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Comparison between Normal Air and CO_2-enriched Air for Municipal Wastewater Treatment by *Chlorella vulgaris*

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Abstract Microalgae present very good option for treatment of various kinds of wastewaters with the aim to harvest the biomass for different purposes like biogas or biodiesel production, feed supplements for animals, etc. Algae utilize organic carbon present in municipal wastewater for their metabolic growth and multiplication. In addition, these also take up nutrients such as nitrogen and phosphorus from wastewater. In the present study, axenic culture of *Chlorella vulgaris* ATCC 13482 was used for treatment of primary municipal wastewater. All experiments were conducted in cylindrical glass bottles of 1 liter working volume in batch mode maintained at 25°C and 4000 lux light intensity with 14 hr/10 hr of light/dark cycle for a period of 10 days. *Chlorella* inoculum was grown in municipal wastewater with supply of normal air and air enriched with 5% CO_2 (vol./vol.). The aim of this study was to compare the effects of normal air and CO_2-enriched air on the efficiency of wastewater treatment by the algae. The supply of 5% CO_2 gave better result than that of normal air for wastewater treatment wherein COD, ammonia-N and phosphate decreased by 76.3%, 94.2% and 94.8% respectively.

Keywords Municipal wastewater, *Chlorella vulgaris*, Nutrients uptake, Treatment efficiency

1. Introduction

1.1. Background

The conventional method of municipal wastewater treatment has been the aeration in activated sludge process. Hence, a major contribution to greenhouse gas emissions comes from power consumption at mechanically aerated wastewater treatment plants. Together with that, handling sludge is also a challenge for these treatment plants. During the last decade, use of algae has gained lot of importance in research because of their extensively wide applications ranging from wastewater treatment options (Carlsson et al., 2007; Chevalier et al., 2000; Garcia et al., 2006; Guzzon et al., 2008; Woertz et al., 2009), CO_2 utilization (Packer, 2009; Kumar et. al, 2011) to production of protein-rich edible neutraceuticals (omega3 fatty acids) (Costa et al., 2003; Lee, 2001; Olaizola, 2003) and for green energy source e.g. biodiesel, methane and ethanol (Chisti, 2007; Milledge, 2011).

1.2. Goals and Objective

From the view point of measures to contain environmental pollution and resource recovery, cultivation of green algae is expected to cater to various needs: (i) algal mediated uptake of organic content and nutrients from municipal wastewater and hence, its treatment earns positive water footprint for the project (ii) bioremediation of CO_2 in flue gas through algal growth (production of 1 kg of algal biomass consumes nearly 2 kg of CO_2) (Herzog and Golomb, 2004) (iii) use of recovered O_2 for aerobic treatment of wastewater (1 kg of CO_2 utilized by algae produces nearly 0.73 kg of O_2 as per the stoichiometry of photosynthesis) (iv) production of biodiesel as sustainable source of eco-friendly and green fuel from lipid in algae gains carbon credits (v) use of left over algal dry mass for biogas generation under anaerobic condition. The objective of the current research work was to compare the effects of normal air and CO_2-enriched air on the efficiency of municipal wastewater treatment by the algae.

2. Materials and Methods

2.1. Wastewater Sampling and Analysis

Municipal wastewater (WW) was collected from the primary sewage tank at Water Reclamation Plant at Ulu Pandan in Singapore and stored at 4°C until further analysis. Wastewater sample was filtered through 0.45 μm filter to

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remove suspended particles and its characteristic parameters, given in Table 1, were estimated in triplicates (mean ±s.d.) as per Standard Methods for the Examination of Water and Wastewater (APHA, 2005). pH of wastewater sample was analyzed using Multi-Parameter Analyzer (3200M Agilent Technologies, USA). Chemical oxygen demand (COD), total-N, ammonia-N, nitrate, total-P, phosphate and sulphate were analyzed using the chemical kits and DR900 colorimeter (Hach, USA). Total inorganic carbon (TIC) and total organic carbon (TOC) were analyzed using TOC-V_{SH} Analyzer (Shimadzu, Japan). Presence of few metals (Ca, K, Mg, Fe, Mn, Co, Cu and Zn) in wastewater sample necessary for growth of green algae was analyzed by ICP-OES (Perkin Elmer Optima 5300DV, USA).

Table 1. Characteristics of municipal wastewater

Parameters	Value
pH	6.6 ± 0.05
COD (mg/L)	293 ± 5.72
TOC (mg/L)	85.0 ± 1.31
TIC (mg/L)	1.5 ± 0.34
Ammonia-N (mg/L)	43.7 ± 1.24
Nitrate-N (mg/L)	1.6 ± 0.18
Total-N (mg/L)	46.7 ± 0.47
Orthophosphate-P (mg/L)	18.5 ± 0.09
Total-P (mg/L)	19.5 ± 0.4
Cl^- (mg/L)	117.7 ± 1.73
SO_4^{2-} (mg/L)	38.3 ± 1.47
Ca (mg/L)	16.1 ± 2.15
K (mg/L)	16.5 ± 0.12
Mg (mg/L)	4.8 ± 0.19
Fe (mg/L)	0.7 ± 0.02
Mn, Co, Cu, Zn (mg/L)	n.d.*

* n.d.: not detectable

2.2. Microalgae Culture

Stock culture of *Chlorella vulgaris* ATCC 13482 was obtained as generous contribution from Prof. Loh Kai Chee's lab at National University of Singapore. This microalgae species has been used for biological treatment of different kinds of wastewaters (Ji et al., 2013; Abou-Shanab et al., 2013). The algae was maintained in Bold's Basal medium (URL1) in 3-litre conical flask at 25°C and fluorescent light of 4000 lux for 14 hr/10 hr light and dark cycle and agitated at 100 rpm on magnetic stirrer. Sterile air at flow rate 0.2 vvm was bubbled through 0.2 µm filter.

2.3. Acclimatization Study

Chlorella grown in Bold's Basal (BB) medium for 10 days was acclimatized to municipal wastewater in cylindrical glass bottles of 2 litre working volume (inoculum to wastewater ratio as 1:20 i.e. 5% vol./vol.) before starting the treatment study. Fluorescent light of 4000 lux was supplied for 14 hr/10 hr light and dark cycle with normal air flow at 0.2 vvm. Wastewater sample was diluted with DI water in respective ratios of 30%, 60% 90% and 100% (vol./vol.) for acclimatizing algae to the wastewater. Controls with BB medium but without wastewater were also used to grow the algae. Fig.1 shows reactors for acclimatizing *Chlorella* to municipal wastewater.

Figure 1. Acclimatizing algae to municipal wastewater

Fig.2 shows algae growth during the acclimatization study. Absorbance was recorded as optical density measurement at 680 nm wavelength after every 24 hr until 10th day.

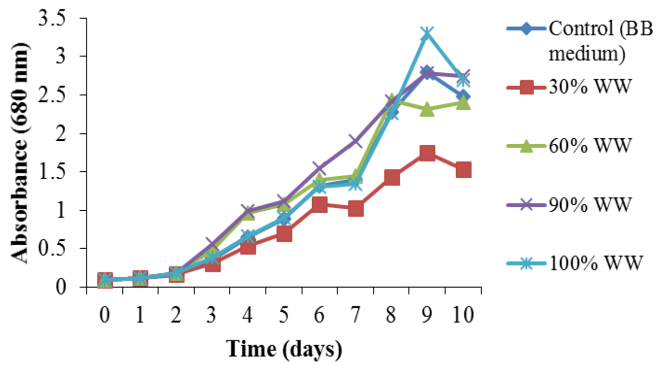

Figure 2. *Chlorella* acclimatization study

As can be seen from Fig.2, *Chlorella* could adapt well even in undiluted municipal wastewater.

2.4. Experimental Setup

Experimental setup for treatment of municipal wastewater by *Chlorella* is shown in Fig.3. Working volume of the reactors was one-liter with sterile air supply maintained at 0.2 L/min over a period of 10 days. Filtered samples of the wastewater were autoclaved at 121°C for 15 minutes before being fed into the reactors. 950 mL municipal wastewater mixed with 50 mL algae inoculum was used in each reactor. Controls (without *Chlorella*) were also used to know the evaporative loss of nutrients and COD (in case if any) by air stripping.

Figure 3. Experimental setup for treatment of wastewater by *Chlorella*

3. Results and Discussion

3.1. Supply of Normal Air

Controls (without *Chlorella vulgaris*) showed lower removal efficiency than those with the algae (Fig.4). Alkaline condition (7 < pH range < 8.5) remained during the test period which has been also reported in literature to be good for growth of green algae (Olaizola et al., 2004; Suryata et al., 2010). *Chlorella* showed removal efficiency of 71%, 89.2% and 90.9% for COD, ammonia and phosphate respectively.

3.2. Supply of Normal Air Enriched with 5% CO_2

Use of 5% CO_2-laden air enhanced process of wastewater treatment by *Chlorella* with COD removal efficiency of 76.3% as shown in Fig.4 (a). With supply of 5% CO_2, it also proved to be a better choice for uptake of ammonia and phosphate removal efficiency of 94.2% and 94.8% respectively as shown in Fig.4 (c) and Fig.4 (d).

Data for decrease of organics and nutrients (COD, TOC, ammonia and phosphate) for the treatment process over 10 days have been given as summary in Table 2. Here, supply of CO_2 aids in the efficiency of the algae to treat wastewater. CO_2 utilization has been reported to enhance wastewater treatment by microalgae under mixotrophic condition (Cuellar-Bermudez et al., 2015).

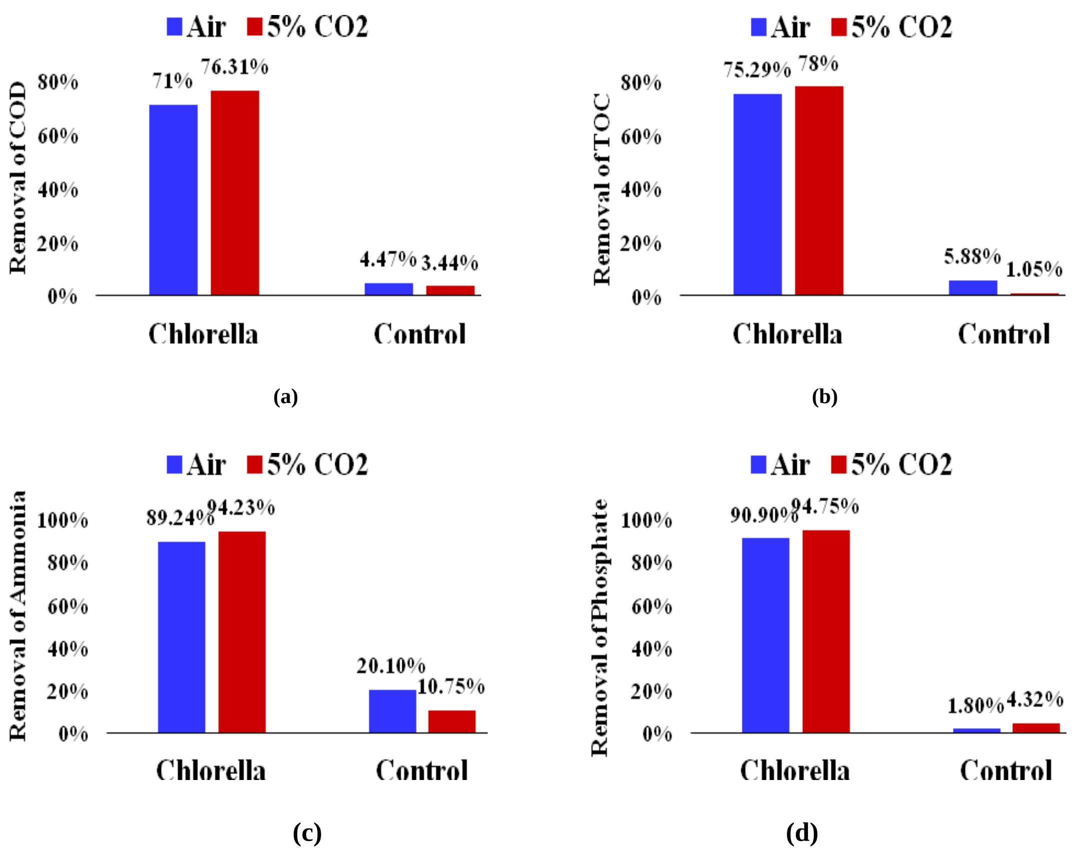

Figure 4. % Decrease of (a) COD (b) TOC (c) ammonia and (d) phosphate with normal air and air enriched with 5% CO_2

Table 2. Decrease of organics and nutrients with normal air and enriched 5% CO_2 supply for 10 days

	COD (mg/L)		TOC (mg/L)		Ammonia-N (mg/L)		Orthophosphate-P (mg/L)	
With Air	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Chlorella	293	84.97	85	21	43.7	4.7	18.5	1.67
Control	293	279.9	85	80	43.7	34.9	18.5	16.7
Air with 5% CO_2								
Chlorella	293	69.4	85	18.7	43.7	2.52	18.5	0.97
Control	293	282.9	85	84.1	43.7	39	18.5	17.7

For the present study, final effluent characteristics (pH, COD, TOC, ammonia and phosphate) for the treatment by *Chlorella* after 10 days are shown in Table 3. Values for pH, COD and phosphate are within the allowable limits for trade effluents discharge into watercourse/controlled watercourse set by Public Utilities Board Singapore (URL2).

Table 3. Water quality of the effluent after treatment with *Chlorella vulgaris*

Parameters	Treatment with *Chlorella* using 5% CO_2	Discharge Standards in Singapore	
		Watercourse	Controlled Watercourse
pH	8.11	6-9	6-9
COD (mg/L)	69.4	100	60
TOC (mg/L)	18.7	**	**
Ammonia (mg/L)	2.52	**	**
Phosphate (mg/L)	0.97	5	2

**not shown

4. Conclusions

Microalgae have shown tremendous potentials in treating wastewater from different sources. Algae utilize nutrients available in the wastewater through metabolic assimilation and hence it is possible to remove and recover nutrients in form of algal biomass from wastewater. Growing microalgae with wastewater is one of the best options as control strategy for wastewater management along with CO_2 utilization. For the present study, only 5% vol./vol. algae inoculum was used and 95% vol./vol. was primary sewage municipal wastewater. Primary sewage was efficiently treated by *Chlorella vulgaris* and the process was enhanced with supply of air enriched with 5% CO_2. COD, TOC and nutrients load (nitrogen and phosphorus) were significantly removed from the primary sewage. Further, algal biomass can be harvested and put to anaerobic digestion for methane production or lipids from algal biomass can be channelized into biodiesel production.

Abbreviations

ATCC: American Type Culture Collection
COD: Chemical Oxygen Demand
ICP-OES: Inductively Coupled Plasma-Optical Emission Spectroscopy
PTFE: Polytetrafluoroethylene
PUB: Public Utility Board Singapore
TOC: Total Organic Carbon
TN: Total Nitrogen
TP: Total Phosphorus
vvm: volume of gas supply/volume of liquid/minute (aeration rate)

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