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Potential of microalgal biodiesel production and its sustainability perspectives in Pakistan

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ABSTRACT

Pakistan has a strong potential of biodiesel production if the available feedstock resources are used sustainably and implementable policies are made in appropriate direction. To meet the energy demands and to find alternative and non-conventional resources of energy different challenges like research and development, infrastructure development, decentralized type of power delivery system, commercialization, market development, education and outreach programs, public awareness, monitoring, subsidies, government participation, technology transfer and evaluation must be considered and a comprehensive policy must also be made to systematically control and integrate them at national level. Pakistan is enriched with a wide variety of feed stocks which can be used for biodiesel production. Pakistan has an enormous potential of biodiesel production from jatropha, plants seed oil and microalgae which needs more consideration and practical applications. Harvesting the potential of microalgae for biodiesel production in Pakistan can be helpful to make it self-sufficient for energy demands. Pakistan is also facing several challenges like climate change, lack of financial resources, state of art technology and absence of appropriate government policies, which limit the commercialization of biodiesel. Although Government of Pakistan has established different institutions to promote and develop alternative energy technologies and to achieve 10% share of bioenergy in the energy sector by 2020, but still the targets are to be achieved on practical grounds. In this article, we have reviewed the potential of biodiesel in Pakistan, feed stocks, biodiesel production process, barriers and future developments. Future policies on biofuels, trends, recommendations, and the implication of existing policies are also discussed with research and developments goals for the promotion of biodiesel in Pakistan.

1. Introduction

The world is facing serious energy crisis in this century due to increased industrialization and overuse of natural resources such as fossil fuels. Fossil fuels comprise 88% of the global energy consumption [1]. The shares of oil, coal and natural gas are 35%, 29% and 24% respectively. It was estimated that there would be approximately 53% increase in energy demands by the year 2030. It is expected that in USA alone, the petroleum demands may escalate to 116.00 million barrels per day by 2030. With the same pattern of consumption for coming 40 and 60 years may result in depletion of most of the oil and gas reserves [2]. The use of fossil fuel resources imparts serious negative impact on the environment. Burning of fossil fuels generates greenhouse gases which aggravate the global warming. It is estimated that the burning of fossil fuel contributes the maximum share in the emission of greenhouse gases [3]. By 2006, the fossil fuels associated CO_2 emissions were 29 billion tones. CO_2 emission also affects the ecosystem biodiversity [4]. For example, 1/3rd of emitted CO_2 is absorbed into the oceans, which change the water pH. The change in water pH results in the death of marine species. Depletion of fossil fuels is essential to be addressed for the energy security, climate changes and sustainable development [5]. In view of extensive dependence on non-renewable sources for energy, there is dire need to utilize the alternative sources of energy. These sources should hold the trait of sustainability and green economy. In this perspective, biofuels have emerged as potential alternatives [6,7].

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Biofuels are promising alternative source of energy as they are prevalent in nature. They are renewable and available throughout the world. Biofuels can be obtained from existing biological resources [8]. In coming decades the share and utilization of biofuels in the motorised fuel market is more expectedly to grow rapidly. Therefore, biofuels production is expected to grow fast in coming years [9]. European countries have made reasonable policies in this context and planned to utilize about 5.75% of biofuels at the end of 2010 and 10% till 2020. High crop productivity can also play an important role in the energy budget. The global biodiesel production was estimated as 5.8 billion litters in 2006 [10]. As far as the global biofuel production is concerned, different countries differ in biofuels production shares they add i.e. Germany (48% of total), other European countries (30%), USA (15%) and several other countries, such as Brazil, China, India, Canada, Colombia, and Malaysia, almost have 7% share [6].

According to the estimates of 2007, about 7% of global vegetable oil production was required for biodiesel plants in European Union [11]. The global vegetable oils production reached about 115 million tonnes in 2006 of which the basic share was among few countries i.e. Brazil, China, India, Indonesia, Nigeria, Philippines, Pakistan, Thailand, USA, and Uzbekistan contributing about 80% of the total production. USA and Brazil were the leading producers in the same years producing about 8 million tonnes of tallow which is the most important animal fat [12].

Biofuels production has a rich history. Through past few decades, a wide range of feed stocks have been examined for biofuels productivity. For biodiesel production, feed stocks are grouped as first generation (G1), second generation (G2), and third generation (G3) biofuels. In the following section, we will discuss these feed stocks in detail [13,14].

This article is a review of the barriers in production, future developments and potential of biodiesel production in Pakistan. Future policies on biofuels, trends, recommendations, and the implication of existing policies are also discussed with research and development (R & D) goals for the promotion of biodiesel production in Pakistan. The review overviews the feed stocks i.e. G-1, G-2 and G-3, suitability and technology implications for biodiesel production in Pakistan.

The current review presents up-to-date information on biodiesel production from various feed stocks with special emphasis on micro-algae as substrate. Structurally, the paper comprises of 11 sections. The overview of the biodiesel feed stocks is presented in Section 2. In Section 3, the mechanism of microalgal biodiesel production is elaborated. Sections 4 and 5 describe advancements in methods of biotechnology and lipids metabolism in microalgae, respectively. Sections 6, 7 and 8 are based on the future policy on biofuels, midterm policy for biofuels and biodiesel policy recommendations for Pakistan, respectively. Detailed biodiesel policy recommendations are given in details in Section 9 and the impacts of biofuels on socioeconomics and Pakistan's potential of biodiesel and bioethanol are elaborated in detail in Sections 10 and 11 respectively.

2. Biodiesel feed stocks

2.1. First generation feed stocks

First generation feed stocks for biofuels are mainly oil seeds and food crops. Feed stocks such as soybeans, rapeseed, sunflower and palm oil comprise first generation feed stocks because they were primarily used to produce biofuels [15]. Biodiesel production from aforementioned first generation feed stocks is easy as these are obtained by simple pressing of oil-bearing biomass [16]. Energy generation is not restricted by technological limitation; instead it could be increased by increasing feed stocks. For improving domestic energy security, first generation biofuels can offer some benefits concerning carbon sequestration. Different energy demands by source are expected till 2030 worldwide (Fig. 1). On annual basis, almost 50 billion liters of

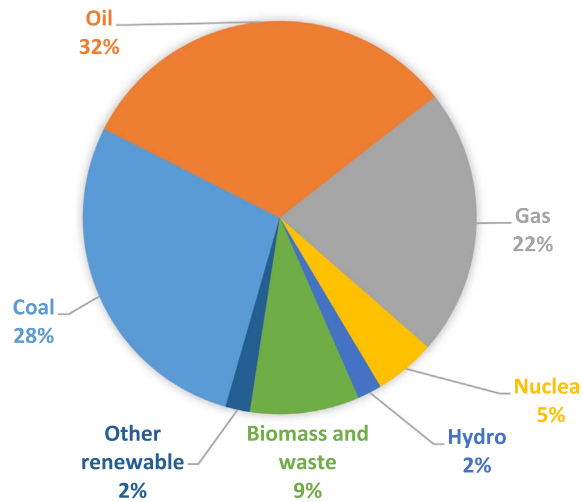

Fig. 1. Different energy demand by source expected till 2030, worldwide [22].

1st generation biofuels are produced [17]. First generation biofuels can be categorized on the basis of either their potential to be blended with petro-fuels or to be combusted directly in engines. These can be circulated through existing infrastructure, or through utilization in alternative vehicle technologies like Flexible Fuel Vehicle (FFVs) or in vehicles using compressed natural gas (CNG) [18].

Based on their macromolecular composition, the first generation feed stocks consisting of vegetable oils and animal fats are mainly composed of triglycerides and di-glycerides as major constituents and mono-glyceride as a small fraction. The vegetable oils are long chains molecules with multiple alkyl branches with increasing molecular size. The relative molecular weight of vegetable oils ranges from 850 a.m.u. to 995 a.m.u. which is much higher than diesel (168 a.m.u. on average) [19,20]. The chemical formula for common diesel fuel is $C_{12}H_{23}$. After reducing the viscosity and specific gravity of vegetable oils they can substitute petro-diesel. For this purpose different techniques are examined and employed. Among these, transesterification is commonly employed technique, which is the most reliable, most feasible, and can easily be used for biodiesel production. It has many advantages over other processes e.g., it is performed under normal conditions and it returns good quality and quantity of biodiesel [21].

Trans-esterification is a catalytic chemical method in which the triglycerides are converted into di-glycerides which are then converted into mono-glyceride in the presence of methyl or ethyl alcohol and from an ester linked molecule called biodiesel [19]. The production of biodiesel from oil through trans-esterification mainly depends upon the nature of feedstocks exploited, quantity and kind of catalyst, alcohol, operational temperature, and chemical reaction time [23]. Various procedures have been investigated for the production of biodiesel from vegetable oil and upon the chemical analysis of the product. It was evident that the chemical properties were similar to that of petro-diesel. The methods of biodiesel production are: transesterification, microemulsification, cracking, blending, and pyrolysis. Trans-esterification is the chemical conversion of the oil into fatty acid methyl esters (biodiesel). The viscosity of vegetable oil is also reduced through the process of trans-esterification, therefore it is widely used [24].

The transesterification reaction occurs in the presence of suitable homogeneous catalysts i.e. base catalyst such as potassium hydroxide (KOH) or sodium hydroxide (NaOH) and base catalyst such as sulfuric acid, or heterogeneous catalysts such as metal oxides or carbonates. NaOH is well-known and widely employed because of its low-cost and high product yield efficiency [25]. The following factors influence trans-esterification process: reaction temperature, ratio of alcohol to vegetable oil, amount of catalyst, mixing intensity, raw oils used, and catalyst [26]. Among first generation feedstocks, rapeseed oil (especially) has the highest prospective to be utilized as a fuel for diesel

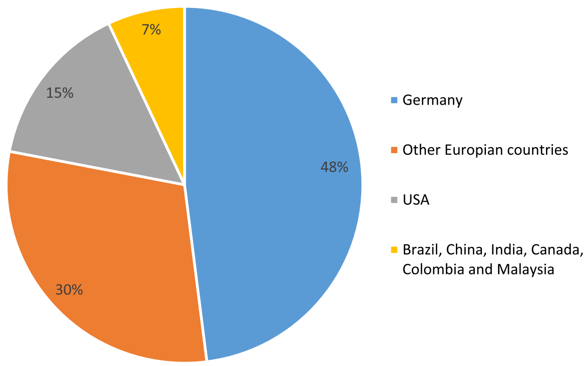

Fig. 2. Worldwide biofuels production share.

engines. From the studies on rapeseed based biodiesel, it is evident that it has the lowest emission levels and brake specific fuel consumption rates [27]. Keeping in view the concept of environmental protection, bio-resource sustainability and economic viability, food processing wastes, especially in waste edible oils, seem to be attractive. Oil produced from food waste has low carbon, sulfur and nitrogen contents. Thus, rapeseed oil causes less pollution and health risk as compared to traditional fossil fuels [1,28] (Fig. 2).

Despite sustainable character of first generation biofuels, there are some disadvantages associated with them. Scale-up of first generation biofuels can result food insecurity, water scarcity, soil degradation, deforestation and biodiversity loss [29]. Deforestation in tropical countries such as Indonesia and Malaysia were investigated, and it resulted from the production of biofuels after about 80% the world's supply of palm oil [30]. In past few years, this trend was assessed after the large-scale deforestation mainly caused by the over increase of oil crops production to meet the world's biofuels demand. Ultimately, the application of biofuels as a temporary fuel for petro-diesel could lead to widespread harm to the environment and wildlife [31]. Although the biofuels are the alternatives of the petroleum based fuels but their wide spread usage has caused a lot of problems like global food security, depletion of fresh water resources and deforestations [32].

One of the major issues that need to be addressed is the food versus fuel crises. A vital part of human food is based on palm and soy oils. An imbalance to the global food market could be observed if diverting food crops to harvest oil on large scale biodiesel production [33]. These oils have the limited ability to achieve targets for biodiesel production. In the edible oil market their extensive use as biofuels may cause competition, which increases the prices of both edible oils and biodiesel. Hereafter, biofuels production from palm oil is not a sustainable option [34].

2.2. Second generation feed stocks

The second generation biofuels feed stocks comprise whole plant tissue, including energy crops or agricultural remains, wood residual wastage. There are few energy crops which represent few examples of second generation feed stocks such as *Jatropha* sp., *Madhuca longifolia*, salmon oil, tobacco seed, jojoba oil and sea mango. Waste from cooking oils, restaurant grease, animal fats, beef tallow and pork lard can also be named as second generation biofuels feed stocks [35]. Over the past few years, non-edible oil crops have also been investigated for the extensive biodiesel production [36].

The second generation feed stocks are more efficient and environmentally friendly than the first generation feed stocks [37]. Animal fat methyl esters have some advantages over first generation feed stocks, such as higher octane numbers, non-corrosiveness, cleaner and renewable properties. They eliminate competition for food and feed. They require lesser land area and mixed crop cultivation can be used. Non-edible oil crops can be cultivated in non-farm lands which are not appropriate for food crops. Because of the presence of few toxic compounds in non-edible oils, they are not suitable as humans food [38].

The problem with second generation feed stocks is the lack of efficient technologies for the commercial exploitation of wastes for biofuels production. Furthermore, the majority of animal fats contain high concentration of saturated fatty acids, which renders trans-esterification, difficult [39]. Due to comparatively low performance of biodiesel in cold temperatures they are not able to fully displace the present day transport fuels [40]. The usage of animal biodiesel feedstock from contaminated animals can also cause bio-safety issues.

Biodiversity in forests is affected by the extensive wood extraction and forest residues that remove nutrients from soil and also cause runoff which perhaps has a negative influence on the water availability [10]. The energy potential of second generation biofuels will be significantly low if the biomass resources are excluded from bioenergy source selections [41].

2.3. Third generation feed stocks

Microorganisms are considered as third generation biodiesel feed stocks. A wide range of microorganism can be usedfor this purpose. Microalgae are being considered as the most promising choice for biodiesel production. Algae are divided into two major groups based on their size and morphology: macroalgae and microalgae based on their thallus size [42]. Kelps serve an example of marine macro-algae which have multiple cells, resembling roots, stem and leaves of higher plants. On contrary, microalgae are prevalent both in fresh and marine water [43]. Microalgae are photosynthetic autotrophs, mixotrophic and heterotrophic microscopic organisms [7]. Their life style may be colonial or as free living; their photosynthetic ability makes these organisms fascinating for using them in industrial processes to produce special chemicals and nutritional products [44]. On the basis of carbon source, autotrophic microalgae use inorganic carbon while heterotrophs use organic carbon source. Both microalgae (autotrophs & heterotrophs) vary in their biodiesel yield [7]. Table 1 shows the oil yield of different microalgae species [45].

Microalgae possess some unique properties. They are eco-friendly, demand less area to grow. They are also rich in oil contents. In comparison to growing food crops fodder and similar products, microalgae don't require huge land area [46]. Microalgae are the only organisms known so far capable of both oxygenic photosynthesis and hydrogen production. Microalgae are grown in artificial as well as natural environments. Wild algae i.e. natural inhabitants are required for this purpose. Microalgae have simple growth requirements i.e. use light, carbon dioxide and other inorganic nutrients efficiently and are capable of growing in diverse environments [47]. Microalgae also have

Table 1
Oil contents of different microalgae strains [7,8,50].

Microalgal species	Oil content composition (%)
Ankistrodesmus TR-87	28–40
Botryococcusbraunii	34–75
Chlorella sp.	50
Chlorella protothecoides (autotrophic/ heterotrophic)	40–55
Dunaliellatertiolecta	33
Hantzschia DI-160	66
Nannochloris	25
Nannochloropsis	35–47
Nitzschia TR-114	28–42
Phaeodactylumtricornutum	20–28
Scenedesmus	34
Stichococcus	32–40
Tetraselmissuecica	20–35

Table 2
Advantages and disadvantages of first, second and third generation feed stocks for biofuels production.

Feed stock	Advantages	Disadvantages	References
First generation (G1) (Vegetable oils, corn, sunflower oil etc.)	• Environmentally friendly • Economic and social security	• First-generation biofuels are directly related to a biomass that is generally edible • Limited feed stocks (food vs fuel crises) • Blended partially with petroleum diesel	[8,60,122]
Second generation (G2) (Agriculture and forest residues, grass, aquatic biomass, Waste Vegetable Oil (WVO), *Jatropha* and *Eshornia* etc.)	• Not competing with food • Reduced cost of conversion • Environmentally friendly • They are perennial and so energy for planting need only be invested once • They are fast growing and can usually be harvested a few times per year • They have relatively low fertilizer needs • They grow on marginal land • They work well as direct biomass • WVO can decrease engine life if not properly refined	• Grasses are not suitable for producing biodiesel • They require extensive processing to made into ethanol • It may take several years for switch grass to reach harvest density • The seeds are weak competitors with weeds. So, even though they grow on marginal land, the early investment in culture is substantial • They require moist soil and do not do well in arid climates	[8,60,123]
Third generation (G3 [124]) (Algal biomass)	• Algae can use a diverse array of carbon sources • Total carbon emissions would be reduced substantially • Ease of scale up Technology readily available • Low water use • High flexibility to strain selection (closed system cultivation) • No caustic chemicals needed in oil separation from algal biomass	• A minor drawback regarding algae is that biofuel produced from them tends to be less stable than biodiesel produced from other sources • The oil found in algae tends to be highly unsaturated. Unsaturated oils are more volatile, particularly at high temperatures, and thus more prone to degradation • The cost of algae-base biofuel is much higher than fuel from other sources. • Scalability (depends on PBR type) • Technology no demonstrated on large-scale	[4,125,126]

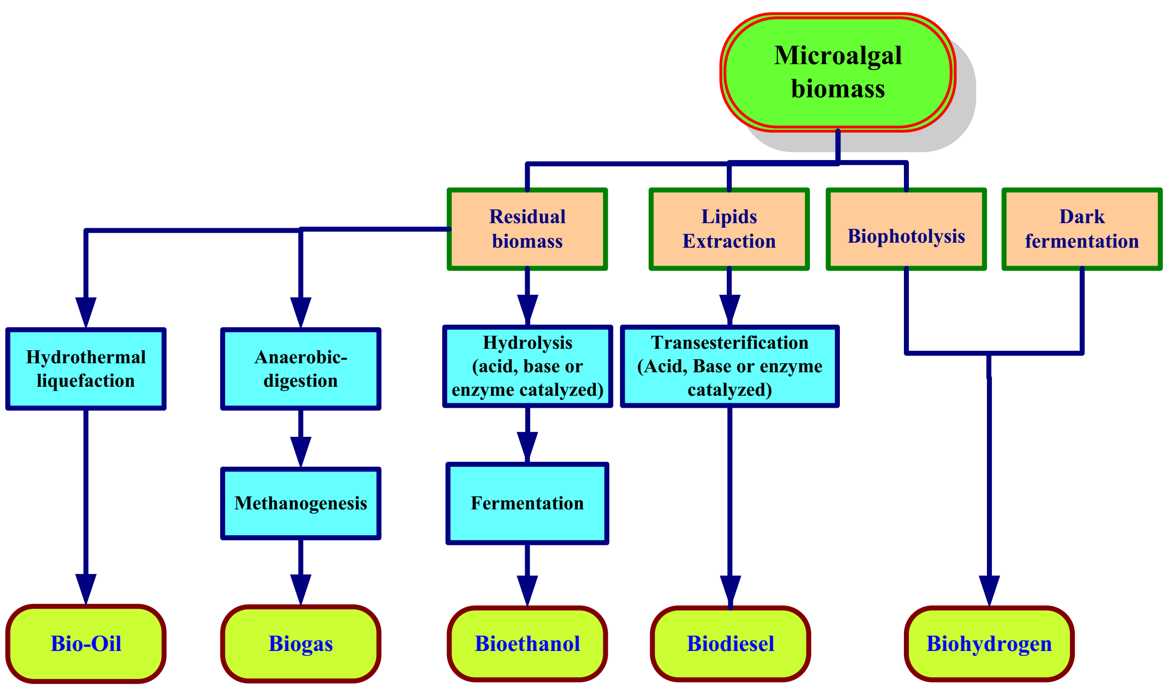

Fig. 3. Biofuels production process in comparison with biodiesel production.

the ability to mitigate air pollution by reducing CO_2 level in the atmosphere. They use CO_2 which is acting as greenhouse gas in the atmosphere. It is estimated that 1.8 kg of CO_2 is required for producing 1 kg of algal biomass [38].

The oil content of some microalgae exceeds 80% of the dry weight of algal biomass and according to Oilgae (2010) some have about 15–40% (dry weight). In comparison, the oil content of palm kernel is about 50%, copra has 60%, and sunflower contains 55%. In fact, microalgae have the highest oil yield among various plant oils. It can produce up to 100,000 l of oil per hectare per year, whereas palm, coconut, castor and sunflower are reported to produce 5950, 2689, 1413 and 952 litter per hectare year, respectively [6,48]. Various biofuels can be produced using microalgae. They can produce methane, hydrogen, bio-ethanol and biodiesel through various processes. Microalgae-based biofuels can be used in existing fuel engines without any modification [49]. Some advantages and disadvantages of different feed stocks for biofuel production have been described in Table 2.

Properties of petroleum based diesel like cold filter plugging point, density, flash point, heating value, solidifying point and viscosity are almost similar to the microalgal biodiesel. Most of the parameters fulfill the requirement of American society for testing and materials (ASTM) for biodiesel quality [50]. The requirement of international biodiesel standard for vehicles is also fulfilled by microalgal diesel. The comparison between fossil oil and bio-oil produced by fast pyrolysis of wood and microalgae has shown that microalgal bio-oil has high heating value, low density and low viscosity as compared to the bio-oil (obtained from wood) [51]. Microalgal bio-oil is preferable to use than lignocelluloses-based oil due to its high quality. The presence of higher concentration of polyunsaturated fatty acids in microalgal oil as compared to vegetable oil will results in the oxidation during storage which will limit the oil utilization [52].

The potential of photosynthetic cyanobacteria and microalgae to produce biofuel is both economical and environmental friendly. It can decrease our dependence on fossil fuels as energy source [53]. Many eukaryotic microalgae have the ability to store significant amounts of energy-rich compounds, such as triacylglycerol (TAG) and starch that can be used for the production of several distinct biofuels; including biodiesel and ethanol [52]. A scheme of biofuels production process has been shown in Fig. 3.

Microalgae can remove nitrogen (N) and phosphorus (P) from sewerage wastewater mainly through uptake into cells. Microalgae have higher tendency to remove inorganic nutrients from wastewater and to produce higher quantities of green biomass. Naturally this process occurs in lakes as eutrophication. Eventually, microalgal biomass can be harvested for lipid extraction and biofuels production. If cultivation is coupled with wastewater treatment then a considerable amount of biofuels can be produced to overcome the energy crises.

There are few fresh water microalgal species that are investigated for wastewater treatment i.e. *Scenedesmus* and *Chlorella*. They can potentially remove excess of N and P from wastewater. Microalgae have many advantages over other feed stocks, in the removal of nitrogen and phosphorus. There are the following advantages:

- They are low-cost due to solar energy requirement
- Can simultaneously fix CO_2
- As compared to biological nitrification and denitrification, they do not need extra organic carbon source
- They have very less problems of sludge handling problems
- They increase the concentration of oxygen in the water bodies i.e. increase dissolved oxygen level

As compared to petro-diesel, biodiesel properties are similar to normal diesel so it can be used directly in diesel-burning engines with less emission of carbon monoxide (CO) or sulfur oxides (SOx). Besides, microalgae have high photosynthetic ability and can efficiently produce high lipids content. As compared with conventional crops, the lipid productivity per unit dry biomass of microalgae is about 15–300 times. Therefore, microalgae are recognized as promising substitute for petro-fuels in the future.

Biogas is made by anaerobic digestion of organic biomass i.e. methanogenesis. Therefore, it needs specific cellulosic and hemicellulosic sources. For this reason, microalgae are the promising source. The anaerobic digestion of microalgae was firstly examined in the 1950s by Oswald and Golueke in California, USA. The researchers then used microalgal biomass from different sources i.e. high rate ponds and harvested the biomass for biogas production. And they obtained considerable results.

In context of anaerobic digestion microalgae is a potential resource that seems to be the most directly energy producing process. The most important parameter for anaerobic digestion is the cell wall characteristics that can determine the methanogenic efficiency of the substrates. (Table 3)

As compared to other substrates like sewage sludge the literature on microalgae usage is very limited. However one of the major drawbacks of microalgal anaerobic digestion is the hydrolysis of cell walls (cellulose and hemicellulose). In recent years, the pre-treatment of substrates to enhance the anaerobic biodegradation needs very intensive research. Various pretreatment processes are effectively investigated i.e. chemical, thermal and mechanical processes (i.e. ultrasounds and microwave). They provide positive results for efficient disintegration and anaerobic biodegradability of sludge. They also provide a positive energy balance of the reported processes.

Yuan et al. [8] showed that anaerobic digestion is a promising method to treat the blue green algae from eutrophic lakes. In addition, some scientists [9,10] from countries with cold climate are also interested in microalgal cultivation for biogas production. As shown by Collet et al. [3], the coupled process of microalgae cultivation and succeeding biogas production is a better option compared to microalgal biodiesel production. However, it still needs fertilizer supplements and the investment of biogas infrastructures [3].

3. Mechanism of microalgal biodiesel production

Light and nutrients are the essential components of microalgal growth. Microalgae consume nutrients in the presence of light and convert them into organic compounds. Microalgal cultivation is affected by aeration, carbon source, light, nutrients composition, temperature, pH and photoperiod. A number of culture media have been introduced for microalgal cultivation. Nutrients pose high cost on microalgal cultivation. Alternatively, wastewater can be used as a growth medium for microalgae cultivation and growth [52]. Microalgae can use wastewater as a nutrients source and the use of wastewater for microalgal cultivation can serve dual purposes i.e. pollutants removal and biofuels production [53]. Sea water is considered as one of the economical way for microalgal cultivation. Sea water contains the major nutrients for their growth. Although there are more than 70 elements present in sea water but six constitute over 99% of all dissolved salts. All of these occur in ionic form including Na^+, Cl^-, Mg^{2+}, K^+, SO_4^{2-} and Ca^{2+} [54].

Light requirement of microalgae is species dependent. Generally, majority of the microalgal species grow well at 4000 to 20, 000 lx. Light can be supplied through fluorescent tubes, light emitting diodes (LED) and optical fibre. For externally illuminated photo-bioreactors optical is reported to show high performance, whereas LED are suitable for externally illuminated photo-bioreactors. In natural environment, sunlight is the energy source for photosynthesis [55]. Light permeation in microalgal culture decreases with time. As a result, the cells suffer light limitation in exponential phase of microalgal growth. To overcome light limitation, different mode of light supplement can be applied [4]. A schematic diagram of microalgae cultivation in Fig. 4 explains various parameters which affect microalgal cultivation and growth.

Slow growth rate is a major bottleneck towards the application of microalgae-based biofuels. Slow growth prolongs the total time of microalgae cultivation, and thus increases the cultivation cost. Attempts are made to overcome this limitation by manipulating the effect of light and nutrients [55]. Growth rate could be reduced; however, it causes to decrease the lipids contents. As there is a trade-off between microalgal growth rate and oil yield. Therefore it is needed to search such microalgal strains which are capable of growing fast and accumulating more oil content [53]. The details of downstream process for microalgal biodiesel production are described in Table 4.

The primary concern is the selection of suitable algal species which can grow in a specific culture and which will be able to produce desired products. Harvesting of microalgae can be done by centrifugation, flocculation, membrane filtration and micro screens. The water from harvested cells could be dried under vacuum conditions until constant dry weight is achieved [49]. The oil content of microalgae during drying remains in cells which can be extracted out of cells using oil press and solvent extraction methods. Chemicals like hexane and benzene can be used for microalgal oil extraction coupled with press methods. This combination of chemicals and physical methods can result in extraction of more than 95% of the total oil content is present in the cells.

Table 3
Advantages and disadvantages of biodiesel from microalgae oil [63,127,128].

Advantages	Disadvantages
• Higher growth rates • Grows in diverse areas • Has higher per-acre yield than other best crops i.e. palm oil • There is no need to grow crops like palms to obtain oil • There are few algal species which can be harvested on daily basis • Biodiesel obtained from algae biofuel have no sulfur • Algal biodiesel is non-toxic in nature • Algal biodiesel is bio-degradable in nature • Oil extracts from algae can be used both in bioethanol and biodiesel production • Algal biodiesel can reduce carbon emissions • Biodiesel produced from microalgae has similar chemical composition as compared with petro-diesel	• With respect to many polyunsaturated fatty acids molecules, the obtained biodiesel is unstable • Relatively new technology, difficult to be adopted in developing countries • The technologies are expansible • In dry biomass oil extraction the major problem is extensive use of organic solvents i.e. hexane • The scale up technology is very expensive • In lipids extraction procedures from wet algal biomass the presence of water medium possess a great challenge • The total cost on biodiesel production from algae is much higher as compared with same amount of petro-diesel • Difficult to be installed at industrial scale

Fractionation of oil is very easy when enzymatic withdrawal to disrupt cell wall is carried out [56].

Pulverization of dried biomass is done using chemicals and supercritical fluid. Using supercritical fluid is another technique to extract oil. In this technique, CO_2 is liquefied using pressure and heat until it attains the properties of both liquid and gas. CO_2 in this liquid state acts as a solvent for oil extraction. Soxhelt extraction is one of the most commonly used methods for oil extraction [57]. This method uses hexane as a solvent. The extracted oil is converted into biodiesel by trans-esterification. Biorefinery or co-product strategy is an emerging way to produce variety of biofuels and chemicals from microalgae by integrating environmental friendly chemical technology and bioprocessing [58].

4. Genetic engineering to improve microalgae cultivation

Recently, genetic engineering is a powerful tool to improve microalgae cultivation. The growth rate, biomass yield, and oil contents can be enhanced by using this technique. In recent past, major advancement in the field of microalgal genomics and genetic engineering enabled scientists to develop methods for genetic modification of microalgae [49]. The development of microalgal model systems is one of the major advancement in genetic tools. These systems can be used to engineer the carbon metabolism of microalgae. These advancements can be extended to industrial levels by using potential organisms [59].

Deep understanding of genetic manipulation of metabolic networks which are involved in production of several important compounds is crucial. The claim of Arizona state researches of finding a method to produce biofuels using genetically programmed microbes, which can self-destruct themselves after photosynthesis. Therefore it will be an easy and cheap way to produce biofuels [60]. These self-destruction genes were taken from bacteriophage through recent developments like metabolism, proteomics and systems biology. Structural and functional genomics are being applied. Higher quantity of genomic sequencing involved in microalgae metabolism is one of the examples which permits to catch several pathways. These metabolic pathways are mainly comprised of inorganic carbon fixation, fermentation, protection expression and vitamin synthesis. These pathways can be designed in direction to enhance the production of desired product [18].

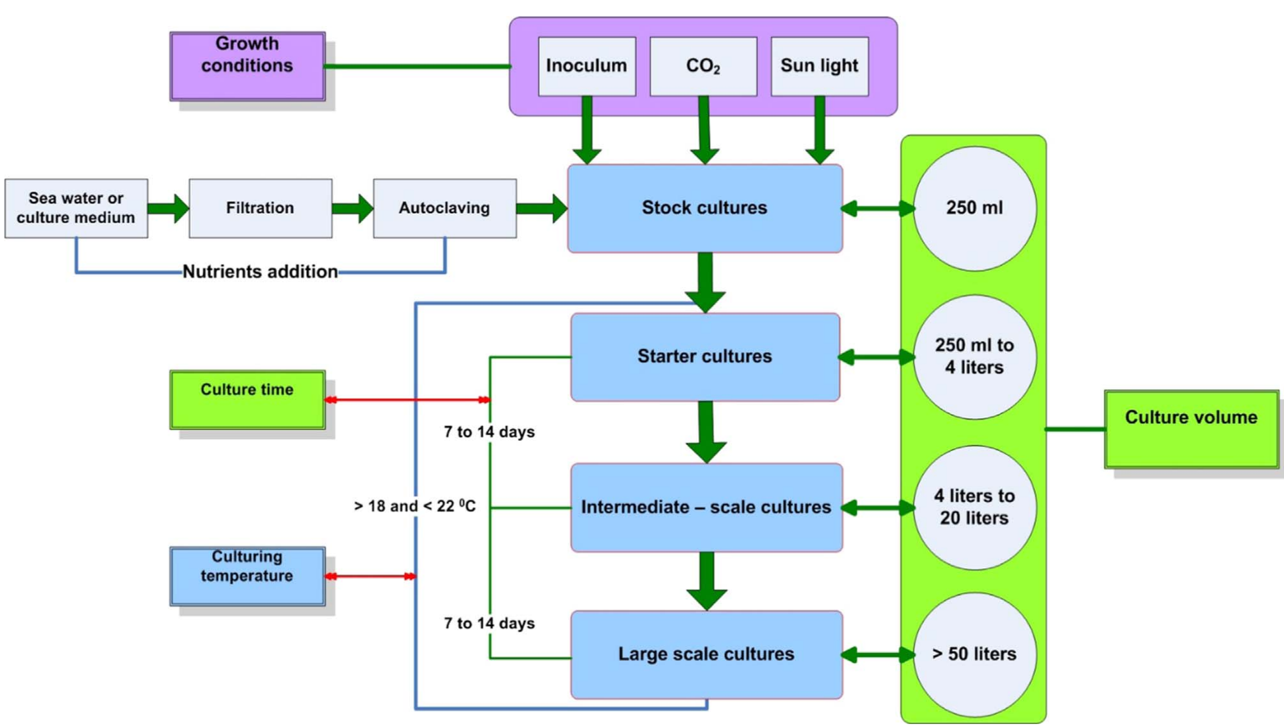

Fig. 4. Microalgae cultivation.

Table 4
Downstream processing of microalgae through cultivation, harvesting and lipids extraction for microalgal biodiesel production.

Process	Technology	Macroalgae cultivation plants	Construction and designs	Operating parameters	Type of microalgae	Culture medium/ nutrients	Efficiency	References
Cultivation	● Raceway ponds	● Paddle wheel-driven ponds	● 4875 ha of raceway ponds ● 1463 MLD (385 MGD) of water handling capacity ● 15 g m^{-2} d^{-1} microalgal growth rate ● Harvesting rate (10%)	● Harvesting rate ● Biomass lipid content ● Daily oil production ● Extraction efficiency ● Required daily biomass (DW) ● Total biomass in raceways ● Required growth surface area for daily production ● CO_2 recovery to culture	● *Chlorella vulgaris*	● Redfield ratio elemental composition of $C_{106}H_{181}O_{45}N_{16}P$ for g^{-1} -algae DW a) 525.1 mg C g_{-1} b) 91.9 mg N g_{-1} c) 12.7 mg P g_{-1}	● Production of 585 t of lipid extracted algae (LEA) per day	[129]
	● Photo-bioreactor	● Bubble-column photo-bioreactor	● cylindrical glass column ● 4.8 mm wall thickness ● 10.5 cm inner diameter ● 70.5 cm height ● Total volume of 6.1 Litter ● 20 W, cool white fluorescent lights	● Light ● CO_2 ● Nutrients ● Water	● *Cyclotella sp.*	● Modified Harrison's artificial seawater medium	● 51% lipids productivity increase in 72 h	[130]
	● Photo-bioreactor	● Tubular photo-bioreactor	● Inner diameter (D) = 80 mm ● Length (L) = 1000 mm ● Blank zone = 50 mm	● Light/dark cycles ● CFD	● *Chlorella sp.* ● *Porphyridium cruentum*	● BG11	● 7% increase in surface productivity ● 37.26% increase in biomass productivity	[131]
	● Batch mode cultivation	● Aluminum crimp sealed serum bottles ● Incubator shaker	● 500 mL volume capacity ● 200 mL filtrate ● 150 rpm shaking speed ● 27°C temperature ● 15% CO_2 ● Alternate light/dark periods of 16 h/8 h	● N/P molar ratio ● TN (mg L^{-1}) ● TP (mg L^{-1}) ● Total carbon (mg L^{-1}) ● Cell dry weight (g L^{-1}) ● Cultivation time (day)	● *Chlorella vulgaris* ● *Scenedesmus obliquus* ● *Ourococcus multisporus*	● Modified BG–11 ● Modified Bristol ● Municipal wastewater ● Modified DM ● Modified f/2-Si	● > 99% of nitrogen and phosphorus removal within 4 days ● lipid productivity (0.164 g-lipids g-$cell^{-1}$ day^{-1})	[132]
	● Mixotrophic batch cultivation ● Autotrophic batch cultivation	● 200-mL Erlenmeyer flasks ● 6-L bench-top stirred bioreactor	● Automated turbidimeter	● pH ● Total suspended solids (g L^{-1}) ● Total dissolved solids (g L^{-1}) ● Total COD (g L^{-1}) ● Soluble COD (g L^{-1}) ● Total sugars (g L^{-1}) ● Total nitrogen (g L^{-1})	● *Chlorella vulgaris*	● MBM ● SW ● TS	● 43% (g oil/100 g biomass)	[133]
	● Mixotrophic growth	● Plackett–Burman experimental design	● 250 mL Erlenmeyer flasks ● 1 L Roux culture bottles ● 100 rpm ● 25 ± 2 °C ● Illumination at 100 µmol/s m^2	● Light intensity (µmol/s m^2) ● Lighting period (h) ● Biomass concentration ● FAME content ● COD ● TP ● TN	● *Chlorella* ● *Haematococcus* ● *Scenedesmus* ● *Chlamydomonas,* ● *Chloroccum*	● BG–11 ● Centrate wastewater	● 2.01 g/L net biomass accumulation ● 25% of TVSS as FAME content ● 100% ammonia removal ● 85% heterotrophic growth	[134]
	● Photoautotrophic–	● Orbital shaking	● 250 mL-Erlenmeyer	● optical density (OD)	● *Chlorella sp.*	● Brewery wastewater	● 90% total nitrogen (TN)	[135]

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Table 4 *(continued)*

Process	Technology	Macroalgae cultivation plants	Construction and designs	Operating parameters	Type of microalgae	Culture medium/ nutrients	Efficiency	References
	photoheterotrophic/ mixotrophic cultivation	incubator	flasks ● orbital shaker agitation at 150 rpm ● 25°C ● Illumination at 100 l µmol/s m^2	680–680 nm ● Time (day) ● Cell count (10^6) ● Total nitrogen (TN) concentration (mg/L) ● Total phosphorus TP concentration (mg/L)		(BWW)	and phosphorus (TP) removal ● 1.5 g/L of maximum biomass production ● 18% maximal lipids content production	
Harvesting	Flocculation	● Flocculation followed by sedimentation	● 1.5–6 h time ● 1 g/L of $Al_2(SO_4)_3$and $ZnCl_2$	● Time ● Concentration ● pH ● Optical density (OD 600–680)	● *Chlorella minutissima*	● BG–11	● 60% Recovery efficiency of biomass	[136]
		● Batch mode chemical flocculation	● Aluminum sulfate and ● pH adjustment using HCl	● Time ● Concentration ● pH ● Optical density (OD 600–680)	● *Chlorella vulgaris*	● BG–11	● Concentrates up to 357 times that of the original dry weight	[137]
		● Aminoclay-induced humic acid flocculation	● Mg-aminoclay and Fe-aminoclay dosages ● 180 min time	● 5 g/L aminoclay loading ● UV–Vis absorbance ● Optical density (OD 600–680)	● *Chlorella sp.*	● BG11	● Approximately 100% harvesting efficiency	[138]
		● Batch mode jar test chemical and bio-flocculation	● Metal salts (alum, ferric chloride) ● Cationic polymer ● Anionic polymer (E–38) ● Natural coagulants (*Moringa Oleifera* and *Opuntia ficus-indicacactus*)	● Productivity (g/m^2/day) ● Biomass density (g/L as TSS) ● TN removal efficiency (%) ● Effluent TN (mg/L) ● NH_4^+ ● Removal efficiency (%) ● TP removal efficiency (%) ● Effluent TP (mg/L) ● COD removal efficiency (%)	● *Chlorella sp.*	● Centrate from the anaerobic digestion of municipal sludge	● > 91% harvesting efficiency	[139]
	Gravity sedimentation	● Batch mode gravity sedimentation	● Settlement of biomass, ● Optical Density in range of 0.620–0.820 at 685 nm and took ● 1 h time	● Time ● Concentration ● pH ● Optical density (OD 685)	● *Chlorella vulgaris*	● BG–11	● 60% of biomass recovery	[49]
Pre-Treatment: cell disruption	Ultrasonication	● Batch mode experiments ● Ultrasonic disruption of microalgae cells	● Sterile tissue culture flasks ● 16 min sonication ● 20 °C algal growth temperature ● 12 h photoperiod ● A 20 kHz VCX 600 Probe ● 40% amplitude ● 30 °C sonication temperature	● Frequency ● Temperature ● Time ● Haemocytometer, ● Optical density, UV–Vis Spectrophotometer ● Fluoro-spectrophotometer ● Confocal microscopy	● *Dunnaliella salina* ● *Nannochloropsis oculata*	● f/2 growth media	● ~90% reduction in cell numbers ● ~5–10% reduction in chlorophyll levels ● 5–40% declumping	[140]
	Microwave	● Batch mode experiments ● Cell disruption	● 30 mL universal vessel ● Operational frequency of 2.45 GHz	● Cycles per minute ● Thermal radiations intensity ● duty cycle	● *Nannochloropsis oculata*	● Guilliard medium ● Modified F/2 medium	● 94% cell disruption	[141]

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Table 4 (*continued*)

Process	Technology	Macroalgae cultivation plants	Construction and designs	Operating parameters	Type of microalgae	Culture medium/ nutrients	Efficiency	References
			● 90 °C temperature ● 25 s time	● microwave power				
Lipid extraction	Wet lipid extraction	● Batch mode experiments ● Harvesting and centrifugation for harvesting	● GE Plant ● Aquarium Ecolux lights ● 100 mg dry mass equivalent samples of wet algal biomass ● 1 M sulfuric acid solution ● 5 M sodium hydroxide ● Solution ● 90 °C ● 30 min time	● Type of solvent (hexane) ● Biomass to solvent volume ration ● Methanol for transesterification ● Direct Transesterification (positive control) ● Wet extraction (total)	● *Chlorella* ● *Scenedesmus sp.*	● modified SE media	● 79% extraction of transesterifiable lipids	[142]
	Extraction yield and properties	● DME extraction method ● Bligh–Dyer's (BD) method	● Extraction column (diameter 2.6 cm, length 18 cm) ● Liquefied DME storage tank (volume: 100 cm^3) ● Flow rate of 10 cm^3 min^{-1}	● Lipids / dry weight of the microalgae [%] ● Molecular weight ● DME amount / total lipids ● Ash yield (± 0.1) ● Volatile matter (± 0.4) ● Fixed carbon (± 0.4)	● *Oscillatoria agardhii* ● *Monoraphidium chlorophyta* ● *M. aeruginosa* ● *Microcystis wesenbergii*		● 99% lipids extraction from dry weight	[143]

5. Lipid metabolism in microalgae

Understanding microalgae lipid metabolism is crucial for producing biodiesel. Lipid metabolism control influence both the quality and quantity of biodiesel. As compared to the terrestrial plants the lipid production and metabolism, and also, those pathways which after modification results in fatty acids saturation are not clearly understood in microalgae. Fortunately, the genes in terrestrial plants which take part in lipid metabolism are homologous to microalgae genomes. Due to this homology, it is possible to use the same strategies which are used for lipid contents modification in microalgae [18].

Nutrients stress is among the most effective methods to increase lipids content in green microalgae and diatoms. In the past, microalgae with nitrogen depletion medium resulted in production of high lipids in the form of triacylglycerides while cultures deficient in phosphorous and sulfur resulted in production of neutral lipids from membrane phospholipids [61]. In order to make lipid profile compatible to biodiesel some of the strategies can be used to engineer fatty acids biosynthesis in microalgae e.g. lipid secretion from cells to media. Over expression of those enzymes which are involved in fatty acids biosynthesis, increase in the precursor molecule availability e.g. acetyl CoA, inhibition of β-oxidation or lipase hydration for down regulating fatty acids catabolism, introduction or regulation of *denatureses* by saturation profiles alternation, using *thio-estterases* for optimization of fatty acids chains length [27,62].

ACC gene overexpression alone might not result in enhancing the pathways for whole lipid biosynthesis. In eukaryote, β-oxidation is the primary metabolic pathways that degrade fatty acids. Therefore, blocking this pathway could results in enhancing TAG biosynthesis. Some researchers recommend the over expression of more than one enzymes are involved in TAG pathways. Rate of genes transcription may become higher when there is an overexpression of the factors which interact with specific enzymes. Regulation of the genes responsible for lipid synthesis may up or down due to transcription factor in genetic engineering [63].

6. Future policy on biofuels

According to IEA, alternative policy scenario in 2030 about 7% of biofuel production will contribute to future fuel. Although this increase in biofuel production is very high but in agriculture sector biofuels contribution is not much. According to the European Union's Biomass Action Plan, it is necessary to ensure the certification, which proves that bioethanol imported is made from land crops that were grown in sustainable environment.

Due to limited contribution of biofuel in agriculture, it is predicted that the liquid biofuel production will increase from 14 million hectares in 2004 (which is only 1% of world cultivated land area) to 53 million hectares in 2030 which is 3.8% of land used for agriculture and it depends highly on feedstocks [8,9]. Incentives should be given to the biofuel producers. In the global trading regime, social and environmental standards are now considered at first stage. The linkage of such compulsions with sustainable development may affect the link to trade. For bioethanol import Netherlands and UK are implementing these certification schemes [64].

For the commercial applications of biofuels, government policies are the major barriers. So it must be based on freedom. There should be no politics in free market for biodiesel, as it happened in the past regarding petro-fuels. For the producers from all sectors and of all capacities, the market should be open and there must not be any discrimination. For the commercial production and utilization of biodiesel, there must be subsidy on cultivation of no-food crops that will definitely facilitate the growth and production of feedstocks [65]. The cost of biodiesel can be decreased by the developing new and advanced technologies, feedstock yield increase and by growing economic returns on glycerol production. Investors show interest in

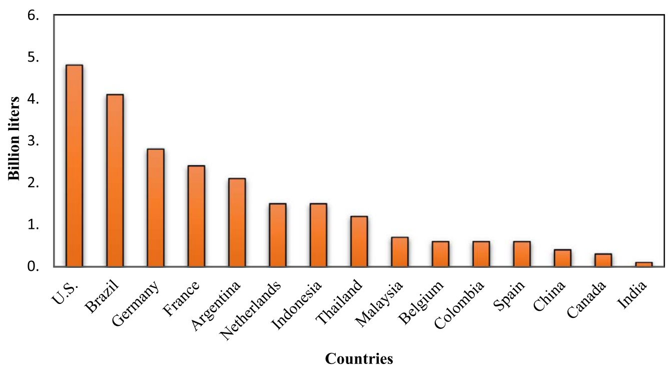

Fig. 5. Biodiesel Production in Leading Countries of the World, 2015 [68].

energy crops for business growth. Therefore, the biodiesel project may be made successful by taking forward initiatives.

Biofuels and bioethanol stood undisputed issues in the World Trade Organization (WTO), which complicated trade of products. According to experts, few energy producing countries have been members of the WTO which never truly investigated energy issues. As biofuels constitute a small percentage of the world's energy supply so it got warranted even less consideration. Biodiesel is still classified as an industrial product [66]. But there is uncertainty about bioethanol for its uses. As according to WTO bioethanol classifies as an agricultural product. Therefore according to different classification approaches there are two types of biofuels, i.e. bioethanol and biodiesel, because there is a wide range of materials from which they are made. Moreover, there are many advantages of using bioethanol i.e. reduced greenhouse gaseous emissions from automobiles and increased vehicle performance and efficiency [67]. The worldwide production share of biodiesel is summarized in Fig. 5.

For bioethanol also, there is no clear policy and custom classification. For example, industrial alcohol is traded and sold under the code 22-07 which is same for both of denatured alcohol i.e. HS-22-07-20 and HS-22-07-10. For bioethanol production, both kinds of alcohol can be utilized. [63]. The European Union (EU) and the USA have their trade agreements which allow different countries to trade under differentiated market access conditions. Consequently, the local industries have begun to bear the losses, many of them have stopped their bioethanol production [69].

Moreover, there is no trade-off for bioethanol and food production. The environmental benefits of using biofuels have been documented internationally. In the distilleries, a closed carbon cycle is employed for bioethanol production, which appears to be environment friendly [70]. Under the revised General System of Preferences (GSP), European Union imposed tariffs leading to the closure of distillery plants. Further, the development and global growth of the industry may become complicated due to institutional uncertainties and unresolved issues that are still present for bioethanol classification. Future growth of bioethanol as a renewable energy source is compromised due to domestic biased policy and export barriers [71].

In European countries like Germany and France, biodiesel is exempted from fuel tax. Europe is on the top regarding the biodiesel production in which Germany, France, and Italy are the leading countries. Whereas in Europe, Spain is leading in the production of bioethanol. Beside these countries other countries in different continents i.e. North America, South East Asia and Brazil have put forward new policies. Therefore, the scientists are getting more opportunities for projects scale-up and commercialization [72]. In 1999, USA has accepted a comprehensive standard policy on industries biodiesel. From this policy the consumer confidence was increased on biodiesel as it is a comparatively innovative trend. In USA, the role of Environmental Protection Agency (USEPA) is remarkable as, it played a key role in promoting biodiesel to the substitution of petro-diesel [73,74].

According to Mukherji and Sovacool [75] environmental and socioeconomic factors have strong impacts on the biofuels production from palm oil in Indonesia, Malaysia and Thailand. According to the policy recommendations palm trees must be planted as potential feed stock in favor of biofuels production according to land laws. Similarly, Indonesia has made policy to produce biofuels till 2025 to achieve 5% increase in basic energy supply [76].

After Brazil, Pakistan is the second largest industrial alcohol exporter to the EU under the system of GSP. Pakistan and Guatemala were indicted by the Commission of Industrial Ethanol Producers of the EU (CIEP), for dumping ethyl alcohol in the European markets, which caused material damage to domestic producers in May 2005. After a year, the European Union forced tariffs on imports from Pakistan. The major tariffs were particularly on bioethanol and feedstock (raw molasses in case of Pakistan) which differentiates against the final product [77].

In energy crops business and commerce investors find the opportunities, but in Pakistan institutional weaknesses, investment cost, management indiscretions, and absence of appropriate government policies are the main hurdles in the development of renewable energy sector. In order to achieve long term socioeconomic benefits, it is necessary to promote renewable energy technologies with comprehensive policy structure and implementation strategies. In recent decades, government of Pakistan has realized the importance of renewable energy sector for the improvement of socioeconomic conditions and green energy sector. In this regards, following policy recommendations were given to improve renewable energy sector in Pakistan [78]:

- On the long term, renewable energy technologies must be included in national energy policy
- Development of lab scale models and commercial level for the provision of adequate resources
- Provision of incentives to the entrepreneurs in the field of renewable energy
- Subsidies must be provided in the form of loans to end users
- The provision of subsidies and loans to the end users
- Provision of proper training and education to urban and rural communities
- Increasing the use of biofuels in blended and pure forms
- Allocation of federal and provincial budget for the promotion of bioenergy sector
- Integrating biofuels with conventional energy resources

7. Biofuels perspectives in Pakistan

Pakistan is an agriculture based country as it shares 24% in GDP. The population of country is more the 180 million and has the 2.05% annual growth rate. It will be the fourth largest country with respect to population in 2050, as estimated by economic survey of Pakistan in 2010. In Pakistan fuel poverty has increased due to low per capita GDP of Pakistan (US$ 2600). Goods have become out of reach for the majority of population due to un-expected increase in fuel prices. Due to this situation unsustainability in the society is increasing [79]. The economic and solid stability of a county is directly related to the energy availability and its progress depends on the per capita energy consumption. And in this scenario, Pakistan appears to be energy deficit country [80,81].

For the promotion of renewable energy in Pakistan various initiatives were taken in past decades, although their results are still pending due to lack of sound policy and corruption. In 1975 Pakistan Council of Appropriate Technology (PCAT) was established for the development and improvement of food and energy sector, residencies, solar cookers and water desalination for health improvements [82]. In 2001, The Pakistan Council for Renewable Energy Technologies (PCRET) was established with an aim to enhance research and development in renewable energy sector of Pakistan. The main objectives of PCRET are to develop carbon free energy technologies for cleaner environment. PCRET is involved in the research activities in the areas of biogas, solar PV, solar thermal, micro-hydel and wind energy. Research and development activities of PCRET mainly cover; community scale solar dryers, laboratory for solar thermal and PV products testing, in remote areas of Pakistan establishing solar electrification, solar lights and establishment of energy training centers. Similarly, Alternative Energy Development Board (AEDB) established in 2003, is also working in Pakistan for the improvement of green technologies which can result in the reduction of greenhouse gases emissions and for the promotion of renewable technologies by means of different projects which are also recognized at international level by International Solar Energy Society (ISES) and the World Wind Energy Association (WWEA) [78].

As compared to its current supplies energy demand has exceeded radically in Pakistan. Due to extensive growth in population and increasing energy demands over last few years Pakistan is facing energy crisis. No effort is done to raise the energy supply in order to meet the energy demands due to which energy supply and demand gap is increasing every year [80,83]. According to an assessment the energy demand in the country will increase three times up to the year 2050. Unfortunately, considering the demand, the supply is not adequate. In Pakistan, oil and gas reserves are insufficient and can only meet the demands for the next 19 and 10 years, respectively. Due to the global trend in the last 5 years the price of fossil fuels have increased exaggeratedly, and commercial energy and electricity generation of Pakistan relies heavily on fossil fuels [84,85]. In Pakistan, the exploitation of microalgal biomass for biofuels production has an enormous potential which could effectively minimize the energy dependence on conventional sources. A brief overview of strength, weaknesses, opportunities, and threats (SWOT) analysis is given in Table 5 for microalgal biofuels production.

About 80% of energy needs of Pakistan is met by importing petroleum oil. Gas, oil and hydro power electricity are the primary energy sources, whereas small fraction of energy is shared by means of coal and nuclear energy. According to the estimates of 2005-06, the commercial energy sector shares 50.3% gas, 29.8% oil, 11.01% hydro, 7.6% coal and 1.2% of nuclear energy [86]. As it is evident from the breakdown of Pakistan electricity supply, thermal power plants have the share of energy consumption of 64% of the total energy demand; whereas, hydropower share about 33% and nuclear power share about 2.4% of the total energy shares. With respect to domestic energy sector it utilizes 44.2%. Industrial sector uses 31.1% and agriculture sector uses 14.3% of total energy produced in the country [87].

The rapid increase in energy prices has become a serious problem for the common people of Pakistan. For example, the price of petrol in year 2007 was $0.9 per liter. In the fiscal year 2006, Pakistan has imported petroleum products which cost nearly 3.1 billion USD. This appeared to be near to 85% of total oil consumption. In 2008, Pakistan was facing 40% of total electricity shortage which is near to over 4000 MW. In order to fill the existing energy gap between energy supply and demand, there is an interim need to take serious and meaningful steps to increase the power generation capacity in Pakistan [88]. Definitely shifting the trend towards ethanol fuel will save the considerable foreign exchange of the country. The blending of 10% ethanol with petrol can save 300 million USD of foreign exchange. Pakistan State Oil (PSO) and the Hydro Carbon Development Institute of Pakistan (HDIP) have launched a pilot project to check the applicability of blended fuel in order to meet the energy shortfall within the country [81,89].

Pakistan has a unique geographical location and suitable climate, so

Table 5
Strength, weaknesses, opportunities, and threats (SWOT) analysis for microalgal biofuels production.

Strength	Reference	Weaknesses	Reference
• Carbohydrates and proteins are the main components • Multiple purpose applications • Disposal is Environmental friendly • Their pretreatment is easy as compared with cellulosic biomass • Due to high energy recovery they can decrease the biofuel cost • They can be exploited at large scale • The frequent use of firewood and charcoal can be reduced by biogas • Approximately up to 20% burden on the forest resources consequently can be reduced • A wide range of food and feed products can also be produced from algae as co-produced • Fresh water usage can be avoided • Several GHGs can be captured and their emissions reduced • Foreign investment could lead to revenues leaving the country	[8,60,144,145]	• Pretreatment of biomass is a complicated process, because it depends upon upstream processes • Pretreatment biomass is specie dependent process • Due to lack of in-depth information there are always chances of uncertainty • For greater energy outputs the routes of SMAB use are not particularly optimized • Downstream processing of microalgal biomass is very least studied for industrial scale up • Processes are not cost effective at industrial scale • Downstream processing is not cost effective • Most of the technological barriers are of fundamental importance • Accessibility of energy is reduced at higher fuel prices • Access to this technology to the poor community may be difficult • A renewable source of nutrient is needed	[145,146]
Opportunities	Reference	**Threats**	Reference
• Multipurpose applications in various directions are possible • For bio-sorption there is no need of surface modification • in combination with primary production process they can easily be integrated • they are widely and abundantly available • in anaerobic processes they can easily be digested • The sites and areas of the production system is also of importance for the economics • Can effectively reduce CO_2 load in atmosphere • Lacks eutrophication can also be converted into algal biofuels production • Several waste streams can be treated • Sewerage wastewater can also be treated and grown algal biomass be used in biofuels production • Large amounts of land with a low economic and ecological value can be used • There is a high potential of synergy with fish cultivation • In developing countries there is a large potential in territorial waters	[62,147]	• Complicated and diverse nature of pretreatment processes • Their future is uncertain • Numerous studies of all procedures are not available • Complicated downstream processes can reduce the efficiency • Competition with the agricultural sector for inorganic fertilizers is expected to increase • Food security is a major threat • Water is a limited resource and a shortage • The availability of energy becomes of crucial position to economic growth if used in algal biofuels production	[147,148]

there are many options for the solar and wind energy production. But due to lack of state policies, presently the production of wind and solar energy is very low. The efficiency of biomass energy is low due to non-scientific traditional methods which are mostly practiced in rural areas. Crop waste, animal waste, and tree wood are most commonly used domestic fuel for cooking and heating purposes. Huge coal reserves can play a major role in overcoming energy crisis but unfortunately, they are not utilized yet. Over the last two decades the development of new hydropower generation projects have increased, and unfortunately nuclear power contribute only 3% to the total electricity supply of country [90,91].

To overcome the energy needs in Pakistan, biofuels can be better option. However, the possibility of biofuels production and utilization has not been exploited yet. Industrial production of biofuel has been not given much attention. Bioethanol can be produced by using molasses sugar. Unfortunately out of more than 70 sugar mills in Pakistan, only six of them have got the facilities until 2007 to produce bioethanol from raw molasses. Over 400000 t of ethanol can be produced annually in Pakistan using sugarcane crop. However, for bio-ethanol production in Pakistan there is no sound policy. Biodiesel could be another potential biofuel source in Pakistan [85,92].

Ethanol is being blended with gasoline in a 1:9 ratio (E10) in three PSO petrol pumps (one each in Karachi, Lahore and Islamabad) on trial. Public sector is concerned over the molasses exports. Therefore, molasses exports should be banned. Beside this subsidy on bioethanol production can overcome the fluctuation of molasses prices. But unfortunately government has not made any reform on the real grounds. Pakistan State Oil (PSO) has been directed by the government to carry out a background study on the viability of bioethanol usage [89,90,93]. It is evident from the government decision that bioethanol promotion mandate was situated within the Ministry of Petroleum and Natural Resources rather than the Ministry of Environment or the Ministry of Industries. Pakistan presently earn around 100 million USD in foreign exchange exports after exporting 160,000 t of industrial bioethanol and alcohol, which is well lower than the potential incomes [86,94].

Nevertheless, the recent increase in fossil fuel exports have exerted a pressure on the economy of the country. Molasses continues to be exported in bulk while industrial alcohol and fuel-ethanol are the value-added components, having considerably higher price. In the form of a high central excise duty and sales tax on fuel alcohol an obstinate domestic policy subsidizes to this suboptimal presentation [80].

At least 5% of biodiesel as a blend is mandated by 2015 in Pakistan. In Pakistan, the biodiesel industry cannot totally depend upon the food crops. One of the potential source for biodiesel production is plants oil. Castorbean is a plant which grows in arid and semi-arid areas of Pakistan. It is among the highest oil content crops that grow in the country. It is suitable due to its solubility in alcohol and it requires less energy for conversion through trans-esterification into biodiesel. So, it is considered as one of the most suitable and un-explored source for biodiesel production in the country [95]. Likewise in Pakistan, microalgal biomass could potentially be used for biodiesel as compared to other plants oil. The details of microalgal biodiesel production are given in previous Sections 2 and 3. With little attention, this resource can become one of the major biodiesel source. Pakistan should also promote the technical requirements for commercializing biodiesel from miceoalgae, so that the industrial sector could be made up to date and aligned with the exact technical necessities. From this the adaptation towards biodiesel can be accelerated by creating an environmental awareness, Pakistan can work on the parallel lines. To achieve sustainable development by the government, organization and mobilization of the environmental groups is helpful [63,85]. Therefore, an efficient cultivation of lipid rich microalgal species in raceway ponds and photobioreactors is required to achieve the sustainable supply of biodiesel for coming decades so that food consumption may not be effected. In this senario, the government would have to arrange microalgal cultivation systems in the coming years, so that later on microalgae could be cultivated instead of growing plants. This will pay to the country in the shape of cost-effective energy system [90,96].

8. Short term policy for biofuels in Pakistan

In 2006, the Mid Term Policy (MTP) was subjected for updates. So that MTP will succeed the current short term Renewable Energy policy (REP) in present year. So that the tools of this policy will assist to improve the expansion of the household renewable energy industry next to 2014, and ahead, which upcoming strategy route can then be settled. The MTP is the product of a many years progression, which implicated the expert, consultants and participants from all over the Pakistan, and also, from many other countries to share the best practices and learned lessons [97,98].

This Policy was a good at the start of 2006 and emphasized on solar, wind and small hydropower projects. Beside this, other Asian countries were much forward than Pakistan. Moreover, in the beginning of 2007, world oil prices increased to a dramatic rise. Meanwhile, incentives for renewable energy investment were provided to those countries which have already settled their policies with implementation of rules and regulations [99,100]. Later on in late 2008, the world trade and industry conditions began to change so that the oil prices were declining. Followed by main troubles in the banking and finance sector the United States housing market collapsed. Direct foreign Investments decreased as the credits begin to tighten. Simply the policy was a good at the beginnings 2006 but later on it was overtaken by unexpected events. In 2007-08 the MTP formulations started in and experts from the USAID, Asian Development Bank, and GTZ helped to revise the new future policy [98,101].

To increase the renewable energy resources MTP aimed to assist the work of different government departments. For this reason, the energy crisis increased the use of renewable technologies in Pakistan was initiated. The main objectives were to facilitate markets and to attract private sector investment by giving incentives and investment; and to endorse the productive use of energy resources and income generation actions. It was intended to help in broad technical, institutional and equipped competence building [97,102]. In short term the Medium Term Policy was built through expanded description of alternatives and renewable. Which deal with the concern of stakeholders, determined policy conflict, included the teachings learned and developed the concept that alternative and renewable energy are essentially promoted. For biofuels, it also presents a policy which expanded incentives through inventive finance i.e. the alternative energy development fund [103,104].

The main incentives given comprises of partial resource risk coverage, tariff on the basis of a premium rate of return for are projects, mandatory use (biofuels), mandatory purchase requirements, mandatory grid connection, AEDF, ADB loan guarantee facility, SBP small ARE facility (< 10 MW), credit market facility and 100% carbon credits to IPP [103,105]. For the profitable and commercial application the government policy on biofuels is a main obstacle. Based on autonomy the government strategy and policy must be devised. There should be no politics implicated as which had happened in past concerning petroleum, so it is important to make a policy which is helpful in free marketplace for biodiesel [106].

For all capacities and potential of the producers the market should be open. On the bases of farm, regulations there should be no favoritism and discrimination. For the production, manufacture and also for the utilization of biodiesel the subsidy on cultivation of no-food crops would be given. The cost of biodiesel could be decreased through the advances of novel and fresh technologies, by increasing feedstock capitulate and by rising the economic returns and profits on glycerol manufacturing [9,63,106].

9. Biodiesel policy recommendations

Pakistan has a strong potential of biofuels production if the natural resources are used sustainably and policies are made in proper direction. According to a study based on testing Environmental Kuznets Curve (EKC) hypothesis for analyzing the potential of renewable energy in Pakistan based on the data encompassing the period between 1970 to 2012, a fanatical support was found for EKC. According to the previous results renewable energy plays a dominant role in the reduction of CO_2 emissions and alternatively nonrenewable practices are the main contributors of CO_2 emission. Therefore, government must encourage the expansion in the investments on renewable energy projects for reducing and mitigating the causes of global warming and climate change. [107]. The main economic dependence is over agriculture in Pakistan by 70% of the population in the country. The living standards of people can be uplifted by production of oil seed crops. At small scale less investment is required for production facilities that can be helpful in the biodiesel production [96]. For oil crops production, the barren land areas could be exploited that can overcome the problems of water shortage and soil salinity. For the benefits of farmers as well as for the country's economy growing energy crops can be beneficial. For the successful commercial and mainstream biomass energy technology establishment in the rural areas of Pakistan, the use of single technology is required instead of multiple energy technologies [90].

To ensure biomass feedstock availability in the country, there is need to invest in infrastructure, equipment and in research and development sector to shift energy mix in Pakistan. In order to solve the issue related to energy crisis, climate change and sustainable development in Pakistan, the adaption of the clean and renewable energy is essential [81,108]. Serious and extensive research is required to promote biomass energy production in the country. In Pakistan energy needs like electricity generation, vehicles fueling, home heating, and industries can be fulfilled by the efficient use of available biomass resources. Due to growing population and increased per capita electricity consumption, fast urbanization and strong economic growth, Pakistan in the last 20 years has become the rapid growing power markets in the world [88].

The major contributors of energy crisis of Pakistan are fast energy demands, low competence of energy resources, high costs of energy imports, rapid industrial demands and increased population growth rate. Due to rapid population growth the electricity demands for housing and industries had increased. Rural area of the country which comprises about 62% of total country population depends mainly upon on non-commercial resources [81]. Economic decline have occurred in the country due to unexpected energy crisis during the last five years, the resulting. There is less efficiency while high losses due to the lack of scientific technologies while utilizing the energy sources [96]. By utilizing the renewable energy sources in the developing countries the long term energy issues faced can be tackled. It is significant to enlarge the present resources and to discover new sources in order to make these resources sustainable. In Pakistan, the utilization and conversion into useful energy is quite low from the bio-resource potentials. There is an indirect impact on the environment because of the low efficiency in the form of high carbon emissions [80].

Ministry of Water & Power Policy in Pakistan gave the recommendations based on its use biodiesel as an alternative fuel in February 2008, which was considered by The Economic Coordination Committee (ECC) of the Cabinet. Then they approved the proposal and mentioned in Para 4 of their summary. According to this summary The National Bio-Diesel Program will be coordinated by Ministry of Water & Power along with AEDB to provide the facilities. The biodiesel will be blended up to 5% of the total volume till 2015, and up to 10% by the year 2025. To reach the fuel quality standards for B-100 and blends up to B-20 Ministry of Petroleum & Natural Resources will assist [98,109]. To ensure the cost-competitiveness of biodiesel with Petroleum Diesel OGRA will be responsible for the pricing mechanism of various blends of Biodiesel (B-5, B-10 etc.). To make it compulsory for public sector vehicles running on petro-diesel to use biodiesel, at a price determined by OGRA, the Government shall provide buy back guarantees to biodiesel producers. All the imported plants, equipment, machinery and specific items shall be exempted from customs duty, income tax and sales tax that are used in biodiesel production [77].

Like solar energy implications, bioenergy sector is also suffering from lack of funding and appropriate policy making and implementation. Likewise, AEDB, Higher Education Commission of Pakistan (HEC), Ministry of climate change Pakistan (MOCC), Environmental Protection Agency Pakistan (Pak-EPA), and PSO must take initiatives to collaborate with academia and research institutions and allocate funds for research and development in bioenergy sector. They must review and revise Midterm Policy (MTP) and locate the bottlenecks in achieving the targets set in 2015 and make a comprehensive policy to achieve 10% (B-10) to 20% (B-20) blend of biodiesel in petrodiesel in 2025 at PSO stations in Pakistan and the biodiesel must be in accordance with the ASTM standards [89,107]. To meet the energy demands and to find alternative and non-conventional resources of energy different challenges like research and development, commercialization, infrastructure development, decentralized type of power delivery system, market development, education and outreach programs, public awareness, subsidies, government participation and technology transfer and adoption, monitoring, and evaluation must be considered and a comprehensive policy must also be made to systematically control and integrate them at national level [78].

10. Biofuel impacts on socioeconomics

Burden on local economy can be reduced by the replacing of conventional energy sources with renewable energy options. There are many socio-economic impact on investments over in a new power plant i.e. increase in job opportunities, increased output and investment in local and countries economy. By dropping costs of renewable liquid fuels and by preservation of few fuel resources the utilization of the fuel can be significantly condensed. There are many ecological, health and safety benefits linked to the development of renewable resources [110]. After textiles the sugar industry is the second largest industry in Pakistan. According to government records there are 76 operational sugar mills in Pakistan. Since the 1990s sugar manufacture in Pakistan has shown a rising trend. Production reached up to 4 million tonnes in 2003–04 as compared to a level of 2.89 million tonnes in 1991–92. Primarily due to impulsive sugarcane growth sugar production has increased over last few years [91,111].

The given reports of Government of Pakistan and IUCN 1992, through production increases consequently increase in sugarcane production are possible. But in contrast, the scope for area expansion is limited factor. Approximately, Pakistan domestically produced sugar was consumed, therefore, exceeding demands was fulfilled through imports. In 2004–05, Pakistan imported 0.27 million tonnes of sugar and as a result in 2005–06 faced a domestic deficiency. Which were again met by imports [96].

Sustainability of bioethanol production appears to be another major concern. In Pakistan, as a by-product of sugarcane crushing, bioethanol is formed from generated molasses in sugar mills. Although sugarcane is a major crop, but severe sugarcane shortages may be caused by lowered yields of the crop in the future due to lack of availability of fertile land and surplus supply of water [112]. As a result, bioethanol production will therefore be suffered. In this context, sugar beet has the potential to cover up the sugar shortage but it will be effective after a long term. Considerable foreign exchange would be saved by shifting towards indigenous fuel ethanol consumption the country rather than coal burning and petro-fuels import. Decrease in oil consumption will also decrease in foreign exchange earnings or government revenue. The main reasons are reduced molasses exports, or subsidies and tax breaks. To give incentives to the fuel, ethanol industry may necessarily be promoted [85,98].

Economics and production conditions for bioethanol both are encouraging. As compared with petrol ethanol is highly price-competitive. Although detailed estimates have not been made, but according to few rough estimate the unit cost of production for bioethanol is half than that of petrol by keeping the consideration of crude oil import and processing costs. Molasses as raw material is available abundantly. Also, the potential for the production of bioethanol production from major crops like cereals, wood pulp and forest products has not been nominated [62].

11. Pakistan's potential of biodiesel and bioethanol

In Pakistan, there is an enormous potential of biodiesel production from from J*atropha, Pongamia* and other plants based oil which needs more consideration and practical applications. Government of Pakistan has established the Alternative Energy Development Board (AEDB) in 2003 based on renewable energy. Its main responsibility was to develop and promote alternative energy technologies and it was targeted to achieve 10% share of bioenergy in the energy sector by 2015 [78]. In 2006 first initiative was taken by AEDB to run a pilot scale project aimed for commercial purposes. The main targets were to make B-10 biodiesel filling station from plant seed oil. Beside this awareness was aimed to be made related to public confidence over biodiesel production, purification and utilization [89]. There are certain institutions in Pakistan which are promoting cultivation of J*atropha* at nursery level at different sites of Baluchistan, the Punjab and Sindh. These cultivated plants obtained an age of several weeks to 18 months in nurseries. However, by the efforts of private sector the cultivation of oil bearing crops have increased from 2 acres to more than 400 acres after 3 year efforts in 2008. In 2008 PSO (Pakistan State Oil) took an initiative in this direction and transplanted 20,000 saplings in farms. Recently they have more sampling for each transplantation reaching up to 20,000 plus. According to these initiatives of PSO more than 6 million trees were targeted to be planted, 24 million kg of oil bearing seeds were estimated to be produced and about 7.2 million L of biodiesel having worth of 345 million PKR with unit price of PKR 48 L^{-1} was targeted to be achieved [89]. However, the cost of biodiesel from *Jatropha* oil could be varied on the basis of investment, labour, land area cost and prevailing policies. Beside commercial implications, biodiesel research in various universities of Pakistan has resulted in a significant impact on the innovation and commercialization in this regard. One of research group has reported a production of about 560 mL of biodiesel from 1 kg of spent tea leaves in the presence of nano-catalyst [113]. In some other institutes the effort is being made to convert molasses and non-edible oil into biodiesel.

For the biodiesel production in Pakistan, the presence of surplus stock of ethanol makes it a suitable source. As biodiesel is produced after catalytic activity so it needs an acid or base catalyst. Sodium hydroxide or caustic soda can be produced from sodium chloride. And Pakistan has one of the largest sodium chloride reserves of the world. So it can be exploited on large scale for biodiesel production [114]. Biodiesel can be potentially produced by the chemical reaction of oils with methanol or ethanol in the presence of suitable catalysts. In this direction methanol is cheaper than ethanol. To produce coal is one of the available sources of methanol. Fortunately, in Pakistan there are about 180 billion tons of coal reserves which stands about are world's fifth largest reserves. There are about 76 sugar mills in Pakistan which have the capacity to process about 300,000 t of sugarcane per day and subsequently can make high volume of ethanol at high rate [115].

According to a study, marine macroalgal specie i.e. *Ulva fasciata* (green seaweed) was used as a potential strain for renewable energy resource in Pakistan for oil extraction and biodiesel synthesis by utilizing waste industrial dusts as catalysts in transesterification step. According to their findings about 88% biodiesel yield was obtained from *U. fasciata* oil in the presence of brown dust of steel converter as a catalyst [116]. Rozina et al., 2017 have enlisted a wide range of non-edible seed oils for the synthesis of biodiesel in Pakistan. They have reported 35 plants for their seed oil content i.e. from 8.5% to 70% (wt/wt) and gathered information about lipids profiling and fatty acid analysis. According to their review a series of steps are related to the biodiesel production from non-edible seed oils i.e. preparation of seeds, solar drying, roasting by heating or solar heating, oil extraction methods (mechanical and solvent extraction methods), purification (sedimentation, boiling and filtration), oil quantification, oil analysis, fatty acid profiling, biodiesel synthesis and characterization [76]. According to a research conducted by Shah et al., 2016 on mixed cultures of microalgae grown on sodium bicarbonate and diammonium phosphate were exploited for maximum lipids extraction by employing various physical and chemical methods. According to the finding there is a great potential of microalgal growth and biomass production in Pakistan as the climatic conditions and indigenous natural resources are suitable and well enough to support 3rd generation (G-3) biofuels feed stocks production [7]. Likewise, in different universities and research institutions in Pakistan, various efforts have been made to test the efficiencies of biodiesel made from soybean and sunflower oil in diesel engines [117,118].

In Pakistan bioethanol production has increased rapidly from 3% in 2000 to 14% in 2003. Molasses is the major source of bioethanol production. Molasses is produced after the sugarcane processing. Sugarcane is one of the major crops in Pakistan after cereal crops. The cost of bioethanol production from sugarcane is considerably lower than other available sources. A positive aspect of sugarcane use is that it will not compromise food crops or cause food scarcity for making bioethanol [115,119]. Therefore, bioethanol provided the right type of policy incentives. In terms of contribution to the country's economy, bioethanol production has remained very small despite of the fact that the sugar and molasses are produced at higher quantity in the country. Until now, only minor quantities were converted to industrial alcohol from the bulk of the raw molasses and more was exported. Therefore in recent policies the emphasis has been made over the increased use of molasses in the country for bioethanol production [88,120].

During 2002-03 the number of distillery industries has increased from 6 to 21 and they also raised molasses exports during the years 2003-04. But the distilleries become idle due to stricter tariff measures initiated by EU. As a result, 2 distilleries have shut down [88]. In the global trading regime Pakistan has supported the standards. As being the member of the Southern block, Pakistan has many times opposed the measures that were taken by North which were protective devices against free trade. In Pakistan, the promotion of bioethanol is suitable. If gasoline is substituting with bioethanol it could generate substantial foreign exchange savings as the annual oil import bill cists up to 3.1 billion US dollars. Rather than appreciating inducements, the private sector is loaded with domestic taxes on industrial alcohol sales. And a lid on private sector involvement was covered by Ministry. Country's export potential was compromised by such domestic biasness, which have been compounded by import restrictions abroad [119].

Over the years the export of molasses has stayed between 0.70 million and 1.75 million tonnes. However, in last five years, a considerable quantity of molasses was converted into three grades of alcohol, i.e. anhydrous or fuel alcohol, neutral or extra-neutral alcohol (ENA), and rectified ethanol (REN) or industrial alcohol. From Pakistan two grades of alcohol can be exported i.e. ethyl alcohol-spirit and un-denatured ethyl alcohol [77,91,96]. In fiscal year 2006, Pakistan has imported petroleum products that cost over 3.1 billion USD. It was equivalent to 85% of the total oil consumption, and a large proportion of the country's trade deficit was also created. The country's considerable foreign exchange could be saved by switching to fuel ethanol. Approximately 300 million USD could be saved if 10% of blend is incorporated and it would be doubled if it reaches to 20%. To meeting the energy shortfall Pakistan State Oil (PSO) and the Hydro

Carbon Development Institute of Pakistan (HDIP), both have launched a pilot project for introducing blended fuel [111,121]. About 400.000 t of ethanol can be produced in Pakistan through 21 distillery units which have the potential for the processing of 2 million tons of molasses. For production of ether biodiesel or gasoil this volume of ethanol can be used. Out of 400,000 t of ethanol produced remaining 318,000 t is surplus because only 80,200 t is spent and exported.

12. Conclusions

Exploration of alternative feed stocks inherited with renewable character to produce biofuels can reduce the dependence on conventional fuels in Pakistan. Pakistan has a strong potential of biodiesel production as there are multiple feedstocks which could be exploited for its synthesis. However, biodiesel productions from 1st and 2nd generation stocks have proved unviable due to global politics, biased policies, social barriers, and more importantly their use as food source. Microalgae could be the ultimate and sustainable energy source. Microalgal based biodiesel and bioethanol which meet American society for testing and materials (ASTM) standards can be used in existing vehicle engines without any modification. In Pakistan, there is a tremendous potential to produce biodiesel even from microalgae along with 1st and 2nd generation feedstocks. But global sanctions, limited access to international market, unclear fuel policy, lack of technical expertise and political will are the major constraints to promote biodiesel in Pakistan. Thus, the attention must be given to encourage the biodiesel production by providing incentives at local level and convincing the global partners through diplomatic efforts. Although Government of Pakistan has initiated different programs to promote biofuels in Pakistan, but still the goals are to be reached on real grounds. The infrastructure development, commercialization, education and outreach programs, public awareness, monitoring, subsidies, government participation, technology transfer and adoption, evaluation and lack of appropriate policies should be the hallmark of the mentioned technologies and recommendations.

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