# Production of Bioethanol from Corn Straw by Co-Immobilization of Saccharomyces Cerevisiae and Aspergillus Niger in Na-Alginate

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# Production of Bioethanol from Corn Straw by Co-Immobilization of *Saccharomyces cerevisiae* and Aspergillus *niger* in Na-Alginate

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**Abstract.** Bioethanol from corn straw was produced using *Saccharomyces cerevisiae* and *Aspergillus niger* co-immobilized on Na-Alginate. Corn straw was mechanically and chemically pre-treated using NaOH before hydrolysis and fermentation. Cell of *S. cerevisiae* and *A. niger* were immobilized individually and simultaneously on Sodium Alginate gel beads. These immobilized cells were utilized to proceed the corn straw into bioethanol. The effect of using co-immobilized cells of *S. cerevisiae* and *A. niger* will be compared with the usual technique where the immobilized cells were applied separately and sequentially. The composition of *S. cerevisiae* and *A. niger* were also studied. Hydrolysis and fermentation were carried out at 30°C, pH 4 and 150 rpm in shaker incubator. Yield and quality of bioethanol were studied. Reusability of immobilized cells were also studied. The co-immobilisation of *S. cerevisiae* and *A. niger* will made proses simpler, inexpensive and less energy due to its less sequential process and the reusability of cell.

**Keywords:** *Aspergillus niger*, Co-immobilization, Corn straw, *Saccharomyces cerevisiae*

## 1 Introduction

Indonesia has high dependence on fossil energy, The primary energy matrix is still largely dominated by fossil fuels, oil (33.9%), coal (25.4%), and natural gas (15%), while renewable energy only covers 25.7%[1]. The continuous exploitation of fossil energy can cause pressure on the oil supply, $CO_2$ emissions to the atmosphere, and environmental pollution due to climate change[2]. Meanwhile, government policies No. 79 of 2014 concerning the National Energy Policy states that the target for renewable energy in 2025 and 2050 is at least 23% and 31%, respectively. one of the renewable energies is bioethanol. It is considered as the main source of renewable energy in the future, with economic and environmental benefits[3]. The first generation of bioethanol did not preferable because it requires a lot of raw materials that also used as foods. Therefore, the manufacture of bioethanol from edible biomass raw materials is replaced with non-edible biomass, namely lignocellulosic (bioethanol 2$^{nd}$ generation)[4]. Lignocellulosic biomass is the most abundant in nature and is one of the low-cost renewable resources with cellulose content (35–50%)[5]. The cellulose fraction contained in lignocellulose is hydrolyzed to produce glucose levels which play a role in the fermentation process to produce bioethanol[6]. Lignocellulose can be obtained from agricultural waste such as corn straw. Based on data by Badan Pusat Statistik (2018), corn production continues to increase and reaches 30,055.623 tons in 2018. So that the availability of these raw materials is abundant and has the potential to be used as raw material for bioethanol.

The ethanol fermentation process from lignocellulosic materials usually involves two main process[2]. The first stage is starch liquefaction by amylase and enzymatic saccharification and the next stage is glucose fermentation into ethanol. Adelabu (2019) converted lignocellulosic to bioethanol using cell immobilization techniques that have been widely used previously [7]. With the immobilization technique, the cells can be reused after the fermentation is complete by separating the cells from the product so that the production of bioethanol can run continuously [8]. it also can increase the yield and quality of bioethanol. However, two different processes were still involved. Therefore, The Co-immobilization technique is employed because it can be carried out in one stage where the microorganisms for hydrolysis and fermentation are mixed. This method can reduce energy input and increase substrate utilization efficiency[2]. Furthermore, the reusability of immobilized cell made this process more efficient. in this study, the co-immobilization technique was employed in the production of high yield and quality bioethanol from corn straw with less time, less energy, and efficient costs.

## 2 Material and Method

### 2.1 Material

The materials needed are corn straw, potato dextrose agar (PDA), *A niger*, *S cerevisiae*, yeast extract, $(NH_4)_2SO_4$, $KH_2PO_4$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MnSO_4.H_2O$, $FeSO_4.7H_2O$, NaCl 0.9 %, NaOH 1%, Na-Alginate, $CaCl_2$ 3% (w/v), $H_2SO_4$ 1 N, $H_2SO_4$ 72%, acetate buffer.

### 2.2 Culture on Solid Media

*Aspergillus niger* or *Saccharomyces cerevisiae* were cultured on PDA media aseptically and then incubated at 30°C for 72 hours. *Aspergillus niger* colonies are marked in black while *Saccharomyces cerevisiae* colonies are marked in white.

### 2.3 Culture on Liquid Media

Liquid media were composed of yeast extract 5 g/L, $(NH_4)_2SO_4$ 0.7 g/L, $KH_2PO_4$ 1 g/L, $CaCl_2.2H_2O$ 0.2 g/L, $MgSO_4.7H_2O$ 0.15 g/L, $ZnSO_4.7H_2O$ 2.5 g/L, $MnSO_4.H_2O$ 0.8 g/L, and $FeSO_4.7H_2O$ 0.7 g/L and corn straw. *S. cerevisiae* and *A. niger* were incubated at 120 rpm 30°C for 72 hours.

### 2.4 Yeast Harvest

The yeast was filtered using filter paper to remove corn straw. The yeast cells were then separated by centrifugation at 10000 rpm for 15 minutes. The results were then precipitated in sterilized 0.9% NaCl[9].

### 2.5 Pre-treatment

Corn straw was washed, cut, dried in an oven at 100°C for 24 hours, and crushed into 60 mesh. Corn straw (50 grams) is mixed with 800 mL 1% NaOH. The mixture was stirred and heated at 80°C for 2.5 hours. It was cooled and filtered; the solids were washed with hot water until neutral (pH 7). The solids obtained were oven at 100°C to constant weight.

### 2.6 Co-Immobilization

Na-Alginate 2% (w/v) and yeast 2% (w/v) with certain composition was mixed in 150 mL water. It was added into the 3% (w/v) $CaCl_2$ solution to form a bead gel with an average diameter of 3-4 mm. The bead gel granules formed were separated from the $CaCl_2$ solution using filter paper. Bead gel is stored at 4°C for 24 hours before use[10].

### 2.7 Analysis of Cellulose, Hemicellulose and Lignin Contents

A total of 1 gram of sample (weight a) was put into a 250 ml round bottom flask. Add 150 ml of $H_2O$ into the flask. The mixture was refluxed at 100°C with a water bath for 1 hour. The result was filtered with filter paper, the residue obtained was washed with 300 ml of hot water. The solid obtained was dried in an oven at a temperature of 60°C to a constant weight (weight b). Solid b was put back into the 250 ml round bottom flask then added 150 ml of $H_2SO_4$ 1 N. It was refluxed with a water bath for 1 hour at 100°C. The result was filtered and washed until the pH was neutral and the residue was dried to a constant weight in an oven at 60°C (weight c). Solid c was put back into the 250 ml round bottom flask then added 10 ml $H_2SO_4$ 72% and left at room temperature for 4 hours, then added 150 ml $H_2SO_4$ 1 N. Refluxed with a water bath for 1 hour at 100°C. The result was filtered and washed until the pH was neutral and the residue was dried to a constant weight in an oven at 60°C (weight d). The solid was ashed and weighed (weight e)[11].

### *2.8* Fermentation

Corn straw 10 (%, w/v) and co-immobilized bead gel (5% of corn straw) were mixed and incubated at pH 4 at 30°C, and shaker speed was 150 rpm. Fermentation was carried out for 96 hours[2].

### 2.9 Analysis

The concentration of ethanol from fermentation is determined by gas chromatography method. The reducing sugar was determined by the DNS method. Bead gel's Morphological analysis was carried out using SEM. [2, 12]. Testing of fuel consumption is done by determining the fuel consumption of the test vehicle with a chassis dynamometer using cycles. Meanwhile, in the exhaust gas emission test, it is measured with a gas analyzer whose results are known to be no more than 20 minutes after the test cycle[12].

## 3 Result and Discussion

He (2018) produce bioethanol from cellobiose substrate using co-immobilization of β-glucosidase and yeast cells. the maximum bioethanol yield was 98,6% and it can reuse until 7 times retaining bioethanol yield at 60%. Arun Beniwal (2018) produce ethanol using Cheese-way substrate and obtained a yield of 46,267% with co-immobilization of *K. Marxianus* and *S. Cerevisiae* at a ratio of 3:1 for 36 hours. After 5 cycles, the yield of bioethanol became 42,6%. According to Kirupa Sankar Muthuvelu (2018), *Ipomoea carnea* substrate was fermented using co-immobilization of Laccase, cellulase, and β -glucosidase at a ratio of 1:1:1. The yield of bioethanol was 63,43% for 72 hours. 52,15% was obtained after 6 cycles. Wen-Shiang Lee (2012) conducted a study using the sweet potato test in producing bioethanol by co-immobilizing *S. cerevisiae* with *A. Oryzae* or *M. Purpureus* at a ratio of 1:2 resulting in a maximum bioethanol yield of 40,8% for 9 hours. Besides the co-immobilization technique, there is an immobilization technique that is also used in the production of bioethanol. Blessing Adebola Adelabu (2019), who obtained 55,27 g/L bioethanol from corn straw for 96 hours by using cell immobilization of *W. Chambardii*, *Saccharomyces diastaticus*, *C. Shehatae*, and *C. Tropicalis*. The immobilized yeast can be used 5 times with a retaining yield of 12% bioethanol.

Based on the literature study, the co-immobilization of two microorganisms was studied before. *S. cerevisiae* and *A. niger* co-immobilized on Na-alginate was expected to produce high yield and quality of bioethanol. The co-immobilized cell was also projected to perform better than an immobilized cell. This immobilized and co-immobilized cell was predicted can be reused within five to seven cycles. The bioethanol produced was anticipated as a mixture of vehicle fuel along with gasoline.

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