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Effect of sonication pretreatment on hydrogen and acetone-butanol-ethanol coproduction from *Chlamydomonas mexicana* biomass using *Clostridium acetobutylicum*

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ABSTRACT

Acetone-butanol-ethanol (ABE) fermentation produces acetone, butanol, and ethanol, which are clean and sustainable energy sources with high energy densities. The ABE fermentation encounters butanol toxicity, by-products formation, and generation of lignin-derived inhibitors. The implementation of the low-lignin substrate, effective pretreatment, and biofuel coproduction would enhance the fermentation efficiency. Thus, this study investigated the use of sonication pretreated *Chlamydomonas mexicana* biomass as a cost-effective substrate to produce biohydrogen (bioH_2) and ABE using *Clostridium acetobutylicum* to demonstrate effective production of biofuels through ABE fermentation. Sonication enhanced the recovery and bioaccessibility of carbohydrates (74%) and proteins (52.4%), and their efficient utilization for bioH_2 and volatile fatty acid (VFA) production. The bioH_2 yield from sonicated *C. mexicana* biomass (2.9 mL/g-carbohydrate), was ~1.5 times higher than that attained from non-sonicated biomass (1.97 mL/g-carbohydrate). Subsequently, VFAs generated in the acidogenic phase (1.5 g/L acetate, and 6.05 g/L butyrate) were used to produce 0.54 g-ABE/g-carbohydrate through ABE fermentation. Thus, this study demonstrates that both soluble carbohydrates (28 g/L) and proteins (14 g/L) from pretreated microalgal biomass were efficiently converted to 110.2 mL bioH_2 and 20.84 g/L ABE. These soluble carbohydrates and proteins could be used for high-energy biofuel production through ABE fermentation with minimum waste generation.

1. Introduction

Carbon-rich fossil fuels supply about 80% of the world's energy. However, the immense utilization at the current rate would lead to its depletion within 35–107 years and emanation of carbon dioxide (CO_2) [1,2]. Fossil fuels consumption contributed to environmental deterioration, global climate change, and consequent increased pollution-related health problems [3]. Therefore, alternative fuels (sustainable/renewable) with high energy content have been developed to replace fossil fuels, overcome CO_2 emissions, and overcome the global energy crisis [4]. Biomass has been widely studied for its use in the production of several biofuels, including biohydrogen (bioH_2), bioethanol, biodiesel, and higher alcohols [5,6]. Acetone-butanol-ethanol (ABE) fermentation is attracting interest to produce reliable and affordable energy, mainly bioH_2 & biobutanol that are potentially better substitutes than bioethanol. Biobutanol has high energy density, low volatility, affinity with aqueous solutions, and has already been employed as a fuel additive and as pure fuel instead of gasoline [7,8].

The ABE has been achieved through biomass fermentation employing various microorganisms, including *Clostridium beijerinckii* and *C. acetobutylicum*. These fermentative microorganisms have shown their potential for laboratory and commercial applications because of their high competencies [9]. Besides, they can utilize a diversity of carbohydrates in biphasic fermentation processes (acidogenesis and solventogenesis) [10]. Acidogenesis involve the production of organic acids (including acetate and butyrate) and gases (bioH_2), while solventogenesis involves the ABE synthesis [11]. The ABE production includes feedstock selection, pretreatment, and fermentation [12].

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