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A preliminary study of cost and energy analysis of bio-fuel production from microalgae cultivated in parboiled rice mill wastewater

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Abstract

Microalgae industry is rapidly growing industry with high potential for value added products. Especially microalgae derived biofuels provide a sustainable solution for continuously rising energy crisis, food security and greenhouse gas emission. However, at present economical limitations make this green fuel not commercially viable. Integrating wastewater treatment with the microalgae cultivation can significantly reduce the biofuel production cost. Parboiled rice mill is rich in nutrients and capable of providing inexpensive water resources. Nevertheless, energy and cost analysis are required in order to ensure the cost effectiveness of such approaches. Thus, the study evaluates the cultivation of microalgae in rice mill wastewater in the outdoor uncontrolled conditions for the cost and energy effective, biofuel production. Microalgae biomass was cultivated in low-cost poly-bags of 3L volume using parboiled rice mill wastewater in outdoor. Cultivation was done under ambient temperature with 0.2vvm aeration without any optimization of the natural environmental

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conditions. This can be rationalized to reflect a realistic scenario in terms cost and energy estimation of production of lipid and bio-ethanol at laboratory scale. The obtained bioethanol and lipid yield were 1.4% and 5.81% respectively. Cultivation of the microalgae consumed 2.16 MJ of energy per gram. The required energy content for production of bioethanol and lipid from a gram of biomass were 3.61764 MJ and 3.5336 MJ respectively. Cost of microalgae cultivation to produce gram of bioethanol and lipid were $ 22.91 and $ 5.52 respectively. Total production cost per gram of bioethanol and lipid were $ 64.73 and $ 55.91 respectively. These values are comparatively exorbitant. Since the production was at very small scale and the yield was very low due to the microalgae cultivation in outdoor under natural condition in laboratory scale. The production of biofuel could become economically feasible if the yield could optimally increase and simultaneous production of bioethanol and lipid along with other value added products such as biofertilizer, aquaculture feed etc. could be achieved. Therefore, the method resulted in the lower cultivation cost can be incorporated with the residual biomass utilization and could produce sustainable microalgae biofuel.

Keywords: Microalgae; Rice mill wastewater; Lipid; Bioethanol; Energy; Cost analysis

Introduction

Microalgae production is a rising industry with very high potential. Even during COVID -19 pandemic in 2020 the global market was estimated as 3.4 billion USD and projected to reach 4.6 billion USD by the year of 2027(Show, 2022). Microalgae contain range of valuable bioactive compounds including: lipids; proteins; carbohydrates; carotenoids and vitamins (Tan *et al.*, 2020). Thus microalgae biomass has great value as bio-plastics, bio-fertilizer, pharmaceutical and cosmetic active ingredients, food source, aquaculture and animal feed, CO_2 capture, wastewater treatment system and biofuels (Daneshvar *et al.*, 2019; Sakarika *et al.*, 2020). Especially microalgae derived biofuels provide a sustainable solution for continuously rising energy crisis, Greenhouse Gas (GHG) emissions, and food security issues due to using food crops for biofuel production. Hence the biofuel from microalgae is a renewable and sustainable alternative energy source to secure the future energy demand (Pokoo-Aikins *et al.*, 2010; Zewdie and Ali, 2022).

Microalgae typically have high growth and productivity, ability to grow on wastewater, saline or seawater and potential for high lipid or carbohydrate contents which make it an excellent feed stock for range of biofuels (Branco-Vieira *et al*., 2020). Algal lipids are composed of glycerol, sugars or bases which are trans esterified to saturated or unsaturated fatty acids to produce biodiesel (Halim *et al*., 2012). Carbohydrates is another major component which can be readily used as feedstock to produce bioethanol. Since they are characterized by high concentration of easily fermentable cellulose and starch, along with low concentration of hemicelluloses and absence of lignin (Sakarika *et al*., 2020; Tan *et al*., 2020). The whole biomass can be subject to anaerobic digestion to produce biogas. Further it can be done using residual biomass after the lipid extraction as well. Finally, the production of bio hydrogen is a clean energy through direct biophotolysis or dark fermentation (Sakarika *et al.*, 2020). If more than one biofuel or another value-added product with biofuel could be produced with wastewater treatment, then the whole approach will be highly sustainable, economically feasible and environmentally friendly (Rafa *et al*., 2021).

However, at present commercial facilities producing microalgae are limited to high-value applications such as food, cosmetics, and pharmaceuticals active components etc. Because, the current high microalgae production cost cannot be afforded by other low value market products such as biofuel (Acién *et al*., 2016). The cost for microalgal biomass production is currently much higher than the cost for production of other energy crops (Bušić *et al*, 2018; Zewdie and Ali, 2022). Therefore, in the recent decade numerous studies have explored various technologies and implementation techniques to make the microalgae biofuel production cost effective enough to be competitive to traditional fuels in the global market (Chen *et al*., 2018). Among them, integration of microalgal cultivation for biofuel production with wastewater (WW) treatment for both low cost nutrient and environmental pollution reduction was one of the promising process that resulted in significant reduction in the production cost (Zewdie and Ali, 2022). The ALL-GAS project, led by FCC Aqualia is an excellent example of this. The project demonstrates the sustainable large-scale production of biofuels based on low-cost microalgae cultures using municipal wastewater (https://www.all-gas.eu/).Wastewater is rich in growth nutrients like nitrogen, carbon and phosphorous and trace elements that are essential for micro algae growth, which consequently result in the phytoremediation of the wastewater. In addition, the ability of microalgae to remove, organic and inorganic pollutants including heavy metals and emerging contaminants is well documented in the literature (Kanaujiya *et al*., 2019; Li *et al*., 2022; Sakarika *et al*., 2020).

Parboiled rice mills are one of the important and oldest industry in the developing countries like Sri Lanka where rice is the staple food crop. Worldwide rice milling production is approaching about 500 million metric tons in 2021 (Mukherjee *et al*., 2016). They play key role in both the national and rural economy and of the food security. However, they produce huge amount of wastewater which is rich in nutrients and typically have very high chemical oxygen demand is reported to be discarded without any remediation treatment into environment. This results in significant eutrophication, surface and ground water pollution, and wastage of large quantities of utilizable water (Umamaheswari *et al*., 2021). Integrating this wastewater treatment with microalgae production system could provide a win-win situation. In our previous study microalgae, recovered 88% of total nitrogen and 75% of phosphate. Further the chemical oxygen demand was reduced by 87%, emphasizing the enhancement of the parboiled rice mill wastewater (Ketheesan *et al*., 2021).

However, experimental validations are still required for converting wastewater cultivated microalgae biomass into value added products such as biofuel. Energy and cost analysis should have been carried out in order to determine the feasibility of the system (Judd *et al*., 2017). This is one of the most significant issues for any industrial application of research output as economic feasibility is the major concern of commercial execution of any product. There are several life cycle assessment studies, Techno economic analysis and energy balance studies have been performed to investigate the commercial scale, real world application of biodiesel production from microalgal biomass (Branco-Vieira *et al*., 2020; Dutta *et al*., 2016; Hossain *et al.*, 2019). These studies have certain limitations, that is, typically they consider very optimal conditions for microalgae growth and higher lipid, carbohydrate and biomass productivity for microalgae. Moreover, the studies include theoretical assumptions, and often lack original data (Hossain *et al.*, 2019; Show, 2022).

Thus the present study is a novel approach to produce microalgae biomass by using inexpensive nutrient and water sources obtained from rice mill factory in outdoor natural conditions without any control measurement. Through this, the study investigates more realistic answers in terms of cost and energy estimation for the production of lipid and bio ethanol at laboratory scale. Further, the study analyses whether the cost reduction by the utilization of wastewater and lack of control measurement could provide enhanced cost and energy efficiency.

Materials and method

Microalgae cultivation

Chlorella sp. which was isolated from polluted lakes in Jaffna district and grown in the Environmental laboratory of the Civil Engineering department, Faculty of Engineering, University of Jaffna, Killinochchi was selected and obtained for the research purpose. Stock culture was grown inside the laboratory in bold basal medium under ambient temperature. Culture was maintained under light intensity of 500 PAR (16h: 8h) and mixing of 18 RPM.

Collection of rice mill wastewater

Parboiled rice mill wastewater was collected from the rice mill situated in Killinochchi town area. As a pretreatment, sedimentation of rice mill wastewater was done. The characterization of the wastewater, recovery of nutrients and treatment efficiency of the rice mill wastewater by microalgae was done. The rice mill wastewater was characterized by very high COD (>2500mg/L), Phosphate (471 mg/L), Sulphate (650 mg/L) and Nitrate (340 mg/L) and at average microalgae recovering more than 70% of nutrients. The full results were published in the previous work (Ketheesan *et al*., 2021).

Cultivation and harvesting of microalgae in rice mill wastewater

Microalgae were cultivated outdoors in about six 3-L high gauge polythene bags (Polybags). Microalgae were inoculated with an initial biomass concentration of approximately 20-30 mg/L and grown for 7 days. No additional lighting and CO_2 supply was provided. Aeration at 0.2 vvm was provided to ensure the mixing of the culture. At the end of cultivation period biomass was allowed to settle and effluent was removed. Biomass was harvested by centrifugation, dried at 70 ℃ and ground to fine particles.

Valorization of microalgae biomass

Bioethanol

Five gram of microalgae biomass was dissolved in 100 mL distilled water and sonicated. The obtained supernatant was fermented to ethanol by *Saccharomyces cerevisiae.* It was cultured in LB medium. The dichromate oxidization method was used to determine the amount of ethanol extracted. The extraction was done in triplicates.

Lipids

One gram of biomass was added to hexane/methanol 7:3 (v/v) and sonicated. Extraction of lipids was done by Soxhlet extraction method (Buchi B-811 system). Extraction was carried out for 2 hours followed by 10 minutes of rinsing and 5 minutes of drying in the rotatory evaporator. The extraction was done in triplicates.

Cost analysis

Economic evaluation typically consists of capital cost, variable operation costs and operational cost (Zewdie and Ali, 2022). The study didn't include capital cost and operational cost as this is a laboratory scale experiment and established systems were used. Therefore, only the variable operation cost that includes power and raw materials was considered. Cultivation of microalgae biomass, Harvesting, Bioethanol production, Extraction of lipids were identified as a major stage for the cost analysis.

Results and discussion

Bioethanol and lipid yield

Microalgae ethanol and lipid yield were 1.4 ± 0.04% and 5.81 ± 0.69% respectively. These values are in the very lower range compared to similar studies. Typically, 12%-32% of lipid content and 4-20% ethanol content were recorded in the literature (Bušić r *et al*., 2018; Hossain *et al*., 2019; Li *et al*., 2022). Uncharacteristically low value recorded in the study may be due to the fact that the cultivation was done in outdoor under natural conditions in very small scale. Higher yield was often obtained in indoor cultivation and large scale outdoor cultivation where the impact of environment conditions can be diluted or diffused (Branco-Vieira *et al*., 2020).

Further, microalgae have been known to survive under a wide range of conditions. Compared to unfavorable conditions, favorable conditions result in lower storages of lipid and carbohydrate. Thus, various studies emphasized that nutrient starvation, especially phosphorus limitation, significantly enhance the carbohydrate and lipid content of the biomass (Hanifzadeh *et al.,* 2018, Rehman and Anal 2019, Shrestha *et al.*, 2020). Hence, it is ideal to cultivate microalgae under optimal conditions and later expose them to unfavorable conditions such as nutrient starvation in order to increase lipid and carbohydrate content (Pokoo-Aikins *et al*., 2010).

Energy and cost analysis

Energy required for the production of bioethanol and lipid were 3.62 MJ and 3.53 MJ respectively. The value was obtained for the production and processing of gram of microalgae in a single cycle. Energy requirement for the mixing was the highest (Table 1) in this study. In order to prevent the sedimentation, mixing was employed here. To small scale production such as this manual mixing could reduce this cost. However, in large scale production such as raceways and photo bioreactors as well as mixing utilize significant energy that impact the production cost.

Table 1: Energy consumption at various stages of the process.

Production stages	Process	Equipment	Energy used for required capacity	
			kWh	**MJ**
Microalgae Cultivation	Mixing	Air pump	0.600	2.16
	Harvesting	Refrigerated centrifuge	0.12375	0.4455

Table 1: Energy consumption at various stages of the process. (Continued)

Raw material preparation (microalgae)	Drying	Incubator (BI-BSP-100)	0.02400	0.08640
Bioethanol production	Cell destruction	SONIC water bath	0.00047	0.00169
	LB medium preparation	SN 30 sterilizer	0.00330	0.01188
	Storing	Bio Base refrigerator	0.00005	0.00018
	Yeast culture	Incubator (BI-BSP-100)	0.00013	0.00047
	Yeast culture mixing	Shaking Incubator (BSD-250)	0.03720	0.13392
Lipid extraction	Cell destruction	SONIC water bath	0.00022	0.0008
	Extraction	Buchi B-811 Soxhlet	0.01758	0.0633

The second highest energy intensive process was harvesting. This is especially true for large scale production as centrifugation is very energy intensive (Bušić *et al.*, 2018). This account for about 30% of their expenditure ($2.92–3.06/L biodiesel) (Chen *et al*., 2018; Rafa *et al*., 2021). However, the biomass cultivated in the rice mill wastewater showed high settle ability. Thus, tropical countries like Sri Lanka can employ solar drying for the cost-effective harvesting of the biomass (Pokoo-Aikins *et al*., 2010).

Chemical cost is the highest for processing 1g microalgae for both bioethanol and lipid production (Table 2).

Table 2: Cost of cultivation and processing of a gram of microalgae

Production stages	Supplies	Total cost Rs.	$
Cultivation	Polyethylene bags	5.00	0.014
	aeration tubes and pump (NS L-15)	110	0.3056
	Energy cost	8.83	0.025
Harvesting	Energy cost	2.17	0.006
Bioethanol production	chemicals	210.275	0.584
	Energy cost	0.61	0.0017
Extraction of Lipids	Chemicals	205.92	0.572
	Energy cost	0.26	0.00073

In terms of total production, cost of lipid was lower than the bioethanol since the lipid yield was higher (Table 3).

Table 3: Production cost of bioethanol and lipid

	For 1g of bioethanol	For 1g of Lipid
Required Microalgae biomass	71.43 g	17.21 g
Microalgae cultivation cost	$22.91	$5.52

Table 3: Production cost of bioethanol and lipid (Continued)

production cost	$ 41.82	$ 50.39
Total cost (Cultivation cost + Production cost)	$ 64.73	$ 55.91

The cultivation cost is lower in the studies. This may be attributed to the integration of the process to wastewater treatment. To cultivate 1g of microalgae biomass $ 0.34 is required in the present study. If we extrapolate this to produce 1kg of the biomass, it will be about $ 6.42 since the energy and raw material cost remains fixed up to certain amount of microalgae biomass. This value is comparable to other studies which reported production cost of microalgae biomass grown in wastewater between $ 2 to $ 15 (Branco-Vieira *et al*., 2020; Rafa *et al*., 2021).

Energy and cost values for the production and processing of 1g microalgae is comparable to the other studies. However, the production cost of bioethanol and lipid is very high in the present study. Similar studies reported production cost of $ 2.8 to 6.7 per liter of biofuel (Judd *et al*., 2017). In the study by Branco-Vieira *et al*.(2020) production cost of biodiesel was estimated as $ 0.33/L and of biomass $ 0.0022 per g, in a 15.247 ha facility size. The high production cost in the present study is due to very low yield obtained in the study. Low yield results in the requirement of large amount of microalgae biomass and consequently the production cost is increased several fold for the processing of this large amount of biomass (Table 3).

Average price of liter of ethanol is $ 1.16 that means one gram of ethanol is $ 0.0015 in 2022. Current price of algae biomass is $ 19000 per ton means $ 0.021 per gram (Show, 2022). Biodiesel price is $ 5.34 per gallon (United States, Department of Energy). Biofuel from microalgae to be economically feasible, even the lowest price reported in the literature necessitates to substantially reduce their cost and to operate them near their optimum values.

Therefore, in addition to integrating wastewater treatment, simultaneous production of different biofuels along with other value added products such as: biofertilizer; aquaculture feeds; proteins; enzymes etc. It I s recommended by several authors (Bielsa *et al*., 2016; Dutta *et al*., 2016; Rafa *et al*., 2021). The study by Gupta *et al*., (2016) reported that when the protein is also extracted, cost of biodiesel production could be reduced from $17.26/L to $13.73 US/L. In another study by Prieto *et al*. (2017), production cost of biodiesel from microalgae was found to reduce from $ 3.90/L to a staggering $ 0.54/L when astaxanthin and polyhydroxy butyrate are coproduced. Further, Chen *et al*. (2018) reported that despite the importance of the lipid content of the microalgae, often it does not significantly affect the parameter of cost estimation if the microalgae residues are assigned an economic value (Rafa *et al*., 2021).

Regardless of these, there are numerous hurdles that need to be handled related to the cost of current technologies for the widespread commercialization of microalgae-based biofuel. The present study shows that even at laboratory scale with integrating wastewater, the production cost is extremely high. To bring microalgae biofuel production to be competitive in the global market alternate technologies and novel approaches are required. However, still microalgae are having very high potential in the biofuel field. Dwindling fossil fuel reserve and controversy over utilization of food crops for biofuel when the food security is at stake with ever increasing population makes the microalgae an inevitable alternate source.

Conclusion

Bioethanol and lipid yield obtained from microalgae biomass was 1.4% and 5.81 % respectively. This uncharacteristic in this study may due to the fact that the cultivation was done in outdoor under natural conditions in very small scale. Comparatively lower cultivation cost was achieved in the study ($ 6.42 per Kg) as no lighting systems and additional CO_2 supply were provided and cultivation was carried out in wastewater medium using low cost poly bags. However, due to the low yield, production cost of bioethanol and lipid increased several folds compared to other studies. The present study shows that even at laboratory scale with integrating wastewater, the production cost is extremely high. However, since the cultivation cost is lower if the residual biomass could be utilized to produce value added products such as proteins, biofertizer, aquaculture feed could make the process more cost effective. Further simultaneous production of various biofuel such as bioethanol, biodiesel, biogas and bio hydrogen can also make the microalgae derived biofuel production more sustainable, energy and cost effective and feasible in the commercial scale applications. Therefore, the method resulted in the lower cultivation cost can be incorporated with the simultaneous production of various value added products and could produce sustainable microalgae biofuel.

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