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Review article

Biological pretreatments of microalgal biomass for gaseous biofuel production and the potential use of rumen microorganisms: A review

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ABSTRACT

Pretreatments to break down complex biopolymers in microalgae cells are a key process in the production of gaseous biofuels (methane and hydrogen) from such biomass. Biological pretreatment implies cell degradation by purified enzymes; enzymatic cocktails or by microorganisms with enzymatic activity capable of hydrolyzing the microalgae cell wall. This review presents relevant results using those methods that are less energy intensive and, in some cases, more specific than other strategies, such as chemical and physical pretreatments. Enzymatic pretreatments are specific and efficient, with cellulase, hemicellulase, pectinase, protease and amylase being the most explored enzymes. For biomass pretreatment, enzymatic cocktails have been more effective than single enzymes, as it is more feasible to obtain enzymatic extracts of one or more hydrolytic microorganisms than their purified enzymes. The potential use of hydrolytic cultures for cell disruption to breakdown complex biopolymers has been demonstrated. Their use is less specific than that of enzymatic extracts, but more cost-effective. Pure cultures of hydrolytic bacteria, most of which have carbohydrase activities, have increased the biofuel conversion efficiency from microalgae and from bacterial consortia. The use of natural microbial consortia with hydrolytic activities, such as ruminal microorganisms, represents a potential pretreatment for microalgae. In this review, common hydrolytic activities are highlighted and compared, and the use of ruminal microorganisms as a cell disruption strategy is discussed. Understanding the operational conditions applied to natural consortia, such as ruminal microorganisms, will favor a suitable system for microalgae cell disruption that may increase the biological hydrogen and methane recovery from microalgae.

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1. Introduction

Microalgae-derived biofuels have several advantages over second- and first-generation biofuels; they can potentially produce 50 times more biomass than switchgrass which is the fastest growing terrestrial plant and require less water, even though they grow in aqueous media [1]. Indeed, nutrients for their cultivation can be obtained from wastewater with an organic content reduction, thus maximizing water use efficiency. On the other hand, the algae's capability to fix CO_2 (1.83 kg of CO_2 per 1 kg of dry algal biomass) can relieve the effects of global warming, using sunlight energy in photobioreactors that are easy to operate [2,3].

The processes for harvesting and drying the lipid-rich microalgae are energy intensive, compromising the efficiency of algae-derived biodiesel production [4]. In addition to biodiesel production, strategies for the production of other biofuels, such as biogas, bioethanol and biohydrogen, through the fermentation of wet algal biomass are being developed and have promising energy efficiency [5–7]. The full-scale application of these technologies depends on the optimization of algal biomass production, harvesting and downstream processes. A limiting step in the downstream processes for microalgae utilization is the release of high-value constituents, such as carbohydrates that could be used in fermentative biofuels production.

The relevance of algae pretreatments to biofuels production has been reviewed for bioethanol production [8] and anaerobic digestion for methane production [9]; it has also been reviewed from the microalgae biorefinery approach [10]. The purpose of pretreatment is the disruption of the cell wall in order to improve the availability of biomolecular constituents in microalgae. Different pretreatment technologies have been suggested. Pretreatments are classified as biological, mechanical or chemical, based on the force applied and energy consumed in the process. Biological pretreatments refer to cell degradation by purified enzymes, enzymatic cocktails (either a mixture of purified enzymes or extracts produced by hydrolytic microorganisms), and to the use of cultures of microorganisms with hydrolytic activities in direct contact to microalgae. Regarding energy consumption, chemical and enzymatic pretreatments are not energy intensive and have a good selective product recovery, thus having a positive impact on steps in the downstream process. However, biological treatments take longer than mechanical treatments, and the chemicals and enzymes are costly [10]. Some applications of enzymatic microalgae treatments for bacteria and yeast cell disruption have been demonstrated in medicine, agriculture and the food industry [11], suggesting their possible application in producing microalgae-based bioproducts. The major roadblocks are the higher cost of enzyme production and handling, high enzyme-to-substrate specificity, and enormous diversity in algal cell envelope composition and structure [12].

On the other hand, using direct microalgal cell disruption by hydrolytic microorganisms has been tested as a pretreatment for different purposes, such as hydrogen [13,14], methane [15,16], and bioethanol production [17], and for lipid release in biodiesel production [18]. Using hydrolytic bacterial cultures can be less expensive than purchasing purified enzymes; however, the recovery of specific products can be threatened. In this sense, using hydrolytic strains or consortia is closer to consolidated bioprocessing (CBP), which has been detailed for lignocellulosic materials; in brief, it includes the hydrolysis, saccharification and fermentation of the substrate to biofuel, all in the same reactor [19]. The present review compares the performance of different biological pretreatments for algal cell disruption, underlining the potential use of natural consortia – such as ruminal microorganisms – with hydrolytic and saccharification capabilities.

2. Microalgae cell composition

Microalgae are composed mainly of carbohydrates (4–64%), proteins (6–61%) and lipids (2–40%) [20]. The fact that some species store carbohydrates instead of lipids makes these species attractive for fermentative biofuels production [21]. However, microalgae composition can vary according to environmental conditions, affecting the production of different macromolecules [6].

One of the most exceptional characteristics of microalgal composition is the presence of a complex, dynamic and polysaccharide-rich cell wall [22]. This extracellular matrix affords the cell physical and microbial protection and helps with cell-cell adhesion in some cases. Among some different classes of green algae (Trebouxiophyceae, Chlorophyceae, Ulvophyceae), the structure of the cell wall differs in composition. However, a common component is cellulose, resembling that of higher plants; it also typically contains other polysaccharides such as pectin and hemicellulose [23]. Moreover, the presence of some biopolymers such as sporopollenin and algaenans give extra resistance to the algal cells. Sporopollenin composition is not well understood because of its unusual chemical stability and resistance to degradation by chemicals under very harsh conditions. Analyses have revealed a mixture of biopolymers, mainly containing long chain fatty acids, phenylpropanoids, phenolic compounds and trace carotenoids [24]. Algaenans have been characterized as aliphatic biomacromolecules in which the main building blocks are described as C30–34 mono- or diunsaturated ω-hydroxy fatty acids that are joined through a combination of ester and ether linkages. This composition creates a strong and recalcitrant structure that is similar to cutin and lacking in carotenoid moieties and is reported to be resistant to acids, bases, enzymes and detergents [24,25].

These abovementioned characteristics make microalgae resistant to harsh environmental conditions and to the energy recovery processes of fermentation and anaerobic digestion. In this sense, a biomass pretreatment is needed in order to disrupt, release and solubilize the main components of algal cells [9]. This requirement is a bottleneck determining the efficiency of subsequent processes.

3. Enzymatic hydrolysis of microalgae

Biological pretreatments involve the use of enzymes and/or microorganisms to carry out the hydrolysis of algal biomass. The most exploited enzymes for these purposes are those with cellulase, hemicellulase, pectinase, protease and amylase activity [26], due to the ease of their extraction and purification from fungi [27]. Cellulases encompass a group of enzymes that hydrolyze the crystalline structure of cellulose into small oligosaccharides and subsequently to glucose. They consist of at least three major enzymatic components: 1) endoglucanases (EC 3.2.1.4), which randomly hydrolyze glycosidic bonds in amorphous regions of the cellulose, leading to a reduction in chain length and generation of reducing ends, 2) exoglucanases or cellobiohydrolases (EC 3.2.1.74), which act on both reducing and non-reducing ends, releasing glucose or cellobiose, and 3) β-glucosidases (EC 3.2.1.21), which hydrolyze cellobiose or oligosaccharides to glucose [28,29]. Cellulase of *Trichoderma reesei*, a white rot fungus, is one of the most commonly used enzymes because it has one of the highest reported activity levels for wild microorganisms; moreover, this fungus has