Novia Sumardi <novia@ft.unsri.ac.id>

[Fermentation] Manuscript ID: fermentation-1848684 - Submission Received

3 pesan

Editorial Office <fermentation@mdpi.com> 19 Juli 2022 pukul 21.47
Balas Ke: fermentation@mdpi.com
Kepada: Novia Novia <novia@ft.unsri.ac.id>
Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>

Dear Dr. Novia,

Thank you very much for uploading the following manuscript to the MDPI submission system. One of our editors will be in touch with you soon.

Journal name: Fermentation
Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
E-mails: winta_efrinalia@unsri.ac.id, novia@ft.unsri.ac.id, eldamelwita@ft.unsri.ac.id
Submitted to section: Industrial Fermentation, https://www.mdpi.com/journal/fermentation/sections/Industrial_fermentation
Fermentation Processes to Obtain Value-Added Products from Agro-Industrial Residues
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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>

Thank you for your response.
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Novia Sumardi <novia@ft.unsri.ac.id> 19 Juli 2022 pukul 21.52
Kepada: "Ms. Mayora Li" <mayora.li@mdpi.com>

FYI

[Kutipan teks disembunyikan]

Novia Sumardi <novia@ft.unsri.ac.id>

[Fermentation] Manuscript ID: fermentation-1848684 - Major Revisions

2 pesan

Fermentation Editorial Office <fermentation@mdpi.com> 29 Juli 2022 pukul 09.09

Balas Ke: aileen.zhang@mdpi.com
Kepada: Novia Novia <novia@ft.unsri.ac.id>
Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Dear Dr. Novia,

Thank you again for your manuscript submission:

Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
E-mails: winta_efrinalia@unsri.ac.id, novia@ft.unsri.ac.id, eldamelwita@ft.unsri.ac.id
Submitted to section: Industrial Fermentation, https://www.mdpi.com/journal/fermentation/sections/Industrial_fermentation
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Novia Sumardi <novia@ft.unsri.ac.id> 31 Juli 2022 pukul 10.08
Kepada: aileen.zhang@mdpi.com
Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

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[Kutipan teks disembunyikan]

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[Fermentation] Manuscript ID: fermentation-1848684 - Manuscript Resubmitted

2 pesan

Fermentation Editorial Office <fermentation@mdpi.com> 6 Agustus 2022 pukul 18.57

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Kepada: Novia Novia <novia@ft.unsri.ac.id>
Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>

Dear Dr. Novia,

Thank you very much for resubmitting the modified version of the following manuscript:

Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
E-mails: winta_efrinalia@unsri.ac.id, novia@ft.unsri.ac.id, eldamelwita@ft.unsri.ac.id
Submitted to section: Industrial Fermentation, https://www.mdpi.com/journal/fermentation/sections/Industrial_fermentation
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A member of the editorial office will be in touch with you soon regarding progress of the manuscript.

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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>

Thank you for your response.
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[Fermentation] Manuscript ID: fermentation-1848684 - Revised Version Received

2 pesan

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Kepada: Novia Novia <novia@ft.unsri.ac.id>
Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Dear Dr. Novia,

Thank you very much for providing the revised version of your paper:

Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
E-mails: winta_efrinalia@unsri.ac.id, novia@ft.unsri.ac.id, eldamelwita@ft.unsri.ac.id
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We will continue processing your paper and will keep you informed about the status of your submission.

Kind regards,
Ms. Iris Zhang
E-Mail: iris.zhang@mdpi.com

--
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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Thank you for your response.
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[Fermentation] Manuscript ID: fermentation-1848684 - Minor Revisions

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Fermentation Editorial Office <fermentation@mdpi.com> 11 Agustus 2022 pukul 08.11

Balas Ke: aileen.zhang@mdpi.com
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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Dear Dr. Novia,

Thank you again for your manuscript submission:

Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
E-mails: winta_efrinalia@unsri.ac.id, novia@ft.unsri.ac.id, eldamelwita@ft.unsri.ac.id
Submitted to section: Industrial Fermentation, https://www.mdpi.com/journal/fermentation/sections/Industrial_fermentation
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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Well received with thanks.
[Kutipan teks disembunyikan]

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[Fermentation] Manuscript ID: fermentation-1848684 - Revised Version Received

2 pesan

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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Dear Dr. Novia,

Thank you very much for providing the revised version of your paper:

Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
E-mails: winta_efrinalia@unsri.ac.id, novia@ft.unsri.ac.id, eldamelwita@ft.unsri.ac.id
Submitted to section: Industrial Fermentation, https://www.mdpi.com/journal/fermentation/sections/Industrial_fermentation
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Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>

Thank you for the update.
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Novia Sumardi <novia@ft.unsri.ac.id>

[Fermentation] Manuscript ID: fermentation-1848684 - Accepted for Publication

1 pesan

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Kepada: Novia Novia <novia@ft.unsri.ac.id>
Cc: Winta Efrinalia <winta_efrinalia@unsri.ac.id>, Elda Melwita <eldamelwita@ft.unsri.ac.id>, Fermentation Editorial Office <fermentation@mdpi.com>, Aileen Zhang <aileen.zhang@mdpi.com>

Dear Dr. Novia,

Congratulations on the acceptance of your manuscript, and thank you for submitting your work to Fermentation:

Manuscript ID: fermentation-1848684
Type of manuscript: Article
Title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks
Authors: Winta Efrinalia, Novia Novia *, Elda Melwita
Received: 19 July 2022
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Submitted to section: Industrial Fermentation, https://www.mdpi.com/journal/fermentation/sections/Industrial_fermentation
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Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-Treated Rice Husks

Fermentation **2022**, *8*(9), 417; **https://doi.org/10.3390/fermentation8090417 (https://doi.org/10.3390/fermentation8090417)**

by **Winta Efrinalia (/search?authors=Winta%20Efrinalia&orcid=)** [1],
Novia Novia (/search?authors=Novia%20Novia&orcid=0000-0002-0046-6076) [1,2,*] **(mailto:novia@ft.unsri.ac.id)**
(https://orcid.org/0000-0002-0046-6076) and
Elda Melwita (/search?authors=Elda%20Melwita&orcid=) [1]

Reviewer 1: Anonymous

Reviewer 2: Mattia Gelosia

Reviewer 3: Anonymous

Fermentation **2022**, *8*(9), 417; **https://doi.org/10.3390/fermentation8090417 (https://doi.org/10.3390/fermentation8090417)**

Received: 19 July 2022 / Revised: 17 August 2022 / Accepted: 19 August 2022 / Published: 23 August 2022
(This article belongs to the Special Issue **Fermentation Processes to Obtain Value-Added Products from Agro-Industrial Residues (/journal/fermentation/special_issues/ferment_residues)**)

Round 1

Reviewer 1 Report

Manuscript title: Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks

Manuscript number: fermentation-1848684

General comments: The manuscript addresses the kinetics of enzymatic hydrolysis of cellulose from pretreated rice husk using a semi-mechanistic model. The release of reducing sugars was evaluated at various enzyme concentrations and hydrolysis times. The manuscript is well-written, and the results are presented and discussed clearly. Also, the conclusions are supported by the results. Although the manuscript

lacks novelty regarding the advances in the enzymatic conversion of biomasses into reducing sugars, it shows relevant aspects regarding mathematical modeling of the hydrolytic process. Some aspects are detailed below to help authors to improve the manuscript for possible publication:

Abstract:

Line 14 "at various enzyme concentrations of 10, 15, and 20% (v /w) […]" and line 19 "The highest reducing sugar content was obtained (2.21 g/L) at an enzyme concentration of 30% (v/w) […]": I suggest the authors correct the interval of enzyme concentration (i.e., 10 to 50%) in line with the best result of the study.

Introduction:

The Introduction section is well-written and theoretically well-founded.

Line 53: Please correct “and –glucosidase β” to “β-glucosidase”.

Material and methods:

Line 99: The sentence seems to be missing information regarding how the enzyme extract was obtained. Please check carefully.

Line 120: Please insert references to methods used to quantify cellulase activity. Also, specify whether enzyme quantification analyses refer to total cellulases or accessory enzymes (e.g., endo-β-glucanases) as shown in Table 3.

Line 125: Please change "20 g of rice husk" to "20 g of pretreated rice husk".

Line 126, 128: Why was a medium solution added to enzymatic hydrolysis? The reasons for inserting a growth medium into the enzymatic hydrolysis reaction medium are unclear. In addition, the authors need to clarify which type of enzyme fraction was added (i.e., filtered crude extract or a crude extract containing cells).

Line 128: According to Figure 6, the effect of enzyme loading was also evaluated above 20%. Please clarify which enzyme concentration ranges were used in the study.

Results:

Line 194: Table 3 shows the total cellulase and endo-β-glucanase activities in the crude extract of *Aspergillus niger*. Why did the authors not quantify the activity of β-glucosidase and other accessory enzymes in the crude extract?

Line 192/198: Please standardize the enzyme unit throughout the manuscript (U/mL or $U.mL^{-1}$).

Line 198 to 200: The results should be compared with other studies addressing cellulase production by *Aspergillus* species, not commercial extracts or crude extracts from other fungi. Furthermore, the cited studies must use the same quantification methods as this manuscript for comparison in terms of enzymatic activity.

Line 213: How was cellulose quantified in enzymatic hydrolysis assays? Please specify it in the "Materials and Methods" section.

Lines 214 and 215: "There is no cell division in the depletion phase of microorganisms, in which the number of cells remains constant and growth slows down." I could not complain about how this explanation fits with enzymatic hydrolysis since there are no microorganisms in the hydrolysis medium but only the cell-free crude cellulase extract. The authors need to revise this sentence or clarify what composes the hydrolysis reaction medium.

Line 274: Why was the hydrolysis time set at 5-h and not 10-h? As shown in Figure 2, the reducing sugar concentration increases between 5-h and 10-h of hydrolysis and then tends to stabilize after 15-h of reaction.

Line 291: "For enzyme concentrations of more than 30%, the lowest reducing sugar concentration obtained was 0.89 g/L." What would be the explanation for this behavior? I suggest the authors insert a brief explanation about the low release of reducing sugars at high enzyme loadings.

Tables and Figures:

Table 2/Figure 1: EDS analysis is not mentioned in the "Material and Methods" section. Please briefly explain how EDS analysis was performed in the methodology.

Author Response

Thank you for giving us the opportunity to submit a manuscript titled “Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks” for publication in the Journal of Fermentation. We appreciate the time and effort that you dedicated to providing feedback on our manuscript and are grateful for the insightful comments and valuable improvements to our paper. We have incorporated the suggestions made by the reviewers. Those changes are written in red highlight text within the manuscript.

Abstract:

Point 1:

Line 14 "at various enzyme concentrations of 10, 15, and 20% (v /w) […]" and line 19 "The highest reducing sugar content was obtained (2.21 g/L) at an enzyme concentration of 30% (v/w) […]": I suggest the authors correct the interval of enzyme concentration (i.e., 10 to 50%) in line with the best result of the study.

Response 1:

Thank you for pointing this out. In accordance with kinetic data, we corrected to “the highest reducing sugar content was obtained (1.80 g/L) at an enzyme concentration of 25% (v/w)” (in red-line 19-20).

Introduction:

Point 2:

The Introduction section is well-written and theoretically well-founded.

Line 53: Please correct “and –glucosidase β” to “β-glucosidase”.

Response 2:

Thank you for bringing it to our attention. It was already revised (in red-line 54).

Material and methods:

Point 3:

Line 99: The sentence seems to be missing information regarding how the enzyme extract was obtained. Please check carefully.

Response 3: We thank the reviewer for pointing this out. We have incorporated the revision in the manuscript. *Aspergillus niger* was obtained from the Microbial March Gallery, Indonesia. The culture was routinely maintained for 4-6 days at 30 ^{o}C on a Potato Dextrose agar plate. The harvested spores were suspended in distilled water to achieve a final concentration of 1 x 10^{6} spores mL^{-1} (in red-line 100-104).

We completed the enzyme extract procedure (in red-line 126-128).

The fermented product was extracted with 100 mL of deionized water, shaken at 150 RPM for 1 h. The sample was then centrifuged at 4000 RPM for 30 min at 4 ^{o}C. The crude enzyme solution was used for hydrolysis and evaluated for Endoglucanase and Exoglucanase activity.

Point 4:

Line 120: Please insert references to methods used to quantify cellulase activity. Also, specify whether enzyme quantification analyses refer to total cellulases or accessory enzymes (e.g., endo-β-glucanases) as shown in Table 3.

Response 4: Thank you for bringing it to our attention. We added the procedure of the enzyme quantification and reference used (in red-line 129-152). We also revised table 3.

2.2.1. Evaluation of Endoglucanase activity (Hu et al., 2018)

Approximately 0.1 mL of cellulase enzyme filtrate and 0.1 mL of 1% CMC solution were added to a citrate buffer (pH 4.8) and incubated at 50 ^{o}C for 30 min. The addition of 3 mL of DNS reagents was followed by 10 min of heating in boiling water (T = 100 ^{o}C) to stop the reaction. After cooling to room temperature, absorbance was measured at a wavelength of 540 nm. On a glucose standard curve, absorbance results were plotted. While citrate buffer and DNS reaction were used to create blanks and determine the zero-absorbance point. Based on the glucose released by the cellulase enzyme, 1 unit of CMCase enzyme activity corresponds to 1 mole of glucose produced per minute. If the incubation lasted 30 min, the production of 1 mg of glucose per mL was: 1/ (30x0.180) = 0.185 units. Where 0.180 is glucose (µmole). One unit of CMCase (IU mL^{-1}) equals mg glucose multiplied by 0.185 per mL. The test was carried out three times with each condition.

2.2.2. Evaluation of Exoglucanase activity (Hu et al., 2018)

After placing approximately 0.1 mL of cellulase enzyme filtrate into a test tube that contained (1 cm x 6 cm) of Whatman No. 1 filter paper (50 mg) and 1 mL of citrate buffer with a pH of 4.8, the mixture was allowed to incubate at a temperature of 50 oC for 30 min. Approximately 3 mL of DNS reagents are added, and the mixture is then heated for 10 min in boiling water to end the reaction. Furthermore, the absorbance was measured at a wavelength of 540 nm after it had been cooled. The amount of glucose that was produced per minute was used as the basis for the calculation of the FPase enzyme activity. One unit of FPase activity was equal to 1 µmole of glucose produced per minute. If the incubation was for 30 min, 1 mg of glucose produced every mL was: 1/(30x0.180) = 0.185 units. Where 0.180 = glucose µmole. It means, one unit of Fpase (IU mL^{-1}) = mg glucose x 0.185 / mL. The test was carried out three times with each condition.

Reference:

Point 5:

Line 125: Please change "20 g of rice husk" to "20 g of pretreated rice husk".

Response 5:

Thank you for pointing this out. It was already changed (in red-line 155).

Point 6:

Line 126, 128: Why was a medium solution added to enzymatic hydrolysis? The reasons for inserting a growth medium into the enzymatic hydrolysis reaction medium are unclear. In addition, the authors need to clarify which type of enzyme fraction was added (i.e., filtered crude extract or a crude extract containing cells).

Response 6:

We thank the reviewer for pointing this out. We realized that we were wrong in writing the procedure, there should be no addition of a growth medium into the process of enzymatic hydrolysis. We deleted it and made the revision in the manuscript. It was already revised (in red-line 156). The type of enzyme fraction was added (in red-line 129-152).

Point 7:

Line 128: According to Figure 6, the effect of enzyme loading was also evaluated above 20%. Please clarify which enzyme concentration ranges were used in the study.

Response 7:

Thank you for pointing this out. For the kinetic calculation, we had data in ranges 10-20% enzyme concentration. Figure 6 is for 5 h hydrolysis time with range 10-50% enzyme concentration. This figure displayed the effect of enzyme loadings on the release of reducing sugars. The optimal enzyme loading for cellulase-mediated enzymatic hydrolysis of rice husk fibres is 30 %, which maximizes soluble sugar contents at all cellulose loadings (in line: 319-321).

Results:

Point 8:

Line 194: Table 3 shows the total cellulase and endo-β-glucanase activities in the crude extract of Aspergillus niger. Why did the authors not quantify the activity of β-glucosidase and other accessory enzymes in the crude extract?

Response 8:

Thank you for pointing this out. The crude extract of *Aspergillus niger* was evaluated for Endoglucanase and Exoglucanase activity (in red-line 129-152). We apologize not quantify the activity of β-glucosidase. However, the enzyme activities were compared to other study in table 3.

Point 9:

Line 192/198: Please standardize the enzyme unit throughout the manuscript (U/mL or U.mL-1).

Response 9:

Thank you for pointing this out. It was already revised (in red-line 16; 228-230; 339).

Point 10:

Line 198 to 200: The results should be compared with other studies addressing cellulase production by Aspergillus species, not commercial extracts or crude extracts from other fungi. Furthermore, the cited studies must use the same quantification methods as this manuscript for comparison in terms of enzymatic activity.

Response 10:

Thank you for pointing this out. We compared this study with other studies using cellulase from *Aspergillus* species (in red-line 228-231) and revised Table 3.

Point 11:

Line 213: How was cellulose quantified in enzymatic hydrolysis assays? Please specify it in the "Materials and Methods" section.

Response 11:

Thank you for pointing this out. We mentioned in manuscript that the composition of the rice hull (cellulose, hemicellulose lignin) before and after treatment was analyzed using the Chesson method (Datta, 1981) (in red-line 112-113).

Reference:

Datta, R. (1981). Acidogenic fermentation of lignocellulose-acid yield and conversion of components. Biotechnology and Bioengineering, 23(9), 2167–2170. https://doi.org/10.1002/bit.260230921

Point 12:

Lines 214 and 215: "There is no cell division in the depletion phase of microorganisms, in which the number of cells remains constant and growth slows down." I could not complain about how this explanation fits with enzymatic hydrolysis since there are no microorganisms in the hydrolysis medium but only the cell-free crude cellulase extract. The authors need to revise this sentence or clarify what composes the hydrolysis reaction medium.

Response 12:

We thank the reviewer for pointing this out. We deleted the sentence as it is not related to this study (in red-line 245-247).

Point 13:

Line 274: Why was the hydrolysis time set at 5-h and not 10-h? As shown in Figure 2, the reducing sugar concentration increases between 5-h and 10-h of hydrolysis and then tends to stabilize after 15-h of reaction.

Response 13:

Thank you for pointing this out. The hydrolysis time at 5-h is sufficient to describe the effect of enzyme loading on the reducing sugar concentration.

Point 14:

Line 281: "For enzyme concentrations of more than 30%, the lowest reducing sugar concentration obtained was 0.89 g/L." What would be the explanation for this behavior? I suggest the authors insert a brief explanation about the low release of reducing sugars at high enzyme loadings.

Response 14:

Thank you for pointing this out. As the enzyme loading was increased to between 35 and 50%, the reducing sugar concentration decreased dramatically. This biphasic response of sugar content to varying enzyme loading is due to the fact that hydrolysis is an enzyme-limited process at a low enzyme to substrate loading (i.e., enzyme loading 25%), while excess enzymes at enzyme loading > 30% accelerate the hydrolysis rate and produce products (glucose and other reducing sugars) that form inhibitory complexes by binding to the free dissolved enzymes in the liquid phase. Depending on whether the glucose and cellobiose have bound to the active and/or non-active sites of the cellulase, these complexes inhibit the cellulase's catalytic activity through competitive/uncompetitive/non-competitive inhibition and reduce the concentration of measurable free glucose and reducing sugars in the liquid phase (Paul & Chakraborty, 2019). The optimal enzyme loading for cellulase-mediated enzymatic hydrolysis of rice husk fibres is 30 %, which maximizes soluble sugar contents at all cellulose loadings by ensuring that the system is neither enzyme-limited nor significantly product inhibited. (in red-line 309-321).

Reference:

Paul, S. K., & Chakraborty, S. (2019). Mixing effects on the kinetics of enzymatic hydrolysis of lignocellulosic Sunn hemp fibres for bioethanol production. Chemical Engineering Journal, 377(October 2018), 120103. https://doi.org/10.1016/j.cej.2018.10.040

Tables and Figures:

Point 15:

Table 2/Figure 1: EDS analysis is not mentioned in the "Material and Methods" section. Please briefly explain how EDS analysis was performed in the methodology.

Response 15:

Thank you for pointing this out. It was already revised (in red-line 114-115).

Author Response File: **Author Response.docx (https://susy.mdpi.com/user/review/displayFile/28686875/Ep61Tn3c?file=author-coverletter&report=21145216)**

Reviewer 2 Report

Please see attached file

Comments for author File: **Comments.pdf (https://susy.mdpi.com/user/review/displayFile/28701936/nMXhHY2Z?file=review&coverletter&report=21145579)**

Author Response

Thank you for giving us the opportunity to submit a manuscript titled “Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks” for publication in the Journal of Fermentation. We appreciate the time and effort that you dedicated to providing feedback on our manuscript and are grateful for the insightful comments and valuable improvements to our paper. We have incorporated the suggestions made by the reviewers. Those changes are written in red highlight text within the manuscript.

The paper debates a well-discussed topic about enzymatic hydrolysis kinetics of cellulosic biomass, using the Michaelis-Menten model to investigate it. The novelty of the paper is the use of the model to describe the hydrolysis of pre-treated rice husks with H_2O_2-NH_4OH and the production of cellulolytic enzymes from *Aspergillus niger*. Anyway, the paper showed exactly omission in those aspects.

It’s not clear which model was used by the authors, since they don’t precisely declare it, and how they derived it from the common Michaelis-Menten. Equations 11, 12, and 13 need to be explained more accurately and correctly (e.g. equation 11, the ln[A0]/[A] is not equal to [A0] - [A]). The experimental results are very little discussed, they are only shown, and there is no comparison with other research works on rice husks hydrolysis and enzymatic hydrolysis kinetics’ models. For these reasons, it is not easy for a reader to understand and verify the statements made by the authors in their conclusion.

All that said, I truly recommend a major revision of the article before its publication.

The following are my suggestions and comments to improve the manuscript.

English needs to be revised, especially in materials and result sections.

Give more importance to the enzymes production, making an experimental comparison with commercial enzymes cocktail (e.g. Novozymes), and/or discuss the result obtained by other authors on rice husks hydrolysis using different enzymes cocktail. It is advisable (not mandatory) used pre-treated rice husks, instead of rice husks, for the growth of Aspergillus spores. Since the substrate is the same used in hydrolysis test, the enzymes performance could be improved. Please, define well the meaning of U/ml. How much (g) glucose is released? What are the assay conditions used?

A new section could be added in materials and methods for describing both the assay conditions used to determine the glucose and the method used to determine the glucose after the enzymatic hydrolysis. For example “Analytical methods”.

Response:

We thank the reviewer for pointing this out. We inserted the theory as reviewer suggestion (in red-line 63-74). We revised the equations as reviewer suggestion (in red- equation number 1-14). We also compared this study with other studies using cellulase from *Aspergillus* species (in red-line 258-266) and revised Table 3. We also made the “Analytical methods” in materials and methods (in red-153-198). We compared the cellulose enzymatic hydrolysis kinetic constants between this study and previous work as presented in table 4. We also inserted table 5: Comparison of total reducing sugars concentration reported for various subtrates, pre-treatments and microorganisms with the present study.

Point 1:

Line 18: Replace content with concentration

Response 1:

Thank you for pointing this out. We replaced content to “concentration” (in red-line 19; 34; 266; 339; 343; 353).

Point 2:

Line 25: Check the numbers. The annual world production of rice is about 741 milion tons and the rice husks account for 20% in weight.

Response 2:

Thank you for bringing it to our attention. We corrected the numbers. Indonesia produces 2.743 million tons of rice, with about 549 tons of waste rice husks [1] (in red-line 26).

Point 3:

Line 40: Insert the first name of the author followed by et al.

Response 3:

Thank you for pointing this out. The first name of the author was inserted (in red-line 41).

Point 4:

Line 47: Replace “from the husk” with “from the pre-treated husk”

Response 4:

Thank you for pointing this out. We revised as reviewer suggestion (in red-line 49).

Point 5:

Line 48: Replace lowering with reducing

Response 5:

Thank you for pointing this out. We revised as reviewer suggestion (in red-line 50).

Point 6:

Line 67: It is advisable to move the mathematical argument in the materials and methods section (e.g., “Kinetic model for enzymatic lignocellulose hydrolysis”). Please, use the more common letter S instead of A for the substrate. Again, revise and explain the equation 11, 12, and 13.

Response 6:

We thank the reviewer for pointing this out. We revised as reviewer suggestion (in red- equation number 1-14).

Point 7:

Line 88: check the word “gastric”.

Response 7:

Thank you for pointing this out. It was revised to “enzymatic hydrolysis” (in red-line 106).

Point 8:

Line 99: Did you buy the crude extract or the spores of Aspergillus niger?

Response 8:

We thank the reviewer for pointing this out. We have incorporated the revision in the manuscript. *Aspergillus niger* was obtained from the Microbial March Gallery, Indonesia. The culture was routinely maintained for 4-6 days at 30 ^{o}C on a Potato Dextrose agar plate. The harvested spores were suspended in distilled water to achieve a final concentration of 1 x 10^{6} spores mL^{-1} (in red-line 117-120).

We added the procedure of the enzyme quantification and reference used (in red-line 157-190). We also revised table 3.

The procedure of the enzyme quantification:

2.2.1. Evaluation of Endoglucanase activity (Hu et al., 2018)

Approximately 0.1 mL of cellulase enzyme filtrate and 0.1 mL of 1% CMC solution were added to a citrate buffer (pH 4.8) and incubated at 50 ^{o}C for 30 min. The addition of 3 mL of DNS reagents was followed by 10 min of heating in boiling water (T = 100 ^{o}C) to stop the reaction. After cooling to room temperature, absorbance was measured at a wavelength of 540 nm. On a glucose standard curve, absorbance results

were plotted. While citrate buffer and DNS reaction were used to create blanks and determine the zero-absorbance point. Based on the glucose released by the cellulase enzyme, 1 unit of CMCase enzyme activity corresponds to 1 mole of glucose produced per minute. If the incubation lasted 30 min, the production of 1 mg of glucose per mL was: 1/ (30x0.180) = 0.185 units. Where 0.180 is glucose (µmole). One unit of CMCase (IU mL^{-1}) equals mg glucose multiplied by 0.185 per mL. The test was carried out three times with each condition.

2.2.2. Evaluation of Exoglucanase activity (Hu et al., 2018)

After placing approximately 0.1 mL of cellulase enzyme filtrate into a test tube that contained (1 cm x 6 cm) of Whatman No. 1 filter paper (50 mg) and 1 mL of citrate buffer with a pH of 4.8, the mixture was allowed to incubate at a temperature of 50 ^{o}C for 30 min. Approximately 3 mL of DNS reagents are added, and the mixture is then heated for 10 min in boiling water to end the reaction. Furthermore, the absorbance was measured at a wavelength of 540 nm after it had been cooled. The amount of glucose that was produced per minute was used as the basis for the calculation of the FPase enzyme activity. One unit of FPase activity was equal to 1 µmole of glucose produced per minute. If the incubation was for 30 min, 1 mg of glucose produced every mL was: 1/(30x0.180) = 0.185 units. Where 0.180 = glucose µmole. It means, one unit of Fpase (IU mL-1) = mg glucose x 0.185 / mL. The test was carried out three times with each condition.

Referense:

Hu, Y., Du, C., Leu, S. Y., Jing, H., Li, X., & Lin, C. S. K. (2018). Valorisation of textile waste by fungal solid-state fermentation: An example of circular waste-based biorefinery. Resources, Conservation and Recycling, 129(June 2017), 27–35. https://doi.org/10.1016/j.resconrec.2017.09.024

Point 9:

Line 125-126: Why did you use a culture medium in a solely enzymatic hydrolysis process?

Response 9:

We thank the reviewer for pointing this out. We realized that we were wrong in writing the procedure, there should be no addition of a growth medium into the process of enzymatic hydrolysis. We deleted it and made the revision in the manuscript. It was already revised (in red-line 146).

Point 10:

From line 176 to 185: The information should be moved in the materials and methods section.

Response 10:

Thank you for pointing this out. It was already removed to the materials and methods section (in red-line 153-156).

Point 11:

Line 203: Please cited the work, otherwise, it has to be specified that is an unpublished work.

Response 11:

Thank you for pointing this out. We revised the references.

Point 12:

Line 250, 251, 253, 256, and 259: There is an inconsistency between the equations used in the figures and in the text.

Response 12:

Thank you for pointing this out. The slope value represents the reaction rate constant (k_2) and the interception represents the Michaelis-Menten constant (K_M). We revised and added the value of k_2 (in red-line 322-323). V_M is k_2 multipied by $[E]_0$.

Author Response File: **Author Response.docx (https://susy.mdpi.com/user/review/displayFile/28701936/nMXhHY2Z?file=author-coverletter&report=21145579)**

Reviewer 3 Report

I think that the paper can be considered for publication after revision. Please consider the following comments:

English needs extensive revision.

Double check the manuscript to correct typos (e.g. “corn corn corn” at page 3).

Abstract

Avoid acronyms in the abstract. For instance, use “Michaelis constant” instead of Km in the abstract.

Introduction

Introduce Michaelis-Menten model before providing equations. You can use:

doi: 10.1080/07388551.2021.1873241

doi: 10.1016/j.ymben.2014.03.007

Results.

Avoid materials and methods in results (e.g. “The reducing sugar concentration was analyzed using a Spectrophotometer with the 206 Dinitrosalic Acid (DNS) method”)

Use past tenses for results (e.g.“The reducing sugars level rise sharply”)

Discussion

Improve the discussion. Is Km comparable with other studies?

References

In the main text, use the name of the authors when needed. For instance, replace “according to [x]…” with “according to Author1 et. Al [x]”.

Conclusions

Reformulate the first sentence.

Author Response

Thank you for giving us the opportunity to submit a manuscript titled “Kinetic Model for Enzymatic Hydrolysis of Cellulose from Pre-treated Rice Husks” for publication in the Journal of Fermentation. We appreciate the time and effort that you dedicated to providing feedback on our manuscript and are grateful for the insightful comments and valuable improvements to our paper. We have incorporated the suggestions made by the reviewers. Those changes are written in red highlight text within the manuscript.

I think that the paper can be considered for publication after revision. Please consider the following comments:

Point 1:

English needs extensive revision.

Response 1:

Thank you for pointing this out. We revised as reviewer suggestion.

Point 2:

Double check the manuscript to correct typos (e.g. “corn corn corn” at page 3).

Response 2:

Thank you for pointing this out. We revised as reviewer suggestion (in red-line 106).

Abstract

Point 3:

Avoid acronyms in the abstract. For instance, use “Michaelis constant” instead of Km in the abstract.

Response 3:

Thank you for pointing this out. We revised as reviewer suggestion (in red-line 18-19).

Introduction

Point 4:

Introduce Michaelis-Menten model before providing equations. You can use:

doi: 10.1080/07388551.2021.1873241

doi: 10.1016/j.ymben.2014.03.007

Response 4:

Thank you for pointing this out. We inserted the theory as reviewer suggestion (in red-line 64-75).

Results.

Point 5:

Avoid materials and methods in results (e.g. “The reducing sugar concentration was analyzed using a Spectrophotometer with the 206 Dinitrosalic Acid (DNS) method”)

Response 5:

Thank you for pointing this out. We revised as reviewer suggustion (in red-line 154-156; 158-167; 192-197).

Point 6:

Use past tenses for results (e.g.“The reducing sugars level rise sharply”)

Response 6:

Thank you for pointing this out. We revised (in red-line 272; 321; 328; 343; 346; 348; 350; 352; 355).

Discussion

Point 7:

Improve the discussion. Is Km comparable with other studies?

Response 7:

Thank you for pointing this out. We compared the cellulose enzymatic hydrolysis kinetic constants between this study and previous work as presented in table 4.

References

Point 8:

In the main text, use the name of the authors when needed. For instance, replace “according to [x]…” with “according to Author1 et. Al [x]”.

Response 8:

Thank you for pointing this out. We revised (in red-line 42; 97; 123).

Conclusions

Point 9:

Reformulate the first sentence.

Response 9:

Thank you for pointing this out. We revised (in red-line 372; 378).

Author Response File: **Author Response.docx (https://susy.mdpi.com/user/review/displayFile/28765707/n2UeTKhV?file=author-coverletter&report=21214064)**

Round 2

Reviewer 2 Report

Please see attached file

Comments for author File: **Comments.pdf (https://susy.mdpi.com/user/review/displayFile/28701936/nMXhHY2Z?file=review&coverletter&report=21585991)**

Author Response

Point 1: Line 57: Please add this reference to better support your statement:

https://doi.org/10.3390/en15072600

Response 1: We thank the reviewer for pointing this out. We added the reference in manuscript **[22]** (red-line: 58).

To reduce the cost of enzyme production, the pre-treatment of lignocellulose should provide more efficient and cost-effective hydrolysis [20]–[22].

Reference:

22. Fabbrizi, G.; Giannoni, T.; Lorenzi, Leonardo; Nicolini, A.; Iodice, P.; Coccia, V.; Cavalaglio, G.; Gelosia, M. High Solid and Low Cellulase Enzymatic Hydrolysis of Cardoon Stems Pretreated by Acidified Γ-Valerolactone/Water Solution. Energies 2022, 15 (7), 1–12, doi: 10.3390/en15072600.

Point 2: Line 96: Add author name

Response 2: Thank you for pointing this out. We added the author name (in red line: 95).

Point 3: Line 136: The enzyme does not grow since they are not living beings. Please rewrite the sentence.

Response 3: We thank the reviewer for pointing this out. We revised as reviewer suggestion (in red-line 136-137).

Point 4: Line 137: The sentence is not very clear. Please rewrite it.

Response 3: Thank you for pointing this out. We rewrote: “The medium was inoculated with 10 mL of the prepared inoculum. A flask was incubated at 30 ^{o}C for 96 h.” (red-line 137).

Point 5: Line 148: Add (mL) after fraction if the concentration is expressed as volume of crude extract per gram of biomass.

Response 5: Thank you for pointing this out. We added as reviewer suggestion (in red-line 148).

Point 6: Line 157: Which type of enzymes? Does the statement refer to generic enzymes or the cellulase family?

Response 6: Thank you for pointing this out. It refers to the cellulase enzyme family (in red-line 158).

Point 7: Line 158: Add the words “of enzyme” after amount

Response 7: Thank you for pointing this out. We revised as reviewer suggestion (in red-line 159).

Point 8: Line 161: Which substrate was it used to assess the enyme activity?

Response 8: Thank you for pointing this out. We used a pretreated rice hull as substrate (in red-line 135).

Point 9: Line 180: The FPase assay is used for determining the total cellulase activity. How did you get to only quantify the exo activity?

Response 9: Thank you for pointing this out. Exo-β-glucanase activity is also known as Filter paper activity (FPase). It is utilized to determine the total cellulase activity in the culture filtrate using the standard method described by **[41].**

The procedure:

After placing approximately 0.1 mL of cellulase enzyme filtrate into a test tube that contained (1 cm x 6 cm) of Whatman No. 1 filter paper (50 mg) and 1 mL of citrate buffer with a pH of 4.8, the mixture was allowed to incubate at a temperature of 50 ^{o}C for 30 min. Approximately 3 mL of DNS reagents were fed, and the mixture was then heated for 10 min in boiling water to end the reaction. Furthermore, the absorbance was measured at a wavelength of 540 nm after it had been cooled. The amount of glucose produced per minute was used to calculate the FPase enzyme activity. One unit of FPase activity was equal to 1 µmole of glucose created per min. If the incubation was for 30 min, 1 mg of glucose produced for every mL was: 1/(30x0.180) = 0.185 units. Where 0.180 = glucose µmole. It means, one unit of Fpase (IU mL-1) = mg glucose x 0.185 / mL. The test was carried out three times with each condition (red-line 183-194).

Reference:

41. Darwesh, O.M.; El-Maraghy, S.H.; Abdel-Rahman, H.M.; Zaghloul, R.A. Improvement of paper wastes conversion to bioethanol using novel cellulose degrading fungal isolate. Fuel 2020, 262, 116518, doi: 10.1016/j.fuel.2019.116518.

Point 10: Line 180: It should be 39. Please check all in-text citations and renumber them.

Response 10: We thank the reviewer for pointing this out. We realized that we were wrong in writing citations. It should be 41 of Darwesh et al. (2022). We checked all in-text citations and renumbered them.

Reference:

41. Darwesh, O.M.; El-Maraghy, S.H.; Abdel-Rahman, H.M.; Zaghloul, R.A. Improvement of paper wastes conversion to bioethanol using novel cellulose degrading fungal isolate. Fuel 2020, 262, 116518, doi: 10.1016/j.fuel.2019.116518.

Point 11: Line 190-191: Please use the same formatting to define Fpase and CMcase. See lines 177 and 178.

Response 11: Thank you for bringing it to our attention. We revised as reviewer suggestions (in red-line 193).

Point 12: Line 201: Please be consistent with the name. Use husks or hulls.

Response 12: Thank you for pointing this out. We revised as reviewer suggestions (in red-line 201).

Point 13: Line 203: Plese make explicit the acronym

Response 13: Thank you for pointing this out. We removed the sentence and revised HWS to “Hot Water Solubility” (red-line 202 and table 1).

Point 14: Line 203: You did not showed the cellulose recovey, please add this infromation in the text. The cellulose recovery is different from cellulose content.

Response 14: Thank you for pointing this out. We added cellulose recovery and lignin removal (red-line 214-215).

Point 15: Line 212: Which components?

Response 15: Thank you for pointing this out. We added components “carbon, oxygen, and silicon” (in red-line 220,221).

Point 16: Line 217-218: Please discuss this statement and add a reference to support it.

Response 16: Thank you for pointing this out. We discussed the statement and added some references to support it (in red-line 226-232).

Before pre-treatment, the lignocellulosic surface is protected from cellulose degradation by silica frames resistant to breakdown **[43]**. Silica is a problem in industrial processes because it forms insoluble precipitates as a physical barrier to cellulase action **[44]**. In order to obtain realistic hydrolysis yields from silica-rich bio-masses like rice husks, it is essential to employ effective pre-treatments to remove silica, which is solubilized in an alkali medium (pH > 9) **[45].**

Reference:

43. Kim, T.H.; Taylor, F.; Hicks, K.B. Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pre-treatment. Bioresour. Technol. 2008, 99(13), 5694–5702, doi: 10.1016/j.biortech.2007.10.055.
44. Moreira, B.R.; Breitkreitz, M.C.; Simister, R.; McQueen-Mason, S.J.; Gomez, L.D.; Rezende, C.A. Improved hydrolysis yields and silica recovery by design of experiments applied to acid-alkali pre-treatment in rice husks. Ind. Crops Prod. 2021, 170, 113676, doi: 10.1016/j.indcrop.2021.113676.
45. Le, H D.M.; Sørensen, R.; Knudsen, N.O.; Meyer, A.S. Implications of silica on biorefi neries – interactions with organic material and mineral elements in grasses. Biofuels, Bioprod. Biorefining 2014, 9(2), 109–121, doi: 10.1002/bbb.

Point 17: Line 224: Substantially, figure 1 and table 2 show the same results in different forms. Please, choose one of them and discuss it accordingly. The table is more readable than the figure.

Response 17: We thank the reviewer for pointing this out. We deleted Figure 1 and removed sentences related to this figure (in red-line 208-215; 236-248). We also revised the number of other figure captions (in red-line 269; 271; 298; 310; 313; 416; 319; 336-339;).

Point 18: Line 237-246: The same paragraph is shown in materials and methods, so it should be removed.

Response 18: Thank you for pointing this out. We removed the paragraph (in red-line 158-168).

Point 19: Line 247: Which sample?

Response 19: Thank you for pointing this out. The sample is pretreated rice husk (in red-line 53).

Point 20: Line 251-253: Again, the enzyme does not grow since they are not living beings. Where does the high water content promote the production of cellulase? In the culture medium? Please add a reference to support the statement.

Response 20: Thank you for pointing this out. We deleted this sentence, because we did not find the reference to support the statement (in red-line 253-354).

Point 21: Line 254: Please briefly discuss table 3. in the text.

Response 21: Thank you for pointing this out. We discussed table 3 in the manuscript (in red-line 252-261). We deleted the paragraph in lines 265-272.

Point 22: Line 264: Add name author and reference number.

Response 22: Thank you for pointing this out. We added the name author and reference number (in red-line 260). Although using a crude enzyme, the value of cellulase activity in this study tends to be higher than in the previous work, Kaur et al. [46].

Reference:

46. Kaur, B.; Oberoi, H.S.; Chadha, B.S. Enhanced cellulase producing mutants developed from heterokaryotic Aspergillus strain. Bioresour. Technol. 2014, 156, 100–107, doi: 10.1016/j.biortech.2014.01.016.

Point 23: Line 264: Add the word “cellular” before biomass

Response 23: Thank you for pointing this out. We added as per reviewer suggestion (in red-line 261).

Point 24: Line 272: Better use the word “increase”

Response 24: Thank you for pointing this out. We revised as per reviewer suggestion (in red-line 277).

Point 25: Line 273-277: The sentence is not clear, please rewrite it.”

Response 25: Thank you for pointing this out. We revised as per reviewer suggestion (in red-line 281).

Due to the cellulase enzyme’s activity entering the moderate phase and depletion, there is a decline in the levels of reducing sugar. At an intermediate stage, cellulose is degraded into reducing sugar on a large scale. The concentration of enzymes increases proportionally to the concentration of reducing sugar as enzyme activity increases. Previous researchers [34] demonstrated a comparable pattern of findings (in red-line 283-288).

Reference:

34. Makarova, EI; Budaeva, V.V.; Kukhlenko, A.A.; Orlov, S.E. Enzyme kinetics of cellulose hydrolysis of Miscanthus and oat hulls. 3 Biotech 2017, 7 (5), 1–96, doi: 10.1007/s13205-017-0964-6.

Point 26: Line 278: Which model? Please add this information in materials and methods section. For example: “The enzymatic kinetics was studied using the equation 14”.

Response 26: Thank you for pointing this out. We revised as per the reviewer's suggestion (in red-line 150-151).

Point 27: Line 289: You first state that the findings from other research works are similar or comparable to yours, but that it implies that a direct comparison of different models is difficult due to differences in reaction schemes and model structures. Therefore, how can they be comparable to yours if a direct comparison is difficult to perform?

Response 27: Thank you for pointing this out. We deleted the sentence to avoid confusion (in red-line 300-303).

Point 28: Line 301: It should be 39

Response 28: Thank you for pointing this out. We corrected the reference. It Should be **[23]** (in red-line 314).

Reference:

23. Bisswanger, H. Enzyme Kinetics: Principles and Methods. In Psychology in the Brain, third ed.; Wiley-VCH: Weinheim, Germany, 2017, pp. 1–21.

Point 29: Line 309: It should be 14.

Response 29: Thank you for pointing this out. We corrected as per the reviewer suggestion (in red-line 320).

Point 30: Line 310: It’s not the same equation. The dependent varibale of (14) is [P]/ln{[P]∞/([P]∞- [P])} and the indipendent is [E]0*t / ln{[P]∞/([P]∞ - [P])}

Response 30: Thank you for pointing this out. We corrected as per the reviewer suggestion (in red-line 321).

Point 31-33: Line 309-318: The dependent variable is different from (14) and the one shown in line 310. The same thing in figure 4 and 5

Response 31-33: We thank the reviewer for pointing this out. We realized that we were wrong in writing the variable. However, the kinetic calculation was based on equation 14. So we revised (in red-line 329-333). We corrected Figures 2, 3 and 4.

Point 34: Line 340: Insert (v/w). Please add this information near all the percentage concentrations in the text.

Response 34: Thank you for pointing this out. We revised as per the reviewer suggestion (in red-line 354-365).

Point 35: Line 352-354: How could you state that if you just tested a 10% weight ratio percentage concentration of pretreated rice husks?

Response 35: Thank you for pointing this out. For the kinetic calculation, we had data in ranges 10-20% enzyme concentration and 5-25 h hydrolysis time. Figure 6 is for 5 h hydrolysis time with a range 10-50% enzyme concentration. Figure 6 displays the effect of enzyme loadings on the release of reducing sugars. The optimal enzyme loading for cellulase-mediated enzymatic hydrolysis of rice husk fibres is 30 %, which maximizes soluble sugar contents.

Point 36: Line 354-355: The sentence is not very clear, please rewrite it. Add a ref. that confirms your statement.

Response 36: Thank you for pointing this out. We deleted the sentence because we didn’t find any supporting libraries.

Point 37: Table 5: The references 58, 59, 60, 61 are not listed in the reference section.

Response 37: Thank you for pointing this out. We revised references 58, 59, 60, and 61 in table 5.

Point 38: Line 374-375: The sentence is not very clear. Please rewrite it.

Response 38: Thank you for pointing this out. We revised references as per the reviewer suggestion (in red-line 381-382). The time dependence of the amount of reducing sugars was investigated by varying the amount of enzyme.

Point 39: Line 375-377: The sentence is not very clear. Please rewrite it.

Response 39: Thank you for pointing this out. We revised references as per reviewer suggestions (in red-line 388-392). The findings of this study led to an improved method for the enzymatic hydrolysis of pretreated rice husk, which was shown to follow the Michaelis-Menten kinetic model and for which the kinetic parameters K_M and V_M were determined to be 0.001 to 0.0007, Mol L^{-1} and 1.3 x 10^{-7} to 2.7 x 10^{-7} Mol L^{-1} s^{-1}, respectively.

Point 40: Line 378: Another optimal reducing sugar level (2.21 g/L) was obtained at enzyme concentrations of 30% with an hydrolysis time of 5 h.

Response 40: Thank you for pointing this out. We used various hydrolysis times (5 -25 h) and enzyme concentrations (10 -20 % v/w) for the kinetic calculation. So we decided the optimal reducing sugar level is 1.80 g/L.

Author Response File: **Author Response.docx (https://susy.mdpi.com/user/review/displayFile/28701936/nMXhHY2Z?file=author-coverletter&report=21585991)**

Reviewer 3 Report

The authors considerably improved the manuscript compared to the previous version. Moreover, they toke into account all my suggestions. Therefore, I think that the paper can be accepted. I just have few further suggestion before publication:

-Better to add a sentence reporting future directions in the conclusion section

- Typo in line 341: Table 4 is Table 5

- In the Michaelis menthen theory part, consider adding this DOI: 10.1080/07388551.2021.1873241

Author Response

The authors considerably improved the manuscript compared to the previous version. Moreover, they toke into account all my suggestions. Therefore, I think that the paper can be accepted. I just have few further suggestion before publication:

Point 1: Better to add a sentence reporting future directions in the conclusion section

Response 1: We thank the reviewer for pointing this out. We added the sentence in manuscript (red-line: 367-369).

Additional research is necessary to develop a comprehensive model of enzymatic hydrolysis of cellulose to provide a valuable tool for future engineering applications.

Point 2: Typo in line 341: Table 4 is Table 5

Response 2: We thank the reviewer for pointing this out. We revised it (red-line: 348).

Point 3: In the Michaelis menthen theory part, consider adding this DOI: 10.1080/07388551.2021.1873241

Response 3: We thank the reviewer for pointing this out. We added the theory in manuscript (red-line: 63-68).

The kinetic models predict substrate degradation, biomass growth, and product formation [22]. The objective of kinetic models is to simulate biomass growth, substrate utilization, and product formation processes while considering their time dependence. Therefore, a system of ordinary differential equations derived from a mass balance equation represents the processes. Under suitable initial conditions and given kinetic and stoichiometric parameters, these equations can be solved by numerical integrations.

Reference:

22. Policastro, G.; Luongo, V.; Frunzo, L.; Fabbricino, M. A comprehensive review of mathematical models of photo fermentation. Crit. Rev. Biotechnol. 2021, 41(4), 628–648, doi: 10.1080/07388551.2021.1873241.

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