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Saccharification of carbohydrates in microalgal biomass by physical, chemical and enzymatic pre-treatments as a previous step for bioethanol production

D. Hernández [a], B. Riaño [a], M. Coca [b], M.C. García-González [a,*]

[a] Agricultural Technological Institute of Castilla y Leon, Ctra. Burgos, km. 119, 47071 Valladolid, Spain
[b] Department of Chemical Engineering and Environmental Technology, University of Valladolid, C/Doctor Mergelina s/n, 47011 Valladolid, Spain

HIGHLIGHTS

- Several pre-treatments were applied for carbohydrate saccharification of three microalgal species.
- Cell wall composition of microalgae determined pre-treatments efficiency.
- Microwave, autoclave and alkaline hydrolysis resulted in poor sugar release.
- Combination of pre-treatments enhanced monosaccharides release.
- Cell wall disruption was essential for enzymatic attack improving sugar release.

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ABSTRACT

Fermentation of carbohydrates to produce bioethanol is one of the pathways to produce biofuels from microalgae. This process currently needs many stages that are complex and energy consuming. Cell wall disruption and hydrolysis are two of the stages that must be carried out, since most carbohydrates are entrapped within the cell wall or intracellularly as energy storage in the form of starch. In the present work, physical, chemical, and enzymatic pre-treatments were performed on three microalgal species to disrupt and break down complex carbohydrates into simple sugars, as a preliminary stage to produce bioethanol. Pre-treatments were carried out alone and combined with each other. According to the results obtained in the present work, the highest concentration of monosaccharides per g of microalgae dry weight was achieved by the combination of pre-treatments; for *Chlorella sorokiniana* and *Nannochloropsis gaditana* the combination of acid hydrolysis followed by enzymatic hydrolysis produced 128 and 129 mg/g, respectively. In the case of *Scenedesmus almeriensis* the highest monosaccharide concentration (88 mg/g) was obtained after acid hydrolysis with sulphuric acid for 60 min at 121 °C. The results obtained proved the effectiveness of the combination of acid pre-treatment and enzymatic hydrolysis to enhance complex carbohydrates break down into simple sugars in bioethanol production process from microalgal biomass.

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1. Introduction

Currently, bioethanol is produced at commercial scale via fermentation of different carbohydrate-rich feedstocks such as corn, sugarcane and beets [1]. The main producer countries are United States and Brazil [2]. Bioethanol is particularly important since it can substitute gasoline in combustion engines, which makes it one of the most promising biofuels; expecting to reach a production of 100 billion litres in 2015 [3]. However, concerns over food safety and human demand for food poses a major challenge to the use of agricultural stocks. More sustainable fuel production alternatives are thus needed to overcome these problems. In this context, although in optimal conditions the most significant species of microalgae and cyanobacteria contain 15–25% [4] of carbohydrates, different works have identified microalgae as a suitable source of carbohydrates for bioethanol production since they are able to exhibit high carbohydrate content [5] under stress conditions like nutrient starvation [6], high salinity [7] or

* Corresponding author. Tel.: +34 983 317388; fax: +34 983 414780.
E-mail addresses: dhgon@hotmail.com (D. Hernández), gargonmi@itacyl.es (M.C. García-González).

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light deficit-excess [7,8]. Indeed, algae offer a number of potential advantages compared to higher plants. Microalgae have shown to be more efficient than terrestrial plants in converting sunlight to biochemical energy being its production tenfold higher [9,10]. Microalgae consume CO_2, reducing greenhouse gas emissions and their growth is not dependent on arable land availability [11,12]. However, microalgal growth requires high amounts of nutrients, mainly nitrogen (N) and phosphorous (P), being the supply of P limited and N production requires high fossil energy. One alternative to synthetic culture media is to use agro-industrial wastewater which usually presents high N and P concentration. In this sense, the interference with conventional fertilizers is avoided as fertilizers are not required [13–15]. In recent years, different microalgal genera like *Anabaena*, *Chlamydomonas*, *Chlorella*, *Porphyrydium*, *Scenedesmus*, *Spirogyra* and *Spirulina*, among others, have been used to produce bioethanol. These microalgae have been grown in synthetic medium achieving carbohydrate concentration from 8% up to 64% [4,16].

In order to produce bioethanol, a disruption of the cell wall must be carried out since most carbohydrates are entrapped within the cell wall (cellulose and hemicellulose), or intracellularly as energy storage in the form of starch [8,17]. In a further stage, it is necessary the hydrolysis of polysaccharides to release monosaccharides for its later fermentation into bioethanol. Biomass pretreatment is also a necessary stage to increase the surface area, to enhance sugars solubility and to improve substrate digestibility [18]. Pre-treatments have been viewed as one of the most crucial and expensive processing stages in biomass conversion to fermentable sugars [19].

Different methods have been tested to disrupt and to hydrolyze cell wall carbohydrates into monosaccharides. Among others, physical methods include high-pressure homogenization, microwaving, sonication and heat [20–22]. In addition, chemical lysis using alkaline or acid reagents have been also applied to hydrolyze microalgal biomass into its constituent monosaccharides [18,23,24]. Enzymatic pre-treatment has shown to be an efficient tool to get cell wall hydrolysis in some microalgae [19]. These physical, chemical and enzymatic pre-treatments have a particular economic cost that depends on many parameters as: (i) electricity cost; (ii) alkaline or acid reagent; (iii) temperature and time reached during thermal pre-treatment; (iv) type of enzymes used; (v) addition of surfactants during enzymatic hydrolysis and (vi) type of raw material used, among others. According to pre-treatment costs, they may be ordered (from low to high costs) as (i) physical: microwaving, sonication, high-pressure homogenization and heat; (ii) chemical: alkaline and acid; and (iii) enzymatic pre-treatments: cellulases and amylases [25–27]. Pre-treatments have a great potential for improving the efficiency of fermentation and lowering costs throughout research and development. Thus, optimizing cell disruption and sugar extraction methods is essential to produce bioethanol in a cost-effective and environmentally sustainable manner. Despite of the many cell disrupting methods tested in literature for microalgal cell wall disruption, a standard pre-treatment has not been identified to treat most of microalgal species. Furthermore, data in literature concerning biomass pretreatments are not comparable, because quite different microalgae strains, conditions and techniques are used, making it difficult to compare these results between microalgae.

In the present work, different physical, chemical, and enzymatic pre-treatments were performed on three microalgal species to study disruption and break down of complex carbohydrates into simple sugars, as a preliminary stage to produce bioethanol. *Chlorella sorokiniana* and *Scenedesmus almeriensis* were selected due to their ability to grow in wastewater containing high organic matter concentrations [15], and *Nannochloropsis gaditana* was selected because its ability to grow in high salted mediums [28]. Both characteristics minimize the appearance of competitive microalgal species and predators like rotifers and protozoa in open pond cultures, resulting in a higher growth of the selected microalgae. The methods evaluated in the present work covered physical, chemical and enzymatic processes. Sugar release (SR) was determined for each microalga under the different experimental conditions studied, analyzing monosaccharides and sugar degradation products in hydrolysates (furfural, 5-hydoxymethylfurfural (HMF), propionic acid, acetic acid, formic acid and lactic acid) to determine the effectiveness of the pre-treatment processes and the possible inhibition of the sugar degradation products on fermentative microorganisms in further sugar fermentation.

2. Materials and methods

2.1. Microorganisms

Chlorella sorokiniana was obtained from the culture collection of the University of Goettingen (Goettingen, Germany). Microalgae inoculum was cultivated in a mineral medium according to Guieysse et al. [29]. Biomass was centrifuged at 10,000 rpm (Beckman Coulter, Avanti centrifuge J-30I) for 10 min, washed with distilled water and dried in an oven (Selecta, Digitronic) to a constant weight at 80 °C. The biomass was stored at 4 °C for further use.

Nannochloropsis gaditana B-3 and *S. almeriensis* were obtained in lyophilized form from the Food Innovation and Sustainability Center (Almería, Spain). *N. gaditana* was cultured following González-López et al. [28]. *Scenedesmus almeriensis* was isolated from a fresh water pool in Almería (Spain) and cultured following Sánchez et al. [30]. Lyophilized biomass was washed with distilled water, dried in an oven to a constant weight at 80 °C and stored at 4 °C for further use.

2.2. Methods for cell disruption and sugar extraction

2.2.1. Acid hydrolysis

Acid hydrolysis of microalgal biomass was carried out using H_2SO_4 (purity greater than 96%, VWR International, Radnor, USA) at different concentrations (4%, 7%, and 10% (v/v)) at 121 °C for 30 min. The assays were carried out in 250 mL Erlenmeyer flasks (Simax, Prague, Czech Republic). The H_2SO_4 concentrations and temperature were selected according to Harun et al. [31] and Miranda et al. [24]. Acid pre-treatments were performed using 100 mL of microalgal biomass at a concentration of 30 g volatile suspended solids (VSS)/L. A control was performed using 0% H_2SO_4 followed by autoclave treatment (121 °C for 30 min).

2.2.2. Alkaline hydrolysis

The biomass was suspended in 100 mL of NaOH (purity greater than 98%, Panreac Química SLU, Barcelona, Spain), 1 M and 5 M, to set a final concentration of 30 g VSS/L. After that, samples were incubated at 90 °C for 30 min with constant agitation at 60 rpm with a magnetic stirrer (RH Basic 2, IKA, Staufen, Germany) using 250 mL Erlenmeyer flask (Simax, Prague, Czech Republic). The alkaline hydrolysis parameters in terms of NaOH concentration, temperature and incubation time were adapted from Ellis et al. [32] and Harun et al. [18]. A control was set using biomass suspended in 0% NaOH at 90 °C for 30 min.

2.2.3. Autoclaving hydrolysis

Autoclave hydrolysis assays were performed using microalgal biomass suspended in a volume of 100 mL to set a concentration of 30 g VSS/L. Two sets of experiments were carried out. In the first set, biomass was suspended in water and samples were autoclaved at 121 °C for 30, 45, 60 and 90 min. A control was performed

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