A Review on Indigenous Freshwater Microalgae Isolated from Natural Habitats of Arunachal Pradesh, India, as a Biodiesel Source

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1 Introduction

With increase in global energy demand due to change in lifestyle, heavy industrial development and increase in population, the dependence on fossil fuels is rising exponentially. Fossil fuels are unsustainable energy sources and the widescale use of fossils might exhaust world fossil oil reserves [1, 2]. Also as per European Environmental Agency (EEA), the energy and transport sectors are responsible for around 80% of the greenhouse gas emission in the European continent [2]. And as per International Energy Agency (IEA) in the future, the countries with the fastest-growing major economy such as India and China will further raise energy consumption which may lead to major release of greenhouse gases [3]. Greenhouse gas contributes to climate changes, global warming, respiratory diseases and changes in pH level of ocean, affecting the safety of both environment and human life [4]. Therefore, due to energy crisis coupled with environmental pollution has forced several nations to consider alternate source of energy which is clean and renewable [5].

1.1 Energy Demand of India

India is highly dependent on imports of crude oil, the country imported an estimated 80.6, 81.7, 82.69 and 83.3% of oil it consumes in year 2015–16, 2016–17, 2017–18 and 2018–2019, respectively [6]. As per IEA 2007, India's crude oil consumption may rise to 6 million barrels/day (2.2 billion barrels/year) [3]. Apart from energy insecurity, India has to deal with environmental safety which is caused by pollution generated by fossil fuels. Therefore, the need of sustainable fuel supplies and rise of crude oil prices have led the Indian government and other economic experts to search

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for an alternative source of fuel supply. Presently, the Government of India found their interest in biofuel as an alternative source of energy and issued a comprehensive national policy on biofuels created by the Ministry of New and Renewable Energy (MNRE). In 2003, The National Policy on Biofuels encouraged the blending of 5% ethanol with petrol in 9 states of India and later included 20 states in year 2006. And by year 2017, they called for blending at least 20% of biofuels as an additive with petrol and diesel fuel [6].

1.2 Biofuel Feedstock

Biofuel is derived from biomass which can be replenished readily like plant, animal waste and algae material. It is considered to be the largest source of renewable energy and has potential to replace petroleum-based oil. The most common biofuel is bio-ethanol, biodiesel, methane, biogas, etc. Depending upon feedstock from which biofuel is obtained, they are divided into four generations (Fig. 1). First-generation biofuels are derived from food-based feedstock like corn, sugarcane, soybean, and animal fat. Second-generation biofuels are obtained from non-edible oil-based feedstock, agricultural waste, forest residue and plant waste biomass like Jatropha, Nahar, rubber seed, castor, and waste cooking oils. Third-generation biofuels are produced from algae (popular algae species like *Chlorella vulgaris, Botryococcus Braunii,* etc.). And fourth-generation biofuels are just extensions of third-generation biofuels where algae are genetically modified to alter its cellular metabolism for higher CO_2 capture ability and higher lipid production rate [7]. Currently, most of biofuel production comes from first-generation feedstock except for Jatropha. However, extensive use of first-generation feedstock may raise a scarcity of food for human beings,

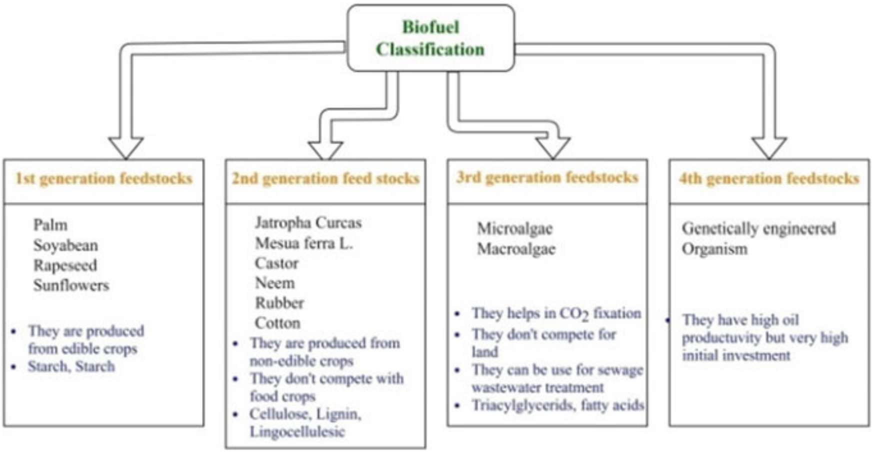

Fig. 1 Biofuel classifications

and it will be very expensive compared to conventional diesel fuel. First-generation feedstock may dominate biofuel production in the future, since industrial production is well-established for them but for developing countries like India, Indonesia and Malaysia producing biofuel from edible crops are not economically feasible [8]. To avoid competition with food, biofuel policy of India (2018) has encouraged the production of biofuel from second-generation feedstock [9]. Nevertheless, the available quantities of second-generation feedstock are not enough to meet the demand of biofuel and growth of plant-based crops needs large amount of land which can lead competition with land and has bad impact on biodiversity [10]. Therefore, recently many researchers have shown interest in third-generation feedstock, algae, because of their ability to remove heavy metals and harness nutrients from the wastewater, ability to capture CO_2 from atmosphere, can be grown in wastelands, high oil content and required less land [7].

1.3 Algae: As Potential Source for Biofuel Production

The use of algae for wastewater treatment using solar energy through photosynthesis subside harmful heavy metal and nutrients, also entrap CO_2 emissions of fossil fuels that have powered machineries like IC engine and power plant. The algae have higher oil yield per acre of land than most terrestrial crop plants, i.e. around 500 to 1000 times more that crop-based plants. Some of algae have high oil content which is around 25–50% of dry weight, and most of them have high triglycerides which is currently thought to be feasible and the higher values for projecting a possibility in the future as a potential source of biofuel production [11]. However, there are severable shortcoming, production of biofuel from algae is not cheap and its production cost surpasses the price of conventional fuel price by vast margin. Use of fertilizers and harvesting accounts for 60% of total production cost, and microalgae cultivation is a water-intensive process which required around 11–13 million litre of water per hectare of land for cultivation in open ponds [12]. The loss of freshwater is a huge economical drawback, also the excess unfiltered fertilizer in a wastewater production from algae cultivation leads to bioaccumulation in the ecosystem harming the surviving flora and fauna. Considering the scope and issue related to microalgae biodiesel production, this review focuses its attention on biodiesel production from naturally available wild algae strains and stresses out its importance [13].

Present study also focuses on scope of freshwater microalgae growth in North-eastern region of India, including their biomass growth, biomass productivity, lipid content, and compared with other microalgae species which are common and not detected in Arunachal Pradesh. The review also discuss performance, emission and combustion characteristics of diesel engine fuelled with microalgae species which were isolated from local water bodies.

2 Biodiesel Productions from Microalgae

Many studies have found that triglycerides have potential to replace conventional diesel fuel in diesel engine and vegetable oils primarily consist of triglycerides. However, the direct use of vegetable oils or its blends can lead to injector choking, plugging and gumming of filter and fuel lines because of unfavourable properties like high viscosity, free fatty acid, density and acid composition of such oils. To overcome the hindrance caused by direct use of vegetable oils in diesel engine, a considerable amount of effort has been made by researchers to develop a technology that could bring properties and performance of vegetable oils similar to conventional diesel fuel. Till now four main processes have been investigated to overcome the drawbacks of vegetable oils, and they are micro-emulsification, pyrolysis, dilution and transesterification [14]. As per Murugesan et al. 2009 and Fukuda et al. 14, transesterification is the most promising technology available right now for biodiesel production and biodiesel derived from third-generation feedstock is one of the best possible options because of its high yield productivity per hectare, CO_2 absorbing capability and act as wastewater purifier [15].

2.1 Recent Development on Microalgae Biofuel

With increase in popularity of microalgae as a source of renewable energy, researchers have underwent several studies, and recently, they have shown their interest towards algae which can grow in dark. Algae which can be cultivated under heterotrophic and mixotrophic modes have higher lipid content than in a photoautotrophic mode of cultivation; though they have lower biomass productivity than latter one, the overall energy consumption of heterotrophic and mixotrophic was observed to be less than phototrophic [16]. Lately, microalgae–microbial fuel cell technology (MMFC) piques the interest of scientist as they can be utilized for bioelectricity production without damaging environment unlike lithium-based battery [17]. There are many studies where cultivation algae in a wastewater were carried, and it was observed that a very few algae species were able to thrive on it. *Chlorella sp.* was able to survive in biochar which was derived from residue of hematite and produce perceptible amount of biomass which can be used for biofuel production [18]. Rasouli et al. cultivated co-culture of green microalgae and methanotrophs in an industrial wastewater and were able to recover nutrient and heavy metal, which aided the cleaning of wastewater [19]. Zainan et al. studied the quality of bio-oil of microalgae *C. vulgaris* which underwent catalytic pyrolysis process and found that the quality was significantly improved compared to other extraction processes [20]. Karthikeyan et al. improved the diesel engine emission by adding Ni-doped ZnO and ZrO_2 nanoparticles in a *B. braunii* [21].

2.2 Prospects of Biodiesel Production from Algae in India

India's dependency on imported crude oil is country's greatest economic vulnerability but despite being energy deficit, many researchers have claim that India has potential to develop promising sustainable energy sources. Researchers claimed that southern part of Asia mainly India is suitable for mass algae cultivation due to its tropical climate which naturally serves as advantages over other countries and vast availability of usable wasteland in India can be utilized for algae biomass production with reasonable effort [22]. Developing countries like India lack proper technique to treat wastewater, and at the same time, there is a growing disquietude on the depravity of wastewater generation. Study showed that the concentration of nutrient level in both municipal and agriculture wastewater was high, and algae grow rapidly in nutrient-rich environment which makes it most suitable for low-cost wastewater treatment [23]. Raceway open pond algae cultivation is considered as satisfactory for tropical countries like India due to its cost-effectiveness and superior flexibility [24]. Back on days India has been mass cultivating *Spirulina* algae in a raceway pond for food and other medical supplements now several institutions and organization have carried work on various aspects of algae [25]. University of Madras, Chennai, cultivated *Sargassum, B.Braunii* microalgae species in an open raceway pond for biogas production, *C. Vulgaris* were extensively studied and cultivated to estimate its biomass production and its resilience in Indian tropical condition by Central Rice Research Institute (CCRI), Cuttack [26]. Life cycle analysis (LCA) of several diatoms species and its potential as an additional biofuel sources were research by Vivekananda Institute of Algal Technology (VIAT), Chennai [27]. VIAT is also developing a technology to treat industrial wastewater using microalgae [28]. Alternate hydro-energy centre, IIT Roorke, successfully converted microalgae oil into biodiesel using several conversion methods [29].

3 Feasibility of Growing Freshwater Microalgae in North-Eastern Region of India

Factors which are associated to selection of site for mass microalgae cultivation are:

(a) *Availability of land for algae cultivation*
With growing population and high demand of fertilized land, it has become impractical to use fertilized land for algae biomass cultivation. Though algae have high oil yield per area as compared to terrestrial crops, still it is not economically viable to compete for fertilized land with food crops especially for developing countries like India. Therefore, wasteland is considered to be attractive options for algae cultivation. The GOI classifies 90 million hectares as wasteland which can be utilized for commercial production of algae biomass. Arunachal Pradesh accounts for total 21.7% of land as wasteland (18,175.95

km^2) which if taken advantage of than it can help out reduce CO_2 from the atmosphere, reduces current diesel consumption and creates job for native [30].

(b) *Availability of water*
The main obstacle for cultivating freshwater microalgae is vast usage of freshwater, as per study 12 million litre of water is used for cultivation of one hectare of land which and around 80% of water are wasted during microalgae cultivation [12]. It is a hefty price to cultivate microalgae when usage of water is considered. Nevertheless, the average rainfall recorded for Arunachal Pradesh is 450 cm, ranging from 100 to 990 cm. The vast majority of rainfall is at mid altitude to low altitude. Arunachal Pradesh has large freshwater sources for mass cultivation and usage of freshwater won't be foremost problem for domestic consumption [31].

(c) *Nutrient sources and supply*
Arunachal Pradesh is filled with vast amount of biomass where animals and birds excreta and fallen leaves can be used as a bio-fertilizer for algae cultivation. Using biological wastages will also help cleaning of environmental.

(d) *Wastewater composition*

Wastewater has lot of nutrients, and few of them can thrive on wastewater. According to Debnath, composition of waste found in Arunachal Pradesh is mostly organic which was 53% in average and can be used for heterotrophic and mixotrophic cultivation as those mode of cultivation tend to deliver higher lipid productivity than photoautotrophic mode [32].

3.1 List of Indigenous Algae Strains Detected in Arunachal Pradesh

Selecting suitable microalgae species as an ideal source of biodiesel is one of the most crucial steps. The important characteristics of selected microalgae species are to have high biomass growth rate, high lipid content with desirable fatty acids, robust in nature, ability to compete against other microorganisms. A very few researchers have studied the diversity of algae in natural habitats of north-eastern region of India, Das and Adhikary documented total of 86 freshwater species which includes 8 Cyanophyta, 30 Chlorophyta, 8 Euglenophyta, 39 diatoms and 1 Xanthophyta from different locations of Arunachal Pradesh [33]. Out of 86 freshwater species which were documented by Das and Adhikary only 13 of them were found to be studied by researchers for their cell growth, lipid content and their potential as a feedstock for biofuel (Table 1).

Table 1 List of Indigenous algae reported in the literature

Freshwater algae species	Ref
Cynophyta (Blue-green algae)	
Oedogonium sp	[34]
Spirogyra sp	[34]
Botryococcus Braunii	[12,35–39]
Scenedesmus Bijugatus	[40 41]
Scenedesmus quadricauda	[42–45]
Euglenophyta	
Euglena sanguinea	[46]
Diatoms (Bacillariophyta)	
Tabellaria fenestrate	[47]
Fragilaria capucina	[48]
Synedra ulna	[49, 50]
Diadesmis confervacea	[51]
Navicula sphaerophora	[52]
Nitzschia species	[47]
Navicula sp	[47]

4 An Overview of Growth Dynamics and Lipid Content of Freshwater Microalgae Found in Arunachal Pradesh

The biomass productivity, specific growth rate, oil content and culture medium used for the growth of same algae species found in local water bodies are collected from several literatures and placed in Tables 2 and 3. From this study, we will get the general idea of growth behaviour, nature and biofuel potential of locally available algae strains. Since every algae is different and using fourth-generation algae is presently not practical; therefore, opting for naturally available biomass is economical viable for now. Oedogonium as a genus of filamentous green algae are generally found in freshwater pond and lakes. Lawton et al. (2013) studied three types of freshwater macroalgae of genus *Oedogonium, Spirogyra* and *Cladophora.* The biomass productivity of Oedogonium was at least 20% higher than *Spirogyra* and *Cladophora* when they are treated with low air supply and no external CO_2 supply. *Oedogonium* was able to dominate in mixed species culture and grew exponentially within a week [53]. *Oedogonium* were able to withstand varying range on environmental conditions and demonstrated that natural occurring species of *Oedogonium* can be domesticated which provides first crucial steps to determine suitability of algae as an energy source [54].

B. braunii strain is a green, pyramid-shaped planktonic microalgae which grow in cluster and can be found in tropical and temperate lakes and estuaries [55]. It was claimed by many researchers as one of the most promising feedstock out of all microalgae and macroalgae because of their high lipid content and high biomass

Table 2 Biomass productivity of freshwater algae [12, 34–51]

Algae species	Nutrient	Biomass productivity/ Biomass yield*
Mycrocystis sp	Modified Murashige and Skoog Basal Medium	20.30 ± 1.79 mg/L*
Oedogonium species	BB medium	0.478 g/L*
Botryococcus braunii	Chu13	0.026 mg/L/d
	na	0.035 mg/L/d
	Modified Chu13	0.026 mg/L/d
	BG11	0.037 mg/L/d
	Secondary domestic wastewater	0.034 mg/L/d
	na	0.19 mg/L/d
	na	0.26 mg/L/d
	na	0.345 mg/L/d
	BG11	0.03 mg/L/d
	Modified Chu13	0.114 mg/L/d
Scenedesmus bijugatus	BG11 medium	28.67 mg/L/d
	BBM	0.26 g/L/d
Scenedesmus quadricauda	Aquaculture wastewater	4.85 ± 0.02 mg/L/h 10.44 ± 0.14 mg/L/h
	BG11medium	68.92 mg/L/d
	na	0.19 g/L/d
	BG11 medium	81.79 mg/L/d
	Sewage wastewater	79.17 mg/L/d
	BBM	0.19 g/L/d
Euglena sanguinea	0.5 mL seaweed + 0.25 mL poultry extract + 19 NPK fertilizer	0.927 g/L/d
Tabellaria sp	Modified Murashige and Skoog Basal Medium	16.90 ± 0.80 mg/L
Fragilaria capucina	Modified WC medium (NaCL & Si added)	0.61/ d
Synedra crystalline		
Synedra species	Modified Murashige and Skoog Basal Medium	21.30 ± 1.53 mg/L
Synedra ulna	na	0.75/ d
	sD11 medium with 540 μg $NaSiO_3$	0.64/d (6.4 g lipid/ml)
	Modified Csi medium	0.245/ d
Diadesmis confervacea	modified f/2 media	
Navicula sphaerophora	Chu13 medium	0.508 g/L

(continued)

Table 2 (continued)

Algae species	Nutrient	Biomass productivity/ Biomass yield*
Navicula species	Modified Murashige and Skoog Basal Medium	38.20 ± 1.13* mg/L
	CSi medium	0.17–0.221/ d
Nitzschia species	Modified Murashige and Skoog Basal Medium	20.50 ± 0.36* mg/L
Spirogyra species	BG medium	0.469 g/L

productivity. Some algae strain of *B.braunii* were able to produce about 86% of hydrocarbons [40, 42, 43, 56]. *B.braunii is* lipid composed of saturated and polysaturated fatty acids and also triacylglycerols (TAGs) which makes it suitable for biodiesel production [56].

Scenedesmus is a genus of green algae which grow into colony and can exist in unicells. Many researchers have studied the species of *Scenedesmus* for wastewater treatment, biomass estimation and their potential as a feedstock for biodiesel. Among several species of genus *Scenedesmus*, *Scenedesmus quadricauda* and *Scenedesmus bijugatus* were reported in Arunachal Pradesh, India [33]. Han et al. were able to obtain 28.67 mg/L/d of algae biomass productivity when *S. bijugatus* were cultivated with BG11 culture medium; he also reported that *S. bijugatus* content 24.3% of total lipid dry weight basis (DWB) which makes it good competitor as a biodiesel feedstock [40]. Ashok et al. reported that they were able to obtain 0.26 g/L/d of *S. bijugatus* biomass productivity. Halfhide et al. cultivated *S. quadricauda* freshwater algae in a filtered wastewater. He used two different culture environment, axenic culture and non-axenic culture. They obtained maximum biomass productivity of 4.85 ± 0.02 mg/L/h for axenic culture and 10.44 ± 0.14 mg/L/h for non-axenic culture [42]. Ahmad et al. reported 0.19 g/L/d biomass productivity with 18.4% of total lipid content on DWB [43]. Han et al. (2015) studied the survivability and change in chemical composition of *S. quadricauda* in different environments. They cultivated *S. quadricauda* in two media: sewage water and BG culture medium. They reported that by using sewage water as a nutrient medium, they were able to obtain 79.17 mg/L/d biomass productivity with total lipid content 27.36% DWB, and by using BG culture medium, they obtained 81.79 mg/L/d biomass productivity with total lipid content 31.31% DWB [44].

Euglena Sanguinea is unicellular species which belongs to family of *Euglenaceae* and order of *Euglenales*. They are motile in nature and their shape changes depending upon motion. They are extremely robust in nature and can survive in sewage wastewater. They are also good in breaking down the nutrient and can be used to minimizing the harmful chemicals in wastewater. They have biomass productivity of 0.927 g/L/d and can store 25% DWB lipid on their cell [45].

Tabellaria is a genus of freshwater diatoms or Bacillariophyta. They have cuboid shape and siliceous cell walls are attached at the corners due to which colonies are

Table 3 Freshwater algae lipid content

Algae species	Lipid content (% DWB)	Ref
First-generation biodiesel feedstock		
Soyabean	18	[60]
Sunflower	40	[60]
peanut	45–52	[60]
Rapeseed	41	[60]
Oil palm	40–52	[60]
groundnut	66	[60]
Second-generation biodiesel feedstock		
Jatropha curcas	20–60	[61]
Rubber	40–60	[61]
Cotton	17–25	[61]
Neem	25–45	[61]
Mahua	35–50	[61]
Pongamia Pinnata	27–39	[61]
Castor	45–50	[61]
Tung	35–40	[61]
Mesua ferra L	58–75	[61]
Soapnut	51.8	[61]
Third-generation biodiesel feedstock		
Mycrocystis sp	16.5	[47]
Oedogonium species	9.2	[62]
	11.4	[34]
Botryococcus braunii	25.7	[35]
	13.5	[12]
	36.14	[37]
	18.4	[38]
	21.1	[38]
	17	[38]
	40	[39]
	19	[63]
Scenedesmus bijugatus	24.3	[40]
Scenedesmus quadricauda	18.4	[43]
	31.31	[44]
	27.36	[44]
Tabellaria sp	10.90 ± 1.71	[47]
Fragilaria capucina	14 (30 Low N)	[48]

(continued)

Table 3 (continued)

Algae species	Lipid content (% DWB)	Ref
Synedra crystalline	na	
Synedra species	20.30 ± 2.01	[47]
Synedra ulna	6.4 * (g lipid/mL)	[50]
	29	[50]
Diadesmis confervacea	14.6	[51]
Navicula sphaerophora	13.8	[52]
Navicula species	27.20 ± 1.37	[47]
	24	[50]
Nitzschia species	16.50 ± 1.24	[47]
Spirogyra species	7.3	[62]
	4.93	[34]
Euglena sanguinea	25	[46]

shaped in zig-zag order [57]. Saputro et al. isolated total 8 freshwater microalgae from Wonorejo River and Surabaya River of Indonesia. They cultivated *Tabellaria sp* using modified Murashige and Skoog Basal culture medium and obtained 16.90 ± 0.80 mg/L total biomass yield. They also reported that *Tabellaria sp* content 10.90 ± 1.71% of total lipid on dry weight basis [47].

Fragilaria capucina is filamentous freshwater diatoms/ Bacillariophyta. *F. capucina* can accumulate lipid even at high concentration level of N and Si. Justin et al. reported that the maximum specific growth rate of *F. capucina* was 0.61 per day, and total lipid content at low N level was more than 30% DWB [48]. *Synedra ulna* comes under family of Fragilariaceae. Specific growth rate of *S. ulna* is reported to be 0.245 per day to 0.75 per day and has oil content around 29% DWB [34, 58, 59]. Saputro et al. studied the specific growth rate and lipid content of 8 isolated algae using modified Murashige and Skoog Basal culture medium and reported that Synedra species have total biomass growth 21.30 ± 1.53 mg/L and content 20.30 ± 2.01% of oil in its cell on DWB [47].

Navicula is a genus of boat-like diatoms, and they have been identified as high lipid-producing algae. They have biomass productivity of around 38.2 mg/L/d and specific growth rate around 0.17–0.22 per day. As per report, they are able to produce 27.2–24% of lipid [47]. Vinayak reported that they have discovered a strain of diatom *Diadesmis confervacea* which can accumulate lipid in its cell up to 14.6% DWB and also extract oil naturally when cells attain maturity in 31st day of culturing in vivo [51].

5 Performance and Emission Analysis of Diesel Engine Fuelled with Algae Biodiesel

There are various physicochemical properties of biodiesel which contribute to any changes in performance and emission characteristics of a diesel engine. Biodiesel is an oxygenated fuel, and it often contains extra oxygen atoms which influence performance, emission and combustion significantly. Most of the cases, use of biodiesel in diesel engine results in reduction of unburned hydrocarbons (UHC), Carbon monoxide (CO) and particulate matter (PM) as shown in Table 5. Brake-specific fuel consumption (BSFC) and brake thermal efficiency (BTE) are two important parameters to study the performance characteristics of an engine. BTE is a measure of % of chemical energy in a fuel which transform into useful work. There are numerous publications on engine analysis using biodiesel and its blend as shown in Table 4. For most of the crop-based biodiesel, the BTE decrease with increase in volume percentage of biodiesel in a blend which is due to low energy density of biodiesel fuel as compared to diesel fuel [64]. BSFC and BTE have an inversely relationship as because mathematical formula of BTE is almost reciprocal of BSFC mathematical formula. Therefore, brake-specific fuel consumption (BSFC) is generally higher for biodiesel and its blends than conventional diesel fuel as the quality of biodiesel depends upon the composition of oil. *C. vulgaris* biodiesel and its blends increase the BTE of diesel engine and similar reports were found for *B. Braunii* and *Scenedesmus Obliquus* biodiesel [65, 58]. This may be due to higher cetane number of algae biodiesel which improves the ignition delay and enhance the combustion process, thus lowering the fuel consumption or due to higher oxygen molecules in biodiesel which help burning the fuel efficiently. The result of *C. vulgaris, B. Braunii* and *Scenedesmus Obliquus* displayed the potential of algae biodiesel as an alternative fuel which even improves the engine performance.

NO_x is formed due to ignition delay, oxygen content in the fuel and temperature of combustion chamber. Unsaturated fatty acids and longer hydrocarbon chain in biodiesel are correlated to the formation of NO_x. Except for *Scenedesmus Obliquus,* all other algae biodiesel tested in diesel engine increase the NO_x emissions which could be result of extra oxygen atoms which improves combustion and thus increases the in cylinder temperature. Subsequently, it enhances The NOx formation [59] and CO are formed due to incomplete combustion of air and fuel mixture, this happens due to insufficient oxygen supply to complete the combustion reaction. The ability to provide extra oxygen atoms helps in peroxidising the air-fuel mixture and biodiesel being an oxygenated fuel reduces the harmful emission of HC and CO. Patel et al. also reported the reduce in particulate matter (PM) when using *C. vulgaris* biodiesel in a diesel engine [65]

Table 4 Performance of diesel engine using crop-based and algae biodiesel [12, 52, 58, 59, 61, 64, 65]

Biodiesel	Testing unit	Engine performance	
		BTE	BSFC
Crop-based biodiesel			
Soyabean	na	↓	↑
Sunflower	na	↓	↑
Waste cooking oil	na	↓	↑
Mesua ferra L	Single cylinder, water cooled (6.5 hp)	↓	↑
Algae biodiesel			
Chlorella Vulgaris	Single cylinder, water cooled (5 hp)	↑	↓
Crypthecodinium cohnii	Four cylinder, turbocharged, intercooled, (134 hp)	↓	↑
*B.braunii**	Single cylinder, air cooled	↑	↓
*Scendesmus Obliqus**	Single cylinder, water cooled (6.5 hp)	↑	↓
*Navicula sphaerophora **	Single cylinder, water cooled (4.69 hp)	↓	↑
*Euglena sanguinea**	Single cylinder, water cooled (4.69 hp)	↓	↑

*Algae species found in Arunachal Pradesh

6 Combustion Analysis of Diesel Engine Fuelled with Algae Biodiesel

In Table 6, all the report on combustion analysis of microalgae biodiesel in diesel engine stated and compared with each other. Islam et al. reported that cylinder pressure (CP) of *Crypthecodinium cohnii* biodiesel was slightly less than diesel fuel at 25% engine load and after further increase in load there was no significant difference between blends and diesel fuel. The decrease in cylinder presser (CP) at lower engine load may be because of high viscosity of biodiesel [59]. Heaik et al. tested *Ankistrodesmus braunii* and *Nannochloropsis sp* biodiesel in single cylinder Ricardo engine and observed that slight in-cylinder pressure, Rate of pressure rise (RPR) and net heat release rate NHRR [35] and reported just opposite result of Islma et al.

Table 5 Emission of diesel engine while running using crop-based biodiesel and algae biodiesel [52, 58, 59, 64, 65]

Biodiesel	Testing unit	Emissions				
		NO_x	HC	CO	PM	EGT
B.braunii	1-cylinder, air cooled	↑	↓	↓	na	na
Scendesmus Obliqus*	1-cylinder, water cooled (6.5 hp)	↓	↓	↓	na	na
*Navicula sphaerophora**	1-cylinder, water cooled (4.69 hp)	↑	↓	↓	na	na
*Euglena sanguinea**	1-cylinder, water cooled (4.69 hp)	↑	↓	↓	na	↑
Chlorella Vulgaris	1-cylinder, water cooled (5 hp)	↑	↓	↓ (↑at max load)	↓	na
Crypthecodinium cohnii	1-cylinder, turbocharged, intercooled, (134 hp)	↑	↓	na	na	na

*Algae species found in Arunachal Pradesh

Table 6 Combustion characteristic of diesel engine fuelled with algae biodiesel [35, 59]

Biodiesel	Test unit	Combustion		
		CP	RPR	NHRR
Ankistrodesmus braunii	Single cylinder Ricardo-E6 engine (12 hp)	↑	↑	↑
Nannochloropsis sp	Single cylinder Ricardo-E6 engine (12 hp)	↑	↑	↑
Crypthecodinium cohnii	Four cylinder, turbocharged, intercooled, (134 hp)	↓ (same after 25% load)	↑ (↓ after 50% load)	na

*Algae species found in Arunachal Pradesh

7 Conclusions

To find an ideal sustainable source for biodiesel production, the feedstock should have sufficient amount of oil content, high productivity and suitable fatty acid chains for having good biodiesel properties. Most of recent researches on algae species were on *C. vulgaris* and *Chlamydomonas reinhardtii*, now recently many researchers have shifted their attention towards locally available wild algae species. This review described the potential of 13 indigenous algae species which were found in Arunachal Pradesh and all algae species have found to yield biomass productivity and content high % of oil (DCW). Four wild freshwater algae species *B. braunii, Scenedesmus obliquus, C. vulgaris and E. sanguinea found to be most favourable among other species,* these have ability to utilize for wastewater treatment and carry out nutrient breakdown as well as aid the production of algae biodiesel. By using sewage wastewater for algae biomass production is not only advantageous for environmental safety and CO_2 reduction from atmosphere but also lower the cost of biodiesel production by avoiding fertilizers.

7.1 Scope for Future Research Work

1. Chemical and physical composition changes for algae depending upon their environment. Therefore, lipid content for the same species differs as per report because researcher has used different environment for algae cultivation and lipid content generally varies with several different parameters like temperature, culture medium, pH value, nutrient level of N, K, Ni, P, CO_2 concentration level and air turbulence. Thus, there is a scope to further study the suitability of algae species for more biomass growth rate and higher lipid productivity.
2. Microalgae biodiesel generally depends upon TAGs and free fatty acids of lipid. Not many reports have been found on fatty acid composition of microalgae and the report varies for same species depending upon extraction method, culture medium and cultivating environment. Therefore, there is a scope to further study the range of fatty acid compositions of algae lipid
3. Many publications have reported the potential of algae biodiesel as a replacement of conventional diesel fuel in a diesel engine but a very few report have been found studying the actual engine characteristics when fuelled with algae biodiesel due to the limited amount of fuel. A complete mapping of engine performance, emission, and combustion characteristics with microalgae biodiesel needs to be done.

References

1. Chandrasekhar, K., Lee, Y.-J., & Lee, D.-W. (2015). Biohydrogen production: Strategies to improve process efficiency through microbial routes.*International Journal Molecular Science*, *16*, 8266–8293.
2. European Environmental Agency (EEA) .(2004). Greenhouse gas emission trends and projections in Europe 2004: Progress by the EU and its Member States towards achieving their Kyoto Protocol targets. Report N85.
3. International Energy Agency (IEA). World Energy Outlook (2007). China and India Insights, Paris, France.

4. Ormerod, W. G., Freund, P., Smith, A., & Davison, J. (2002). Ocean storage of CO_2. IEA greenhouse gas R&D programme. International Energy Agency
5. Ministry of Petroleum and Natural Gas, Government of India. (2020). http://petroleum.nic.in.
6. Basavaraj, G., Rao, P., Reddy, C., Ashok, A., Pinnamaneni, S. R., & Reddy, B. V. S. (2012). A review of national biofuel policy in India: A critique need for promotion of alternative feedstock. *Journal Biofuels*, *3*(2), 65–78.
7. Kasturi, D., Achlesh, D., & GawLin, J. (2014). Evolution retrospective for alternative fuels: First to fourth generation. *Renewable Energy, 69*, 114–122.
8. Ravindranath, N. H., Lakshmi, C. S., Manuvie, R., & Balachandra, P. (2011). Biofuel production and implications for land use, food production and environment in India. *Energy Policy, 39*(10), 5737–5745.
9. Ministry of Petroleum and Natural Gas. (2018). National Policy of Biofuel, New Delhi.
10. Reinhardt, G., Rettenmaier, N., Ko¨ppen, S. (2008). How sustainable are biofuels for transportation? In *Bioenergy: Challenges and opportunities. International Conference and Exhibition on Bioenergy*. ISBN 978–92–5–107414–5.
11. Lundquist, T. L., Woertz, I. C., Quinn, N. W. T., & Benemann, J. R. (2010). A realistic technology and engineering assessment of algae biofuel production. *EBI, 2010*, 1–178.
12. Chinnasamy, S., Bhatnagar, A., Hunt, R. W., & Das, K. C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology, 101*, 3097–3105.
13. Mahapatra, D. M., Chanakya, H. N., & Ramachandra, T. V. (2011). Assessment of treatment capabilities of Varthur Lake, Bangalore India. *International Journal Environment, 14*, 84–102.
14. Fukuda, H., Kondo, A., & Noda, H. (2001). Biodiesel fuel production by transesterification of oils. *Journal of Bioscience and Bioengineering, 92*, 405–416.
15. Srivastava, A., & Prasad, R. (2000). Triglycerides-based diesel fuels. *Renewable Sustainable Energy Review, 4*(2), 111–133.
16. Papu, N. H., Lingfa, P., Dash, S. K. (2020). *Euglena Sanguinea* algal biodiesel and its various diesel blends as diesel engine fuels: A study on the performance and emission characteristics. *Energy Sources*, *Part*. https://doi.org/10.1080/15567036.2020.1798566
17. Costa, C., & Hadiyanto, A. (2018). Bioelectricity production from microalgae-microbial fuel cell technology (MMFC). *MATEC Web Conference, 156*, 2–4.
18. Ashokkumar, V., Chen, W.-H., Kamyab, H., Kumar, G., Al-Muhtaseb, A. H., & Ngamcharussrivichai, C. (2019). Cultivation of microalgae *Chlorella sp.* in municipal sewage for biofuel production and utilization of biochar derived from residue for the conversion of hematite iron ore (Fe_2O_3) to iron (Fe)– Integrated algal biorefinery. *Energy, 189*, 116–128.
19. Rasouli, Z., Valverde-Pérez, B., D'Este, M., De Francisci, D., & Angelidaki, I. (2018). Nutrient recovery from industrial wastewater as single cell protein by a co-culture of green microalgae and methanotrophs. *Biochemical Energy Journal, 134*, 129–133.
20. Zainan, N. H., Srivatsa, S. C., Li, F., & Bhattacharya, S. (2018). Quality of bio-oil from catalytic pyrolysis of microalgae *Chlorella vulgaris. Fuel, 223*, 12–19.
21. Karthikeyan, S., & Prabhakaran, T. D. (2018). Emission analysis of *Botryococcus braunii* algal biofuel using Ni-Doped ZnO nano additives for IC engines. *Energy Sources, Part A433A: Recovery, Utilization, and Environmental Effects+A465, 40*, 1060–1067.
22. Beer, L. L., Boyd, E. S., Peters, J. W., Posewitz, M. C. (2009). Engineering algae for biohydrogen and biofuel production. *Current Opinion Biotechnology*, *20*(3), 264–271.
23. Chanakya, H. N., Karthick, B., Ramachandra, T. V. (2006) .Nitrogen and carbon flows through Bellandur lake-role of Bellandur lake as a natural wetland treating Bangalore wastewater. http://wgbis.ces.iisc.ernet.in/energy/lake2006
24. Pulz, O. (2001). Photobioreactors: production systems for phototrophic microorganisms. *Applications Microbiology Biotechnology*, 287–293.
25. Venkatamaran, L. V. (1986). Blue-green algae as biofertilizer. In A. Richmond (Ed.), *CRC Handbook of microalgal mass culture* (pp. 455–471). Boca Raton: CRC Press.
26. Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A., & Das, K. C. (2009). Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO_2 and temperature. *International Journal of Molecular Sciences, 10*(2), 518–532.

27. Ramachandra, T. V., Durga, M. M., & Karthic, B. K. (2009). Milking diatoms for sustainable energy: Biochemical engineering versus gasoline-secreting diatom solar panels. *Industrial and Engineering Chemistry Research, 48*(19), 8769–8788.
28. Sivasubramanian, V., Subramanian, V. V., Raju, P. A., Muthukumaran, M. (2009). In *Phycoremediation of Oil Drilling Waste at Kakinada International Conference on Algal Biomass,Resources and Utilization*, Stella Maris College, Chennai.
29. Rajvanshi, S., & Sharma, M. P. (2012). Micro Algae: A potential source of biodiesel. *Journal of Sustainable Bioenergy Systems*, *02*(03).
30. Government of India .(2011). Wasteland atlas of India, Arunachal Pradesh. https://dolr.gov.in/documents/wasteland-atlas-of india.
31. Dhar, O. N., & Nandargi, S. (2004). Rainfall distribution over the Arunachal Pradesh Himalayas . *Weather, 59*(6), 155–157.
32. Debnath, A., & Mitra, A. (2013, December 4–8). Challenges of solid waste accumulation and management in a growing urban area: A study of Itanagar town of Arunachal Pradesh. *Indian Society for Ecological Economics-Global Change, Ecosystems, Sustainability*.
33. Das, S. K., & Adhikar, S. P. (2012). Diversity of freshwater algae in Arunachal Pradesh and their distribution in different altitudes. *Journal Indian Bot Society, 91*(1–3), 160–182.
34. Munir, N., Imtiaz, A., Sharif, N., & Naz, S. (2015). Optimization of growth conditions of different algal strains and determination of their lipid contents. *Journal Animals Plant Science, 25*(2), 546–553. ISSN: 1018–7081
35. Haik, Y., Selim, Md. Y. E., & Abdulrehman, T. (2010). Combustion of algae oil methyl ester in an indirect injection diesel engine. *Energy, 36*, 1827–1835.
36. Lee, J. Y., Yoo, C., Jun, S. Y., Ahn, C. Y., Oh, H. M. (2010). Comparison of several methods for effective lipid extraction from microalgae. *Bioresourcing Technology, 101*(1), S75–S77.
37. Sydney, E. B., da Silva, T. E., Tokarski, A., Novak, A. C., de Carvalho, J. C., & Wojciechowski, A. L. (2011). Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. *Applied Energy, 18*(10), 3291–3294.
38. Ahmad, A. L., Yasin, N. H. M., Derek, C. J. C., & Lim, J. K. (2011). Microalgae as a sustainable energy source for biodiesel production: A review. *Renewable Sustainable Energy Review, 15*, 584–593.
39. Abomohra, A.E.-F., Wagner, M., El-Sheek, M., & Hanelt, D. (2012). Lipid and total fatty acid productivity in photoautotrophic fresh water microalgae: Screening studies towards biodiesel production. *Journal of Applied Phycology, 25*(4), 931–936.
40. Han, L., Pei, H., Hu, W., Han, F., Song, M., & Zhang, S. (2014). Nutrient removal and lipid accumulation properties of newly isolated microalgal strains. *Bioresourcing Technology*, *165*(C), 38–41.
41. Ashokkumar, V., Salam, Z., Tiwari, O. N., Chinnasamy, S., Mohammede, S., & Ani, F. N. (2015). An integrated approach for biodiesel and bioethanol production from *Scenedesmus bijugatus* cultivated in a vertical tubular photobioreactor. *Energy Conversation Management, 101*, 778–786.
42. Halfhide T, Åkerstrøm, A., Lekang, O. I., Gislerød, H. R., & Ergas, S. J. (2014). Production of algal biomass, chlorophyll, starch and lipids using aquaculture wastewater under axenic and non-axenic conditions. *Algal Research, 6*(B), 152–159.
43. Ahmad, A. L., Yasin, N. H. M., Derek, C. J. C., & Lim, J. K. (2011). Microalgae as a sustainable energy source for biodiesel production: A review. *Renewable Sustainable Energy Review, 15*, 584–593.
44. Han, L., Pei, H., Hu, W., Jiang, L., Ma, G., Zhang, S., & Han, F. (2015). Integrated campus sewage treatment and biomass production by *Scenedesmus quadricauda* SDEC-13. *Bioresource Technology, 175*, 262–268.
45. Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., & Bonini, G. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photo bioreactor. *Biotechnology and Bioengineering, 102*, 100–112.
46. Kings, A. J., Raj, R. E., Miriam, L. R. M., & Visvanathan, M. A. (2017). Growth studies on microalgae *Euglena sanguinea* in various natural eco-friendly composite media to optimize the lipid productivity. *Bioresource Technology, 244*, 1349–1357.

47. Saputro, T. B., Purwani, K. I., Ermavitalini, D., & Saifullah, A. F. (2019). Isolation of high lipid content microalgae from wonorejo river, Surabaya, Indonesia and its identification using rbcL marker gene. *Biodiversitas, 20*(5), 1380–1388.
48. Chaffin, J. D., Mishra, S., Kuhaneck, R. M., Heckathorn, S. A., & Bridgeman, T. B. (2012). Environmental controls on growth and lipid content for the freshwater diatom, *Fragilaria capucina*: A candidate for biofuel production. *Journal of Applied Phycology, 24*, 1045–1051.
49. Tilman, D., Mattson, M., & Langer, S. (1981). Competition and nutrient kinetics along a temperature gradient: An experimental test of a mechanistic approach to niche theory. *Limnology and Oceanography, 26*(6), 1020–1033.
50. Graham, J. M., Graham, L. E., Zulkifly, S. B., Pfleger, B. F., Hoover, S. W., & Yoshitani, J. (2011). Freshwater diatoms as a source of lipids for biofuels. *J. Ind., 39*, 419–428.
51. Vinayak, V., Gordon, R., Gautam, S., Rai, A. (2014). Discovery of a diatom that oozes oil. *Advanced Science Letters*, *20*(7–9), *Special Issue: National Conference on Nanotechnology and Renewable Energy*, New Delhi: Jamia Millia Islamia.
52. Papu, N. H., & Lingfa, P. (2018). Isolation biomass estimation and characterization of the biofuel potential of diatom *Navicula Sphaerophora. AIP Conference Proceedings, 10*(1063/1), 5032022.
53. Lawton, R. J., de Nys, R., & Paul, N. A. (2013). Selecting reliable and robust freshwater Macroalgae for biomass applications. *PLoS ONE, 8*(5), e64168.
54. Lawton, R. J., de Nys, R., Skinner, S., & Paul, N. A. (2014). Isolation and identification of *Oedogonium species* and strains for biomass applications. *PLoS ONE, 9*(3), e90223.
55. Metzger, P., & Largeau, C. (2005). *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids. *Applications Microbiology Biotechnology, 66*(25), 486–496.
56. Kalacheva, G. S., Zhila, N. O., & Volova, T. G. (2002). Lipid and hydrocarbon compositions of a collection strain and a wild sample of the green microalga Botryococcus. *Aquatic Ecology, 36*(2), 317–331.
57. Hoek, C., Mann, D. G., Martin, J. H. (1995). *Algae: An introduction to phycology*. Cambridge University Press. ISBN 0-521-31687-1.
58. Farouk, K. El-B., Gad, M. S., Abdoc, S. M., Abed, K. A., Matte, I. A. (2016). Performance and exhaust emissions of a diesel engine burning algal biodiesel blends. *International Journal Mechanics Mechatronics, 16*(3), 151–158. 165803–9797-IJMME-IJENS
59. Islam, Md. A., Rahman, M. M., Heimann, K., Nabi, Md. N., Ristovski, Z. D., Dowell, A., Thomas, G., Feng, B., Alvensleben, N., & Brown, R. J. (2014). Combustion analysis of microalgae methyl ester in a common rail direct injection diesel engine. *Fuel, 143*, 351–360.
60. Hoekman, S. K., Broch, A., Robbins, C., Ceniceros, E., & Natarajan, M. (2012). Review of biodiesel composition, properties, and specifications. *Renewable Sustainable Energy Review, 16*(1), 143–169.
61. Dash, S. K., & Lingfa, P. (2018). Performance evaluation of Nahar oil–diesel blends in a single cylinder direct injection diesel engine. *International Journal of Green Energy, 15*(6), 400–405.
62. Sharif Hossain, A. B. M., Salleh, A., Boyce, A. N., Chowdhury, P., & Naqiuddin, M. (2008). Bodiesel fuel production from algae as renewable energy. *American Journal of Biochemistry and Biotechnology, 4*(3), 250–254.
63. Ashokkumar, V., & Rengasamy, R. (2012). Mass culture of B braunii Kutz. Under open raceway pond for biofuel production. *Bioresource Technology, 104*, 394–399.
64. Jahirul, M. I., Brown, R. J., Senadeera, W., O'Hara, I. M., Ristovski, Z. D. (2013). The use of artificial neural networks for identifying sustainable biodiesel feedstocks. *Energies, 6*(8), 3764–3806.
65. Patel, J. S., Kumar, N., Deep, A., Sharma, A., Gupta, D. (2014). Evaluation of emission characteristics of blend of algae oil methyl ester with diesel in a medium capacity diesel engine. SAE Technical Paper 2014–01–1378.