Chapter 15
Integrating Microalgae Cultivation with Wastewater Treatment for Biodiesel Production

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15.1 Introduction

Microalgae are responsible for more than half of the world's primary production of oxygen. They are the simplest and most abundant form of plant life on the earth (Energy from algae (2010). These photosynthetic organisms are categorized under third generation biofuels and are known to have high oil and biomass yields, can be cultivated with wastewater, do not need arable land for cultivation, do not compete with common food resources and very efficiently use water and nutrients for growth (Hannon et al. 2010). There are various routes of metabolism which microalgae have adopted for their growth and survival viz., autotrophic, heterotrophic and mixotrophic. They are capable of shifting their metabolism in response to changes in the environmental conditions (Devi et al., 2012). Algal cultivation for biodiesel production is considered more amenable a technology than the cultivation of oil crops (Chisti, 2007) because the yields of algae-derived oils are much higher (Abou-Shanab et al., 2010). Autotrophically algae gain energy through light by fixing atmospheric CO_2 (Devi and Venkata Mohan, 2012). However, low biomass yields, requirement of cultivation systems with large surface area and shallow depth for better access of light are some of the disadvantages associated with autotrophic mode of nutrition. In the absence of light, the photosynthetic process gets suppressed and algae gain energy from alternative organic processes using heterotrophic pathways that convert sugar into lipids (Perez-Garcia et al. 2010). This pathway leads to significantly denser biomass, facilitating greater lipid yields.

Integrating biodiesel production with CO_2 mitigation from industrial flue gases and wastewater treatment is considered as a viable strategy in algal cultivation and gives an additional offset towards waste remediation. Algae can be cultivated in

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both open raceways and closed systems. Closed systems reduce the chances of contamination with other bacteria/microalgae and thus are more suitable for pure strains. Various bioprocesses and downstream processing technologies are used extensively for recovery of lipids and various co-products from microalgae. They also include physical, thermo-chemical, biochemical and biological treatments to create energy-rich products from the source biomass (Demirbas, 2011). Integration of the biorefinery concept with wastewater treatment will ensure efficient utilization of algae biomass and reduces overall residual waste component of biomass favouring sustainable economics. Algae biodiesel research and development is gaining pace in biofuels markets and has vast scope for advancements in future (Bracmort, 2013). In this chapter, an attempt has been made to summarize the basic and applied aspects of nutritional modes employed by microalgae, algal-cultivation strategies, downstream processing technologies and biorefinery concepts by analyzing the contemporary literature in concurrence with recent developments.

15.2 Nutritional Modes

Nutritional modes significantly influence the carbon assimilation and lipid productivity of microalgae (Devi et al., 2013). Three types of nutritional modes—autotrophic, heterotrophic and mixotrophic—are reported to produce algal fuel in the presence of light. Autotrophic organisms can convert physical (light) and chemical (CO_2 and H_2O) energy into carbohydrates, which forms the basis for all other carbon containing biomolecules (Yoo et al., 2010). Autotrophic organisms are relatively self-sufficient because they obtain their energy from sunlight (Nelson et al., 1994). Photosynthetically fixed CO_2 in the form of glucose serves as the sole energy source for all the metabolic activities of the algal cells (Chang et al., 2011). Major advantage of the autotrophic nutritional mode is that the algal oil production occurs at the expense of atmospheric CO_2. Large scale microalgae cultivation systems (such as open/raceway ponds) are usually operated under photoautotrophic conditions (Mata et al., 2010). However, low biomass yields, requirement of cultivation systems with large surface area and shallow depth for optimum light availability are some of the inherent disadvantages. In the absence of light, the photosynthetic process gets suppressed and algae gain energy from alternative organic processes that convert sugar into lipids (Venkata Mohan et al., 2014a). Heterotrophic organisms utilize organic carbon produced by autotrophs as energy for their metabolic functions because they cannot utilize atmospheric CO_2.

Microalgae use organic molecules as primary sources of energy and carbon through heterotrophic nutritional mode and facilitate high biomass productivities, which are economically feasible for large scale production (Behrens, 2005; Perez-Garcia et al., 2011a). The growth of algae can be significantly denser resulting in greater yield because light is not a limiting factor. Heterotrophic nutrition takes place both in the presence and absence of light. This unique ability is shared by several species of microalgae (Perez-Garcia et al., 2011a). Light and carbon act as an energy source in photo-heterotrophic nutrition mode whereas the sole source of energy during dark conditions is organic carbon. Photo-heterotrophic nutritional mode avoids the limitations of light dependency which is the major obstruction for gaining high cell density in large scale photo-bioreactors. The major advantage of heterotrophic nutritional mode is the facilitation of wastewater treatment along with lipid production, which gives this nutritional mode an edge over the other two (Venkata Mohan et al., 2014b). Moreover, cost effectiveness, relative simplicity of operation, and easy maintenance are the main attractions of the heterotrophic growth approach (Perez-Garcia et al., 2011a, b). However, heterotrophic systems suffer from contamination problems (Olguin, 2012).

Microalgae can also function under mixotrophic nutrition by combining both the autotrophic and heterotrophic mechanisms thus assimilating available organic compounds as well as atmospheric CO_2 as carbon source (Chandra et al., 2014). Since mixotrophs can utilize organic carbon, light energy is not a limiting factor for biomass growth (Chang et al., 2011). Mixotrophism is often observed in the ecological water bodies where the homeostatic structure and function of a living system is supported by chemical, physical and organic activity of the biota, which balances the ecological status (Venkata Mohan, 2010). Depending on the species, growing conditions and growth stages, microalgae have been shown to produce various types of lipids including triacylglycerides, phospholipids, glycolipids and betaine lipids (Greenwell et al., 2010). The intracellular lipid granules stored under stress conditions act as precursors for fatty acid biosynthesis.

15.3 Wastewater Treatment vs Lipid Production

Algae based wastewater treatment involves photosynthetic conversion of solar energy into useful biomass utilizing nutrients such as nitrogen and phosphorus along with organic carbon. This wastewater, if discharged into water bodies, would lead to eutrophication. Algae are the main biocatalysts in oxidation ponds, raceway ponds and high rate algal ponds where they are used to remove concentration of nutrients, especially for polishing purpose. Heterotrophic/mixotrophic algae cultivation particularly requires carbon, water and inorganic salts. The availability of the nutrients affects the growth of algae as well as the lipid profile. Along with the ability to grow heterotrophically in nutrient-rich and organic environment, algae also remove toxins and heavy metals from wastewater. Levels of several contaminant heavy metals are significantly reduced by the cultivation of microalgae (Munoz and Guieysse, 2006). Moreover, the presence of photosynthetic component in treatment setup makes the whole process eco-friendly (Stephenson et al., 2010; Venkata Mohan et al., 2014a). The following studies were performed with different wastewaters for enhancement of lipid productivities along with nitrogen and phosphate removal.

Cultivation of *Botryococcus braunii* in secondarily treated sewage as treatment operation showed 17 % lipid accumulation (Orpez et al. 2009). Hu et al. (2012)

reported lipid productivity of 20 % with *Auxenochlorella protothecoides* on municipal wastewater. *Scenedesmus* sp. cultivation in fermented swine wastewater yielded good lipids and other value added products in association with nutrient removal (Kim et al., 2007). Organic content-rich wastewaters like diary wastewater are highly amenable for heterotrophic cultivation. The biomass and lipid productivities of 2.5 and 5.8 g L^{-1} were obtained for soya whey and ethanol thin stillage in heterotrophic mode using *Chlorella vulgaris* (Mitra et al., 2012). In the study conducted by Beevi and Sukumaran (2014), diary wastewater was supplemented with 4 % and 6 % of glycerol as carbon source. Lipid productivities of 39 % and 42 % were reported using *Chlorococcum* sp. RAP13 in heterotrophic mode (Beevi and Sukumaran, 2014). Acidogenic effluents rich in volatile fatty acids from fermentative hydrogen producing process were evaluated as substrate for lipid accumulation by heterotrophic cultivation (Venkata Mohan and Devi, 2012). Acetate gets easily assimilated by the algal cell, as a part of the acetyl- coenzyme A (acetyl-CoA) metabolism in a single-step reaction catalyzed by acetyl-CoA synthetase (Boyle and Morgan, 2009). TAG accumulation in response to environmental stress is likely to occur as a means of providing an energy that can be readily assimilated on return of favourable environment, to allow rapid growth (Devi et al., 2012). Thin layer culture systems are well-known for high biomass productivities. Attached mode growth of *Chlorella* sp. with dairy manure wastewater showed high biomass growth as well as fatty acid yield (Johnson and Wen, 2010).

15.4 Cultivations Methods

Cultivation of microalgae influences both biomass growth and lipid productivity. Culturing of algae requires the input of light as an energy source for photosynthesis with a sufficient supply of macronutrients (carbon, nitrogen and phosphate) and micronutrients (sulphur, potassium, magnesium) in dissolved form (Mata et al., 2010). These nutrients play a critical role in biomass enrichment by facilitating cell growth, maintenance and synthesis of different kinds of lipids (Table 15.1). The main options for algae cultivation on a commercial scale are open-ponds or closed systems called photobioreactors (Chisti, 2007; Robert et al., 2012). There are also hybrid configurations that include a combination of the two growth options. Innovations in algae cultivation aim towards increasing lipid productivity of algae while consuming resources that would otherwise be considered waste (Campbell, 2008).

15.4.1 Open Ponds/Raceway

Open ponds mimic the ecological niche of algae and can be categorized into natural waters (lakes, lagoons, ponds, etc.) and artificial ponds or containers (Pearson, 1996; Chisti, 2007). Artificial systems include shallow ponds (large in size),

Table 15.1 Effect of macro and micronutrients on algae growth.

Carbon	Major source of energy for heterotrophic algae (i.e. auxotrophs, mixotrophs, etc.). Higher rates of growth and respiration are obtained with glucose.
	High concentrations can lead to product inhibition and reduced growth rate
Nitrogen	Major constituent of protein, nucleic acids, vitamins, amino acids, purines, pyramidines and porphyrins
	Low nitrogen causes increase in polyunsaturated fatty acids (PUFAs)
Phosphorous	Helps in biosynthesis of nucleic acids and phospholipids and TAGs at low concentration. Constituent of cell membrane and is required for phosphorylation reactions
	Deficiency leads to decrease in phospholipids of cell membranes
Potassium	Helps in osmoregulation, ion exchange across intracellular membranes, protein synthesis and also helps in activation of certain enzymes.
	Deficiency leads to increase in respiration
Calcium	Major component for cell wall, important in spindle formation during mitotic cell division. Helps in regulation of certain metabolic activities, improves growth and maintains membrane stability
Magnesium	Helps in activation of enzymes related to photosynthesis and respiration. It's the central atom in chlorophyll. Assists in DNA and RNA synthesis
	Deprived conditions results in low turnout in photosynthesis.
Sulphur	Main component of amino acids like cysteine and methionine. Constituent of several cofactors, vitamins and ferredoxin
Iron	Plays a major role in formation of protein like ferrodoxin and cytochrome. Major component responsible for assimilation of N_2 also important in activation of enzymes of photosynthesis and respiration.
	High concentration leads in inhibition of algae growth
Manganese	Activates many enzymes related to photosynthesis, respiration and nitrogen metabolism. Actively participates in splitting water molecule into oxygen during photosynthesis.
	Decreases the efficiency of photosynthesis
Zinc	Essential for activation of carboxylases for fatty acids synthesis
Molybdenum	Active component in nitrogenase and nitrate reductase which help in nitrogen metabolism
	Deficiency results in low nitrogen absorption

raceway ponds, tanks and circular ponds. These ponds are usually constructed in shallow dimensions as light penetration is a major limiting factor that can slow down biomass growth of algae. Moreover, when these systems are operated in a continuous mode, CO_2 and nutrients have to be fed continuously to the pond (Chisti, 2007). Ponds are divided by a series of baffles, and water is moved through the ponds for proper mixing of nutrients thus ensuring uniform algae growth. Commercial production of single-cell protein, health food, and beta-carotene from algal biomass is one of the oldest industrial large open-pond cultivation systems since 1950s (Chisti, 2007; Perez-Garcia et al., 2011b). Open-pond cultivation incurs low construction and operating costs, which invariably results in low production costs (Stephenson et al., 2010). Open-pond cultivation inherits some drawbacks such as poor light diffusion, not-so-efficient mixing, losses due to evaporation, poor diffusion of atmospheric CO_2, uncontrolled pond temperature, contamination by predators and other fast-growing heterotrophs and the requirement of large areas of land (Harun et al., 2010; Perez-Garcia et al., 2011b). Uncontrolled environments in and around the pond pose a multitude of problems that can directly or indirectly stunt algae growth (Kazamia et al., 2012; Mata et al., 2010).

15.4.2 Photobioreactors

Photobioreactors (PBRs) provide a more controlled environment than open ponds, due to pre-set conditions. Everything that the algae need to grow (carbon dioxide, water and light) can be supplied within the system (Weissman, 1987; Pulz, 2001). PBRs facilitate better control of culture environment, such as carbon supply, water supply, optimal temperature, efficient exposure to light, pH levels, gas supply rate, mixing regime, etc. and can achieve high growth rates (Mata et al., 2010; Sierra et al., 2008). Higher biomass productivity can be obtained in closed cultivation systems where contamination can also be prevented to major extent (Ramanathan et al., 2011). High mass transfer is one of the important criteria for PBR design, especially for CO_2 sequestration (Ugwu et al., 2008).

Various types of closed cultivation systems or PBRs have been reported for algae cultivation. Tubular photobioreactors with diverse configurations are being used viz., horizontal, serpentine, vertical, near-horizontal, conical and inclined with suitable illuminated surfaces. Ventilation and mixing is performed by pump or ventilation systems. Sparger is attached at the bottom of the reactor to diffuse small bubbles of gas. Good mixing, mass transfer of CO_2 and effective removal of the O_2 produced during photosynthesis are major advantages with this configuration. Vertical column photobioreactors are low cost, easily constructed and compact systems which are suitable for large scale production. Air-lift photobioreactors comprise two interconnecting zones called the riser, where the gas mixture is sparged, and the down comer, which does not receive the gas. Mixing in the system is done by bubbling the gas through a sparger in the riser tube, with no physical agitation. Gas held up in the down comer significantly influences the fluid dynamics of the airlift reactor. Airlift reactors have the characteristic advantage of creating circular mixing patterns in which liquid culture passes continuously through dark and light phases, giving a flashing-light effect to algal cells. Tubular photobioreactors made from polyethylene tubes (plastic bags) are being used commercially for algal cultivation which provide light ranging in the visible and near-infra red region (low UV transmission) of spectrum and is associated with low cost. Stirred-tank photobioreactors are the conventional reactor setup in which agitation is provided mechanically with the help of impellers or baffles by providing illumination externally. Internally illuminated photobioreactor incorporates both solar and artificial lighting systems and switches to the artificial lighting system in the absence of solar light.

Flat-plate or flat panel photobioreactors comprise transparent flat plates made of transparent materials for maximum utilization of solar energy to achieve high photosynthetic efficiencies. Accumulation of dissolved oxygen concentrations is low compared to horizontal tubular photobioreactors. Lack of temperature control and gas engagement zones are some of the disadvantages observed with these systems. A coiled transparent and flexible tube of small diameter with separate or attached degassing unit is the basis for the helical type of bioreactor. A centrifugal pump is used to drive the culture through a long tube to the degassing unit. CO_2 gas mixture and feed can be circulated from either direction, but injection from the bottom gives higher photosynthetic efficiency. A degasser facilitates removal of photosynthetically produced oxygen and residual gas of the injected gas stream. The energy required by the centrifugal pump in recirculating the culture and associated shear stress and fouling on the inside of the reactor are some of the challenges encountered while working with this system.

15.5 Downstream Processing

Downstream process of algal biomass includes harvesting, dewatering, drying, cell disruption and extraction of product followed by biodiesel preparation by transesterification (Fig. 15.1).

15.5.1 Dewatering and Drying

Dewatering and drying of algae are most important steps in downstream processing of microalgae. Dewatering involves separating the extracellular water from the algal suspension. A subsequent drying stage removes all water from the biomass (~95 % by mass). The water content must be substantially reduced especially while preparing the algae for storage, in order to avoid rapid decay and decomposition (Bruton et al., 2009). The intracellular water remaining in the algal suspension can only be removed by thermal processes. Reducing the mass delivers considerable savings on transport costs, especially over long distances. Different drying techniques such as rotary drying, spray drying, solar drying, cross-flow drying, vacuum shelf drying, flash drying and freeze drying are used (Shelef et al., 1984). Drying by solar energy is the simplest and most economical method of drying although it depends on climatic conditions, and involves the risk of algal paste decay during the process at high light intensities (Becker, 1994). Drying is performed either by direct sunlight or by means of a circulating air flow heated by solar energy. Spray drying is frequently used in the production of algae for food purposes, because a large number of constituents are retained. Similar to flash dryers, it is a continuous process wherein the paste is dried in a few seconds (Becker, 1994).

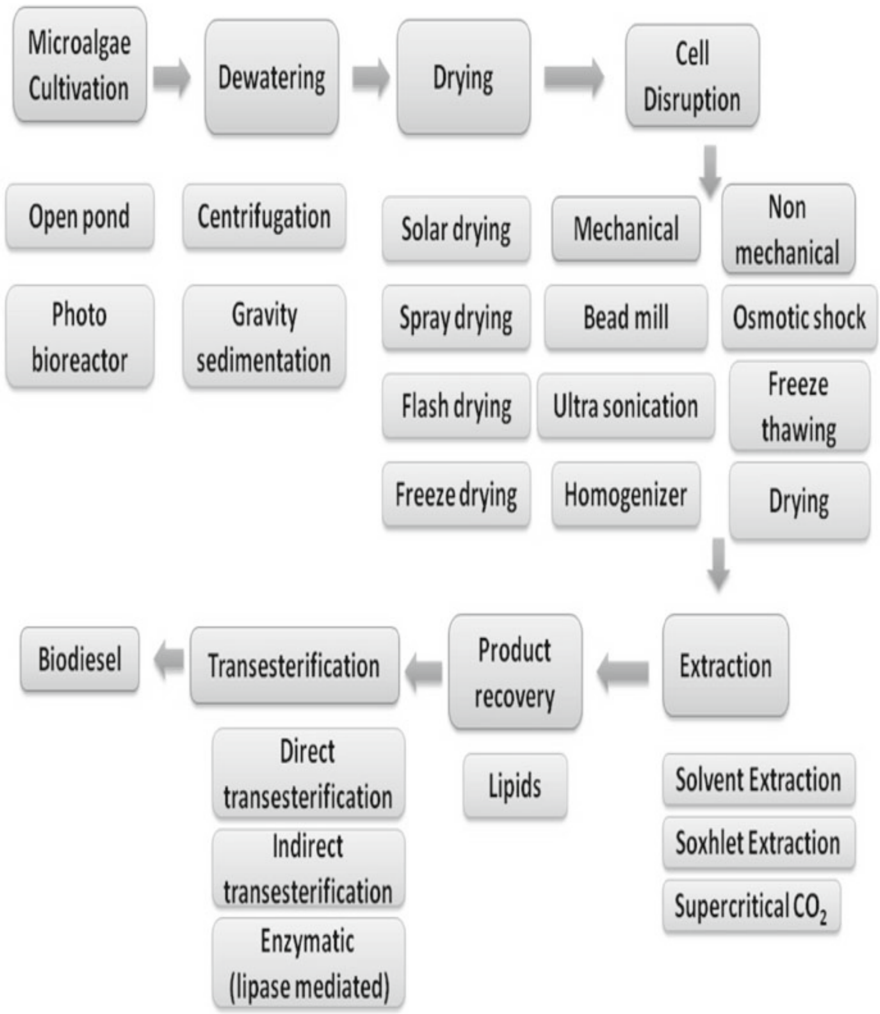

Fig. 15.1 Flow diagram of different unit operations during downstream processing of microalgae.

15.5.2 Cell Disruption

The disruption of algae cells prior to extraction is of particular importance because the contents of the extracted lipids are determined according to the disruption method and equipment employed. The selection of appropriate device for disruption is the key factor for enhancing the lipid extraction efficiency (Lee et al., 2010). Cell disruption methods are classified as mechanical and non-mechanical. Mechanical cell disruption includes bead mill, high pressure press, homogenizers, ultrasonication, autoclave, lyophilization and microwave while non-mechanical methods often involve lysing the microalgae cells with acids, alkalis, enzymes, or osmotic shocks.

Expeller press (or oil pressing) is a mechanical method applied for the disruption of algae cell membranes by squeezing the cells under high pressure (Mercer and Armenta, 2011) which can recover nearly 75 % of the oil from algae cells in a single step.

The advantages of this method include elimination of a solvent requirement and easy operation. This method involves the application of beads for the disruption of the algal cell wall. Continuous exposure of biomass to beads leads to cell-wall rupture, resulting in the release of intracellular contents into the solvent medium. Similar to expeller pressing, this method can also be applied for both disruption and extraction. Non-mechanical cell disruption uses chemical solvents, enzymes, solvent fluids and osmosis. Extraction solvents used are mainly hexane, acetone, chloroform and methanol. Organic solvents and supercritical carbon dioxide is used extensively to extract lipids from microalgal biomass. Both technologies have their own merits and demerits. Despite having low reactivity with lipids and being directly applicable to wet biomass, organic solvent extraction is slow and uses a large amount of toxic solvents.

15.5.3 Extraction of Lipids

Several methods have been employed for extracting microalgae lipids (Table 15.2). Among the processes described, solvent extraction is suitable for extracting lipids from mass cultures but requires large volumes of solvent. The existing methods of lipid extraction usually involve selective solvent extraction, and the biomass material may be subjected to drying prior to extraction (Lee et al., 2010). Lipids are soluble in organic solvents but sparingly soluble or insoluble in water. Selection of solvent systems is an important criterion for lipid extraction and typically depends on the type of lipid present (total/neutral lipids) and the proportion of non-polar (neutral) lipids (commonly known as triacylglycerols) and polar lipids (mainly phospholipids and glycolipids) in the sample (Huang et al., 2010). Recovery and reusability of the solvent are possible with this method. Ultrasonication extraction method is specifically used when dealing with small volumes of biomass, but can perform well when coupled with the enzymatic treatment, but both methods lack cost effectiveness and feasibility for large-scale applications. The combination of 'ultrasono-enzymatic treatment' causes faster extraction and facilitates higher oil yields as compared to ultrasonication and enzymatic extractions individually (Fajardo et al., 2007). Supercritical carbon dioxide extraction (SC-CO_2), pulse electric field procedure, osmotic shock, hydrothermal liquefaction, and wet lipid extraction require more optimization efforts for large-scale applications.

Each method has its own advantages and disadvantages for practical applicability. High cost, power consumption and difficulty involved in scaling up are some of the persistent limitations of many methods. Supercritical carbon dioxide extraction is a green technology that can be potentially used for large-scale microalgal lipid extraction. However, this process has high capital cost and energy requirement for supercritical fluid compression. For extraction of high valued products, bead mill is

Table 15.2 Various extraction techniques employed for oil extraction from microalgae.

Extraction Method	
Solvent (selective)	Biomass subjected to drying prior to extraction
	Selection of solvent systems is an important criterion for lipid extraction
	Hexane is used with Soxhlet and Goldfish methods
	Chloroform/methanol or chloroform/methanol/water with Folch or modified Bligh and Dyer procedures
	Best suited to extract non-polar lipids
Soxhlet	A semi-continuous process that allows the buildup of a solvent in the extraction chamber
	Solvent surrounding the sample is recycled back
	Provides a soaking effect and does not permit channelling
	Polar and membrane bound lipids are not recovered
Wet lipid	Uses wet algae biomass by using solvent proportionately
	Elimination of drying step
Hydrothermal liquefaction	Biomass converted in hot compressed water to a liquid biocrude.
	Processing temperatures range 200–350 °C with pressures of around 15–20 MPa
	Ideal for the conversion of high-moisture-content biomass (microalgae) as drying step is not necessary
Ultrasonic	Ultrasonic-assisted extractions recover lipids through cavitation
	Ultrasound breaks the cell wall by cavitation shear forces
Supercritical carbon dioxide (SC-CO_2)	Relatively low temperature and the stability of CO_2 allow most compounds to be extracted with little damage or denaturing
Pulse electric field (PEF)	Short pulses of a strong electric field enlarges the pores of the cell membranes and expels lipid contents
Enzymatic treatment	Enzymes degrade the cell walls by water acting as the solvent
	Oil fraction much easier to recover
Osmotic shock	Osmotic shock causes a release in the cellular contents of microalgae
	It is also induced to release cellular components for biochemical analysis

used along with chemical solvents (Chisti and Moo-young, 1986). Hexane and methanol are used in combination for high extraction efficiency and with economic viability (Neelma et al., 2013).

15.5.4 Biodiesel Preparation

After lipids are extracted, constituents of the lipids (TAG's) are subjected to transesterification. The triglyceride composition of algae upon transesterification with an alcohol can produce algae-derived biodiesel (alkyl esters). Different types of transesterification reactions viz., acid/base catalyzed, direct and enzymatic were used.

The transesterification process consists of the reaction of triglyceride molecules with alcohol in the presence of a catalyst to produce glycerol and mono-alkyl fatty acid esters also known as biodiesel (Harrison et al., 2012). The fatty acids react with methanol to form diacyl glycerides, monacyl glycerides, and finally, fatty acid methyl esters (FAMEs) (Gong and Jiang, 2011). In this process glycerol is formed as by-product. The transesterification process reduces the viscosity of the FAME as compared to the parent oil, whereas the fatty acid composition will not be altered. Alcohols are the key substrates in transesterification. The commonly used alcohols are methanol, ethanol, propanol, butanol and amyl alcohol, but methanol is widely applied in the transesterification of microalgae oils because of its low cost and physical and chemical advantages.

15.5.4.1 Direct Transesterification

Direct transesterification or in-situ transesterification is receiving much attention in current industrial processes. This technique combines lipid extraction and transesterification in a single step, further reducing the overall number of downstream processes required for biodiesel production (Wahlen et al., 2011). In this process, acid catalyst and pure methanol are added simultaneously to microalgal biomass (dried powder) wherein, methanol extracts the lipids which in the presence of acid catalyst get transesterified to produce fatty acid methyl esters (FAMEs). The lipid extraction and transesterification reaction takes place in a single step in this process (Sathish and Sims, 2012; Ehimen et al., 2010). The algae biomass can be effectively converted to fatty acid methyl esters through this process in relatively less time. Minimization of solvents and requirement of less time for reactions are the advantages of this method whereas the lipid productivity and success rate of the reactions are the associated drawbacks. Direct transesterification is feasible at pilot and large scale systems.

15.5.4.2 Acid and Base Catalyzed Transesterification

Acid catalysis (H_2SO_4/HCl) is usually performed at high alcohol-to-oil-molar ratios, low to moderate temperatures and pressures, and high acid-catalyst concentrations (Zhang et al., 2003). Base-catalyzed transesterification of microalgae oil is used most frequently and involves the presence of a base catalyst (hydroxides/carbonates) to precede the reaction (Meher et al., 2006; Vargha and Truter, 2005). In this reaction, the triglycerides are readily transesterified in the presence of the catalyst at an atmospheric pressure and temperature of 60–70 °C in the presence of excess methanol (Srivastava and Prasad, 2000). The main drawback with this process is the formation of soap at high free fatty acid concentrations (Furuta et al., 2004). Compared to base catalysts, acid catalysts are less susceptible to the presence of free fatty acids in the source feedstock (Helwani et al., 2009), but the reaction rates of converting triglycerides to methyl esters are too slow (Gerpen, 2005). Repeated application of catalyst in the reactions increases the acid value of the microalgae oil.

15.5.4.3 Enzyme-mediated Transesterification

This kind of reaction is catalyzed by the enzyme lipase, whereby total triacylglycerides (both extracellular and intracellular) can be converted to biodiesel (Bisen et al., 2010). The conversion process requires complex processing instruments and the expensive nature of enzymes makes the process limiting. As a solution to overcome the limitations, immobilization was employed but the low technical feasibility of the process makes the reaction complex (Helwani et al., 2009; Watanabe et al., 2001). A novel method has been established for microalgal lipid extraction (Origin Oil, 2010) which performs three simultaneous functions (dewatering, cell disruption, and lipid extraction) in a single downstream step. This method substantially reduces the energy expenditure required to produce biodiesel from microalgal biomass. The downstream technologies required for industrial-scale production of microalgal biodiesel helps overcome the economic hindrances.

15.6 Algal Biorefinery

Extensive studies on microalgae biomass have revealed that there is a huge potential for co-products which can be recovered using biochemical and thermo-chemical technologies. The extraction of more than one type of product from single biomass source increases the value of biomass and offers additional uses to end products (Mussgnug et al., 2010; Yang et al., 2011; Ehimen et al., 2011). The major process after biomass growth is extraction of lipids and conversion to biodiesel. The residual biomass (deoiled cake) can be subjected to a range of bio/thermo chemical processes like fermentation, anaerobic digestion or pyrolysis (Fig. 15.2). The microalgae biomass residues after biodiesel production can be used to generate biomethane which can be burnt to produce electricity. Recovery of methane and biohydrogen by using lipid extracted microalgae pulp as fermentative feedstock after proper pretreatment by biomethanization or acidogenic process accounts for renewability of biomass and increases economic outcomes (Subhash and Venkata Mohan, 2014). Thermo-chemical conversion of algae biomass can also be performed for synthesis of bio-oil and biochar (Sarkar et al., 2014). This will make the production of biodiesel from algae more competitive by reducing the overall production costs and energy needed for downstream processes (Harun et al., 2010). The high input costs will be the major limitation but integrated biorefinery concept can overcome the technical and economic constraints.

Polysaccharides are also produced by microalgae which are used as feedstocks for biofuels production. Carbohydrates derived from photo-synthesis are either accumulated in the plastids as reserve materials (e.g. starch) or become the main component of cell walls (e.g., cellulose, pectin and sulphated polysaccharides) (Ho et al., 2011). Microalgae that contain glucose-based carbohydrates are the most feasible feedstock for bioethanol production. On the other hand, the microalgae can improve the environmental load with respect to pollution and achieve more sustain-

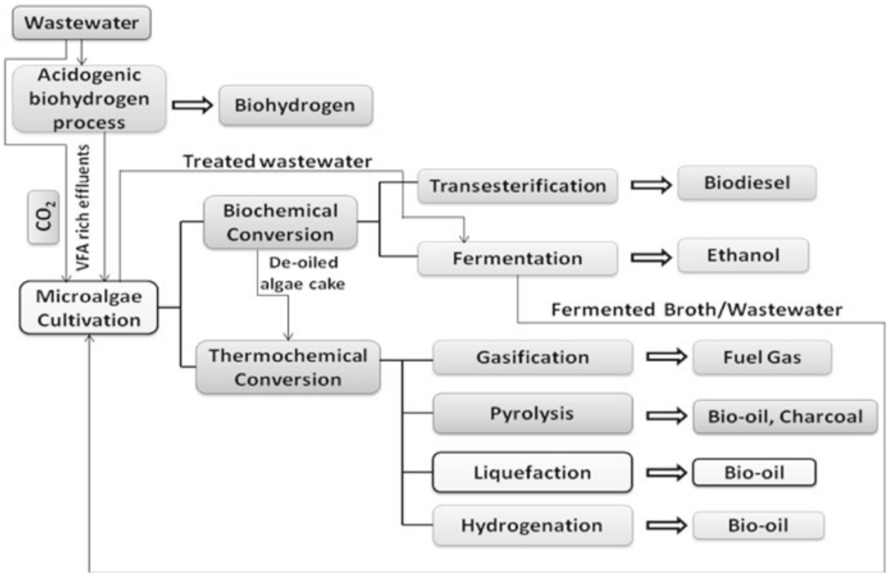

Fig. 15.2 Integrated algal biorefinery.

able lifestyles using CO_2 and wastewater (Sivakumar et al., 2012). Wastewater treatment integration with biofuel production is gaining attention in the current generation of sustainable fuels synthesis (Venkata Mohan et al., 2011). Integrating algal biorefinery concept with wastewater treatment will provide efficient utilization of algae biomass and reduces overall residual waste component of biomass and favours sustainable economics (Venkata Mohan et al., 2014b). A conceptual design of Green Wisdom Inc., USA, using microalgae for integrated bioremediation and biofuel production depicted the potential of microalgae for rural communities leading to economic acceleration and sustainability.

15.7 Future Scope and Challenges

In the future, microalgal biodiesel can be used directly or blended in appropriate ratios for internal combustion engines. The improved screening and selection enables the cultures to grow rapidly in wastewater containing elevated nutrient loads and flue gases. By osmosis technology, cleaned water will be separated from microalgae that will help in feasible production of biodiesel and alleviation of air and water pollution. Innovative cultivation systems and modification of the biochemical composition by simple changes in their cultivation conditions (nutrients, light intensity, temperature, pH, mixing etc.) can lead to higher productivities of the targeted products. On the other hand, their ability to synthesize and accumulate various high value products (e.g. biopolymers, proteins, polysaccharides, pigments) will help to reduce the production costs.

Intensive research needs to be focussed on advanced downstream and bioprocess technologies to reduce the scale up and production costs. Novel separation and extraction systems with higher efficiencies can provide promising results for future applications. The advances in material science and technology will also help producers utilize the proper equipment with lower cost. Employing photo bioreactors can increase the productivities of various species by improved process control and elimination of contaminants. Real time projections for prediction of cost per volume models are required for accurate extrapolation of lab scale data for development of marketable biodiesel technologies. Microalgae based biofuels should be deployed sensitively with the ability to forecast the trend of future technologies.

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