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A Review on the Use of Sugar Mills Wastewater Used as a Growth Medium for Microalgae Feed Stock And Bio Diesel Production in Pakistan

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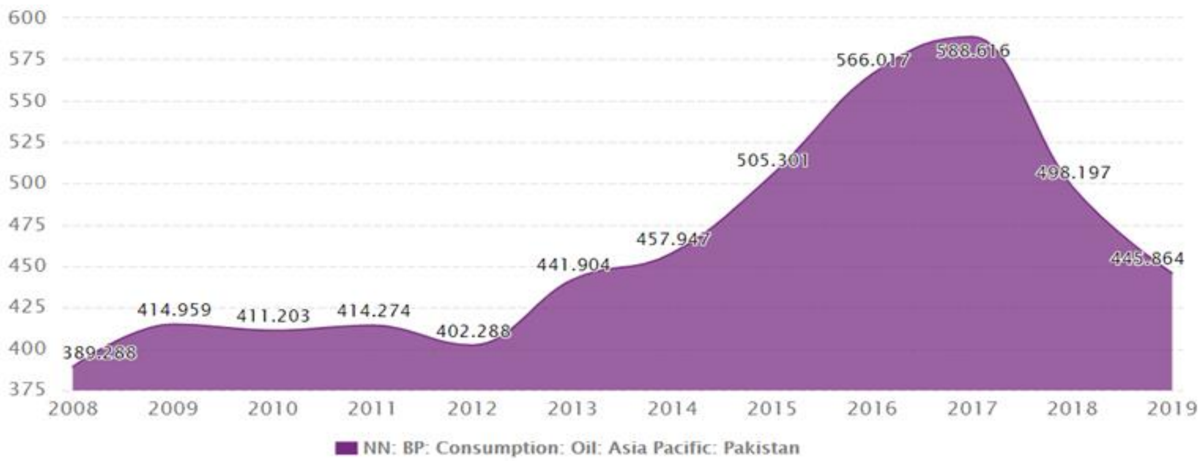
ABSTRACT

This review article assesses existing investigation on sugar industry wastewater for cultivating microalgae and its potential for biodiesel production. Pakistan is an agro based industrial economy in developing world. Energy crisis and second largest agro based processing industrial wastewater with high COD and BOD and other nutrients discharge directly in to natural water bodies without treatment due to complexity of process, high in energy consumption. Pakistan is currently serving demographic, economic and energy crisis, due to high growing demands, the situation is worsened to supply non-renewable fuel sources, which are the major cause of global warming and climate change. In this senerio, the study aims to provide sustainable low cost and energy-efficient solutions for sugar mills wastewater treatment and to find renewable viable options which are driving force for country in this developing world. To accomplish these objective, Bio based wastewater treatment system with biofuels production is promising' alternative and renewable energy source based idea are launched in this review paper. Various feedstock's has been reviewed for production of biofuels, but much more production efficiency has resulted in micro-algae, the most compelling source of biofuel, and its potentials for brewing wastewater. Pakistan is a tropical archipelago, which can grow microalgae as a reproductive source of biomass, will use wastewater from the sugar industry as a source to produce biofuels. Finally, the BOD, COD and other Toxins are removed and biomass product from microalgae is used as bio energy feedstock in this whole process

INTRODUCTION

Pakistan is known as an agriculture country. The second largest agro based sector in Pakistan is sugar industry. Currently, Pakistan is one of the leading sugar producer countries around the agricultural world. Sugar industry is a largest source of wastewater, generated during processing and manufacturing of sugar, that wastewater directly discharge into natural water bodies now it become threat for natural environment (Panhwar *et al.*, 2019). On other hand Pakistan known as developing country, Energy is one of the vital factors responsible for the development of any nation (Zaidi *et al.*, 2018). That is suffering from energy crisis due to its growing demands (Tareen *et al.*, 2020). The energy demands have increasing constantly throughout the years (Figure 1). Simultaneously, exploration of nonrenewable fossil fuel sources are decreasing, with growing population, the situation is worsened by insufficient fuel supplies to energy consumer(Azimatun Nur and Hadiyanto, 2013).Crisis in non-renewable fuel sources have made them an un-reliable energy source. Excessive pollution from greenhouse gases and the effects on global change have become important natural, economic and social issues. In this aspect, the advancement of renewable energy sources is inevitable; biofuels are known to be a viable option and a driving force for the developing world. Various feedstock's have been studied for production of biofuels, but much more production efficiency has resulted in micro-algae, the most compelling source of biofuel, and more inefficient quality of sustainable food and its potentials for brewing with brackish or wastewater(Gill *et al.*, 2013). Pakistan is a tropical archipelago, which can grow microalgae as a reproductive source of biomass. However, only few microalgae will use wastewater from the sugar industry as a source to produce biofuels(Gill *et al.*, 2013). Finally, the BOD, COD and other Toxins are removed and biomass product from microalgae is used as bio energy feedstock in this whole process(Manzoor et al, 2016).



Methodology of Literature Review

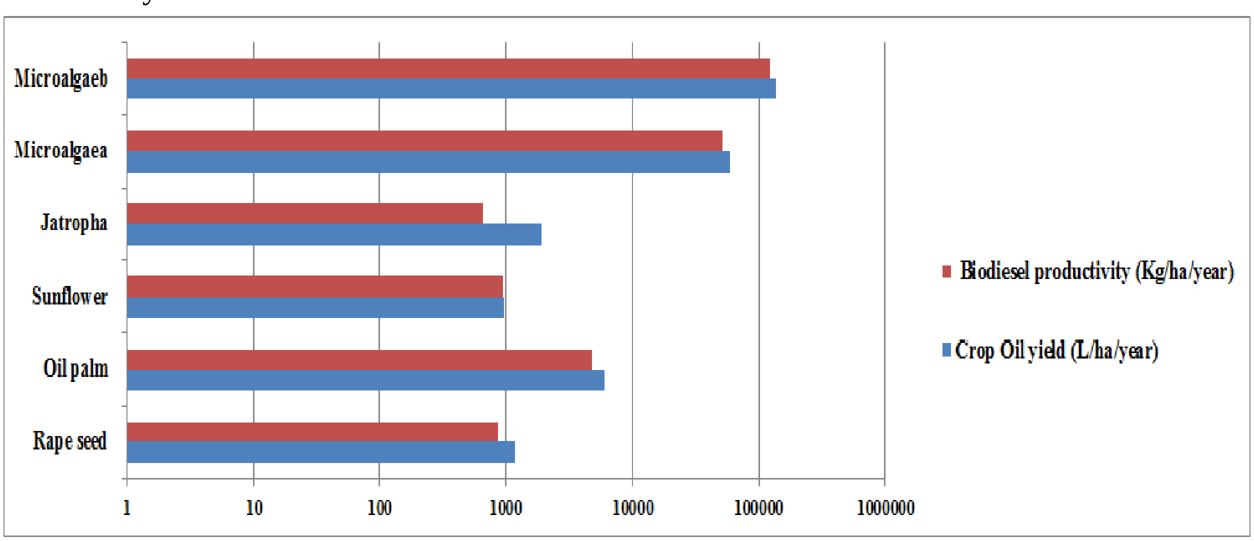
The review article is based totally on the research analysis of published research articles, reports, and case studies from scientific database such as Science Direct, Google Scholar, and Scopus. The focus of review on cultivation of microalgae in sugar mills effluents in the context of Pakistan.

Microalgae Potential Feed Stock for Biodiesel

Microalgae are bio-based feedstock that has countless prospective for the replacement of fossil fuels. The increased fossil fuel consumption is alarming, and use a microalgae biofuel as an alternative with high production potential and less competition is slowly becoming more common with food processing. Biodiesel output from feedstock, which will reduce greenhouse emissions and will not clash with other sustainable crop production(Karmakar, Karmakar and Mukherjee, 2010).Microalgae species is used as feedstock for biofuel that can be grown with minimal input, their nutritional requirements are simple because photoautotrophic organisms.

In the last decades, Algae-derived biofuels also attained popularity. Microalgae can be used for different types of renewable fuels and sustainable, such as methane(Chisti, 2007a)., biodiesel (Ahmad *et al.*, 2011) (Gavrilescu and Chisti, 2005) and bio hydrogen(Kapdan and Kargi, 2006). Different microalgae species can be modified in various environment conditions for cultivation. Species living in the local environment(s) can be quickly cultivated under the growing conditions and should therefore be screened. Such provision is not therefore necessary for biodiesel feedstock of first generation, such as palm oils, soybean oil and sunflower because of their obvious food competition reasons.(Mata, Martins and Caetano, 2010).Third generation biodiesel feed is micro-organisms and can be used for production in a wide range of fields. Microalgae is one of the best highest ratio third generation feedstock source for Biodiesel production then obtained from traditional feedstocks (Karmakar, Karmakar and Mukherjee, 2010). Microalgae are considered to be potentially very good sources of biodiesel production through high oil production, lowest-cost source and timely biomass production(Karmakar, Karmakar and Mukherjee, 2010). Biodiesel production from microalgae, as comparison crops are much greater as shown in table 1 (Lim and Teong, 2010). Algal biomass can be grown in marginal soils, compact bioreactors and saline water bodies as comparison to oil producing crops (Lim and Teong, 2010)

Table 1 Oil yield of some biodiesel feedstocks



Crop	Crop Oil yield (L/ha/year)	Biodiesel productivity (Kg/ha/year)
Rape seed	**1190**	**862**
Oil palm	**5950**	**4747**
Sunflower	**952**	**946**
Jatropha	**1892**	**656**
Microalgaea	**58,700**	**51,927**
Microalgaeb	**136,900**	**121,104**

a Algae contain 30 % oil (/wt) in biomass
b Algae contain 70 % oil (/wt) in biomass (Manzoor *et al.*, 2015).

Table 2 some of the important advantages of microalgae as feedstock for biodiesel production

S. NO:	Advantages	References
1	1 Ability to sequester atmospheric CO2	(Manzoor *et al.*, 2015)
2	2 Highly efficient in biomass production	(Huesemann *et al.*, 2009)
3	3 Capable of growing in saline/wastewaters (industrial effluents and municipal sewage)	(Raja *et al.*, 2008)
4	4 Can be cultivated on non-arable land	(Demirbas, 2009)(Spolaore *et al.*, 2006)
5	5 Several microalgal species produce valuable co-products including proteins, natural dyes, antioxidants, pigments, fats, and sugars	(Raja *et al.*, 2008)(Li *et al.*, 2008)
6	6 They require less labor, are easy to handle and have a short life cycle as compared to other crop oils	(Schenk *et al.*, 2008)

Biomass Conversion

The biomass produced from microalgae can also be used for the production of biodiesel, bioethanol, bio hydrogen, methanol and even electricity(Wang *et al.*, 2008). However, the most technically reasonable and commercialized sustainable forms of green energy in the domestic sector are biodiesel and bioethanol(Francisco *et al.*, 2010). Many studies also observed that direct (insitu) trans esterification was carried out using supercritical and micro wave approach(Patil *et al.*, 2011a)(Babajide *et al.*, 2010), and ultrasound methods(Ehimen, Sun and Carrington, 2012). Micro algae may be converted to biodiesel(Cardozo *et al.*, 2007), The trans esterification process by using solvents and a catalyst to produce biodiesel is one of the most promising technologies due to its simplicity in handling, and consume low amount of energy in conversion of microalgae biodiesel(Patil *et al.*, 2011b)(Ehimen, Sun and Carrington, 2010)(Xu *et al.*, 2011). In fact, researchers want the possibility of converting microalgae into biodiesel that is grown in wastewater. Penglin (Xu *et al.*, 2011), has reported that direct trans esterification is feasible for microalgae cultivated in rice straw wastewater. (L. F. Wu *et al.*, 2012)worked on the production of bio diesel from micro algae medium as a industrial wastewater, concludes that 18, 4 % of the microalgae absorb lipid through ammonium extraction and that their structure is ideal for the production of biodiesel. Most of these factors also effect on lipids in microalgae. Widjaja et al. Concludes lipid increases factors such as nitrogen depletion, concentration of CO2, time of harvesting, and finally extraction process in chlorellavulgar(Singh and Gu, 2010). In addition, the

specific strain inoculum also plays an important role in producing a high volume of biofuel based on microalgae. In fact, if the weight of the 30% oil and biomass costs is approximately $1,40 and $1,81 for the provision of the bioreactors and the runs of photography for one liter of oil, respectively(Chisti, 2007a).

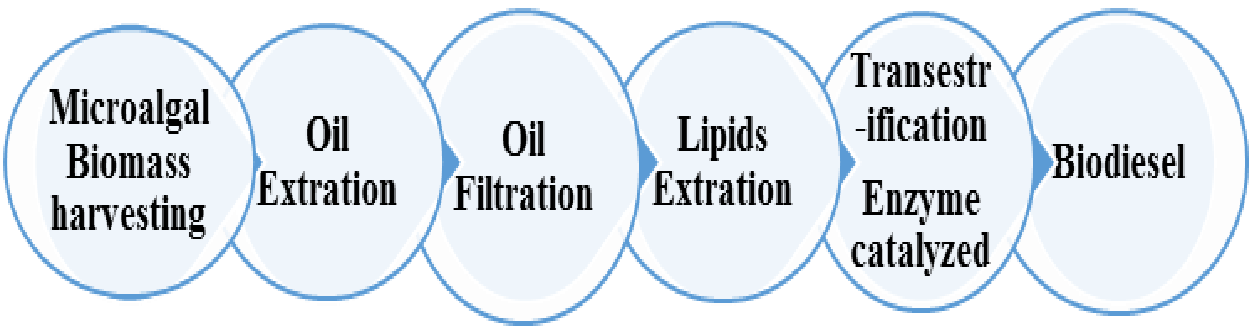
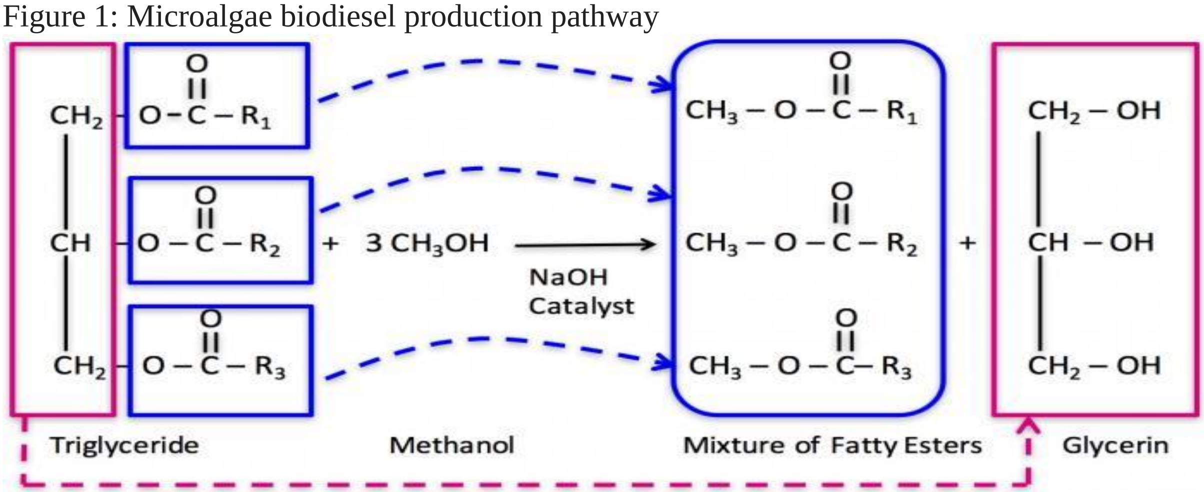


Figure 1: Microalgae biodiesel production pathway



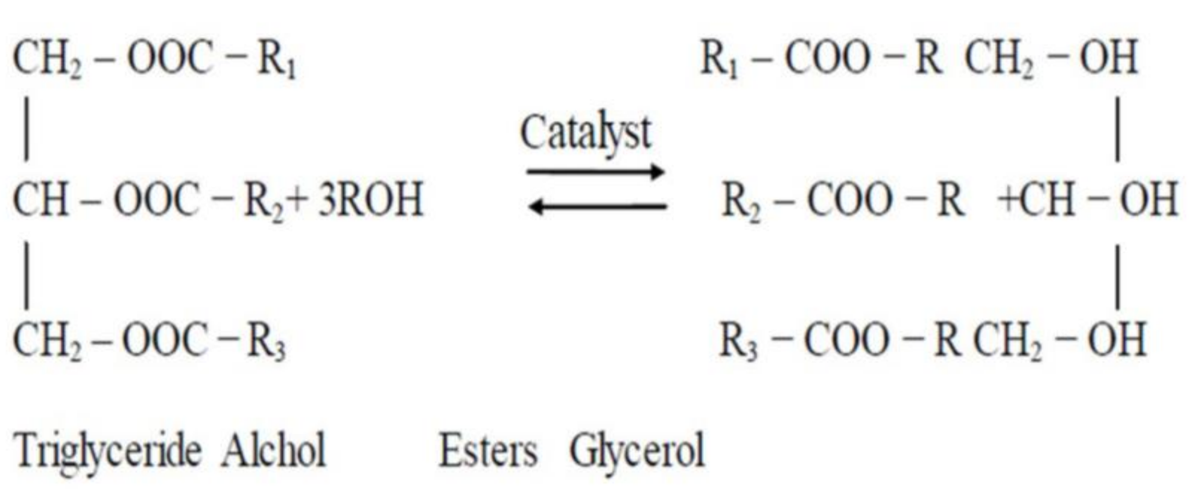


Figure 2: Chemistry of esterification.

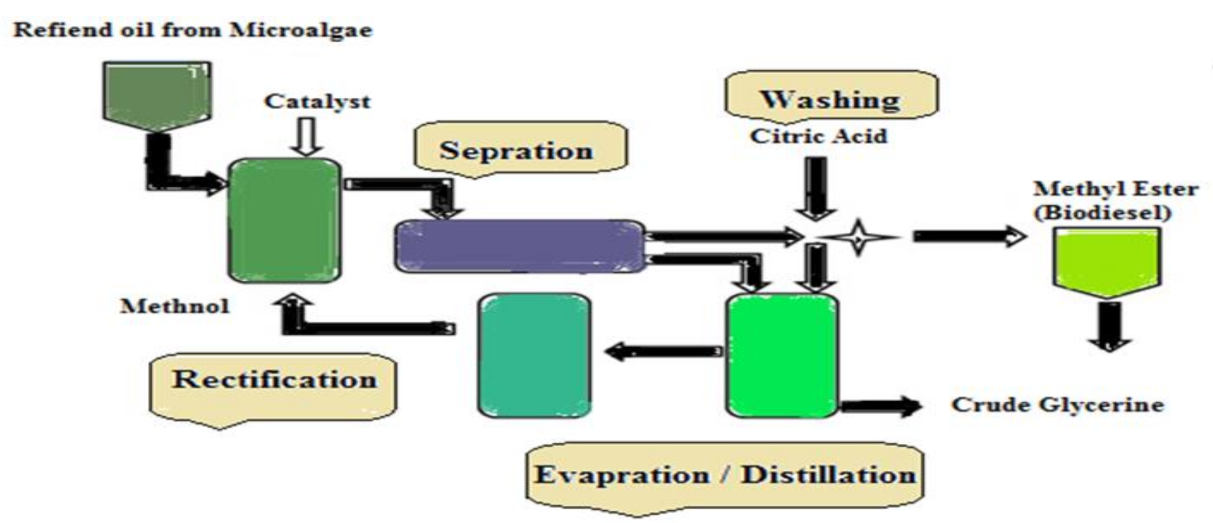


Figure 3: Schematic of esterification process of microalagl oil to biodiesel.

Microalgae Cultivation in wastewater

Microalgae species are different are in their forms can be grown according to their specific required nutrient media (Papazi, Assimakopoulos and Kotzabasis, 2012), such as are carbohydrates, starch, cellulose hemicellulose, organic compounds (Lara *et al.*, 2003). In controlled environmental conditions microalgae is high efficient in removal in nitrogen phosphorus toxic metals, during growth of microalgae in wastewater at varying level if nitrogen and phosphorus are tolerated. Phytoplankton growth can also lower the risk of harm through microalgae cultivation (Cai, Park and Li, 2013). Algal species for cultivation depends on the type of wastewater. Wastewater as cost-effective media substrates may be used as a medium for production of liquid biofuel. The production of organic fuels in agro-industrial and municipal waste water through oleaginous microalgae is a more cost-effective and more promising sustainable production (Cai, Park and Li, 2013). In different types of waste / waters the microalgae will grow efficiently. Several of the most frequently available forms of wastewater are listed below, for low cost algal growing in the near future.

Table 2: Microalgae grown from different waste water sources

SNO:	**Waste water**	**Microalgae strain**	**References**
1	Domestic wastewater	Mixed algae growth	(Shyue Koong Chang and Schonfeld, 1991)
2	Domestic wastewater	*Botryococcusbraunii*	(Nurdogan and Oswald, 1996)
3	Wastewater from textile industry	*Chlorella vulgaris*	(Sydney *et al.*, 2011)
4	Synthetic wastewater	*Chlorella vulgaris*	(Lim, Chu and Phang, 2010)
5	Primary wastewater	*Haematococcus pluvialis*	(Kang *et al.*, 2006)
6	Soybean processing wastewater	*Chlorella pyrenoidosa*	(Kang *et al.*, 2006)
7	Primary and secondary wastewater	*Desmodesmus communis*	(Hongyang *et al.*, 2011)
8	Urban waste water	*Chlorella*sp.and*Chlamydomonas* sp.	(Samorì, Samorì and Pistocchi, 2014)

Wastewater treatment along with algal biomass Production

In the last four decades, the bioremediation based microalgae has been researched extensively to remove pollutants from water(Press and Sciences, 2011)(Kuyucak and Volesky, 1988)(Romero-Gonzalez, Williams and Gardiner, 2001)(Fu and Wang, 2011). A practical alternative approach to bio remediating algae was recently studied in which algae are directly cultivated in the wastewater stream. Three microalgae species were grown in water contaminated with heavy metals coupled with nutrients. All species achieved high heavy metal concentration (to 8% dry weight). Interestingly, fairly, in contaminated water growth rates have doubled compared to freshwater (Saunders *et al.*, 2012). Another study analyzed the ability to grow algae using wastewater as a nutrient source. The algal species consortium, including Scenedesmus sp. Chlorella and Chlorella sp, respectively. The average growth productivity achieved in this case was 3.3±1.5 g dry wt m-2 d-1 (Dalrymple *et al.*, 2013). Another study reported similar results in which Chlorella sp's rate of growth was 3 g dry wt m-2 d-1, grown on wastewater(Woertz *et al.*, 2009). In addition, 13 g dry wt m-2 d-1 was reported to be significantly higher growth rates. It has been shown that 94% ammonia, 89% total nitrogen and 81% total microalgae were removed at the end of the 14 day batch cultivation(Li, Chen, *et al.*, 2011). In a recent study, anaerobic sludge centrate algae showed a rate of 12.8 g dry wt m-2 d-1 increase in productivity(Li, Chen, *et al.*, 2011). The lipid content reported was nearly 11 percent in both studies. Generally, *Chlorella* sp. can generate lipid contents up to 30% (Table 1) But in high-strength media the lipids, in particular Chlorella sp., were reduced by two folds in the growing algae. The caloric content associated with lipid production is believed in such situations to be significantly reduced (Illman, Scragg and Shales, 2000). Generally, the microalgae "starve" in nitrogen produces high lipid content (Chisti, 2007a). In order to assess their lipid content and the lipid productivity, several microalgae species have been studied in various experimental designs for starvation and heterotrophic conditions. However, the most suitable *Chlorella* sp. has been found for such systems(Chu, See and Phang, 2009)(Bhatnagar *et al.*, 2010) Chlorella kessleri produces a very high density of biomass among them (2.01 g L−1) when it is cultivated in municipal wastewater(Li, Zhou, *et al.*, 2011). Naturally, a group of microalgae genera (*Chlorella*, *Micractinium* and *Actinastrum*). During wastewater treatment, a maximum lipid productivity of 24 mg L−1d−1 was reported(Woertz *et al.*, 2009). Some microalgae, such as Botryococcus braunii, are commonly found on all continents in wetlands, brackish waters, and salt-water ponds, and are able to absorb 15-75% dry weight of insaturated long-chain hydrocarbons (An *et al.*, 2003)(Órpez *et al.*, 2009). But for each source of water, we still need to optimize the desired conditions. A very complex type of Scenedesmus sp strain. LX1 are showing biomass yield (0.11 g L−1), and lipid productivity (8 mg L−1 d−1) usage of secondary effluent as a batch culture growth medium (Xin, Hong-ying and Jia, 2010). which are encouraging results. In addition, in another study, it was shown that LX1 had significant removal of total nitrogen (90.4 percent) and total phosphorus (nearly 100 percent). Using ammonium as the source of nitrogen, LX1 obtained a very high growth rate of 0.82 d−1(Xin *et al.*, 2010). Similar results were reported in a more recent study(Zhou, Li, *et al.*, 2012)(Zhou, Hu, *et al.*, 2012). *Chlamydomonas reinhardtii* Is another microalga with potential of wastewater treatment and oil production. It was cultivated in wastewaters taken from three different stages of a municipal wastewater treatment plant (influent, effluent and centrate). In this scenario, a lipid productivity of 505 mg L−1d−1 was achieved which can be the highest recorded lipid productivity in wastewater for microalgae (Kong *et al.*, 2010). Keeping the view of bio-extraction ability of contaminant microalgae along with the production of biomass, such Integrated systems which use microalgae for wastewater treatment and the production of oil for biodiesel and chemical products are of interest. Recently, microalgae cultivation in the wastewater treatment and biofuel production were observed as an attractive option for reduced energy, and freshwater costs and reduction of greenhouse gas emissions(Pittman, Dean and Osundeko, 2011)(Park, Craggs and Shilton, 2011)(Menger-Krug *et al.*, 2012). A multi-national organization has also indicated that the combination of biomass production and waste water treatment with microalgae mediated CO_2 fixation and biofuel processing was more feasible(Kumar *et al.*, 2010).

Table 1: Lipid contents in the dry biomass of various species of microalgae (X. Wu *et al.*, 2012)

S.No	Microalgae	Lipid Contents (% Dry mass basis)
01	*Anabaena cylindrical*	4–7
02	*Botyococcus braunii*	25–80
03	*Chlamydomonas reinhardtii*	21
04	*Chlorella emersonii*	28–32
05	*Chlorella protothecoides*	57.9
06	*Chlorella pyrenoidosa*	2
07	*Chlorella vulgaris*	14–22
08	*Crypthecodinium cohnii*	20

09	*Cylindrotheca* sp.	16–37
10	*Dunaliella bioculata*	8
11	*Dunaliella primolecta*	23
12	*Dunaliella salina*	6
13	*Dunaliella tertiolecta*	35.6
14	*Euglena gracilis*	14–20
15	*Hormidium* sp	38
16	*Isochrysis* sp	25–33
17	*Monallanthus salina*	>20
18	*Nannochloris* sp	30–50
19	*Nannochloropsis* sp	31–68
20	*Neochloris oleoabundans*	35–54
21	*Nitzschia* sp	45–47
22	*Phaeodactylum tricornutum*	20–30
23	*Pleurochrysis carterae*	30–50
24	*Porphyridium cruentum*	9–14
25	*Prymnesium parvum*	22–38
26	*Scenedesmus dimorphus*	16–40
27	*Scenedesmus obliquus*	12–14
28	*Schizochytrium* sp	50–77
29	*Spirogyra* sp	11–21
30	*Spirulina maxima*	6–7
31	*Spirulina platensis*	4–9
32	*Synechoccus* sp	11
33	*Tetraselmis maculata*	8
34	*Tetraselmis sueica*	15–23

PROSPECTS FROM PAKISTAN

The area of Pakistan is highly appropriate for cultivation of microalgae (Fig. 3) In Pakistan, all major sources including nutrients, CO2 and sunlight, which are needed to form a micro algal-bacterial consortium, are commonly available throughout the year. Whereas Fig. 4 depicts the sunlight availability in Pakistan throughout the year(Manzoor et al, 2016). reported that the ability to produce high oil revenues of some indigenous oleaginous species of Pakistan (Table I). (Manzoor et al, 2016). There exists about 72,500 algal species in the world and approx¬imately 33% of these algal species have been explored for different purposes to this day [28]. Pakistan holds a distinctive geographical, geological and environmental position in its region which promotes biodiversity(Zaidi et al., 2018). In agro based world Pakistan is the 8th largest with 7.42 metric ton sugar producer country. Sugar industry is a large water consuming as well as largest wastewater producer industry during the manufacturing of sugar. An average of 30,000 - 40,000 liters of effluent generate from per tons of processed sugar (Sahu, 2018)[3].

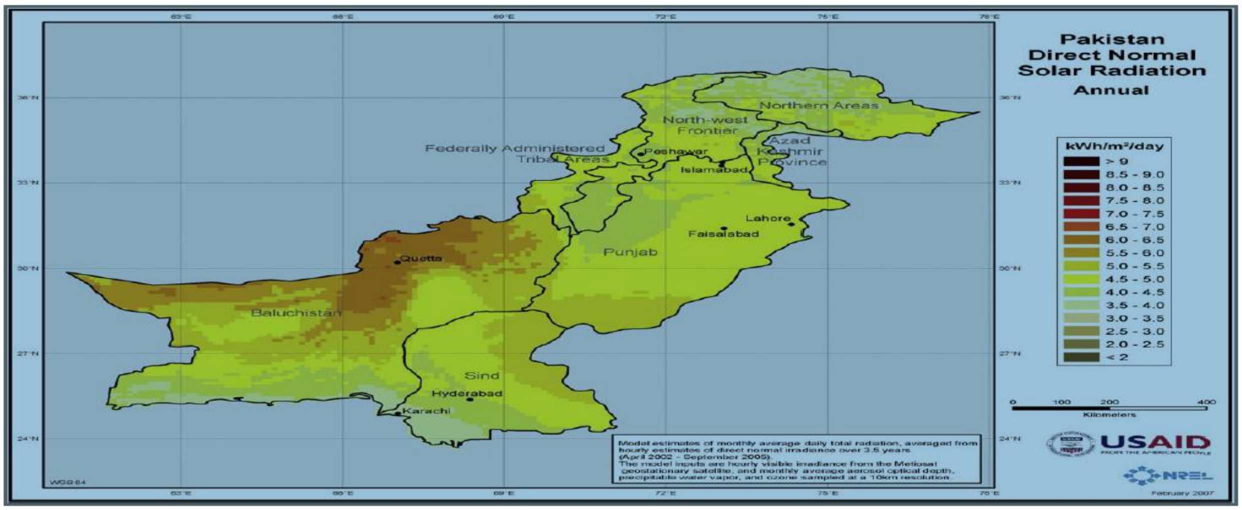


Figure 4: Map showing sunlight distribution in Pakistan developed by USAID and NREL (NREL,2010)

Integrated Processing of Sugar Industry Wastewater for Biomass Production

Almost all sugar industry wastewater contains high amount of BOD and COD(Alayu and Yirgu, 2018). These pollutants can cause greenhouse gas emissions and environmental damage. Microalgae however grow at their highest growth rate use as a growth medium sugar industrial wastewater under low COD and BOD conditions, by removing nitrogen , phosphorus and micronutrients (Molazadeh *et al.*, 2019) . By anaerobic digestion methods this COD and BOD concentration could be reduced (Wu *et al.*, 2019). Methane, carbon dioxide and other compounds are produced in this process (Figure 3). Wastewater influential will be processed into a digestive reactor to make biogas and then combusted to generated electricity and flue gas will be used as CO2 feedstock for the second cultivation of microalgae(Singh and Dhar, 2011). The bio digester effluent usually contains remaining nutrient and has low COD and BOD content used as a medium for microalgae growth.

In this integrated process two cultivation systems used. In the first cultivation, to lower heavy metals, dye color, COD and BOD content wild microalgae are used as phytoremediation. Biomass which are generated from this cultivation could be used as fertilizer, while in the second step cultivation residual filtrate is used as growth medium for microalgae based bioenergy(Cheirsilp and Torpee, 2012). In the second phase for increasing lipid content cultivation of microalgae take place in low concentration nitrogen as well for carbon source the flue gas as a sporting element will be injected into the medium. The generated biomass used for energy recovery and A filtrate material will be discharge in the river (i.e. biodiesel, bioethanol)(Azimatun Nur and Hadiyanto, 2013).

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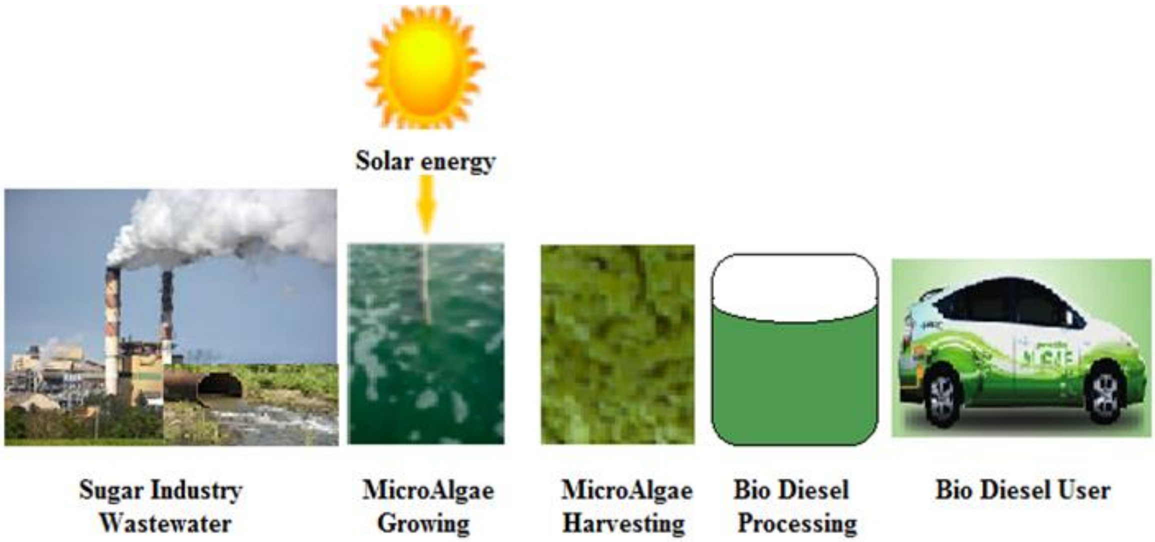

Results and Discussion

The reviewed studies in this paper indicate that sugar industry wastewater contains nutrients such as nitrogen and phosphorus, which can promote growth of microalgae. Chlorella vulgaris cultivated in high – strength wastewater has achieved removal rate of ammonia, total nitrogen, and phosphorus of 94%, 89% and 81%, respectively(Li, Chen, etal.2011). Organisms such as Botryococcus braunii and Scenedesmus sp. Hydroc /and / Acutodesmus obliquus/ are another example of a microalveolate and microcyanobacterium, respectively, which exhibit increased lipid productivity under nitrogendeprived conditions, up to 80% dry weight for nitrogen deprived cells of /CX1/ (Órpez et al., 2009; Xin et al., 2010). Summary They are thus useful for biodiesel manufacture. From your Table 1 we can see that microalgae are capable of producing as much as 136,900 L/ha per year of oil—that's much more than sunflower or jatropha, for example. Moreover, Table 2 illustrates that microalgae are capable of growing in different types of wastewaters such as domestic and textile industry effluents. Pakistan sugar industry generates 30,000 to 40,000 liters of waste water per ton of cane that is processed (Sahu, 2018), therefore this could be an important resource. Due to ample sunlight and native strainsous algae, Pakistan, has great prospect in the inclusion of microalgae in the wastewater treatment system (Manzoor et al., 2016; Zaidi et al., 2018). Therefore, microalgae cultivation in sugar industry effluent provide two benefits: as an environmental remediation and as a renewable energy source. Implementing these systems can contribute to decreasing the reliance on fossil fuels and sustainable treatment of industrial wastewater.

Environmental benefits of Microalgae

Microalgae appear to be the only highly efficient renewable energy-friendly means of producing biofuels(Gill *et al.*, 2013) and removing pollutants from waste wastewater (Chisti, 2007b), Because of lipid production, microalgae as a biofuel feedstock is much higher(Kalwar *et al.*, 2019)(Ahmad *et al.*, 2015). It produces biomass by the photosynthesis process, which converts the chemical energy by using solar energy for growth and by discovering a proactive environment for growth. Water, sunlight and simple nutrients, for example, can thrive. This approach allows microalgae to be cultivated under harsh local conditions that are not suitable to other existing feedstocks. Conventional carburization produces large quantities of CO_2, NO_2 and S pollutants and other toxic gasses, while sulfate- free algae-derived biodiesel decreases the pollution of particulate matter, CO, hydrocarbons and SOx. The production of microalgae for biofuel use also may play an important role in reducing CO_2 from industrial exastes by bio-fixation (K. and Shukl, 2011). Furthermore, waste water-based algae will contribute towards reducing wastewater pollutants by utilizing NH_4^+, NO_3 and PO_4^{3} as nutrients for the production of the microalgae (Eida, Darwesh and Matter, 2018)(Chisti, 2007a). The residual biomass will be further converted and changed into nutritional supplements to include protein, pigments, antioxidants, natural dyes and carbohydrates(Patel *et al.*, 2019). The production of bio fuel from algae is valuable, because biomass products are rapidly produced within just a few days. Finally, various studies have reported that micro algae-based biofuels, compared to fossil fuels, are more environmentally sustainable (Chia *et al.*, 2018).

Conclusion

The study discussed in this review proposes that microalgae are one of the best candidates for the sugar mills wastewater treatment and biodiesel production in Pakistan. Pakistan sugar production is predicted to continue increasing in the future. The potential wastewater associated with it could be a major problem in the process. Microalgae as future biofuel feedstock cultivated in sugar industrial wastewater is an interesting technology to be adopted in the country. Climatic zones of Pakistan, nature and abundance of agro/food industrial wastes and the wastes containing effluents and endogenous microalgal cultivatable species give much hope to develop bioprocess for efficient biodiesel production. Microalgal treated wastewater may not be perfect for drinking purpose but may be more suitable for irrigation purposes. Combination of wastewater treatment and microalgal CO_2 fixation provides additional economic incentives due to the savings from chemicals (the nutrients) and the environment benefits. It provides a pathway for removing nitrogen, phosphorus, and metal from wastewater, and producing algal biomass, which can further be exploited for biofuel production, without using freshwater. The cultivation of microalgae in sugar mill wastewater is a twofold solution; it simultaneously works as an environment treatment process and a form of renewable energy production. This combined approach of bio-based integrated concept is well suited for Pakistan's climate and RBDL needs. Subsequent work need to be aimed at the commercial production of this technology and its investible potential in factories. However, it is necessary in the future to generate a challenge which finds sustainable and feasible process to produce microalgae biofuel in sugar industry wastewater to reach rational cost of production.

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