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Table of Contents

< PREV

NEXT >

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SESSION: KEYNOTE SPEAKER

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Practical development of continuous supercritical fluid process using high pressure and high temperature micromixer

Shin-Ichiro Kawasaki, Kiwamu Sue, Ryuto Ookawara, Yuichiro Wakashima, and Akira Suzuki

AIP Conference Proceedings 1699, 020001 (2015); <https://doi.org/10.1063/1.4938285>

SESSION: FOOD AND BIOPROCESS ENGINEERING (FBE)

December 2015

Effect of extrusion temperature and moisture content of corn flour on crystallinity and hardness of rice analogues

Faleh Setia Budi, Purwiyatno Hariyadi, Slamet Budijanto, and Dahrul Syah

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Kinetic Study of Enzymatic Hydrolysis of Acid-Pretreated Coconut Coir

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Abstract. Biomass waste utilization for biofuel production such as bioethanol, has become more prominent currently. Coconut coir is one of lignocellulosic food wastes, which is abundant in Indonesia. Bioethanol production from such materials consists of more than one step. Pretreatment and enzymatic hydrolysis is crucial steps to produce sugar which can then be fermented into bioethanol. In this research, ground coconut coir was pretreated using dilute sulfuric acid at 121°C. This pretreatment had increased the cellulose content and decreased the lignin content of coconut coir. The pretreated coconut coir was hydrolyzed using a mix of two commercial cellulase enzymes at pH of 4.8 and temperature of 50°C. The enzymatic hydrolysis was conducted at several initial coconut coir slurry concentrations (0.1-2 g/100 mL) and reaction times (2-72 hours). The reducing sugar concentration profiles had been produced and can be used to obtain reaction rates. The highest reducing sugar concentration obtained was 1,152.567 mg/L, which was produced at initial slurry concentration of 2 g/100 mL and 72 hours reaction time. In this paper, the reducing sugar concentrations were empirically modeled as a function of reaction time using power equations. Michaelis-Menten kinetic model for enzymatic hydrolysis reaction is adopted. The kinetic parameters of that model for sulfuric acid-pretreated coconut coir enzymatic hydrolysis had been obtained which are V_m of 3.587×10^4 mg/L.h, and K_M of 130.6 mg/L.

Keywords: kinetic, enzyme, hydrolysis, coconut, lignocelluloses.

INTRODUCTION

Current world problem in energy sector is the strong increase in demand and more limited supply of petroleum fuel. This steady demand increase resulted from population and industrial growth. In Indonesia, the demand increase of petroleum fuel had achieved 1.9% each year from 2000 to 2012. Transportation sector had shown the biggest growth and industrial sector showed the biggest consumption [1]. The other problem that the world as well as Indonesia has been facing as the petroleum consumption increase is air pollution problem caused by gas emission resulted from petroleum fuel combustion.

The above problems have driven researches and policies focusing on renewable and more environmentally friendly biofuel such as bioethanol. Government of Indonesia through Ministry of Energy and Mineral Resource had set regulations to encourage renewable energy including biofuel utilization for transportation in 2013 and 2014. The Ministry had set target of bioethanol utilisation for electricity up to 20% by 2025 [1].

Lignocellulosic waste biomass is promising material to be used as source for bioethanol production. The process of production involves three steps which are biomass pretreatment, hydrolysis and fermentation. The pretreatment process is aimed at removing lignin content of biomass and hence increasing cellulose content and enzyme access to the cellulose. Without pretreatment step, hydrolysis reaction cannot be accomplished successfully [2, 3, 4, 5]. This step can be accomplished using many methods such pyrolysis, steam ammonia fiber or CO₂ explosion, ozonolysis,

acid pretreatment, alkaline pretreatment, hydrogen peroxide oxidative pretreatment and organosolv pretreatment using methanol, ethanol, acetone, ethylene glycol and several organic acid solvents. None of the treatment methods can be said the best among the others. Every method has advantages and disadvantages. However, the key criteria of pretreatment selection can be energy use and practical viability. The best method and the optimum condition of pretreatment depend on the lignocellulose materials being processed [6, 7, 8].

Hydrolysis is reaction step where long cellulose chain reacts with water to produce smaller glucose molecule. Fermentation process converts glucose, produced in the hydrolysis step, into bioethanol by utilizing microbial whole cell or enzyme as catalyst. The hydrolysis reaction proceeds by the action of catalyst which can be enzyme or acid. The enzyme responsible for cellulose hydrolysis catalysis is named as cellulase. It consists of three enzyme types which are exo-1,4-β-d-glucanases or cellobiohydrolases (CBH) (EC 3.2.1.91), endo-1,4-β-d-glucanases (EG) (EC 3.2.1.4), and 4-β-d-glucosidases (EC 3.2.1.21). The first enzyme breaks cellulose from the ends and move progressively along the cellulose chain. The second enzyme breaks the internal cellulose bond randomly. The last enzyme hydrolyzes cellobiose into glucose [6, 7; 8]. Hydrolysis kinetic study is also important as the kinetic model required in reactor design. The widely used kinetic model for enzymatic hydrolysis reaction is Michaelis-Menten model as follows:

$$r = \frac{V_m \cdot S}{K_M + S} \quad (1)$$

The reaction rate (r) can be expressed as follows:

$$r = \frac{dP}{dt} = -\frac{dS}{dt} \quad (2)$$

In this paper the Michaelis-Menten kinetic parameters (V_m and K_M) are determined for the hydrolysis of sulfuric acid-pretreated coconut coir.

This research focused on the kinetic study of acid-pretreated coconut coir enzymatic hydrolysis. Coconut coir is the fiber part of coconut husk which can be obtained easily after immersing in water. The fiber contains lignin, cellulose and hemicelluloses [9, 10]. Indonesia is rich in coconut (*Cocos nucifera*) tree. It is the biggest coconut producer in the world with 21.5 million tons production or 34.9% of the total world production in 2009 [11]. Such number shows very promising potential utilization of coconut fruit parts to produce value added products. Indeed the fruit parts have been used to produce several value added products. The water content of young coconut is used for healthy refreshing drink; the husk (mesocarp) is used as directly burned fuel, mulch, coir and peat. The hard shell (endocarp) can be used for handicraft, charcoal, and activated charcoal. However, big piles of wasted coconut husk are still frequently seen in big market. This led to odor problem resulted from anaerobic decay. Therefore, another type of utilization can help to overcome this problem. Since the husk contains lignocellulose, it can be used for bioethanol raw material.

MATERIALS AND METHOD

Materials

Coconut husks were collected from local market. Immediately after collection, the material was immersed in water for 24 hours. After immersion, the exocarp part was easily cleaved from the fibrous coir or mesocarp. The coir was then sun-dried. The dry coir was then cut into ± 5 cm long and milled using milling machine (FFC type 23A with rotation velocity of 5800 rpm and power of 3 kW). Fine particles of coconut coir with size of 200 mesh were collected by screening the milled coconut coir. In this research, two commercial cellulase enzymes were used: Celluclast® 1.5L (Sigma Aldrich) and Novozyme 188 (Sigma Aldrich).

Pretreatment Method

Coconut coir particles were pretreated using 1.5% sulfuric acid solution in autoclave at 121°C for 1 hour. The solid concentration at pretreatment step was 7.5% w/v. Afterwards the pretreated particles were washed with distilled water and filtered until the filtrate achieve neutral pH. Then the particles were oven-dried and ready for weighing.

Enzymatic Hydrolysis

The hydrolysis reactions were performed in a series of 250 mL Erlenmeyer flasks. The pH of reaction systems were adjusted 4.8 using 100 mL sterile sodium citrate buffer. The reaction temperature was controlled at 50°C by performing reaction in an incubator shaker equipped with temperature control. Agitation speed used was 150 rpm.

The concentrations of coconut coir slurry were varied at 0.1, 0.2, 0.4, 1.0, and 2.0 g/100 mL. The reaction time was varied at 2, 4, 8, 12, 16, 24, 36, and 72 hours. The volumes of enzyme added were 0.33 mL Celluclast® and 0.33 mL Novozyme 188. Each of reaction conditions was carried out in triplicates. In order to prevent microbial contamination during hydrolysis, 40mg/mL tetracycline antibiotic was added to the flask. At the end of reaction, the flasks were immersed in 95-100°C water to inactivate the enzymes prior to analysis.

Chemical Analyses

The concentrations of reducing sugar produced from hydrolysis reactions were analysed using 3,5-dinitrosalicylic acid (DNSA) reagent [12]. The cellulose and lignin contents of pretreated coconut coir were analysed using Chesson method [13].

Kinetic Parameters Determination

According to the experiment variables mention above, the result data contain five reducing sugar concentrations as the function of reaction time data. Each of the experimental data was then empirically fitted to obtain relationship between sugar concentration and reaction time. The empirical formula used was:

$$S = a.t^b \quad (3)$$

Afterwards, the initial reaction rate can be obtained using the equation (3). Parameters of Michaelis-Menten which are V_m and K_M were then obtained by nonlinear fitting of the initial reaction rates versus initial cellulose concentrations data.

RESULTS AND DISCUSSION

The cellulose and lignin content of raw coconut coir and sulfuric acid pretreated coconut coir had been determined. The raw coconut coir contained 25.36% cellulose and 38.62% lignin. Meanwhile, the pretreated coir contained 37.12% cellulose and 33.21% lignin. It shows that the sulfuric acid pretreatment at the experimental condition had increase the cellulose content of coconut coir.

The hydrolysis reaction of coconut coir using two commercial cellulose enzymes had yielded reducing sugar. The reducing sugar concentration profile as the function of reaction time is shown in fig 1. The profile shows that the reducing sugar increase with time and initial cellulose concentration. However, as can be noticed from the curve, the reaction rate decreased when the reactions are performed longer and then tended to achieve constant reducing sugar concentration. The reaction rate increased with initial cellulose concentration. The highest reducing sugar concentration achieved in this experiment was 1,152.567 mg/L. This was achieved at initial slurry concentration of 2 g/100 mL which corresponded to initial cellulose concentration of 7,424 mg/L.

The relation between hydrolysis reaction rates and cellulose concentration is depicted in fig 2. The experimental data is represented with blue star marker while the model as a result of fitting process is represented with red continuous line. Table 1 shows the values of kinetic parameters obtained from initial reaction rate and initial cellulose concentration data to Michaelis-Menten kinetic model fitting. The result is quite satisfying as we can see the blue star markers are not far scattered from the line. This is also indicated by the value of correlation coefficient (R^2) which is 0.9793.

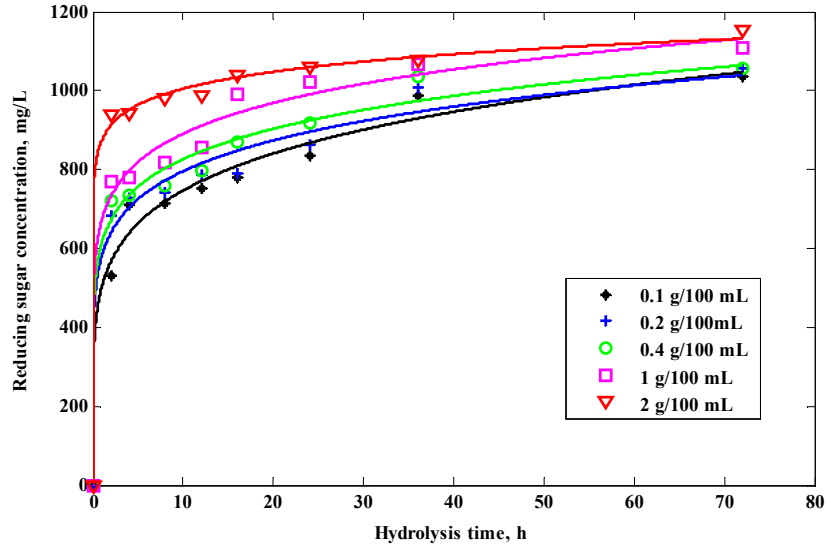


FIGURE 1. Profile of reducing sugar concentration resulted from coconut coir hydrolysis at several coconut coir initial slurry concentration

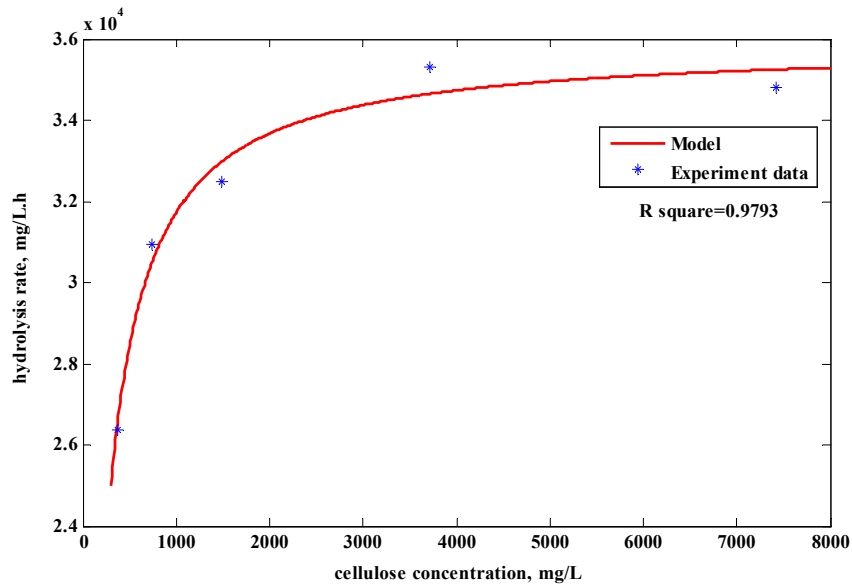


FIGURE 2. Hydrolysis rate profile as a function of cellulose concentration

TABLE 1. The kinetic parameters of Michaelis-Menten model obtained from reaction rate fitting

Kinetic Parameters	Values
V_m	3.587×10^4 mg/L.h
K_M	130.6 mg/L

CONCLUSION

Reducing sugar concentration profile had been produced from acid pretreated coconut coir hydrolysis experiment. The initial rates as empirical function of initial cellulose concentration had been obtained with satisfying correlation coefficient. The reaction rates had been used to determine the kinetic parameters of Michaelis-Menten enzymatic hydrolysis reaction kinetic model. The fitting also had resulted satisfying correlation coefficient.

NOTATION

- a = Empirical equation coefficient mentioned in equation 3
 b = Empirical equation coefficient mentioned in equation 3
 K_M = Substrate concentration at which reaction rate is half of maximum reaction rate (mg/L)
 P = Product concentration (mg/L)
 r = Reaction rate (mg/L.h)
 S = Substrate concentration (mg/L)
 t = Reaction time (h)
 V_m = Maximum reaction rate (mg/L.h)

ACKNOWLEDGEMENT

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Full paper submission due: September 15th, 2015

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ABOUT

Bringing **green technology towards sustainable chemical process** as the theme, ICCME 2015 triggers the development of innovation in chemical processes. This dream is aimed to create sustainable use of sources without depleting them, reduce, reuse/reclaim, and recycle of residual sources, to promote technology innovation, and to make viable economic activities around technology implementation and products. Implementation of this topic can be seen in general chemistry, energy, food, health, agriculture and materials.

The objectives of the ICCME 2015 are to be an international forum for the academia, researchers, students, industries and government in communicating their research and development results on fundamental and application of chemical and material engineering, to share extensively and exchange of ideas, thoughts and discussions on all aspects of chemical and material engineering, to facilitate the formation of networks among participants of the conference for improving the quality and benefits of the research.

Depending on the areas and scope, selected papers from this conference will be considered for publication in some Scopus-indexed journals. However the respective journals have their privilege to peer-review the papers according to their regular publication procedures. The affiliated journals are **Bulletin of Chemical Reaction Engineering and Catalysis** (ISSN 1978-2993, indexed by Scopus), and American Institute of Physics (AIP) Conference Proceedings (ISSN: 0094-243X/E-ISSN: 1551-7616, indexed by Scopus).



ABSTRACT

Abstract should be written in decent English and submitted online by April 30th 2015 according to abstract preparation guidelines. The acceptance of the abstract is based on scientific merit with regard to originality, depth, and relevance to conference's theme. The full-paper of the accepted papers should be submitted online no later than June 30th 2015.

SCOPES

We kindly accept papers in accordance with conference's theme **green technology for sustainability in chemical processes**, especially those in the aspects on chemical and material engineering, but not limited to:

- Heat and mass transfer,
- Chemical reaction engineering and catalysis,
- Thermodynamic,
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