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Algal Biofuels – Production, Cultivation and Lipid Extraction For Biofuel Production

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ABSTRACT

Due to industrialization and modernization, more amounts of fossil fuels are being exhausted on daily basis and they are at the verge of complete depletion. Due to increased consumption of fossil fuels, the problem of environmental pollution is on an increasing scale. To combat the pollution issues and to alternate the depleting fossil fuels, the researchers have concentrated on fuels of biological origin called Biofuels. The biofuels have evolved through 4 generations from utilizing the food crops to modifying the metabolic pathways of microbes. Algal biofuels are very important and feasible owing to their inherent capacity to accumulate high concentrations of lipids. This review article, focuses on various methods that favour algal cultivation and methods of lipid extraction for efficient biofuel production.

Key words: Algal biomass, Biofuels, Fossil fuels, Lipid extraction, Oil extraction

INTRODUCTION

Anaerobic decomposition of dead plants and other organisms buried beneath multiple layers of rocks and rich in Carbon leads to the formation of Fossil fuels, which may take a million years to form [1]. Fossil fuels include petroleum, natural gas, coal etc. which contain a very high percentage of carbon. They have low carbon to hydrogen ratio and range from volatile fuels such as methane, liquids like petroleum, to non-volatile materials containing

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pure carbon such as coal. The most commonly used fossil fuels include propane and kerosene. Burning of fossil fuels is one of the major reasons that contribute to global warming. The oil that is economically viable is said to be eventually exhausted in around 20-25 years. The natural gas and coal are also no exceptions. Everyday consumption of diesel and petroleum is also considered as a threat for the future availability of the fossil reserves. Although renewable energy sources such as solar energy, hydrothermal energy, wind energy etc. may contribute to reduce the dependency on fossil fuels, they are of minimal help [2]. Solar, wind, hydroelectric, and nuclear energy have become suitable substitutes for the depleting fossil fuels in power generation. However, to reduce our dependence on fossil based fuels, the need for low-carbon sources of transportation, through adoption of electric vehicles in a large scale is the need of the hour. Since the fossil fuels are depleting at a faster rate and its usage leads to pollution, researchers and environmentalists are focussing on eco-friendly biofuels to replace the fossil fuels which are at the verge of extinction. Biofuels are those fuels that are derived from biological materials such as agricultural wastes, crops, trees etc. which are able substitutes for conventional fossil fuels. Biofuels are free of sulphur and produce very less carbon monoxide and other toxic emissions. As an effective alternative for petroleum and diesel, biofuels have become the easy solution for the fuel scarcity and environmental problems

There are four generations of biofuels. First generation biofuels are produced from food crop sources such as corn, barley, wheat etc. This demands fertile and cultivable lands for crop production and reduces the availability of lands for human and animal food use, which also has a negative impact on food security and also leads in degradation of environment. Since these biofuels require large quantities of water for crop cultivation and compete with food crops causing food scarcity, First generation of Biofuels are nor much viable. Therefore the researchers and scientists plunged into identifying non-food feedstocks for biofuel production. The other aim was to improve the quality and performance of the biofuel as well as to bring down its costs especially in the transport sector [3-4].

Second generation biofuels include biodiesel and bioethanol, which are considered as an improvement in biofuel production from non-food materials as feed stocks which include baggase, straw, forest residues and feedstocks that are grown on purpose on marginal lands. They are also called as lignocellulosic biofuels as they are derived from hemi-cellulose, cellulose and lignin. However, second generation biofuels require expensive technologies and

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are not profitable commercially. Therefore there was a need for a better and advanced biofuels existed [5-6]

Third generation biofuels are algal biomass based which includes microalgae and other microbes. The advantage of this generation of biofuels is that microalgae can adapt to any kind of habitat and a variety of biofuels can be obtained from them [7]. The biofuel production using microalgae is almost 15-300 times higher than the ones produced from food crops [8-9]. Microalgae are even more advantageous since it reduces the depletion of fossil fuels. Third generation biofuels are under extensive scientific research to improve the quality and production of biofuel along with enhanced separation process to remove non-fuel components with reduced production costs.

Fourth generation biofuels is photobiological and uses solar cells and also are electrofuels which is expected to bring about a breakthrough in the field of biofuels. This generation of biofuels uses a technology to directly convert solar energy into biofuels utilizes inexhaustible, cost effective and easily available raw materials. This process can be accomplished by the developments in metabolic engineering and synthetic biology. However, Synthetic biology is still at its infancy and for concrete successful metabolic manipulations, in-depth research is constructing synthetic living microbial factories and designer microbes which have the ability to directly convert solar energy to fuel needs to be conducted [10]. This review gives an account of the processes and approaches by which algae are cultivated and harvested and an over view of lipid extraction process by which the final biofuel is produced.

PRODUCTION OF ALGAL BIOMASS

Algal biomass can be cultivated by any of the below processes:

Photoautotrophic Production:

Algae, undergoes the process of photosynthesis by utilizing light as the energy resource and inorganic carbon as the source of carbon and produces carbohydrates. Photoautotrophic algal biomass production is the most common strategy to cultivate algae with appreciable lipid content. It is also the most preferred method to cultivate algae for oil production because it utilized CO_2 as its carbon source [11].

Heterotrophic Production:

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The algal biomass is grown on a carbon substrate such as glucose thereby eliminating the requirement of light energy. This production process is simple and feasible in a way that even a bioreactor with small surface to volume ratio will perform the requirement. This process produces high density cells with higher lipid content, which in turn lowers the harvesting budget. This is advantageous over autotrophic algal production as the carbon source is readily available rather than it being produced during the photosynthetic process [12].

Mixotrophic production:

Some algae satisfy their nutritional requirements through autotrophic and heterotrophic means. They are called mixotrophs. The light energy is not the primary requirement for them and they can feed on utilizing organic materials as well. Owing to the fact that mixotrophs utilize both photosynthetic and heterotrophic components for its growth, the biomass loss and the quantity of the consumed substrate is reduced. This is said to the reason for the mixotrophs to have decreased photo inhibition and enhanced growth rate in comparison with the autotrophs or heterotrophs [12].

Photoheterotrophic production:

The growth of the algal biomass requires light energy and also uptakes carbon from external organic sources. Without light energy, the photoheterotrophs do not grow. Although this method leads to the enhanced production of light regulated metabolites, this method of cultivation is usually not suitable for certain biofuel production, ex. Biodiesel [13]

ALGAL CULTIVATION SYSTEMS

The algal biofuels may be grown using various cultivation systems as discussed below:

(i) Open ponds:

Natural water bodies such as lakes, ponds, lagoons etc. or open tanks together can be denoted as open ponds which are easy and feasible to construct and maintain. Its average volume yield may be around 0.06 to 0.42g/L/day. The pond's efficiency depends mainly on its configuration and the algal species being cultivated. The ponds being shallow, favour easy penetration of sunlight as the nutrients and water are being circulated continuously [14. The biomass produced on a daily basis in unit area of the pond is calculated as its output. These artificial open ponds are further classified as:

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(a) *Unmixed Open Ponds* - Unmixed open ponds simply do not get mixed. Because of this, the system does not have a control of factors that favour the cultivation process. Since the pond remains unmixed, the algal cells have a tendency to settle down to the floor due to gravity and hence, the availability of carbon-di-oxide and light is significantly reduced. Due to the decrease in the carbon-di-oxide content, the algal biomass yield also seems to be compromised [14].

(b) *Circular Ponds* – Circular ponds is the first design that is available commercially for fostering algae. However, the system poses a drawback that it has a limited scale to a maximum range of approximately 1000 m^2 at which the core mixer becomes unmanageable [15].

(c) *Open Raceway Ponds* – Open raceway ponds also known as high rate algae ponds (HRAP) is used for the waste water treatment by supporting and maintaining a symbiotic relationship between active aerobic bacteria and the algal biomass. Circulation of nutrients and broth is ensured using a paddle fitted in looped channels. These types of ponds are made of PVC or clay or concrete and is about 0.2 to 0.5 meter in depth enabling deep sunlight penetration. CO_2 is usually taken directly from the air water interface; however, installing aerators inside the pond can help increase the Carbon-di-oxide levels. Although open raceway ponds are very well developed, cross-contamination and infection with undesirable algal species pose a drawback to this method of algal cultivation [15].

(ii) **Closed Photobioreactors:**

Closed photobioreactors do not let the algae to be exposed to the external environment. All favourable conditions are provided to the algal biomass to grow in a closed and contained environment.

(a) *Tubular Photobioreactor* – Tubular Photobioreactor (PBR) is an outdoor algal cultivation unit constructed by linearly arranged tubes made of glass or plastic. PBRs have an ability to expose the growing algal culture to maximum sunlight enabling it as a suitable strategy for outdoor cultivation of algal biomass. A small disadvantage of algal settling at the bottom of the tubes has been reported. However, it can be minimized by using an airlift propeller [16].

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(b) *Flat Plate Photobioreactor* – Flat plate Photobioreactor consists of slender rectangular containers made up of transparent materials tilted at a certain angle allowing maximum exposure to sunlight. The algal biomass cultivated by using this method is said to have high densities. This method is advantageous since it allows low dissolved oxygen accumulation and maximum photosynthesis. However, limitations such as poor temperature control and algal cell adherence to the walls of the bioreactor still exists [16]

(c) *Column Photobioreactors* – The column PBRs allow aeration from the base and the translucent walls of the reactor provide it the maximum sunlight it requires for its growth. There is also a possibility of internal illumination. The optimal conditions of mixing, cultivation and volumetric mass relocation rates are ensured by the configuration of the column PBRs [17]

(d) `*Continuously Stirred Tank Reactors* – Continuously stirred tank reactors has a hollow, broad and capped duct which is cylindrical and has the ability to function both indoors and outdoors effectively. The set-up is stirred and illuminated from above and the gas injectors and drainage are located at the mid and bottom. On comparison with the open ponds, PBRs exhibit extremely low risk of contamination from external pollutants, minimal loss due to evaporation and also allows effective management of cultivation factors [17]

(iii) **Hybrid Systems**

This cultivation method consists of two phases and utilizes both open ponds and photobioreactors at different stages of the growth of the algal biomass. The first phase of algal biomass cultivation occurs in a PBR where continuous biomass growth occurs under controlled optimal conditions which provide a contaminant free environment. Following this the biomass is scaled up in open ponds where they are exposed to nutritional and ecological stress which enhances the production of lipids which is the desired product [18].

The open pond cultivation system is very much advantageous by being able to be set up easily and a cheaper method to cultivate algae which requires lesser energy consumption. However, chances of contamination, evaporation losses, inadequate mixing and aeration, limited availability of light are some of the factors that affect the efficiency of the open ponds.

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HARVESTING OF ALGAE

Harvesting of the maximum biomass is required for efficient biofuel production, along with the reduction in operational costs and maintenance [19]. Harvesting of the algal biomass can be of two methodologies: the first is a two-step process where initially the biomass suspension is thickened to obtain maximum Total suspended solids followed by dewatering where the biomass is obtained in form of a cake. The second process is a single step concentration of the biomass suspension [20, 21]. The properties and value of the end product defines the appropriate harvesting step [22]. Generally microalgal harvesting involves the two-step thickening and dewatering [23-24].

(i) Screening

Screening is performed as a pre-processing step with microalgal cultures. The efficiency of screening depends on the spaces available between the screen openings and the size of the algal cells. Vibrating screens and microstrainers are used for primary screening process [25]. Microstrainers consist of a straining fabric over a rotary drum to capture the algal biomass. Although this method is simple and cost effective it suffers the problem of backwash into the medium [21]. Larger algae however, are harvested with strainers of larger mesh size [25]. Additionally, the fabric or mesh may require constant maintenance as chances of algal and bacterial biofilm formation may cause detrimental effects [21].

(ii) Thickening

In order to increase the concentration of the total solids in the suspension and to reduce the volume of biomass to be processed, the process of thickening is very much required [25]. Basically, the thickening is a process consists of coagulation and flocculation methods followed by gravity sedimentation, floatation and electrical approaches [21].

(a) *Chemical coagulation/flocculation* – This is the most economical approach for efficient microalgal harvesting. This process is used when larger volumes of microalgal cultures are being processed. This is applicable and feasible for a variety of algal species [21]. This process concentrates the biomass suspension 20 to 100 times [26]. The energy demand required for the process is highly reduced by improving the effective particle size prior to dewatering [22, 27].

Coagulation involves adjustment of pH or addition of electrolytes to the culture broth

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[28]. Flocculation can be carried out in the following ways (i) *electrostatic patch* – where charged polymer upon binding to an oppositely charged particle, reverses the charge locally thereby creating a patch that connects to the oppositely charged patches, (ii) *bridging* – occurs when colloids or particles binding to two different particles forms a bridge in between, (iii) *sweep flocculation* – when particles are trapped within a huge mineral precipitation [26].

Salts of multivalent metals such as Ferrous sulphate, Aluminium sulphate, Ferric chloride etc. have been widely used as coagulants for microalgal harvesting. These salts in solution decrease the electrostatic repulsion between the negative charges on the cell surface, favoring aggregation of the cells [28-29]. The more the ion is electronegative; rapid the coagulation process is [28]. As mentioned above, the coagulaton/ flocculation step followed by gravity sedimentation is a cost effective process, however cost of the coagulants represent a very significant portion of the entire process. Therefore, natural coagulants and flocculants such as calcium, carbonates, magnesium, phosphates etc. can be effectively used for algal harvesting [30].

(b) *Auto and bioflocculation* – Auto and bioflocculation can readily be referred to as the same concept. *Autoflocculation* simply occurs by an increase in pH. It is cost effective, non-toxic, low energy consuming, does not need chemical flocculants, and also allows reuse of the culture medium [31]. In this process the algal cells in the presence of sunlight and utilize carbon-di-oxide thereby increasing the pH of the medium causing the cells to auto-flocculate. *Bioflocculation* is caused by interaction with secreted polymers such as EPS [32]. For sustainable microalgal biofuel production, the contribution of bacterial flocculants is much economically significant. Like auto-flocculation, Bioflocculation also does not require the use of chemical flocculants. Flocculation occurs by the attachment of the EPS produced by the bacteria to the algal cells thereby forming flocs [33]. Microalgal bacterial flocs are aggregates that are larger in size and have the ability to settle at a faster rate than the microalgae [34]. However, algae-bacteria co-culture may lead to contamination lowering the biomass application as feed or food [26]. This process has some advantages such as (i) the microbes added may contribute to the increase in lipid yields [35-36]. (ii) Reuse of the medium is facilitated there by cutting down on the cultivation costs [37]. The flocculation process may be enhanced by the addition of chemical flocculants like calcium chloride. This leads to the reduction in the quantity of chemical coagulants used. Fungi assisted microalgal flocculation lead to the formation of pellets that sediments to the bottom [38]. This approach is widely

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used in waste water treatment and furthermore, the fungi contribute to almost 30% of the total lipid content of the biomass favoring biodiesel production [39].

(c) *Gravity sedimentation* – Although being a simple and basic approach, sedimentation works effectively for different microalgal species. Gravity sedimentation is works best when the end product is of extremely low value. The method is not very much reliable since deterioration of most of the algal biomass happened while settling [32]. In order to prevent this deterioration, application of coagulants and flocculants before gravity sedimentation is preferred [22]

(d) *Flotation* – Often termed "inverted sedimentation", floatation induces air bubbles to be fed to the culture broth which provides the lifting force to drag the biomass to the surface. This approach is efficient and feasible than sedimentation process when used along with flocculants. It has the advantage of utilizing minimal space and short operation. Since smaller sized bubbles have low rise viscosity and high surface area to volume ratio, microfloataion is a very much appreciable approach for fragile flocs [40].

(e) *Electrical based processes* – Electrical based approaches for microalgal harvesting is a versatile and eco-friendly approach which does not use any kind of chemicals [21]. On application of the electric field, the negatively charged microalgal cells tend to separate and form precipitates on electrodes and also flocculate on the bottom of the container (electro-flocculation). Bubbles can also be introduced leading to electro-floatation to happen [25].

(iii) Dewatering

Microalgal slurry is dewatered using mechanical means such as filtration and centrifugation. The efficiency of the dewatered cake is then improved by the drying process and the lipids are extracted.

(a) *Filtration* – Filtration is carried out usually following the coagulation/ flocculation process. A drop in pressure is created to allow the system to force the fluid flow through the filtration membranes. Due to the continuous accumulation of the deposits, the membranes suffer fouling which is a major drawback of the process requiring constant cleaning and maintenance of the membranes [32]. Therefore optimization to operate on the sub critical levels is very much required for the maximum recovery of the biomass. .

(b) *Centrifugation* – Considered as the fastest method of biomass recovery, centrifugation is

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also the most expensive method with high energy consumption which has limited application to only products of high value [22, 29, 32, 39]. Centrifugation can separate maximum amount of microalgae from the slurry, however, shear forces and high gravitational forces may result in damage to the cellular structure [23, 29]. Figure 1 is a schematic representation of various processes involved in algal cultivation, harvesting for biofuel production.

LIPID EXTRACTION

Based on the chemical composition of the feedstock, various biofuels such as bioethanol, biodiesel, biomethane, biohydrogen, biobutanol, jet fuel etc. can be produced [41]. Therefore extraction of lipids is a very essential process for the microalgal biodiesel production. Various strategies such as ozonizing the microalgal biomass in methanol leading to rupturing of the cells, usage of various polar and non-polar solvents, transesterification are employed to recover the valuable lipids from the algal biomass. Recently cheap and eco-friendly, virus mediated cell rupture to extract lipids has also been reported [42]

Total Lipid Extraction Methods

(i) *Folch method* – being one of the oldest and frequently used methods of lipid extraction, Folch method utilizes chloroform: methanol in the ratio 2:1 to extract lipids from the algal biomass. The homogenized suspension is equilibrated with saline solution and allowed to separate. Lipids are extracted in the upper phase [43]. This is an easy and rapid method to process huge quantities of samples but less sensitive in comparison to the recent available techniques.

(ii) *Bligh and Dyer method* – This is very similar to the above mentioned Folch method but differs in the solvent ratios used. Bligh and Dyer Method utilized 1:2 ratios of chloroform and methanol. And lipids are then extracted from the chloroform phase [44]. Many modifications have been implemented to improve the quality and quantity of lipids extracted. Addition of 1M Sodium chloride instead of water was observed to inhibit binding of acidic lipids to denatured ones. Addition of hydrochloric acid and 0.2 M phosphoric acis was observed to improve the lipid recovery in shorter time duration and HCl [45-46].

(iii) *Extraction of all classes of lipids* – this method proposed by Matyash et al. (2008) is a combination of modified Folch/Bligh and Dyer method. This method uses Methyl-tert-butylether (MTBE) as a solvent and is considered as an accurate method to provide the lipidome profile. Lipid containing, low density organic upper phase is formed which is then

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extracted and processed to obtained lipids. Methanol and MTBE were added to the sample and incubated at RT, which is followed by the addition of water and the allowed for the phases to be separated. Lipids were extracted by centrifuging the upper organic phase [47].

(iv) *Superior Solvent extraction methods* – Despite effective lipid recovery, most of the extraction methods utilize harmful solvents that pose a health risk. Since the solvent used completely depend on the type of lipid to be extracted less-toxic substitutes such as butanol, isopropanol, MTBE, ethanol, acetic acid esters etc. have been investigated [48]. Recently, for superior recovery of the lipids 2-ethoxyethanol has been suggested instead of chloroform-methanol combination [49]. Use of heat/pressure has also been used for accelerated solvent extraction process to achieve better lipid extraction [50]. However when organic solvents are used in a larger scale, disadvantages and health risks can be expected.

(v) *In situ lipid hydrolysis and supercritical In situ transesterification* – Levin et al. (2010) has described a technique called *in situ* lipid hydrolysis and supercritical *in situ* transesterification (SC-IST/E) process for extraction of lipids from algal biomass that is wet. In this method, the wet algal biomass is kept within a steel reactor, which is immersed in a hot isothermal fluidized sand bath and is removed after a specified time. In this process simultaneous dehydration and drying of the biomass converts it to a solid state which facilitates hydrolysis reaction. The solids and the liquid phase were separated upon cooling by light vacuum. Extensive research is however required for analysing the economic feasibility of this process [51]

Mechanical methods of Algal Oil Extraction

Despite the above mentioned methods, there are various other mechanical methods for algal oil extraction has been proposed for both pilot scale and at commercial levels. These are pretty much effective because the microalgal species or the type of microalgae does not affect the process and possibility of contamination is quite less.

Expeller press or simply oil press is one of the ancient methods for oil extraction from dried seeds. The dried algal biomass which retains oil is subjected to mechanical crushing in the oil press to extract oil from them [52]. The basic principle is to apply high mechanical pressure to crush and break the cells which enable the oil to come out of the biomass. But care needs to be taken when pressure is applied since, high pressure can result in decreased lipid recovery [53]. In the *Bead beating process*, the biomass suspension is subjected to spinning at

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high speed with fine beads which directly damages the cells releasing the lipids [54]. The bead mill may contain multiple shaking vessels on a vibrating platform or a better version that contains beads along with the agitator. Cooling packs are fitted in order to combat the heating effect caused [55].

Ultrasound mediated lipid extraction is an alternative method to overcome any drawbacks of the conventional mechanical cell disruption methods. This method that uses ultrasound waves is a simple, eco-friendly and more economical, with high reproducibility and easy working conditions with very less energy input [56]. *Microwave* mediated cell disruption was introduced for isolating lipids and pesticides from seeds, feeds, food and soil. This has also proved to be a safe, rapid and economical method for lipid extraction from algal biomass [57]. The algal cells may be subjected to *electroporation techniques* where the cell membranes and cell wall were rendered permeable upon application of electric current facilitating the inner lipid contents to be extracted in the medium at a shorter time without compromising on the quality of the lipid extracted [58].

Other non-solvent extraction methods by altering the osmotic pressure in the aqueous media, using ionic liquids for isotonic extraction of lipids, use of enzymes to disrupt the cell walls using enzymes can also lead to significant lipid recovery from algal biomass.

CONCLUSION

Algae play a major role in the current scenario in the production of biofuels, which are now proving to be effective alternates for the fossil fuels that are at the verge of extinction. Although the algal biomass, prove to be efficient and cost effective, the problems encountered during cultivation of algal cultures needs to be addressed. The pH, sunlight and temperature of the culture media need to be periodically monitored, in order to obtain maximum growth of biomass. The chances of contamination though external sources should be minimized as much as possible for effective biomass recovery which in turn directly affects the quality of the biofuel obtained. The micro and macro nutrients required for the maximum growth of biomass needs to be provided on a regular basis an exposure to any inhibitory ions should be prevented. By utilizing the natural products available the production costs can be cut down without compromising the quantity of biomass and lipids obtained and subsequently the quality of the biofuel. When suitable conditions are provided for the algal growth, maximum biomass can be achieved which eventually leads to production of high quality biofuels.

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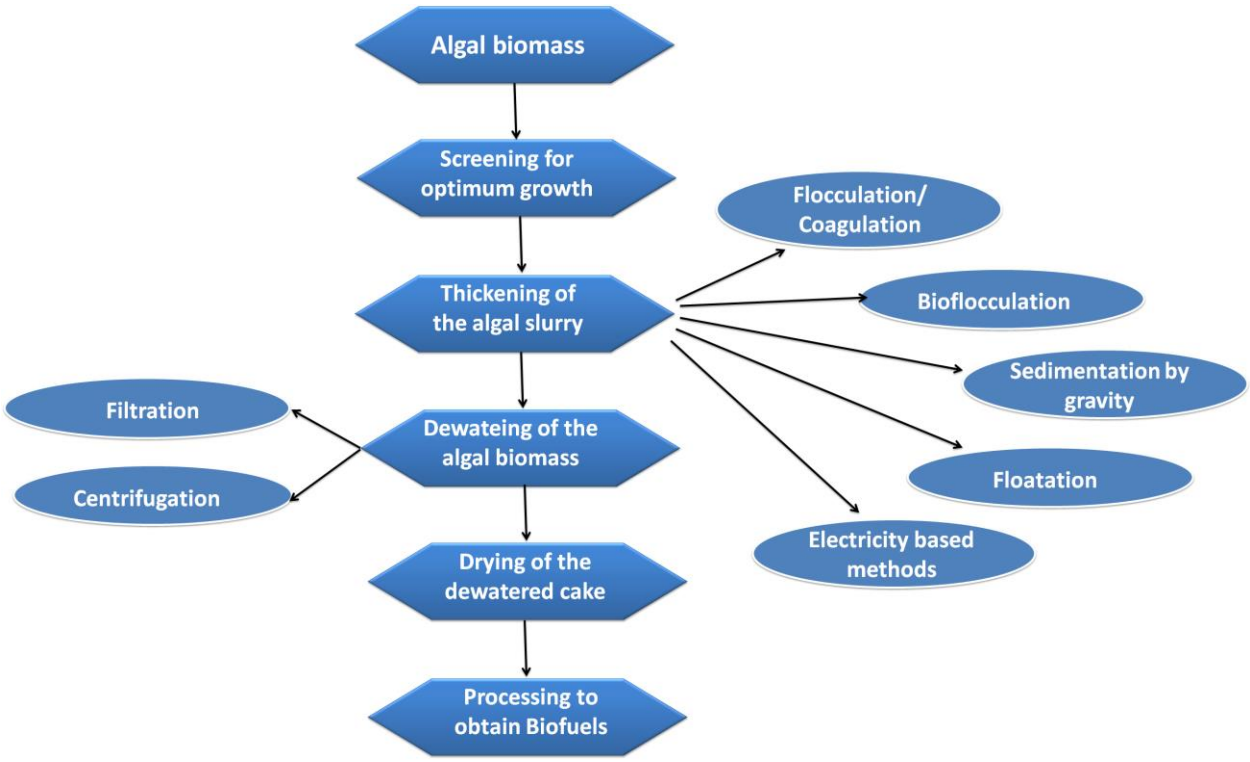

Fig.1 represents the various processes involved in algal cultivation, harvesting for biofuel production