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Phycoremediation of aquaculture wastewater and algal lipid extraction for fuel conversion

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Abstract

In this review, it is discussed the prominent effect generated from aquaculture wastewater considered as the major water polluting crisis in the entire world. The cause rose due to intense development and improvement in aquaculture by the aquatic habitat species triggering quite a challenge in the environment. Scrutinizing this problem, researchers have found a way to tackle it by cultivating algal species in aquaculture wastewater in order to remove its high content of organic and inorganic pollutants. The theory proves wastewater serves as a nutrient source for algal growth and development such as phosphorous, nitrogen, and other trace elements. Besides harvesting the algal biomass from aquaculture wastewater, the extraction of lipid is also processed for biofuel production. Hence, the discussion includes conversion of wastewater into organic and inorganic pollutant-free water with low cost-effective method *via* algal cultivation in wastewater and high lipid yield for biofuel with a carbon-free and sustainable environment.

Keywords: Algae, aquaculture wastewater, harvesting, lipid extraction, transesterification

Introduction

Ever increasing global population and continuous dependence of fossil fuels, increased urbanization and industrialization posing a major threat to energy security and environmental concerns to both developed and developing nations. With the accelerated speed of increasing population growth, wastewater treatment is considered as one of the solutions to control the environmental issues. And the additional challenges for water scarcity bring out the crucial problem related to wastewater. Hence, much of the emphasis has been given now a days for wastewater treatment [1]. The anthropological activities such as sewage, industries, agriculture, medical, research laboratories etc., are pointed to be the sources of wastewater which have tremendously polluted the water resources. Wastewater from various sources comprises both organic and inorganic pollutants. Organic pollutants include proteins, carbohydrates, lipids, etc., whereas inorganic mostly have chemicals and solvents [2], and in industrial wastewater even heavy metals or toxic elements are reported [1]. Nowadays the aquaculture wastewater is quite prominent globally due to its intense development and improvement in fish, marine species, algae and aquatic plant farming. Thus, this rapid increase of aquaculture effluents poses a serious threat to environment [3]. Mariculture other name of aquaculture can be seen their progresses in parallel way with high human demands. The impact performances to the environment for food production has been reported as the fastest growth [4]. Aquaculture wastewater contains a high number of pollutants and chemicals that lead the residing aquatic flora and fauna to die due to eutrophication. The toxic algal blooms not only disturb the aquatic life but it also interferes with the sustainable marine aquaculture development. The wastewater treatment via biochemical methods is not economical and further removal of those chemicals from water poses another challenge. Hence, wastewater treatment by algae is an ecofriendly and cost-effective approach over other physical and chemical methods [5–7].

Earlier aquaculture wastewaters were treated with bulks of antibiotics which later have evolved to antibiotic-resistant [8]. But in an investigation, green microalgae *Tetraselmis sp.* removed nitrogenous and phosphorous compound from aquatic wastewater within 48 hours [9]. Removal of nutrients from livestock wastewater was also reported via, *Desmodesmus sp.* a microalga with potential benefactor [10]. The wastewater treatment *via* microalgae not only removes the pollutants but also shows positive effect towards carbon fixation (1.83 kg CO_2/kg of biomass), high amount of biomass generation within a short period of time. The microalgal biomass can be further utilized for biofuel and valuable bio-products production and can also act as substrate in bio-refinery. Thereby, it provides a sustainable and ecofriendly approach to many of the problems related with wastewater [11].

Since the middle time of 20th century, development in aquaculture growth has risen globally in all over countries providing huge profit to commercial hatcheries and farming system. The wastewater discharge from chemical and other industries has high toxicity level, that poses serious environmental issues [12]. The aquaculture production is kept on increasing due to high demand in the market. Hence large-scale production has been carrying out enormously [13]. Yang *et al.*, [14] has mentioned in his studies the aquaculture effluent treatment with microalgae is highly efficient in absorbing nutrients and value-added biomass generation.

Alga-aquaculture has led to many advantages such as compared to other plants, algae has proved to be better in nutrient removal. The construction and operational costs are low with consistent to high nutrient removal efficiency. The microorganisms consortia like algal-bacterial consortia are highly efficient in solid and other waste treatment into low molecular weight compounds [15]. The large quantity of algal biomass can be produced from aquaculture, that can be further utilized for high demand valuable product generation *Spirulina* and *Chlorella* cultures are commonly used as aquaculture feed, as both has very minimal toxicity level and helps in preventing algal blooms as well. On the other way, addition of expensive chemicals and antibiotics for industrial effluent treatment are not economical and poses severe threat to the environmental [16]. It has also been reported that excess use of chemicals in aquaculture affects the food safety and quality of meat produced *via* aquaculture. In most of the research studies, microalgae such as *Chlorella sp.* is found experimenting in every aspect of wastewater treatment. Biofilms are another slimy and foul in nature found on the surface of the algae or any solid surface attached. Microalgal biofilms mostly have succeeded in reducing the nutrients of phosphorous starting initially from 15 mg L^{-1} within 24 hours[12]. The biofilm has succeeded in more production of biomass production for biofuel [17].

This review summarizes our efforts towards various aquaculture wastewater treatment via microalgae and use of algae for feed purposes. It further highlights the biofuel and value-added products generation from the algal biomass.

Micro and macro algae as a nutritive aquaculture feed

Aquaculture has been rapidly developing in industrial sector resulting large quantity of polluted effluents being discarded into clear water line. Remaining solid residues mostly contains hazardous chemicals and metal elements causing severe incurable diseases [1]. Huge amount of cost and labor are invested upon various physical and chemical techniques. Electro adsorption and electro-reaction coupling process is one of physical technique to clean the wastewater, removing 99% of solid. But it is reported algae is the most efficient and advanced method with low cost benefits in treating wastewater [18]. In aquaculture, many of the microalgae *viz., Nannochloropsis, Chaetoceros, Thalassiosira, Tetraselmis* etc. are known for essential food sources including marine species such as clams, molluscs, oysters and *Spirulina sp.* for providing high protein diet for freshwater fishes and other invertebrate species [17]. Treatment of wastewater with microalgae has been guaranteeing good outcome and have led to great advantages without harming the environment.

Macroalgae universally known as seaweed is easily visible through naked eye. Its habitats are mostly native to marine or other river bodies. *Saccharina latissima* also known as kelps are generally found in river depth. Macroalgae are well-known for their nutritional and bioactive components. Aquaculture with macroalgae production has a strong demand in the market, according to industrial vision. It is a valuable source of aquaculture feed. However, most examples of eutrophication in the marine environment are caused by the deposition of flowing waste in the sea, which includes high nitrogen and phosphorus nutrients. Macroalgae also aids in the bioremediation process by preventing wastes from impacting the marine environment in terms of pH change, turbidity, and increased BOD content, as well as causing marine life death and encouraging toxic algal blooms [19–21]. According to Brakel *et al.*, [22] macroalgae depicts as fastest growing aquaculture development even in poorest coastal regions. Nowadays with advanced facilitation and support of genetic resources, seaweeds such as red algal genera *Eucheuma* and *Kappaphycus* proved economically in many tropical countries.

Microalgae based biorefinery for aquaculture wastewater treatment

Wastewater treatment is rising as fundamental priority. The removal of nutrients and solids, as well as the acceptance of environmentally friendly remediation techniques, play a significant role in this. The most photosynthetic machinery technique that we can ever expect is phycoremediation, or treating wastewater with algae. It extracts all unwanted parameters from wastewater and improves water quality to meet environmental standards. [15]. Compared to conventional wastewater treatment this method is cheap and also has the involvement in biomass production for biorefinery purposes. *Chlorella sorokiniana* is observed as the most utilized objective both for phycoremediation and biomass production [23].

Open pond system

Algal cultivation is the basic necessary condition for more quantity of biomass for biofuel production. Depending on cost, labor and time vast kinds of techniques are available. Open pond is the common system for large scale algal production exposed directly to environment. Here generally, algae species are cultivated in an open pond area covering as much acres of land directly under the sunlight due to their phototrophic nature [24]. Open pond is named as raceway because it resembles with racetrack. This raceway pond system takes less space of land for growth. It requires continuous movement of paddle wheel in the pond to prevent sedimentation of cultures at the bottom level [25]. Generally, paddle wheels depicts the main principle base for the open pond system where the speed of the wheel helps to cover the light intensity for all over the algal growth within the system [26].

Nutrient removal efficiency of aquaculture wastewater with microalgae

The most efficient process and cost-effective method for culturing algae is via wastewater sources; rather than cultivating in expensive amounts of chemicals. Aquaculture wastewater contains required nutrients such as nitrogen, sulphur, phosphorous which alga feeds on for growth. Its composition is mentioned in **Table 1** , pointing its physico-chemical properties such as its pH, Chemical Oxygen Demand (COD), nitrate, chloride, sodium, potassium, magnesium, nitrite, ammonium and phosphorous were depicted in aquaculture wastewater. In **Table 1**, the content found under those properties extremely higher compared to normal *i.e.* these wastewater has the capability to cause diseases.

Wastewater cultivation is positively progressing both in bioremediation and biomass production for biofuel. It is either way sustainable to environment as budget friendly way. *Ulva sp., Codium sp., Ecklonia sp., Saccharina sp., Gracilariopsis sp.* have experimented in fish seaweed aquaculture waste for bioremediation that have removed high concentration of ammonia and phosphorous within 30-40% nutrient removal converting into less polluting [30]. Whereas for microalgae *Tetradesmus obliquus* has removed 99.3% of ammonia and 99.2% of phosphorous concentration from swine manure wastewater [31]. In **Table 2**, various algal species cultivated in different types of aquaculture wastewater are shown. The inoculated culture in the wastewater is mentioned parallel to the algal species name. The remaining columns are about the results of removal of nutrients described in percentage that found after cultivating in aquaculture wastewater. It specifies how algae worked as bio-remediation.

Table 1. Composition of various types of aquaculture wastewater.

Types of aquaculture wastewater	pH	COD (mg/L)	Nitrate (mg/L)	Chloride (mg/L)	Sodium (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Nitrite (mg/L)	Ammonium (mg/L)	Phosphorous (mg/L)	Reference
Fishery	7.86	32.4	0.35	NA	NA	NA	NA	24.7	6.25	1.83	[7]
Fishery	8.1	2.25	NA	19,400	10,790	387	1293	NA	NA	1.21	[27]
Seawater marine	7.75	7.84	8.02	NA	NA	NA	NA	0.25	0.48	4.56	[28]
Oreochromis niloticus aquaculture	5.22	64.3	52.0	24.5	28.5	8.3	3.3	0.01	12.8	11.2	[23]
Fishery	7.2	NA	96.60	655	540	21	69	0.006	0.010	1.98	[29]

NA Not Available

Table 2. Nutrient removal in aquaculture wastewater with different algal species

Types of aquaculture wastewater	Algae species	Type of algae	Amount of inoculation (g/L)	Time of treatment (Days)	Removal compounds COD (%)	NH4 (%)	Nitrate (%)	Nitrite (%)	Phosphorous (%)	Total nitrogen (%)	Reference
Fishery	*P. kessleri TY*	Microalgae	10	3	94.4	96.2	94.3	99	96.6	NA	[7]
Salmon farming	*Chlorella minutissima*	Microalgae	NA	10	NA	NA	88.6	74.3	99	88	[27]
Shrimp culture	*Gracilaria tenuifrons*	Macroalgae	1.75	8	NA	35.1	NA	71.7	33.2	2.8	[28]
Fish-seaweed aquaculture	*Codium fragile*	Macroalgae	1000	28	NA	0.07	NA	NA	0.22	0.56	[23]
	Ulva pertusa		1000	28	NA	0.04	NA	NA	0.15	0.56	
	Ecklonia stolonifera		1000	28	NA	0.11	NA	NA	0.26	0.57	
	Gracilariopsis chorda		1000	28	NA	0.11	NA	NA	0.23	0.50	
	Saccharina japonica		1000	28	NA	0.15	NA	NA	0.21	0.56	
Oreochromis niloticus aquaculture	*Chlorella sorokiniana*	Microalgae	NA	14	NA	99.9	75.2	NA	77	78	[29]

NA Not Available

Recent advances in microalgae harvesting and lipid extraction

After cultivation, harvesting which means collecting or gathering of algal cultivation determines as most difficult and important out of all process work. For large scale harvesting of biomass, it requires quite expensive technique, maintenance of time, man power and so on. In case of microalgae harvesting techniques such as centrifugation, sieving, filtration, sedimentation, flotation, flocculation are predominantly utilized [33]. whereas for macroalgae simple technique such as drying and storing is basically preferred but however few techniques from microalgae harvesting techniques are also operated [34]. Thermo reversible gel transition [35,36] characterized with either agar or sol gel for harvesting of algae where clustered cells are settled at bottom and collected the biomass. Flocculation is another technique of harvesting. Nanocellulose is an insoluble substance where bonding of polysaccharide and glucose monomers occurs with the concept of more concentration of nanofibril more increase of flocculation [37]. Bacterial cellulose *Gluconacetobacter xylinus* has found to be successfully harvested with 90% of clump formation [38]. *Pleaurotus ostreatus* [39] and *Scenedesmus obliquus* [40] are another flocculating process. According to Leite [41], pH modulation through Dissolved Air Flotation can be harvested at higher biomass. Magnetic nanoparticles is another better technique for harvesting [42]

Mostly utilized lipid extraction method is Bligh and Dyer as said to be quickly approachable to quantification outcome within less timing but more hazardous to environment as well as self-health. But MTBE *i.e.,* Methyl-tert-butyl ether is the better method than the previous method with non-hazardous effect and increase in the extracted lipid [43]. For future perspective role such as to study the characterization from extracted algal biomass production, high resolution nuclear magnetic resonance spectroscopy (HR NMR) or mass spectroscopy technique is used to study the changes of various composition kept in different storage conditions were found in the algal sample. Such techniques are extremely advanced in analytical process [34]. On the other hand, Dimethyl ether is a gas type where the liquefied gas is passed with the help of Nitrogen gas and proceeded for algal extracting [44]. Super high hydrostatic pressure technique is even utilized for extracting lipid maintaining pressure 100 MPa to 1000 MPa [45]. Another process of lipid extraction solvent is ionic liquid that comprises of ion solvents of non-volatile substance including bubbling CO_2 gas for extraction [46]. In the given **Table 3**, it basically determines the biomass productivity and lipid growth found after algal cultivation in aquaculture wastewater. In the same table various algal species names and according to that in the left column the types of wastewater are given where following that horizontally we can read the biomass and lipid found after cultivating the algae in that same wastewater. Within these three table tables (**Table 1, 2 and 3**) it gives the idea about reading the physico chemical properties before algae cultivating and harvesting the biomass, lipid measuring and lastly with the remaining water and can be proceeded with re-reading the physico chemical properties determining removal of nutrients from the wastewater.

Microalgae as a sustainable future biofuel approach

Developing with rapid high rise of industries by regular use of natural resources are leading us into depletion of fossil fuels sooner creating havoc in environment. It surges carbon dioxide till peak point making possibly prone to global warming similarly threatening wastewater globally. At the bright side, the microalgae have several unique features like ability to fix CO_2 and convert it into valuable components *via* photosynthesis, robust growth with high lipid contents. The microalgae harvesting, qualitative and quantitative estimation of lipid has been reviewed [43,47]. The availability of molecular approaches to increase lipid accumulation and recovery has been extensively discussed [48]. Biofuel is the breakthrough for solution. Among generation after generation there has been change into biofuel production. Initially beginning with edible plants such as soybean, maize, brassica comes under first generation and had a great deal with alternative fuel. The lipid yield was good but in case of other matters like production of biodiesel from food crops during the time of world war period was a huge downfall. In second generation Jatropha plant being the non-edible is another alternative fuel production which is a good source compared to first one [49]. The life cycle, production in large scale, huge mass of land for cultivation is the major demerit. Third generation i.e., microalgae is currently the most successfully running lipid yield production out of all. Cultivation of microalgae is only 14 days where this microorganism can be grown in even a small tub or artificial huge ponds. The biomass with high production of yield can be grown in any suitable environment with different stress conditions changing physiological condition. *Monoraphidium sp.*is cultivated in BG 11 media and transferred into high ammonia content wastewater with 44% of stress condition present [50].

Transesterification, in case of biodiesel it can be termed as conversion of a 3-methyl glyceride when it reacts with methanol in presence of catalyst to form Fatty acid methyl esters to form ethanol, likewise shown in **Figure 1**. For conversion into biofuel after harvesting method and weighing dry biomass, lipid extraction process is followed. It basically consists of two types mechanical and non-mechanical, the previous type usually relates with solvents and the later describes extraction through instrumental techniques. Triglycerides act as main components, these are fatty acids extracted from algal species and converted into fatty acid methyl esters through direct transesterification method. This method depicts reaction of triglycerides with mono alcohols in presence of catalyst were analyzing solvent as hexane with better results compared to chloroform and methanol where pointing a strong line selection of solvents affects in lipid yielding after purification [51].

Biodiesel has inherent sustainable transportation fuels for future mostly to reduce increasing pollutants emitted from exhaust cylinder. Many modernized machine learning techniques and renewable feedstock are emerging rapidly for biofuel conversion compared to chemical catalysts. The main source of biodiesel is manufactured basically from renewable oil derived microbes or plants which causes zero-effect in ecosystem accompanying with carbon reduction. Enzyme mediated undertakes non-toxic transesterification compared to same old process of chemical utilization [52,53].

From many processing experiments, microbes such as microalgae is the leading aspect, *Euglena sanguinea* due to its presence of superior combustion characteristics were able to produce biodiesel that blends with the regular agricultural diesel engine till 40% by extracting lipid from the algal biomass [27]. A heterogeneous nano-catalyst $Ca(OCH_3)_2$, a novel reactive distillation column is experimented for algal biodiesel production optimized by maximizing biodiesel purity by NSGA-II, non-dominated sorting genetic algorithm, designed both for low cost production and CO_2 emissions [28]. For another substitute yield of biodiesel an experiment conducted between *Chlorella sorokiniana* and *Monoraphidium sp.* where the biomass, fatty acid pro-

Table 3. Nutrient removal in aquaculture wastewater with different algal species

Aquaculture wastewater	Algal species	Type of algae	Total duration days of treatment method (mg/L)	Biomass concentration /productivity (%)	Lipid growth (%)	Reference
Fishery	*P. kessleri TY*	Microalgae	5	26	NA	[7]
Sea bream factory	*Tetraselmis suecica*	Microalgae	10	68	25	[27]
Salmon farming	*Chlorella minutissima*	Microalgae	10	55	46.37	[13]

NA Not Available

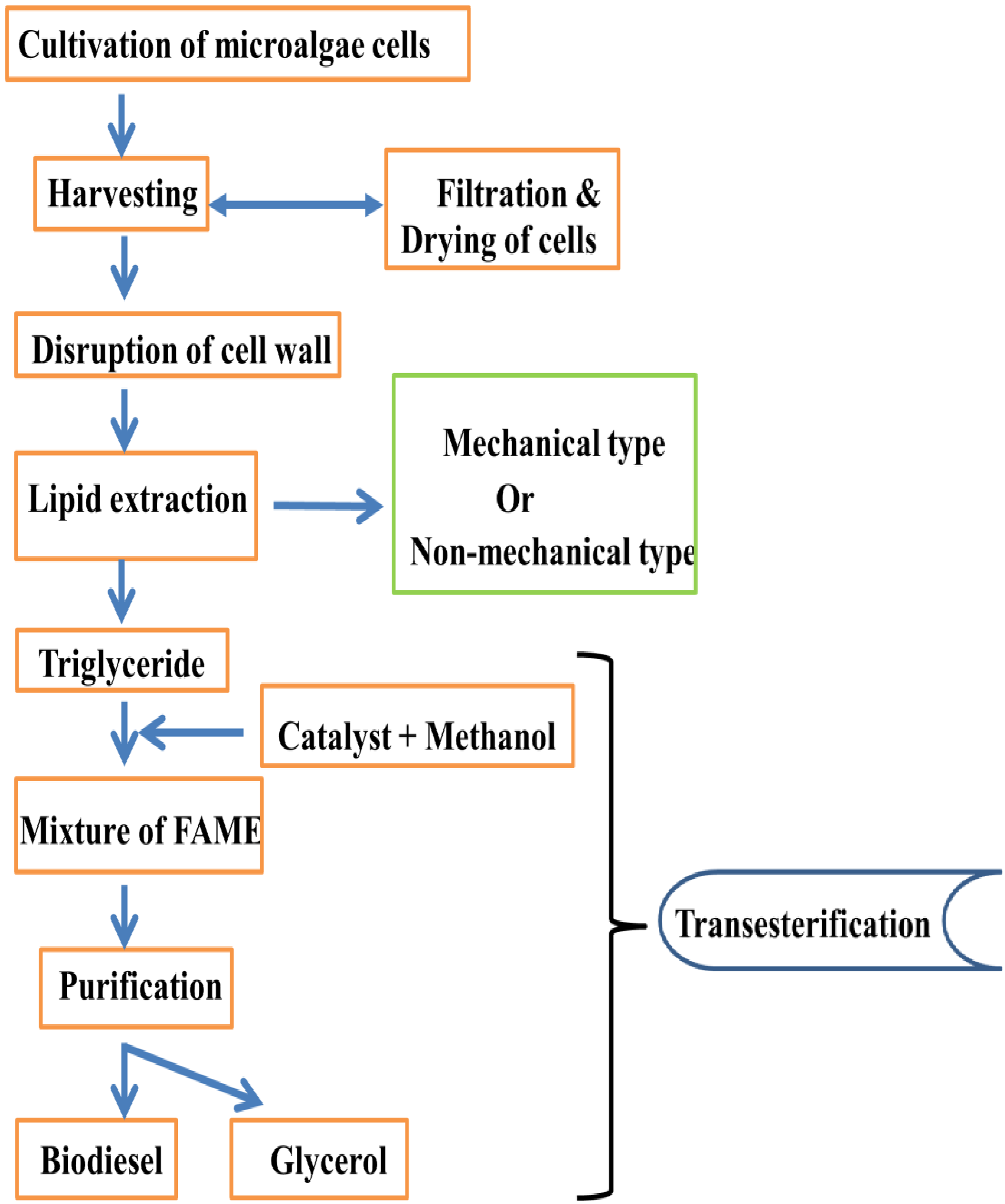

Figure 1. Flowchart diagram for biodiesel production through use of transesterification.

file studies were compared showing better outcome from *Chlorella sp*. In the mentioned study, lipid analyzing, its thermal efficiency all were recovered higher for biodiesel benefitting with low emissions of CO and HC [51]. Lipid extraction through microwave assisted in situ transesterification technique for the biodiesel yield was achieved from algae such as *Ulothrix sp.* 88% dry weight (DW), *Cladophora sp.* 80% DW, *Oedogonium sp.* 73% DW and *Spirogyra sp.* 67% DW. This technique for high biodiesel yield utilizes solvent free method [29].

Conclusion

From this study it reveals aquaculture wastewater is being the significant source for cultivating algae proceeding with sustainable environment in simple, cost effective way with zero waste reassurance. Both microalgae and macroalgae plays vital role in aquaculture production and wastewater remediation. The presence of nutrients in wastewater reveals necessary requirements for their growth. The emitted aquaculture effluent contains highly nutritive source for algae that blends into it recommending as bio or phyco remediation in process. Addition to that biomass produced from aquaculture wastewater can also be converted into biofuel through transesterification process with recent ideas of harvesting techniques. From the reported articles, it is known not much work have been proceeded in aquaculture wastewater co-related with micro and macroalgae. It still needs to be explored in order to achieve higher biomass and lipid for biofuel production where a solution is required for further research as there is huge gap in laboratory work and large-scale production. Hence, this study needs to be taken to further simplified step by investigating more into it.

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