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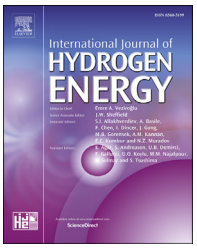

# Dark fermentative hydrogen production following the sequential dilute acid pretreatment and enzymatic saccharification of rice husk

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ABSTRACT

This study examined the effect of sequential dilute acid hydrolysis and enzyme saccharification conditions of rice husk on hydrogen ($H_2$) fermentation. The rice husk was hydrolyzed using dilute $H_2SO_4$ and then further enzymatically hydrolyzed with various concentrations (0.1, 0.25, 0.5, 0.75, and 1.0 mg protein $mL^{-1}$) of Celluclast 1.5 L®. The sequential pretreatment significantly enhanced sugar recovery, up to 174% for 1.0 mg $mL^{-1}$ concentration of the enzyme. Increasing the concentration of enzyme slightly increases sugar recovery. While the production of furfural and 5-hydroxymethylfurfural occurred during dilute acid hydrolysis, it was not observed during enzymatic saccharification. At the following $H_2$ fermentation of the hydrolyzates, $H_2$ yield was improved up to 150% by using the dilute acid and enzymatic hydrolyzate as substrate, as compared when only dilute acid hydrolyzate was used. The highest $H_2$ yield of 473.1 mL $H_2$ $g^{-1}$ rice husk was achieved with enzyme concentration of 0.75 mg protein $mL^{-1}$.



## Introduction

Biorefinery is the series of various conversion processes which harness renewable energy from carbohydrate-rich materials by thermochemical conversion of biomass [1,2]. A wide range of technologies is used to break down biomass resources to their building blocks, which can be converted to fuels and chemicals. Biomass, a renewable energy source, is a promising source for generation of biofuels, like biogas, biomethane, syngas, charcoal, bioethanol, and biodiesel. There is currently a great interest to biomass due to the increasing demand for energy and the adverse environmental effects caused by limited energy sources, such as fossil fuels.

One of the most important biorefinery products is hydrogen ($H_2$). $H_2$ is a promising alternative energy carrier due to its high energy efficiency, recyclability, and environment-friendliness [3,4]. The energy yield of H2, 122 kJ $g^{-1}$, is found to be 2.75 times higher than that of fossil fuel [5]. Interest in biohydrogen production is currently high because conventional physico-chemical $H_2$ production methods require non-

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renewable sources, such as coal, oil, and natural gas, high energy consumption and high costs. In recent, a highly significant portion of biohydrogen is produced by dark fermentative $H_2$ production [6–8]. Dark fermentation of $H_2$ occurs when fermentative microorganisms break carbohydrate-rich biomass down to $H_2$ [9,10]. Among these $H_2$-fermentative microorganisms are bacterial populations belonging to the orders *Enterobacteriales* and *Clostridiales* [11]. While this process is done at less energy-intensive and eco-friendly mild operating conditions, anaerobic biohydrogen production from lignocellulosic biomass has yet to be fully explored [12].

Lignocellulosic biomass, which include all terrestrial plants and energy crops, is a promising feedstock for biohydrogen production, due to its abundance and low cost. This type of biomass has a complex surface structure of cellulose, hemicellulose, lignin, and other components, making it recalcitrant in nature [10,13]. Pretreatment processes are therefore required to break the polysaccharide-lignin complex interactions, making the carbohydrates in the biomass more utilizable for biorefinery processes [12,14,15]. Pretreatment is highly essential for the use of lignocellulosic biomass to mitigate the recalcitrance by alteration of the composition and structure of the biomass and improve enzymatic accessibility. Various pretreatment methods have been tried for lignocellulosic biomass in previous studies involving generation of $H_2$. Among the aforementioned pretreatment methods, most previous studies dealt with mechanical, physical, chemical, and biological processes, or a combination of any of these processes, to overcome the recalcitrance of lignocellulosic biomass at a sustainable and economical manner [16–19]. The hydrolysis of lignocellulosic biomass is often regarded as the rate-determining step in fermentative $H_2$ production [12]. Once soluble monomeric sugars have been released and recovered from the lignocellulosic biomass, fermentation can be performed.

Dilute acid pretreatment is one of the most widely performed pretreatment methods for lignocellulosic biomass. Hydrolysis with dilute acid is normally preferred over concentrated acid because the former is more cost-effective and environmentally-friendly compared to the latter [19]. Gonzales et al. [20,21] have previously studied the production of $H_2$ via dark fermentation using dilute acid-pretreated lignocellulosic biomass. The dilute acid pretreatment conditions were optimized for the biomass under study and the efficiency of pretreatment in harnessing the simple sugars were primarily assessed, followed by the use of the hydrolyzate solutions as substrate for fermentative $H_2$ production. During dilute acid pretreatment, the biomass is hydrolyzed in harsh physicochemical conditions resulting to a solution rich with monomeric sugars. The lignin-carbohydrate complexes are disrupted, effectively releasing the carbohydrates, making these molecules easily accessible to enzymes and microorganisms [19–21]. The cellulose and hemicellulose backbones of the polysaccharide components of lignocellulosic biomass are stable only up until a particular acidity and thermal condition. Beyond this acidity and thermal condition, the polysaccharides break down and become highly soluble in the acid, converting the cellulose and hemicellulose to monomeric sugars, which are easier to extract [22].

While dilute acid pretreatment is effective in breaking down the complex structure of the lignocellulosic biomass, the sequential acid and enzyme hydrolysis method is considered to be more efficient in further breaking the biomass down and recovering monomeric sugars [12]. Most studies involving the sequential acid hydrolysis and enzyme saccharification of various lignocellulosic biomass are on bioethanol production, and not primarily for $H_2$ fermentation by dark fermentation [23]. The conditions of enzyme saccharification must also be optimized, since it is highly important to recover as much sugar as possible from the biomass, especially those which cannot be recovered by simply doing dilute acid hydrolysis. Enzyme saccharification parameters such as enzyme concentration must be optimized to result to an efficient saccharification [12].

Rice husk, an agricultural byproduct of the rice industry, was used as the substrate for this study. This particular biomass was chosen since South Korea is a major consumer of rice and this biomass is just disposed as waste, left unutilized or incinerated. Hydrolysis of lignocellulosic biomass, such as rice husk, leads to production of various useful biofuels, which include biohydrogen, as compared to combustion, which results to only heat as a product [24].

The objective of this study was to investigate the effect of sequential dilute acid hydrolysis and enzymatic saccharification on soluble sugar recovery from rice husk, a lignocellulosic biomass material, and the following fermentative $H_2$ production. Optimization of enzyme loading during the saccharification was also performed and $H_2$ fermentation performance was evaluated by both sugar yield and $H_2$ production.

## Materials and methods

### Biomass

Untreated rice husk acquired from a local rice milling company was used as the lignocellulosic biomass in this study. The glucan, xylan, arabinan, lignin, ash, and extractives composition of the biomass were measured by following the NREL laboratory analytical procedure [25]. The biomass composition is presented, on dry basis, in Table 1. The rice husk was air-dried and initially milled to reduce the particle size to 1–2 mm prior to use.

### Sludge

Anaerobic granular sludge obtained from an upflow sludge blanket reactor treating brewery wastewater in South Korea was used as the inoculum in this study. The anaerobic granular sludge is known to contain more strains of $H_2$-producing bacteria [4]. The anaerobic granular sludge obtained from the brewery wastewater treatment facility was found to contain the following mesophilic genera: *Clostridium* and *Enterobacter*, which are both known $H_2$-producing bacteria [4]. The pH, volatile suspended solids (VSS), and total suspended solids (TSS) concentration of the sludge were 6.8, 12.6 g $L^{-1}$, and 22.6 g $L^{-1}$, respectively. To harvest only anaerobic spore-forming $H_2$-producing bacteria, the sludge was heat-treated

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