microalgal membrane reactor for energy and microalgal biomass production from wastewater

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Minh T. Vu[a], Hang P. Vu[a], Luong N. Nguyen[a*], Galilee U. Semblante[b], Md Abu Hasan Johir[a] and Long D. Nghiem[a,c]

[a] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia

[b] Technical Services, Western Sydney University, Kingswood, NSW 2747, Australia

[c] NTT Institute of Hi-Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

[*]Corresponding author:

Luong N. Nguyen: Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, NSW 2007, Australia

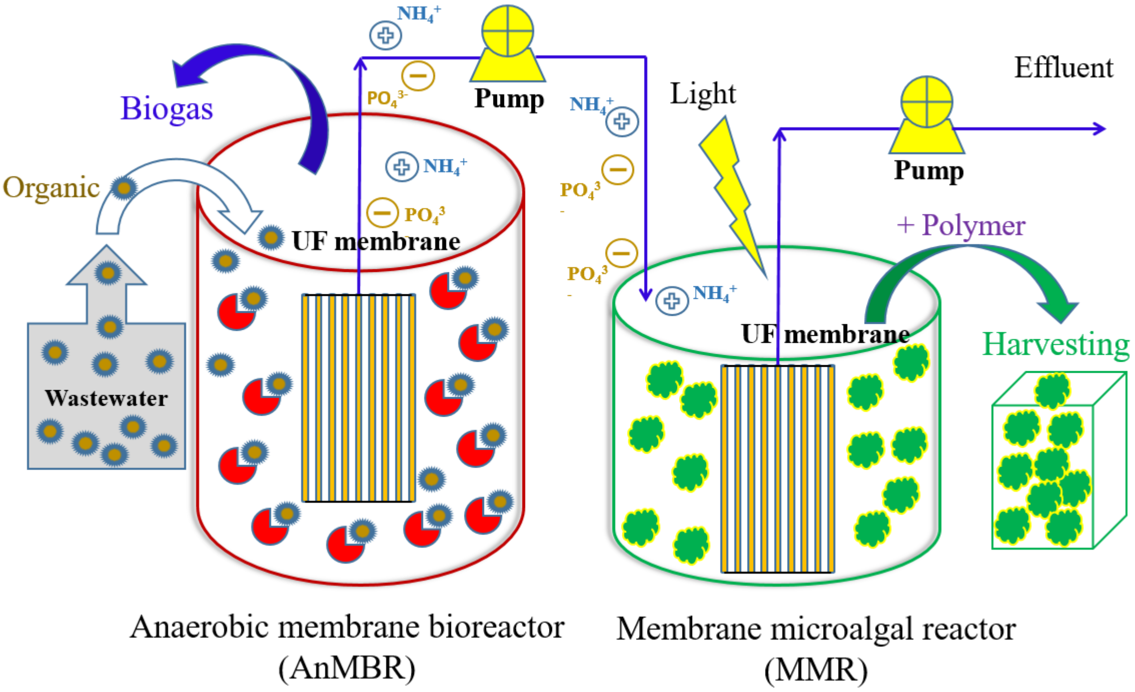
Phone: (+61) 468863865 E-mail: luongngoc.nguyen@uts.edu.au

Abstract

In the concept of a circular economy, wastewater is no longer waste but a resource for water, energy and nutrients. In this study, a hybrid system containing an anaerobic membrane bioreactor (AnMBR) and a microalgal membrane reactor (MMR) was developed to harvest energy, nutrients, and microalgal biomass from food and agribusiness industrial wastewater. The AnMBR removed over 97% of chemical oxygen demand (COD) and generated 4.7 ± 0.15 L (*n=80*) of biogas equivalent to 2.4 kWh kg^{-1} COD $_{(feed)}$ d^{-1}. Through anaerobic metabolism, the microorganism in AnMBR generated NH_4^+ and PO_4^{3-} -rich effluent. Their effluent concentrations were 1.9 and 1.4 times of that in the influent, respectively. NH_4^+ and PO_4^{3-} -rich effluent was directly used (i.e. without filtration or sterilization) to culture microalgae *Chlorella vulgaris* in the MMR. . Microalgal biomass production reached up to 700 mg/L after 6 days of operation and nutrient removal rates of above 75% were achieved. However, biomass production and nutrient removal declined toward the end of experiment. The generated biomass was completely harvested using cationic polyacrylamide at the dose of 36 mg g^{-1} dry weight. Overall, the AnMBR has great potential to produce energy. Future research is needed to intensify the microalgal growth (e.g. genetic modification of strains, addition of plant hormones) in the MMR for continuous operation of the hybrid system.

Keywords: Anaerobic membrane bioreactor; Microalgal membrane reactor; Algae harvesting; Nutrient removal; Biogas production; Polyacrylamide

Graphical abstract



Highlight

- AnMBR achieved over 97% COD removal
- AnMBR generates nutrient-rich effluent (i.e. NH_4^+ and PO_4^{3-}) for microalgal cultivation
- Microalgal cultivation in MMR was achieved using AnMBR effluent in short term
- Flocculation by cationic polymer is effective to harvest microalgal biomass

1. Introduction

The recovery of clean water, energy, and nutrients from wastewater is an important component of a circular economy. Water reuse gives an extra level of certainty and security to water supplies in the face of a changing climate. There has been an upward trajectory in both technology development and full-scale implementation. For example, NEWater, the trade name of reclaimed water produced in Singapore, now operates five full-scale plants that supply up to 40% of Singapore's water demand for industrial activities. Recently, there have also been efforts to develop technologies that can recover energy and nutrient from wastewater [1; 2]. Although results to date are still limited, they highlight the significant potential and economic merit of energy and nutrient recovery especially from wastewater from food and agribusiness industries that has high amount of organic content (i.e. high strength wastewater).

Anaerobic membrane bioreactor (AnMBR) combining the anaerobic digestion process with a membrane separation (i.e. independent of sludge settleability) provides a number of benefits.

For example, the AnMBR is considered as a sustainable alternative to aerobic membrane reactor since it produces renewable energy in the form of biogas [3]. AnMBR is particularly suitable for high organic wastewater due to the anaerobic metabolism’s high tolerance to loading and solid free effluent [4; 5]. Through anaerobic metabolism (i.e. without oxygen), microorganisms assimilate organic carbon to grow and produce biogas [4; 5; 6; 7]. The produced biogas is heat and energy source to fuel the AnMBR. While this concept has been touted to result in “energy neutral wastewater treatment”, there is limited literature on anaerobic energy output. Moreover, the main drawback of AnMBR is low nutrient removal efficiency due to inherent anaerobic metabolisms that release free ammonia and orthophosphate from protein and organic phosphorus compounds. Thus, additional technologies are often required to either remove or recover nutrients from AnMBR effluent [7; 8; 9].

The available nutrients in the AnMBR effluent are potential source to grow microalgae for renewable biomass. During the cultivation, microalgae assimilate dissolved nitrogen and phosphorous [10; 11; 12]. Some microalgae have been successfully cultivated in non-sterile environments such as wastewater [13; 14; 15] for removal of nitrogen and phosphorus [16; 17]. Collectively, the nutrient rich AnMBR effluent is suitable to cultivate microalgae. Microalgal cultivation using widely available waste streams without economic value can vastly reduce operating cost in microalgal biomass production. Microalgal biomass is a renewable feedstock for biofuel and biochemical production [18; 19; 20; 21; 22; 23]. Therefore, microalgae cultivation is potentially an environmentally sustainable solution in the concept of circular economy [24]. The utilisation of anaerobic digestion process to produce energy and nutrient rich effluent as well as microalgal culture will provide multidimensional benefit such as (i) high effluent quality, (ii) nutrient recycle and reuse, and (iii) renewable biomass [25; 26; 27]. However, there only a few studies reported the integration of AnMBR and microalgal cultivation in a batch experiment. Experimental results from continuous culture of microalgae will facilitate and enhance the readiness of microalgal cultivation from wastewater.

This study aims to evaluate the performance of a hybrid system consisting of AnMBR to produce biogas and microalgal membrane reactor to remove nutrient from high organic and nutrient wastewater in food and agribusiness activities. The performance of AnMBR in terms of organic carbon removal and energy production (i.e. biogas) was evaluated. Nutrient-rich effluent from the AnMBR was directly fed to a microalgal membrane reactor. A microalgal harvesting method was used. The technology developed in this study provides a stepping stone to valorize resources from high organic and nutrient wastewater.

2. Materials and methods

2.1 Microalgae species and cultural conditions

The freshwater *Chlorella vulgaris* (CS-41) was obtained from the Australian National Algae Culture Collection, CSIRO Microalgae Research (Hobart, TAS, and Australia). This robust green microalgae can resist some variations in the ambient environment and invading microorganisms. This species was grown in the AnMBR effluent in 1-L flask, aerated at 1.5 L min^{-1} at ~20 °C and ~100 µmol photons m^{-2} s^{-1} light in a 20:4 hour light:dark cycle. The 4 hour darkness allows microalgae cell respiration and repair of their photosynthesis system. Light intensity was selected based on our preliminary assessment of culture conditions as well as reported value from literature [28]. This experimental step provides adaptation period to microalgae to AnMBR effluent. The species *C. vulgaris* was selected due to its high photosynthetic efficiency and high productivity as well as its resilience to bacterial contamination [29].

2.2 Anaerobic membrane bioreactor

The AnMBR consisted of a MINIFOR fermenter (Lambda Pty Ltd, Czech Republic) and a hollow fiber membrane module. The MINIFOR fermenter consisted of a 3 L glass reactor, two peristaltic pumps (i.e. feed and effluent pump), an overhead mixer, a redox-temperature-pH probe and temperature control unit (Fig. 1a).

The membrane unit comprised of 20 PVDF fibers (Evoqua Water Technologies, Australia) potted using epoxy resin. The length and pore size of the fibers were 30 cm and 0.04 µm, respectively, provides a surface area of 0.02 m^2.

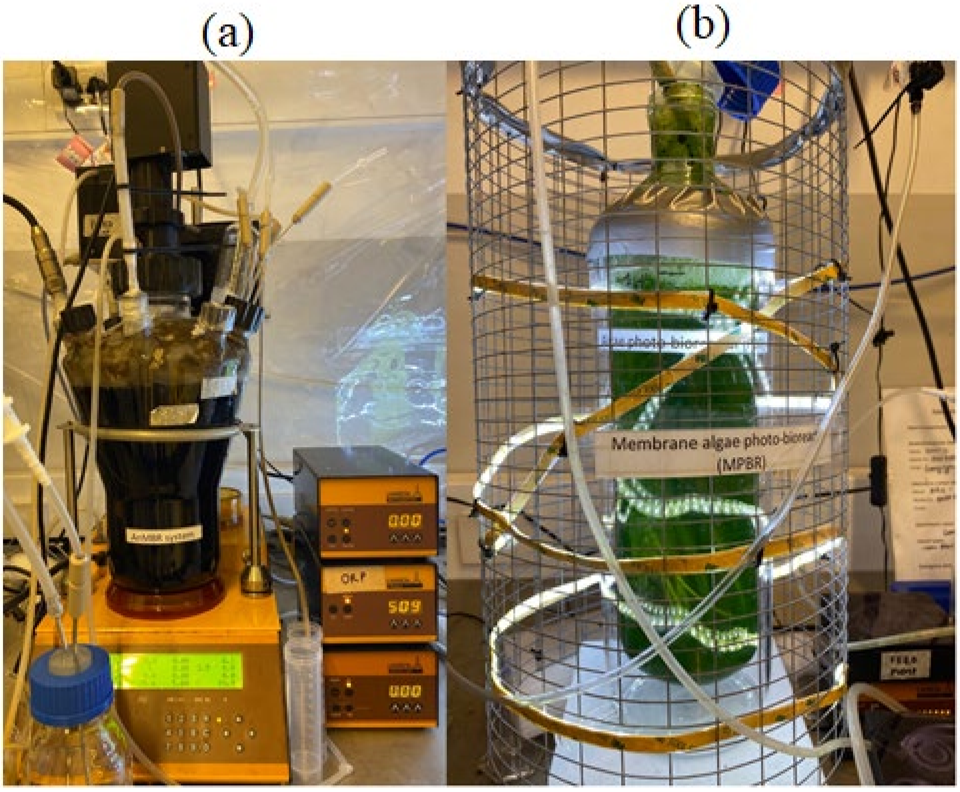


Figure 1. The hybrid (a) anaerobic membrane bioreactor and (b) microalgal membrane reactor

Anaerobic digested sludge taken from a full-scale mesophilic anaerobic digester (i.e. operates at 35 °C) of a domestic wastewater treatment plant (NSW, Australia) was used to seed the AnMBR with active volume of 2 L. A synthetic wastewater solution that simulated high organic and nutrient wastewater from food and agribusiness activities (i.e. high-strength wastewater). This wastewater contained per liter: glucose (1.875 g); peptone (3 g); KH_2PO_4 (220 mg); urea (540 mg); $MgCl_2$ (210 mg); $CoCl_2.6H_2O$ (6 mg); $FeSO_4.6H_2O$ (40 mg); $MnCl_2.4H_2O$ (1.8 mg); $Na_2MoO_4.2H_2O$ (1 mg); $NiCl_2.6H_2O$ (1 mg). The synthetic wastewater has COD, NH_4^+ and PO_4^{3-} of 7500, 164 and 66 mg/L, respectively equivalent to C: N: P ratio of 112: 3: 1 [30] and pH 7.

The hydraulic retention time of the AnMBR was maintained at 24 h. On the daily basis, 2 L of synthetic wastewater was fed in to the AnMBR at the flow rate of 1.4 mL min^{-1}. The membrane module was submerged in the reactor and was operated at constant flux of 4.2 L $m^{-2}h^{-1}$. It was operated with 10 min on and 1 min off cycles to provide relaxation time. The flux was periodically measured every two days to confirm no significant membrane fouling over the 80 days operation. The sludge retention time was 80 days (i.e. no sludge withdrawal excluding small volume for mixed liquor suspended solid analysis). The mixed liquor suspended solids was in the range of 10 to 14 g L^{-1}. The AnMBR was kept at 35 ± 0.1 °C. The produced biogas was continuously collected in 5 L gas bag daily. The gas volume and biogas content were measured using a portable GA5000 gas analyser for CH_4, CO_2 and H_2 (Geotechnical Instruments, UK).

2.3 Microalgal membrane reactor

A laboratory scale microalgal membrane reactor (MMR) system was used (Fig. 1b) including a 1.5 L cylindrical glass tank, influent and effluent pumps as well as air compressor. Another membrane module (section 2.2) was submerged in the MMR and operated at constant flux of 3.15 L m^{-2} h^{-1}. It was operated with 10 min on and 1 min off cycles to provide relaxation time. The MMR was aerated at the rate of 100 L min^{-1} via a diffusor located at the bottom of the reactor. Before entering the reactor, air was filtered through a 0.45 µm PES syringe filter (Sigma Aldrich, Australia).

The MMR was started by diluting the microalgae culture (Section 2.1) at a ratio of 1:50 (v/v) with AnMBR effluent (without any pre-treatment) to obtain an initial biomass concentration of 300 mg/L. The MMR was kept at room temperature (i.e. 22-23 °C) and illuminated on the side at ~100 µmol photons $m^{-2}s^{-1}$ light intensity in a 20:4 hour light:dark cycle.

The AnMBR effluent was continuously supplied to the MMR at a flow rate of 1.5 mL/min, resulting in a hydraulic retention time of 24 h. Fifty (50) mL of biomass solution (i.e. 1/30 of the biomass in the reactor) was removed from MMR daily at midday during, resulting in a cell retention time of 30 days. The MMR was operated for a period of 13 days and repeated twice.

2.4 Analytical methods

2.4.1 Organic carbon, nutrient measurement and energy recovery calculation

COD was measured using a Hach colorimetric method after filtering the samples through a glass fiber filter (0.45 µm). Ammonium (NH_4^+) in the AnMBR and MMR effluent were measured by using ammonia TNTplus vial kits with the DR3900 spectrophotometer (Hach Australia).

An ion chromatography (Thermofisher, Australia) was used to measure phosphorus (PO_4^{3-}) in the AnMBR and MMR effluent. The system includes a Dionex AS-AP auto sampler and Dionex AS19 IC column (7.5 µm pore size, 4 mm diameter and 250 mm length). A 10 µL sample was delivered in an isocratic mode with the hydroxide gradient (Time [min]: concentration [mM]) (0 – 10: 10; 10 -25: 45; 25-27: 45; 27-30: 10; 31) [29].

The potential energy recovery from the AnMBR (kWh kg^{-1} $COD_{(feed)}$ d^{-1}) was calculated by the following assumption and equations. Biogas has a calorific value of 22 MJ per 1 m^3 (equivalent to 6.1 kWh per 1 m^3) [31]. The electrical conversion efficiency is about 35% [32]. Therefore, 1 m^3 produces 2.14 kWh electricity. Accordingly, the energy yield (MJ/day) equalled daily biogas production (m^3/day x 22 MJ/ m^3) and the daily biogas production (m^3/day) equalled total biogas production per gram COD x total $COD_{(feed)}$ d.

2.4.2 Microalgal growth and harvesting method

Optical density was measured daily by the absorbance of a 2 mL of microalgal cell suspension at 680 nm using a UV spectrophotometer (UV 6000 Shimadzu,, Australia). Dry weight was determined by gravimetric analysis. The sample (50 mL) was filtered through a 1.1 µm pre-weighed glass fiber filter. The resulting fiber with microalgae deposition was dried at 60 °C to a constant mass over 4 h.

Flocculation using two cationic polyacrylamide polymer was used to harvest microalgal biomass from the MMR solution. Two polyacrylamide polymers namely BASF Zetag 3815 (SNF Pty Ltd; Corio, VIC, Australia) and Folpam FO 4808 (SZF Shanghai, China) were investigated. The polymers are high charge (>80% charge) and high molecular weight (>15 MegaDalton). A stock solution of the flocculant (0.2% w/v) was mixed at 100 rpm and 1 h in

Milli-Q water until fully dissolved. The stock solution was used within 4 hours of preparation to prevent hydrolysis. The polymer solution was added in the microalgal suspension in a dose-response fashion with gently mixed for one minute and then allowed to settle for another minute. Then, supernatant sample (10 mL) was pipetted from a height of one- and two-thirds from the bottom of the culture for evaluating the flocculation performance.

The flocculation efficiency was calculated based on the change in the optical density at wavelength of 680 nm (Equation 1) [33].

$$\text{Flocculation efficiency (\%)} = \left(\frac{OD_{i-OD_f}}{OD_i}\right) \times 100 \qquad \text{Equation 1}$$

Where OD_i and OD_f is the optical density before and after flocculant addition, respectively. Each polymer dosage was repeated three times.

3. Results and discussion

3.1 Potential energy recovery from high-strength wastewater

The AnMBR effectively removed COD from high-strength wastewater (Fig. 2). The results (> 97%) are in agreement with the consensus in literature that anaerobic treatment is effective for organic carbon removal [1; 34]. The main removal mechanism of anaerobic treatment involves different microbial groups that symbiotically use organic matter for energy and produce biogas (i.e. CH_4 and CO_2) [6].

The AnMBR produced 4.7 ± 0.15 L (*n = 80*) of biogas per day containing 64% CH_4 or 0.3 - 0.5 L biogas per gram of COD removal (Fig. 2). The theoretical methane potential (0.35 L

CH_4/g COD) is widely used to indicate the maximum methane yield from the anaerobic digestion. In this study, an average 0.19 L methane per g of COD removal was achieved. This value is higher than those commonly reported in the anaerobic digestion of primary or mixed primary and secondary sludge [1; 35; 36]. This is likely because the synthetic wastewater used in this study contained readily biodegradable organic carbon (i.e. glucose and peptone). The AnMBR in this study produced an average of 2.4 kWh/kg COD $_{(feed)}$ day (section 2.4.1). The theoretical the potential energy in wastewater is estimated as 14.7-17.8 mJ /kg COD or 4.5 kWh/kg COD [37]. A conventional wastewater treatment plant has an estimated energy consumption of 0.88 kWh/kg [38]. Based on these calculations, through the AnMBR treatment, positive energy production from wastewater may be achieved. This is reinforced by the study of Van Zyl et al. [5], which reported that biogas production could compensate seven times the energy required for AnMBR operation. Another recent study on a pilot scale AnMBR suggested that biogas could generate 73% of the energy consumption [39]. The results confirm the feasibility of AnMBR to treat high-strength wastewater and produce energy. This study is in line with the increasing interest in AnMBRs relates to resource recovery and the circular economy. Anaerobic treatment allows energy recovery through conversion of the organic carbon to methane gas, rather than the energy-intensive aerobic process. While integrating membrane separation into the anaerobic reactor provides similar effluent quality (i.e. turbidity, suspended solid free and low organic content) to the aerobic MBR, membrane fouling and subsequent cleaning requirements is one key technical challenge, limiting its widespread applications.

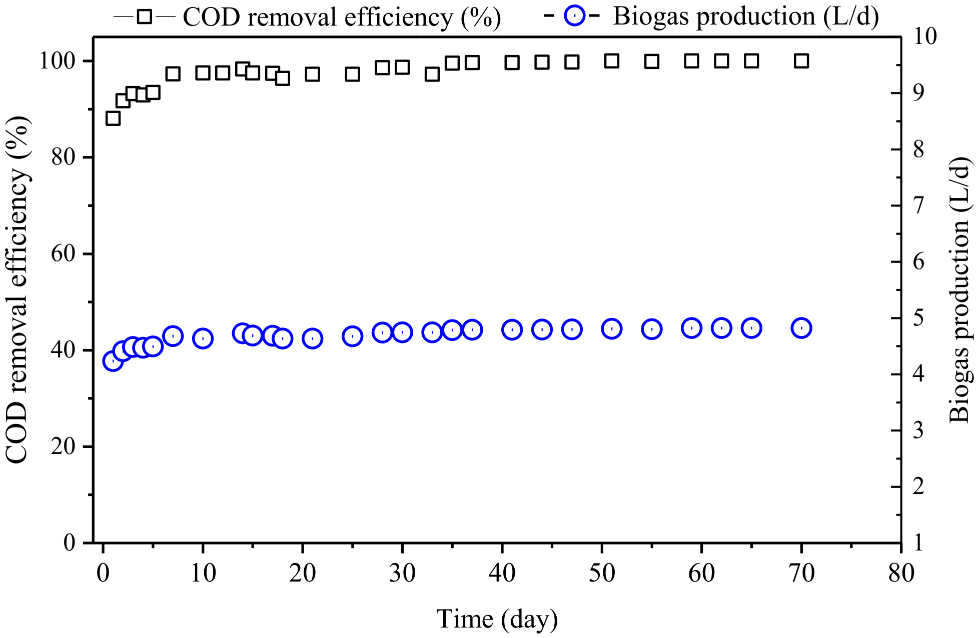


Figure 2. COD removal efficiency (%) and relative biogas volume production (L/d) by the AnMBR

3.2 Nutrient-rich AnMBR effluent

The AnMBR produced effluent with high level of ammonium (NH_4^+) and phosphate (PO_4^{3-}) (Fig. 3). NH_4^+ and PO_4^{3-} concentrations in the effluent were 1.9 and 1.4 times than that in the influent, respectively. Indeed, NH_4^+ and PO_4^{3-} concentrations gradually increased along experimental time (Fig. 3). This is because NH_4^+ and $PO_4^{3-}$is released from degradation of amino acids during acidogenesis and organic phosphorus, respectively. Conventionally, anaerobic effluent is returned as feed of aerobic treatment. Additional treatment such as physicochemical approaches might be necessary if nutrient loading were too high or addition of organic carbon to promote nitrification and denitrification [40] . It appears that the composition of the AnMBR effluent (i.e., low COD but high nutrient levels) is well suited for microalgal cultivation. Utilizing AnMBR effluent, which is plentiful and has little economic value, is a stepping stone towards cost-effective microalgal biomass production.

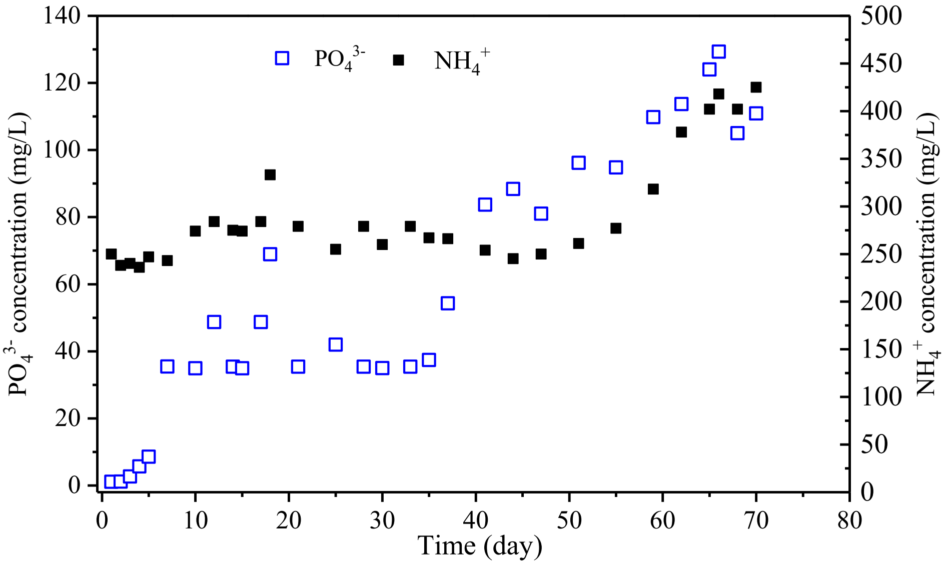


Figure 3. Nutrient-rich effluent generated from the AnMBR

3.3 Performance of membrane microalgal reactor

3.3.1 Biomass production and nutrient removal from AnMBR effluent

Biomass production in the MMR oscillated in the range of 450 to 700 mg/L over the 12 days operation (Fig. 4a). During the first six days, biomass production steadily increased (ca. 8.6 to 50 mg/L d). After this period, biomass production in the MMR decreased (Fig. 4a). This observation is consistent with the growth phase of microalgae (e.g. lag, exponential growth, declining growth, stationary and death phase) in batch photobioreactor studies [18; 41]. Previous studies have suggested that lag phase is critical especially when using nutrient-rich aqueous feed such as wastewater [26; 42]. The consequence can be the collapse of the microalgae culture [18; 42]. In this study, a high inoculum-to-reactor volume ratio was used (i.e. initial biomass 300 mg/L) to alleviate the impact of lag phase.

The declining growth phase of microalgal culture is one challenge for high throughput biomass production as well as a continuous MMR operation for nutrient removal (Fig. 4). In the MMR, nutrient removal is mainly contributed by microalgal uptake during growth phase. Ammonium can be absorbed through active transport and directly utilized to produce amino acids, while nitrate and nitrate can be converted to ammonium by nitrate reductase and nitrite reductase before further assimilation process. As such, the ammonium in the AnMBR is preferable nitrogen source for microalgal growth. Likewise, when entered into the cells, phosphorus is used for energy transfer and cell membrane formation as well as nucleic acid metabolism [10]. Of note, the MF membrane in this study does not retain soluble NH_4^+ and PO_4^{3-} ions in the solution. The pH of MMR ranged from 7 to 8, hence ammonia stripping could not possibly occur. Nitrogen and phosphate elimination in the MMR were due to biomass growth. Overall, this study confirms the feasibility of using AnMBR effluent for microalgal cultivation in short period (13 days). Long-term culture of microalgae resulted in the collapse of the microalgae culture. This is one possible limitation of microalgal-based wastewater treatment technique since, wastewater requires continuing operation. Another limitation is the requirement of a large reactor volume. The microalgal culture conditions requires aeration, light, homogenous cellular distribution and mass transfer of nutrients. These conditions are influenced by the reactor volume [43]. However, having a large microalgal reactor is counterproductive to the compact design of MBRs and wastewater treatment facilities in space-deficient locations [29].

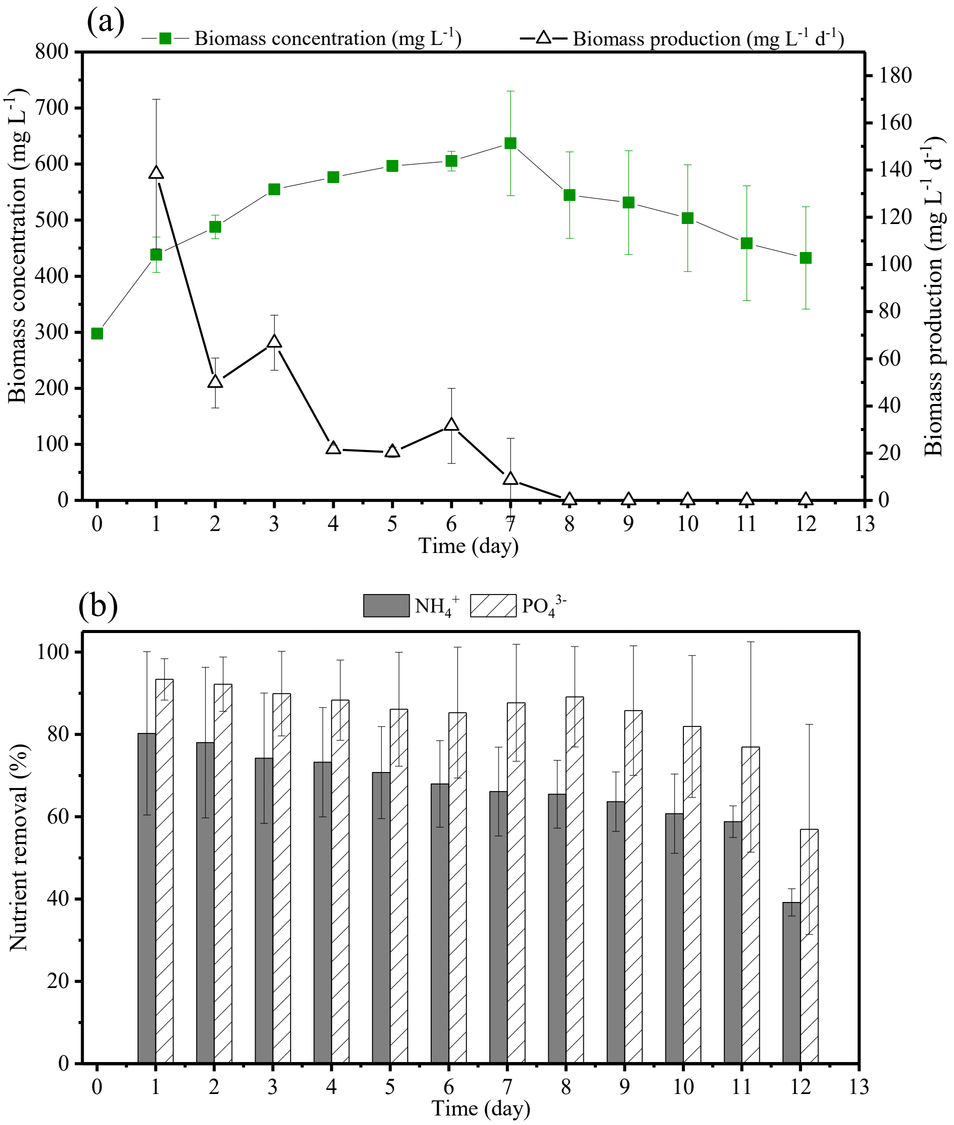


Figure 4. (a) Biomass concentration and production (dry weight mg L^{-1}) (b) nutrient removal efficiency (%) by the MMR. Values and error bars are mean and standard deviation of two identical MMRs.

3.3.2 Biomass harvesting

The microalgal biomass was effectively harvested from the reactor (Fig. 5). The dose-response relationship indicated that at 36 mg/g dry weight, an 80 ± 4.5 to 95 ± 5.0 % of biomass could be harvested using BASF Zetag 8185 and Flopam FO 4808, respectively. The negatively charged microalgal cells were neutralized by cationic polymer causing the formation of microalgal flocs. This mechanism is widely used in microalgal harvesting process [44]. Accordingly, high efficiency was achieved at a relatively small dosage compared to the previous studies using inorganic flocculants (e.g. $FeCl_2$, $Al_2(SO_4)_3$ and organic flocculants (e.g. cationic starch, chitosan) [45; 46; 47]. Optimisation of flocculant dose is an important step in algal biomass harvesting process. Over flocculant dose could be counterproductive [44] and increase operating cost. Microalgae harvesting has been identified as a major constraint in microalgae biotechnology development at industrial scale [47]. Operational cost of harvesting step attributes for 20 to 35% of total production cost. Thus, this study has identified two effective flocculants (i.e. low flocculant dose and simple method) for future microalgal harvesting process.

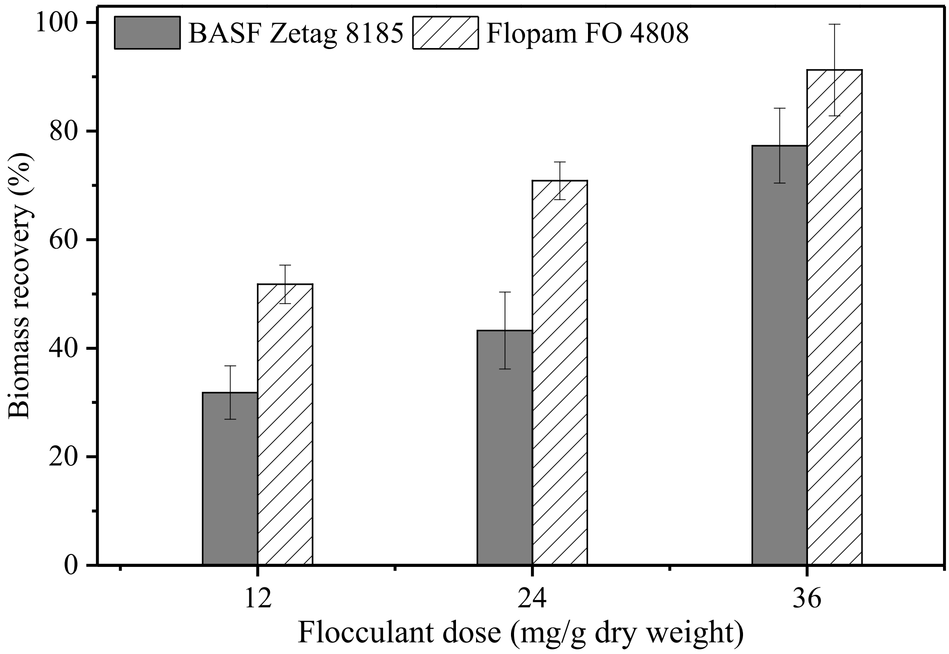


Figure 5. Performance of flocculant BASF Zetag 8185 and Flopam FO 4808 on microalgal solution. Values and error bars are mean and standard deviation of triplicate samples.

4. Conclusions

This study demonstrated that high-strength wastewater could be source for energy and microalgal biomass production through a hybrid AnMBR-MMR system. An equivalent of up to 2.4 kWh kg^{-1} COD$_{(feed)}$ d^{-1} could be achieved in the form of biogas through the AnMBR system that removed above 97% of COD. Nutrient removal was relatively low due to the liberation nitrogen and phosphorus via anaerobic metabolism. The nutrient-rich AnMBR effluent can be directly used for microalgae culture in MMR. However, further study is needed to optimise the MMR for continuous operation and high throughput microalgal biomass production. Biomass was effectively harvested (85-95%) using two cationic polymers, which can be used for future research.

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Declaration of author contributions

Minh T. Vu: Data curation, Formal analysis. **Hang P. Vu**: Data curation, Formal analysis. **Luong N. Nguyen**: Conceptualisation, Writing- review & editing. **Galilee U. Semblante:** Writing - review & editing**. Md Abu Hasan Johir:** Writing- review & editing**. Long D. Nghiem:** Supervision, Writing - review & editing and funding acquisition.

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