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Nutrient utilization and oxygen production by *Chlorella Vulgaris* in a hybrid membrane bioreactor and algal membrane photobioreactor system

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Abstract

This work studied oxygen production and nutrient utilization by *Chlorella Vulgaris* at different organic/inorganic carbon (OC/IC) and ammonium/nitrate (NH_4^+ -N/ NO_3^- -N) ratios to design a hybrid aerobic membrane bioreactor (MBR) and membrane photobioreactor (MPBR) system. Specific oxygen production by *C. vulgaris* was enough to support the MBR if high growth is accomplished. Nearly 100% removal (or utilization) of PO_4^{3-} -P and IC was achieved under all conditions tested. Optimal growth was achieved at mixotrophic carbon conditions (0.353 d^{-1}) and the highest NH_4^+ -N concentration (0.357 d^{-1}), with preferable NH_4^+ -N utilization rather than NO_3^- -N. The results indicate the potential of alternative process designs to treat domestic wastewater by coupling the hybrid MBR - MPBR systems.

Keywords: Algae; Membrane bioreactor; Membrane photobioreactor; Nutrients; Specific oxygen production

1. Introduction

An imbalanced relationship between urban growth and available fresh water supply is creating a worldwide demand for sustainable technologies involving the reuse of treated wastewater. Membrane bioreactor (MBR) process, which combines microfiltration (MF) or ultrafiltration (UF) membrane

technologies with biological treatment, is widely used due to its high removal efficiency compared to the conventional activated sludge process given that it operates at higher mixed liquor suspended solid (MLSS) concentrations, hence removing more organic and inorganic pollutants. In addition, sludge is retained by a physical barrier generating high quality permeate eliminating the need for settling with conventional clarifiers. Submerged membrane filtration configurations are typically used, either in a separate membrane tank as a sidestream unit or directly in the bioreactor, which further reduces the footprint of MBRs compared to conventional plants (see **Fig. S1**) (Kazemzadeh-afshar et al. 2012).

In submerged MBRs, the aeration performs three essential tasks: i) delivering dissolved oxygen (DO) to the microorganisms for consuming the wastewater pollutants, ii) mixing, and iii) providing the scouring to prevent the membrane surface from fouling (Kazemzadeh-afshar et al. 2012). However, energy for aeration accounts for over 40% of the operational cost of the MBR (Kazemzadeh-afshar et al. 2012; Meng et al. 2008). During the biological wastewater treatment process, the oxygen consumption by microorganisms is mainly for treating carbonaceous oxygen demand and nitrogenous oxygen demand, along with negligible traces of inorganic compounds that are in their reduced forms and require oxidation (Seong-Hoon Yoon 2015). The most dominant mechanism in carbon-rich wastewater treatment is the conversion of carbons to heterotrophs and CO_2 while O_2 is consumed. Meanwhile, the nitrogen is oxidized to nitrate (NO_3) by autotrophs while utilizing energy from the oxidation reaction for fixing CO_2 to generate its building blocks from the carbon. The volumetric oxygen consumption rate by microorganisms is represented as the oxygen uptake rate (OUR). If divided by the MLSS, the specific activity of microorganisms is measured as specific oxygen uptake rate (SOUR). Typically, the SOUR values range from 2 to 5 $\text{mg O}_2/\text{g MLVSS/hr}$ (Ng et al. 2006). These rates are directly proportional to the food/volume ratio (F/M ratio) as naturally, the amount of decomposed substrates is correlated to the oxygen amounts needed in steady state. This indicates that the SOUR does not solely depend on the

sludge concentration, but can also be controlled by operational parameters such as the hydraulic retention time, organic loading rate, total nitrogen concentrations, and solid retention time (Seong-Hoon Yoon 2015).

Over the years, microalgae have gained attention from an industrial perspective. These little light-harvesting “cell factories” offer unique abilities in many applications including the recovery of nutrients and energy from wastewater. Although many of these microbes can grow heterotrophically, they can also grow autotrophically needing only water, sunlight, and inorganic nutrients. There is an active interest for large-scale cultivation systems, which can be divided into open pond systems and closed photo-bioreactors. The more common open pond systems mainly consist of circular ponds with rotating arms for mixing or multiple loops with paddle wheels for stirring, although other simple configurations do exist (Carvalho et al. 2006). Despite being an economical option, these systems often face limitations, as they are open to the environment, making it impossible to avoid contamination, pollution, and water evaporation. At times harsh environmental conditions (e.g. high pH or salinity) are applied to guarantee certain strain dominance, however, most microalgal species cannot thrive in such conditions thus achieving lower cell density. In addition, temperature differences due to seasonal variations and the need for large land areas to maintain optimal pond depths around 15-50 cm to maximize sun exposure, make these open configurations impractical.

Closed systems (i.e. photobioreactors) have been developed as an alternative to overcome the above challenges, and by controlling the environment can achieve up to three-fold cell density compared to open ponds. Algal membrane photobioreactor (MPBR) may be a more suitable alternative to conventional high-rate open ponds, which are less efficient due to light penetration limitations, a larger bioreactor footprint, and lower biomass concentrations (Wang et al. 2012). An additional advantage is that photobioreactors can be operated continuously at low hydraulic retention time (HRT) without

risking biomass washout (Marbelia et al. 2014). Moreover, photosynthetic oxygenation by algal MPBR is considered as one method to overcome the high costs of MBR plants (Muñoz & Guieysse 2006; Oswald et al. 1985). Microalgae can generate high rates of oxygen (O_2) (up to 10 mg/L min) via photosynthesis for the aerobic organic pollutants degraders in MBRs (Yusuf 2007). During photosynthesis, microalgae can utilize carbon dioxide (CO_2) produced by aerobic bacteria from organic degradation in the MBR process. Thus, the use of microalgae is also capable of providing climate change mitigation (Monteith et al. 2005). Normally, CO_2 is released into the environment and thus causing to the greenhouse gas effects. Furthermore, microalgae can serve as a tertiary treatment after MBRs to further remove nutrients (Nitrogen; N and Phosphorus; P) that were not degraded in the MBR process through assimilating nutrients into the microalgal biomass (Alcántara et al. 2015). Hence, incorporating MPBRs into wastewater treatment is rapidly gaining attention, specifically in cost-effective symbiotic systems with the MBR utilizing aerobic sludge and O_2 for organic degradation. Another benefit to use the algal MPBR is the potential to add value of the biomass if further processed to feedstock, production of biofuels, human and animal nutrition, biofertilizers, pharmaceuticals, cosmetics, etc. owing to the special chemical composition of microalgae.

To fulfill a novel conceptual hybrid system, sequential coupling of an aerobic MBR and a microalgal MPBR is needed to gain a better understanding of oxygen production behavior on carbon source and nutrient type and concentration or ratio, essential information for the design process. However, oxygen production rates by microalgae treating MBR effluent when exposed to various substrate concentrations has not yet been studied. Therefore, this proof-of-concept study was aimed to assess the effect of nutrient ratios of synthetic wastewater effluents on growth rates, which is directly correlated to the specific oxygen production rate (SOPR) of the selected strain *Chlorella vulgaris* (*C. vulgaris*) in the following stoichiometric equation (Eq. 1) (Xu et al. 2014; Tang et al. 2014).

Variations tested in this study were i) organic/inorganic carbon (OC/IC) ratio and ii) ammonium/nitrate (NH_4/NO_3) ratio to evaluate the optimal growth conditions and nutrient utilization rates. This green eukaryotic species was selected for its fast growth characteristic, high photosynthetic efficiency, and ability to fixate CO_2 and assimilating nutrients and organics by mixotrophic growth combining both autotrophy and heterotrophy (Xu et al. 2014; Safi et al. 2014).

2. Materials and methods

2.1 Algae inoculum and pre-cultivation conditions

The microalgae *C. vulgaris* (UTEX 259) used in this study was purchased from UTEX Culture Collection of Algae (US). The seed was pre-cultivated autotrophically in Modified Bold's Basal Medium (BBM) purchased from Sigma-Aldrich with the addition of 750 mg/L of sodium bicarbonate (NaHCO_3) as an IC source at 24 °C on an orbital shaker with a rotational speed of 160 rpm, under continuous white LED illumination at photosynthetic photon flux density (PPFD) 135 $\mu\text{mol photons/m}^2/\text{s}$. During the logarithmic growth phase, algal cells were collected for the batch tests. They were then centrifuged at 8,000 rpm for 5 min, the supernatant decanted and the pellet re-suspended with Milli-Q water 2-3 times to eliminate any residual nutrients.

2.2 Batch test

In this study, the main substrates tested were the following macronutrients: carbon (C), nitrogen (N), and phosphorus (P), applying six different ratios (T1-T6) as shown in **Fig. 1**. The batches examined the effect of carbon source type both (i) in organic form, as it is ubiquitous in primary wastewater, and (ii) in

inorganic form, the carbon form required for photosynthetic reactions. Glucose and sodium bicarbonate were used as the OC source and the IC source, respectively. T1 contained no IC but 100 mg/L of OC, whereas T2 contained 100 mg/L of IC and no OC source, and lastly T3 contained 50% of each (50 mg/L OC and 50 mg/L IC). T1/T2/T3 had fixed N ratios of 40 mg/L NH_4^+ -N, 10 mg/L NO_3^- -N, respectively. In the second batch tests, two N sources as (i) ammonia (NH_4^+ -N), which is found in primary wastewater, and (ii) nitrate (NO_3^- -N), found in MBR effluent as a result of nitrification, were examined. T4 included NH_4^+ as the sole N source at 100 mg NH_4^+ /L, T5 contained 100 mg NO_3^- /L, and T6 contained mixed N sources (50% of each). Carbon ratios of IC:OC were fixed at 50:50 for T3/T4/T5 at 100 mg/L in total. Trace micronutrients ($\text{MnCl}_2 = 1.448\text{mg/L}$, $\text{ZnSO}_4 = 0.178\text{mg/L}$, $\text{CuSO}_4 = 0.079\text{mg/L}$, $\text{Co}(\text{NO}_3)_2 = 0.049\text{mg/L}$ and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O} = 0.312\text{ mg/L}$) were added to each of the replicates.

Multiple batch experiments were carried out in 250 mL Erlenmeyer flasks with cotton buds (semi-open system) at a working volume of 150 mL, as shown in **Fig. S2**. The flasks were incubated in an isolated environmental growth chamber under the same physical conditions as of the pre-cultivation. Basal nutrients were added to all tests to eliminate any external nutrient deficiencies, 25mM of HEPES buffer was injected to maintain a neutral pH (7-8), and DO was sustained at a saturation level at 24 °C.

2.3 Analyses

2.3.1 Specific growth rates

C. vulgaris cell density was monitored by measuring the optical density (OD) at a wavelength of 683 nm (OD_{683}) using an ultraviolet-visible spectrophotometer. Measurements were taken at Day 0, Day 0.5 (12 h), Day 1 (24 h), Day 2, Day 4, Day 7, and Day 10. A linear regression analysis was carried out to create a relationship between OD_{683} and MLSS as mg/L according to standard methods (Apha et al. 1999). An additional analysis of OD_{683} against cell number per microliter (N) was conducted, calculating cell

number using an Accuri C6 Flow Cytometer (BD Biosciences). The regression analyses are given in Eqs. 2 and 3, respectively:

$$OD_{683}=0.0017MLSS-0.0525, R^2 = 0.991 \quad (2)$$

$$OD_{683}=(3 \times 10^{-5}) N-0.3779, R^2 = 0.999 \quad (3)$$

C. vulgaris was tested in six substrate conditions yielding different specific growth rates (μ) over 10 d. Eq. 4 was applied to calculate the μ from the start of the experiment to the end of lag phase (Day 0-1) and from the start of the experiment to the end of the exponential phase (Day 0-7):

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (4)$$

where, X_2 is the final cell concentration at time t_2 and X_1 is the initial concentration at time t_1 .

2.3.2 Specific oxygen production rate (SOPR)

During the pre-cultivation, *C. vulgaris* was cultivated with commercial BBM media in batch in 250 mL PYREX™ flasks but filled to a working volume of 300 mL in order to reduce headspace using airtight flasks sealed with silicon and rubber, equipped with a DO probe and continuously mixed with a magnetic stirrer (at 280 rpm). SOPR was calculated using a similar method as reported in a previous study by (Tang et al. 2014). Prior to the SOPR assay, the media was purged with nitrogen (N_2) gas to eliminate the initial DO. Sodium bicarbonate was then added to the mixed liquor at 0.75 g/L under continuous light conditions in order to ensure CO_2 -independent photosynthetic reactions. The inoculum was finally injected into flask at 10% of the working volume and DO concentration was then monitored for 24 h using an online bench meter.

2.3.3 Sampling and water quality analysis

During the experimental phase, monitoring of the pH and DO on the all replicates were carried out at

Day 0, 4 and 10 using the Multi 9430 Digital Meter (WTW, Germany). Samples were taken from the replicates at Day 0, 0.5 (12 h), 1 (24 h), 2, 4, 6, 7, and 10. Samples were filtered using 0.45 μm pore size syringe filters (Fisher Whatman puradisc-25 mm) to remove the microalgal cells and any other particles prior to chemical analysis. The supernatants were then properly diluted and analyzed for $\text{NH}_4^+\text{-N}$ using the Salicylate method, $\text{NO}_3^-\text{-N}$ using the Dimethylphenol method, and $\text{PO}_4^{3-}\text{-P}$ using the Ascorbic method with HACH analytical kits and Hach DR 5000 spectrophotometer (Hach Co., Loveland, CO, USA). OC and IC were also analyzed in a Total Organic Carbon analyzer (TOC-V CPH, Shimadzu, Japan).

3. Results and discussions

3.1 SOPR

Although CO_2 enriched air is normally used for microalgae cultivation (Arudchelvam & Nirmalakhandan 2012; Duong et al. 2012; Ortiz Montoya et al. 2014; Abreu et al. 2012), here sodium bicarbonate was directly added to the medium as a more convenient way to test SOPR, since the typical growth environment during algal cultivation is dominated by bicarbonate within the carbonate species due to pH-dependent CO_2 solubility (Van Den Hende et al. 2012).

Fig. S3 displays the DO profile as a function of time during algal cultivation. Once the culture was inoculated, automatic measurements were recorded for 24 h and analyzed. The oxygen production rate (OPR) was hence calculated by taking the linear polynomial model's slope of the increase in DO concentration over time. For instance, if the following equation ($y = 0.0102x + 1.425$) is obtained with fitting (with 95% confidence bounds) with an R-square: 0.9993, the linear slope of the graph (0.0102) equals the OPR. The specific oxygen production rate (SOPR) was determined by dividing OPR by the MLSS concentration in mg/L, which was 35.21 mg/L. The calculated SOPR was 17.31 mg O_2/g MLSS/h.

3.2 Growth rate of *C. vulgaris*

C. vulgaris was tested in six different conditions yielding different specific growth rates (μ), showing the growth in terms of optical density (OD) over time for a duration of 10 d (**Fig. 2**). In the following sub-sections (3.2.1 and 3.2.2), the effect of different nutrient source ratios on the algal growth is discussed.

3.2.1 Carbon source ratio

T1, T2, and T3 were compared against one another with different C sources at the same final concentrations (100 mg of C/L – either OC or IC), while N and P sources were fixed. **Fig. 2** shows the growth trends of the three conditions, T1 experienced the slowest growth rate at 0.223 d^{-1} , inferring that carbon in organic form alone (i.e. heterotrophic growth) may not be the optimal carbon substrate for microalgae under continuous light conditions, with a stationary phase prematurely initiated after Day 4 as opposed to the other carbon conditions. T2, supplemented with IC, initially exhibited a similar growth trend to T1, however, after Day 4 a significantly better growth than T1 at 0.303 d^{-1} was observed. T3 showed the optimal μ_{max} (D_{1-7} : 0.353 d^{-1}) and higher biomass productivity out of the three conditions tested. This represented the mixotrophic growth combining both OC and IC, and is in agreement with reports from previous studies (Liang et al. 2009; Bhatnagar et al. 2011). This indicates that IC is required to yield a fast growth rate with *C. vulgaris*, however, more importantly the ability to alternate between the two modes provided a higher growth yield and hence could potentially produce higher DO concentrations if such significant growth can be accomplished.

3.2.2 Nitrogen source ratio

In sets T4, T5, and T6, comparisons with different N sources at the same final concentrations (100 mg of N/L) were carried out, where C and P sources were fixed. Of the three conditions, T5 resulted in the

slowest $\mu_{\max}$ overall at 0.114 d^{-1} , implying that NO_3^- -N was not efficiently supporting the growth of *C. vulgaris* and as the only N source failed to deliver the necessary substrate, showing that N is the growth limiting substrate for the chosen strain (**Fig. 2**). Conversely, T4 showed the highest μ out of all tests at 0.357 d^{-1} with NH_4^+ -N at 100 mg/L validating its vital role in enhancing growth. Finally, T6 operated with both N sources at maximum growth of 0.243 d^{-1} , demonstrating a lower growth than T4 due to lower NH_4^+ -N availability (i.e. 50 mg/L).

3.3 Nutrient removal and utilization

While achieving maximum growth is essential in finding the optimal O_2 production, they are independent of nutrient and substrate concentrations. Hence, utilization rates must be determined to find a balanced system that can achieve both nutrient removal and to boost O_2 production.

3.3.1 IC utilization

IC was measured at Day 0 and Day 10. Good removal was achieved for all test conditions indicating that utilization of IC for *C. vulgaris* was maximum and required for their growth with nearly complete removal at more than 96% for T2 to T6 (final concentrations of 2.05, 0.78, 1.01, 1.70, and 1.06 mg/L in descending order) (**Fig. S3**). Phototrophic growth is heavily dependent on IC, further validating the photosynthetic reaction presence. OC removal was not targeted during this experiment. It was tested in order to detect any interference from OC on the specific growth rates or N/P utilization efficiencies (see discussion in **section 3.2.**).

3.3.2 Nitrogen utilization

Nutrient removal (or utilization) trends of NH_4^+ -N and NO_3^- -N over time are demonstrated in **Fig. 3a** and **b**, respectively. Overall, an inclination towards NH_4^+ -N consumption by *C. vulgaris* over NO_3^- -N is clearly demonstrated. At the initial stage between day 0 to 2, all tests (T1-T6) exhibited subtle to no

NO_3^- -N removal. Interestingly, T1 was at complete stagnation in terms of NO_3^- -N utilization exhibiting absolutely no removal throughout the full duration of the experiment, despite being supplemented at a concentration of 10 mg/L of NO_3^- -N apart from 40 mg/L of NH_4^+ -N as N sources similarly to concentrations provided to T2 and T3. As for T3, T5, and T6 the NO_3^- -N removal rates were very low with an average of $13.07 \pm 0.0076\%$. T2, however, could achieve much higher removal at nearly double the rate (24.5%).

As for NH_4^+ -N consumption rates, there was a positive correlation with the growth of *C. vulgaris*. **Fig. 3a** presents the NH_4^+ -N uptake as a function of time. NH_4^+ -N removal patterns demonstrated a similarity for T1-T3, and between T4 and T5 with definite decrease in concentrations and leveling off. **Fig. 3a** showed that T1 achieved the least NH_4^+ -N removal at efficiency of 39.6% (**Table 1**), in the presence of OC, presenting a linear relationship to its maximum specific growth rate. T2, which was supplemented with equal amounts of NH_4^+ -N and NO_3^- -N to T1, exhibited a higher 63.6% removal of NH_4^+ -N, supporting the NH_4^+ -N utilization preference for *C. vulgaris* and its direct contribution to biomass growth increase. T3 and T6 showed the sharpest drops in NH_4^+ -N concentration at 74.6% and 73.6% removal. T4, with the highest NH_4^+ -N initial concentration at 100mg/L NH_4^+ -N could remove 59.4% of the overall supplement.

3.3.3 Phosphorus utilization

By day 4, in the midst of the logarithmic growth phase, all PO_4^{3-} -P was able to be consumed by *C. vulgaris* in all six conditions, some experiencing sharper drops than others (**Fig. 3c**). T3, T4, and T6, the conditions able to achieve the highest maximum growth, reduced PO_4^{3-} -P to zero by Day 2. These results confirm that regardless of the nutrient conditions given to *C. vulgaris*, PO_4^{3-} -P is required as a basic macronutrient source with a rapid uptake.

3.4 Possible utilization mechanisms

In the scope of this work, the abiotic factors that affect algal growth conditions were predetermined (pH, light intensity, and temperature) to isolate the effect of C and N concentrations. pH variations did not occur in the trials due to the addition of HEPES buffer that maintained a pH 7-8. However, in real continuous systems, studies have shown that during algal cultivation pH can increase thereby making an alkaline environment, which leads to negative effects on algal productivity (Yun et al. 1997; Scherholz & Curtis 2013).

The OC:IC ratio was varied in trial T1 (100:0), T2 (0:100), and T3 (50:50), to accurately investigate the effect on *C. vulgaris* with N/P loads fixed at concentrations simulating MBR effluents at a low HRT (~approx.6-8 h). Short HRTs do not have enough time to enrich slow-growing nitrifiers hence achieving partial nitrification (at $\text{NH}_4^+\text{-N} = 40 \text{ mg/L}$, $\text{NO}_3^-\text{-N} = 10 \text{ mg/L}$, and $\text{PO}_4^{3-}\text{-P} = 5 \text{ mg/L}$).

The envisaged metabolic pathway (in **Fig. S4**) represents the HCO_3^- (IC) and N assimilation activity in *C. vulgaris* based on the results and previous biochemical studies (Hammed et al. 2016; Sanz-Luque et al. 2015; Rigobello-Masini et al. 2006; Scherholz & Curtis 2013). The trials (T2-T6) confirmed that IC was fully acquired by the biomass under continuous illumination. The photoautotrophic growth regime of microalgae involves photosynthetic reduction cycle (Light reaction), CO_2 concentration, and dark reaction. Photons are absorbed by the algal pigments to generate electrons for the photosystems to later convert ADP^+ and NADP^+ to their energetic form ATP and NADPH, respectively (Wang et al. 2014). Some of the reduced energy produced is allocated to drive the dark reaction for CO_2 assimilation through the Calvin cycle, and some is used for N assimilation in the chloroplast. The carbon concentration mechanism (CCM) in microalgae uses IC transporters and carbonic anhydrase in elevated CO_2 concentrations to facilitate uptake of dissolved IC and CO_2 . Microalgal carbonic anhydrase is a zinc-metalloenzyme that catalyzes the reversible hydration of CO_2 by rapid interconversions of IC

species ($\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}^+$) and concentrates C around ribulose-1,5-bisphosphate carboxylase / oxygenase (RuBisCO), the carboxylating enzyme of the Calvin cycle (Rigobello-Masini et al. 2006; Moroney et al. 2011).

The $\text{NH}_4\text{:NO}_3$ ratio was varied in trials T4 (100:0), T5 (0:100), and T6 (50:50), with fixed OC and IC set at equal concentrations at 50 mg/L. T4 experienced the highest growth rate but NH_4^+ -N removal rates of T6 was more successful at 76.3% compared to T4 at removal rates of 59.6%. Trial observations display high NH_4^+ -N utilization against NO_3^- -N, and results are in agreement with other studies done previously. A recent study reported that *C. vulgaris* assimilates both NH_4 and NO_3 but NH_4 is a preferred N source in supporting growth due to the lower energy cost (Tang et al. 2014). This could be because the cell surface of algae is electronegative, so NH_4 is attracted to the cells rather than NO_3 (Hadjoudja et al., 2010) as NO_3 is in an oxidized N species, and it must be reduced to ammonium prior to incorporation into organic molecules in the form of glutamate in the chloroplast (Dortch, 1990). NO_3 uptake and reduction process is mediated by an enzyme called NO_3 reductase, consumes reducing power (i.e. NADPH) provided by photosynthesis to be converted into nitrite (NO_2) in the cell's cytoplasm prior to being transported into the chloroplast which is even further reduced in the stroma into NH_4 for final conversion to amino acids through glutamine synthetase/glutamine oxoglutarate amino transferase or glutamate synthase (GS/GOGAT) pathway, the necessary protein building blocks of the cells. Nitrate Reductase (NR) is inhibited by high NH_4 concentrations so NO_3 is the secondary option as energy expenditure is required as opposed to NH_4 , which is less actively transported into the cell and chloroplast. The enzymatic activity of NR is directly associated to the inorganic carbon assimilation process, where low concentrations of IC decreases NR activity (Rigobello-Masini et al. 2006). This theory verifies the reason why T1 was unable to consume NO_3 ; as energy was not provided from the photosynthetic reactions, which was needed by IC as it was operating in the heterotrophic mode (only

OC was provided). Correspondingly, T2 utilized more NO_3 than T3 as higher IC concentrations were available so competition for electrons was reduced.

In addition, all trials utilized 100% PO_4 confirming that inorganic phosphate is a required macronutrient as it plays a central role in cellular metabolic processes, particularly in energy transduction, nucleic acid and DNA biosynthesis, and synthesis of other structural and functional components needed for the growth and development of the algal cells. Since inorganic carbon acquisition in microalgae is energy-dependent, PO_4 limitation could indirectly affect assimilation. Photosynthesis supplies reducing power, carbon skeletons, and adenosine PO_4 needed for assimilating N (Rigobello-Masini et al. 2006). The compounds of the Calvin cycle require phosphorylation, and low PO_4 would impair the process (Noctor & Foyer 1998; Makino & Mae 1999). PO_4 uptake in microalgae is controlled by transporters that drive the affinity and rate of PO_4 uptake (Pitt et al. 2010).

3.5 Suggested configurations

This research tackled the basic biochemical mechanisms of N and P utilization efficiencies in six different conditions, varying carbon and nutrient loading rates. Accordingly, the results can reasonably be correlated to an outcome of an engineered process design to treat domestic wastewater using coupled algal-bacterial system. The trials showed that NH_4^+ -N was required for *C. vulgaris*' growth more than NO_3^- -N, hence the basic algal tertiary treatment process may not be the best possible design. It also demonstrated the need for IC if high nutrient removal is required. In addition, OC does not disrupt the growth and utilization efficiencies, but on the contrary the growth rates demonstrated superiority when different carbon sources were supplemented. The SOPR study also showed notable O_2 production rates that can facilitate the bacterial growth in a conventional MBR.

Fig. 4 represents three configurations suggested and currently being studied to design the bridging the gap between the batch results achieved and the potential of *C. vulgaris* in a wastewater treatment process.

Configuration one treats the algal MPBR as a tertiary treatment process after the conventional MBR, which lacks the anoxic tank. A recirculation loop is created from the MPBR effluent back into the aeration tank for providing the necessary DO. In this process, the HRT of the aeration tank and the recirculation ratios play crucial roles in providing balance to the system and high effluent quality.

Configuration two employs the different sequences and the MPBR as a “Pre-denitrification” process where the microalgae can consume most of $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ before entering the conventional aeration tank. In this process, the considerations are the high organic loading rates (OLR) on the microalgae and possible NH_4 passing through into the aeration tank.

Configuration three maintains the same order as *Configuration two*, however, the wastewater is fed into both tanks to reduce or divide the OLR and decrease the algal sensitivity while maintaining the stream from the MPBR to the aeration tank to provide the OPR required. This configuration has a challenge in figuring out the appropriate NLR (Nitrogen loading rate)/OLR/PLR (Phosphorus loading rate) ratios. All configurations can be tested with and without the addition of IC.

4. Conclusion

C. vulgaris was cultivated at various nutrient concentrations in order to gain an understanding on the effect of different wastewater constituents on the O_2 production and nutrient utilization. Overall, the importance of IC source for the growth of *C. vulgaris* was validated and its P utilization was superior for all conditions tested. The *C. vulgaris* was favorably grown with $\text{NH}_4^+\text{-N}$ as an N source and mixotrophic C conditions. This shows algal MPBR will be helpful in either supplying the O_2 by recirculating the O_2 -

rich effluent produced into the MBR or improving water quality by reducing the nutrients before entering into MBR.

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List of Tables

Table 1. Nutrient removal efficiency (RE) from Day 0 to Day 10 with the following equation:

$$\text{RE (\%)} = (C_0 - C_{10}) / C_0 \times 100\%.$$

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Table 1. Nutrient removal efficiency (RE) from Day 0 to Day 10 with the following equation:

$$\text{RE (\%)} = (C_0 - C_{10}) / C_0 \times 100\%.$$

Nutrients	T1	T2	T3	T4	T5	T6
IC	n.a.*	97.8%	98.3%	97.9%	96.8%	97.9
NH ₄ ⁺ -N	39.6%	61.3%	74.6%	59.4%	n.a.*	73.6%
NO ₃ ⁻ -N	0%	24.5%	11.6%	n.a.*	14.2%	13.4%
PO ₄ ³⁻ -P	100%	100%	100%	100%	100%	100%

*n.a.: not applicable.

List of Figures

Figure 1. Six different substrate conditions tested (T1-T6) with two different nutrient sources as organic carbon (OC) in form of glucose and inorganic carbon (IC) in the form of sodium bicarbonate, two N sources as ammonium ($\text{NH}_4^+\text{-N}$) form and nitrate ($\text{NO}_3^-\text{-N}$) forms at different nutrient ratios with a 100 mg/L as a baseline concentration. The bars display concentration ratios at mg/L for each substrate. $\text{PO}_4^{3-}\text{-P}$ concentration was fixed at 5 mg/L for all trials.

Figure 2. Changes in DO concentration with time (oxygen production rate (OPR) by photosynthesis). *C. vulgaris* samples were prepared (in triplicate) in airtight flasks where the DO concentrations were measured every min for 24 h. The linear phase only counted for the OPR (until reaching full oxygen saturation).

Figure 3. (a) Ammonia ($\text{NH}_4^+\text{-N}$), (b) nitrate ($\text{NO}_3^-\text{-N}$) and (c) phosphate ($\text{PO}_4^{3-}\text{-P}$) uptake by *C. vulgaris* at different concentrations as a function of time.

Figure 4. Schematic diagrams of three process train of the coupled MBR-MPBR system eliminating mechanical aeration.

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Figure 2. Growth rate curves displayed of optical density at 683nm (OD₆₈₃) as a function of incubation time (Day 0 - Day 10).

(a)

(b)

(c)

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Highlights

- Microalgal membrane photobioreactor (MPBR) is tested for O₂ production.
- Microalgae (*C. vulgaris*) is cultivated at various nutrient conditions.
- *C. vulgaris* favorably uses mixotrophic carbon condition and ammonia in its growth.
- Possible nutrient utilization mechanisms are discussed.
- MPBR hybrid system configurations are suggested based on nutrient utilization.