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Commentary on Succinic Acid and Poly-γ-glutamic Acid Production using Lignocellulosic Waste Biomass

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Short Commentary

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ABSTRACT

In this short commentary, the possibility of exploiting lignocellulosic waste biomass for succinic acid and poly-γ-glutamic acid production via fermentation were explored with several typical examples. We mention, in addition, the traits of expanding production scale with fibrous-bed bioreactors. We hope to discuss the potential and prospect of utilizing lignocellulosic waste biomass for the production of valuable chemicals.

INTRODUCTION

Lignocellulosic biomass, which contains cellulose (40-50%), hemicellulose (25-50%) and lignin (10-40%), is the most abundantly available raw material on Earth [1]. Broadly, it is classified into three categories: virgin biomass, waste biomass and energy crops. Owing to the rapid growth of global population and corresponding agricultural activities, the amount of lignocellulosic waste biomass is increasing, resulting into a big question to deal with it. The traditional handling methods include landfilling, incineration and composting. Unfortunately, these methods suffer from either environmental contamination or economic limitations [2-4]. Recent studies have reported the utilization of lignocellulosic agricultural waste for bioconversion into high valued chemicals [5,6]. The access to this bioconversion is by turning lignocellulosic agricultural waste into fermentable sugars. In this short commentary, we discuss the utilization of lignocellulosic waste biomass for producing succinic acid and poly-γ-glutamic acid, as well as fibrous-bed bioreactors for productivity improvement is also referred to.

Succinic Acid and Poly-γ-Glutamic Acid Production with Different Lignocellulosic Waste Biomass

Both succinic acid and poly-γ-glutamic acid can be produced from sugars such as glucose, xylose, maltose and fructose. Sugars can be natural intermediates in the biological conversion of lignocellulosic waste biomass, but access to sugars is often hindered by the recalcitrance of plant cell walls [7]. As a result, different physical, physic-chemical, and chemical pretreatment methods were explored, including mechanical comminution, pyrolysis, stream explosion, and acid or alkaline hydrolysis.

Jiang et al. pretreated sugarcane bagasse with dilute H_2SO_4 and then used cellulase to convert cellulose and hemicellulose into cellobiose. Their work verified that cellobiose can be used as a potential carbon source for succinic acid fermentation with *A. succinogenes*, and a final succinic acid concentration of 30.3 g/L with a yield of 67.8% was achieve via batch fermentation in anaerobic bottles [8]. Liu et al. used sugarcane bagasse hydrolysate containing 50.7 g/L of reducing sugars to produce succinic acid in a 3-L fermenter. After 120 h fed-batch fermentation using *E.coli* BA305, the succinic acid concentration was 39.3 g/L [9]. Our group also gathered much experience in waste biomass conversion to valuable chemicals. The hydrolysates of corn straw, corn core, rice straw and wheat straw were respectively investigated as carbon resources for succinic acid fermentation by *A. succinogenes*. Results indicated that the hydrolysate of corn stover was better than that of rice or wheat straw in fermentation of succinic acid [10]. Moreover, different pretreatment methods of acid hydrolysis, alkali hydrolysis, and aqueous-ammonia soaking and steam explosion were compared with corn stover used as the model lignocellulosic waste biomass. Results showed that 0.1% (v/v) of dilute alkali pretreatment was the most favorable method for succinic acid production. Furthermore, simultaneous saccharification and fermentation technique was applied by *A. succinogenes* in a 5-L fermenter, and the succinic acid concentration and yield could reach 47.4 g/l and 72%, respectively [11]. In another work, pretreated sugarcanecane bagasse was enzymolyzed by a multi-enzyme "cocktail" containing cellulase, xylanase, β-glucanase and pectinase to improve the yield of glucose and xylose. At the end of a fed-batch fermentation conducted in a 3-L bioreactor with *A. succinogenes*, a succinic acid concentration as high as 70.8 g/L can be obtained with a yield of 81.5% [12].

Among the trials of poly-γ-glutamic acid production with lignocellulosic waste biomass, Xu et al. treated corncob fibers with NaOH and HCl to obtain hydrolysates consisting of glucose, xylose and arabinose, which can be taken up by *B. subtilis* and led to 24.92 g/L of product concentration [13]. *E. coli* was engineered to be endowed with the ability of co-fermenting a glucose-xylose mixture in sugarcane bagasse hydrolysate [14]. Poly-γ-glutamic acid production with *B. subtilis* was also carried out through solid-state fermentation with a mixture of dry mushroom residues and monosodium glutamate production residues, achieving an outcome of 115.6 g/kg poly-γ-glutamic acid and 39.5 x 10^8 colony forming unit's g^{-1} cells [15]. Tang et al. also used *B. subtilis* for poly-γ-glutamic acid production. They found xylose was more suitable for cell growth but not for biosynthesis compared with glucose, and thus introduced a two-stage hydrolysis process of rice straw to extract glucose and xylose separately. Afterwards, a co-fermentation strategy was applied to obtain a higher product accumulation with a shorter cell growth period [16].

Succinic Acid and Poly-γ-Glutamic Acid Production Using Fibrous-Bed Bioreactor

Fibrous-bed reactors, which developed with cells immobilized in a fibrous matrix, were reported to offer the advantages of high cell density, long term stability and high production, thus were favorable to pilot or industrial scale production. Yan et al. first fabricated a fibrous-bed reactor with cotton terry cloth for repeated-batch and fed-batch fermentations of succinic acid by *A. succinogenes*; results showed a succinic acid concentration of 98.7 g/L with a yield of 89% and productivity of 2.77 g/L/h were achieved during repeated fed-batch fermentation [17]. Furthermore, similar bioreactor was examined for continuous fermentation, exhibiting a long-term stability for 18 days with no obvious fluctuations in both succinic acid and cell density [18]. In comparison, an immobilized and suspended-cell system using plastic composite support was designed to retain high biomass concentration, leading to the maximum succinic acid concentration of 34 g/L with a yield of 88% in 38 h of incubation [19]. And by using an external membrane cell recycle system, *A. succinogenes* 130Z cell concentration in continuous culture increased to 16.4 g/L at a dilution rate 0.2 h^{-1} [20].

Xu et al. developed an aerobic plant fibrous-bed bioreactor for poly-γ-glutamic acid production. Notably, sugarcane bagasse was used as cell immobilization carrier, which shortened fermentation time from 72 h to 48 h. The average product concentration and productivity of 71.21 g/L and 1.246 g/L/h were achieved by cells immobilized in bagasse, which was reused eight times over a period of 457 h [21]. According to their research, the cells adapted in this fibrous-bed bioreactor have lower cell membrane permeability and higher key enzyme activities than the original cells, thus possessing greater tolerance for substrates and final product.

CONCLUSION

Although the utilization of lignocellulosic waste biomass for valuable chemicals has the benefits of resource recycling as well as eliminating competition for using starch-based food, two problems are also associated with the prevalent methods of pretreatment methods, especially acid and alkaline hydrolysis. One is the introduction of side products of soluble phenolic compounds in the sugar-containing hydrolysate, which is toxic to cells. The other is the generation of waste water. The former can be solved by a detoxication process before fermentation process. However, up to now, there was still no report about the alkali treatment wastewater in the production of succinic acid and poly-γ-glutamic acid, which cast a shadow on industrial application. In order to improve the utilization of lignocellulosic waste biomass for valuable chemicals production, the treatment methods of lignocellulosic waste biomass should still be optimized to adapt to practical application, and there should be a systematic pathway from raw material processing to final effluent treatment. Moreover, as for the fabrication and operation of fibrous-bed reactors, emphasis is still put on the bioreactor structure and support for cell adsorption to overcome mass transfer limitation, stabilize cells and bring operational feasibility.

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