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Bioethanol production from taro waste using thermo-tolerant yeast *Kluyveromyces marxianus* K21

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HIGHLIGHTS

- Bioethanol production from taro waste medium using *Kluyveromyces marxianus* K21.
- Corn gluten meal can be used as an organic nitrogen source.
- Simultaneous saccharification and fermentation (SSF) was recommended.
- The scaling-up experiment in a 5 L bioreactor was performed.
- A 94.2% theoretical ethanol yield was obtained.

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ABSTRACT

In the present study, evaluation and optimization of taro waste (TW), which was mainly composed of taro peels that contain many starch residues, as the main carbon source in medium were studied. The flask studies showed the optimal medium was using 170 g/L of TW which is about 100 g/L of glucose and 9 g/L of CGM as alternative nitrogen source. Simultaneous saccharification and fermentation (SSF) exhibited higher bioethanol productivity toward separation hydrolysis and fermentation (SHF). The optimal condition of SSF was 5% of *Kluyveromyces marxianus* K21 inoculum at 40 °C resulting in the maximum ethanol concentration (48.98 g/L) and productivity (2.23 g/L/h) after 22 h of cultivation. The scaling up experiment in a 5 L bioreactor demonstrated that K21 can still maintain its capability. After 20 h of cultivation, 43.78 g/L of ethanol (2.19 g/L/h of productivity) was achieved corresponding to a 94.2% theoretical ethanol yield.

1. Introduction

Since the Industrial Revolution, the increased demand for fossil fuels took place as the world population grew and many countries are industrialized. Massive combustion of the fossil fuels would adversely increase the content of greenhouse gas resulting in greenhouse effect and global warming (Aditiya et al., 2015). In order to prevent the air pollution from burning fossil fuel and energy crisis, many countries begin to develop renewable energy source (Aditiya et al., 2015).

Renewable energy such as wind, water, solar, geothermal and biomass receive much attention not only for them to protect the environment, but also to meet the energy demand instead of fossil fuels (Cherubini et al., 2009). Bioenergy, such as bioethanol, biodiesel and biogas, has become a feasible and economical solution. Among them, bioethanol demonstrates higher burning efficiency and lower environmental impact (Balat et al., 2008). According to the Renewable Fuels Association (RFA), the U.S. and Brazil produced 13.3 and 6.2 billion gallons of ethanol fuels in 2014. It is estimated that feedstock accounts for about 20–55% of total production costs (Aditiya et al., 2015). Countries with agricultural-based economy are therefore suitable for bioethanol production (Aditiya et al., 2015; Sindhu et al., 2016). Among the bioenergy crops, sugarcane is the main feedstock used for bioethanol production in tropical countries such as Brazil and India

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(Raghavi et al., 2016). In United States, the bioethanol production is mainly made from starchy materials, such as corn and potato (Chintagunta et al., 2015; Richelle et al., 2015; Zhang et al., 2011). Other sources of biomass are also reported for bioethanol production such as algae, paper pulp waste and agricultural composite (Ho et al., 2013; Lee et al., 2015; Dwiarti et al., 2012; Zhao et al., 2015).

Taro (*Colocasia esculenta*), a member of the Araceae family, is a tropical perennial plant cultivated mainly in tropical and subtropical regions such as Asia, Oceania and Africa (Hsieh et al., 2015). Taro in Asia is used to produce a variety of desserts, snacks and confectionery products (i.e. taro cake, taro pastry, taro ball) (Hsieh et al., 2015). The production of these food products leaves huge chunks of taro peel available for further utilization (Hsieh et al., 2015). The high starch content of taro peel serves as a great feedstock for glucose production, providing raw materials for bioethanol production (Davis et al., 2006).

Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are two dominating process configurations that use enzymes for saccharification (Wingren et al., 2003). SHF allows the hydrolysis and fermentation processes to conduct separately. However, the enzyme and microorganism inhibition caused by the hydrolysate will reduce the ethanol productivity (Tomás-Pejó et al., 2008). Contrary, SSF can hydrolyze the biomass and ferment them to bioethanol simultaneously reducing the hydrolysate inhibition. SSF requires a compromise between the optimum condition of enzymatic hydrolysis and microorganism fermentation (Ishola et al., 2013; Dwiarti et al., 2012; Horita et al., 2015; Jang et al., 2012; Zhang et al., 2011; Zhao et al., 2015).

The present work aims to establish an effective process to produce bioethanol from taro waste (TW) using thermo-tolerant yeast *Kluyveromyces marxianus* K21. Fermentation parameters, such as alternative nitrogen sources, TW loading amount, inoculum sizes, temperature and fermentation strategies (SHF and SSF) were also evaluated.

2. Methods

2.1. Raw material supply

Fresh TW was kindly provided by O-Nongs Co. Ltd (Taichung, Taiwan). The species of taro was *C. esculenta* var. *esculenta*, which is a variant of *C. esculenta*. According to O-Nongs, fresh taro corm was first cleaned by washed with water to remove dirt, roasted and only 40% of the aroma-rich core was extracted by taro extractor. TW includes mostly of the outer core of taro corm with residual taro starch and cork. The TW was dried, blended and sieved by a stainless steel # 20 mesh. TW mash was stored at −20 °C until use. Bone meal (BM) and chicken meal (CM) were obtained from Foray Forage, Inc. (Yunlin, Taiwan). Fish meal (FM), soy meal (SM), corn gluten meal (CGM) and dried distillers grains with solubles (DDGS) which were obtained from Morsun Co. Ltd. (Kaohsiung, Taiwan).

2.2. Microorganism and medium

K. marxianus K21 (BCRC 21363) was purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan). For inoculum preparation, *K. marxianus* K21 was grown at 40 °C for 16 h in YM medium containing 3 g of yeast extract, 3 g of malt extract, 5 g of peptone and 10 g of glucose per liter of deionized water. For YMA, 20 g/L of agar was added in the medium.

2.3. Hydrolysis of starch

2.3.1. Liquefaction

According to the previous results, 8.05 g of TW powders (equal to 40 g/L of glucose) with 100 mL of deionized water and 0.9 mL of α-amylase (thermostable α-Amylase from *Bacillus licheniformis* with a declared activity of 500–1500 units/mg protein; Sigma–Aldrich, St. Louis, MO) were used (Hsieh et al., 2015). The mixture was agitated at 120 rpm in a shaker water bath for 5 h at 79.2 °C. The optimal condition of TW content, α-amylase content and reaction time was further adjusted based on the original condition.

2.3.2. Saccharification

Liquefied slurry was adjusted to pH 4.2–4.5 by 1% H_2SO_4. The mixture was subsequently added with 30 μL of amyloglucosidase (amyloglucosidase from *Aspergillus niger* with a declared activity of 300 units/mL aqueous solution; Sigma–Aldrich, St. Louis, MO) and agitated at 120 rpm in a shaker water bath for 3 h at 60 °C (Hsieh et al., 2015). The optimal condition for amyloglucosidase content and treatment time was further adjusted based on the original condition.

2.4. Fermentation medium

The basal fermentation medium contained 40 g of glucose, 5 g of yeast extract, 4 g of $(NH_4)_2SO_4$, 0.3 g of $MgSO_4{\cdot}7H_2O$, 0.3 g of $CaCl_2{\cdot}H_2O$ and 1 g of KH_2PO_4 per liter of deionized water. For medium studies, the hydrolyzed TW was used as the carbon source instead of glucose. The rest of the ingredients were kept the same as in baseline medium. For TW loading test, TW concentrations ranged from 85 to 107 g/L was added in order to evaluate the effect of glucose concentration on ethanol production. Moreover, six different alternative nitrogen sources were evaluated to investigate an economical substitute of yeast extract. BM, CM, FM, SM, CGM and DDGS as dry powder were used as alternative nitrogen sources to replace yeast extract and ammonium sulfate in the medium at the same concentration (9 g/L).

2.5. Ethanol fermentation

Two hundred and fifty mL flasks (working volume of 100 mL) were used for medium optimization. Seed culture was grown in YM medium for 16 h at 40 °C. After inoculation, 24–72 h of fermentation was carried out at 40 °C, 150 rpm and samples were taken every two or four hours. To evaluate the fermentation strategy, SHF used saccharification liquid as carbon source and SSF used liquefaction liquid as carbon source. The liquefaction liquid mixed with nitrogen source and mineral salt was directly sterilized at 121 °C for 15 min. The SHF medium was inoculated with *K. marxianus* K21 at initial stage. The SSF medium was inoculated with *K. marxianus* K21 and added with amyloglucosidase at the same time at initial stage.

For scaling up experiment, stirred tank bioreactor (Major Science, New Taipei City, Taiwan) with 6.0 L vessel (working volume of 5 L) equipped with temperature, aeration and agitation controls. The fermentation conditions were based on the optimal conditions obtained in the flask study. The optimal parameters obtained from SSF were applied. The aeration rate and agitation rate were set at 0.01 vvm and 300 rpm.

2.6. Residual glucose and ethanol production

Glucose and ethanol concentrations were measured using HPLC with a column Rezex R0A-Organic Acid H^+ (Phenomenex Inc., Torrance, CA) kept at 80 °C. The eluent for separation was 5 mM H_2SO_4 applied at an elution rate of 0.6 mL/min. The column was coupled