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Hydrolysis of *Golenkinia* sp. biomass using Amberlyst 36 and nitric acid as catalysts

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ABSTRACT

Golenkinia sp. is a microalga with potential commercial value because of its high carbohydrate content (more than 40%). The major challenge is to find hydrolytic processes that can efficiently convert these carbohydrates into fermentable sugars. Hydrolysis of microalgal biomass generally uses a dilute liquid acid catalyst, such as hydrochloric acid, sulfuric acid, or nitric acid. However, neutralization and desalting processes are needed after this procedure for production of a hydrolysate that is suitable for use as a fermentable broth. These processes require large amounts of energy and are, therefore, very expensive. In this work, we hydrolyzed 10 g/L of *Golenkinia* sp. using 9.2 g/L of Amberlyst 36 with 0.01 N of nitric acid at 150 °C for 160 min, a process that required no desalting. 88% of total sugar yield mainly glucose and galactose was obtained after a 120 min reaction. Our results also showed that these 2 catalysts had a prominent synergistic effect, in that the yield when both catalysts were used together was much greater than the sum of the yields of the individual catalysts. The small amount of nitric acid reduced the particle size of the *Golenkinia* substrate, and this led to a greater total surface area and 135% increase in the hydrolysis efficiency of Amberlyst 36. The *Golenkinia* sp. hydrolysate (3.7 g/L sugars) was used as a carbon source for 2,3-butanediol fermentation by *Klebsiella oxytoca*, with no need for further detoxification or deionization processes. The yield was 0.19 g of 2,3-butanediol per g of sugar.

1. Introduction

Microalgae are a potential alternative source of renewable energy because of their high rate of carbon dioxide fixation and their high content of lipids [1], which can be converted to biodiesel and can account for as much as 30–50% of dry biomass [2]. Microalgal biofuels are a potential substitute for petroleum-based energy sources, but there are challenges in the large-scale production of oil from microalgae [3]. This problem can be resolved by using lipids extracted from microalgal biomass as a feedstock for microbial fermentation to produce high value-added products [4]. In addition to lipids, microalgae also contain huge amounts of carbohydrates, up to 80% of dry biomass [5], which are synthesized from photosynthesis and the metabolism of atmospheric carbon dioxide [6]. These carbohydrates are mainly stored in the cell walls, soluble polysaccharides, and cellulose [4].

Several methods are currently available to hydrolyze microalgal biomass, and these methods may be classified as thermal, mechanical, biological, or chemical processes [7]. Thermal hydrolysis employs heat to disintegrate cell walls at 50 °C to 270 °C, especially above 180 °C [8]. Significant energy input is needed for thermal hydrolysis, and undesirable side reactions frequently occur. Mechanical hydrolysis employs physical force, such as ultrasound or microwaves, to directly break apart cells [9]. Mechanical processes are time consuming, require large amounts of electrical energy, and are not suitable for large–scale operations. Biological hydrolysis is widely used to decompose cells by use of appropriate enzymes [10]. The advantages of enzymatic hydrolysis are that enzymes have high selectivity for the cell wall, there is minimal production of undesirable compounds, there is little energy consumption, and operating conditions can be variable [11]. However, enzymatic hydrolysis is unsuitable for disruption of microalgal cell walls because they have complicated structures, with various monosaccharides and sulfated polysaccharides, in contrast to lignocellulosic biomass, which mainly contains glucose. Also, the cost of the enzymes needed for biological hydrolysis is greater than the catalysts needed for chemical hydrolysis [12]. Chemical hydrolysis employs chemical compounds such as detergents, acids, or alkalis, with acid treatment being the most widely used method [13-16]. Acid hydrolysis can produce high yields of fermentable sugars in about 1 h, but it operates at relatively high temperature (80 °C to 150 °C) and leads to the production of undesirable toxic compounds. Thus, after acid hydrolysis,

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the hydrolysate must be neutralized and desalted to make the broth fermentable [14]. Several new technologies can also be used for hydrolysis of microalgal cells. For example, laser treatment can disrupt microalgal cells [17], and a combination of acidic and enzymatic hydrolysis is also effective [18]. However, further research is required to develop these methods so that they are energy-efficient and can be operated at a large scale.

In this study, we report the hydrolysis of microalgal biomass using a solid acid. There has been no report to date on the use of solid acids, either with the aid of liquid acid or not, for this purpose. We used *Golenkinia* sp., which contains about 43% carbohydrates based on dried cell weight, as a substrate. Our initial results indicated that the sole use of a solid catalyst was not feasible due to the low efficiency of microalgal cell wall hydrolysis, which is limited by interaction between the insoluble microalgal biomass and the interface of the solid acid catalyst. Thus, we sought to overcome this problem by studying the effect of simultaneously adding a small amount of liquid acid.

2. Materials and methods

2.1. Materials

Nitric acid and Amberlyst 36 (polystyrene-*co*-divinylbenzene sulfonic acid resins), a solid catalyst, were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Hexane, sulfuric acid, and hydrochloric acid were obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). Table 1 shows the physicochemical properties of Amberlyst 36. Microalgal biomass of *Golenkinia* sp. was supplied by NLP (Natural Live Plankton, Busan, Republic of Korea). The initial biomass contained 68% water. It was dried in a thermal oven at 70 °C for 72 h, and then ground into powder (< 1 mm) using a mortar and pestle. The ground powder was used as a substrate for hydrolysis experiments.

2.2. Methods

2.2.1. Hydrolysis of microalgal cells with acid catalysts

Microalgal biomass was added to deionized water so the resulting suspension had a biomass concentration of 10 g/L (10% w/v). Then, Amberlyst 36 (1 to 13.8 g/L) was added to the suspension. To study the possible synergistic effects of nitric acid and Amberlyst 36, liquid acid was supplied to the biomass-deionized water and Amberlyst 36 mixture, so the final mixture had a nitric acid content of 0 to 0.01 N.

Table 1
Characteristics of Amberlyst 36[a], HCl[b], H_2SO_4[b], HNO_3[a], used in this study.

Solid acid		Liquid acid			
Properties	Characteristics	Properties	HCl	H_2SO_4	HNO_3
Physical form	Opaque beads	Molecular weight [g/mol]	36.5	98.1	63.1
Functional groups	SO_3H	Acidity (pKa)	− 8.0	− 3.0, 1.99	− 1.4
Acid capacity [meq H^+/g]	≥ 5.40	Acid capacity [meq H^+/g]	27.4	20.4	15.8
Surface area [m^2/g]	33				
Particle size [mm]	0.600–0.850				
Average pore diameter [Å]	240				
Maximum operating temperature [°C]	150				

[a] Provided by Sigma-Aldrich Datasheet.
[b] Provided by JUNSEI Datasheet.

The reaction was performed in a 250 mL continuously stirred autoclave reactor with a working volume of 150 mL at 150 °C for 180 min (Hanwoul Eng. Co., Korea). To avoid undesirable reactions with reactive gas, N_2 gas was purged into the reactor, and pressure was maintained at 345 kPa for sampling. Samples (2 mL) were taken at 20 min intervals, centrifuged at 13,200 rpm for 2 min, and then stored at − 20 °C until subsequent analysis. The microbial fermentation broth had 10 g/L of microalgal biomass suspension that was hydrolyzed with 9.2 g/L Amberlyst 36 and 0.01 N nitric acid.

2.2.2. Recycling of catalyst

After completion of hydrolysis, Amberlyst 36 was separated from the hydrolysate using a strainer (100 μm pore size), washed with deionized water, and dried in a thermal oven at 70 °C for 24 h. The recovered Amberlyst 36 was added to new fresh medium, with 10 g/L *Golenkinia* sp. biomass in deionized water, and the reaction was allowed to proceed. This process was repeated for 4 times, and the hydrolytic activity of the solid acid catalyst was evaluated by measuring the sugar yield from each reaction.

2.2.3. Extraction of lipid

Before microbial fermentation, lipids in the hydrolysate that were released during acid hydrolysis were extracted using hexane. Briefly, 50 mL of microalgal hydrolysate was mixed with 50 mL of hexane, followed by vigorous vortexing for 20 min. The sample was then centrifuged at 4000 rpm for 10 min for phase separation. The upper hexane phase contained lipids, and was removed using a syringe. The extraction steps were repeated 3 times. The remaining aqueous phase contains sugars, and was used as a fermentation broth.

2.2.4. 2,3-BDO (2,3-Butanediol) fermentation

Wild type of *Klebsiella oxytoca* (ATCC8724) was purchased from the Korean Collection for Type Cultures. These cells were precultured in a glucose medium on a shaker (150 rpm) at 37 °C (initial pH 5.5), until the optical density at 600 nm reached 4.0 to 4.5. The glucose medium consisted of 9 g/L glucose, 5 g/L yeast extract, 6 g/L $(NH_4)_2SO_4$, 8.7 g/L K_2HPO_4, 6.8 g/L KH_2PO_4, 0.25 g/L $MgSO_4{\cdot}7H_2O$, and 2% (v/v) trace metal solution (2.5 g/L $FeSO_4{\cdot}7H_20$, 0.05 g/L $ZnSO_4$, 0.05 g/L $MnSO_4{\cdot}H_2O$, and 0.05 g/L $CaCl_2{\cdot}2H_2O$) [19,20]. The main culture medium contained lipid-extracted hydrolysate as a carbon source, which replaced the glucose in the preculture medium. Then, 1% (v/v) of the preculture medium was inoculated into a 250 mL Erlenmeyer flask containing 50 mL of the main culture medium, and incubated at 37 °C for 14 h (initial pH 5.5). Samples were taken at 1 h intervals. All experiments were performed in triplicate.

2.2.5. Analytical methods

Golenkinia sp. biomass contains organic and inorganic chemical compounds, including carbohydrates, proteins, lipids, and inorganic minerals (ash). The amount of total carbohydrate was measured by a phenol-sulfuric acid assay, as described in Laurens et al. [21]. The weight percentages of nitrogen and other elements, such as carbon, hydrogen, and sulfur, were determined by an element analyzer (FLASH 2000 series, Thermo Scientific, USA). Protein content was estimated using nitrogen-to-protein conversion factor of 4.78 for marine microalgae [21]. Total lipids were extracted by modified Bligh-Dyer method, and quantitated gravimetrically [22,23]. Ash was analyzed as described in Laurens et al. [21].

Cell growth was monitored by measuring optical density at 600 nm (OD_{600}) using a UV–Vis spectrophotometer (UV-2550, Shimazu, Japan). Sugar concentration was determined by high-performance liquid chromatography (Waters, USA), and an evaporative light scattering detector (ELSD, Sedex, France). Organic acids, 5-hydroxymethylfurfural (HMF), total furfurals, and 2,3-butane diol (BDO) were measured using high-performance liquid chromatography (Ultimate3000, Dionex, USA), and a UV/Vis and refractive index detector (RID, Shodex, USA). Organic