



# Pre-treatment methods for production of biofuel from microalgae biomass

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## ABSTRACT

Microalgae biofuel is one of the most promising renewable energy sources that can contribute to the replacement of fossil fuels globally because of its sustainability and its ability to reduce the carbon dioxide emission in the atmosphere. However, the rigidity of microalgae of microalgae cell wall inhibits the extraction of lipids for biofuel production. To improve microalgae biofuel production, different pre-treatment techniques have been studied to evaluate their effectiveness on microalgae cell wall disruption. The main objective of this paper is to review the different pre-treatment technologies used in biofuel production from microalgae biomass and to critically discuss the current limitations and promising perspectives towards achieving economic and industrial scale production. Pre-treatment methods reviewed are categorized into mechanical techniques (e.g. high-pressure homogenizer and bead mills), physical techniques (e.g. ultrasonic and microwave methods), thermal pre-treatment techniques (e.g. autoclave and steam explosion), chemical techniques (e.g. catalytic and enzymatic), and combined techniques. Furthermore, comparisons of these techniques are discussed. The overall effect of the applications and methods on biofuel production together with energy consumption are critically examined.

## 1. Introduction

The sustainable sources of energy are rapidly increasing due to the increase in the world's population and industrialization. Traditional sources of energy, such as oil, natural gas, and coal, are non-renewable. The usage of these traditional sources often leads to tremendous damage to the environment by increasing the atmospheric load of carbon dioxide; also, greenhouse gases (GHGs) [1–4]. Challenging goals for these “new” supply options to meet our energy demands globally have been set, e.g. at European level by the commitment of meeting 20% of the overall energy demand from RE sources by 2020 [5]. Thus, it is an imperative issue to explore alternative energy sources to reduce the pollution caused by fossil fuel consumption [6]. The use of renewable energy is already growing. About 140 GW of the 300 GW of the new electricity generation capacity built globally between 2008 and 2009 were generated from renewable sources, while in 2005, renewables produced 16.5% of the world primary energy [7]. The use of renewable energy sources has been increasing rapidly in recent years and this trend is set to continue over the near future due to a 30% rise in global energy demand by 2040 [8]. With this development, it entails that all EU to fulfil at least 20% of its total energy needs with renewables by 2020 – to be achieved through the attainment of individual national targets, and at least 10% of their transport fuels should come from renewable sources by 2020 [9]. Renewable energy sources such as wind,

biomass, geothermal, solar and hydropower can provide sustainable energy services, based on the use of routinely available and indigenous resources. The rapid development of microalgae biomass as an alternative source of energy resources has been developing over a period of time [10]. The interest in microalgae development is due to the following points; lipid accumulation, not contending with agricultural land and can grow easily during culture period [1,11]. Though,

microalgae production process is still at a developing stage as compared to fossil fuel, as there are still some production bottlenecks that inhibit industrial process efficiency. The full-scale process and development consumes a lot of energy during lipid extraction process [12]. Even though, microalgae cells contains large amount of lipids, there is a significant need that the cell wall need to be disrupted or broken to enhance extraction efficiency [10]. Microalgae cell disruption is an integral part of the downstream operation required to produce biofuel from microalgae biomass, as biofuel produced from microalgae cells is non-toxic when compared to fossil fuels [13]. The cost of processing and dewatering of microalgae cells has tremendously affected the full-scale production of biodiesel and still remains the major bottleneck during the production process [14]. Recent advances in recombinant DNA technology have brought about the development of new techniques to produce useful enzymes, protein, and bioethanol from renewable biomass through cell disruption. Also, most fermentation and recovery operations require breaking of the cell wall by

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mechanical and non-mechanical techniques for separation and purification. The advancement of these biotechnological techniques have increased the demand for cell disruption on a very large scale in order to enhance lipid production from biomass as sustainable energy resources [15,16]. The potential use of lipids extracted from microalgae biomass for biofuel generation is increasing rapidly, and it is essential to consider the interaction of the disruption operation with downstream units and to clearly demonstrate the cost benefits of other alternative pre-treatment techniques [17]. However, extraction of lipids from microalgae has not met the required standard, and the entire production process is still at a developing stage even with the addition of solvents during lipid production. Though mechanical disruption has been widely used for microalgae cell disruption, the amount of water on microalgae cells reduces the rate of shear force which enhances cell wall rupture. Therefore, a pragmatic cell wall breakage method should accommodate materials that are in a high moisture slurry-phase environment. In recent years, various methods of cell disruption techniques, such as autoclaving, bead beating, microwaving, steam explosion, autoclave, osmotic shock, sonication, microwave, and high-pressure homogenizer, enzymatic, catalytic and ultrasonic pre-treatment, have been studied to evaluate their efficiency in microalgae cell disintegration, but the economic efficiency of the techniques still affects the industrial scale production. However, appropriate cell rupture is being selected on the basis of the durability of the cell wall, size of the process stream, the risk of sub-cellular destruction of important products, costs of the process and the safety of the method used [18]. Although cell pre-treatment improves lipid yield during extraction [19], the disruption efficiency has not been fully considered. Biomass pre-treatment is a very complex process, increases cost and can be used to increase process efficiency resulting in an ideal energy balance when compared with non-pre-treated biomass [20]. This study aims to review past research work that critically evaluate the pre-treatment of microalgae for biofuel production, in which mechanical, physical, thermal, chemical, and combined pre-treatment techniques were reviewed. The significance of cell disintegration using microalgae biomass to enhance biofuel will be detailed, and a comparison of different pre-treatment techniques used for the same purpose will be evaluated and summary of discussion will also be highlighted. Fig. 1 indicates a diagrammatic representation of the pre-treatment techniques used during cell disruption.

## 2. Mechanical pre-treatment technique

Mechanical techniques such as; bead milling and high-pressure homogenizer have been proven and utilized at large scale for cell disruption and pre-treating *Laminaria* spp. biomass for enhanced methane production [21,22], where its objective is mainly the reduction of particle size and crystallinity during biomass cell disintegration [23].

Cell disruption using mechanical technique seems to be more advantageous as compared to other disruption techniques, as it protects the microalgae cells from being contaminated and also protects the functionality of the material during cell rupture [24], as it can be used as a combination of different pre-treatment techniques which are not energy efficient. Finding efficient microalgae of cell disruption, multiple options should be applied, which include identification of biological features of the organisms that will improve the efficiency of cell disintegration using a mechanical technique. Although mechanical pre-treatment increases disruption efficiency of microalgae cells, the production process can also be inhibited by some inert materials in the substrate; such as stones and metal pieces. This technique also increases cell surface areas which makes the substrate easy [25]. Conclusively, the significant disadvantage of using this technique for cell rupture is the high-cost and energy consumption during cell pre-treatment process. In this study, the most popular mechanical techniques will be described as the high-pressure homogenizer and the bead mill.

### 2.1. High-pressure homogenizer

High-pressure homogenization has been widely used in microalgae cell disruption processes due to its scalability, continuous operation and the ability to process wet biomass [26], while most microalgae cells have been shown to be more resilient towards mechanical cell rupture [27]. However, high-pressure homogenization has also been shown to be highly effective in processing wet microalgae concentrate of up to 25 w/w% solids as a precursor of lipid recovery without incurring excessive energy demands [12]. In industries, the high-pressure homogenizer is linked to the production of stable emulsions and widely used in cell disruption of yeast cells and seaweeds, its effectiveness during cell rupture leads to an increase in lipid extraction [28]. However, a high-pressure homogenizer can also be used both in small and large-scale production during microalgae cell disruption, and its mechanism comprises of inlet and outlet chambers, external cavitation, shear force, a pressure regulator and a valve seat. The homogenizer valve seat consists of one or two positive displacement pumps which force the cell suspension under a high pressure of 150 MPa (but can vary up to 400 MPa) through an orifice which then collides against the seat valve [12]. The cells are then spread across the seat surface and eventually collide with the impact ring. A study, conducted by Donsi et al. [29] on microbial inactivation by using a high-pressure homogenizer, considered the effects of using valve geometry, piston micrometric valve and orifice valve for cell disruption process. The piston and orifice valve has a diameter of 3–14 m and 130 mm length respectively. They observed that using multiple high-pressure homogenizers at a pressure of 100 and 300 MPa showed that a piston micrometric valve system is more effective as compared to orifice valve due to cell interactions

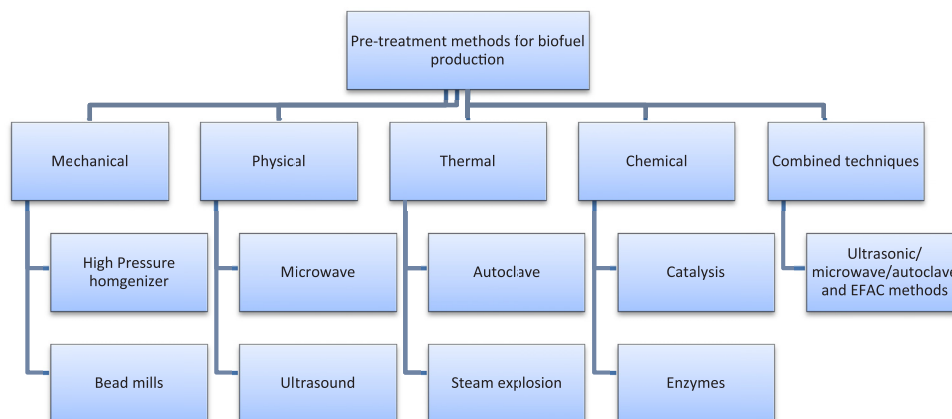


Fig. 1. Classification of cell Pre-treatment methods (Modified as requested).

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