Prospects of microalgal biodiesel production in Pakistan- a review

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Abstract

Biodiesel is an alternative, renewable, biodegradable and environmentally friendly fuel for transportation, with properties like petroleum-derived diesel, and can be used directly in a compression ignition engine without any modifications. The world's fossil fuel and crude oil reserves are going to dry up in the next few decades, but, contrariwise, an attractive, high quality, readily available and economically extractable oil from microalgae is a substitute feedstock to produce alternative biodiesel fuel for the transportation sector in the future. Microalgae have a higher biomass productivity (tons/ hectare / year) and lipid yield (kg/ kg of algal biomass) as compared to vegetable oil crops. To overcome the problem of energy deficiency in developing countries, like Pakistan, and boost their economic growth, alternative fuels are proving very important for environment-friendly and sustainable development, especially in the last few decades. Different research studies on microalgae cultivation, characterization of microalgae oil (lipids), and evaluations of its socio-economic feasibility to produce renewable biodiesel have been conducted in the past in Pakistan for its future prospects. This review paper includes the overall summary and compilation of the microalgae research conducted in Pakistan on biodiesel production and includes the algal biodiesel production cost analysis. The studies showed promising results for harnessing microalgae and using its lipids to produce biodiesel with favourable properties that were comparable to the conventional diesel in Pakistan. The information related to the microalgae research will help stakeholders and governmental organisations working in the renewable energy sector to consider its cultivation on a large scale, using waste water as a feedstock to produce biodiesel to meet the target set by the Government of Pakistan of using 10% blended biodiesel by the year 2025 in Pakistan.

Key words: Microalgae, Biodiesel, Pakistan, Socio-economic analysis, Alternative energy

1. Introduction

Pakistan is an agricultural country with 45.2 million hectares of marginal land [1] and according to the Survey of Pakistan; the country's pollution in 2015 was 192 million [2] and increased to 194 million in 2016 with a 2% population growth rate [3] and is predicted to double in 50 years. Pakistan Electric Power Company (PEPCO) quoted that the country has an electricity generation capacity of 13,240 MW, while the average demand is around 18,000 MW with a shortfall of approximately 4,760 MW[4]. Moreover, the Hydrocarbon Development Institute of Pakistan showed that the shares of resources in energy consumption are oil (29 %), coal (10 %), gas (44 %), liquid petroleum gas (1 %) and electricity (16 %) [5]. In Pakistan, the biggest consumer of petroleum products is the transportation sector, which accounts for about 48%, followed by power generation units (36%), industrial sector (12%) and residential users (4%) [6]. Pakistan is a developing country, trying to reduce its dependence on imported fossil fuels to save its valuable foreign exchange reserves. It imports 346,400 barrels of oil per day into the country with a total bill amount of US$ 10.7 billion. The transportation and industrial sector has a consumption of approximately 8.22 million tonnes of petroleum diesel per year.

Fossil fuel reserves are finite and currently we consume the equivalent of over 11 billion tonnes of oil in fossil fuels globally. Crude oil reserves are vanishing at the rate of 4 billion tonnes a year and if we carry on at this rate without taking account of our growing population or aspirations, our known oil deposits will be gone by 2052 [7]. As a result, there is a pressing need for alternative, domestic sources of fuel. Biofuels are ready to meet that need and are one way to ensure adequate fuel supplies at a time when yields from existing oil fields are declining and new fields are not yet up and running. Biofuels can do much to help fill the gap between limited fuel supplies and increasing worldwide demand that is almost sure to widen in the coming years [8]. Furthermore, frequent and increased usage of biomass as a renewable energy resource could minimize our reliance on fossil-derived fuels, overcome their CO_2 gas emission and lessen their greenhouse effect [9]. The non-edible feedstock's can be cultivated on 350,000 acres of uncultivated tracts of semi-arid land in Pakistan to produce biofuels [10], instead of utilising arable land used for food crops (wheat, rice, sugarcane and cotton), because if biofuel crops are grown on existing arable land, instead of food crops for food, Indirect Land Use Change (ILUC) emissions would occur because of the necessity of maintaining food production, and therefore do not compete with the food supply [11]. With the increase in demand of energy, the shortage of petroleum-derived fuels and increasing prices, the existence of potential biomass feed stocks of biofuel paves ways for Pakistan to promote policies for biodiesel production and it's blending with conventional fuel to save foreign exchange reserves of the country [9].

Biodiesel is an environmentally friendly liquid fuel that can be used in the transportation sector [12, 13]. Other than microalgae, the sources of biodiesel could be vegetable oil, animal fat, or recycled cooking oils containing triglycerides [14] and is made by combining methyl alcohol with a base or acid catalyst. It is biodegradable in nature, non-toxic, and safer from a storage point of view because it has a higher flash point as compared to petroleum diesel. It has a same or higher cetane number that required for good fuel property [15]. Biodiesel is not aromatic and has 10% oxygen content (by weight). Compared with diesel fuel, biodiesel reduces the discharge of gases like Carbon monoxide (CO), Hydrocarbons (HC) and Particulate matter in the evolved gases to the environment [9].

Apart from microalgae, other promising feedstock in Pakistan could be indigenous vegetable seed oils [13]. The techno-economic analysis of biodiesel production showed favourable economic benefits with job opportunities for farmers to cultivate the non-edible crops such as: castor, jatropha, pongamia, and eruca sativa on their marginal lands and auspicious properties as a substitute for petro-diesel. The oil extracted from the vegetable oil seed is converted into biodiesel while its de-oiled cake is thermally treated with slow pyrolysis [16] to convert it into an energy-dense, low moisture content, solid fuel for heating, cooking and power generation. It can also be anaerobically digested to produce gaseous biofuel (i.e. bio-methane) via fermentation [17]. Anaerobic digestion is a process in which organic matters are decomposed into biogas in the absence of oxygen. Biogas is a mixture of methane, carbon dioxide, hydrogen sulphide and traces of other gases. This mixture of gases can be used directly for cooking and lighting purposes. The municipal solid waste generated from 10 major metropolitan cities of Pakistan contains approximately 2423 tonnes of organic waste per day with an energy potential of producing 242 million cubic-meters of bio-methane gas [18]. Animal dung is also a potentially large biomass resource. It has the same energy content as wood and can be used for biogas production. In Pakistan, around 172.2 million livestock animals produce 652 million kg dung per day and the country's electricity production will be approximately 35.62 million kWh per day from animal dung [19].

In the past, a lot of research and development has been made in Pakistan to produce biodiesel from vegetable oil seeds. In continuation to the development of biodiesel, the Government of Pakistan [20], took a few initiatives to tackle the problem of environmental degradation through automobile emission control and mitigating measures. Therefore, it launched the National Biodiesel Programme to reduce dependence on imported crude oil and to meet environmental guidelines by blending 10% biodiesel in petro-diesel for use in the transportation sector by the year 2025. Moreover, a plant has been installed by Alternative Energy Development Board at Karachi in collaboration with Pakistan State Oil (PSO) with a capacity of producing 18,000 tonnes per annum biodiesel from jatropha feedstock. The Government of Pakistan has exempted custom duties and general sales tax on biodiesel processing equipment and materials to encourage stake holders to produce alternative fuels in Pakistan [5].

Microalgae is a potential feedstock to produce biofuels, if lipid yields are between 30-50% (on dry weight basis) [21] with favourable compatible properties to convert it into economically feasible biodiesel [22]. Microalgae has a higher lipid productivity yield per area (roughly between 20,000-80,000 L/acre/year), which is 7-31 times more than the conventional vegetable seed crops [23]. It's de-lipid cake (de-oiled residue) can be used as a fodder for livestock with nutritional contents for their growth and also to produce bio-ethanol by fermentation [22]. The transportation sector accounts for 21% of the global fossil fuels carbon dioxide (CO_2) emissions to the atmosphere, causing climate change and global warming issues [24]. Microalgae absorbs carbon dioxide (CO_2) from the atmosphere and thus helps in CO_2 sequestration and produces up to 20 times more oil per unit area than palm oil and also does not compete for land use with food crops cultivation [25].

Most of the microalgae research is focused on photoautotrophic growth of algae, which uses energy from sunlight to fix carbon from CO_2 in the presence of water and produce biomass. Oil-rich microalgae strains are capable of producing the feedstock for a number of transportation fuels, such as biodiesel, gasoline and jet fuel, while mitigating the effects of CO_2 released from various sources that contribute to climate change [26]. Microalgae have great potential as a renewable fuel source, but, for ensuring its economic and environmental feasibility, there is a need for high-level academic and industrial research into its growth and bioprocessing. New algae strains that efficiently use CO_2 and wastes as nutrients, novel oil extraction methods, and industrial-scale designs for fuel production are imperative for long-term energy sustainability [27].

Regarding biodiesel production in Pakistan, detailed research has been conducted previously on indigenous edible and non-edible vegetable oil seeds as a feedstock and its prospectus scope in Pakistan regarding social and environmental benefits [15]. However the research investigations on the cultivation of microalgae in the country to produce biofuels are in preliminary stages. So far, there has been quite a demand on commencing large-scale algal biodiesel projects in Pakistan by different stake holders, but very little has been done to date. This is due to the high costs and economic constraints associated with farming algae on a large scale. If a cost-effective method of producing microalgae biomass on both saline lands and sewage (waste water) is developed, economically viable algal biodiesel could become a major success in the renewable energy sector [28]. But one can only find limited information related to the current research and future prospects of microalgae cultivation in Pakistan as a potential candidate to produce biodiesel. This review paper is an attempt to compile the research conducted on microalgae, and its future prospects to produce microalgal biodiesel in the country, to reduce the burden on the imported crude oil for energy requirements, focussing on different types of indigenous microalgae species with favourable characteristics to grow in the local climatic conditions. Moreover, the supply chain mass balance to produce biodiesel in Pakistan is studied from the field (raw seeds) to the final product (biodiesel) having compatible properties with petro-diesel. Furthermore, the microalgae biodiesel production feasibility study in the country is also studied and presented in the paper based on more realistic assumptions. This study will be helpful for the stake holders and decision makers working on renewable biodiesel fuel production to tackle the future challenges faced by the country, make future policies and action plans.

2. Microalgae biodiesel production

The microalgae lipids contains fatty acids (triglyceride molecules), which are reacted with methyl alcohol in the presence of an acid or base catalyst to form methyl esters (also called biodiesel) and glycerol as a by-product [29] as shown in **Equation 1.** The cultivated microalgae biomass is harvested, dried, grounded and then the oil is extracted by organic solvent extraction method and is transesterified at 60^{o}C for 90 mins (reaction time) in a closed reactor. After the reaction, two biphasic layers are formed with a supernatant layer of biodiesel, with a bottom, dark brown layer of glycerol as a value added by-product, after the mixture is allowed to settle at room temperature. The applications of glycerol includes cosmetics, pharmaceuticals and the soap industry [22, 30]. Approximately, 1 part of glycerol is produced for every 10 parts of biodiesel. Biodiesel can be used as an additive (typically 20%) to reduce vehicle emissions or in its pure form as a renewable alternative fuel for diesel engines, without any engine modifications, when it is blended with mineral diesel in a neat composition [31].

$$Triglycerdide\ (fatty\ acids) + Methyl\ Alcohol \overset{catalyst}{\Longleftrightarrow} Biodiesel\ (methyl\ ester) + Glycerine\ (as\ a\ value\ added\ byproduct) \quad (1)$$

3. Comparison of physical and chemical properties of microalgal and vegetable oil seeds derived biodiesel

Comparison of physical and chemical properties of vegetable seed oil and microalgae oil biodiesel is shown in **Table 1**. It was found that the microalgae oil's physical and chemical properties are comparable with soybean oil, and can be used as a feedstock to produce biodiesel. The kinematic viscosity was found to be higher in algal lipids compared with soybean oil due to difference between the oil and lipid's (fat's) melting points [32]. Moreover, the microalgal oil is found having an increased 4% higher heating value as compared to the soybean oil. The viscosity and density values of methyl esters produced via the transesterification process [14], decrease considerably for both the feedstocks (i.e. microalgal oil and soybean oil), bringing the values within the permissible range of biodiesel standard ASTM D6751. The presence of long chain saturated and unsaturated fatty acids (octadecanoic acid (C-18:0), oleic acid (C-18:1) and linoleic acid (C-18:2) affects biodiesel combustion quality with higher cetane number due to shorter ignition delay period [33]. The microalgal biodiesel is found having higher cetane number as compared to soybean biodiesel as presented in **Table 1.** The cold flow properties of soybean biodiesel such as cloud and pour point were found to be lower than microalgal biodiesel. This is mainly due to the lower level of palmitic acid (saturated fatty acid) in the microalgal oil [34]. Although the presence of some unsaturated fatty acids increased the pour point and cloud point of the microalgal biodiesel, microalgae with its high biomass productivity rate can be considered as a proper source of biodiesel production [35]. The flash point of biodiesel produced from microalgae was found to be lower as compared to the soybean oil [30], but it is acceptable keeping in view the safety and storage of the fuel with minimum value of 130^{o}C as prescribed by the ASTM D6751.

4. Comparison of engine performance and emission testing of microalgae and vegetable seed biodiesel:

Environmentally friendly biodiesel fuel provides significantly reduced emissions of carbon monoxide, particulate matter, unburned hydrocarbons and sulphur oxides compared to conventional petroleum derived diesel. The emission analysis observed combustion of B20 mixed (jatropha and pongamia) biodiesel fuel blend with 60% and 35% lower emission of carbon monoxide and sulphur dioxide respectively in contrast to petroleum diesel. The result showed that NO_x emissions were 10% higher from biodiesel fuel, as compared to conventional diesel fuel. This is due to the high exhaust temperature of biodiesel combustion combining the oxygen content emitted in the exhaust with atmospheric nitrogen (79% in the atmosphere); however, this drawback of biodiesel emissions may be reduced by EGR (Exhaust Gas Recirculation) technology. The research also revealed that B20 mixed biodiesel fuel can be used, without any modification, in a compression ignition engine [36].

In another study, the power (hp), torque (N-m) and brake specific fuel consumption (g/MJ) across a range of engine speeds was also compared, favourably to values determined for soybean biodiesel. Analysis of exhaust emissions (unburned hydrocarbon HC, CO, CO_2, O_2 and NO_x) showed that all biofuels produce significantly less CO and HC than petroleum diesel. Surprisingly, the microalgal biodiesel was found to have the lowest NO_x output, even lower than petroleum diesel. The low prevalence of shorter chain length fatty acids present in microalgal oil likely contribute to its low NO_x emissions, which makes it an attractive and competitive option in contrast to petroleum diesel [37]. **Table 2,** depicts the comparison of emissions from petroleum diesel and microalgae biodiesel fuels, showing reduced emissions of CO, unburned HC, and NOx from microalgae biodiesel in contrast to petroleum diesel. Oxygen content present in biodiesel is about 11%, due to which, it has better combustion properties and therefore has less exhaust emissions [15].

Furthermore, the engine performance test showed maximum power at 3500 rpm with 8.5 hp recorded with petroleum diesel, followed by soybean biodiesel with 8.2 hp and the least with microalgal biodiesel with 7.8 hp. The torque generated from petroleum diesel was 15.5 N-m, while soybean biodiesel having 15.2 N-m and microalgal biodiesel 14.8 N-m. The engine power and torque generated by algal biodiesel is approximately 7-10% lower than the petroleum diesel, but these values are negotiable in terms for achieving the environmental and sustainable development. The brake specific fuel consumption (BSFC) from petroleum diesel combustion was 105-120 g/MJ, while soybean biodiesel was 122-145 g/MJ and microalgal biodiesel was 120-148 g/MJ, having the highest value [37]. The particulate matter emissions were significantly reduced by blending 20% with biodiesel as compared to petroleum biodiesel, due to the high amount of long chain and level of unsaturation fatty acids [38]. Moreover, a life cycle assessment on GHG (greenhouse gases) emissions from microalgae biodiesel, based on detailed mass and energy balances, showed 70% lower than those of petroleum diesel, meeting the minimum 60% GHG reduction requirements for the European Union Renewable Energy Directive [39].

5. Closed carbon cycle and energy balance of biodiesel

Biodiesel reduces net carbon dioxide (CO_2) emissions by 78 % compared to petroleum diesel. This is due to biodiesel's closed carbon cycle as the CO_2 released into the atmosphere when biodiesel is burned is re-absorbed by growing plants, which are later processed into fuel [40] or, in other words, the plantation of energy crops is helpful in mitigating CO_2 present in the atmosphere due to the combustion of biodiesel [13]. Its environmental benefits are shown during the combustion in the engines, given that their emissions of CO_2 correspond to the amount that was sequestrated from the atmosphere during the growth of these plants, resulting in a closed carbon cycle [41].

Moreover, another advantage of biodiesel is a positive energy balance compared to other alternative fuels and also in terms of effective use of fossil energy resources, i.e. biodiesel yields around 3.2 units of fuel product energy for every unit of fossil energy consumed in the life cycles. By contrast, the petroleum diesel life cycle yields 0.83 units of fuel product energy per unit of fossil energy consumed for its production [42].

6. Microalgal oil fatty acid compositional analysis

The oil's fatty acid composition has an effect on the performance of the biodiesel fuel produced. The algal and vegetable seed oils show more or less similar fatty acid composition (see **Table 3**). Increasing the carbon to hydrogen ratio increases the duration of ignition delay. The number of double bonds present in the fatty acid have more impact on ignition delay than an average carbon chain length [33]. Algal oil has quantitatively less fatty acids with double bonds, thus, with low C:H molar ratio, has a shorter ignition delay period (thus having higher cetane number with complete fuel combustion property) and is more suitable as compared to vegetable oils such as soybean, palm, sunflower and rapeseed [43]. Palmitoleic acid is the most commonly occurring fatty acid in the microalgae lipids, while C15:0, C16:0, C14:1 and C18:1 are the next commonly occurring acids. The unsaturated acids were found in larger proportion (46.50-70.46%) than saturated FAs (16.82-39.20%) [44]. The major fatty acids present in the composition of the algal lipids contain palmitic (C16:0), oleic (18:1) and linoleic (18:2), which has a direct impact on the biodiesel fuel properties. Higher oleic acid (C18:1) content in the algal oil improves the oxidation stability and decreases cold filter plugging problems during winter conditions [45].

7. Algal biodiesel research (studies conducted on indigenous species of micro and macro algae found in Pakistan)

The cultivation of microalgae in Pakistan to produce biofuels is in its preliminary stages. So far, there has been quite a demand on commencing a large-scale algal biodiesel project in Pakistan by different stake holders, but very little work has been done to date; this is due to the high costs and economic constraints associated with farming algae on a large scale. If a cost-effective method of producing algae on both saline lands and sewage waste water is developed, algal biodiesel could become a major success in Pakistan [28]. The potential of biodiesel production can be made economical by growing microalgae (also called 'kai' in Urdu, the national language of Pakistan) in the ponds build on marginal land, using saline water or waste water. The amount of land required for growing algae is also minimal as compared to other vegetable oil yielding crops. There are many species of microalgae identified, which are suitable for cultivation in Pakistan due to the favourable climatic conditions [46]. In the last decade, biodiesel production in Pakistan from microalgae is getting importance and research is being conducted to cultivate microalgae on marginal land or saline land to avoid competition with the arable land used for growing edible crops [1]. In the country, 28 million hectares of land is unutilized due to water scarcity, high temperature, and soil salinity and can also be utilised for microalgae cultivation. Researchers at HEJ Institute of Chemistry investigated six different species of microalgae and macroalgae to produce biodiesel, namely *Scenedesmus quadricauda*, *Scenedesmus acuminatus*, *Nannochloropsis* sp., *Anabaena* sp., *Chlorella* sp. and *Oscillatoria* sp. They were isolated from fresh and marine water resources of southern Pakistan and the GC-MS/MS analysis of their fatty acid methyl esters (FAMEs) were compared. *Scenedesmus acuminatus* contains the highest oil content (17 % by weight) among all six microalgal strains, whereas *Oscillatoria* sp., was found to have highest biomass productivity rate of 2166 mg/ day/ L. Fatty acid profiling of the biodiesel produced from the microalgal oil from all these six species showed high content of saturated and monounsaturated FAMEs with favourable properties as a diesel fuel. The physical and chemical properties such as density, kinematic viscosity, iodine value, higher heating value and cetane number of the biodiesels were found to be within the permissible range of the international biodiesel standard ASTM D 6751 [33]. The Department of Botany at the Government College University conducted a research investigation, comparing the lipid extraction yield from the macroalgae species *Cladophora* sp. with *Oedogonium* sp. and *Spirogyra* sp. The results showed that *Cladophora* sp. produces a higher quantity of biodiesel than *Oedogonium* sp., and that the biomass productivity rate of *Spirogyra* sp. was also higher than both the algal specimens in comparison. After oil extraction, the biomass may be used for livestock fodder, ethanol production and also in the paper manufacturing industry [22]. In another study, the Sustainable Development Study Centre at the Government College University investigated the biodiesel production efficiency of indigenous *Chlorella vulgaris*, *Rhizoclonium hieroglyphicum* and mixed algae cultures by a transesterification process. The biodiesel yield from extracted oil was calculated as *C. vulgaris* (95%), followed by *R. hieroglyphicum* (92%) and the least yield was by the mixed algae culture (91%). The biodiesel produced from *C. vulgaris, R. hieroglyphicum* and the mixed algae culture samples were characterized by determining kinematic viscosity, flash point, specific gravity, cetane number, iodine value, acid number, carbon residue, sulfated ash, sulphur and water contents. Properties of biodiesel were compared and were found to be compatible with ASTM D 6751 standard [30].

Furthermore, a Pakistani researcher at Mie University of Japan has claimed that the nation could benefit by harnessing its 27–28 million acre saline lands for algal farming, creating employment opportunities and bring benefit to the rural community [47]. Other researchers have identified four strains of algae suitable for cultivation in Pakistan's deserts [28] with satisfactory lipid yields i.e. ~ 40 % by weight: *Haematococcus pluvialis, Microcoleus vaginatus, Chlamydomonas perigranulata*, *Synechocystis*.

However, it was observed that total lipid contents of the microalgae cultivated for biofuels in wastewater may be lower than the one observed in synthetic medium. So a cost-benefit analysis should be undertaken to justify cases in which the nutrients might not be sufficient for supporting algae growth and nutrient supplementation may be required, and the left-over biomass may be characterized for its use in bio-methane production as a carrier for bio-fertilizers, direct application as bio-fertilizers, solid bio-char fuel and mixing with animal feed (especially biomass with higher protein contents). Different species of microalgae have been studied under various experimental designs such as nutrient starvation and heterotrophic conditions to evaluate lipid yield and lipid productivity rate. However, *chlorella* sp. and *Scenedesmus* were found to be the most suitable for cultivation in waste water systems to produce alternative fuels such as biodiesel and biogas [48]. Additionally, agricultural and industrial waste water along with different animal wastes (poultry and cow) as growth medium (rich with nutrients) were used for microalgae cultivation and significant increases in lipid content and biomass yield were observed in mixotrophic conditions [49].

A hypothetical study suggested the cultivation of microalgae in Pakistan with agricultural/ industries waste water effluent containing nutrients such as nitrogen, phosphorus and potassium. The temperature in most parts of the country does not drop below 15 ^{o}C throughout the year and Pakistan is blessed with excellent sunlight intensity ranging from 5-7 kWh/m^2/day during 300 or more clear days per annum and these climatic conditions are ideal for microalgae cultivation. Extensive studies have been carried out to use microalgae for wastewater treatment and simultaneous energy production by utilising microalgal biomass as a feedstock to produce biofuels. The nutrient requirement can be reduced by using wastewater as a growth medium. However, pre-treatment of wastewater is necessary before feeding it to microalgae in order to remove contamination, which is a quite expensive step. Research should be carried out to characterize different types of wastewaters suitable for microalgae cultivation. Moreover, low-cost methods should be devised to control contamination [50].

A research study at Pakistan Council for Scientific and Industrial Research (PCSIR) was initiated on the biofuel production from microalgae. Microalgae samples were collected from both marine and fresh water. A total of sixteen species were identified up to the generic level and isolated for cultivation, out of which twelve belong to marine and four to fresh water. The marine genera identified were *Chaetoceros*, *Thalllassiosira*, *Nitzschia*, *Fragilaria, Cyclotella, Skeletonema, Dunaliella, Chlorella, Nannochloropsis, Spirulina*, and *Oscillatoria*, whereas the fresh water genera identified were *Chlamydomonas*, *Scenedesmus, Zygonema*, and *Stichococcus.* The cultures of the species mentioned above were maintained in the laboratory. The mass culture of only four genera was successful under outdoor conditions. The genera include two marine viz. *Chaetoceros* and *Cyclotella* and two fresh water viz. *Chlamydomonas* and *Scenedesmus* (see microscopic images in **Figure 1**). All genera mentioned above had a rapid growth and wide tolerance to the environmental parameters. The cultivated microalgae was harvested, filtered and dried to analyze lipid yields.
The unpublished results from PCSIR showed that the lipid content of some indigenous microalgal strains, namely *Chaetoceros* sp., *Cyclotella* sp., *Scenedesmus* sp., *Chlamydomonas* sp., and mixed green algae (various species) under normal growth conditions in an open pond was found to be between 1.5 to 16.6 % by weight. Algae can accumulate 1% to 84 % of lipids by weight, which greatly depends upon the type of algal strain and combination of suitable growth conditions such as nutrient level, light intensity, temperature and type of production system. It has been observed that the microalgae species of cold climatic conditions are typically considered higher lipids producers within

temperatures of 15- 25 ^{o}C [51]. However, further investigation is required to get the optimum conditions for higher biomass productivity and lipid yields for outdoor cultivation in the cold season.

Scientists at the National University of Sciences and Technology (NUST) cultivated *Chlorella vulgaris* in a closed photo-bioreactor (20 L) in a controlled environment to produce biodiesel and characterised its properties. Higher biomass productivity was obtained, and the dried biomass was then subjected to supercritical carbon dioxide extraction. The maximum biodiesel yield was achieved at 5,000 and 9,000 psi at 50 and 80°C. The yield of more than 99% is achieved under both conditions. The quality of the biodiesel produced was found to be in accordance with the ASTM D6751[52].

An open pond (approximately 15000 gallons) is used to treat domestic waste water at the NED University of Engineering and Technology for irrigating and watering the plants in the campus. The influent Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values were 260 mg/L and 371 mg/L respectively, while the treated effluent was having values of 256 mg/L BOD and 366 mg/L COD. The results were found to be satisfactory in terms of reducing the organic pollutant load in treated waste water according to the NEQS (National Environmental Quality Standards, Pakistan). The influent and effluent dissolved oxygen levels were 0.42 mg/L and 0.36 mg/L respectively. The quantitative analysis showed algal biomass yield as 0.381 g/L and the total lipid content was 10-15 % by weight, extracted by the Bligh and Dyer method (unpublished results).

The fresh water species such as *Chlorella vulgaris* and *Scenedesmus* are the most promising species of microalgae that can be cultivated in Pakistan, with favourable climatic conditions for their growth [49] and suggest potential to produce biodiesel [53]. It was observed that *chlorella vulgaris* has high flexibility to adapt to diverse culture conditions and was tested as a biofuel feedstock that can be cultivated under phototrophic and heterotrophic conditions, and the biodiesel produced from it complies with international biodiesel standard ASTM D6751 and European standard EN-14214. It can accumulate 63% lipid content when cultivated at 25^{o}C under nutrient depleted conditions [54].

8. Socio-economic analysis of microalgae biodiesel for Pakistan

Pakistan is blessed with a lot of natural resources and those resources can be utilized to bring economic and sustainable environmental stability in the country through renewable microalgal biodiesel production. The economic benefits of a biodiesel industry would include value added feedstock, an increased number of rural manufacturing jobs, increased income taxes, increased investments in plants and equipment, an expanded manufacturing sector, an increased tax base from plant operations, improvement in the current account balance and reductions in health care cost due to improved air quality and greenhouse gas mitigation [14].

The microalgae cultivation on the marginal land of Pakistan (~ 350,000 acres) would not compete with the arable land to grow food crops such as wheat, sugarcane, cotton, and rice, which together account for more than 75% of the value of total crop output. This will bring employment opportunities in the rural areas having around 70% of the whole country's population, facilitating farmers to get their earnings based on utilizing their idle non-arable lands productively. The extracted microalgae oil will be used as a feedstock to produce biodiesel, whilst its de-oiled cake can be used to produce bio-methane by anaerobic digestion for heating and cooking purposes in domestic use. This will provide sufficient support to the energy sector to meet the domestic requirements of the farmers, improve their well-being and bring socio-economic progress to the entire community. Moreover, the microalgae biomass contains high value added products such as carbohydrates and proteins that can be extracted for their use as health/nutritional supplements in the nutraceutical and pharmaceutical industries.

The additional benefit of the biodiesel manufacturing process is the recovery of glycerol as a major by-product (one part of glycerol is produced for every ten parts of biodiesel) [14]. It is used as a feedstock in pharmaceutical, cosmetics, soap manufacturing and for the production of hydrogen fuel. Attempts are also made to use the crude glycerol to cultivate microalgae as a source of carbon, and it can be directly used in combustion, composting, anaerobic digestion, animal feeds, and thermochemical/biological conversions to produce value-added products.

Sustainable development in the renewable energy sector is necessary to reduce dependence on imported fossil fuels, to cut down the crude oil import bill. The importance of environmentally-friendly biodiesel fuel has been realised in the last few decades to reduce burden on the foreign reserves for producing petro-diesel from crude fossil oil. The current daily import of 346,400 barrels/day of crude oil in the country produces 4,156,800 gallons/day petro-diesel (for every 1 barrel of crude oil, approximately 12 gallons of petro-diesel is produced). Using 10% blended biodiesel fuel with petro-diesel as the set target under the National Biodiesel Program by the Government of Pakistan will proportionally reduce the quantity of diesel consumption by 415,680 gallons/day (i.e. saving 34,640 barrels/day of imported crude oil at US$32/barrel and in return saving around valuable foreign reserves of US$1,039,200/day for the country [55].

Developing a focused programme to harness microalgae biodiesel can be extremely beneficial for a developing country like Pakistan, which consumes approximately 8 million tonnes of diesel annually in the transportation and agricultural sectors and in various other industries. Autotrophic microalgae, which are capable of synthesising their own food from inorganic materials using light and producing oil as a by-product, can be cultivated on 350,000 acres of uncultivated tracts of marginal semi-arid land to make biodiesel production commercially viable in Pakistan. The country's solar radiation (5.1-6.2 kilowatt hour/square metre/day) is also best suited for microalgae cultivation which optimally requires 4.52 kilowatt hour/square metre/day. The microalgae are also quite versatile in their use, with its value added by-product in the form of glycerol having a market value of $36/litre (Rs3, 582/litre), and its prime consumers being pharmaceutical, cosmetic and soap industries. Similarly, worthy carbohydrates $14.25/kg (Rs1, 418/kg) and proteins $82/kg (Rs8,159/kg) found in microalgae are used as nutritional supplements [10]. The residual de-oiled cake is biodegradable and can be fermented in an anaerobic digestion reactor to produce environmentally friendly bio-methane which can be used as a cheap burning fuel in the rural areas of Pakistan, where about 70% of the total population of Pakistan lives [15] and can use it for domestic heating and cooking purposes [55].

Approximately, one acre of sunflower produces 350 litres of biodiesel, while one acre of microalgae produces 92,000 litres of biodiesel [56], with glycerol as a by-product. The protein and carbohydrates extracted from microalgae can be used as animal feed and nutritional supplements [57]. The biodiesel fuel provides sustainable benefits to the rural communities such as improved energy security and can be used to run agricultural farm machinery, transporting feedstock, livestock, etc [55].

The potential socio-economic advantages of microalgae cultivation in Pakistan will be [46]:

- As a local feedstock to locally produce biodiesel.
- Can be grown in saline and waste water ponds.
- Its biomass can be burned to produce heat and energy.
- Algae culture grown material can be transformed into bio-methane.
- Biological hydrogen production by algae is also possible for use in fuel cells.
- Pollution-free, greener Pakistan.
- Bring employment opportunities for farmers and labourers.
- Boosting prosperity in the rural areas of the country.
- Reduction in fossil fuel import, saving foreign exchange reserves.
- Bring socio-economic and environmental benefits to the consumers.
- Cheapest raw material to produce liquid and solid fuels for cooking and heating purposes.
- Utilization of uncultivated areas for indigenous energy generation resources.
- Extraction of protein and carbohydrates as dietary supplements.

A simplified process for biodiesel production from jatropha cultivation farm field to supply chain mass balance is calculated, excluding production consumables such as energy, and is presented in **Table 4**. The vegetable oil seed feedstock (100 kg) grown by farmers is crushed to recover oil (approximately 43 % by weight) which comes to 43 kg with a useful by-product i.e. de-oiled cake (57 kg). The resultant extracted crude oil (43kg), along with methyl alcohol (3.57 kg) and 0.43kg caustic soda as catalyst (i.e. 1% by weight of oil), is then used asa feedstock for the biodiesel plant producing biodiesel (38.7 kg) and the co-product glycerol (4.3kg), because the principle byproduct of biodiesel production is crude glycerol, which is about 10% by weight of vegetable oil as mentioned by Yang *et al* [58]. The methyl alcohol and the catalyst used for the conversion of vegetable oil into biodiesel is recovered, i.e. 4 kg as shown in the table, and can be recycled. Furthermore, in order to reduce the production cost of jatropha biodiesel, its de-oiled cake is converted into gaseous combustible fuel with the fermentation of the biomass in a closed reactor in the absence of oxygen, converting the organic matter into bio-methane (biogas) for cooking/heating, lighting and power generation purposes in domestic use. It offers a promising alternative route for converting biomass into bio-gas (whose major constituent is methane gas; approximately 50-75 percent by volume) with an energy value between 20-26 mega joules/cubic meter (whereas the natural methane gas has an energy value of 40MJ/kg). About 20kg of wet de-oiled biomass can produce one cubic meter of biogas/day which is approximately equivalent to generating 6kWh power. The 6kWh power is sufficient energy to power three 100W incandescent bulbs for 20 hours. The residue sludge after producing biogas is then used as a natural organic fertiliser, rich in nitrogen, phosphorus and potassium, for farming edible crops [55].

However, microalgal oil is not affordable at its current price. Thus, there is an urgent need to improve the economics of microalgal biofuels by optimizing the whole process of biofuels production. The cost of microalgae biofuels mainly depends on its cultivation and during cultivation, carbon supply is the most important nutrient source which controls the growth rate (recycling of biodiesel production by-product glycerol as a carbon source for its cultivation can reduce its production cost) and increase its lipid productivity [50]. The cultivation, harvesting and lipid extraction cost can be further reduced by introducing solar thermal as a source of energy to make algal biodiesel production economically viable with respect to petroleum-derived diesel, bringing positive socio-economic impacts on the local economy from microalgal biodiesel production.

9. Cost assessment on microalgae cultivation in an open pond to produce biodiesel

The microalgae biodiesel production includes the following sub processes [59]:

1. Growing the microalgae in engineered ponds.
2. Harvesting the biomass in settling ponds.
3. Extracting the microalgae oil from biomass.
4. Converting the microalgae oil into biodiesel.

Based on the processes listed above by Gallagher [59], the economics of biodiesel production (costs in $ per litre) based on microalgae cultivation in an open pond with dimensions 40 feet length x 20 feet width x 1feet depth (~ 22653 L) capacity for 21 days at PCSIR, and on some realistic assumptions based on its processing, were calculated and are depicted in **Table 5**. The biodiesel production cost analysis does not include the depreciation of machinery and equipment and its maintenance charges. The locally available fertilizers di-ammonium phosphate and urea (nitrogen containing fertilizer) are used as nutrients in the ratio of (1:5) per L respectively. The locally available fertilizers' (nutrients') cost is calculated by multiplying the unit price per kg of urea (Rs. 36/kg) and di-ammonium phosphate (Rs. 40/kg) with the quantity required for the 22653 L pond i.e. 22.65 kg urea and 113.25 kg di-ammonium phosphate respectively. At a biomass saturation stage, approximately after 30 days, the microalgae were allowed to settle under the action of gravity to reduce the energy demand of harvesting [32], and then a few days later the thick algal biomass layer that had settled at the bottom was harvested by cloth filtration through a pump **(**JTP-8500, Sun Sun, China),used for aquarium and swimming pools. The pump flow rate was 141L/min and the time required to empty the 22653 L tank was 160.65 min (2.67 hrs). The pump power consumption (0.330 kW) is multiplied by its operational time and multiplied by the electricity tariff charges to get the harvesting energy consumption. The actual biomass productivity was found to be 30 g/L per 30 days i.e. ~ 680 kg wet biomass by weight was collected, with a moisture content of 75%.

Then the wet algal biomass was dried under the sun (with zero energy demand) and 170 kg dry biomass was recovered. Approximately 35 % by dry weight (i.e. 170 x 35/100= 59.6 kg), the lipids are extracted by a small screw mechanical oil press (YZS-100, ABC Machinery, China). Considering the algal oil density 0.932 kg/L [32], the extracted oil volume was found by dividing mass of lipid extracted with its density (59.5/0.932 = 63.84 L). The processing time to extract lipids from the collected algal biomass was calculated by dividing the quantity of biomass (170 kg) with the processing speed (150 kg/hr) of the mechanical screw oil press. The power consumption of the mechanical screw oil press (7.45 kW) was multiplied by its processing time (1.13 hr) and the electricity tariff rate to calculate oil extraction power requirement as presented in the table. Transesterification was done to convert the extracted lipids into algal biodiesel, its energy demand was calculated by considering methanol usage as 20 % by volume of lipid (12.68 L) and potassium hydroxide (KOH) catalyst as 1 % (0.59 kg) by weight of lipid, plus the assumption of 10% methanol (1.27 L) and catalyst (0.059kg) wastage after the reaction were multiplied with their respective market values (i.e. methanol Rs.1000/L and KOH Rs. 1000/kg). Total amount of energy required for stirring, mixing, heating and in-line heaters was assumed to be 0.28 kWh per L of biodiesel produced by transesterification as presented in previous research work [60]. The total biodiesel produced was around 90% by volume of lipids (57.6 L), while 10 % glycerol was generated (6.4 L) as a byproduct. Therefore, the energy charges for transesterification of 64 L of algal lipids were multiplied with its production utility charges (0.28 kWh per L). The total charges for cultivation, harvesting, lipid extraction and biodiesel processing was calculated based on the wages per month of one skilled worker (Rs.25000/ month), plus a technician with wages (Rs.15000/ month). In order to calculate the revenue generated by de-oiled cakes which were about 65% of the microalgal biomass (i.e. 170 kg dry biomass – 59.5 kg algal lipid = 110.5 kg de-oiled cake), is multiplied with its market value Rs.30 /kg (i.e. cotton seed de-oiled cake price in Pakistan, known as khali in local language, as a fodder for animals). Similarly, the revenue generated by glycerol, that is the quantity of glycerol produced that is 10% of the lipids after transesterification, is multiplied by its market value (i.e. 6.4 L x Rs.3500/L).

The de-oiled cake can be used as animal fodder, while the value added glycerol in pharmaceutical, cosmetics and soap industry to reduce the biodiesel production cost with the revenue generated. The biodiesel processing labour, utilities, fuel and chemicals' costs were included in the input cost. The total biodiesel produced cost was subtracted by the revenue generated and divided by the total quantity of biodiesel produced (57.6 L) to get the total microalgal biodiesel cost in Pak Rupees per litre. The current biodiesel production cost was found to be higher (Rs.371/L) as compared to the price of petroleum derived diesel (Rs.84/L) in Pakistan. This is due to the low microalgae biomass productivity in an open pond and the economic viability of liquid biofuels can be further improved using solar power for its production.

The major economic factor to consider with respect to the input costs of biodiesel production is the feedstock, which is 80% of the total operating cost [14]. Therefore, microalgae biomass yield can be improved to reduce biodiesel production cost by introducing carbon dioxide from the atmosphere into the pond, which also keeps the microalgae in suspension. A study conducted by National Renewable Energy Laboratory (NREL) to establish the feasibility of large-scale algae production in open ponds, introducing CO_2, maintaining temperature, and pH conditions showed increase in biomass productivity. The single day biomass productivities reported over the course of one year were as high as 50 g of microalgae per square meter per day. [61]. Similarly overall production cost of biodiesel could be reduced by utilizing solar thermal energy for different unit operations and, additionally, reducing the facility's carbon footprint. The industrial production of biodiesel is not profitable without government subsidies [14], such as reduction in taxes on equipment and machinery required for biodiesel production and provision of technical knowhow to the farmers to grow microalgae, by arranging training and learning sessions to enhance their working skills.

Extensive research and development has to be done before the production of micro-algae biofuels can play a competitive role in the fuel industry. One requirement to achieve the potential of the micro-algae industry is the development of long-term large scale demonstration projects so it can be shown that it is commercially viable to produce biofuels through micro-algae. Nevertheless, if algae oil can be produced as a by-product of higher valued algal products and, additionally, carbon credits can be used to increase the revenue side of these businesses, algae oil can play vital role in the future as a transportation fuel feedstock [62].

10. Conclusion

Renewable energy technologies can help Pakistan to reduce the gap and overcome the shortfall between energy supply and demand for its growing industrial economy. The indigenous microalgae species such as; *Chlorella vulgaris*, *Chaetoceros* sp, *Cyclotella* sp, *Scenedesmus* sp, *Chlamydomonas* sp and mixed green algae (various species) have normal growth conditions in an open pond cultivation with favourable climatic conditions in Pakistan. The less algal biomass productivity and lower lipid content problem can be tackled through further research and development. Moreover government incentives to the farmers will pave a way forward for an economic and sustainable development in rural areas. The cost assessment analysis showed a five times higher cost of microalgal biodiesel production in Pakistan as compared to the petroleum derived diesel. The high production cost of biodiesel which includes cultivation of microalgae as a feedstock to produce biodiesel can be further reduced by using waste water and marginal land of Pakistan. While the harvesting and lipid extraction processes, can be made feasible by utilizing solar power through naturally gifted sunlight for bringing its cost competitive with petroleum diesel.

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Table 1: Comparison of physical and chemical properties of vegetable and microalgae oil derived biodiesel.

Properties	**Microalgal oil**	**Soybean oil**	**Microalgal biodiesel**	**Soybean biodiesel**	**Test method**
Density (g/cm^3)	0.932[32]	0.914[14]	0.642[33]	0.884[14]	ASTM D1298
Specific gravity	0.934[32]	0.915[14]	0.643[33]	0.885[14]	ASTM D5355
Kinematic viscosity (mm^2/sec)	70.44[32]	32.60[14]	3.30[33]	4.08[14]	ASTM D445
Higher heating value (MJ/kg)	41.38[32]	39.64[14]	39.28[33]	39.48[14]	ASTM D2015
Cetane number	-	-	49.80[33]	46[14]	ASTM D 976
Cloud point (°C)	-	-	0 [35]	-5 [34]	ASTM D2500
Pour point (°C)	-	-	-11[35]	-9 [34]	ASTMD5853
Flash point (°C)	-	255[14]	145[30]	168[14]	ASTM D93

Table 2 shows the comparison of emissions from petroleum diesel and microalgae biodiesel [37]

Gases	**Petroleum diesel**	**Microalgal biodiesel**
Carbon dioxide %	3.70	3.79
Carbon monoxide %	0.10	0.09
Unburned hydrocarbon ppm	28.96	19.75
Oxides of nitrogen ppm	25.71	21.87
Oxygen %	15.30	21.87

Table 3: Comparison of fatty acids composition of algal oil and soy bean oil [43]

Fatty acids	**Algal oil**	**Soybean seed oil**
C16:0 Palmitic	51.0	8.0
C16:1 Palmitoleic	0	8.0
C18:0 Stearic	2.0	3.0
C18:1 Oleic	39.0	18.0
C18:2 Linoleic	7.0	49.0
C18:3 Linolenic	0	6.0
Molar carbon to hydrogen ratio (C:H)	0.516	0.554

Table 4. Simplified biodiesel production field to supply chain mass balance (all values in the table below are in kgs) [55].

	Inputs	**Crushed seeds**	**Biodiesel production**	**Outputs**
Oil seeds from field	100	100	-	-
Methyl alcohol + catalyst (caustic soda)	4	-	-	4
De-oiled cake	-	57	-	57
Crude vegetable oil	-	43	43	-
Bio-diesel	-	-	38.7	38.7
Crude glycerol	-	-	4.3	4.3
Outputs	104	-	-	104

Table 5. Biodiesel production unit cost from microalgae cultivation in an open pond (unpublished results from PCSIR and NED University are shown here)

Processes	**Sub processes**	**Power x time (kWh)**	***Cost (Pak Rs)**	**Revenue (Pak Rs)**
Cultivation	Nutrients (1N:5P)/L	-	5345	-
Harvesting	Sedimentation by gravity	-	-	-
	Cloth Filtration (pumping)	0.33x2.67=0.88	14.08	-
Drying	Solar thermal	-	-	-
Lipid extraction	Mechanical press/ expeller	7.45 x 1.13=8.44	135.0	-
Transesterification	Methanol @20% by volume of lipid (assuming 10% methanol wasted)	-	1276	-
	Catalyst @1% by weight of lipid (assuming 10% KOH wasted)	-	59.5	-
	Processing utility energy charges of (heater + mixer) per 64 L litre of algal lipids	0.28 x 64 =17.92	286.72	-
Labour charges	Including cultivation , harvesting, oil extraction and biodiesel processing per month	-	40000	-
De-oiled cake	@65% de-oiled cake recovered (170kgs dry biomass)	-	-	3315
By-product glycerol	@10% glycerol produced (i.e. 64L x 10%= 6.4L)	-	-	22400
Total biodiesel production cost i.e. conversion of 90% oil (64L) into biodiesel = 57.45L			47116	25715
Cost of producing 1 L of microalgal biodiesel (in Pak Rupees/L)			Pak Rs.371/L biodiesel produced [US$ 3.56/L]**	

*Cost = (Power x time) kWhr x Rs.16/ kWhr, where electricity tariff rate is 1 kWhr = Pak Rs. 16 [63]

** (1US $= Pak Rs.104)

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