Sequential membrane bioreactor followed by membrane microalgal reactor for nutrient removal and algal biomass production

Accepted version

Environmental Science: Water Research & Technology

November 2019

Luong N. Nguyen[a*], Minh V. Truong[a], Anh Q. Nguyen[a], Md Abu Hasan Johir[a], Audrey S. Commault[b], Peter J. Ralph[b], Galilee U. Semblante[c] and Long D. Nghiem[a,d]

Please cite as:

Nguyen, L.N., Vu, M.T., Nguyen, A.Q., Johir, M., Commault, A.S., Ralph, P.J., Semblante, G.U. and Nghiem, L. (2019) Sequential membrane bioreactor followed by membrane microalgal reactor for nutrient removal and algal biomass production. Environmental Science: Water Research & Technology.

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

[b] Climate Change Cluster (C3), University of Technology Sydney, NSW 2007, Australia

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

[d] 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

A hybrid process combining a single compartment aerobic membrane bioreactor (MBR) and a membrane microalgal reactor (MMR) was evaluated for nutrient removal and microalgal biomass production. When operated without biomass extraction, the microalgal biomass in the MMR reached 920 mg L^{-1} on day 18 then collapsed, rendering nutrient removal ineffective. Stable operation of the MMR was achieved by regular biomass extraction (i.e. 1/30 of the microalgal biomass in the reactor daily). Biomass production at steady state was approximately 26 g $m^{-3}d^{-1}$. NO_3^- and PO_4^{3-} uptakes by microalgae were 4.0 ± 1.1 and 1.5 ± 0.9 g $m^{-3}d^{-1}$, respectively. A facile flocculation and separation technique capable of recovering 98% microalgal biomass was demonstrated. Although the hybrid process can significantly enhance nutrient removal and produce biomass, further research is needed to intensify the microalgal growth rate. At the current microalgal growth rate, a large MMR volume (37 times the MBR) is necessary for synchronous operation.

Keywords: Algae harvesting; Biomass recovery; Nutrient removal; Microalgae; Membrane bioreactor.

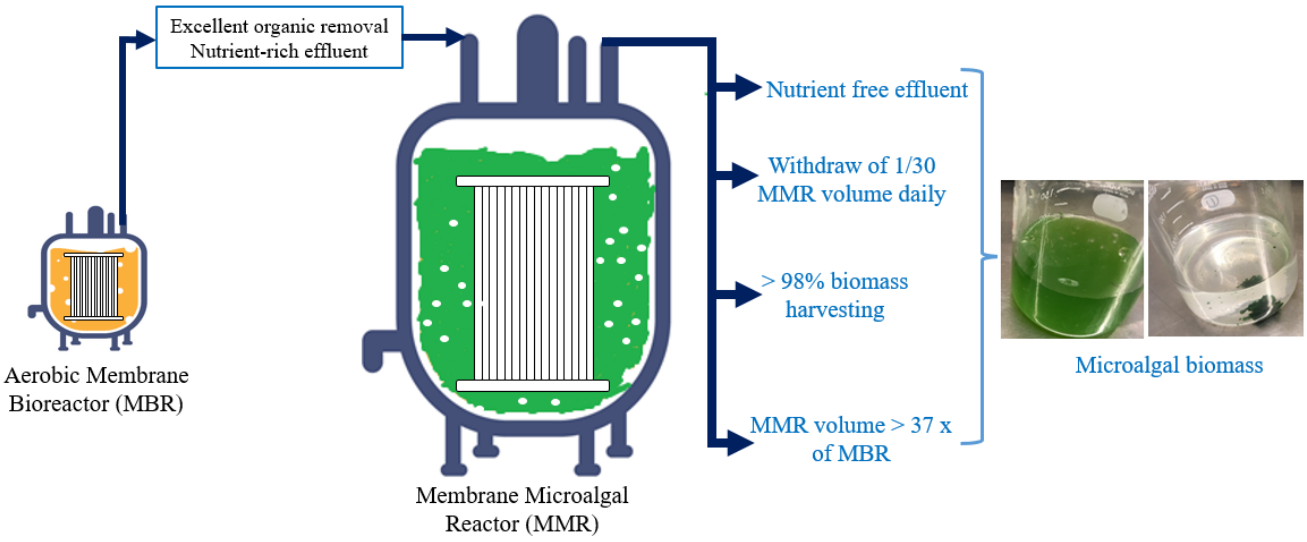
Water Impact Statement

The application of microalgae to simultaneously remove nutrients and produce valuable biomass from wastewater is a stepping stone to water sustainability. This study presents a method combining an aerobic membrane bioreactor and a membrane microalgal reactor to provide excellent nutrient removal and generate biomass. The results suggest that a synchronous operation can be achieved with high volume of the membrane microalgal reactor.

Highlight

- *Chlorella vulgaris* was used to remove nutrients from MBR effluent & produce biomass
- Without regular biomass extraction, the microalgae crashed after reaching maturity
- Daily extraction of 1/30 of the reactor biomass resulted in stable operation
- A simple polymer flocculation technique for microalgae harvesting was demonstrated
- A large microalgal reactor volume is required for synchronous operation

Graphical abstract



1. Introduction

Membrane bioreactor (MBR) is a hybrid between biological treatment and membrane separation (1, 2). In the biological reactor, microorganisms utilise dissolved organic carbon and oxygen to reproduce. The membrane then separate and retain microorganism biomass in the reactor to provide clean effluent free of suspended solids and bacteria. A simple MBR with a single aerobic stage can effectively remove dissolved organic carbon (90-95%) from wastewater (3, 4), but not nitrogen and phosphorus.

To meet nutrient discharge standards, complex biological processes with multiple redox stages are necessary. For example, biological nitrogen removal involves both aerobic (for nitrification) and anoxic (for denitrification) stage to convert ammonia to nitrate then nitrogen gas. Yet, biological nitrogen can only achieve 75% nitrogen removal at optimum conditions (3, 5). Moderate biological phosphorus removal (ca. 60-70%) is also possible under alternating aerobic and anaerobic conditions to enrich a specific microbial group called polyphosphate-accumulating organisms (5, 6). Biological phosphorus removal is a complex process that is energy intensive and difficult to optimise. Indeed, many large scale MBR plants have been built and commissioned over the past few years, the high cost of nutrient removal remains a major limitation (7-9). Additional processes, e.g. physical or chemical treatment, are often used to manage nutrients, resulting in an increase in the cost of wastewater treatment (4).

Nutrients (i.e. nitrogen and phosphorus) are essential for microalgae growth. Previous studies have shown that microalgae can effectively assimilate a variety of nitrogen (e.g. ammonia, nitrate, urea and peptone) (10-12) and phosphorous-bearing compounds (13-15). Microalgae have also been successfully cultivated in non-sterile environments such as wastewater (13, 14, 16, 17) with batch studies showing effective nitrogen and phosphorus removal (18, 19). Microalgal biomass is also a renewable feedstock for the production of animal feed, biofuel, and a range of biochemicals (20-23). Therefore, combining MBR with

90 microalgae cultivation is potentially an environmentally sustainable solution to (i) obtain high
91 effluent quality without use of complex processes or chemicals, (ii) enable beneficial reuse of
92 residual nutrients from the effluent, and (iii) produce valuable microalgal biomass (24-26).
93 However, to date, there have been only a few studies examining the integration of these two
94 processes. Key technical considerations including (a) microalgal biomass production rate and
95 nutrient removal efficiency, (b) separation of microalgae from the final effluent and biomass
96 harvesting, and (c) synchronization of MBR and microalgae process have been recommended
97 in future studies to enhance the readiness of MBR coupled MMR process (24, 25) .
98 This study aims to assess the performance of a combined MBR and membrane microalgae
99 reactor (MMR), focusing on answering initial questions about nutrient removal efficiency,
100 microalgae harvesting, and reactor synchronization. The microalgal biomass growth rate
101 under MBR effluent as well as the microalgal flocculation efficiency using cationic
102 polyacrylamide polymers will be determined. The results of the study will provide important
103 insights on the feasibility of incorporating microalgae to MBR technology and wastewater
104 treatment process.
105 **2. Materials and methods**
106 2.1 Microalgae strains and growth conditions
107 The freshwater green algae *Chlorella vulgaris* (CS-41) was obtained from the Australian
108 National Algae Culture Collection, CSIRO Microalgae Research (Hobart, TAS, and
109 Australia). The culture was maintained in the Climate Change Cluster (C3) culture collection
110 at University Technology Sydney in freshwater MLA media (Algaboost; Wallaroo, SA,
111 Australia). Seed cultures were grown to early stationary phase in 1-L Schott's bottles, bubbled
112 with air at ~20 °C and ~100 μmol photons m^{-2} s^{-1} light in a 16:8 hour light:dark cycle. *C.*
113 *vulgaris* was selected due to its high photosynthetic efficiency and high productivity (20).

114 2.2 Membrane bioreactor

115 A laboratory scale MBR system was employed in this study. The system consisted of an
116 acrylic reactor with an active volume of 3 L, a pressure sensor, air supply compressor, and
117 influent and effluent pumps. A hollow fiber membrane module was made in the laboratory of
118 Centre of Technology for Water and Wastewater, University of Technology Sydney,
119 Australia. The module comprised of 40 PVDF fibers (supplied by Evoqua Water
120 Technologies, Australia) of 30 cm in length and 0.04 μm in pore size. The effective surface
121 area of the membrane module was approximately 0.04 m^2. The module was plotted using
122 epoxy resin (Selleys Araldite Ultra Clear). The membrane module was submerged in the
123 reactor and operated on 9 min suction and 1 min rest cycle mode with an average flux of 6.3 L
124 m^{-2} h^{-1}. The reactor was aerated at air flow rate of 100 L min^{-1} via two diffusors located at the
125 bottom of the reactor. This resulted in a dissolved oxygen concentration of 3 to 6 mg L^{-1}.
126 Transmembrane pressure was continuously monitored using a high resolution pressure sensor
127 (± 0.1 kPa) (Cole-Parmer Pressure meter) to confirm that no significant membrane fouling has
128 occurred during the experimental period. Transmembrane pressure was 35.2 kPa at the end of
129 the experimental period, thus no membrane cleaning or backwashing was performed.

130 Activated sludge taken from an aeration tank of a municipal wastewater treatment plant
131 (NSW, Australia) was used to seed the MBR. The hydraulic retention time was set at 24 h.
132 Apart from samples for mixed liquor suspended solid analysis, no sludge was withdrawn from
133 the MBR at any stage of this study (i.e. 150 days), and thus sludge retention time was closely
134 to 150 days. The mixed liquor suspended solid oscillated in the range of 2.9 to 6.3 mg L^{-1}
135 (5.06 ± 1.02, $n = 45$). The MBR was kept at laboratory room temperature (i.e. 22-23 °C). The
136 synthetic feed contained per liter: glucose (1.83 g), NH_4Cl (30 mg), KH_2PO_4 (340 mg),
137 K_2HPO_4 (600 mg), $MgSO_4$ (270 mg), $FeSO_4$ (10 mg), and 10 mL of 100 × trace element
138 solution ($ZnSO_4 \cdot 7H_2O$ 0.35 mg, $MnSO_4 \cdot H_2O$ 0.21 mg, H_3BO_4 2.1 mg, $CoCl_2 \cdot 2H_2O$ 1.4 mg,
139 $CuCl_2 \cdot 2H_20$ 0.07 mg, $NiSO_4 \cdot 6H_2O$ 0.1 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.21 mg) as described previously

(1). The synthetic feed had a ratio of COD, total nitrogen and total phosphorous (COD:TN:TP) of 80:5:1 represent composition of municipal wastewater. MBR effluent was collected daily into 5 L container and pumped to the membrane microalgae reactor at designed flow rate of 1.5 mL/min (Section 2.3).

2.3 Membrane microalgae reactor

A laboratory scale MMR system was used. The system consisted of a glass-cylindrical tank with an active volume of 1.5 L, air supply compressor, and influent and effluent pumps. Another membrane module (same as described in section 2.2) was submerged in the photobioreactor. The membrane module was operated at an average flux of 3.15 L m^{-2} h^{-1}. The reactor was aerated at air flow rate of 100 L min^{-1} via a diffusor located at the bottom of the reactor.

The MMR was started by diluting the algae culture (section 2.1) at a ratio of 1:50 (v/v) with MBR permeate to a total of 1.5 L. The MMR was kept at room temperature (i.e. 22-23 °C) and illuminated on the side at ~125 μmol photons/m^2 s light intensity in a 16:8 hour light:dark cycle. The MBR permeate (1.5 L) was continuously supplied to the MMR at a flow rate of 1.5 mL/min, resulting in a hydraulic retention time of 24 h. 50 mL of biomass solution (i.e. 1:30 of the biomass in the reactor) was removed of the MMR every day at midday during operation and referred to as “biomass extraction” in the text, equivalent to cell retention time of 30 days.

2.4 Analytical methods

2.4.1 Organic carbon and nutrient measurement

Total organic carbon (TOC) of the MBR feed and permeate and the MMR permeate was measured by using a total organic carbon analyser (Milti N/C 3100, AnalytikJena, Germany). The instrument was calibrated using potassium hydrogen phthalate at a range concentration of 1 to 100 mg L^{-1}.

Nitrite (NO_2^-), nitrate (NO_3^-) and phosphorus (PO_4^{3-}) in the MBR and MMR permeate were measured by using ion chromatography (Thermofisher, Australia). The system was equipped with a Dionex AS-AP Auto sampler, 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 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 stop run).

2.4.2 Microalgal growth

The optical density and dry weight of the microalgal culture were determined daily by a UV spectrophotometer (UV 6000 Shimadzu; Ermington, NSW, Australia) at a wavelength of 680 nm and gravimetric analysis, respectively, to assess microalgal growth. For optical density analysis, 2 mL of homogenous microalgae cells suspension was transferred into a cuvette to measure optical density. For gravimetric analysis, 50 mL of microalgal cells suspension 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. Linear regression coefficient (R^2) of 0.96 was obtained between optical density and dry weight biomass.

2.4.3 Microalgal biomass harvesting

Microalgal biomass was harvested by flocculation with two high charge (>80% charge) and high molecular weight (>15 MegaDalton) cationic polyacrylamide polymers. These polymers are BASF Zetag 3815 (SNF Pty Ltd; Corio, VIC, Australia) and Folpam FO 3808 (SZF Shanghai, China). A stock solution of the flocculant (0.4% w/v) was prepared in Milli-Q water with continuous mixing at 100 rpm for 1 h and stored at room temperature and used within 1 day of preparation.

Microalgal suspension and flocculant solution were gently mixed for one minute and then allowed to settle for another minute. An aliquot (10 mL) of the culture in the bottle was

pipetted from a height of one- and two-thirds from the bottom for evaluating the flocculation performance.

The flocculation efficiency was calculated based on the change in the optical density at wavelength of 680 nm before and after each flocculant addition, as shown in the following equation.

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

Where OD_i and OD_f is the optical density of the culture before and after flocculant addition. Each polymer dose was repeated three times.

2.3.4 Membrane microalgae reactor calculation

MMR system should be synchronized to remove residual nutrient in the MBR effluent. However, the update rate of nutrient by microalgae can be slow, requiring a larger reactor volume. This reactor volume was calculated based on the nutrient concentration of MBR effluent and the nutrient uptake rate by the microalgae during one day of operation.

$$\text{MMR volume (L)} = (C_{01} - C_0)/U$$

Where, C_0 is nutrient concentration in the MBR effluent (mg/L); C_1 in nutrient concentration in the MMR effluent (i.e. with 100% removal) and U is nutrient uptake by microalgae per 1 L volume.

3. Results and discussion

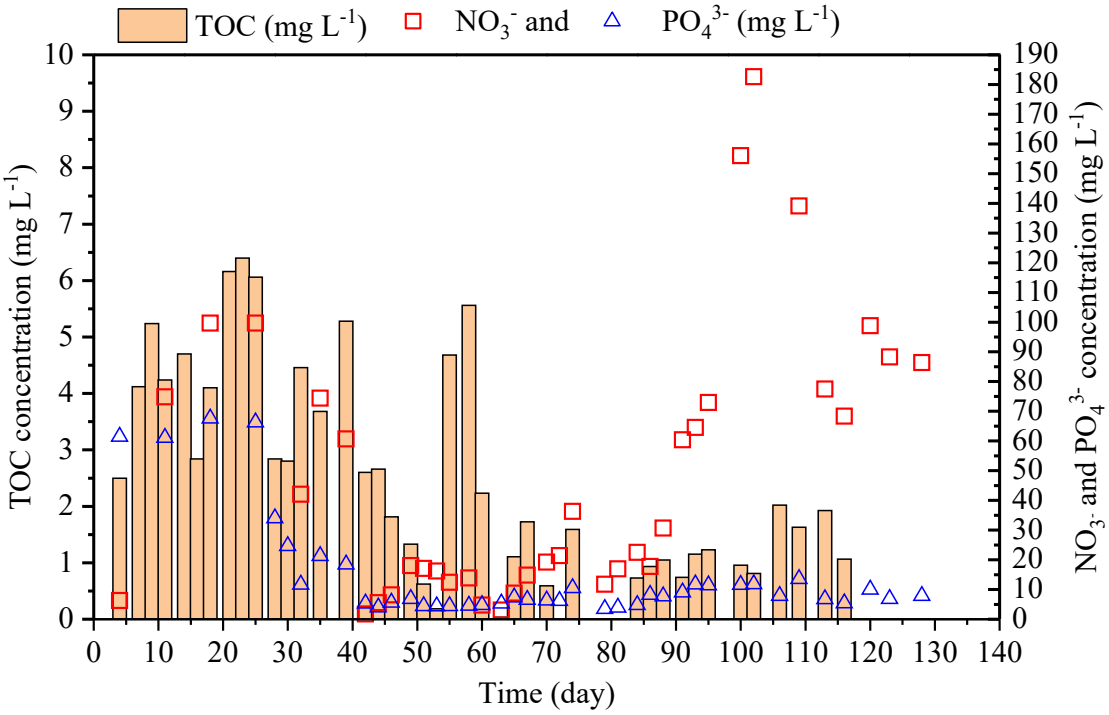
3.1 Bulk organic removal by MBR

The aerobic MBR system consistently removed over 99% of organic carbon, resulting in a final total organic carbon concentration in the MBR effluent of ca 0.5 – 6.5 mg L^{-1} (Fig 1). This result is likely due to the presence of high biomass level (i.e. mixed liquor suspended solid of 5.06 ± 1.02 g L^{-1}, *n = 45*) and aerobic conditions (i.e. dissolved oxygen of 3 -7 mg L^{-1}) in the reactor. Under aerobic condition, activated sludge microorganisms (i.e. heterotrophs) can breakdown organic carbon for their growth and maintenance. These microorganisms

oxidize a wide range of organic compounds and are essential in the removal of carbonaceous materials (3).

Efficient removal of organic carbon by the aerobic MBR limits the carbon source available for bacterial growth in the MMR. Keeping the bacterial level low in the MMR facilitates the growth of microalgae by reducing competition for nutrients and/or preventing bacteria predation on algae biomass. As such, initial removal of organic carbon and bacteria is often required for microalgae cultivation using wastewater (27). A low organic carbon content also promotes microalgae to uptake CO_2, providing additional benefit to the MMR. The aerobic MBR in this study offers a solution for both organic carbon and bacteria removal.

Effluent from the aerobic MBR system could be directly used for microalgal cultivation without any supplements (Fig 1). During aerobic respiration, microbes in the MBR reactor produce up to 182 mg L^{-1} of nitrate (NO_3^-) and 66.3 mg L^{-1} of phosphorus (PO_4^{3-}). The concentration of ammonia and nitrite were negligible, suggesting a full nitrification in the aerobic MBR. Nitrate and phosphorus concentrations fluctuated over the operation period (Fig 1). This variation represents a realistic situation in a full-scale system, resulting in the N/P ratio between 6.3 and 14.4, with the mean value of 10.3. Wastewater N/P ratio is an important parameter for microalgal growth. It has been suggested that the optimal N/P ratio for microalgae cultivation is between 7.5 and 9.6 as it represents the N/P ratio in microalgal biomass (25, 28). The N/P ratio of the effluent in this study was close to that requirement for *C. vulgaris* (16, 25) and was in the optimal range for a number of microalgal species (13).



235

236 **Figure 1**. TOC and nutrient concentrations in MBR effluent. The MMR was coupled with the

237 MBR (i.e. MMR operated in batch mode ("without biomass extraction") from day 40 to 75

238 and continuous mode ("with biomass extraction") from 80 to 120 day.

239

240 3.2 MMR without biomass extraction

241 The growth of microalgae in the MMR was monitored overtime (Fig 2a). The initial lag-

242 phase lasted 8 days. This long lag-phase might correspond to the adaptation of the microalgae

243 to the new culture medium (from MLA media to MBR effluent). After this phase, microalgae

244 grew rapidly and reached stationary phase after 18 days of operation at a biomass of 920 mg

245 L^{-1}. The obtained biomass concentration was similar to the reported biomass from batch

246 photobioreactor studies and continuous photobioreactor using microalgal culture medium

247 (14). This result confirms the feasibility of using MBR effluent for microalgal cultivation.

248 However, prolong operation periods resulted in the collapse of the microalgae culture. At day

249 20, the biomass in the MMR dropped to 800 mg L^{-1} and continued declining to 250 mg L^{-1} at

day 27 (Fig 2a). Ecological collapse of microalgae culture in MMR configuration has been neglected in the literature. This is because most photobioreactor studies were only conducted over a short period (25, 28). To address this issue, continuous operation of the MMR (noted as "MMR with biomass extraction" in this study) was investigated.

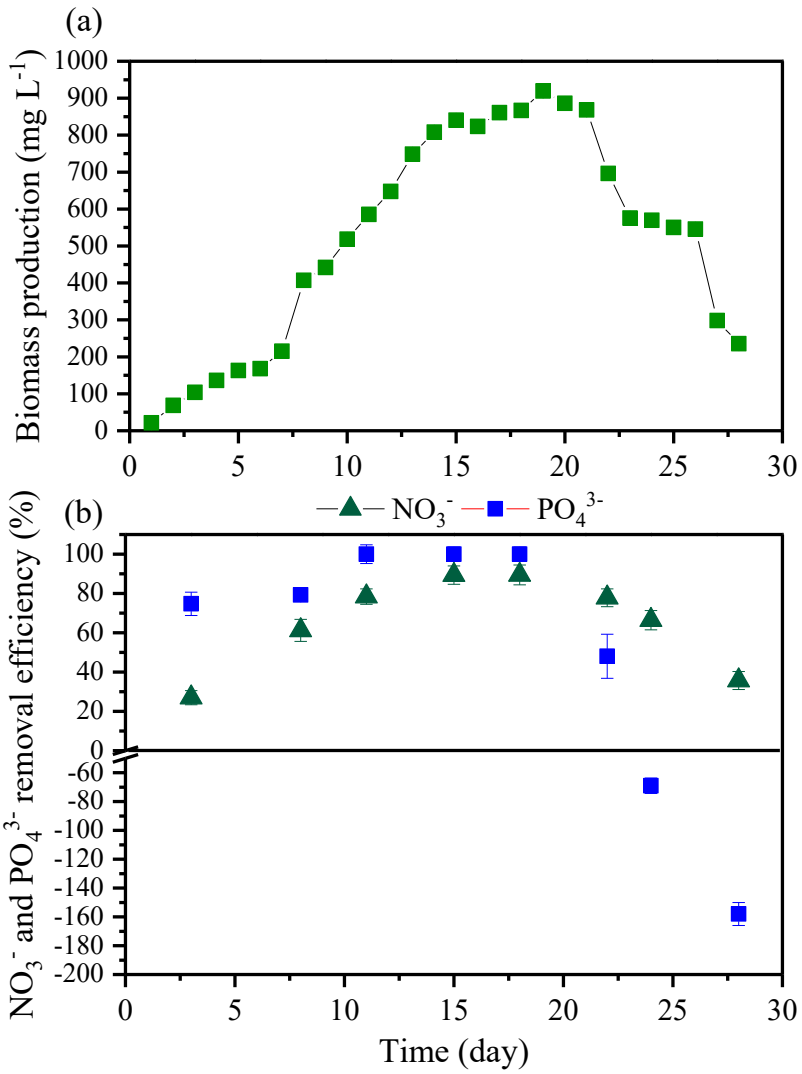


Figure 2. Biomass production (dry weight) (a) and nutrient removal efficiency (b) by the MMR without biomass extraction. Values and error bars are mean and standard deviation of duplicate measurements.

NO_3^- and PO_4^{3-} efficiency values of 75% and 99%, respectively, were achieved at day 11 and retrained stable till day 20 (Fig 2b). This achievement concurred with the microalgae growth phase in the reactor. The removal efficiencies correspond to uptake rates of 15.9 ± 1.6

261 mg L^{-1} for NO_3^- and 4.3 ± 0.9 mg L^{-1} for PO_4^{3-} (*n=5*), respectively. The uptake of nutrient
262 through microalgal assimilation into biomass which is retained by the membrane is a major
263 nutrient removal mechanism by the MMR. It is noted that the MF membrane in this study
264 does not retain soluble NO_3^- and PO_4^{3-} ions in the solution. Previous studies have also
265 suggested that the microalgal assimilation is important for nutrient removal (14). Thus,
266 maximizing microalgal growth is the solution to enhance nutrient removal in MMR. In batch
267 mode, the algal biomass has to be harvested in late exponential, beginning of stationary phase
268 to avoid the release of NO_3^- and PO_4^{3-} into the growth medium due to cell lysis.

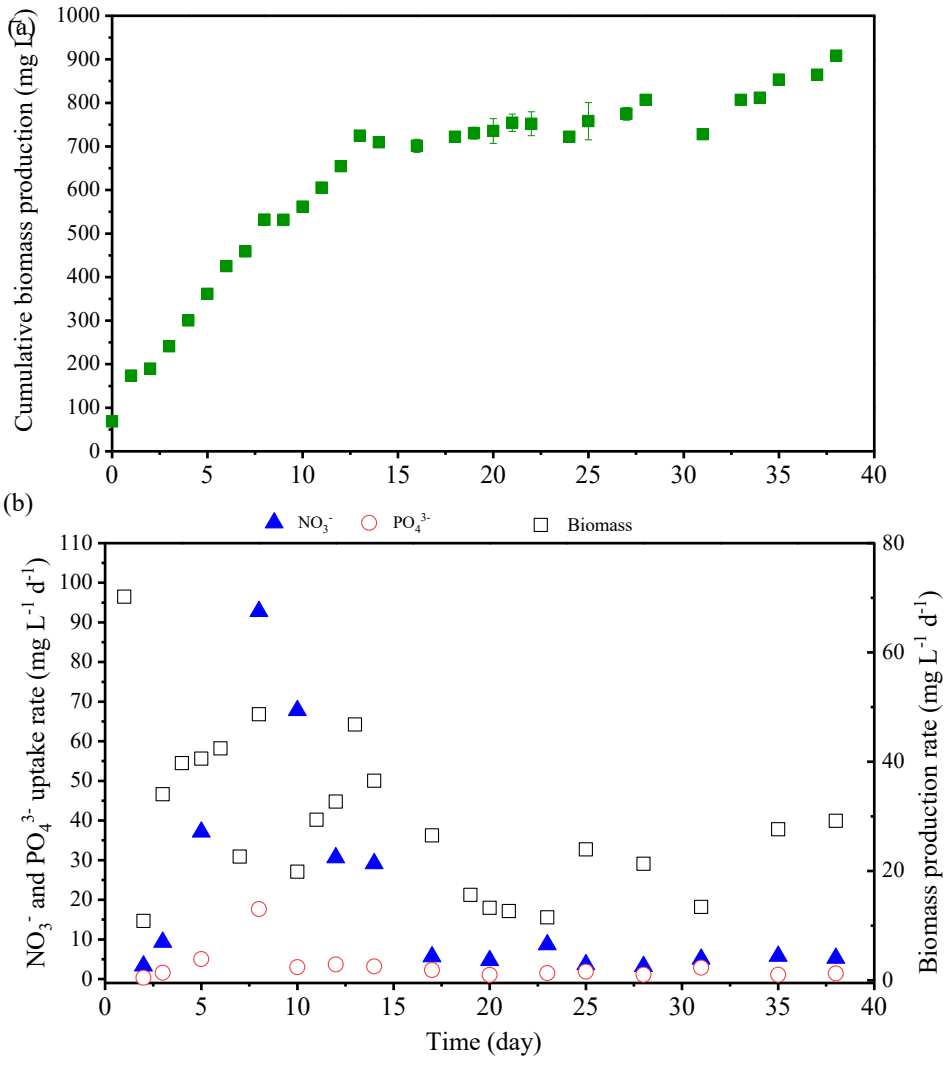
269 3.3 MMR with biomass extraction

270 3.3.1 Biomass production and nutrient uptake

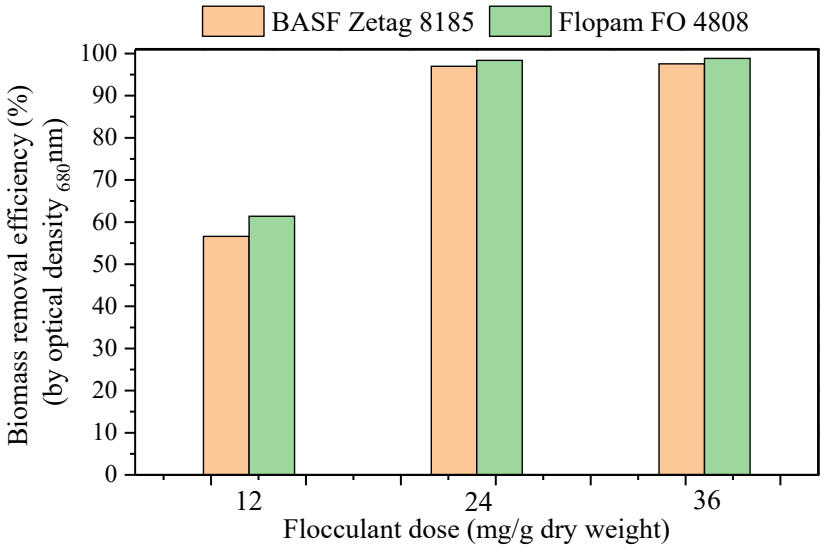
271 Periodical extraction of biomass improved microalgal growth in the MMR (Fig 3a). The
272 biomass in the MMR rapidly increased from 60 to 730 mg L^{-1} within 15 days. A steep
273 increase in biomass in the reactor is likely due to the higher production rate than that of
274 biomass in the 50-mL microalgal culture daily at this stage. There was a short lag phase of 2
275 days, indicating the adaptation of *C. vulgaris* to the MBR effluent (i.e. without adaptation, the
276 lag phase was 8 days). After this period, biomass production oscillated in the range of 730 –
277 900 mg L^{-1}. The biomass withdrew daily could improve the growth of the remaining algae by
278 reducing light and carbon limitation as seen with high cell density culture (29). Overall, the
279 average volumetric biomass productivity in the MMR was 25.8 ± 13.5 g $m^{-3}d^{-1}$ generating
280 1.29 ± 0.69 mg of dried biomass (i.e. from 50 mL microalgal suspension) was harvested per
281 day, using polymers with a 98% harvesting efficiency (Section 3.3.2).

282 As noted in Section 3.2, nutrient removal in the MMR is governed by microalgal
283 assimilation. Accordingly, the consumption of NO_3^- and PO_4^{3-} was high in the first 15 days
284 and then stabilized with an uptake rate of 4.0 ± 1.1 and 1.5 ± 0.9 g $m^{-3}d^{-1}$ (*n=8*) for NO_3^- and
285 PO_4^{3-}, respectively. When assimilated by the cells, phosphorus is used in energy transfer and
286 formation of cell membranes and nucleic acid metabolism (10). This process was found to be

slow, affecting the nutrient uptake and removal in the MMR. Although previous studies reported the variable removal efficiency, the differences in microalgal species, cultivation time, batch and continuous operations as well as the initial nutrient concentration make the direct comparison with literature data impossible (25, 27, 28). For example, Kothari et al. (30) achieved 87% removal of phosphorus by *C. pyrenoidosa* from dairy industrial wastewater after 10 days of batch culture. This removal was achieved in association with the increase in biomass production and cultivation time. The results from this study suggested that nutrient uptake rate was not proportional to the increase of the nutrient's supply. Therefore, the reactor volume of the MMR should be larger to meet the requirement of nutrient removal.



297 **Figure 3**. Cumulative biomass production in dry weight (a), nutrient removal efficiency and
298 biomass production rate (b) by the MMR with biomass extraction. Values and error bars are
299 mean and standard deviation of duplicate measurements.
300
301 3.3.2 Biomass harvesting
302 The microalgal biomass was effectively harvested from the reactor by flocculant cationic
303 polyacrylamide (Fig 4). With a flocculant dose of 24 mg g^{-1} dry weight, the optical density
304 OD_{680} decreased by 98%. Previous studies have established 90% reduction in optical density
305 as the bench mark for effective flocculation for microalgae harvesting (31, 32). Moreover, the
306 flocculant dose was relatively small in this study in comparison to the previous reports (33-
307 35). This is likely because of the high cationic charge density (> 80%) of the polymer that
308 supports the charge neutralization of the negatively charged microalgal cells. The efficiency
309 of microalgal harvesting in this study allows for 98% of the biomass collection as well as the
310 safely discharge of effluent (i.e. prevention of introduction of microalgal species to aquatic
311 environment).

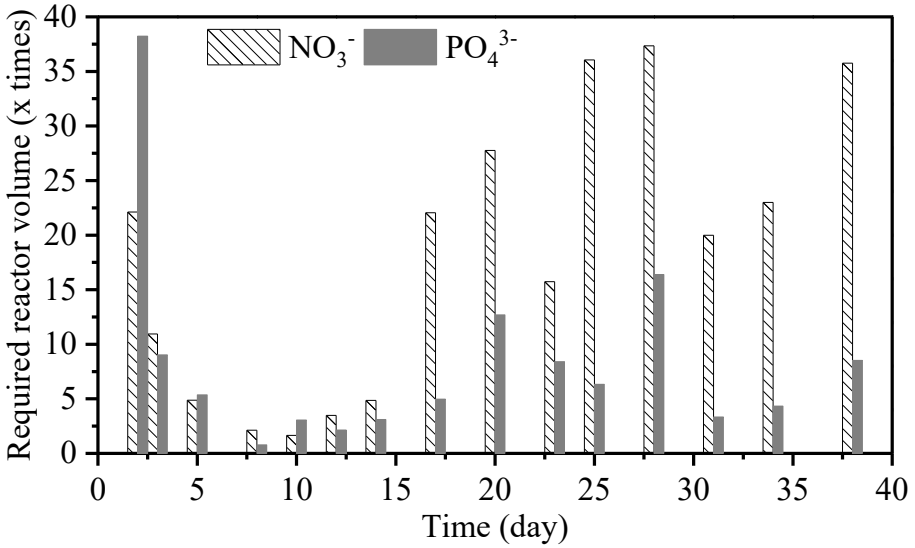


312
313 **Figure 4**. The effect of BASF Zetag 8185 and Flopam FO 4808 flocculant doses on
314 flocculation efficiency indicating by the optical density removal at λ = 680 nm (*C. vulgaris*).

315

316 3.3.3 Synchronization of MMR to MBR treatment

317 The limitation of nutrient uptake (i.e. slow and low level) by microalgae and the
318 requirement of high effluent quality indicate that a large reactor volume of the MMR is
319 required given the growth conditions tested (Fig 5). This required that the reactor volume was
320 calculated by a ratio of nitrate and phosphorus concentrations in MBR permeate and their
321 uptake rate in the MMR (i.e. to achieve 100% removal). The volume of the MMR should be
322 at least 37 times that of the MBR (Fig 5) with an operation of 1 day hydraulic retention time.
323 Large microalgae reactor volume has been suggested in the literature (25, 36) to facilitate
324 light penetration, homogenous cellular distribution, and mass transfer, and others. However,
325 having a large MMR is counterproductive to the compact design of MBRs and wastewater
326 treatment facilities situated in urban and other space-deficient locations. In other words,
327 having a large footprint could hinder the translation of a coupled MBR-MMR technology to
328 industrial-scale level. Therefore, further investigation is recommended to increase the rate of
329 microalgal growth through optimizing key operating parameters reactor shape, irradiation,
330 and retention time to facilitate the implementation of MMR in wastewater treatment
331 processes.



332

333 **Figure 5**. Required reactor volume of the MMR to compensate MBR permeate volume at

334 100% removal of nitrate and phosphorus.

335 **4. Conclusions**

336 This study demonstrated excellent NO_3^- and PO_4^{3-} uptake rate of 4.0 ± 1.1 and 1.5 ± 0.9 g m^{-3}

337 d^{-1}, respectively, by a membrane microalgal reactor (MMR) to further remove nutrients from

338 a conventional MBR treating synthetic municipal wastewater. Stable operation of the MMR

339 was achieved by extracting the produced biomass (1:30 of microalgal biomass in the reactor,

340 cell retention time of 30 days) on a daily basis. A facile flocculation and separation technique

341 for microalgal biomass harvesting was also demonstrated to achieve 98% efficiency. Further

342 research to optimize microalgal production is needed to increase the microalgal growth rate to

343 reduce the MMR reactor volume for scaling-up and practical application of MMR in

344 wastewater treatment.

345 **5. Acknowledgements**

346 The authors acknowledged the funding supports from the Faculty of Engineering and

347 Information Technology, University of Technology Sydney under Tech lab BlueSky Project

funding scheme 2019. Evoqua Water Technologies (South Windsor, NSW 2756, Australia) is thanked for the provision of membrane samples.

References

1. Nguyen LN, Hai FI, Kang J, Price WE, Nghiem LD. Removal of trace organic contaminants by a membrane bioreactor–granular activated carbon (MBR–GAC) system. Bioresour Technol. 2012;113:169-73.
2. Ma J, Dai R, Chen M, Khan SJ, Wang Z. Applications of membrane bioreactors for water reclamation: Micropollutant removal, mechanisms and perspectives. Bioresour Technol. 2018;269:532-43.
3. Menger-Krug E, Niederste-Hollenberg J, Hillenbrand T, Hiessl H. Integration of Microalgae Systems at Municipal Wastewater Treatment Plants: Implications for Energy and Emission Balances. Environ Sci Technol. 2012;46(21):11505-14.
4. Shao S, Qu F, Liang H, Chang H, Yu H, Li G. A pilot-scale study of a powdered activated carbon-membrane bioreactor for the treatment of water with a high concentration of ammonia. Environ Sci Water Res Technol. 2016;2(1):125-33.
5. Zuthi MFR, Guo WS, Ngo HH, Nghiem LD, Hai FI. Enhanced biological phosphorus removal and its modeling for the activated sludge and membrane bioreactor processes. Bioresour Technol. 2013;139:363-74.
6. Zhang H-L, Fang W, Wang Y-P, Sheng G-P, Zeng RJ, Li W-W, et al. Phosphorus Removal in an Enhanced Biological Phosphorus Removal Process: Roles of Extracellular Polymeric Substances. Environ Sci Technol. 2013;47(20):11482-9.
7. Xiao K, Liang S, Wang X, Chen C, Huang X. Current state and challenges of full-scale membrane bioreactor applications: A critical review. Bioresour Technol. 2019;271:473-81.
8. Sun F-y, Wang X-m, Li X-y. An innovative membrane bioreactor (MBR) system for simultaneous nitrogen and phosphorus removal. Process Biochem. 2013;48(11):1749-56.
9. Seib MD, Berg KJ, Zitomer DH. Reduced energy demand for municipal wastewater recovery using an anaerobic floating filter membrane bioreactor. Environ Sci Water Res Technol. 2016;2(2):290-7.
10. Perez-Garcia O, Escalante FME, de-Bashan LE, Bashan Y. Heterotrophic cultures of microalgae: Metabolism and potential products. Water Res. 2011;45(1):11-36.
11. Commault AS, Laczka O, Siboni N, Tamburic B, Crosswell JR, Seymour JR, et al. Electricity and biomass production in a bacteria-Chlorella based microbial fuel cell treating wastewater. Journal of Power Sources. 2017;356:299-309.
12. Hemalatha M, Sravan JS, Min B, Venkata Mohan S. Microalgae-biorefinery with cascading resource recovery design associated to dairy wastewater treatment. Bioresour Technol. 2019;284:424-9.

13. Chiu S-Y, Kao C-Y, Chen T-Y, Chang Y-B, Kuo C-M, Lin C-S. Cultivation of microalgal Chlorella for biomass and lipid production using wastewater as nutrient resource. Bioresour Technol. 2015;184:179-89.
14. Gao F, Li C, Yang Z-H, Zeng G-M, Feng L-J, Liu J-z, et al. Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. Ecological Engineering. 2016;92:55-61.
15. Chew KW, Chia SR, Show PL, Ling TC, Arya SS, Chang J-S. Food waste compost as an organic nutrient source for the cultivation of Chlorella vulgaris. Bioresour Technol. 2018;267:356-62.
16. Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, et al. Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. Appl Biochem Biotechnol. 2010;162(4):1174-86.
17. Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol. 2011;102(1):17-25.
18. Ruiz-Martinez A, Martin Garcia N, Romero I, Seco A, Ferrer J. Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent. Bioresour Technol. 2012;126:247-53.
19. Dickinson KE, Whitney CG, McGinn PJ. Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalga Scenedesmus sp. AMDD. Algal Research. 2013;2(2):127-34.
20. Vo P, Ngo HH, Guo WS, Chang SW, Nguyen DD, Nguyen PD, et al. Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation? Bioresour Technol. 2018;256:491-501.
21. Jacob-Lopes E, Maroneze MM, Deprá MC, Sartori RB, Dias RR, Zepka LQ. Bioactive food compounds from microalgae: an innovative framework on industrial biorefineries. Current Opinion in Food Science. 2019;25:1-7.
22. Nagarajan D, Lee D-J, Chang J-S. Integration of anaerobic digestion and microalgal cultivation for digestate bioremediation and biogas upgrading. Bioresour Technol. 2019;290:121804.
23. Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. Bioresour Technol. 2011;102(1):71-81.
24. Edmundson S, Huesemann M, Kruk R, Lemmon T, Billing J, Schmidt A, et al. Phosphorus and nitrogen recycle following algal bio-crude production via continuous hydrothermal liquefaction. Algal Research. 2017;26:415-21.
25. Luo Y, Le-Clech P, Henderson RK. Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors: A review. Algal Research. 2017;24:425-37.
26. He PJ, Mao B, Shen CM, Shao LM, Lee DJ, Chang JS. Cultivation of Chlorella vulgaris on wastewater containing high levels of ammonia for biodiesel production. Bioresour Technol. 2013;129:177-81.

27. Lian J, Wijffels RH, Smidt H, Sipkema D. The effect of the algal microbiome on industrial production of microalgae. Microb Biotechnol. 2018;11(5):806-18.
28. Choi HJ, Lee SM. Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater. Bioprocess Biosyst Eng. 2015;38(4):761-6.
29. Wang B, Lan CQ, Horsman M. Closed photobioreactors for production of microalgal biomasses. Biotechnol Adv. 2012;30(4):904-12.
30. Chokshi K, Pancha I, Ghosh A, Mishra S. Microalgal biomass generation by phycoremediation of dairy industry wastewater: An integrated approach towards sustainable biofuel production. Bioresour Technol. 2016;221:455-60.
31. Roselet F, Vandamme D, Roselet M, Muylaert K, Abreu PC. Screening of commercial natural and synthetic cationic polymers for flocculation of freshwater and marine microalgae and effects of molecular weight and charge density. Algal Research. 2015;10:183-8.
32. Ma Y, Gao Z, Wang Q, Liu Y. Biodiesels from microbial oils: Opportunity and challenges. Bioresour Technol. 2018;263:631-41.
33. Zhou Y, Franks GV. Flocculation mechanism induced by cationic polymers investigated by light scattering. Langmuir. 2006;22(16):6775-86.
34. Bilad MR, Vandamme D, Foubert I, Muylaert K, Vankelecom IFJ. Harvesting microalgal biomass using submerged microfiltration membranes. Bioresour Technol. 2012;111:343-52.
35. Augustine A, Tanwar A, Tremblay R, Kumar S. Flocculation processes optimization for reuse of culture medium without pH neutralization. Algal Research. 2019;39:101437.
36. Sheng ALK, Bilad MR, Osman NB, Arahman N. Sequencing batch membrane photobioreactor for real secondary effluent polishing using native microalgae: Process performance and full-scale projection. J Cleaner Prod. 2017;168:708-15.