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Assessment of Microalgal Triacylglycerol as Environmental Benign Approach for Biodiesel Production

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ABSTRACT

Energy plays a vital role for the development for the human welfare. The global economy totally runs on energy, or we can say that all living things depends on it, for example, plants takes energy from the sunlight, water and minerals, and this process is called photosynthesis, animals takes energy from plants and from one another, whereas humans takes energy from both. As the population increases, the uses of fossil fuels increased rapidly and because of this supply of renewable resources get affected and global warming makes earth more complicated due to increase in carbon dioxide. The production of biodiesel using microalgae biomass appears to be more viable and cheaper. Microalgae are a good source of lipid and triacylglycerol (TAG) that are sustainable feedstock for biodiesel. The present paper is an attempt to know the potential of microalgae for the biodiesel production.

1. Introduction

The world has been suffering from enormous crisis of energy due to depletion of finite resources of fossil fuels. The continued use of petroleum based fuels is now depleting because of over population and contribution of these fuels to pollute the environment is also increasing [1]. Use of vehicles all over India increasing drastically, and due to this India meets nearly 75%-80% of its total petroleum requirements through import.

Wasting excess amount of fuels adversely affects the environment and the atmosphere of earth, result global warming, which is an international issue all over world. There are many sources of renewable energy i.e. solar energy, wind energy, hydro energy, and biomass energy which produce from the natural sources, in biomass energy we used different microorganisms. At the present time the quantity of biofuel from vegetable oil, animal fat is not enough to meet world energy requirement for transportation fuel [2]. Microalgae as a source of biomass that contain excess amount of lipid is cheap and environment friendly and do not affect the overall balance of CO₂ in the atmosphere [3]. It is a good source of lipid and fat (triacylglycerol) that are beneficial for biodiesel and main important thing is that is biodiesel blended fuel required little or no modifications in diesel engine [4]. With the use of biodiesel, we can greatly reduce environmental pollution as well as dependency on the fossil fuel.

1.1 Microalgae

Microalgae are unicellular, basically found in fresh water and marine water system. Their sizes vary from few micrometers (0.1µm) to (100 µm). Unlike other higher plants, they do not have roots, stems or leaves. They are specially adapted by environment. But microalgae are capable of performing photosynthesis, which converts sunlight, water and carbon dioxide to sugars. They are fastest growing photosynthesizing organism and can produce excess amount of oxygen and have a capability to reduce CO₂ [5, 6], which are responsible for global warming. Microalgae can fix CO₂ by photosynthesis is 10-50 times than plants [7]. The biodiversity of microalgae is huge and they represent an almost unrecognized resource. It has been estimated that about 2-10 lakh species in many different genera exist of which about 50,000 species [8] are described (Table 1). Most of these microalgae species produce unique products like carotenoids, antioxidants, fatty acid, toxins, enzymes etc., [9]. Microalgae also play a key role on food web and provide energy.

Table 1 The four most important group of algae in reference to abundance

Algae	Known species	Storage mass	Habitat
Diatoms	1000,000	Carbohydrate, TAGs	Ocean, fresh water
Green algae	8000	Starch, TAGs	Fresh water
Blue-green algae	2000	Starch, TAGs	Different habitat
Golden algae	1000	Carbohydrate, TAGs	Fresh water

1.2 Advantages of Microalgae to Other Resources

1. Higher algal biomass can grow in the suitable culture vessels i.e. photobioreactor throughout the year [10], and to avoid contamination and increase lipid content [11].
2. Microalgae have an ability to produce; biopolymers, proteins, polysaccharides, pigments, animal feeds, and fertilizers [12].
3. They have an ability to reduce CO₂ concentration, which are produced due to combustion of fossil fuels, and reduces the emission of greenhouse gases [13, 14]
4. Microalgae can be cultivated in saline, blackish water, waste water [15, 16] or coastal or sea water on non-arable land very easily.
5. Microalgae synthesize and accumulate large quantities of neutral lipids or oil as compared to other oil producing crops (Table 2).
6. They require less land (100-200 times) than oil crop plant like Jatropha, palm or soybean [17] and produces more oil (10-20 times). It can also be grown on non-productive land [18]

Table 2 Comparison of algae with different crops for biofuel

Source	Gallons of oil acre per year
Algae	5000-20,000
Palm	635
Coconut	287
Jatropha	207
Canola	127
Peanut	113
Sunflower	102
Soybean	83
Corn	18

1.3 Microalgae as Biofuel Production

Increase in demands of fossil fuels, in today's world, alternate means of fuels is being investigated all over the world. Biodiesel and bioethanol are renewable fuels with much potential in today's current research. Microalgae can be remarkably rich in oils (up to 80% dry weight of biomass) suitable for conversion to fuel. Furthermore, microalgae are

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more productive than land based agricultural crops. Their important role as a good food source is due to the contents of minerals, vitamins and oils, which are rich in polyunsaturated fatty acid [19]. These fatty acids that contain more than one double bond in their backbone, such as α -linoleic acid, eicosapentaenoic acid and docosahexaenoic acids, all fatty acids belongs to omega 3 group of PUFAs. Microalgae having the ability to synthesize triacylglycerol are considered as a generation feedstock for production of biofuel and specially biodiesel [20]. The idea of using microalgae as a source of fuel is not new in today's world, but it is now have been taken seriously because of rising price of petroleum and more emerging or use of vehicle due to overpopulation which causes adverse effect on earth atmosphere and the reason is global warming, which is also associated with burning of fossil fuels [21].

2. Types of Algae Used in Lipid Production

Chlorella zofingiensis is a green algae that can grow well in both photoautotroph as well as heterotrophs, green algae, having higher cellular biomass and are phylogenetic relatives of *C. zofingiensis* such as *Chlorella vulgaris* and *C. protothecoides* were reported to give high amount of lipids [22]. Euglenophyta, have a photosynthetic ability like microalgae, the scientist believe that it gained this ability because of endosymbiosis relationships with photosynthetic green algae. Diatoms and golden-brown algae the most abundant types of unicellular algae estimated for around 100,000 different species. Diatoms consist of silica shells that vary their shape and structure depending from species to species. Fire algae are also unicellular organism and are classified into two types i.e. dinoflagellates and cryptomonads. Dinoflagellates can cause a phenomenon known as a red tide, in which the ocean appears red due to their large abundance. Cryptomonads similar to dinoflagellates and produce algal bloom.

Red algae found in tropical marine locations, their cell wall consists of cellulose, and have an ability to produce different types of carbohydrate. Brown algae, is the largest species among all algae, consisting of varieties of seaweed and found in marine environments. Yellow-green algae, they actually found in fresh water, these algae store carbohydrate as lecosin.

3. Lipid Synthesis in Microalgae

Both inorganic carbon (CO₂) [23] and organic carbon sources (glucose, acetate) are utilized by microalgae for the production of lipid. The contents of lipid in micro-algal cell vary from species to species. The lipids are differentiated into two important parts: Neutral lipids and polar lipids. Triacylglycerol, the long hydrocarbon chains of fatty acids are extraordinarily efficient for energy storage [24]. Because they contain carbon in a fully reduced form and will therefore yield a maximum amount of energy on oxidation. Triacylglycerol or fat are tri-ester of fatty acid and glycerol. They are water insoluble, but they do not even form micelles very effectively fat accumulated in plants cells therefore form as oily droplets in the cytoplasm. Polar lipids i.e. phospholipids, galactolipids molecules that play dominant roles in membrane formation have highly polar head groups and in most cases two hydrocarbon tails. These phospholipids are naturally occurring lipids containing phosphate head groups. These compounds make up a significant fraction of the membrane lipids throughout the bacteria plant kingdom.

The synthesis route of triacylglycerol in microalgae consists of three steps:

1. The formation of acetyl co-enzyme A in the cytoplasm.
2. The elongation and desaturation of carbon chain of fatty acid.
3. Biosynthesis of triacylglycerol in microalgae.

3.1 The Formation of Acetyl Coenzyme A in Cytoplasm

The vast majority of the stored fuel in the most of the plant cells is in the form of fats in the form of oily droplets in cytoplasm. However, a large proportion of the calorie intake of diet of animal or human is carbohydrate but because of reserved storage of carbohydrate is limited; there are efficient mechanisms for converting carbohydrate to fats. Acetyl-CoA is the key intermediate between both the carbohydrate and fat metabolism. Glycolysis is the first metabolic pathway, which takes place in cytoplasm also called EMP pathway, in glycolysis, one molecule of glucose (6C) converts into two molecules of pyruvate (3C). Now this pyruvate goes under mitochondrial matrix convert pyruvate to acetyl CoA by pyruvate dehydrogenase complex by oxidative decarboxylation, thus the process is unidirectional, and then acetyl CoA convert into citrate with help of citrate synthase and the process is called citric acid cycle or Krebs cycle. Now citrate from mitochondria moves to cytosol to form fatty acid and this

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process is called glyoxylate cycle, lipid rich body, where fatty acid oxidation is also operative.

3.2 The Elongation and Desaturation of Carbon Chain of Fatty Acids

The elongation of these fatty acids depends upon two enzyme i.e. acetyl CoA carboxylic enzyme (ACCE) and fatty acid synthase in most of the organism. In the process of fatty acid, acetyl CoA is the main intermediate, and for the elongation of carbon chain acetyl CoA cooperates with malonyl CoA on which enzyme work are acetyl-ACP and malonyl-ACP. The short chain fatty acids (C14-C18) are main components of biodiesel and majority of microalgae with short chain used for the production of biodiesel, but high content of long chain fatty acid exist in some species.

3.3 The Biosynthesis of Triglycerides in Microalgae

Fatty acyl CoA, along with glycerol 3 phosphate, both serve as the major precursors to triacylglycerol. Glycerol 3 phosphate is derived either from the reduction of glycolysis intermediate dihydroxyacetone phosphate, catalyzed by glycerol phosphate dehydrogenase, or from ATP- dependent phosphorylation of glycerol by glycerol kinase. Di-hydroxyacetone phosphate. The pathway involving DHAP predominates in adipose tissue, because adipose lacks glycerol kinase. Diacylglycerol 3 phosphate, also called phosphatidic acid, which is a main precursor of both the phospholipids and to triacylglycerol. The pathway to TAGs involves hydrolytic removal of phosphate.

4. Biodiesel

Biodiesel, is called as the mono-alkyl ester of long chain fatty acid, which is derived from the renewable sources, such as vegetable oil or animal fats etc. biodiesel is one of the most renewable fuels and also non-toxic or nonhazardous and biodegradable [25]. Biodiesel has come to mean a very specific chemical modification of natural oils. One of the biggest advantages of biodiesel to many alternate transport fuels is that it can be used in existing diesel engines without further modification, and can be blended with petroleum diesel [26].

4.1 Chemical Composition of Biodiesel and Transesterification

The plants oils usually contain free fatty acids, phospholipids, sterols, water etc. because of these oil cannot be used directly. To overcome from these problems we need slight chemical modifications mainly transesterification, pyrolysis and emulsification. Among these, the transesterification is the key and foremost important step to produce the safest, cleanest and ecofriendly fuel from vegetable oils, using an alkali catalyst and methanol as a receptor [27-29]. TAGs consists of three long chains of fatty acids attached to a glycerol backbone. By reacting these TAGs with alcohols (a chemical reactions known as transesterification), amino-alkyl ester is formed which is known more generically as biodiesel. Transesterification requires 3 mole of alcohol for one mole of TAG to produce 1 mole of glycerol and 3 mole of methyl esters. In industries, the moles in each reaction increases as they required large amount of methyl esters, and because of the large amount the % wise yield also increase to about 98%. Biodiesels were produced from seed oil, such as sunflower oil, soybean oil, rapeseed, corn etc., by transesterification reactions using methanol, ethanol, 2-propanol, and 1-butanol as alcohol. Potassium hydroxide, sodium hydroxide, and sulfuric acid were used as catalysts. After biodiesel production, ester conversion rates and fuel properties such as viscosity, density, and total and free glycerol were determined. The most suitable alcohol for biodiesel production is methanol. The most suitable alcohol for biodiesel production is methanol. Other alcohols also are used with an acid catalyst for long reaction times such as 48 h. As the alkaline catalyst, potassium hydroxide is more superior to sodium hydroxide. Among the studied, seed oil such as vegetable oils, sunflower oil gave the best results, while cottonseed oil couldn't give better result in terms of practicality and some fuel properties [30].

4.2 Eco-Friendly Biodiesel

Biodiesel, in view of environment is considered as carbon neutral as compared to CO₂, which responsible for global warming and the biggest advantage of biodiesel is it can be used in diesel engine without any modification; we can blend with petroleum diesel in a required ratio. The use of biodiesel in today's life maintains the balance between agriculture, economic development and the environment. Biodiesel performs as well as petroleum diesel while reducing emissions of particulate matters, carbon monoxides (CO), hydrocarbons and oxides of sulphur (SO_x). Emissions of oxides of nitrogen (NO_x) are however, higher for biodiesel in many engines [31]. Biodiesel eliminates all the notorious black soot and

particulates matter emission in a very low contents. The other benefits of biodiesel include the fact that it is highly biodegradable and appear to reduce air toxics and carcinogens. The use of biodiesel will allow a balance to be sought between agriculture, economic development and the environment.

5. Conclusion

Bio-diesel production in India is under development basis as compared to foreign countries. The major reason behind this is commercialization, outreach programs, public awareness and technologies transfer. Biodiesel contribution in India is still on a working position as compared to other countries, like USA, Germany, Europe, Brazil, Indonesia etc. The economic growth of India, has the potential of biodiesel production as there are enormous feedstock such as (seed oils, micro-organism, waste material etc.), which could be the exploited for its synthesis. The government has been initiating, and motivating new research and technologies for biodiesel production which yield positive results and to reduce environmental pollution.

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