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Microalgae digestive pretreatment for increasing biogas production

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ABSTRACT

Microalgae have many advantages for the production of biogas by anaerobic digestion process. However, the anaerobic digestion process has been reported to be limited in the hydrolytic stage due to the specific characteristics of the cell wall components thus resulting in an inefficient conversion of biomass to biogas. Pretreatments aim to achieve an increase in the biogas production by increasing solubilization. Enzymatic pretreatment is described as an environmentally-friendly process, due to the low energy consumption, great yield of freed, fermentable sugars from the biomass under light operational conditions, the absence of corrosive problems, and few derivatives produced. Within the category of enzymatic pretreatments, it might identify two types, which are related to the origin of the enzymes and which may be classified as endogenous enzymes, and commercial exogenous enzymes. It should also be considered that enzyme production costs for commercial enzymes might be a negative factor in the process. The objective of the present review is to analyze and discuss the application of digestive pretreatments on the solubilization of microalgae, with a focus on the cell wall, and its relation to biogas production increase.

1. Introduction

There are numerous efforts evaluating different alternatives for generating renewable energy that can supply current demands. Some examples of these alternatives include solar energy, wind energy, and those produced from biomass, among others.

Microalgae have been studied for their use in the production of different types of biofuels, as an alternative for meeting the demand of fossil fuels. It is possible to produce bio-hydrogen by means of a photobiological process, biodiesel from microalgae oils [1–3], bioethanol [4] from carbohydrate fermentation, or biogas from anaerobic digestion (Table 1).

The usage of microalgae in the production of biofuels has many advantages over other types of biomass; these include not competing with agriculture for soil usage in producing foodstuff, a high growth-rate in comparison to higher plant life, and the ability to cultivate them in arid zones or those with low water quality [5].

Particularly, the generation of biogas by means of anaerobic digestion is currently an alternative with low operational costs compared to other processes [4]. Anaerobic digestion consists of the transformation of the organic material through a series of biochemical processes: hydrolysis, fermentation, acetogenesis, and methanogenesis. These processes are carried out by different microorganisms [6]. The biogas produced is made up mainly of methane, with some 55–70% v/v, and carbon dioxide, 30–40% v/v. Depending on the substrate utilized in the process, it is possible for other compounds to be present, such as nitrogen, < 2%; hydrogen and/or oxygen, < 1%; hydrogen sulfide, 0–50 ppm; other sulfide compounds, volatile organic compounds, aromatics, and halogens, 10–270 mg/m^3; and siloxanes, 80 – 2500 μg/m^3 [5].

Different biogas yields have been reported for microalgae biomass, and vary in a wide range from 50 to 800 mL CH_4/gVS (volatile solids), values that represent some 40–72% of maximum theoretical biogas production [7]. This wide range of production and of biogas percentages are possibly due to different factors: microalgae composition and cell-wall structures; operational microalgae cultivation factors, such as cultivation photoperiodicity, light intensity, cultivation media, or others [8]; microalgae species used, due to species-specific cellular characteristics, differences in structure, cellular wall synthesis and cellular composition, and the carbohydrate, protein, and lipid content.

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Table 1
Methane yield increase after digestive pretreatment applied to microalgae biomass for biogas production.

Microalgae	Enzyme	Solubilization (%)	Methane yield (CH_4/ g. SV)	Increase (%)	Reference
Acutodesmus obliquus	Enzymatic cocktail (cellulase, protease, β-glucanase and xylanase)	36.3	218	14	[63]
Arthrospira máxima	Lipomod™ 957 (esterase, protease)	–	1545	672	[12]
Chlorella sp.	Hydrolytic enzyme from bacteria *Bacillus licheniformis*	43.4	415	22.7	[64]
Chlorella vulgaris	Alcalase 2.5 L (protease)	52	600	500	[65]
Chlorella vulgaris	Viscozyme (β-glucanase, cellulase, xylanase)	40	150	200	[65]
Undeterminated	Bacterial strain *Pseudobutyrivibrio xylanivorans*	–	279.9	13	[66]
Oocystis sp.	Cellulase®	218	203	7.6	[67]
Oocystis sp.	Enzymatic cocktail (cellulase, glucohydrolase and xylanase)	243	217.3	15.2	[67]
Oocystis sp. *Diatoms, bacteria and other microorganisms*	Laccase commercial	–	100	20	[43]
Oocystis sp. *Diatoms, bacteria and other microorganisms*	Laccase from *Trametes versicolor*		144	74	[43]
predominanted by *C. vulgaris*	*Bacillus* 252 sp	6.2	0.08[a]		[45]
Scenedesmus obliquus	Depol™ 40 L (cellulase, endogalactouronase)	–	1425	403	[12]
Nannochloropsis gaditana	*Raoultella ornithinolytica from Mytilus chilensis*	–	262.84[b]	140.32%	[17]
Nannochloropsis gaditana	*Raoultella ornithinolytica from Mesodesma donacium*	–	282.92[b]	158.68%,	[17]
Botryococcus braunii	*enzymatic extract of Anthracophyllum discolor 1000 U/ L*	62	521	60	[42]

[a] (g COD/g. COD).
[b] mL $gVSS^{-1}$.

These factors strongly affect biogas production [5,6].

We should also consider that different stages of the anaerobic digestion process might be limited by different factors. For example, at high concentrations of fatty acids, the methanogenesis stage is limited, since the methanogenic archaea are considerably diminished in their capability to remove hydrogen and VFA (Volatile Fatty Acids) [9]. During the hydrolytic stage, the microalgae structural complexity and cell wall composition can be limiting factors. In this stage, the bacteria degrade microalgae with exo-enzymes. First, the bacteria internally produce exo-enzymes to be liberated to the media. Once these exo-enzymes degrade the substrate, the latter is absorbed by the bacteria, which continues to be digested by the bacteria with endo-enzymes [10]. Some relevant aspects of this process are that the production of exo-enzymes and the substrate solubilization may take several hours or days. In addition, not all bacteria produce exo-enzymes; this type of bacteria requires even longer periods for the degradation process [10]. Similarly, it should be noted that enzymes are substrate specific. In this sense, we know that the structure and composition of the cell wall are species-specific. As such, it is possible to describe a species without a cell wall, such as *Dunaliella salina*, while other species with rigid structures might vary from a simple polysaccharide wall to those with a trilaminate structure composed of external, intermediate, and internal walls [5]. The most structurally and compositionally complex cellular walls are difficult to solubilize with bacterial anaerobic digestion since the exo-enzymes freed by these types of hydrolytic bacteria might not be specific for the mentioned cell wall components. This situation results in small available amounts of hydrolyzed organic material, which limits the entire process, and consequently the production of biogas [11,12].

Given the above, hydrolytic stage becomes fundamental when looking to undertake microalgae anaerobic digestion, since it produces the liberation and intracellular organic material that will later be digested by the bacteria and finally synthesized into biogas by the archaea. Said hydrolysis is also necessary for the production of other types of biofuels, such as biodiesel, bioethanol, and hydrogen, as well as the production of commercially high-value macromolecules, such as lutein, astaxanthin, and lipids, among others [13].

This type of biotechnological challenge in biogas production has been tackled with several strategies, from among which microalgae biomass pretreatment has been one of the most outstanding [5,11,14–17]. The purpose of this application is to condition and increased the digestibility of the microalgae by hydrolyzing the cell wall, which can increase biogas productivity.

During recent years, there has been an increasing amount of research related to the application of different pretreatments to microalgae cultivation in order to obtain valuable sub-products. In this context, there have been descriptions of physical pretreatments that look to reduce the size of the macromolecules found in the cellular wall by means of physical force or high temperatures and/or pressure [18]. Nevertheless, this type of pretreatment is associated with high energy requirements and, additionally, do not digest the cell wall components. Further alternatives are found in biological pretreatments which, for example, utilize enzymatic digestion in order to tease out the components of the cell wall, for which the energy requirements are much lower [12].

The objective of the present review is to analyze and discuss the application of digestive pretreatments on the solubilization of microalgae, with a focus on the cell wall, and its relation to increases in biogas production.

2. Microalgae cell wall composition and structure

As mentioned previously, the microalgae cell wall plays a fundamental role in anaerobic digestion. In photosynthetic organisms, the cell wall exercises a central biomechanical function in relation to environmental interactions. As such, it is possible to differentiate among wide variations of structure and composition (Table 1) that all depend on multiple factors (growth stage, cellular shape, environmental factors). This variation can also be due to phylogenetic factors since this category includes functional genes; these latter are responsible for the synthesis and formation of the cell wall, as well as any subsequent modifications thereof [19] (Table 2).

Studies related to the formation, structure, and composition of microalgae cell walls by means of phylogenetic analysis have made these differences available, and have mentioned that they have been acquired from different phylogenetic lines associated with several cellular functions, such as, for example, environmental sense perception, cellular shape, cellular motility, intercellular connections, etc. [19].

In relation to the formation of microalgae cell walls, differences have been reported among species of the same genus. For example, among *Chlorella* microalgae, widely utilized in the production of biofuels, two different formation manners have been described. These are