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Removal of Nutrients and COD in Wastewater from Vietnamese Piggery Farm by the Culture of *Chlorella vulgaris* in a Pilot-Scaled Membrane Photobioreactor

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Abstract: The treatment of nutrients and organic contaminants in wastewater using microalgae has drawn significant interest thanks to its advantages of environmental friendliness, low cost, CO_2 emission reduction, and recycling of valuable biomass. Among other algae species, *Chlorella* sp. showed good vitality, simplicity in cultivation, and high nutrient accumulation in harsh conditions of wastewater. In this study, *Chlorella vulgaris* was inoculated in a membrane photobioreactor (MPBR) with piggery digestate to investigate the *C. vulgaris* growth rate and the removal efficiency of nutrients and chemical oxygen demand (COD). The results indicated that the cultivation of *C. vulgaris* in an MPBR system exhibited continuous and simultaneous removal of NH_4^+, PO_4^{3-}, and COD from two-fold diluted piggery wastewater. Both the algae growth rate and nutrient removal depended on the liquid hydraulic retention time in the MPBR. The highest removal efficiency of NH_4^+ (74.55%), PO_4^{3-} (70.20%), and COD (65.85%) was obtained in the longest HRT of 5 days with the highest microalgae biomass concentration of around 1.1 g/L. The algae washout phenomenon was negligible in the continuous cultivation in the MPBR system. Compared to the cultivation in batch mode, the MPBR could achieve a similar algae growth rate and treatment efficiency with a much shorter hydraulic retention time.

Keywords: *Chlorella vulgaris*; membrane photobioreactor; piggery wastewater; nutrients removal; COD removal

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1. Introduction

In recent years, the cultivation of microalgae has become an attractive topic to researchers due to its high potential for biofuel production, CO_2 fixation, and wastewater treatment [1]. In terms of wastewater treatment, besides the ability to assimilate nutrients and organic matter from wastewater, it is considered one of the pathways toward a sustainable circular bio-economy [2]. The advantages are namely environmentally friendly, low-cost, CO_2 and carbon fixation, CO_2 reduction, and recyclability of valuable biomass which is scientifically preferable to other conventional methods [3]. Among the various alga species, *Chlorella* sp. is popular in the basic research for wastewater treatment due to its high growth rate, good survival in harsh conditions of wastewater, short reproduction time, and high nutrient removal efficiency [4–6].

Several studies have attempted to grow the *Chlorella* sp. culture in aquaculture wastewater [3,7–9]. Mujtaba et al. (2016) found that the maximum growth of *C. vulgaris* in a batch experiment achieved 0.76 g/L and achieved 99% and 90% of nutrients and COD removal, respectively [3]. Wang et al. (2021) recorded the maximum microalgae biomass concentration of 1.62 g/L in diluted digested wastewater with the NH_4^+-N of 110.5 mg/L [7]. The authors also found that increasing the concentration of the wastewater inhibited the growth of biomass due to the harsh condition and larger turbidity in the batch experiments. Oil wastewater was also investigated as aquaculture for growing *C. vulgaris* for biomass production in the study by Silva et al. (2022) [8]. A 26-day pre-cultivation with BG-11 medium was performed before feeding the produced water. Their results indicated that the 1.69 g/L of biomass concentration could be achieved in produced water while the control group reached 3.86 g/L. Cultivation of *C. vulgaris* in the batch experiment was also tested in bioremediation and nutrient removal from municipal wastewater in the study of Znad et al. (2018) [9]. The maximum biomass concentration was recorded at 1.6 g/L after 13 days of cultivation with 80% and 100% of total nitrogen (TN) from the primary wastewater. The work also confirmed that the algal growth was promoted from the wastewater carbon sources although susceptible to possible toxicity in the influent. However, it should be noted that the batch cultivation in these studies was operated under a relatively long retention time (more than 10 days). An extra time for sedimentation was also indispensable for liquid separation and algae harvestation. The batch-mode cultivation provided valuable information about *C. vulgaris* growth in different medium; however, the prospective of practical application would be comprehensively investigated in an advanced system.

A membrane-submerged photobioreactor (MPBR) has been widely employed in various studies related to algae cultivation due to its advantages such as continuous-flow operation, easy separation of algae and liquid, and absence of liquid washout from large liquid flow rate [10–16]. With a submerged membrane module, continuous cultivation could be performed appropriately without the risk of biomass washout with the liquid outlet. Moreover, algae harvesting would be simplified without long sedimentation time. Besides the characteristics of wastewater, the hydraulic retention time (HRT) was also taken into consideration in the studies using the MPBR system [12,15]. Gao et al. (2018) discussed that HRT has obvious impacts on biomass production and nutrient removal in the MPBR system with the stimulated secondary effluent [12]. Their results indicated that an HRT of 2.0 days was optimal to achieve a stable algae concentration of 1.035–1.524 g/L. A shorter HRT of 1 day might also be suitable for algae growth but not sufficient for nitrogen and phosphorous removal. On the other hand, the longer HRT of 4 days to 6 days ensured lower nutrient concentration at the effluent but was not ideal for algae growth. The variation of HRT was also investigated in a study using the MPBR system in the co-cultivation of *C. vulgaris* and *A. platensis* for the treatment of winery wastewater [15]. The longer HRT (4.6 days) was proven to exhibit better algae growth (6 g/L to 4 g/L) and higher COD removal (90% to 75%) than others (2.0 days and 1.4 days of HRT). These results indicated that the use of the MPBR system provided independent control of HRT, adjustable intake dosage of medium and wastewater, and a much shorter HRT (a maximum 4.6 days compared to the more than 10 days of the batch mode) while maintaining a relatively good growth of microalgae.

In Vietnam, with intensive agriculture production, there is a massive amount of generated wastewater from pig farms. According to the statistic of the Ministry of Natural Resources and Environment (MONRE), the amount of piggery wastewater was estimated at around 5.6 million m^3 per day, much larger than that of cow and buffalo farming [17]. According to a case study by Giang et al. (2021), the wastewater characteristic from randomly selected farms in Vietnam contains a considerably high level of TN (126.7 ± 259.7 mg/L) and COD (505.3 ± 706.9 mg/L) [18]. The wastewater treatment process composed of biological process and physiochemical process is often required to ensure the acceptable discharge from a typical pig farm [19]. From the biological process, waste-activated sludge is abundantly generated as a hazardous solid waste from the wastewater treatment plant [20]. The biodegradable organic contaminants and nutrient content of the pig farm wastewater, which was conventionally removed by biological process, could be ideal nutrition for the growth of microalgae [21]. Despite extensive research related to the cultivation of microalgae for pollution removal from the wastewater, the idea of and information about wastewater treatment combined with microalgae cultivation are still unfamiliar for both research and practical implementation.

By mid-2022, the Vietnamese Government had set several targets for the Circular Economy development scheme [22]. One of the targets was to contribute to CO_2 emission reduction, waste reduction, and the production of renewable energy from waste [23]. In terms of wastewater treatment, especially piggery wastewater, the application of a microalgal-based system could be a promising alternative for biological treatment thanks to its biomass recovery, energy saving, and CO_2 emission reduction. Although the high concentration of pollutants from piggery might inhibit the cultivation of microalgae, *C. vulgaris* has proven to have successful growth in such harsh conditions [16]. Therefore, research related to the cultivation of *C. vulgaris* in piggery wastewater using an MPBR system is mandatory to better clarify the prospect of microalgal-based wastewater treatment technology in practical scenarios for sustainable development and circular economy in Vietnam. In this study, *C. vulgaris* was cultivated in a 50-L membrane photobioreactor (MPBR) system to continuously remove nutrients and COD from piggery wastewater collected from a household pig farm in Vinh Phuc, Vietnam. The growth of *C. vulgaris* and the variation of NH_4^+, PO_4^{3-}, and COD in the effluent was investigated in three different hydraulic retention times (HRT) of 1.25 days, 2.5 days, and 5 days to find out the optimal operation for microalgae growth and treatment efficiency.

2. Materials and Methods

2.1. Microalgae Strain and Wastewater

The microalgae *Chlorella vulgaris* was isolated and cultivated in the Department of Hydrobiology—Institute of Environmental Technology. The *Chlorella vulgaris* was precultivated in a BG-11 (Blue-Green medium) at a temperature of 29–31 °C, under the continuous white fluorescent of 2000 lux for 16 h per day, shaking at 120 rpm.

The piggery wastewater was collected from a household pig farm that was raising around 100 pigs at Vinh Tuong commune, Vinh Phuc Province, Vietnam. The wastewater was generated from the cleaning and feeding activities and was collected after a screening unit with a screening gap of 0.5 mm to get rid of the solid components. The digestate was collected from the outlet stream from the anaerobic digestion system of the facility. Then, it was delivered to the Laboratory of the Institute of Environmental Technology and stored at 4 °C before use. The characteristics of the wastewater is described in Table 1. The wastewater was screened once again with layers of cloth screening (0.05–0.1 mm) and 2-fold diluted with de-ionized (DI) water to simulate the influent of biological treatment which has low turbidity, low total suspended solids (TSS), and good transparency of light for stable algal photosynthesis and wellness of optical measurement.

Table 1. Characteristics of the raw and 2-fold diluted piggery wastewater used in this study.

Parameters	Unit	Raw Piggery Wastewater	Screened 2-Fold Diluted Piggery Wastewater
pH	mg/L	7–9	8.42
COD	mg/L	573.4	287.0
TSS	mg/L	143.3	35.2
NH_4^+	mg/L	367	184.0
NO_2^-	mg/L	0.76	0.34
NO_3^-	mg/L	0.32	0.16
PO_4^{3-}	mg/L	61.7	31.0

2.2. Experimental Procedure

The experiment was conducted in a pilot-scale MPBR with the dimensions (width × length × height) of 0.4 m × 0.2 m × 0.75 m (60 L) and a working height of 0.625 m (50 L), equipped with a 02 submerged membrane module composed of hollow fiber with 0.4 × 0.4 μm pore size. The membrane was physically cleaned at the beginning of each HRT setup.

The schematic diagram of the pilot-scale MPBR system used in this study was presneted in Figure 1. The microalgae were initially pre-cultivated in the BG-11 medium until the biomass concentration reach 0.1 g/L before being seeded to the MPBR. The BG-11 medium is a well-known medium for the cultivation and maintenance of blue-green algae, which supports the autotrophic growth of green algae. According to the successful growth in the pre-cultivation period of other studies that employed *C.vulgaris*, the BG-11 medium was also used in this study [3,7–9]. After the inoculation, the pre-mixed 1:1 mixture of the piggery wastewater: DI water (2-fold dilution) was fed continuously from the primary tank (1) to the MPBR (3) at three different hydraulic retention times of 1.25 days (HRT1), 2.5 days (HRT2), and 5 days (HRT3) for a total of 15 days (Figure 1). The chosen retention times of the MPBR in this study were significantly longer than a conventional MBR system which employed activated sludge and nitrobacteria (5–10 h) [24]. However, it was reported that sufficient microalgal growth required a longer hydraulic retention time from 1 day up to 10 days [25]. The inlet and outlet streams were continuously controlled by two peristaltic pumps (2) (model KS-22-PTC-HWS-S, Cheonsei, Korea). Two 4000-lux LED lamps (7) were installed close to the reactor to maintain the fluorescent illumination throughout the operation. CO_2 gas was supplied from a cylinder (6) and an air compressor (9) with a controlled air flow rate. The gas distributor (5) was also installed at the bottom of the reactor to maintain the agitation, CO_2 supply, and membrane fouling control. The water samples inside the reactor and from the outlet stream were collected daily to examine the COD and nutrients (NH_4^+ and PO_4^{3-}) removal efficiency and biomass production. All experiments were carried out in duplicate.

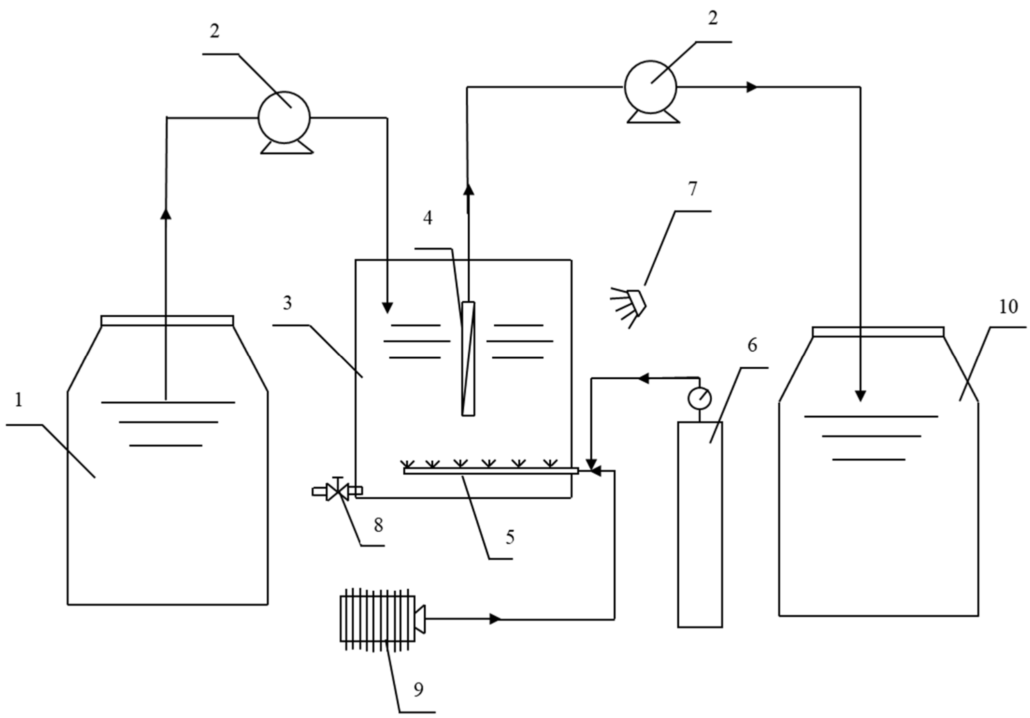


Figure 1. A schematic diagram of the pilot-scale MPBR system. (1)-Primary tank; (2)-peristaltic pumps, (3)-MPBR vessel, (4)-MBR membrane, (5)-air distributor, (6)-CO_2 gas cylinder, (7)-LED lamps, (8)-discharge valve, (9)-Air compressor, (10)-Discharge collector.

2.3. Analysis of Algae Biomass and Water Quality

2.3.1. Algae Biomass Analysis

A volume of 50 mL of the liquid sample taken from the MPBR was analyzed for both optical density at 680 nm (OD_{680}) and dry biomass. The OD_{680} was measured by the UV-Visible spectrophotometer (UVD-3000, Labomed, Los Angeles, CA, USA) at the wavelength of 680 nm. On the other hand, the dry biomass concentration (X; g/L) of the algae was determined by measuring the dry weight of the solid that was filtered from the 0.45-μm cellulose acetate filters and dried at 105 °C for 2 h. Then, the correlation between the OD_{680} and X was described in Equation (1). It is noted that the correlation of this study was calculated from the measurement of dry biomass and OD_{680} of *C. vulgaris* in 2-fold diluted piggery wastewater only.

$$X\left(\frac{\mathrm{g}}{\mathrm{L}}\right) = 0.4302 \times OD_{680}, R^2 = 0.99 \quad (1)$$

The specific growth rate and the productivity of the microalgae were calculated from the biomass on the final day of the experiment. The specific growth rate μ (d^{-1}) was calculated by the Equation (2) [26]:

$$\mu = \frac{1}{t} ln\left(\frac{X_f}{X_0}\right) \quad (2)$$

The biomass productivity p (g/L/d) was calculated according to Equation (3) [27]:

$$P = \frac{X_f - X_0}{t} \quad (3)$$

where t is the total experiment time (days), while X_f and X_0 are the final and starting biomass concentration (g/L).

2.3.2. Algae Biomass Analysis

The water sample from the inlet and outlet stream of the MPBR was taken for analysis of COD, NH_4^+, and PO_4^{3-} to assess the removal efficiency of the system. The COD was measured by the dichromate method according to the ISO 6060 method [28]. The measurement of NH_4^+ and PO_4^{3-} were performed by the ISO spectrometric method using the UV-Visible spectrophotometer (UVD-3000, Labomed, Los Angeles, CA, USA) [29,30]. The removal efficiency (%) of each pollutant was calculated as Equation (4) below:

$$Removal\ efficiency = \frac{C_o - C_f}{C_o} \times 100\% \quad (4)$$

where C_o and C_f are concentrations of the pollutants in the inlet and outlet stream of the MPBR system.

3. Results and Discussions

3.1. Microalgae Growth in Wastewater

The growth characteristic of *C. vulgaris* in the MPBR tank with 50% diluted piggery wastewater under three different hydraulic retention times was investigated. The growth profile of the *C. vulgaris* in 14 days is presented in Figure 2. It can be observed that the biomass concentration grew from around 0.1 g/L to 0.326 ± 0.016 g/L, 0.582 ± 0.019 g/L, and 1.095 ± 0.033 g/L after 14 days at HRT1, HRT2, and HRT3, respectively. The HRT1, HRT2, and HRT3 are corresponding to the hydraulic retention time of 1.25 days, 2.5 days, and 5 days (equivalent to the liquid flow rate of 28, 14, and 7 mL/min). The exponential growth was rapidly achieved after 2 days of lag phase at HRT3 of 5 days. It is noted that the cultivating conditions of all the experiments were under a mixotrophic condition, in which the CO_2 supply was maintained at 60 mL/min. The growth of *C. vulgaris* was found to be at a comparable state with the previous cultivation of *C. vulgaris* in batch mode in artificial wastewater and 10% swine mixed with BG11 (from 0.4 to 3.16 g/L within 2–12 days) [1,3] (Table 2). The difference between continuous-flow cultivation and batch cultivation is the liquid medium is continuously fed to the microalgae culture. The continuous injection ensured the maintenance of sufficient nutrients presented to the microalgae but posed a risk of algae wash-out at the outlet of the system. However, the result of increasing biomass over time proved that the submerged membrane could effectively be equipped to avoid the wash-out of biomass. These results indicated that the growth rate of microalgae in batch mode could be repeated in the continuous flow system with a similar HRT.

In comparison with other MPBR systems, at a similar HRT of 4.6 days in a 20 L photobioreactor, the co-culture of A. platensis and *C. vulgaris* in 20% (*v*/*v*) winery wastewater with Bold's Basal Medium achieved a much larger biomass concentration at Day 15 (above 5 g/L) which was 5-fold larger [15]. It is noted that the 20% winery—BBM mixture had lower COD (119.3) and TSS (0.75) concentrations compared to this study. This could be the result of a much harsher cultivating medium for a single culture of *C. vulgaris* in this study where the piggery wastewater was only 2-fold diluted with DI water. The COD content of piggery digestate often composed the remaining fatty acid from the anaerobic digestion and might require longer effort to be broken down than the alcohol and sugar components in the winery wastewater. The observed lag phase in the early stage of each HRT might be the result of relatively low inoculum (initial biomass was around 0.1 g/L) before direct continuous feeding of the wastewater mixture. This phase was absent in other studies where the larger inoculum and batch-mode cultivation were adapted [6,7,15,31].

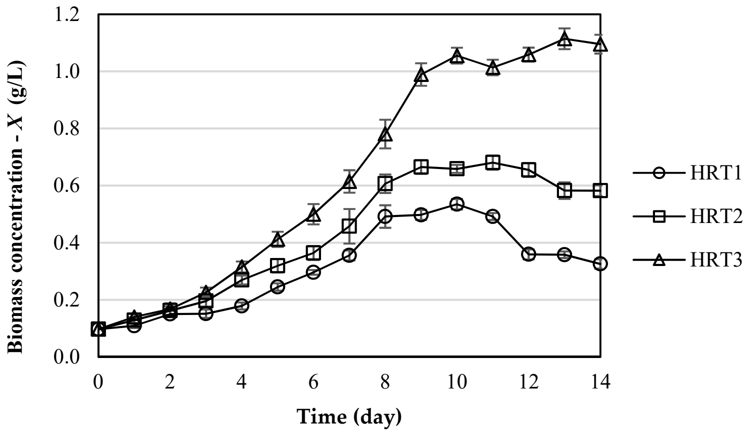


Figure 2. The growth of Chlorella vulgaris in the pilot-scaled MPBR with 2-fold diluted piggery wastewater in 14 days at three hydraulic retention times (HRT1 = 1.25 days, HRT2 = 2.5 days, HRT 3 = 5 days).

The hydraulic retention time was the significant factor that affected the growth of *C. vulgaris* in the wastewater mixture in this study. The longer retention time resulted in higher final biomass concentration (0.326 ± 0.016 g/L to 1.095 ± 0.033 g/L as the HRT increased from 1.25 days to 5 days). The growth characteristics were also improved at longer HRT (Table 2). The specific growth rate and biomass productivity increased from 0.086 ± 0.004 d^{-1} to 0.173 ± 0.002 d^{-1} and 0.016 ± 0.001 g/L.d to 0.071 ± 0.002 g/L.d, respectively. This result is in good agreement with other studies that employed continuous photobioreactors, which observed a similar pattern [15,32]. In contrast, Gao et al. reported that shorter HRT resulted in an increase in biomass in the cultivation of *C. vulgaris* in artificial wastewater [12]. The biomass concentration increased from 0.481 to 0.873 g/L as the HTR was shortened from 6 to 1 day in the artificial wastewater condition of 40 mg/L COD, 5 mg/L NH_4^+, and 10 mg/L PO_4^{3-}. The opposite findings between Gao et al. and this study might be answered by the significant difference between the wastewater conditions. The longer HRT could either exhibit higher or lower growth of microalgae in the MPBR system, and it depended on the characteristic of the wastewater. Larger flowrates presented much harsher conditions of nutritional (NH_4^+) and conditional stress (larger COD and TSS fed) to the *C. vulgaris* culture, resulting in a lower biomass concentration and productivity. Long HRT favors the microalgae growth with the excessive organic content and nutrients and, meanwhile, idles the growth with the inadequate nutrient supply. The much larger reactor volume (50 L) could be another reason for the lower biomass concentration with a similar hydraulic retention time as the lighting could be inhibited compared to a smaller MPBR reactor (4 L) [12,15]. The washout effect could be neglected in the MBR-equipped system.

Table 2. Growth characteristic of *Chlorella vulgaris* in pilot-scaled MPBR with piggery wastewater at three hydraulic retention times in comparison with previous studies.

Cultivation Condition	Experimental Setup	HRT	Final Biomass Concentration, *X*	Specific Grow Rate, *μ*	Productivity, *p*	Ref.
		Day	g/L	d^{-1}	g/L/d	
2-fold diluted piggery ww.	Continuous mode with MPBR	1.25	0.326 ± 0.016	0.086 ± 0.004	0.016 ± 0.001	This study
		2.5	0.582 ± 0.019	0.128 ± 0.002	0.035 ± 0.001	
		5.0	1.095 ± 0.033	0.173 ± 0.002	0.071 ± 0.002	
10% swine with BG-11	Batch mode	12	3.16	-	0.188	[1]
Artificial ww. (with glucose as COD)	Batch mode	2	0.4	-	-	[3]
Simulated ww.	Continuous mode with MPBR	1	0.873	-	0.048	[12]
		2	0.878	-	0.048	
		4	0.603	-	0.033	
		6	0.481	-	0.026	
20% winery ww. with BBM	Continuous mode with MPBR	1.4	6.10 ± 0.05	0.083 ± 0.004	0.240 ± 0.011	[15]
		2.0	3.61 ± 0.04	0.003 ± 0.001	0.154 ± 0.013	
		4.6	2.90 ± 0.02	0.014 ± 0.002	0.136 ± 0.019	

3.2. Pollutants Removal by the MPBR

The 2-fold diluted piggery wastewater collected from a household pig farm was used for feeding after inoculation. As discussed above in Section I, the purpose of this study is to investigate the prospective of using *C. vulgaris*-MPBR system in a practical scenario. Hence, the dilution of the raw wastewater was to simulate the influent of the biological process for the *C.vulgaris*-MPBR system. The characteristic of the inlet stream of the experiment is presented in Table 3 below:

Table 3. Pollutants characteristics of the 2-fold diluted piggery wastewater.

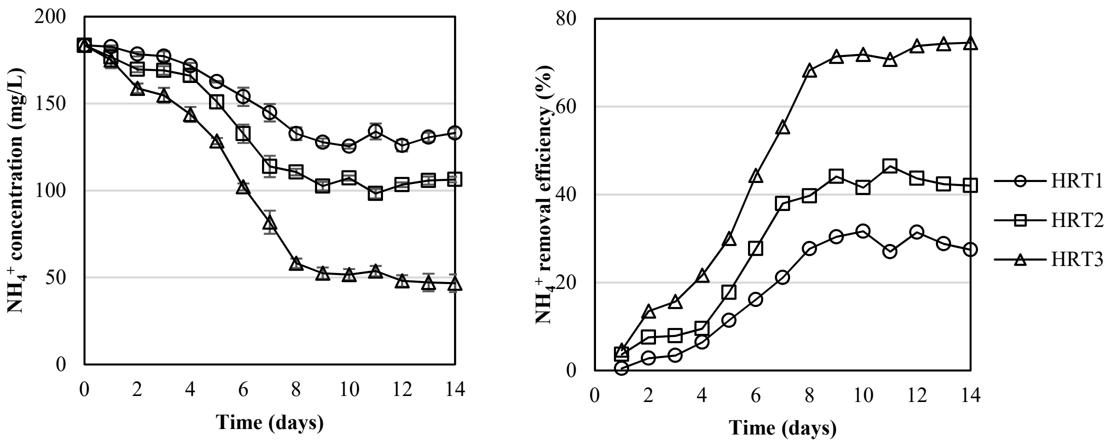
Parameters	Unit	Value
pH	-	8.42
COD	mg/L	287.0
NH_4^+	mg/L	184.0
PO_4^{3-}	mg/L	31.0

The change of ammonium concentration in the outlet stream and the removal efficiency were described in Figure 3. There was a considerable reduction in NH_4^+ concentration in the first week of the experiment at all three HRTs. The concentration dropped from

184 mg/L to 132.7, 110.6, and 58.2 mg/L at HRT1, HRT2, and HRT3, respectively. It can be observed that the rapid reduction in ammonium did not occur during the lag phase of algae growth (from Day 1 to Day 3). From Day 9 to the rest of the experiment, the output concentration of NH_4^+ mostly remained stable for all HRTs. On Day 14, the ammonium removal efficiency was recorded at 27.47%, 42.04%, and 74.55% at HRT1, HRT2, and HRT3, respectively. A similar trend was also recorded for the variation of PO_4^{3-} concentration, where a sharp decrease was recognized in the first half of the experiment period (Figure 4). At the HRT2 of 2.5 days and HRT3 of 5 days, the PO_4^{3-} concentration reduced significantly from 31 mg/L to 17.15 (46.19 %) and 9.61 mg/L (69.85%) from Day 0 to Day 5, respectively. At a shorter time of HRT1 (1.25 days), the decrease of PO_4^{3-} was at a much slower pace, which was only 15.44% (31 mg/L to 26.95 mg/L) reduction on Day 5. After Day 9, the PO_4^{3-} concentration was stable at 19.25 mg/L, 17.35 mg/L, and 8.97 mg/L for HRT1, HRT2, and HRT3, respectively.

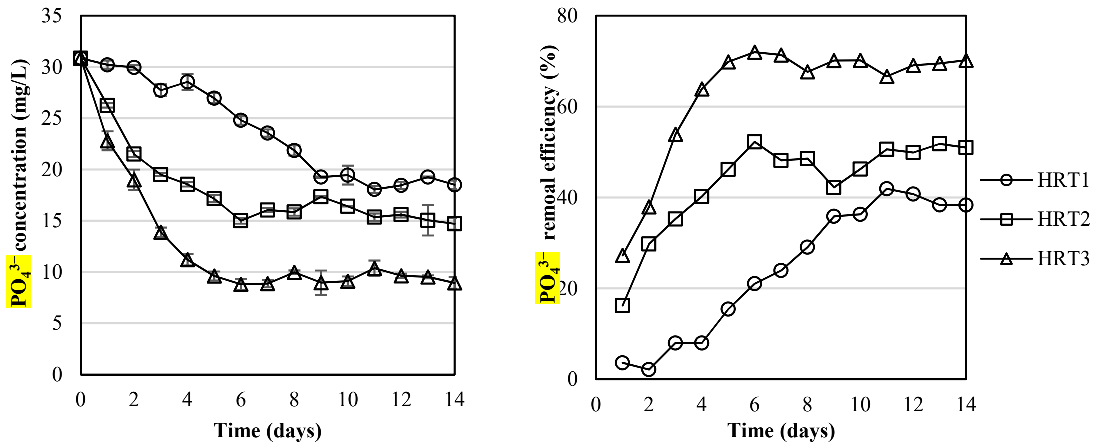
In a batch photobioreactor, the removal efficiency of nutrients by *C. vulgaris* cultivation mostly depended on the initial nutrient concentration [1,33]. Acebu et al. reported that the total nitrogen removal efficiency varied from 45.4% to 29.8% as the dilution ratio of swine in BG-11 varied from 25% to 100% [1]. After 8 days of batch cultivation, the result of Choi and Lee also recorded that the NH_4^+-N removal efficiency drastically decreased from 99.61% to 3.59% as the initial NH_4^+-N concentration increased from 10–200 mg/L [33]. This pattern might have been reflected in the changing of HRT in the continuous flow of the MPBR system. Even though the initial concentration of nutrients was equivalent for various HRT, the nutrient dosage to the system was significantly affected by the liquid flow rate. In detail, shorter HRT was driven by a larger liquid flow rate, which caused a larger mass flux of NH_4^+ and PO_4^{3-} to the *C. vulgaris* culture. A shorter contact time of wastewater with the *C. vulgaris* might also reduce the removal efficiency. It is noted that the maximum removal efficiency of NH_4^+ by *C. vulgaris* in batch photobioreactor was achieved from Day 3 to Day 4, which was 2–3 days sooner than that of this study. The maximum removal efficiency by MPBR was also recorded to be 2–3 days later than the batch photoreactor [10,12,14,15]. The low biomass at pre-cultivation (0.1 g/L) and the large volume of the reactor (50 L) might be the main causes of the longer time to reach maximum algae growth and, consequently, the maximum nutrient removal rate.

Similar to the finding of this study, the variation of HRT on nutrient removal was also reported to have a certain impact on the nutrient removal rate of microalgae culture in other MPBRs. The reduction rate of total nitrogen and total phosphorous drastically varied from 35.9% to 92.1% and 76.9% to 94.0% (maximized and maintained at Days 5–7), respectively, as the HRT increased from 1 to 6 days [12]. It is noted that the initial concentration of TN and TP were much lower (10 mg/L and 0.8 mg/L, respectively) compared to this study. Other research also discussed that elongated HRT led to a reduction of the nutrient concentration in the effluent of the MPBR system [12,14]. However, the nutrient removal rate and algae growth favored the shorter retention time at low nutrient concentration mediums. Therefore, a careful selection for HRTs should be considered for optimum biomass production and nutrient removal should be done in the case of low-nutrient wastewater.



(**a**) (**b**)

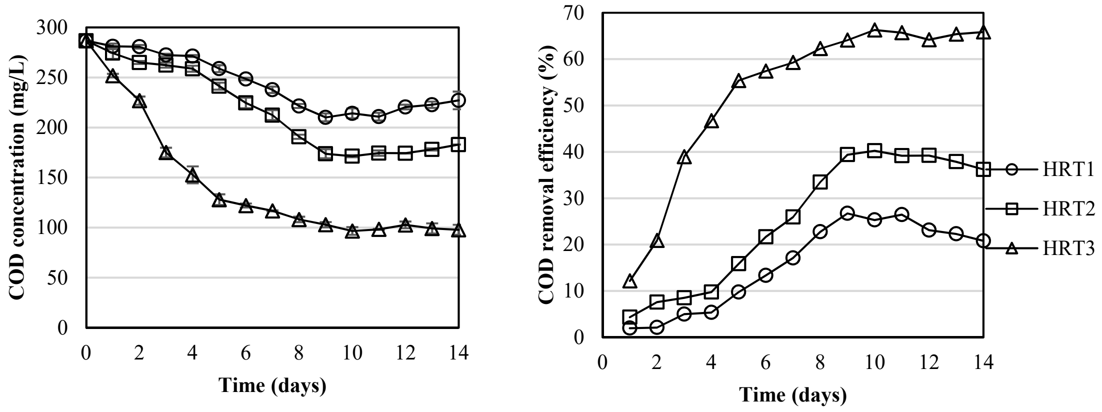
Figure 3. The change of (**a**) NH_4^+ concentration and (**b**) NH_4^+ removal efficiency in 2-fold diluted piggery wastewater over 14 days in *Chlorella vulgaris*-based MPBR at various hydraulic retention times (HRT1 = 1.25 days, HRT2 = 2.5 days, HRT 3 = 5 days).



(**a**) (**b**)

Figure 4. The change of (**a**) PO_4^{3-} concentration and (**b**) PO_4^{3-} removal efficiency in 2-fold diluted piggery wastewater over 14 days in *Chlorella vulgaris*-based MPBR at various hydraulic retention times (HRT1 = 1.25 days, HRT2 = 2.5 days, HRT 3 = 5 days).

The COD removal ability of *C. vulgaris* in the MPBR was also investigated in this study. The variation and the removal efficiency of the COD concentration in the outlet stream of the system are described in Figure 5. At the hydraulic retention time of 5 days, the COD reduction was rapid in the first 5 days, dropping from 287 mg/L to 127 mg/L (reaching 55.37% of COD removal efficiency). This was the same period of the exponential phase of microalgae growth, at which the culture largely consumed the nutrient and carbon source. In contrast, the reduction of COD at shorter HRT was at a much slower pace (9.75% and 15.85%) during the same period for 1.25 days and 2.5 days. It required a longer period for the COD removal efficiency to reach a stable state (around 25% and 40% for HRT1 and HRT2, respectively) than that of HRT 3. At the final stage of the experiment, the COD concentrations in the output stream were 227.05 mg/L, 182.95 mg/L, and 97.90 mg/L (corresponding to 20.81%, 36.19%, and 65.85% of COD removal efficiency) for HRT1, HRT2, and HRT3, respectively.



(**a**) (**b**)

Figure 5. The change of (**a**) COD concentration and (**b**) COD removal efficiency in 2-fold diluted piggery wastewater over 14 days in *Chlorella vulgaris*-based MPBR at various hydraulic retention times (HRT1 = 1.25 days, HRT2 = 2.5 days, HRT 3 = 5 days).

C. vulgaris culture has been proven to remove the organic carbon source in previous studies [3,11,15,34]. The result of the HRT3 was in good agreement with the finding of Mujtaba et al. (2018) in which 60% of COD was rapidly eliminated in the early stage of incubation [3]. However, this COD removal of single *C. vulgaris* culture was still lower than co-culture with the activated sludge in the batch photobioreactor (90% COD removal) [3]. Similar to the finding in nutrient removal, the time to reach maximum COD removal in MPBR (5 days) was longer than that in the batch photoreactor (1 day). During the growth of microalgae, it usually uptakes energy from light and inorganic carbon. However, the algae can also consume organic carbon in conditions that lack inorganic CO_2. This explains the higher COD removal efficiency at the HRT3, where the biomass concentration was significantly higher than that of HRT1 and HRT2 with the same dosage of CO_2 supply. Lower amounts or absence of CO_2 supply might improve the COD removal efficiency in piggery wastewater with high COD in the inlet stream. In a continuous MPBR, the HRT was also a factor that drove the COD removal efficiency. In a similar trend to this study, the COD removal efficiency of the co-culture of *C. vulgaris* and Arthospira achieved >90% at 4.6 days of HRT compared to 75% at 1.4 days and 2 days [15]. Spennati et al. (2021) explained that sufficient retention time was necessary for the co-culture to effectively consume the organic pollutant in the medium. A detailed comparison between the data on nutrient and COD removal using *C. vulgaris* culture using MPBR of this study with other research is presented in Table 4. The removal efficiency of nutrients and COD in a pilot-scaled MPBR system were comparable to other studies in both batch mode and other continuous MPBR systems. The removal efficiency varied with the change in the initial condition of the pollutant and the hydraulic retention time. Hence, the application of the microalgal-based MPBR system should be carefully considered in terms of wastewater characteristics and hydraulic loading for each specific case.

Table 4. Pollutants removal efficiency from 2-fold diluted piggery wastewater of *Chlorella vulgaris*-based MPBR and comparison with other studies.

Wastewater	Experimental Condition	HRT (Days)	Pollutants Removal Efficiency (%)			Ref.
			NH_4^+	PO_4^{3-}	COD	
2-fold diluted piggery wastewater	Continuous mode with MPBR NH_4^+ = 184 mg/L	1.25	27.47	15.44	20.81	This study
		2.5	42.04	46.19	36.19	
		5	74.55	69.85	65.85	

	PO_4^{3-} = 31 mg/L COD = 287 mg/L					
Mixture of swine wastewater (COD = 456 mg/L, NH_4^+ = 470 mg/L) and BG-11	25% swine mixture	12 (Batch mode)	41.7	-	-	[1]
	50% swine mixture		45.4	-	-	
	75% swine mixture		28.6	-	16	
	100% swine		29.9	-	30.4	
Artificial ww.	NH_4-N = 50 mg/L PO_4-P = 10 mg/L COD = 490 mg/L	2 (Batch mode)	92	87	60	[3]
Simulated secondary effluent of municipal ww.	Continuous mode with MPBR NH_4-N = 5 mg/L PO_4-P = 40 mg/L COD = 0.8 mg/L	1	35.9	76.9	-	[12]
		2	76.9	88.0	-	
		4	90.6	94.9	-	
		6	92.1	94.0	-	
Chlorella vulgaris and *Arthospira platensis* in winery wastewater	Continuous mode with MPBR COD = 199.3 mg/L	4.6	-	-	>90%	[15]
		2.0	-	-	~75%	
		1.2	-	-		

4. Conclusions

The cultivation of *C. vulgaris* in a pilot-scaled MPBR system exhibited continuous and simultaneous removal of NH_4^+, PO_4^{3-}, and COD from 2-fold diluted piggery wastewater. Both the algae growth rate and nutrient removal depended on the liquid hydraulic retention time in the MPBR. The highest removal efficiency of NH_4^+ (74.55%), PO_4^{3-} (69.85%), and COD (65.85%) was obtained with the longest HRT of 5 days with the highest microalgae biomass concentration of around 1.1 g/L. The algae washout phenomenon was negligible in the continuous cultivation in the MPBR system. Compared to the cultivation in batch mode, the MPBR could achieve similar algae growth rate and treatment efficiency with a much shorter hydraulic retention time. According to our study, the cultivation of *C. vulgaris* in an MPBR system with wastewater has a high potential for nutrients and COD removal in piggery wastewater. The harvested microalgae from this process can be assessed on biofuel or fertilizer production. The careful selection of influent characteristics and hydraulic retention time is crucial for the effective application of microalgal-based MPBR in wastewater treatment and microalgal production in the future. Moreover, sufficient lighting would be a challenge to be addressed on the large scale for this technology.

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