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Title **Saccharification Kinetics at Optimised Conditions of Tapioca by Glucoamylase Immobilised On Mesostructured Cellular Foam Silica**

Author (s) J. Agustian[1a*], L. Hermida[1b]

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Is the abstract adequate?	**Yes**	No
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Are there significant technical errors?	Yes	**No**
Does this paper adequate reference relevant work?	**Yes**	No
Does English need significant improvement?	**Yes**	No

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(Ask you give as possible full evaluation of article, with the indication on concrete technical and language mistake)

	Reviewer's comment	Author's comment *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	**Abstract** This needs some editing for English, for example: • As ~~the~~ insoluble substrates such as tapioca can be used to make chemical compounds, saccharification of ~~the~~ tapioca by glucoamylase ~~that was~~ immobilised on mesostructured cellular foam (MCF) silica using Box-Behnken Design of experiment was conducted to optimize this process so that the experimental results can be used to develop large-scale operations. • The kinetic constants (Vmax, KM) produced by **the** immobilised enzyme differed highly ~~with~~ **from** the values yielded by free glucoamylase indicating reduction of substrate access to enzyme active sites ~~indeed~~ **had** occurred. **Introduction** Similar minor editing for English required. **Methods** Line 99: centrifugation force should be expressed in 'g' as rpm produces different force dependent on the radius of the centrifuge.	**The abstract has been improved based-on:** 1. The reviewer suggestions (highlighted in yellow). 2. Other parts considered important by authors (highlighted in green). **The Introduction:** Some improvements have been made on this part as given in highlighted words. **The Methods:** We consider it is the line no. 91. The unit has been changed from "3000 rpm" to "1000 g"

	Results and Discussion Minor editing for English required. It is unclear what Figure 6 is demonstrating and from where the values of maximum reaction rate are derived.	**The Results and Discussion**: 1. This part has been modified as highlighted in the texts. 2. Explanations on how to determine the kinetic constants from the applied equation have been added (highlighted texts).
Minor REVISION comments	-	-

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Saccharification Kinetics at Optimised Conditions of Tapioca by Glucoamylase Immobilised on Mesostructured Cellular Foam Silica

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Abstract

As insoluble substrates such as tapioca can be used to make chemical compounds, saccharification of tapioca by glucoamylase immobilised on mesostructured cellular foam (MCF) silica using Box-Behnken Design of experiment was conducted to optimize this process so that the experimental results can be used to develop large-scale operations. The experiments gave dextrose equivalent (DE) values of 6.15–69.50% (w/w). Factors of pH and temperature affected the process highly. The suggested quadratic polynomial model is significant and considered acceptable (R^2 = 99.78%). Justification of the model confirms its validity and adequacy where the predicted DE shows a good agreement with the experimental results. The kinetic constants (V_{max}, K_M) produced by the immobilised enzyme differed highly from the values yielded by free glucoamylase indicating reduction of substrate access to enzyme active sites had occurred.

1. Introduction

Mesoporous silicas, one of the important materials for enzymes' supports, have been used to immobilise free glucoamylase by applying various immobilisation methodologies. The materials prepared from tetraethoxysilane and phenyltriethoxysilane had successfully encapsulated this enzyme where the product was used for many cycles in soluble starch hydrolysis [1]. Functionalised ordered mesoporous silicas were also developed to support glucoamylase covalently to give the immobilised enzyme capable to hydrolyse soluble starch efficiently [2, 3]. Previously, Szymanska et al. [4] immobilised glucoamylase on functionalised mesostructured cellular foam (MCF) silica where the immobilised enzyme was used to saccharify the soluble starches.

Saccharification of insoluble starches based-on the silica supports is not found yet. As ethanol and other chemicals can be made from the insoluble substrates via fermentation, saccharification of tapioca starch using glucoamylase immobilised on the MCF silica is required as knowledge on the process are needed to develop large-scale operations. Variation of the operational factors simultaneously through a factorial experimental design is considered as the correct way to deal with the factors [5]. Since optimisation of process is considered to be a step to define the optimum conditions by evaluating the interactions of the operational factors [6], to optimize the tapioca saccharification using the glucoamylase immobilised on the MCF silica, a response surface methodology based-on Box-Behnken design is herewith reported. The optimized results were used to measure the kinetics of the reaction. Reusability of the immobilised glucoamylase was also evaluated under the optimized conditions.

2. Experimental

2.1. Chemicals and Biochemical

Pluronic® P123 (435465), mesithylene (>98%), tetraethyl orthosilicate (>99%) and sodium acetate (>98%) were purchased from Sigma Aldrich. Ammonium fluoride (>98%), HCl (37%), di-sodium hydrogen phosphate dodecahydrate (>99%), D(+)-glucose monohydrate (>99%) and potassium dihydrogen phosphate (>99%) were supplied by Merck Indonesia. Glucoamylase LYPH170122 (30.000 U g^{-1}) were imported from Xi'an Lyphar Biotech Co. Ltd. (China). Tapioca was bought from a supermarket.