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# Feasibility of acetone–butanol–ethanol (ABE) fermentation from *Amorphophallus* konjac waste by *Clostridium acetobutylicum* ATCC 824

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ABSTRACT

Using *Amorphophallus* konjac waste as a source of fermentable carbohydrate, the feasibility of acetone–butanol–ethanol (ABE) fermentation by *Clostridium acetobutylicum* ATCC 824 was investigated in this study. Two distinct combination strategies for the enzymatic hydrolysis and fermentation, simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF), for konjac waste fermentation were compared. β-Mannanase was added to facilitate the hydrolysis of glucomannan and to increase the fermentability of the substrate. The ABE concentration (6.64 g/L) from SSF was less than that from SHF; the result from the control experiment was 4.34 g/L of ABE. SHF obtained a higher production of total ABE solvents (10.95 g/L) with 7.10 g/L of butanol at a productivity rate of 0.30 g/L/h. The effect of the bioactive alkaloid abundant in the konjac waste on ABE fermentation was evaluated. The results indicated that the alkaloid affected the ABE fermentation results when using *C. acetobutylicum*, and the effective extraction of alkaloids can help to increase the butanol concentration and shorten the fermentation period. Therefore, the utilization of *Amorphophallus* konjac waste (a low-cost agricultural resource) in ABE fermentation provides an alternative to increase the economic viability of ABE production.



## 1. Introduction

In response to the rising price of gasoline, the exhausted fossil fuel resources over the past decades, the emission of greenhouse gases, and the environmental pollution incurred through the global utilization of fossil fuels, renewable and sustainable alternatives, such as biofuels, should be treated as a partial replacement of fossil fuels. Among the available alternatives, biobutanol, a superior option when compared to bioethanol [1], has received considerable attention because of its octane-improving power, low-viscosity, and high energy content [2]. During anaerobic bacterial fermentation performed by microorganisms such as *Clostridium acetobutylicum* [3,4] and *C. beijerinckii* [5], butanol is the predominant fermentation product with additional acetone and ethanol (in a ratio of 6:3:1, respectively). The fermentative acetone–butanol–ethanol production is called AB or ABE fermentation [6]. The ABE bio-based products are expected to replace petrochemical products to an extent. In addition to its potential use as a biofuel, butanol can also be used in the chemical synthesis industry as a neutral C4 primary alcohol in plastic manufacturing [7]; currently, this primary alcohol is derived from petroleum products.

Substrate cost is a major factor affecting the economic feasibility of butanol production [8]. Therefore, low-price substrates are required to control for the effects of the economics of fermentation-derived liquid fuels [8]. Starchy materials, such as cracked corn [9], potato and soy molasses [8], are commonly used in the ABE fermentation industry [10,11]. Cheese-whey [12,13], food waste [14] and lignocellulosic materials, including agricultural residues [15,16] and forestry wastes, are considered as potential additional fermented substrates [17]. However, corn and potatoes are important resources for human foods. Although non-food biomass, such as agricultural byproducts, has been utilized in various methods [18], the complex structures of cellulose and lignin requires pretreatment [19]. This pretreatment adds a mixture of different hydrolyzing enzymes for microbial fermentation [20], increasing the cost of ABE fermentation.

*Amorphophallus* konjac has long been used in China and Japan, and its main product, Konjac Glucomannan (KGM), has been introduced into western nations over the past few decades [21]. Konjac waste, a byproduct of the KGM processing industry, contains a substantial amount of carbohydrates [22]. The attractive

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**Table 1**
ABE fermentation characteristic using sugars present in konjac waste.

| | Total sugar (g/L) | Butanol concentration (g/L) | Total ABE solvent[b] (g/L) | Total acids[c] (g/L) | Sugar utilization (%) | Yield[d] |
|---|---|---|---|---|---|---|
| Glucose | 60 | 9.05 ± 0.55 | 10.97 ± 0.97 | 2.56 ± 0.03 | 98.78 | 0.19 |
| Mannose | 60 | 9.12 ± 0.19 | 11.02 ± 0.44 | 4.12 ± 0.03 | 99.72 | 0.19 |
| Mixed sugar[a] | 60 | 8.90 ± 0.29 | 11.58 ± 0.38 | 4.02 ± 0.08 | 98.26 | 0.20 |

[a] The mixed sugar consists of 30 g/L glucose and 30 g/L mannose.
[b] Total ABE solvent includes acetone, butanol and ethanol.
[c] Total acids includes acetic acid and butyric acid.
[d] Yield is defined in Section 2.

properties of konjac waste include that the material consists of all hexose sugars after hydrolysis and the structures of the components are relatively easy to utilize. Pentose fermentation and pretreatment methods for lignocellulosic materials are not required. In addition, the konjac waste also contains abundant medicinal components such as alkaloids. These components have been reported to display pharmacological activities [23], including antibacterial activity. With the recent growing demand for KGM worldwide, konjac waste amounts to approximately two thousand tons per year in China alone [24]. This waste is abandoned directly or marketed as a low-cost animal feed ingredient. Therefore, this waste not only becomes a source of pollution but also is a loss of a resource; the effective use of konjac waste requires additional attention. Because konjac waste is an economical substrate for ABE production, generating biofuel when reducing waste seems to be an ideal process. Butanol generation from konjac waste can improve the multi-utilization of *Amorphophallus* konjac materials and displays the potential to enhance the economic feasibility of waste treatment and reduce environmental pollution.

Previous studies investigating konjac waste focused on the extraction of components with pharmacological activity from the waste. Few studies have explored the fermentation of the waste to produce alcohol or the solid state fermentation of the waste for fodder. Only limited information is available regarding ABE fermentation using konjac waste as the carbon source. In this study, therefore, the feasibility of butanol production using konjac waste was investigated. The separate hydrolysis and fermentation (SSF) of konjac waste was compared to the simultaneous saccharification and fermentation (SHF) of the waste. β-Mannanase was added to facilitate the hydrolysis of glucomannan and improve the fermentation performance. Konjac waste extraction by an organic solution was used to add extra value to the material and improve the ABE fermentation results. The overall objective of this investigation was to generate acetone–butanol–ethanol from konjac waste using *C. acetobutylicum* ATCC 824.

## 2. Materials and methods

### 2.1. Microorganisms

*C. acetobutylicum* ATCC 824 was maintained as a spore suspension in 6% (w/v) corn mash at 4 °C. Cells were grown anaerobically at 37 °C in the pre-culture medium for 20–36 h before being transferred into the solvent production medium containing sugars or konjac wastes as substrates. The solution was sterilized in an autoclave at 115 °C for 30 min and was then cooled to room temperature. A 10% (v/v) inoculum (5 mL of actively growing cells inoculated into 50 mL of fermentation medium) was used for these studies.

### 2.2. Materials preparation and growth conditions

All konjac wastes used in the investigations were obtained from Hubei Province, China. After collection, the konjac waste was dried and stored at room temperature.

Batch fermentation experiments were performed in a 100-mL serum bottle with a 50-mL actual fermentation volume. The hexose (including glucose and mannose) present in the konjac waste, the enzymatic hydrolysate of konjac waste or the konjac waste after the extraction of alkaloids were fermented as the carbon sources. To evaluate the fermentability of the sugars in the konjac waste, glucose or mannose was used to test the fermentation results (Table 1). The carbon source in corn starch is glucose. Unless otherwise noted, all fermentation studies were conducted using a medium containing the carbon source with the following supplementary chemicals: 6 g/L $(NH_4)_2SO_4$, 1.768 g/L $KH_2PO_4$, 2.938 g/L $K_2HPO_4$, 2 g/L $Ca(OH)_2$, 10 mg/L *p*-aminobenzoic acid, 10 mg/L biotin, and 1 mL/L mineral salts solution.

Enzymatic hydrolysis experiments were conducted using a solid substrate of 60 g/L (w/v) in distilled water and an enzyme loading of 1 mL/3 g solid substrate. The enzyme preparation containing 30 U/mL β-mannanases was provided by Baifu Ltd., China. The pH was adjusted to the optimum of 5.5 using 1 M $H_2SO_4$ or NaOH. The hydrolysis reaction of konjac waste using β-mannanase was conducted at 70 °C for 1 h, and then the hydrolysate and the added supplementary chemicals were sterilized. After cooling to room temperature, the medium was inoculated to begin the fermentation at 37 °C. For the SSF, the prepared medium was first sterilized and then cooled to room temperature; β-mannanase was added into the medium to being the fermentation (at 37 °C).

The initial pH of the medium was adjusted to 6.5 ± 0.2 before autoclaving. After inoculating, the fermentation media were sparged with filtered oxygen-free nitrogen atmosphere to maintain strict anaerobic conditions. The microorganism grew at 37 °C under strict anaerobic conditions for a certain period without agitation, and samples were then collected at intervals during the operational period for the related tests and analyses.

### 2.3. Alkaloid extraction method

In total, 150.0 g konjac waste was extracted with 100 mL 95% (v/v) ethanol at 50 °C for 1 h. The mixed liquid was then filtered, and the liquid was collected. The solid part was extracted using ethanol at the identical conditions three times. The obtained ethanol extract was concentrated using a rotary vacuum evaporator with an aspirator. The rotary vacuum evaporator was fitted with a cold water circulating bath and rotated at 60 rpm. The temperature of the water bath was maintained at 50 °C to evaporate the solvent. The concentrate was then dissolved by 10% $H_2SO_4$. The pH of the aqueous extract was adjusted to 10 using 40% diluted ammonia (v/v). The solution was then extracted using a chloroform wash three times; the flow through was collected. The extracted alkaloids in the chloroform were reacted with bromocresol green prior to analysis at 414 nm on a UV spectrophotometer (UV mini-1240, Shimadzu, Japan). The results were compared against a calibration curve established with alkaloid standards. The extraction rate (%) was then calculated: the extraction amount (g) divided by the material amounts extracted (g).