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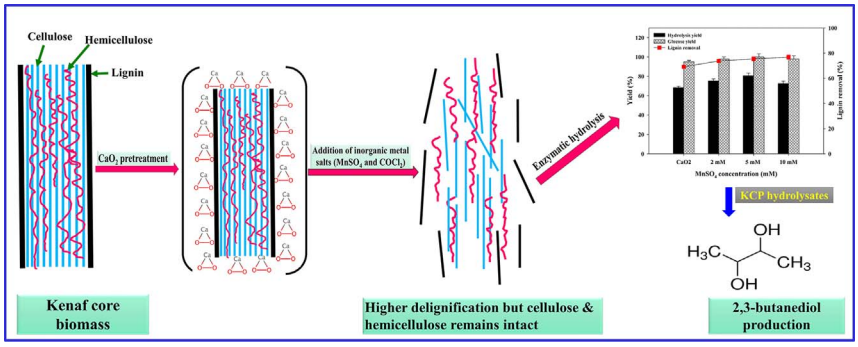
Combined effect of inorganic salts with calcium peroxide pretreatment for kenaf core biomass and their utilization for 2,3-butanediol production

Rijuta Ganesh Saratale[a], Han Seung Shin[b], Gajanan S. Ghodake[c], Gopalakrishnan Kumar[d], Min Kyu Oh[e], Ganesh Dattatraya Saratale[b,*]

[a] Research Institute of Biotechnology and Medical Converged Science, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggido 10326, Republic of Korea
[b] Department of Food Science and Biotechnology, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggido 10326, Republic of Korea
[c] Department of Biological and Environmental Science, Dongguk University, Ilsandong-gu, Goyang-si, Gyeonggido 10326, Republic of Korea
[d] School of Civil and Environmental Engineering, Yonsei University, Seoul 03722, Republic of Korea
[e] Department of Chemical and Biological Engineering, Korea University, Seongbuk-gu, Seoul 02841, Republic of Korea

GRAPHICAL ABSTRACT



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ABSTRACT

This study focuses on development of calcium peroxide (CaO_2) pretreatment that removes major part of lignin but retaining most of sugar components of kenaf core powder (KCP) biomass. In chemical pretreatment, usually higher loss of biomass occurs which was less during this pretreatment strategy. Supplementation of inorganic salts; manganese sulfate ($MnSO_4$) and cobalt chloride ($COCl_2$) in CaO_2 pretreatment resulted in maximum delignification of KCP relative to individual CaO_2 pretreatment. Maximum glucose yield (98%) and hydrolysis yield (80.5%) was achieved after enzymatic hydrolysis (30 FPU/g of KCP) under optimized conditions. Analytical results proved effective lignin removal and significant destruction of KCP with this pretreatment strategy. Finally, utilization of KCP enzymatic hydrolysates by developed strain *Klebsiella pneumoniae* KMK05 resulted in maximum 2,3-butanediol (BDO) production (10.42 g/L) and BDO titer (0.385 g/g of sugar). BDO titer achieved with KCP derived sugars were found comparable with the mixture of standard sugars which is notable.

1. Introduction

Worldwide bio-refinery systems deal to generate energy, fuel, and biologically made chemicals from renewable assets are of the main emphasis because of unusual crude oil reserves, steady increases in its value, and adverse effects on the environment (Saratale and Oh,

* Corresponding author at: Department of Food Science & Biotechnology, Dongguk University, 32 Dongguk-ro, Ilsandong-gu, Goyang-si, Gyeonggido 10326, Republic of Korea.
E-mail address: gdsaratale@dongguk.edu (G.D. Saratale).

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2015a). 2,3-Butanediol (BDO) considered as an important chemical which has been widely used as the solvent, food additive, printing inks, perfumes, and in the pharmaceutical and cosmetics industries (Ji et al., 2011; Sun et al., 2009). In addition to this, dehydration of BDO formed into methyl ethyl ketone can be used as a fuel additive to increase the octane rating of gasoline. However, the existing BDO production methods are costly, which confines its wide applications (Wu et al., 2008; Saratale et al., 2016). Microbial BDO production utilizing cheap, renewable lignocellulosic biomass as a carbon source offers an attractive route of economically feasible and sustainable approach (Um et al., 2017; Mazumdar et al., 2013). Moreover, development of new microbial strains using metabolic engineering approaches to increase the concentration and yield of BDO which is an important aspect. Kenaf (*Hibiscus cannabinus* L., *Malvaceae*) is the substantial rapidly growing crops up to 1.5–3.5 m tall, higher photosynthesis rate, and a short time to harvest (2–6 months) (Chen et al., 2013; Batouli et al., 2014). Kenaf produces considerably higher biomass about 15–20 tons dry weight biomass per hectare but still not exploited as a remarkable feedstock for BDO generation which is first time addressed in this study.

In plant biomass, sugar sources are mainly, cellulose and hemicellulose and are protected by the crystallinity of cellulose, lignin, and with a greater degree of polymerization making it more recalcitrant to hydrolysis (Mosier et al., 2005; Saratale and Oh, 2015a). Many investigators have widely studied the various physical, chemical, and combined physic-chemical pretreatment methods to enhance delignification of biomass and its recalcitrant nature by which it enriches enzyme accessibility and can achieve better reducing sugar production. Most of these methods are costly, less effective and comprise the higher removal of lignin and hemicellulose content and thus cellulose content of biomass remains for further enzymatic saccharification which results in low sugar yield (Sivagurunathan et al., 2017). Considering this, some investigators showed that partial removal of lignin from biomass considerably enhances the enzymatic saccharification of cellulose and hemicellulose component to fermentable sugars (Azelee et al., 2014). Thus a suitable method which can partially fractionate the biomass constituents need to be developed. It was observed that addition of inorganic salts with oxidizing agents results in the formation of hydroxyl radicals and superoxide ions that greatly affects the composition of biomass and can remove lignin effectively (Ramadoss and Karuppan, 2015; Monavari et al., 2011). Utilization of lignocellulosic biomass for BDO production makes the process more cost-effective and practically applicable. However, washout of biomass sugar components (mainly hemicellulose) is one of the major limitation of the process. In this study, we have investigated CaO_2 pretreatment in combination with inorganic salts for partial delignification of KCP biomass, to avoid higher washout of hemicellulose content, not only to enhance the hydrolysis yield but also to achieve better saccharification.

Developing the microbial strain using metabolic engineering strategy found to be useful for the efficient conversion of hexose and pentose sugars of renewable biomass into 2,3-butanediol. In this study, the effectiveness of CaO_2 and combination with inorganic salts pretreatment methods on the delignification and enzymatic digestibility of KCP was systematically investigated. The effects of pretreatment on structural features, lignin removal, crystallinity and chemical composition of KCP biomass were evaluated using various analytical techniques. Lastly, we have employed KCP enzymatic hydrolysates for its efficient conversion to 2,3-butanediol (BDO) using an engineered *K. pneumoniae* strain under batch fermentation.

2. Materials and methods

2.1. Materials

The kenaf core biomass was kindly provided by Professor Jeun Joon-Pyo of Korea Atomic Energy Research Institute, South Korea. The kenaf core was milled and the obtained powder was sieved through a 200 μm size mesh. The obtained kenaf core powder (KCP) was dried in a vacuum oven by keeping the temperature at 50 °C for 1 day. The dried sieved biomass was collected in an airtight container, at room temperature for further use. Calcium peroxide, hydrogen peroxide, Whatman filter paper No.1, manganese sulfate monohydrate ($MnSO_4{\cdot}H_2O$), zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), and cobalt chloride hexahydrate ($CoCl_2{\cdot}6H_2O$), were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the enzymatic hydrolysis experiments, cellulase from *Trichoderma reesei* ATCC 26921 (product number C 2730, Enzyme Commission (EC) Number 3.2.1.4, 700 U/g-cellulase), was obtained from Sigma–Aldrich (St. Louis, MO, USA). The other chemicals employed in this study were of the highest purity available and of analytical grade.

2.2. Chemical pretreatment

Initially, chemical pretreatment was carried out by combining KCP biomass individually with CaO_2 and strong oxidizing agent; H_2O_2 by keeping each concentration at 2% (w/v) in an electrically heated water bath at 100 °C and incubated for one hour of incubation. In each pretreatment, the ratio of the solid phase to the liquid phase was maintained at 1:10. After one hour of incubation, the treated biomass samples were washed with tap water till the pH of the solution remains neutral. The samples were then centrifuged well and dried at 60 °C until a constant weight was achieved and further explored to its chemical composition and enzyme hydrolysis analyses.

2.3. Calcium peroxide pretreatment optimization

The effects of calcium peroxide concentrations (1%, 2%, 3%, 4% and 5%) by keeping KCP concentration (10%) constant, the effect of KCP concentration (5%, 8%, 10%, 12% and 15%) by keeping CaO_2 concentration (4%) constant was studied. The KCP (10%) was pretreated using calcium peroxide (4%) in combination with various inorganic metal salts such as; $MnSO_4{\cdot}H_2O$, $ZnSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$, and $CoCl_2{\cdot}6H_2O$ by keeping the concentration of 2 mM were studied. The effects of operational parameters, for instance reaction time (30, 60, 90, 120, and 180 min), temperature (room temperature (RT), 60, 80, 100 °C and autoclave at 121 °C for 15 min) and using the different molar ratio of $MnSO_4$, and $COCl_2$ (1 mM, 2 mM, 5 mM, and 10 mM) with CaO_2 pretreatment were investigated. After the pretreatment, the pretreated KCP was filtered, washed with distilled water to neutral pH and dried at 60 °C until a constant weight was obtained. The pretreated KCP was stored in a vacuum plastic bag for the subsequent analysis and experiments. The delignification ratio (%) was determined according to the following equation:

$$\text{Delignification ratio (\%)} = D_{KCP} - D_{PT-KCP} / D_{KCP} \times 100$$

where, D_{KCP} is the quantity of lignin present in the raw native KCP, and $D_{PT\text{-}KCP}$ is the amount of lignin in the pretreated KCP, measured in (g/g).

2.4. Enzymatic hydrolysis of pretreated KCP biomass

KCP pretreated using individual H_2O_2, CaO_2 and CaO_2 with different metal salts was subjected to enzymatic hydrolysis. The enzymatic hydrolysis of untreated and pretreated KCP was achieved at 2.0% (w/v) in 20 mL of 50 mM citrate buffer (pH 5.0) comprising 0.005% (w/v) sodium azide. An enzyme solution equal to FPU activity of 30 U/g of untreated and pretreated KCP was put into Erlenmeyer flasks and incubated at 50 °C and 150 rpm for 24 h. Samples were taken from the reaction mixture at different time intervals and instantly heated at 100 °C for 15 min to denature the enzymes and then cooled followed by centrifugation for 10 min (at 4 °C; 10,000 rpm). The resulted supernatant was used for the reducing sugar analysis. Experiments were