Advanced wastewater treatment using microalgae: effect of temperature on removal of nutrients and organic carbon

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Abstract. This study evaluated the use of mixed indigenous microalgae (MIMA) as a treatment process for wastewaters and CO2 capturing technology at different temperatures. The study follows the growth rate of MIMA, CO2 Capturing from flue gas, removals of organic matter and nutrients from three types of wastewater (primary effluent, secondary effluent and septic effluent). A noticeable difference between the growth patterns of MIMA was observed at different CO2 and different operational temperatures. MIMA showed the highest growth grate when injected with CO2 dosage of 10% compared to the growth for the systems injected with 5% and 15 % of CO2. Ammonia and phosphorus removals for Spirulina were 69%, 75%, and 83% and 20%, 45% and 75 % for the media injected with 0, 5 and 10% CO2. The results of this study show that simple and cost-effective microalgae-based wastewater treatment systems can be successfully employed at different temperatures as a successful CO2 capturing technology even with the small probability of inhibition at high temperatures.

1. Introduction
In the near future, improving the overall efficiency of the Carbon Capture and Storage (CCS) chain will become increasingly important, as the implementation of the various CO2 existing capture methods from flue gas are not currently economically feasible. On the other hand, it is to be expected that CO2 will become available from a multitude of different sources. For the GCC (Gulf Cooperation Council) this will include power plants, refineries, various petrochemical processes (methanol, ammonia), and will encompass gas separation at processing plants and at LNG production facilities. CO2 has to be removed from flue gas (CO2-N2) and from natural gas (CO2-CH4) mixtures. The increase in CO2 concentration which considered as one of the greenhouse has been associated to several negative environmental impacts, such as climate change and change in ocean water chemistry. Therefore, it becomes urgent for world economies to reduce their carbon emissions [1-3]. Additionally, since this greenhouse gas presents long residence times [4,5], new strategies to reduce CO2 concentration in the atmosphere and in flue gas emissions are required [6].

The current average costs of CO2 capture and storage, based on chemical absorption, are estimated to be at least 55 US$ per ton of CO2 taking a coal-fired power plant as a reference [7]. In the absorption process, the solvent regeneration step accounts for approximately 75% of the energy consumption (and thus costs) [8,9]. The high energy penalty, which for power plants can be as high as 10% (based on the overall power plant efficiency of 35-45%), is one of the main drivers for research into more economically viable, and ultimately sustainable, approaches to be included in the full CCS chain.

At the same time, with the increase on world population the high volumes of wastewater generated by human was always a permanent problem that requires appropriate treatment. While domestic wastewater treatment plants (WWTPs) is considered an effective treatment for removal of biochemical
oxygen demand (BOD), suspended solids and bacteria, the removal of inorganic nutrients, still limited and challenging in these processes. With the implementation of the new wastewater discharge regulation (e.g. Canada Gazet, 2012), the removal of nutrients, mainly dissolved nitrogen and phosphorus, is becoming one of the essential requirement for the approval of the WWTP performance. The new regulations were based on the fact that the presence of these nutrients in wastewater will leads to eutrophication by stimulating the growth of unwanted plants such as algae and aquatic macrophytes [10-12], and increase the effluents toxicity to fishes and aquatic organisms [13]. Moreover, untreated nutrients in wastewater effluent reduce the performance of disinfection step and increase the chlorine demand [14]. As a result, there is a big need to find a treatment process that can remove these nutrients before the effluents are discharged [15, 16]. Different studies have used algae for treatment of different types of wastewater. Most recently, Zhu et al. [17] reported a removal efficiency of 65-76% of COD, 68-81% of TN and 90-100% of TP from piggery wastewater by fresh water microalgae Chlorella zofingiensis. Wang et al. [13] reported COD removal of 50.9%and 56.5% from primary and secondary effluents by green algae Chlorella sp. The study showed significant nutrient removals efficiencies. A total nitrogen and Phosphorus removal efficiencies of 68.4%, and 50.8%, and 82.8%and 85.6 % were reported for the same previous wastewater samples, respectively. So far, limited attention has been paid to the dual role of microalgae as advanced technology for treatment of different kinds of wastewater and as CO2 bio-fixation technology. Moreover, there are knowledge gaps in understanding the performance of different microalgae strains in the treatment of wastewater and their capacity for CO2 capturing. In particular, according to our literature review, no studies were carried out using mixed indigenous microalgae cultures as opposed to lab-grown pure culture strains. The objective of this study was to investigate and compare the treatment efficiency and CO2 bio-fixation capacity of lab-grown mixed indigenous cultures of microalgae in different wastewaters. Primary effluent, secondary influent and septic tank effluent was used in the study to determine possible applications and locations for the proposed treatment systems. The study was carried out in 1.50 L-Erlenmeyer flasks and CO2 was provided to microalgae mixed with air though air diffuser.

2. Materials and methods

2.1. Culture system

Algae growth experiments were carried out in 1 L- sterilized Erlenmeyer flasks at 36°C. The growth experiments were performed using an incubator equipped with thermostat and two cool white lamps of 1500 lux each. Temperature control was maintained at 8, 20 and 36 °C by a fan connected to thermostatic control. Two fluorescent —daylight lamps were used as light sources. The photon flux intensity inside the incubator was measured by NIST Traccable Radiometer (International light. Model IL 400A) and found to be 1.5 μ W/cm2.

A volume of 850 mL of wastewater (primary, secondary or septic) was mixed with algal strain and incubated at constant temperature and continuous lighting for a period of 28 days. No carbon dioxide (CO2) was supplied to stored cultures other than diffusion of CO2 present in the ambient atmosphere. The flasks were stirred manually 4 times a day.

2.2. Characteristics of wastewaters

Wastewaters were collected from three different sources (1) effluent of primary treatment, (2) effluent of secondary treatment, and (3) septic tank effluent. All wastewaters were filtered using glass microfiber filters (934-AH, Whatman, USA) to remove large particles and indigenous bacteria. Table 1 presents the chemical characteristics of the three wastewaters used in this study.
Table 1: Properties of the Wastewaters

| Parameter          | Primary effluent | Secondary effluent | Septic effluent |
|--------------------|------------------|--------------------|---------------|
| COD$_{s}$ (mg/L)   | 245              | 65                 | 165           |
| NH$_4^+$ (mg-N/L)  | 44               | 40                 | 45            |
| NO$_2^-$ (mg-N/L)  | 0.006            | 1.1                | 5             |
| NO$_3^-$ (mg-N/L)  | 0.33             | 23                 | 12            |
| Total Phosphorus (mg/L) | 15       | 32                 | 10            |

3. Results and discussion

3.1 Algae growth at different temperatures

The growths of mixed indigenous microalgae (MIMA) reported as function of optical density (OD) at 690 nm and at different temperatures (20, 25 and 36 °C) and different CO2 dosage in primary effluent, secondary effluent and Septic effluent were plotted as function of time in Figure 1. The growth curve of cultivated algae follows atypical growth curves consisting of lag phase, exponential growth phase and stationary phase. The length of the lag phases accounts for the algae capacity to adapt to the new wastewater environment. Figure 1 shows that the lag phases of MIMA depend on the type of wastewater and the cultivation temperatures.

The growth trends of MIMA in primary wastewater effluent has rapid growth. It can be seen in the figure 1a that the lag time for MIMA range from 1 to 3 days, and the best growth trend was obtained at a temperature of 25°C. The maximum optical density reported for MIMA at 20, 25 and 36 °C were 0.74, 1.05 and 0.88 A.U. The measured OD was converted into dry cell weight using predetermined conversion factors. The final biomass concentrations for experiments carried out with MIMA in primary wastewater effluent after 24 days of cultivation were found to be 1.91, 2.71 and 2.27 g/L at 20, 25 and 36 °C, respectively.

The growth rates of MIMA were almost comparable for secondary and primary wastewaters and slightly higher in septic tank effluent. Moreover, it was observed that the mixed algal culture reached a stationary phase within 17 days of cultivation time in Septic tank effluent while it requires 22 days in secondary and primary wastewaters. The average specific growth rates in the first 3 days of growth phase (days 3, 4 and 5) were found to be 0.22, 0.20, and 0.40 day⁻¹ for primary effluent, secondary effluent and Septic tank effluent, respectively.

3.2 CO2 capturing

The effect of increasing CO2 concentrations on its fixation rate showed that the lowest CO2 fixation rates were achieved when MIMA was fed with CO2 at atmospheric concentrations. Higher CO2 dosage increased CO2 but is was followed by a decrease in MIMA growth. The obtained results suggest that MIMA can be effective technology for CO2 capturing. In addition, experimental results showed that the evaluation of Ph can be used as an indicator of the CO2 capturing efficiency. Picardo et al. [18] showed that for the pH values in the range of 6.0 to 9.0, bicarbonate (HCO3⁻) is the most common form of inorganic carbon present in solution, suggesting that external can utilized by microalgae in cell formation. On the other hand, for the condition of low pH 5.0 to 7.0, CO2 utilization occurs through mass transfer. Thus, CO2 up take by algal culture is mass transfer controlled process. It was observed also that when the algal culture aerated with atmospheric air enriched with CO2-enriched air the pH of water solution decrease suggesting that CO2 uptake in these conditions may be performed through diffusion.
COD removal efficiency also varied among different wastewaters. The results indicate that cultivation times can be shortened based on the optimum COD removal and desired final effluent qualities. Rapid decrease in COD was achieved during the first 7 days of cultivation for all wastewater samples. Significant COD removals were achieved with the mixed indigenous culture, which were 64.9%, 70.3% and 69.3%, respectively.

3.4 Removal of nitrogen compounds
MIMA showed very good efficiencies in removing ammonia (NH4–N) from wastewater (Figure 2b). ammonia removal efficiencies were 63.2%, 67.5% and 71.7% for primary effluent, secondary effluent and septic tank effluent. In addition to ammonia, nitrate (NO3–N) and nitrite (NO2–N) removals were also measured. MIMA 64%, 66.7% and 60% of nitrate from primary effluent, secondary effluent and septic tank effluent, respectively. The removal of nitrate from the effluents corresponded to the generation of nitrite (Figure 2d). The initial nitrite in primary effluent, secondary effluent and Septic tank effluent were 0.005, 0.89 and 0.14 mg-N/L. The concentration of nitrite did not exceed 0.11 mg-N/L for primary effluent, 0.93 mg-N/L for secondary effluent and 0.99 mg-N/L for Septic tank effluent. Wang et al. [13] reported a similar increase in nitrite and a decrease in nitrate during the cultivation of MIMA. Table 2 provides a summary of the percent removals of ammonia, nitrate as well as total inorganic nitrogen (TIN). TIN removal efficiencies were around 60% on average and above 50% could be achieved by MIMA. The highest TIN removal of 80.8% was achieved in Septic tank effluent by using the mixed culture. The results show that algal strains can be used to effectively remove inorganic nitrogen from different types of wastewater. Some of this removal could be due to the volatilisation of ammonia at elevated pH levels as a result of microalgae activity. Experiments carried out with adding CO2 showed also promising results of nitrogen compounds removals.
3.5 Removal of phosphorus

The percent removal of total dissolved phosphorus in primary effluent, secondary effluent and septic tank effluent with MIMA. Similar to COD and nitrogen removal, phosphorus removal was different in different wastewater samples and also varied with the type of microalgae. The general trend indicated that the highest phosphorus removal was achieved in primary effluent and septic tank effluent, and limited removal was observed in secondary wastewater. Reported phosphorus removal efficiencies were 79%, 49% and 21.9% in primary effluent, secondary effluent and septic tank effluent, respectively. Some of the removal could be caused by the precipitation of phosphorus through the formation of hydroxyapatite due to elevated pH levels occurred as a result of microalgae activity.

![Graphs showing organic matter and nutrient removals](image)

**Figure 2**: Organic matter and nutrient removals in primary effluent, secondary effluent and septic tank effluent by mixed indigenous microalgae culture at 25 °C and without addition of CO2.

**Table 2**: Percent removal of COD, NH4+, NO3-, total inorganic nitrogen (TIN) and total dissolved phosphorus (TDP) by mixed indigenous microalgae in primary effluent, secondary effluent, and septic tank effluent.

| % Removal | Primary effluent | Secondary effluent | septic tank effluent |
|-----------|------------------|--------------------|---------------------|
| COD       | 64.9             | 70.3               | 69.3                |
| NH₄⁺      | 63.2             | 67.5               | 71.7                |
| NO₃⁻      | 64               | 66.7               | 60                  |
| TIN       | 63.2             | 67.3               | 80.8                |
| TDP       | 70.0             | 30.8               | 50                  |

4. Conclusions
The suitability of algae process in treating wastewater from different location in municipal wastewater treatment plant at different temperature was evaluated. Growth rate, COD removal and nutrient removals (N and P) were followed. The results of this study show that MIMA is effective in capturing CO2 and removing ammonia and phosphorus from wastewater. This simple and cost-effective microalgae-based wastewater treatment systems can be successfully employed in different regions in spite of some potential inhibition to microalgae due to high temperatures. MIMA showed a maximum growth for media injected with 10% of CO2 at different temperatures. More than 70% of ammonia and 75 % of Phosphorous was removed by MIMAin the temperature range of 20-30°C. Growth rates of microalgae were different not only between the two temperatures but also for the same microalgae injected with different CO2, which indicates that the operational condition play an important role in determining the potential of algae as CO2-capturing technology and this knowledge can be used to optimize the microalgae based treatment systems.

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**Acknowledgements**

This publication was made possible by the NPRP grant (NPRP No.: 6 - 1436 - 2 - 581) from the Qatar National Research Fund (a member of Qatar Foundation). The statements made herein are solely the responsibility of authors. The authors gratefully acknowledge the support provided by the Qatar University to do this work.