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Research Article

Progress of Microalgal Biodiesel Research in Pakistan

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Abstract

Excessive use of fossil fuels has led to severe energy calamity and environmental pollution in the world. The effect can be mitigated by shifting from conventional fuels to biofuel which may become a replacement of fuels such as diesel, gasoline and Compressed Natural Gas (CNG). Algal biomass is considered as one of the most promising and emerging sources of biodiesel production. Technologies related to biodiesel production using algal biomass have gained initial foothold in Pakistan but have failed miserably in gaining necessary momentum due to lack of government support to technology. The aim of this study is to indicate the progress and future perspectives of biodiesel production in Pakistan through microalgae. The study indicates that a microalgae is one of the best candidates for biodiesel production in addition to other energy crops like Jatropha, Castor and Pongamia Pinnata. There is a need to expeditiously develop biodiesel technology using local resources to lower the burden of imports on country's economy while also bringing security of energy resources.

Keywords: Algal biomass; Biodiesel; Biofuels; Oil percentage, Pakistan

Introduction

Energy is one of the vital factors responsible for the development of a country. National development is very closely linked to the development in energy sector of a country. Pakistan is an energy deficit country [1]. Pakistan is ranked 34th according to the consumption of refined petroleum products and its petroleum consumption per day is 450,000 barrels [2]. Most of the energy resources are consumed in automobiles. In order to bring an end to the energy crisis problem of transport sector, there is a need to increase awareness about using alternate fuel for automobiles [3]. Energy consumption by transport sector is 34.2% of the total of 41.9 billion TOE (Tonnes of Oil Equivalent) of Pakistan's energy consumption [4].

Extensive utilization of fossil fuels not only decreases the sources of their production while also cause environmental pollution, energy crisis and global climatic change. Therefore, scientists and researchers all around the globe are focusing their attention to finding alternative, renewable & sustainable energy fuel sources and developing better energy conversion techniques [5]. Biofuels are sustainable energy resource and they have emerged as an encouraging substitution to conventional energy source [6,7]. They are readily available worldwide while also being renewable and can be acquired from prevailing biological resources [8].

Biofuels cover biodiesel, bioethanol and biogas. Biodiesel is manufactured from multiple feed stock including animal fat, vegetable oils and waste cooking oil. Biodiesel is typically produced through the reaction of vegetable oil or animal fat with methanol or ethanol in the presence of a catalyst to yield glycerin and biodiesel (chemically called methyl or ethyl esters). Biodiesel can be used in neat form, or blended with petroleum diesel for use in diesel engines. As far as operation of diesel engine is concerned, the physical and chemical properties of biodiesel are similar to petroleum based diesel fuel. The comparison of similar properties of biodiesel and petroleum diesel fuels is shown in table 1, biodiesel can also be blended in any ratio with petroleum diesel fuel.

Fuel Characteristics	Diesel	Biodiesel
Kinematic viscosity at 35°C (mm^2/s)	812	865
Density at 35°C (kg/m^3)	2.90	4.9
Heating value (MJ/kg)	42.6	37.3
Cetane number	46	> 49
Mass ratio		
Mass content C	0.860	0.7750
Mass content H	0.136	0.1210
Mass content S	0.003	0.0001
Mass content O	----	0.1040
Stoichiometric ratio (air/fuel)	14.5	12.4

Table 1: Properties of diesel and biodiesel [9].

The stock of biodiesel feed stocks is limited which hampers the exploration of biodiesel production. Feed stocks can be categorized in three generations for biodiesel production. First generation comprises of oil seeds and food crops such as soyabeans, rapeseed, sunflower and palm oil. These feed stocks were primarily used for biofuel production [10]. The second generation feed stock includes whole plant tissues, including energy crops such as *Jetropha* sp., Madhuca longifolia, salmon oil, tobacco seed, jojoba oil and sea mango [11]. Third

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generation biodiesel feed stock is microorganisms and widevariety of these can be employed for this purpose. Microalgae is the most prominent and promising third generation feedstock for biodiesel production [12]. High oil content, the cheapest source and rapid biomass production are the traits which reassure that microalgae is recognized as a potentially good source of biodiesel production [13-15].

In comparison to the oil production from crops, oil production growth rates from microalgae are much greater as shown in table 2 [14]. Algal biomass can be produced in marginal land, compact bioreactors and saline water bodies as compared to oil producing crops [16]. Oil content of different microalgae reported in literature is shown in table 3.

S.No	Crop	Oil Production (L/ha)
1	Corn	172
2	Soybean	446
3	Canola	1190
4	Jatropha	1892
5	Cocunut	2689
6	Oil Palm	5950
7	Microalgae (70% oil (by wt) in biomass)	136,900
8	Microalgae (30% oil (by wt) in biomass)	58,700

Table 2: Comparison of biodiesel sources.

S.No	Microalgae	Oil Content (% dry weight)
1	*Botryococcus braunii*	25-75
2	*Chlorella* sp.	28-32
3	*Crypthecodinium cohnii*	20
4	*Cylindrotheca* sp.	16-37
5	*Dunaliella primolecta*	23
6	*Isochrysis* sp.	25-33
7	*Monallanthus salina*	>20
8	*Nannochloris* sp.	20-35
9	*Nannochloropsis* sp.	31-68
10	Neochloris oleoabundans	35-54
11	*Nitzschia* sp.	45-47
12	*Phaeodactylum tricornutum*	20-30
13	*Schizochytrium* sp.	50-77
14	*Tetraselmis sueica*	15-23

Table 3: Oil content of different microalgae [14].

The objective of this study is to provide state-of-the-art information on microalgal biodiesel research in Pakistan and also to identify the potential opportunity of the feedstocks for biodiesel production.

Biodiesel Production Pathway from Microalgal Biomass

Biodiesel production pathway from microalgal biomass is shown in figure 1. Microalgal growth is obligatory to light, carbon dioxide, organic salts, water and suitable temperature. Microalgae utilize organic salts in the presence of light and transform them into organic compounds. It can be grown in 'open culture system' and in 'close culture system' [13]. The open systems composed of open ponds including growth medium. They are the oldest, cheapest and the simplest system for microalgae mass cultivation. They are constructed in rows and exposed to sunlight. The main drawback of open system is that maintenance of CO_2 supply is difficult and it is also prone to inclusion of various biological contamination from environment. In 'open culture system', the growth culture is isolated from the environment and operated in controlled conditions [17]. A photobioreactor is composed of illuminating light source which is used for growth of microorganisms. They can be classified as horizontal tubular photobioreactors; stirred photobioreactors; airlift and bubblecolumn photobioreactors.

The harvesting consists of recovery of microalgal biomass from culture broth. Harvesting is a costly process and it contributes to 20-30% of the total production cost of biomass [18]. Harvesting can be done by flocculation, sedimentation, filtration and centrifugation. Harvesting is followed by drying to remove water untill dry weight is achieved. Extraction of lipids and fatty acids are required to produced biodiesel. Solvent extraction is commonly applied to extract lipids and fatty acids from microalgal biomass, as it is quick and efficient method. Hexane, ethanol (96%), or hexane-ethanol (96%) mixture are usually used for this purpose [18]. Microalgal lipids undergo transesterification as per the chemical reaction shown in figure 2, in which oil is mixed with alcohol to produce ester and glycerol while ethanol is reclaimed. Schematic diagram of esterification process of microalgal oil to biodiesel is shown in figure 3. Table 4 summarizes production processes of biodiesel from microalgae.

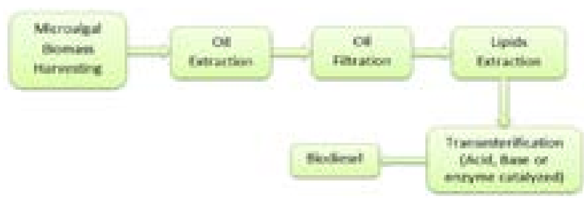

Figure 1: Microalgal biodiesl production pathway.

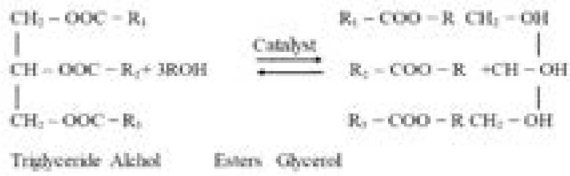

Figure 2: Chemistry of esterification.

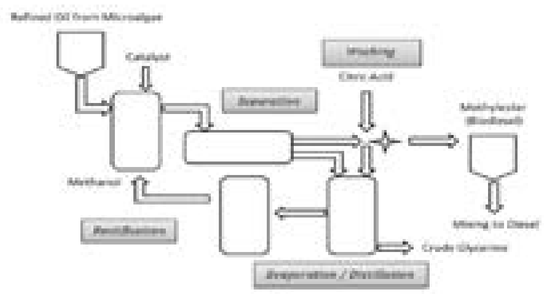

Figure 3: Schematic of esterification process of microalagl oil to biodiesel.

Process	Technique	Microalgae	Result	Reference
Cultivation	Raceway ponds	*Chlorella vulgaris*	585 T of lipid extracted algae per day	[19]
	Photobioreactor	*Cyclotella* sp.	51% lipids productivity increase in 72 h	[20]
	Photobioreactor	*Chlorella* sp.	7% increase in surface productivity	[21]
	Photobioreactor	*Porphyridium cruentum*	37.26% increase in biomass productivity	[22]
	Batch mode cultivation	*Chlorella vulgaris* *Scenedesmusobliquus* *Ourococcusmultisporus*	Lipid productivity(0.164 g-lipids g-cell^{-1}day^{-1})	
Harvesting	Flocculation	*Chlorellaminutissima*	60% recovery efficiency of biomass	[23]
		Chlorella vulgaris	Concentrates up to 357 times that of theoriginal dry weight	[24]
		Chlorella sp.	100% harvesting efficiency	[25]
		Chlorella sp.	> 91% harvesting efficiency	[26]
	Gravity sedimentation	*Chlorella vulgaris*	60% of biomass recovery	[27]
Lipid Extraction	Wet lipid extraction	*Chlorella* *Scenedesmus* sp.	79% extraction of transesterifiable lipids	[27]

Table 4: Summary of production processes of biodiesel from microalgae.

Progress of Biodiesel Research in Pakistan

There exists about 72,500 algal species in the world and approximately 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. Pakistan possess plentiful algal flora because of rich saline habitats and diverse water [16].

The first attempt to produce biodiesel from algal biomass in Pakistan was carried out by Khola et al. [29]. He studied *Cladophora* sp. The study highlighted the biomass after oil extraction, pH and quantitative properties of obtained biodiesel. Khola compared the quality of biodiesel with *Oedogonium* sp. and *Spirogyra* sp. [30]. Obtained results indicated that *Cladophora* sp. produces more quantity of biodiesel as compared to *Oedogonium* sp. and *Spirogyra* sp. Ahmad et al., [31] studied the waste water nutrition for growth of microalgae and its use for biodiesel production. He used mixed algae culture composed of *Microspora* sp., Diatoms, *Lyngbya* sp., *Caldophora* sp., *Spirogyra* sp. and *Rhizoclonium* sp. Results showed that the growth of mixed algae culture was promising and the biodiesel produced was in accordance with the ASTM standards.

Musharraf et al., [16] performed the Gas Chromatography-Mass Spectrometry (GC-MS/MS) analysis of Fatty Acid Methyl Esters (FAMEs) and biodiesel production study for six microalgae strains (*Scenedesmus quadricauda*, *Scenedesmus acuminatus*, *Nannochloropsis* sp., *Anabaena* sp., *Chlorella* sp. and *Oscillatoria* sp.) collected from fresh and marine water resources located in southern region of Pakistan. Results proposed that microalgae found in southern Pakistan has a potentially active growth rate and can be used for biodiesel production. Ahmad et al., [32] performed a study to find out the biodiesel efficiency of *Chlorella vulgaris*, Rhizoclonium hieroglyphicum and mixed culture algae by transesterification process. It was suggested by the study that raw municipal waste water can be used for the growth of algal species. The biodiesel obtained was compared with ASTM standards.

Ahmad et al., [33] studied the treatment of municipal waste water and biodiesel production by employing *Chlorella vulgaris*. Results showed that *Chlorella vulgaris* produced biodiesel of good quality which conformed to the ASTM standards. Further, the study indicated that the treated waste water can be used for irrigation purpose as it has BOD and COD within the limits of national environmental quality standards. Fatima et al., [34] used two freshwater algae species (*Chlorella vulgaris* and *Oedogonium* species) for biodiesel production. The *Chlorella* species produced 6.26 g oil from 38.23 g of dry weight and the *Oedogonium* species produced 8.07 g of oil from 38.23 g of dried weight. Alam et al., [35] inspected 17 species from fresh water sources of Khyber Pakhtunkhwa. Chroococcus turgidus, Sirogonium sticticum, Uronema elongatum and Temnogyra reflexa produced the highest amounts of oil amongst all others. Oil percentages of different microalgae species investigated in Pakistan are presented in table 5.

In Pakistan, there exists vast potential to produce biodiesel from mircoalgal biomass. It has been observed from this study and highlighted in figure 4 that *Chlorella* sp. (fresh water algae) produced the highest amount of oil content percentage followed by *Chlorella vulgaris* which grows in waste water. Rhizoclonium hieroglyphicum and mixed algae culture also produced considerable amount of oil content for biodiesel production.

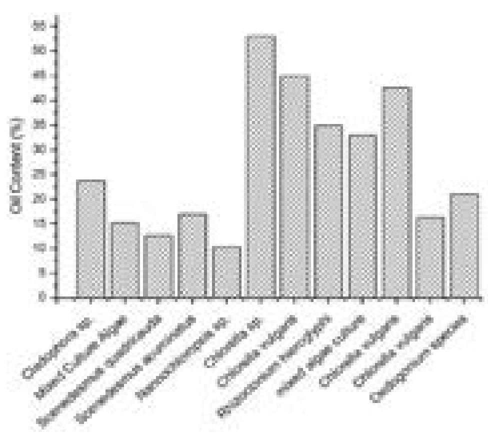

Figure 4: Oil content (%) of microalgal species existing in Pakistan.

Algae Type	Location	Habitat	Oil Percent (%)	Reference
Cladophora sp.	Aquatic ponds of GC University Botanic Garden	Fresh Water	23.8	[29]
Mixed culture algae: *Microspora* sp. *Diatoms* *Lyngbya* sp. *Caldophora* sp. *Spirogyra* sp. *Rhizoclonium* sp.	Aquatic ponds of GC University Botanic Garden	Waste Water	15.13	[31]
Scenedesmus quadricauda	Southern Pakistan	Fresh Water	12.6	[16]
Scenedesmus acuminatus		Fresh Water	17	
Nannochloropsis sp.		Marine	10.4	
Anabaena sp.		Fresh Water	2.98	
Chlorella sp.		Fresh Water	53	
Oscillatoria sp.		Marine	3.69	
Chlorella vulgaris	Fish forms and laboratory of Punjab Fisheries Department, Lahore Pakistan.	Waste Water	45	[32]
Rhizoclonium hieroglyphicum			35	
Mixed algae culture			33	
Chlorella vulgaris	Fish forms of Department of Fisheries near Manawa police station Lahore, Pakistan	Waste Water	42.66	[33]
Chlorella vulgaris	Freshwater sources like tube wells, irrigation channels, aquatic ponds, and fish ponds in Faisalabad	Fresh Water	16.37	[34]
Oedogonium species			21.1	
S. sticticum	Natural springs of Landikotal, Swabi and Swat, water logging of Shabqadar and Mardan, and water tanks of Malakand districts of Khyber Pakhtunkhwa	Fresh Water	7.5	[35]
U. elongatum			7	
C. tergidus			8.5	
T. reflexa			7	
C. glomerata			2	
S. dictyospora			3.2	
E. procera			3	
H. klibsii			5	
H. reticulatum			5.5	
M. abnarmaus			4	
O. angustissimum			6.3	
R. crassiplitum			6	
P. oedogonia			5	
B. gigantea			2.5	
C. cryptica			3.3	
T. cylindrica			4	
O. minima			4.5	

Table 5: Oil percent of different microalgae species investigated in Pakistan.

Inverstment Opportunity in Biodiesel

Biodiesel production is gradually increasing in the world specially in Europe as can be seen from figure 5 that shows total European Union (EU) production of 14,155 million liters in 2017. Several new manufacturers are entering while new varieties of raw materials are being introduced such as microalgae. Biodiesel processing is extensively being undertaken by USA, Brazil, Germany, Indonesia, Argentina, France, Thailand, Spain, Belgium and other countries as shown in figure 6. The world production of biodiesel has grown extensively in the last five years.

Pakistan has been importing diesel fuel every year and despite the fact that local manufacturing of diesel reduced the volume of import during the year 2013-2014, the import of diesel has significantly risen to 3.54 million tons for the year 2016-2017 as shown in figure 7.

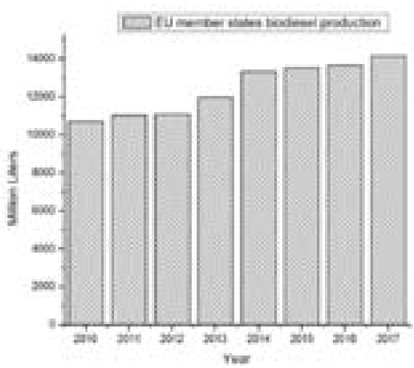

Figure 5: Biodiesel production of EU member states [36].

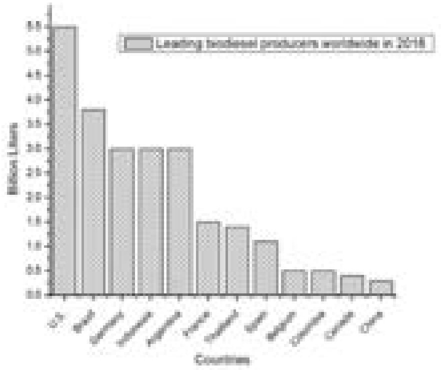

Figure 6: Leading biodiesel producers worldwide in 2016 [37].

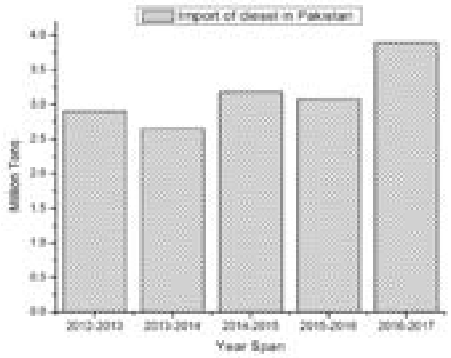

Figure 7: Import of diesel in Pakistan [38].

The production cost of microalgal biodiesel depends on various factor including the harvesting cost, filtration cost, extraction cost and esterification system cost. At the moment, production cost of microalgae biodiesel is considered far more costly when one compares it to petroleum diesel fuels. Chisti [14] calculated the production cost of microalgal oil manufacturing from a photobioreactor for an annual production capacity of 10,000 tons per year. If one assumes the oil content of around 30%, the production cost by the author comes $2.80/L ($10.50/gallon). The costs of esterification process, distribution, taxes and marketing cost for biodiesel are not included in this estimation. Similarly, the petroleum diesel price was estimated to be $2 to $3 per gallon. Following equationis used by Chisti [14] for the estimation of the cost of algal oil where it can be a spirited alternative for petroleum diesel.

$$C_{\text{algal oil}} = 25.9 \times 10^{-3}\, C_{\text{petroleum}}$$

Where, $C_{\text{algal oil}}$ constitutes the price of micro algal oil in $/gallon and $C_{\text{petroleum}}$ is the price of crude oil in $/barrel.

Conclusion and Future Prospects

The study discussed in this review propose that microalgae is one of the best candidates for biodiesel production in Pakistan in addition to other energy crops like jatropha, castor and pongamia pinnata. The country needs to address the problem of shortage of conventional fuels in short span of time and also need to reduce the risk of frequent price escalation. The impact of dependence on conventional fuel can be reduced by gradually shifting to biofuels through favorable strategies and policies and setting up national targets for biomass utilization. Regulations and guidelines are required for utilization of biofuels in transport, power generation, water pumping, remote village esterification etc. Licensing and quality assurance procedures of biofuels must be developed for its better growth. Financing biofuels through initial government support, banking credit and tax incentives can also be helpful for implementing this technology in Pakistan. Provision of land for biofuel cultivation by Government of Pakistan and announcement of National Energy Policy for biofuels can reduce imports & bring security of petroleum resources. These initiatives by government will also encourage the biomass research centers to go for a new startup companies to convert the research into commercial products. These products will serve the purpose of distributed generation of biofuels in different areas of Pakistan which have availability of biomass sources. The quality of production can be then controlled by regulatory agencies.

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