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Treatment of municipal wastewater using *Scenedesmus abundans* and studies on saccharification of grown biomass using ultrasound assistance

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ABSTRACT

Today, numerous research groups are investigating the research on microalgae with the support of government to overcome the dependence on fossil-based fuels, because, the microalgae is the third generation biofuel feedstock. This biofuel feedstock has grasped all the attention due to its capability to grow faster by utilizing the wastewater and consuming the carbon dioxide or flue gas. This study describes the potential of the microalgae, *Scenedesmus abundans* to exploit the nutrients in the municipal wastewater and to yield abundant biomass for biofuel production and sequestering CO_2 in a photobioreactor. During its cultivation, about 75.9% of chemical oxygen demand, 91.6% nitrogen and 90.7% phosphate were removed which results in a good biomass concentration of 3.45 g/L of which the carbohydrate content accumulated was about 57%. Saccharification was carried with mild acid or mild alkali with and without application of ultrasound. On assessment, the ultrasound-assisted mild acid saccharification (at 8% v/v) resulted the highest sugar yield of 139 mg/g dry biomass. These findings were suggested that the municipal wastewater with sufficient nutrients supplement can be directly used for mass cultivation of microalgae which is an essential source of carbohydrate for bioethanol production.

Keywords: Scenedesmus abundans; Municipal wastewater; Sequestration; Carbohydrate; Ultrasound

1. Introduction

Increasing population of this world have risen the environmental challenges like energy crisis, air and water pollution, etc. [1]. Fossil fuels based energy is considered as the predominant source and it is projected to increase by 56% by 2040 [2]. This increasing global energy demand with the depletion of oil reserves have turned attention to develop new alternative energy sources which could help to replace petroleum-diesel. Among the renewable energy alternatives, biofuels are considered as the most promising liquid fuel source and eco-friendly in nature [3].

Many researchers have reported that the concentration of atmospheric CO_2 has reached an alarming level of 400 ppm and is continuously increasing [4]. Further, due to the rapid increase in the population, large amounts of freshwater have been widely used and their subsequent released municipal wastewater (MWW) discharge roots severe water pollution [5]. The MWW contains abundant amount of carbon, nitrogen, phosphorous and other minerals which results in eutrophication and other environmental complications if discharged untreated [6]. Stringent norms have been deployed to the industries treating wastewater due to this generated effluent. Hence, there is a crucial need to reduce the wastewater disposal and greenhouse gas emission. Therefore, the main concern is to discover a greener solution aimed at cleaner air and water [7]. Numerous studies were published on CO_2 sequestration and wastewater treatment through different biological, chemical and physical approaches [8]. However, the mentioned treatment systems are not found to be economic for CO_2 sequestration and wastewater treatment [6].

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Regardless of many current CO_2 mitigation and wastewater treatment technologies, biological treatment process remains the very effective approach due to its energy efficient system [4].

The microalgae-based biological method of simultaneous wastewater treatment and CO_2 sequestration has gathered collective attention than other systems, since the MWW contains sufficient amounts of several nutrients, and the capability of the microalgae to use CO_2 as the carbon source in the process of photosynthesis which plays a pivotal role in algal cultivation [9].

It has been reported that approximately 1.83 kg of CO_2 can be fixed by microalgae through photosynthesis for the 1 kg of microalgae biomass production. This in turn around 54.9–67.7 tons of CO_2 sequestrated corresponding to annual dry weight biomass production rate of 30–37 tons per hectare [10]. Most of the developments have been made to the design and operation of photobioreactors recently to achieve high-efficient CO_2 capture and microalgae yield [11]. Thus, microalgae would be a feasible solution for the treatment of wastewater in an environmentally safe manner and economic, apart from their usage as food product, feedstock for biofuels and sequestration of CO_2 [12].

Microalgal cultivation generally requires organic and inorganic nutrients and carbon dioxide in the presence of sunlight through photosynthesis. According to the study made by Yang et al. [13], to produce 1 kg biodiesel from microalgae without water reuse, about 3.7-ton water, 330 g nitrogen and 710 g phosphorus are required. Since wastewater consists of both these nutritional supplements, microalgae can be cultivated in it and can help in bioremedy of wastewater and make cost-effective production of algal biofuel [14]. Oswald et al. [15] demonstrated the use of microalgae for nutrient removal for the first time and had been shown to efficiently utilize the nutrient source of wastewater [16,17]. In prior studies, the nutrient (nitrogen and phosphorous) removal from a selection of wastewaters including municipal, agricultural and industrial sewage by microalgae cultivation was examined successfully [18]. Thus, combining MWW with CO_2 for microalgal cultivation might provide combined benefits for biomass production as biodiesel feedstocks, wastewater treatment and CO_2 sequestration, which could be the key to resolving above problems [19].

Diverse types of microalgae such as *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Nostoc muscorum*, *Botryococcus braunii*, *Scenedesmus obliquus* and *Spirulina maxima* have been used for mixotrophic, autotrophic and heterotrophic cultivation in sewage [14], and said to have good carbohydrate content (>40% of the dry weight [dw]) [20]. The wastewater degradation carried out by *Scenedesmus* sp. could efficiently remove ammonium, nitrate, phosphate and chemical oxygen demand (COD) to the extent of 70%–98% [21]. In green algae, the starch in chloroplasts and cellulose/polysaccharides on cell wall are the main source of carbohydrate [22], which are not easily fermentable for the production of ethanol. Hence, the polysaccharides of microalgae should be hydrolyzed to fermentable sugars erstwhile to ethanol fermentation [23]. Generally, the chemical (acid and alkaline) and enzymatic hydrolysis are the two common hydrolysis techniques used for this purpose. The acid hydrolysis is the most commonly used technique since it is faster, easier and inexpensive than the enzymatic method which is slower and much expensive [24]. Performing the acid hydrolysis with ultrasound assistance can accelerate the process with much efficiency at shorter time. Ultrasonic-assisted simultaneous saccharification and fermentation of pretreated oil palm fronds for bioethanol production has been carried out effectively [25].

In this study, the SBR pretreated MWW was used to cultivate *Scenedesmus abundans* in a CO_2 aerated photobioreactor, treating the MWW. The final composition of the MWW and the carbohydrate content of the microalgae in the percentage of its dry weight was determined. The effects of various hydrolysis methods (mild acid or mild alkali with and without sonication) and conditions on ultrasound-assisted saccharification of the microalgal biomass were investigated. Subsequently, the sugar yield obtained by various hydrolysis process was compared.

2. Materials and methods

2.1. Microalgae strain and culture

S. abundans was isolated from a nearby water body in Chennai as per standard procedure [26]. Prior to the growth experiments in wastewater, the strain was precultured in BG-11 medium [27]. Composition of the medium (per litre) is: $NaNO_3$ 1.5 g, K_2HPO_4 0.04 g, $MgSO_4$ 0.075 g, $CaCl_2$ 0.036 g, $C_6H_8O_7$ 0.006 g and 1 mL trace metal mix (H_3BO_3 2.86 g, MnO_2 1.81 g and $Co(NO_3)_2$ 0.049 g/L). The strain was maintained in BG-11 agar plates (BG-11 with 15 g/L agar) between the experiments. The microalga was cultivated in a 1-L glass vessel containing 800 mL BG-11 medium, at a light intensity of 2,000 lux using fluorescent lamps with 0.2 vvm CO_2 (2.5%). The culture was incubated for 15 d at room temperature 27°C ± 3°C. Microalgal biomass from the precultures was centrifuged and the developed biomass was used as inoculum to minimize transfer of nutrients from preculture medium to the wastewater used in the microalgal growth experiments.

2.2. Municipal wastewater

The MWW used in this study was taken from the Chennai wastewater treatment plant (Perungudi). The original municipal effluent was first centrifuged to remove insoluble solids. The supernatant was then filtered through 0.45 μm cellulose membranes to remove the suspended solids, and was then diluted for fivefold with distilled water before being sterilized (autoclaved at 121°C for 20 min). As shown in Table 1, the original MWW had a high pH value of about 8.95. The initial pH of the pretreated diluted MWW was adjusted to around 7.5 with 1 N NaOH prior to its use for microalgae cultivation. There was no pH control during the cultivation of microalgae.

2.3. Cultivation of algae in wastewater medium

The microalga was cultivated in a 5-L transparent polypropylene photobioreactor illuminated by external white

Table 1
Characteristics of original and diluted municipal wastewater used in this study

Dilution	pH	COD (mg/L)	NH_3–N (mg/L)	NO_3–N (mg/L)	PO_4–P (mg/L)
Original	8.95	2,250	1,235	14.6	12.9
Diluted	7.5	510	313	4.2	3.8

fluorescent lamps mounted on both sides of the photobioreactor at a light intensity of 200 W/m and 0.2 vvm CO_2 (2.5%) was supplied continuously. Biomass was centrifuged at 6,000 rpm for 10 min, washed with distilled water and dried in an oven to a constant weight at 80°C. The biomass was stored at 4°C for further use. The supernatants after centrifugation was collected and analyzed for nitrogen of nitrite, nitrate and ammoniacal nitrogen and total phosphorous.

2.4. Saccharification of algal biomass

Algal biomass is taken for hydrolysis by mild acid or mild alkali with and without sonication. Due to the lack of lignin and the simple cellular structure of microalgae, only mild reactions are required for simultaneous pretreatment to release carbohydrate from the inner cell wall, and hydrolysis reaction to hydrolyze the complex carbohydrate molecules to simple fermentable sugars [28].

2.4.1. Acid hydrolysis

Acid hydrolysis of microalgal biomass was carried out using H_2SO_4 at concentration of 2%, 4%, 6%, 8%, 10% and 12% (v/v) at 90°C for 30 min. The reaction time of 30 min at 90°C is sufficient since that increasing the reaction temperature and reaction time beyond the optimum point could predominantly degrade the carbohydrate, resulting in the reduction of the bioethanol yield [29]. The assays were carried out in 250 mL Erlenmeyer flasks. The acid pretreatments were performed using 100 mL of microalgal biomass at a concentration of 30 g volatile suspended solids (VSS)/L.

2.4.2. Alkaline hydrolysis

For the alkaline hydrolysis, the biomass is suspended in 100 mL of NaOH, 1–6 M, to set a final concentration of 30 g VSS/L. Afterwards, samples were incubated at 90°C for 30 min with constant agitation with magnetic stirrer at 60 rpm using a 250 mL Erlenmeyer flask. The alkaline hydrolysis parameters in terms of NaOH concentration, temperature and incubation time were adapted from Ho et al. [28] and Ellis et al. [30].

2.4.3. Hydrolysis with ultrasound

The hydrolysis with sonication is carried out by the flask with its content (with acid or alkali content) sonicated at a frequency of 24 kHz and ultrasonic power of 200 W for 15 min. The temperature in the ultrasonic bath was maintained by the recirculation of cold water in the bath throughout the experiment. After the ultrasonic-assisted saccharification process, the residue from the liquid is separated by a filter. The supernatant was further filtered through 0.45 μM regenerated cellulose membranes, diluted and analyzed for glucose concentration.

2.5. Analysis

Every day a volume of 10 mL microalgae suspension was collected from the photobioreactor for growth and nutrient analysis. The growth rate of *S. abundans* was studied by measuring optical density (OD) at 660 nm (OD_{660}) for every 24 h by UV–Visible Spectrophotometer. The initial OD was 0.01 and the growth rate was monitored for 15 d. For nutrient analysis, the samples were centrifuged at 6,000 rpm for 10 min. The collected supernatant was then filtered through 0.45 μM cellulose membrane. The filtrates were analyzed for COD (Hach method), nitrogen of nitrate, nitrite (phenoldisulfonic acid method), ammonia (Nash-reagent spectrophotometric method) and phosphorous (molybdenum antimony anti-spectrophotometric method). For the microalgal biomass composition analyses, the biomass harvested by centrifugation was washed thrice with reverse osmosis treated water. Carbohydrate content of the extracted algal biomass was determined with the modified quantitative saccharification method [31]. The polysaccharides and proteins were detected using phenol-sulfuric method and micro bicinchoninic acid assay protein assay kit, respectively [32].

3. Results and discussion

3.1. Nutrition and COD removal

The MWW was treated with *S. abundans* for a period of 15 d and characterization of the wastewater was carried out every day. Nutrient utilization by *S. abundans* in the wastewater is found remarkable with respect to the microalgal growth. Fig. 1 shows the percentage removal of COD and all nutrients in wastewater treated by *S. abundans.* Ammonia was the main nitrogen source in the diluted MWW (Table 1). As can be seen from Fig. 1, there is a substantial reduction in the ammoniacal nitrogen and nitrate nitrogen in the diluted wastewater compared with the original wastewater which has also been reported by Han et al. [33]. Similarly, Kshirsagar [34] found that in the retention period of 15 d, the removal of nitrate with *Scenedesmus quadricauda* was 70.3%. In this study, initially during first week of treatment the removal of COD and phosphorus were in lesser rate. Beyond seventh day the removal rates were in increasing phase. The removal of ammoniacal nitrogen was 81.9% and 91.6% on

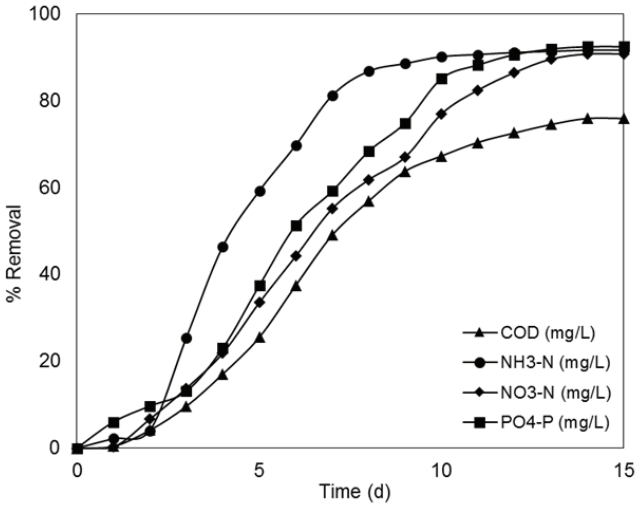

Fig. 1. Percentage removal of COD and various nutrients from wastewater inoculated with *Scenedesmus abundans.*

the seventh and fifteenth days, whereas for the nitrate it was 77% and 90.7% on seventh and fifteenth day, respectively. Phosphate removal is found to be significantly high being 92.9% from diluted wastewater samples with the retention time of 15 d.

Nitrogen is much required for microalga for the synthesis of nucleic acid, protein and phospholipid, and thus the growth of microalgae is believed to be vital for nitrogen removal via the processes of uptake, decay and sedimentation [35]. It should be noted that CO_2 was supplied as an extra carbon source since it was mixotrophic cultivation. As a result, the ratio of C:N:P was reformed due to the CO_2 supplementation. This could be a crucial factor influencing the performance of NH_3–N removal under mixotrophic growth.

The COD removal was 75.9% after 15 d of continuous treatment with algae. The efficiency of COD removal obtained from microalgal growth is negatively dependent on the initial COD concentration. This was previously shown in the study reported by Wang et al. [36], in which diluted wastewater was used to cultivate *Scenedesmus* sp. and was found that the COD removal percentage increased from 30% to 83.7%. It was also discovered that the additional supply of CO_2 for the mixotrophic cultivation thus did not affect the COD removal performance. As shown in Table 2, the performance of the microalgae-based wastewater treatment obtained from this work was compared with those from using other *Scenedesmus* sp. Compared with the associated studies, the microalgae biomass concentration achieved in the present study was relatively higher, while the COD and nutrient removal efficiencies were also higher or comparable with the reported values.

The removal rates of various nutrients and COD level after fifteenth day of continuous treatment with algae is shown in Fig. 2. These rates will be more helpful in understanding the removal of COD and other nutrients from the system by algae in daily basis.

3.2. Growth of algae

The growth characteristics of the *S. abundans* in MWW were investigated. The time-course growth profiles of *S. abundans* under mixotrophic conditions are shown in Fig. 3. The results clearly show that the maximum biomass production was highest during eighth, ninth and tenth day yielding 3.45 g/L. Like other microorganisms, microalgae can undergo four growth phases such as lag, exponential, stationary and lysis [42]. However, in this study, no significant lag phase was observed, since a large inoculum (the initial microalgal biomass was 0.4 g/L in all batches) size was used. The microalgae grown in diluted MWW saw rapid growth, with a maximum productivity of 0.4 g/L/d and also attained the highest cell output under the mixotrophic cultivation. The longer exponential growth phase resulted in a higher biomass concentration. This suggests that providing

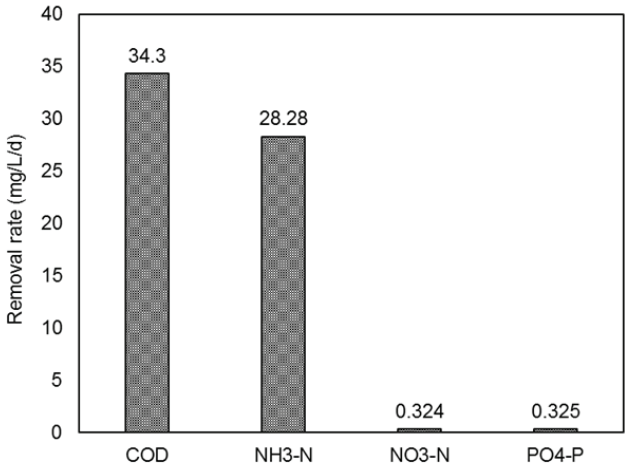

Fig. 2. COD and nutrient removal rates of various constituents of wastewater by *Scenedesmus abundans.*

Table 2
Comparison of the performance of cell growth, COD removal and NH_3–N removal in wastewater treatment using different *Scenedesmus* species

Wastewater	Microalgae strain	Cultivation type	Biomass (g/L)	COD removal (%)	NH_3–N removal (%)	References
Municipal wastewater	*Scenedesmus* sp.	Mixotrophic	0.32	83.7	89.8	[36]
Municipal wastewater	*S. obliquus*	Mixotrophic	1.40	97	98.5	[37]
Piggery wastewater	*S. obliquus*	Mixotrophic	0.83	62	91.2	[38]
Domestic wastewater	*Scenedesmus* sp.	Mixotrophic	1.37	95.9	98	[39]
Swine wastewater	*S. quadricauda*	Mixotrophic	0.33	17	87	[40]
Municipal wastewater	*S. quadricauda*	Mixotrophic	1.09	NA	94	[41]
Municipal wastewater	*S. abundans*	Mixotrophic	3.45	75.9	91.6	In this study

sufficient nutrients (fivefold dilution) is vital in achieving a higher maximum cell concentration for subsequent biomass of *S. abundans*. The productivity was reduced after 6 d of cultivation since there was a significant reduction of nutrients in the medium. Though the productivity was less, the microalgae would have undergone a strong metabolic mechanism during which most of the important biomolecules will be synthesized by digesting the absorbed nutrients.

3.3. Carbohydrate content and productivity

The amount of carbohydrates produced during the growth of microalga in MWW was estimated every day and the results obtained are shown in Fig. 4. From the results, it is evident that the amount of carbohydrates produced during the initial stage of growth was very less. A maximum production of about 56.9% (by dw) was achieved after 14 d of growth. The composition of various monosaccharides was also studied in detail which is also shown in Fig. 4. Based on the results obtained, glucose was predominantly produced during the process. During all the observations, the concentration of glucose was about 90% of the total monosaccharides produced. Since organic nutrients are abundant in the MWW medium and the process being a mixotrophic one, the availability of carbon was ample enough for the microalgae to synthesize carbohydrates in higher composition. If carbon is scarce in the medium, synthesize of penta-carbon monosaccharides would be triggered, but in this system, nitrate was simultaneously removed from the system and carbon was stored in the form of carbohydrate.

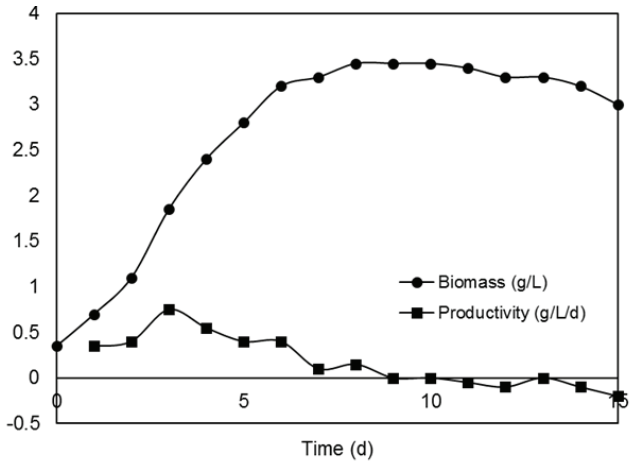

Fig. 3. Biomass and productivity of *Scenedesmus abundans* inoculated in wastewater.

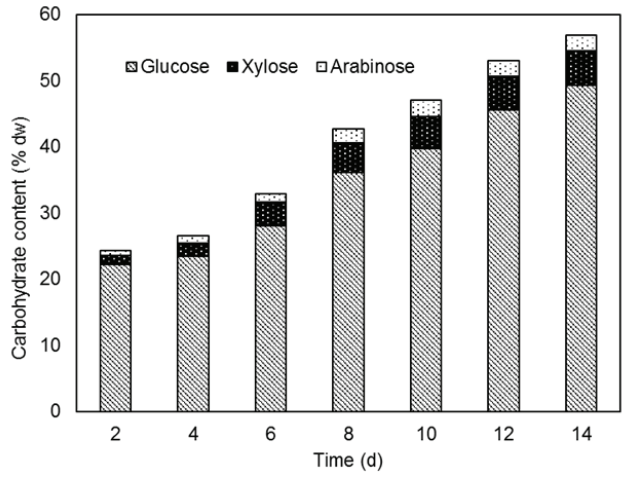

Fig. 4. Availability of carbohydrate content in *Scenedesmus abundans* during growth period.

3.4. Hydrolysis of cellulose

3.4.1. Acid saccharification

Saccharification of cellulose available in *S. abundans* was done by acid hydrolysis method combined with ultrasonication. To emphasize the effect of ultrasound, experiments were also carried out without ultrasonication. The results obtained are shown in Fig. 5. The experiments were carried out with varying acid concentrations of 2%–12%. The sugar yield was increased from 32 to 88 mg/g dw when acid concentration was increased from 2% to 10%, whereas for the acid concentration of 12% the yield slightly decreased to 84 mg/g dw. This can be attributed to the production of furfural in the acid hydrolysis process. During the treatment of biomass with higher acid concentrations (>10%), the glucose formed would be converted to furfural hence reducing the yield of glucose. If the acid concentration is increased further, the formation of furfural will also be increased. Under ultrasonic conditions, the yield of glucose increased significantly. For the acid concentration of 8%, the yield was about 139 mg/g dw which was 52% higher than the maximum yield during the hydrolysis without ultrasonication. Also, the maximum yield obtained in the previous process was for the acid concentration of about 10% whereas for the latter it was for 8%. Furthermore, the increased concentrations (10% and 12%) reduced the yield of glucose which is attributed to the formation of furfural. Since, ultrasonication is an intensification technique; the formation of furfural was triggered for smaller acid concentrations.

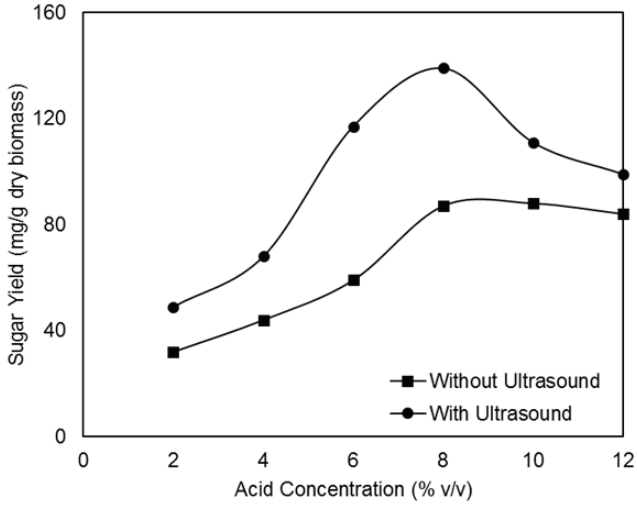

Fig. 5. Amount of glucose produced by acid saccharification of *Scenedesmus abundans*.

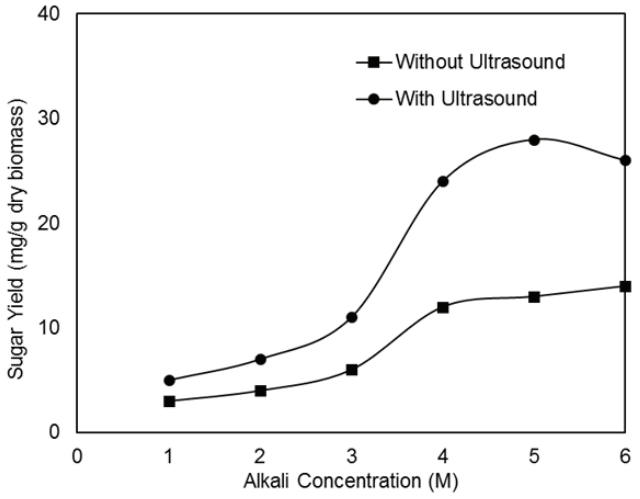

Fig. 6. Amount of glucose produced by alkali saccharification of *Scenedesmus abundans*.

3.4.2. Alkali saccharification

Saccharification was also carried out by alkali hydrolysis with and without application of ultrasound. Fig. 6 shows the results obtained during alkali saccharification. Of all the concentrations studied from 1 to 6 M NaOH, the yield increased from 3 to 14 mg/g dw, which were pretty lower compared with the yield values obtained in acid hydrolysis. With the application of ultrasound the yield values were 5–26 mg/g dw, which were slightly higher than the values obtained by alkali hydrolysis without ultrasonication and much lower than the acid hydrolysis. This shows that the acid hydrolysis is highly efficient for saccharification of algal biomass.

4. Conclusions

S. abundans can efficiently utilize the nutrients and COD in diluted MWW for cell growth and carbohydrate accumulation, with high COD (up to 75.9%), nitrogen (up to 91.6%) and phosphate (up to 90.7%) removal efficiencies. Using diluted wastewater (fivefold dilution) resulted in a good biomass concentration of up to 3.45 g/L. Besides, the carbohydrate content that was accumulated in the microalgal biomass grown in MWW was 56.9% of which glucose accounted for 76%. Furthermore, saccharification of algae was carried out by acid and alkali hydrolysis with the application of ultrasonication. The yield of glucose was higher (139 mg/g dw) during ultrasound-assisted acid hydrolysis with an acid concentration of 8%. These findings suggest that microalgae cultivation in MWW meets multiple objectives of sustainable development that would help in the bioremediation of wastewater, utilizing the sufficient nutrients which are an essential source of carbohydrate for bioethanol production.

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