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Cultivating *Chlorella* sp. in a Pilot-Scale Photobioreactor Using Centrate Wastewater for Microalgae Biomass Production and Wastewater Nutrient Removal

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Abstract This study is concerned with a novel mass microalgae production system which, for the first time, uses "centrate", a concentrated wastewater stream, to produce microalgal biomass for energy production. Centrate contains a high level of nutrients that support algal growth. The objective of this study was to investigate the growth characteristics of a locally isolated microalgae strain *Chlorella* sp. in centrate and its ability to remove nutrients from centrate. A pilot-scale photobioreactor (PBR) was constructed at a local wastewater treatment plant. The system was tested under different harvesting rates and exogenous CO_2 levels with the local strain of *Chlorella s*p. Under low light conditions (25 $\mu mol \cdot m^{-2}s^{-1}$) the system can produce 34.6 and 17.7 $g \cdot m^{-2}day^{-1}$ biomass in terms of total suspended solids and volatile suspended solids, respectively. At a one fourth harvesting rate, reduction of chemical oxygen demand, total Kjeldahl nitrogen, and soluble total phosphorus were 70%, 61%, and 61%, respectively. The addition of CO_2 to the system did not exhibit a positive effect on biomass productivity or nutrient removal in centrate which is an organic carbon rich medium. The unique PBR system is highly scalable and provides a great opportunity for biomass production coupled with wastewater treatment.

Keywords Centrate wastewater treatment · CO_2 · Harvest rate · Microalgae

Introduction

The current method for biofuel production relies on limited arable lands, making it increasingly impossible to meet global biofuel demands without disrupting food production. Oil-producing algae are a promising biofuel feedstock with the potential to meet the world's ambitious goals of displacing fossil fuels without threatening food supplies. However,

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recent environmental life cycle comparison of algae to other bioenergy feedstocks shows that, for production of one functional unit of energy (317 GJ), algae crops have a higher environmental impact than conventional biofuel crops such as corn, canola, and switchgrass with respect to greenhouse gas emissions, water consumption, and energy usage [1]. The same study [1] also identified the critical burden drivers of algae production to be the demands/requirements for fertilizers and CO_2. In order to help reduce this impact, flue gas and wastewater should be used to mitigate the environmental burdens associated with mass algal production systems. On the other hand, management of wastewater and related gaseous emission is very costly and technically challenging. Modifications and improvement of current conventional processes must be made to meet the increasingly stringent regulations and limits on wastewater discharge and gaseous emissions. Growing algae in wastewater streams has a number of benefits. First, it will offset additional costs for nutrient and water supplies for algal growth and help remove nutrients, particularly phosphorous and nitrogen, from wastewater streams. Secondly, this system can assimilate large quantities of organic carbon for biofuel production, which will otherwise be emitted as CO_2 in the atmosphere eventually. Algae are able to capture and utilize this carbon source and convert it into biomass which can then be processed downstream. Coupling algae production with wastewater treatment is a practical way to lower the economic and environmental costs associated with algae based biofuel production.

Cultivating microalgae in wastewater has been studied as an alternative approach for wastewater treatment for more than 50 years [2, 3]. Nutrient removal by active algae has been studied at the University of Kansas since the 1970s [4, 5]. Recently, high rate algal ponds (HRPs) for wastewater treatment have gained great interest [6]. In both systems, oxygen produced by photosynthetic algal was used to support heterotrophic bacteria activity and convert wastewater-borne nutrients into biomass. It was observed that diurnal variations highly affected variations in nutrients removal efficiency, dissolved oxygen (DO), and pH on the daily cycle in the HRPs [3]. In the HRP tested by García et al. [3], the biomass productivity based on total suspended solid (TSS) was 14.8 gm^{-2}, and nutrient removal rates were 73% for total nitrogen and 43% for total phosphorus.

In this project, a collaborated effort was initiated by University of Minnesota and the Metropolitan Council Environmental Services (MCES) in which centrate was used as the nutrient source for mass cultivation of microalgae and removal of wastewater nutrient. Centrate, which is usually generated through dewatering sludge from primary and secondary treatment, is characterized by high carbon, nitrogen, and phosphorus levels. The centrate stream is approximately one million gallons per day (MGD) and recycles approximately 2,000 lb/day of soluble phosphorus back into the liquid treatment train in the Metropolitan Wastewater Treatment Plant (Metro Plant) in Saint Paul, Minnesota. The centrate accounts for approximately 30% of the soluble phosphorus loading to the liquid treatment train. As a result, the removal of soluble phosphorus from the centrate quickly became one of the focuses of the project.

An innovative pilot-scale photobioreactor (PBR) was developed and set up at the Metro Plant. The project made an effort to maximize the PBR's ability for biomass productivity and wastewater treatment. The objective of this study was to determine harvesting rate and CO_2 effects on biomass productivity and nutrient removal. The feasibility to improve the system is also evaluated in this paper. The long-term goal of this project is to develop a cost-effective and energy-efficient mass algal cultivation system that can fulfill the dual purposes of biofuel production and wastewater treatment.

Materials and Methods

Microalgae Strain

A wild-type *Chlorella* sp. strain isolated from a local lake was used in this study. The strain was prepared in Tris–acetate–phosphorus medium [7] with the following solid ingredients (milligrams per liter): NH_4Cl, 400; $MgSO_4 \cdot 7H_2O$, 100; $CaCl_2 \cdot 2H_2O$, 50; K_2HPO_4, 108; KH_2PO_4, 56; Tris (hydroxymethyl) aminomethane, 2420. Liquid chemicals included (milliliters per liter) glacial acetic acid, 1; trace elements solution, 1; consisted of the components listed below (grams per liter) Na_2EDTA, 50; $ZnSO_4 \cdot 7H_2O$, 22; $CaCl_2 \cdot 2H_2O$, 0.05; H_3BO_3, 11.4; $MnCl_2 \cdot 4H_2O$, 5.06; $FeSO_4 \cdot 7H_2O$, 4.99; $CoCl_2 \cdot 6H_2O$, 1.61; $CuSO_4 \cdot 5H_2O$, 1.57; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.10; and KOH, 16. After the strain was inoculated into the pilot-scale PBR at the Metro Plant, centrate wastewater was subsequently introduced until the full scale of 1,500 L was achieved. Thereafter, the strain was maintained in the centrate wastewater.

Municipal Wastewater Source

Our previous studies have shown that better productivity can be obtained with centrate than with primary influent or secondary effluent [8, 9]. This study was conducted at the Metro Plant where centrate was made available to us. MCES operates seven wastewater treatment facilities and treats an average of approximately 250 MGD wastewater. The St. Paul Metro Plant is the largest facility in the system with a 185-MGD treatment capacity. The Metro plant utilizes a biological phosphorus removal system to meet its discharge limit of 1 mg/L total phosphorus on a 12-month rolling average. The sludge generated at the Metro plant are dewatered using centrifuges and then combusted in fluid bed incinerators equipped with heat recovery boilers. This process requires no additional inputs of fuel and creates heat and electricity for the buildings.

The centrate has a high concentration of total suspended solids, thus it has an inherently high turbidity which reduces light transmission. Additionally, residual polymer in the centrate after the centrifugation process may cause undesirable flocculation of growing algae. Treatment to remove the suspended solids and residual polymers is necessary to effectively grow algae. This was accomplished by rapidly mixing the centrate followed by settling. During the rapid mixing process, colloidal particles and residual polymer act on each other. Additionally, intense agitation forced entrained air to escape from the solids. This encourages the solids to settle very quickly [10]. In our process, 5 min of rapid mixing followed by 30 min settling was used to remove colloidal particles and residual polymer.

The nutrient and micro-nutrient (metal ion) profiles were analyzed. Ten centrate samples were collected between 16 Dec 2008 and 6 Jan 2009 and analyzed at the Earth Science Geology and Geophysics Lab at the University of Minnesota using an inductively coupled plasma atomic emission spectrometer (Perkin Elmer Optima 3000, USA).

Pilot Plant at Metropolitan Wastewater Treatment Plant

A pilot plant was set up in the basement of the Solid Management Building at the Metro Plant for easy access to centrate. The pilot plant consists of a centrate treatment tank with mixer, a PBR, a harvest tank and mixer, and a dewatering centrifuge. The PBR is a semi-open system which includes a mixing tank and a recycling peristaltic pump (Masterflex, Cole-parmer Co). Fluorescent growth lights provided an average light intensity of

25 μmol·m^{-2}s^{-1}. Total hydraulic surface area was 11.9 m^2. Total energy input for the lights used in the PBR was 1,280 watts. The PBR had a total hydraulic volume of 1,500 L, of which 1,200 L where in the PBR and 300 L where in the mixing tank. Water depth in the PBR was 10.2 cm. The water flow rate was 38 L/min with plug flow. Due to the pending patent process, a detailed structure of the PBR cannot be disclosed here. However, the information provided is sufficient for readers to understand and analyze the results reported in the paper. Since the centrate can be a significant odor source, the centrate treatment tank and the PBR were enclosed in a sheet metal building. Air is continuously drawn out from the enclosure and passed through an activated carbon treatment system. An immersion heater was employed to regulate the system temperature to around 25°C.

Batch Experiment for Algae Cultivation

A 13-day batch experiment was carried out in May 2009 using the pilot-scale PBR. The purpose of the experiment was to help understand the growth characteristic of the *Chlorella* sp. in the centrate stream. A typical growth curve and optimized harvesting rates were established. A 300-l culture, cultured in centrate with a TSS content of 1,030 mg/L, was inoculated in the PBR followed by addition of 1,200 L of centrate. Water level was 10.2 cm, and the average light intensity was 25 μmol·m^{-2}s^{-1}. During the first 2 days of the experiment, the temperature was 29°C due to heating from the fluorescent lights. By opening the shed enclosure door and increasing the convective ventilation, the average temperature was held to 26±1°C beginning on the third day. The initial status of inoculated algal culture and centrate are listed in Table 1.

Several parameters were monitored to obtain nutrient levels. Chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia (NH_3-N), soluble total phosphorus (STP), and nitride (NO_3^-) were analyzed. Centrifugation followed by filtration through a 0.45-μm cellulose acetate filter (Whatman, USA) allowed us to measure nutrient levels on a soluble basis. In order to assess biomass the productivities, TSS, and volatile suspended solids (VSS) were determined. These parameters were tested at the Metropolitan Council Water Quality Laboratory (Saint Paul, MN) using standard method protocols [11]. In situ measurements of DO and temperature were measured using an oxymeter (Oxi 3400i, WTW, Germany) while the pH was determined using a pH-meter (pH 100, Yellow Springs Instruments, OH).

Biomass growth rates that were described as suspended solid accumulation rate (r, per day) were calculated based on TSS or VSS,

$$r_{TSS} = \frac{Ln(TSS_t) - Ln(TSS_0)}{\Delta t}, \text{ or } r_{VSS} = \frac{Ln(VSS_t) - Ln(VSS_0)}{\Delta t} \quad (1)$$

where r_{TSS} and r_{VSS} are the accumulation rate based on *TSS* and *VSS*. TSS_t, VSS_t, TSS_0, and VSS_0 are the *TSS* and *VSS* at day D_t and D_0. Δt is the time interval (days) between D_t and D_0.

Table 1 The initial status of inoculated algal culture and centrate wastewater (at day 0)

Solution	TSS (mg/L)	VSS (mg/L)	STP (mg/L)	TKN (mg/L)	NH_3-N (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)	COD (mg/L)	pH
Algae culture	1,030	580	183	110	128	0.25	<0.03	1,060	7.36
Centrate	588	496	530	290	264	0.46	<0.03	4,120	6.45

Semi-continuous Algae Cultivation

The system was tested using three different harvesting rates with each rate being tested both with and without additional CO_2 supply. The harvesting rates were one half of the PBR water volume (600 L) every other day (1/4), one third (1/3) of the PBR water volume (400 L) daily, and half (½) of the PBR water volume (600 L) daily. These rates were tested to determine the optimal operation parameters that yielded a maximum production of algal biomass. The effect of additional CO_2 was tested by adding CO_2 (100%) to the mixing tank through a ceramic diffuser. Since the addition of CO_2 affects the pH value of the system, the CO_2 flow rate was controlled to keep the pH in the range of 7.0~7.5. For each test, the running time was ranged from 9 to 29 days. This range was corresponds to three times of the hydraulic retention time (HRT) of that test run. For example, the harvesting of one half every other day has a HRT of 4 days. Thus, the experiment length should be longer than 12 days. Table 2 summarizes the experimental arrangements.

Temperature and pH were monitored daily; these values are listed in Table 2. Although the system's volume and harvested volume were set according the experiment design, the actual volumes might vary slightly from the designed values due to operational errors, evaporation, and leakage during the experiments. Thus, volume of system (V_S), volume of harvested algae (V_h), and volume of added centrate (V_C) were recorded on the harvesting day. Suspended solids (TSS and VSS) and soluble nutrients (COD, TKN, NH_3-N, STP, and NO_3^-) were determined before each harvest. Biomass productivities (grams per square·meter per day) were calculated using Eq. 2. Nutrient removal (grams per square·meter per day) was calculated using Eq. 3 and nutrient removal rates were calculated using Eq. 4.

$$\text{Yield}\left(\text{g}\times\text{m}^{-2}\text{d}^{-1}\right)=\frac{S'V_s^{'}-\left(SV_s-SV_h+S_cV_c\right)}{\Delta t\cdot\text{area}} \tag{2}$$

$$\text{Nutrient}\quad\text{removal}\left(\text{g}\times\text{m}^{-2}\text{d}^{-1}\right)=\frac{\left(SV_s-SV_h+S_cV_c\right)-S'V_s^{'}}{\Delta t\cdot\text{area}} \tag{3}$$

$$\text{Nutrient}\quad\text{removal}(\%)=\frac{S_c-S'}{S_c}\times 100\% \tag{4}$$

where S is the concentration of TSS, VSS, STP, TKN, or COD before harvest at day D_t, S' is the concentration of TSS, VSS, STP, TKN, or COD at following harvesting day D_t' before harvesting, S_c is the concentration of TSS, VSS, STP, TKN, or COD in centrate. V_s

Table 2 Experiments arrangements of harvesting rate and CO_2 effect on algal biomass productivities and nutrients removal rates

Date in 2009	Test run	Days	Harvested volume	Harvesting Rate	CO_2	pH	Temperature (°C)
7/27	5	17	600 L every 2 days	¼	No CO_2	7.5±0.3	28.1±0.7
5/1	2	23	600 L every 2 days	¼	With CO_2	7.3±0.2	26.4±1.3
3/30	1	24	400 L daily	1/3	No CO_2	7.8±0.2	24.9±1.6
8/20	6	17	400 L daily	1/3	With CO_2	7.0±0.4	27.4±0.6
6/14	4	9	600 L daily	½	No CO_2	7.0±0.1	27.9±1.0
5/26	3	17	600 L daily	½	With CO_2	7.1±0.3	24.9±1.1

is the system volume at day D_t, V_h is harvested volume, V_c is volume of added centrate, and V_s' is the system volume at day D_t'. Δt is the time interval (days) between D_t and D_t'. *Area* is the illumination area which is 11.9 m^2.

Since the levels of NO_2^- and NO_3^- were low (<1 mg/L), TKN would be appropriate to reflect the nitrogen removal; thus, we chose TKN as the indicator for nitrogen removal from centrate. All statistical analyses were carried out using JMP 6.0 software. The mean and standard deviations for each test run were calculated. Outliers might occur due to sampling errors, nutrients variation in centrate, and system variations. For these reasons, outliers were removed. The significance of the two factors (harvesting rate and CO_2 supplement) on biomass productivities and nutrient removal were evaluated with analysis of variance at $P \leq 0.05$. The Tukey's HSD mean test at $P \leq 0.05$ was used to compare means with significant differences.

During each test run, several algae samples were collected for analysis of the fatty acid content, British Thermal Unit (BTU) value, and the bacteria biomass. The fatty acid was analyzed using the method described in Wang et al. [12]. The BTU value was tested in the Minnesota Valley Testing Laboratory (Bismarck, ND). The bacteria were separated from algal cells using a centrifuge since bacteria cells were smaller and lighter than algal cells [13]. After centrifugation, the bacteria cells were loosely packed at the top layer above algae cells. By shaking the sample jar, bacteria layer returned to solution while algal cells remained packed. The measurements of TSS and VSS of the bacteria only solution indicate the proportion of bacteria in the total biomass solid.

Results and Discussion

Characteristics of the Centrate

The centrate was a complex mixture with varying chemical and physical properties. High levels of suspended solids in the centrate gave rise to a high turbidity (~1,000 NRTU) and gray color. Tables 3 and 4 describe the various physical and chemical characteristics of the centrate. The presence of the inorganic micro-elements in the centrate suggests the potential metallic inhibitors to algae growth. Compared with TAP medium and other wastewater streams (e.g., primary influent and secondary effluent), the centrate had a higher concentration of phosphorus which might be released when the sludge is kept under anaerobic condition [14]. This condition also encouraged the decomposition of organic

Table 3 Characteristics of the centrate

Parameter	Concentration (mg/L)
Soluble COD	3027±779
TSS	460±276
VSS	349±263
NO_3-N	0.35±0.36
pH	6.15±0.26
PO_4-P	215±135
Soluble TP	392±82
NH_3-N	113±18
TKN	275±151
NO_2-N	<0.03

Table 4 The soluble micro-nutrient contents in the centrate

Element	Concentration (mg/L) average with SD	Element	Concentration (mg/L) average with SD
Aluminum (Al)	0.010±0.006	Molybdenum (Mo)	<0.001
Arsenic (As)	0.009±0.001	Nickel (Ni)	0.022±0.007
Boron (B)	0.354±0.0617	Potassium (K)	144.400±14.854
Barium (Ba)	0.027±0.034	Rubidium (Rb)	0.083±0.008
Calcium (Ca)	109.313±20.038	Selenium (Se)	0.007±0.001
Cadmium (Cd)	<0.0004	Silicon (Si)	17.176±2.864
Chloride	267.549±27.100	Strontium (Sr)	0.247±0.035
Iron (Fe)	3.074±0.023	Sulfate	37.103±4.934
Lead (Pb)	0.009±0.002	Tin (Sn)	0.004±0.0004
Lithium (Li)	0.030±0.027	Titanium (Ti)	<0.001
Magnesium (Mg)	65.104±7.188	Tungsten (W)	0.004±0.001
Manganese (Mn)	2.645±0.499	Zinc (Zn)	0.088±0.071
Mercury (Hg)	0.398 ±0.219	Zirconium (Zr)	0.002±0.0005

Values were obtained from average of ten samples

compounds to soluble organic acids by microorganisms. The organic acids, such as acetic, propionic, and butyric acid contributed to the soluble COD concentration (3,027 mg/L) and lowered the pH value (6.0~6.5) of the centrate [15]. Centrate has an unbalanced N/P ratio and high Ca, Si, Mn, Mg, and sulfate concentrations. A deficiency of Co, Cu, Mo, and Zn were also present. Wang et al. [9] attempted to balance the nitrogen content of centrate by adding nitrogen sources such as NH_4Cl, urea, and Tris; however, no significant difference was found in growth between using raw centrate and centrate with various nitrogen supplements. Based on this observation, raw centrate without nutrient supplementation was used in this present study.

Algae Growth in Batch Experiment

The growth pattern of *Chorella* sp. in centrate is illustrated in Fig. 1. In the first 5 days, the culture was in a lag phase during which algae cells were slowly adapting to the new environmental conditions. This lag phase lasted 3–4 days longer than when algae were cultured under lab conditions [8]. In the first few days, inoculated culture tended to precipitate at the bottom of the PBR probably due to the environmental shock [16]. Around the fifth day, the density of algal biomass started to increase rapidly. The color of the culture broth turned from gray to green color as more algal cells became suspended in the culture. Rapid growth took place during the period of fifth to eighth days after centrate introduction. This growth was characterized by an exponential growth rate of 0.53 day^{-1} on a TSS basis. However, the observed exponential phase under the pilot-scale reactor condition was slower than those cultivated in the lab environment (0.53 vs. 0.95 day^{-1}). This decreased rate is likely due to lower light intensity (40 vs. 200 $\mu mol \cdot m^{-2}s^{-1}$) and deeper water levels (10.2 vs. 3 cm) between the two experimental conditions [8]. Highest algal density was observed on the ninth day with the TSS value of 2,050 mg/L.

The stationary phase was relatively short (1–2 days) when compared with growth under lab conditions (4–5 days). This was followed by the death phase starting on the ninth day,

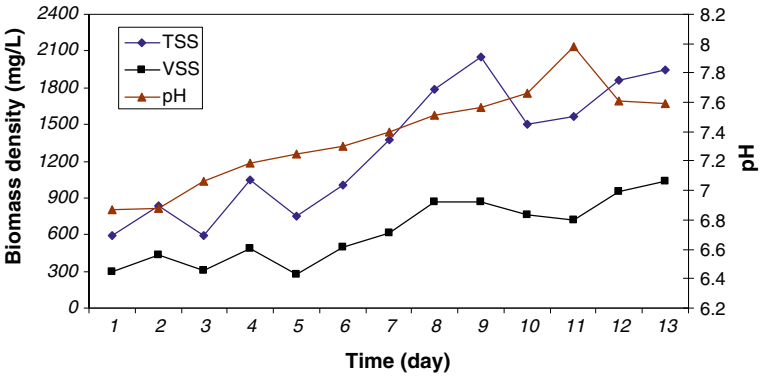

Fig. 1 Thirteen-day batch growth experiment of *Chlorella* sp. conducted in pilot-scale PBR without adding CO_2

during which the lyses of algal cells by bacteria and rupture of dead bacterial cells would diffuse nutrients out to furnish remaining viable cells [17]. A decrease and subsequent increase in TSS and VSS were likely caused by cryptic growth in the endogenous phase.

The accumulation rate of VSS between days 5–8 was 0.49 day^{-1}, which was slightly lower than that of TSS, suggesting that the increase in TSS was not only due to the increase in VSS but also due to mineral precipitations from the centrate. The centrate had an ash content of 15% and the final biomass had a VSS content of 1,040 mg/L with an ash content of 47% of dry basis. On average 50% of harvested biomass was inorganic matter. Algae cultivated in TAP media alone have ash content about 8–10% of the dry weight. Therefore, we concluded that the high ash content was due to the high salinity in the centrate, and the ash compounds were likely outside of algal cells. Table 4 shows that Ca, Si, Mg, sulfate, and STP added up to 620 mg/L. High pH level can convert metal ions into insoluble salts. Interestingly, a thick layer of mineral deposits where later observed in the hoses and around the reactor fittings during the semi-continuous experiments. These deposits needed to be periodically removed to prevent hose clogs and equipment failure.

The exponential phase of the TSS and VSS growth curve last for 4 days (fifth to ninth days) and 3 days (fifth to eighth days), respectively, before reaching the plateau (stationary phase). This suggests that harvesting rate of 1/4 or 1/3 might be the optimal rates for biomass production.

Nutrient Removal in Batch Experiment

Beside inoculated microalgae, other microflora may be present naturally in the centrate. Therefore, activities of these microfloras must be considered in our data analysis. Figure 2 illustrates the nutrients reduction in the batch cultivation. Since the algae culture was still in the lag phase during the first 3–4 days, the activities leading to nutrient reduction are expected to be dominated by non-algae microorganisms. The growth of VSS and the reduction of nutrients during this lag phase were minimal compared with those of the later growth phase, suggesting that bacteria activities were limited. The fluctuation in the TSS, VSS, and nutrient levels might be caused by nutrient uptake by new cells and nutrients decomposed and released from insoluble organic matter and dead cells. Starting on the fifth day, nutrient removal was mainly due to algal biomass growth. The STP, TKN, NH_3-N, and COD reduction from the first to 11th day were 58.1%, 34.8%, 19.5%, 86.3%, respectively.

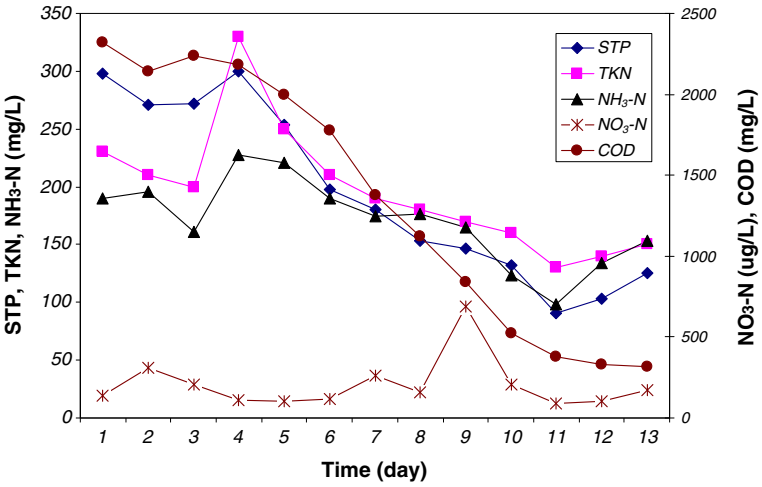

Fig. 2 Nutrient levels of a 13-day batch growth experiment of *Chlorella* sp. conducted in pilot-scale PBR

In general, phosphorus accounts for at most 3.3% of the dry algae cell weight [18], equivalent to about a 40 mg/L phosphorus reduction in the batch experiment. However, the total phosphorus reduction was 210 mg/L, which indicated that the phosphorus mainly reduced through precipitation.

The removal of nitrogen is dominated by nutrient uptake by algae and bacteria during growth. Volatilization of some ammonia at high pH value was another reason for N removal. Soluble nitrogen existed primarily in the form of ammonium (NH_4^+), which accounts for 80% of the total nitrogen (230 mg/L). Nitrate (NO_3^-) remained at a negligible level of 0.35 mg/L. *Chlorella* sp. can utilize both the ammonium and nitrate present in the centrate. Therefore, algal growth rates in the centrate were similar to those on either ammonium or nitrate [18]. Some of the nitrogen released from insoluble organic matter due to bacteria activities may be another nitrogen source for algal growth. However, the amount of nitrogen that was released by bacteria was not clear in this study. The bacteria separated by centrifuge at the end of cultivation are less than 3% of the total biomass. Nitrite levels remained relatively flat during third to eighth days probably due to constant forming of nitrite from other forms of nitrogen and assimilation of nitrite by algae. Between the seventh and ninth days, the ammonium curve flatted out, but the TKN curve kept declining, indicating that there were other types of organic nitrogen compounds being utilized by algae. The average ammonium and TKN consumption rate between days 5–9 were 14 and 20 mg/L/day, respectively. On the ninth day, the increase in nitrite might indicate the death phase of old algae cells and bacteria in which nitrite and other organic forms of nitrogen were quickly released. These compounds were likely taken up by new algal cells but at a lower rate.

Among all the nutrient reduction parameters, reduction of COD, an approximation of carbon levels, was the greatest. This may be attributed to the fact that carbon is a macro-nutrient necessary for algae growth. When an organic carbon source is present in the medium and light is used for energy, algal growth is considered as mixotrophic growth. This type of growth allows assimilated both CO_2 and organic carbon simultaneously. The mixotrophic growth rate is believed to be approximately the sum of both autotrophic and heterotrophic growth rates, which represents the fastest way to grow algae biomass [19]. The average COD consumption rate between the fifth and ninth day was 290 mg/L/day. A lab experiment that compared growth of *Chlorella* sp. in raw centrate and in autoclaved

centrate showed similar COD reduction curves, indicating that bacterial activities were minor with regard to COD removal in batch mode cultivation [20].

Biomass Productivity and Nutrient Removal of Semi-continuous Cultivation

Biomass densities and nutrient levels of the centrate and harvested algae determined from the six semi-continuous testing runs are shown in Fig. 3a–e. Figure 3a–e share common *X*-

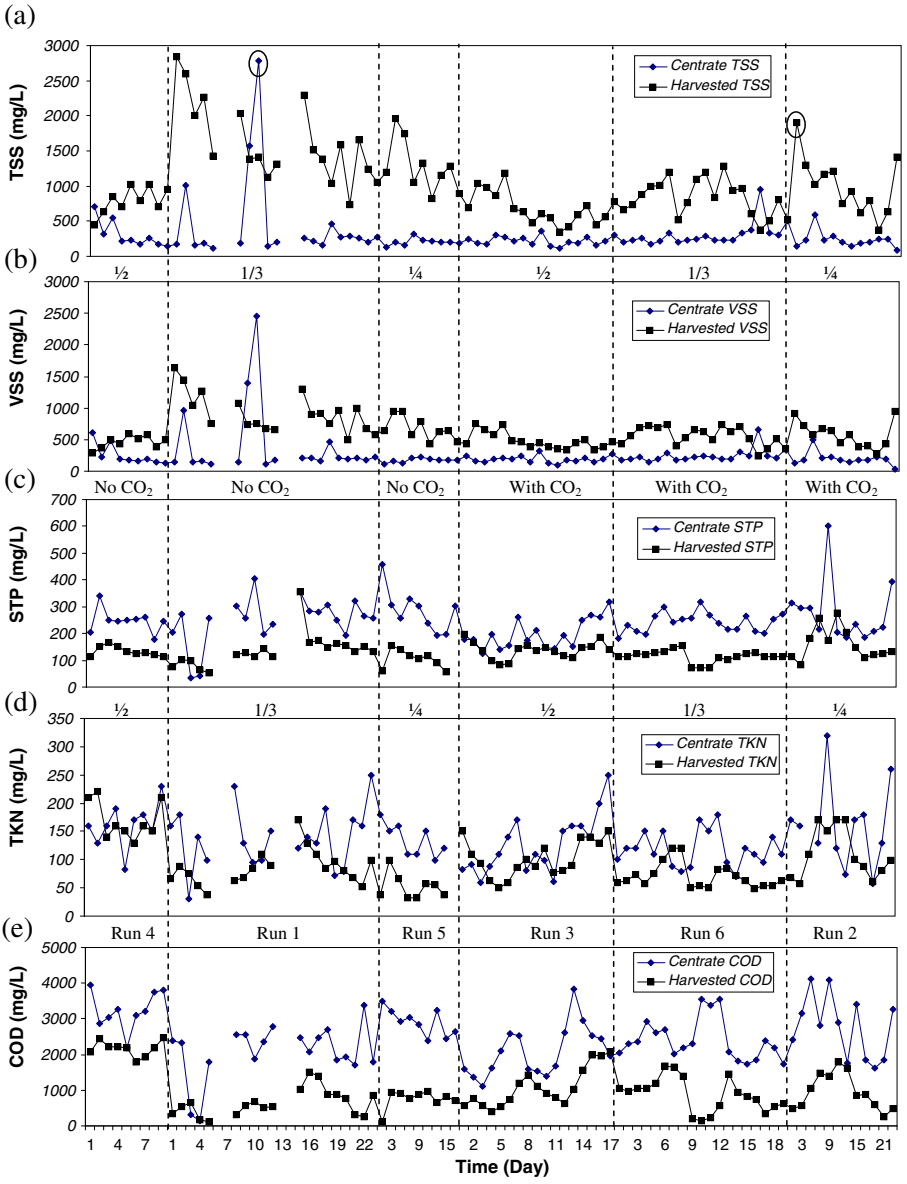

Fig. 3 Biomass density and nutrient levels in harvested algal broth and input centrate of six semi-continuous operations. **a** TSS content, **b** VSS content, **c** STP content, **d** TKN content, **e** COD content

axis, from a to e: harvesting rate, CO_2 supplement, harvesting rate, test run, and cultivation time. Several outliers (but not all) were indicated with circles in the figures. The operation of using 1/3 harvesting rate without adding CO_2 produced average highest volumetric yield of 1,627 mg/L for TSS and 929 mg/L VSS, respectively, or surface areal yields of 34.6 $g \cdot m^{-2} day^{-1}$ for TSS and 17.7 $g \cdot m^{-2} day^{-1}$ for VSS. Richmond et al. [21] reported that raceway system can produce 25 to 27 $g \cdot m^{-2} day^{-1}$ on TSS basis during the summer time and 12 to 14 $g \cdot m^{-2} day^{-1}$ during the winter time. *Oscillatoria* sp. cultured in diluted wastewater using circular ponds had a biomass productivity of around 15 $g \cdot m^{-2} day^{-1}$ on TSS basis [22]. Garcia et al. (2006) [3] used a high rate pond to treat wastewater and produced 12.7 $g \cdot m^{-2} day^{-1}$ TSS. Since the current system was designed to compare with an open pond system under similar conditions, the current system had comparable productivities of those reported in other review articles [23–25].

Due to large variations in the centrate, stream responses of the system were highly dynamic. Although TSS and VSS in centrate were relatively consistent, the nutrient levels of the centrate fluctuated greatly over time (Fig. 3). The complexity of the system was compounded by the unique interactions between the various microorganisms present in the centrate, as well as the interactions between algae and bacteria cells in the reactor. The nutrient reductions in the six runs are listed in Table 5. The best scenario for nutrient removal on a percentage basis occurred with harvesting ½ every other day (1/4 harvesting rate), in which COD, STP, and TKN removal were 70%, 61%, and 61%, respectively, equivalent to 50.8, 4.9, and 2.1 $g \cdot m^{-2} day^{-1}$, respectively. The nutrient removal was comparable with the high rate pond developed by Garcia et al. [3] which showed a 73% TN and a 43% TP removal. Since the biomass productivity and nutrient removal were largely affected by harvesting rate, CO_2 and other factors, more discussion is presented in the next section.

Effect of Harvesting Rate on Biomass Productivity and Nutrient Removal without CO_2 Supplementation

A lower harvesting rate was expected to result in higher algal density. The combined factors of relatively high algal density and harvesting volume gave 1/3 harvesting rate without CO_2 supplementation the higher VSS areal productivities when compared with ¼ harvesting rate (Table 5). However, all three harvesting rates did not significantly affect the TSS productivities.

For nutrient removal, the statistic results show that there was no significant difference in COD removal in terms of grams·per square meter per day among operations using three different harvesting rates. However, when total nutrient removal is presented as percentage, ¼ and 1/3 harvesting rates showed better nutrient removal than ½ harvesting rate. It was easy to understand that lower harvesting rate resulted in longer hydraulic retention time in which higher biomass density would be accumulated, thus, more nutrients would be converted. Therefore, greater nutrient removal in terms of percentage would be obtained. The total COD reduction rate in terms of grams per square meter per day was not significantly different between the three harvesting rates due to the smaller amount of volume that was harvested with the lower harvesting rate. In the view of wastewater treatment for removing the maximum percentage of nutrients, a lower harvesting rate would be a better choice since higher COD reduction (%) can be obtained. However, when considering areal productivity, harvested volume should be considered to obtain higher nutrient removal rates in terms of grams per square meter per day.

Table 5 Effect of harvesting rate (HR) on biomass productivity and nutrients removal rate

CO_2	HR	TSS ($g \cdot m^{-2} day^{-1}$)	VSS ($g \cdot m^{-2} day^{-1}$)	STP ($g \cdot m^{-2} day^{-1}$)	STP (%)	TKN ($g \cdot m^{-2} day^{-1}$)	TKN (%)	COD ($g \cdot m^{-2} day^{-1}$)	COD (%)
No	½	26.4±14.3 a	15.6±3.2 ab	6.2±1.4 a	44.9±8.9 b	−0.5±2.3 b	11.9±7.8 c	44.4±9.6 a	35.2±10.6 b
	1/3	34.6±8.4 a	17.7±4.2 a	4.6±1.8 b	50.0±16.1 ab	2.0±2.7 a	41.2±27.5 b	52.2±12.6 a	69.5±18.4 a
	¼	27.0±10.3 a	12.7±5.3 b	4.9±1.2 ab	60.9±13.8 a	2.1±1.4 a	61.1±14.8 a	50.8±7.1 a	70.3±6.1 a
Yes	½	21.2±10.0 x	14.5±5.5 x	2.9±2.6 x	26.7±21.8 y	0.9±2.3 x	19.7±30.7 y	38.7±10.7 x	51.9±20.0 y
	1/3	17.0±9.9 x	10.9±4.9 xy	2.7±0.9 x	47.2±7.5 x	1.1±1.8 x	45.5±14.7 x	41.4±8.9 x	56.9±16.3 xy
	¼	19.7±11.0 x	8.7±5.0 y	3.1±2.5 x	52.7±14.9 x	0.9±2.0 x	45.3±23.1 x	44.6±6.5 x	70.1±13.5 x

In each comparison, means followed by unlike letters are significantly different at 5%

In test run 4, the removal of TKN in the ½ harvesting rate operation without CO_2 addition was low because the TKN levels of algae broth at several harvest points were higher than those of the added centrate, resulting in negative values in terms of grams per square meter per day for TKN removal. Non-algal activities could also potentially convert the non-soluble forms of nitrogen to soluble forms. Operational errors could also contribute to the negative value. For example, unknown amounts of settled centrate sludge might have accidentally been pumped into the system. This might result in unpredictable increases in the TSS and other nutrient levels because the sludge contains much higher levels of these nutrients.

Removal of STP (grams per square meter per day) was affected more by pH value and harvested volume than by harvesting rate. The greatest removal occurred at ½ harvesting rate without CO_2 addition. This was mainly because precipitation occurred in alkaline conditions.

Effects of CO_2 on Biomass Productivity and Nutrients Removal

Carbon dioxide did not have positive effect on biomass productivity and nutrient removal; furthermore, it reduced the TSS productivity and substantially decreased the STP and COD removal rates (in grams per square meter per day; Table 6). Although statistically, results were not significant due to large variations, it was believed that CO_2 had a negative effect on VSS production and TKN removal in this experimental setup.

In general, pH value can be as high as 8 without CO_2 addition, at which precipitation of Mg, Ca, phosphate, and carbonate salts takes place. Supplementation of CO_2 will cause the pH to drop and prevent minerals from precipitating which is related to TSS accumulation and STP removal. However, excessive CO_2 decreases the pH level severely. This creates an unfavorable condition for algae growth.

It was noticed that the *Chlorella* sp. grew in the centrate mixotrophically. During the lab experiments, when the *Chlorella* sp. grew in centrate with DCMU addition, a sensitive inhibitor of photosynthesis, the growth rate was lower than that without using DCMU, which proved that the microalgae grew in a mixotrophic regime. Moreover, the proportion of heterotrophy or autotrophy in the mixotrophy would be affected by the concentration of the organic carbon and inorganic carbon and light intensity. The centrate wastewater is high in COD (3,000 mg/L), which indicates abundant organic C-substrate. With the presence of light, *Chlorella* sp. can utilize organic C-substrate much faster than CO_2; this process is referred to as photoheterotrophy [26]. Introducing exogenous CO_2 would repress the photoheterotrophy but stimulate photosynthesis. Because the light intensity was relatively low, the increase of the growth rate caused by photosynthesis was not obvious. Hence, the overall growth rate of *Chlorella* sp. was decreased [27]. When extra CO_2 was added, less bacteria population was observed. Since bacteria contributed to COD reduction, less bacteria population would potentially reduce COD removal rate.

Table 6 Effect of CO_2 on biomass productivity and nutrient removal rate

CO_2	TSS ($g \cdot m^{-2} day^{-1}$)	VSS ($g \cdot m^{-2} day^{-1}$)	STP ($g \cdot m^{-2} day^{-1}$)	STP (%)	TKN ($g \cdot m^{-2} day^{-1}$)	TKN (%)	COD ($g \cdot m^{-2} day^{-1}$)	COD (%)
No	29.0±22.0 a	14.7±6.5 a	4.9±2.1 a	49.7±17.0 a	1.3±2.9 a	33.6±34.3 a	49.7±10.8 a	62.4±21.2 a
Yes	19.9±12.1 b	11.9±7.2 a	3.2±2.4 b	39.7±21.1 b	1.0±2.0 a	25.0±35.9 a	42.9±11.9 b	55.8±21.8 a

In each comparison, means followed by unlike letters are significantly different at 5%

Bacteria, Lipid Content, BTU Value of the Algal Biomass

The harvested biomass was composed of not only algae but also of bacteria and other suspended solids. Most of the TSS and VSS in the centrate finally end up in the harvested biomass. The increase of suspended solid was mainly attributed to algae growth and mineral deposit. Bacteria population was affected by temperature, pH, nutrient availability, and competitors. In general, bacteria account for 2–6% of the total biomass. However, bacteria bloom (up to 10–30% of the total biomass) can occur under certain conditions, e.g., with ½ harvesting rate without CO_2 supply. It was observed that CO_2 repressed bacteria population because CO_2 injection helped remove oxygen and therefore hindered the activities of aerobic bacteria. Bacteria population was low when using low harvesting rate because the higher algal cell density was able to out-compete other microorganisms including bacteria.

The lipid (FAME) content was about 4.7–6.3% of dry algal biomass on VSS basis. The BTU value is 9,800±2,200 BTU/lb of dry algal biomass on VSS basis. Electrical energy input from light was 104,900 BTU/day, and only 7% of the electricity was converted to light energy. The energy produced by algal biomass was about 4,600 BTU/day, which was 4% of the electrical energy. Natural sunlight intensity is ten- to 30-fold greater than light intensity of the artificial light sources supplied to our PBR. By converting the current indoor system to an outdoor system, higher productivity and lower operation cost would be achieved by minimizing the artificial light energy input.

Conclusions

In this study, a novel system that combines centrate wastewater treatment and microalgae cultivation was developed and tested under low light conditions with different harvesting rates and CO_2 injection levels by using a locally screened strain of *Chlorella* sp. The system can produce 34.6 $g \cdot m^{-2} day^{-1}$ TSS and 17.7±4.2 $g \cdot m^{-2} day^{-1}$ VSS and remove 70% COD, 61% TKN, and 61% STP which is comparable with or exceeds other systems. The addition of CO_2 did not have a positive effect on biomass productivity or nutrient removal in an organic carbon rich medium. However, CO_2 injection is believed to help repress native bacteria pollution in the medium.

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