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Competitive and Economically Feasible Cell Wall Disruption Techniques for Algal Biofuel Extraction

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Abstract— In the present scenario of fuel and energy crises, attempts for bridging the gap between demand and supply remained ineffective. The environmental damage caused by the existing fossil fuel facing price hike day by day, simultaneously the fossil fuel reservoirs are also exhausting and hence, these alarming energy crises are need to be addressed immediately. Today, the scientific community is running behind the renewable alternative fuel sources, and biofuel is one such alternative. The limitations of first and second generation biofuel have created the way for third generation biofuel technology. Microalgae are the major sources of the third generation biofuel. In order to achieve the high lipid content, we need to modify the pretreatment methods for disrupting the cell wall of microalgae. The classical method of lipid extractions from plants and crops can be followed for third generation biofuel production with trivial modifications in operating conditions. Several cell disruption techniques are known since past, but economically feasible, energy efficient and easily manageable methods are yet to identify, optimize and appraise. In the current review article, we have made an attempt to convey the algal cell wall components which are broadly used in the research and industrial area and focused on the key techniques involved in algal cell disruption.

Keywords— *Microalgae, Biofuel, Cell wall, Lipid, Biomass*

I. INTRODUCTION

Introduction Unsuccessful attempts in fulfilling the purpose of fuel and energy crises still unrelenting in the 20th century. Along with the environmental pollution, continuous ramble in price and demand of these fossil fuel reservoirs are challenging for the future that is needed to be addressed immediately. There are several obstacles and economic challenges need to be tackled in order to meet the fuel crises. Worldwide, as these non-renewable energy resources are running short of supply, the mankind is looking for an alternative source like the natural and renewable energy sources e.g. Biofuel. Earlier, the 1st and 2nd generation biofuels were excavated with the aid of versatile crops and their residues but, unconditionally growing population and the demand for the food supply created a problem in using these first and second generation biofuel sources hence, they failed in filling the gap [1]. Because, in order to cope up with the growing population needs such as energy and food supply, there is a need for considerable agriculture land. As first generation biofuel depended on the sources which are valuable edible sources as well [2]. In case of second-generation biofuel, the non-edible sources were utilized. However, the expenses in obtaining the lingo-cellulosic material in a desired level from raw materials and the processing technology were not fit for further commercial up gradation. The limitations of first and second generation biofuel led the way for emerging of third generation biofuel technology. This led the researchers to find and formulate 3rd generation biofuel from algae and the algal oil. Despite of the facts, that the algae cannot competitive with fossil fuel, they still have many advantages over plants [3].

There are mainly 4 major steps involved in obtaining biofuel from algae viz. cultivating algal biomass, biomass harvesting, lipid extraction and translation of lipids into biodiesel. However, each step has its own challenges. Basically, in order to take out lipids from the microalgae, it needs a massive amount of microalgal biomass and worldwide the techniques for growing algae for biomass production are in research phase. There are ample of parameters need to be taken care of, starting from obtaining microalgal biomass till it is processed. Preliminarily fix between wet biomass and dry biomass is a Challenge. Because in the case of dry biomass, it is characterized that enormous amount of energy is required for drying, whereas wet biomass requires a very less energy input for lipid extraction. Although wet biomass considered as a better source for lipid extraction, the percentage of oil is less compared to that of dry biomass [4]. To exclude the Use of fossil fuel in future, we should search for an alternative feasible and sustainable method. Efforts in growing algal biomass, oil extraction, biofuel production are the interest of researchers.

The classical method of lipid extractions from plants and crops can be followed for third generation biofuel production with trivial modifications in operating conditions. A little number of microalgae are explored for the biofuel production purpose [5] and these microalgae grows on wastewater sources and under extreme conditions as well possesses highest proportion of oil content with respect to its biomass which makes it a complementary source for biofuel production [6]. Microalgal biofuel usually is in the form of neutral lipid [7] and it is still not proven to be a potential source for commercial application because of the lack in effective technology for the extraction of oil from the algal biomass [8]. In order to obtain the high per cent of biofuel or lipids from the microalgae, it is equally necessary to culture the microalgal strains in optimized environmental conditions with suitable technology. Once we ensure the high percentage of lipid from the microalgae by looking at the amount of biomass available. Extraction can be followed by cell disruption techniques. Several cell disruption techniques are known since past, but the economically feasible methods are needed to be standardized.

In this review, an effort has been made in listing and discussing the several microalgal cell wall compositions, which are the well-known resources for biofuel production. As biofuel is the future of energy resource, microalgae plays a vital role in generating cheap and efficient biofuel. It is also important to exploit these microalgal resources in economically cost effective with high yield, for which the possible and efficient mechanical and non mechanical techniques, in the production of biofuel is explained briefly.

II. MICROALGAE AND ITS CELL COMPONENTS

Oil present inside the cell needs to be expelled out from the internal cell structures. For which several cell disruption methods are available (**Figure.1**). All the methods cannot be performed for all types of algal strains. Cell wall of microalgae usually made up of lipid constituents, protein components, polysaccharides glycoproteins and calcium carbonate [9, 10]. However, the cell wall characteristics depended on the various factors of environment in which algae is growing [11]. Hence, the composition of cell wall differs from one species to another (**Table.1)**. Prior to the selection of a method for extraction of lipids, it is always better to understand the components and composition of algae, respectively appropriate technology can be applied for lipid extraction by cell disruption. Presently there are several methods for cell disruption in use generally categorized as mechanical methods and non-mechanical methods. Some of the mechanical methods reported are Shear force, current, Heat and wave energy (**Figure.2**). The purpose of utilizing mechanical energy is to get the high yield. The oil extracted by the mechanical energy is more; however, more energy is spent. It is reported that the mechanical method of cell disruption induce more harshness to the cell environment inurn it may be an obstacle for the researchers while expecting other products [12]. In this review, we focused on the key developments and drawbacks faced by the researchers while extracting of lipid from the algae using cell wall disruption techniques and gives collective information about cell wall contents of various microalgae exploited for the biofuel production.

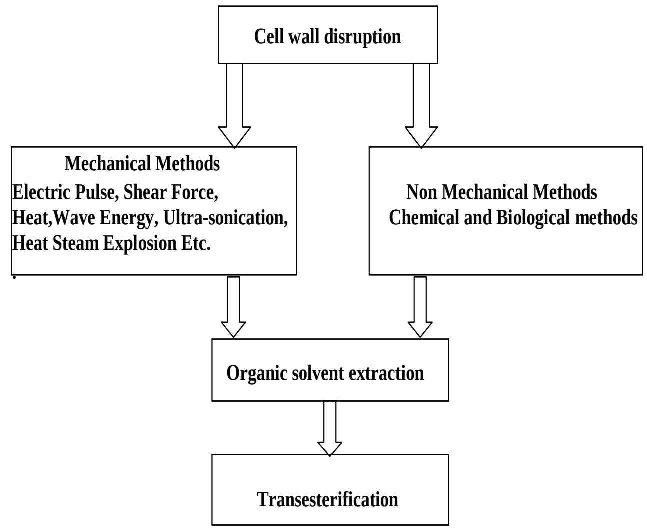

Figure 1. Steps Involved In the Oil Extraction from Microalgae.

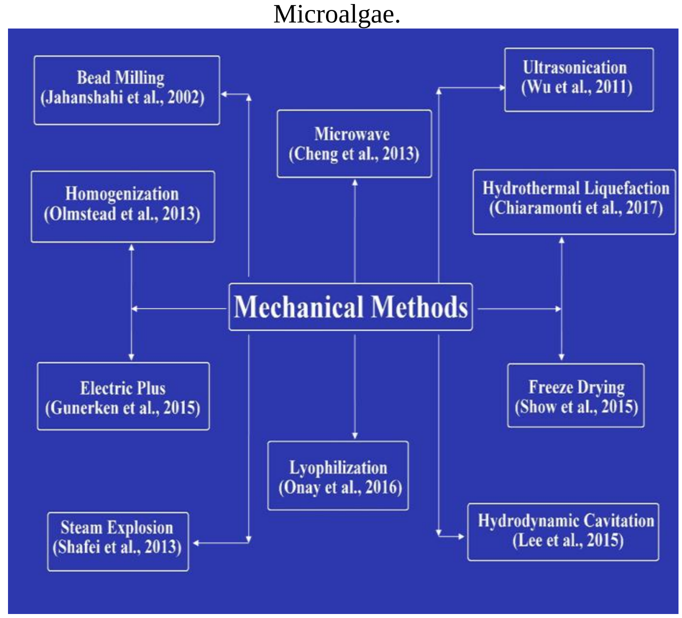

Figure 2. Represents the several Mechanical methods for cell disruption.

{ Onay *et al.*, 2016 [**2**], Jahanshahi *et al.*, 2002 [**13**],Wu *et al.*, 2011 [**14**],Cheng *et al.*, 2013 [**15**], Olmstead *et al.*, 2013 [**16**], Chiaramonti *et al.*, 2017 [**17**], Show *et al.*, 2015 [**18**], Gunerken *et al.*, 2015 [**19**], Lee *et al.*, 2015 [**20**], Shafei *et al.*, 2013 [**21**] **}**

Table 1. Algal cell wall components.

Algae	Cell wall components	References
Acetabularia acetabulum	Mannans	[22]
Anabaena cylindrical	Cellulose fibrils	[23]
Anacystis nidulans	Mannose	[24]
Botryococcus braunii	Variable Algeanan	[25]
Caulerpa ambiguous	Glucose, xylose	[26]
Chlamydomonas reinhardtii	Hyp-rich glycoproteins	[12]
Chlamydomonas sp.	Arabinose + Hyp-rich Glycoproteins.	[27]
Chlorella ellipsoidea	Cls,H-cls,pectin(no algaenan)	[28]
Chlorella emersonii	TLS	[29]
Chlorella fusca	Mannose, glucose, glucuronic acid, Rhaminose	[30]
Chlorella pyrenoidosa	Pectin and cellulose	[31]
Chlorella pyrenoidosa	Rhamnose/galactose	[32]
Chlorella pyrenoidosa	Protein: Lipid: α-Cls: H-cls: Gluamine: Ash(27% : 9.2% : 15.4% : 31% : 3.3% : 5.2%)	[33]
Chlorella saccharophila	Cls,H-cls,pectin (no algaenan)	[28]
Chlorella sp.	Microfibrils of chitin	[34]
Chlorella sp.	TLS + Wall matrix +Rigid walls	[35]
Codium fragile	Cellulose	[26]
Codium fragile	Mannans	[36]
Dunaliella bioculata	No cell wall	[37]
Haematococcus Pluvialis	ketocarotenoid astaxanthin	[38]
Nannochloropsis oculata	cellulosic polysaccharides	[34]
Phormidium foveolarum	Mannose,Glucose	[24]
Tolypothrix tenuis	Mannose,Glucose,Xylose	[24]
Chlorella ellipsoidea	Glucoseamine Blumreisinger	[32]

III. MECHANICAL METHODS

Among the mechanical means of cell disruption bead milling technique is efficiently achieve the purpose and can be easily handled [13]. Other techniques such as electroporation, pulse field electroporation hydrodynamic cavitations are relatively time-consuming and labor intensive techniques. Earlier researchers have achieved highest lipid extraction in *Nannochloropsissalina* by using Hydrodynamic cavitations technique, comparing to the other mechanical methods such as autoclaving and ultra-sonication [20]. However, it has its own limitations. The freeze drying technique is reported as a less significant technique in lipid extraction [4]. Algae with its recalcitrant cell wall material can be broken down easily using the High-pressure homogenization technique [16]. Osmotic shock is not extensively reported. In an experiment, microwave applied for cell wall disruption, pectin and cellulose layers are cut opened and the porosity increased in the stipulated time period [15]. Microwave technique is effective in terms of time and energy. It is also noticed that increase in temperature enhanced the disruption efficiency [15]. Microwaves generate kind of vibrations inside the cell environment and because of the vibrational effects the molecular interactions inside the water molecules there is an increase of temperature occurs. This, in turn assists cell rupture.

Despite all these facts, it consumes high energy [39]. While working with *Nannochloropsis sp.*, Teo and Idris observed the increased irradiation of microwave Technique [40]. Improvement in cell rupture capabilities is observed by the addition of various organic solvents and observed comparatively high lipid yield. This kind of cell disruption techniques are considered as physicochemical methods. Percentage of cell rupture and lipid productivity as observed by Teo and Idris by the microwave technique [40] method is summarized in **Table 2.** The ultrasound is used to rupture the cell wall in *C. protothecoides*[41] obtain significant yield. It is also reported that applying the high-speed ultrasound method can help in enhancing the cavitations in the subjected media. Which in turn result, improved cell wall disruption [42]. Similarly in an experiment conducted by the Pereira, used the ultrasound method along with the presence of Hexane [43], obtained a good yield. In the same way, Araujo, made a sincere attempt using the sonication technique along with the addition of silica by assuming that it may enhance cell disruption by increasing the sheer force [44]. However, unfortunately, the adverse effect of silica was noticed and the efficiency of yield is lowered. Thus, physicochemical method sometimes poses these kinds of challenges to the researchers. These problems need to be tackled.

IV. NON-MECHANICAL METHODS

Due to the disadvantages like harshness, and high energy requirement and low efficiency of mechanical methods alternative and competent methods need to be developed. The chemical method of cell wall disruption is found effective and competent in nature. Basically, oils are soluble in organic solvents and hence, various solvent can be employed for the extraction of oil from microalgae. Solvents such as Hexane, Benzene, Methanol, and Chloroform can be used for the cell wall disruption. Chemical method is effective in terms of economic as well as energy efficiency. But some chemicals with their carcinogenic properties are a drawback. Otherwise, the solvents used for the cell wall disruption are proven good. Integrated approaches, like the combination of mechanical and non-mechanical method works better in most of the cases. In some reports, enzymes are stated to be used for cell disruption. However, enzymes are very specific in their action hence; there is a need for a selection of enzymes for the targeted algal cells.

Hence, it is a kind of chaotic method for working with enzymes; they are relatively costlier under lab scale works and its tedious job [18]. Apart from these mechanical and non mechanical methodologies, trends in extracting elevated amount of lipid and other intracellular composites from microalgae for the production of biofuel is grasping attention.

Table 2. Lipid yield by Microwave technique.

Sl. No.	Solvent	Lipid yield (%)
1.	Chloroform	8.47
2.	Dichloroform	8.47
3.	Hexane	5.86
4.	Chloroform : (1.5%)Sodium sulfate	4.16

V. CONCLUSION

To conclude, techniques discussed in this review article for the extraction of lipid content by disrupting the microalgal cell wall are potential. Different techniques sometimes can be employed by understanding the cell wall composition of microalgal species and sometimes depending on the compound which is targeted. Integrated approaches are economically most effective and energy consumption can be reduced with mechanical approaches by simply employing the useful solvents. The energy required for the mechanical method of cell wall breaking is a major drawback along with which most of the energy is dissipated in the form of heat. In several methods, the algae are allowed to expose for a longer duration, which poses complication with respect to the product quality. Most of the reports says the efficiency of mechanical cell disruption may be feasible at the laboratory scale however, while scaling up of the processes in large scale many times become expensive. Hence, these aspects need to be considered while designing the novel methods for cell wall rupturing, so that the algal products especially the algal biofuel may soon become a sustainable resource for the future fuel crises.

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