Development of Lignocellulase Enzyme Cocktail—A Logical Use of Statistical Experimental Design Techniques

Eugene Marfo Obeng (eugene.obeng@monash.edu)
Monash University https://orcid.org/0000-0001-6525-7577
Clarence M. Ongkudon
Universiti Malaysia Sabah

Research Article

Keywords: cellulases, enzyme cocktail, synergism, statistical experimental design, response surface methodology

Posted Date: September 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-839287/v1

Abstract

The derivation of reduced sugars from lignocellulosic biomass requires an optimum blend of cellulolytic enzymes and reaction conditions that favour high sugar yield. In this respect, statistical design of experiment strategies become useful, but the technique is often misguided such that enzyme redundancy becomes overlooked. Herein, we demonstrate a systematic approach that involves simplex lattice mixture design and central composite design for optimizing enzyme cocktails for the saccharification of lignocellulosic biomass. The simplex lattice mixture design yielded 0.3333: 0.3333: 0.3333 of Celluclast (5%), Hemicellulase (5%) and Laccase (2%), respectively, as the optimum enzyme blend (volume) ratio for the saccharification of hydrothermally pretreated empty palm fruit bunch (EPFB, 10% solid loading). A subsequent application of central composite design resulted in 40 ^{o}C, pH 6 and 24 hrs as the optimum saccharification conditions. The individual Celluclast (5%) and Hemicellulase (5%) yielded a reduced sugar equivalence (RSE) of 1.77 mg/mL and 1.67 mg/mL, respectively. However, the blended enzyme cocktail upon subjection to simplex lattice mixture design and subsequent central composite design yielded an RSE of 2.215 mg/mL and 2.431 mg/mL, respectively. The overall results exemplify the significance of enzyme synergism in lignocellulosic biomass saccharification. The approach herein is intended as an easy-to-copy plan for optimizing enzyme cocktails.

1.0 Introduction

Lignocellulosic biomass remains the most abundant, but underutilized, source of biopolymers on earth (Isikgor and Becer 2015; Dahmen et al. 2018). Mostly in the form of agro-waste, the biomass which is composed of cellulose, hemicellulose and lignin has also been a huge source of pollution to the environment (Michelin et al. 2014). For this reason, the valorization of lignocellulosic biomass has been an important research over the past few decades to (1) derived value-added benefits and (2) mitigate the associated pollution consequences. Among the numerous benefits retrievable from the biomass, the efficient depolymerization of the biomass into reduced sugars for use in the production of essential platform chemicals is highly attractive but remains a pressing challenge due to the recalcitrance of the biomass feedstock (McCann and Carpita 2015; Zhang et al. 2019). Several efforts such as genetic modification of the biomass (Wang et al. 2016; Pazhany and Henry 2019), pretreatment technologies (Mosier et al. 2005; Baruah et al. 2018), the development of enzyme systems (Tozakidis et al. 2016; Obeng et al. 2017, 2018a) and enzyme recycling strategies (Jampana et al. 2021; Arthur et al. 2021; Ying et al. 2021) are currently being studied to overcome these challenges.

For instance, empty palm fruit bunch (EPFB) is a major global agro-waste that results from the production of palm oil (Dungani et al. 2018; Anyaoha et al. 2018). It is a rich source of sugar, with about 75–80 % content of cellulose and hemicellulose (Abdullah and Sulaiman 2013). However, the valorization of EPFB into high titer platform chemicals is yet to be met due, in part, to biomass recalcitrance and inefficient enzyme formulations. Remarkably, the depolymerization of lignocellulosic biomass requires enzymes such as cellulases, hemicellulases and lignases to break down the cellulose, hemicellulose and lignin components, respectively. These enzymes work cooperatively to monomerize the complex polymer.

Of note, enzyme cooperativity is a function of individual enzyme proportion and reaction conditions (Gusakov et al. 2007; Zhou et al. 2009; Obeng et al. 2018b). In this respect, statistical design of experiment strategies become useful, but the use of the technique is often misguided or neglected in enzyme formulations such that enzyme redundancy becomes overlooked. Sometimes, high enzyme loadings are used on dilute substrates which undoubtedly has the penchant to increase process economics exponentially (Badhan et al. 2014). For instance, Agrawal et al. (2021) painstakingly produced and purified recombinant feruloyl esterase D (CE1) and β-xylosidase (GH43) and subsequently concocted them rationally with commercial cellulase CellicCTec2 for the saccharification of pre-treated rice straw and bagasse at 7% (w/v) substrate loading. Similarly, Shen et al. (2021) meticulously produced and purified cellulases from *Trichoderma reesei* and *Aspergillus niger* with similar rational mixing. These impressive efforts and many others could receive a significant improvement with application of statistical enzyme blend strategies.

A crucial bottleneck for existing cellulase biotechnologies is to use low enzyme dosage (e.g., 10 mg/g cellulose) for appreciably high substrate loading (e.g., ≥ 10% w/v insoluble solids) to improve process economics (Hodge et al. 2009; Zhang et al. 2012; Bhagia et al. 2018; Davis et al. 2020). This enzyme-substrate expectation has also been proven to improve enzyme diffusivity, reactivity, and overall mass transfer during saccharification (Kari et al. 2021; Rohrbach and Luterbacher 2021). As such, it is currently part of the 2030 vision of the National Renewable Energy Laboratory to achieve low-enzyme hydrolysis of high substrate loads (Davis et al. 2020). In line with this, Sui et al. (2021) reported over 80 g/L glucose production from a low enzyme [15 filter paper cellulase units (FPU)/g glucan] saccharification of 20% steam exploded corn stalk. The work benefited from rapid room temperature γ-valerolactone delignification. Similarly, Vignesh and Chandraraj (2021) achieved about 134 g/L glucose from 35% pretreated cotton microdust with a cellulase loading of 22 FPU/g glucan. Also, Ying et al. (2021) obtained a maximum of 250.8 g/L glucose at 40% solid loading pretreated poplar using CellicCTec2 (123 FPU/mL) with focus on enzyme recycling. It is apparent that these remarkable achievements could receive a significant improvement with statistical enzyme blend strategies applied, especially since enzyme cost takes a greater part of the overall processing cost.

Herein, we demonstrate an easy-to-copy systematic approach for optimizing enzyme cocktails consisting of cellulase, hemicellulase and laccase for the saccharification of lignocellulosic biomass (Fig. 1). The optimized blend design strategy employs simplex lattice mixture design (Gorman and Hinman 1962; Coteaux 1998; Voinovich et al. 2009) and central composite design (CCD) (Box and Wilson 1951; Box and Hunter 1957; Keskin Gündoğdu et al. 2016) methods to respectively identify optimum enzyme blend composition and operating conditions (i.e., temperature, pH and saccharification time) for the saccharification of hydrothermally pretreated EPFB. The overall aim of this paper is towards serving as a template for researchers in multicomplex enzyme optimization studies.

2.0 Materials And Methods

2.1 Materials and equipment

Celluclast 1.5L (*T. reesei*, Sigma Aldrich), Hemicellulase (*A. niger*, Sigma Aldrich), Laccase (*Trametes versicolor*, Sigma Aldrich), 3,5-Dinitrosalicyclic acid (Acros Organics), Phenol ACS (VWR) and β-glucose (Roth), thermo-shaker (BioSan TS-100), microplate reader (Tecan Infinite m200), microplates (GreinerBio-One 96 flat transparent), thermocycler (MJ Research, PTC-200).

2.2 EPFB Pretreatment

The EPFB was received from Aumkar Plantations SDN BHD (Tawau, Sabah, Malaysia) in a washed dried form. The fibres were pulverized and sieved (≤ 2 mm). About 6 g of the pulverized EPFB (≤ 2 mm) was measured into a ceramic cup and 4 mL of H_2O_{milli} was added. The cup was sealed with aluminium foil and placed in an autoclave at 135 ^{o}C (1 Bar) for 1 hour. The pretreated sample was oven-dried at 60 ^{o}C for 3 hours and cooled to room temperature. It was then stored at 4 ^{o}C for subsequent saccharification experiment.

2.3 Preliminary enzyme dosage screening

Enzyme loading is a critical determinant of saccharification efficiency and overall process economics (Gao et al. 2011; Davis et al. 2020). Herein, different concentrations of Celluclast (1, 2, 5, 8 and 10% v/v) and Hemicellulase (1, 2, 5, 8 and 10% w/v) were prepared using sodium citrate buffer (0.1 M, pH 6) to check for the effective level of each enzyme required to individually saccharify pretreated EPFB (10% solid loading). Celluclast contains about 54.0 mg/mL of proteins with a filter paper activity of 51.4 FPU/mL (Tu et al. 2007). According to the manufacturer, the powdered hemicellulase shows a specific activity of 0.3-3.0 unit/mg solid using locust bean gum as substrate. In this section, about 200 µl of the different concentrations of each enzyme was transferred onto 20 mg pretreated EPFB in 2 mL Eppendorf tubes. The tubes were incubated at 50 ^{o}C and 900 rpm for 12 hours. Supernatants from each test were harvested via centrifugation (14000 rpm, 4 ^{o}C, 2 min) and checked for their reduced sugar equivalence (RSE, mg/mL) using the DNS colorimetric method with microplate (King et al. 2009). The DNS reagent consisted of DNS (1.0 g), phenol (0.2 g), sodium sulfite (0.05 g), Na-K-tartarate (Rochelle salt, 20 g) in 150 mL NaOH (6.7 g/l). Sample blanks consisted of 20 mg pretreated EPFB in 200 µL sodium citrate buffer (0.1 M, pH 6). The standard was β-glucose (1mg/mL). All experiments were conducted in triplicate. The RSE (mg/mL) was computed from absorbance (540 nm) measurement using equations 1 and 2 below.

$$Abs_{Sample_{corrected}} = Abs_{Sample} - Abs_{Blank} \quad (1)$$

$$RSE\ \left(\frac{mg}{mL}\right) = 1\ mg/mL \times \left(\frac{Abs_{sample_{corrected}}}{Abs_{Standard}}\right) \times 1000 \quad (2)$$

2.3 Optimum enzyme mixture composition identification for EPFB saccharification

A (3,2) simplex lattice design of experiment (DoE) was used for the optimum blend identification. From the previous experiment, the concentrations of Celluclast (C) and Hemicellulase (H) were selected as 5% (v/v) and 5% (w/v), respectively, since they yielded the maximum results. Laccase (L; 2% w/v) was used to supplement these enzymes. Specific volumes of these three enzymes were mixed such that the sum of the individual ratios in a run adds up to unity (1). The total volume of the enzyme mixture was fixed at 200 µl. The experimental setup is as indicated in Table 1. Each run was conducted in triplicate and the resulting RSE (mg/mL) averaged for the purpose of analysis. The statistical analysis was done with Statistica (v5.0, StatSoft Inc., USA).

2.4 Optimum EPFB saccharification condition identifications

The optimum temperature and pH for typical mesophilic cellulases ranges from 40–60 ^{o}C and 6–7, respectively (Obeng et al. 2017; Intasit et al. 2021). Also, an equally important condition for saccharification optimization is the hydrolysis time since it has a significant effect on process economics. To explore for promising 'sweet spot', central composite design (CCD) consisting of three factors (i.e., temperature, pH and saccharification time) at three independent levels was employed in addition to the optimized blend ratio obtained from Sect. 2.3. Table 2 shows the details of the experimental runs in uncoded form for the CCD. The statistical analysis was done with Statistica (v5.0, StatSoft Inc., USA). All calculations in systematic order can be found in Supplementary Material 2 (MS-Excel sheet with integrated formulas).

3.0 Results And Discussion

3.1 Preliminary enzyme dosage screening

Figure 2 shows the reduced sugar profiles of Celluclast and Hemicellulase at different enzyme loadings on pretreated EPFB. This experimental setup aimed at selecting the starting stock concentration of Celluclast and Hemicellulase for subsequent blend studies. Using a fixed substrate loading (10% w/v), an increased concentration of both Celluclast and Hemicellulase resulted in an increased corresponding reduced sugar yield. However, the observed increments in RSE yield for both enzymes were not highly significant after the 5% mark. The maximum RSE for both Celluclast and Hemicellulase occurred at 5% enzyme loading and were recorded as 1.77 mg/mL and 1.67 mg/mL, respectively. This implies that for 10% EPFB loading, any enzyme concentration above 5% results in enzyme redundancy and inefficiency. Therefore, 5% each of Celluclast and Hemicellulase were selected for the subsequent blend investigation.

$$RSE\left(\frac{mg}{mL}\right) = 1.36429x + 1.9761x + 1.95448z + 19.45629xyz \qquad (3)$$

3.2 Optimum blend composition for pretreated EPFB saccharification

Table 1 contains the results recorded for the individual blend compositions. The maximum and minimum reduced sugar yields recorded from the simplex lattice experimental runs were 1.229 and 2.370 mg/mL, respectively. The measured RSE (mg/mL; Actual) values were used to fit a reduced special cubic simplex lattice model to data (Equation 3). The optimized model was used to estimate the outcome (Predicted RSE) and associated residuals which were not far from the actual (true) values. The analysis of variance (ANOVA) of the model and its goodness of fit are shown in the supplementary (**Table S1-2)**. The *p*-value of the overall model and that of all the variables in the model equation were < 0.05. The regression coefficient (R^2) of the model was also 0.76. For a model to be statistically relevant, it is recommended that the *p*-values of the variables (herein x, y and z) as well as that for the overall model must be ≤ 0.05. Also, the model must have a high regression coefficient. Therefore, from **Table S2**, the model can be considered as significant and used for subsequent analysis.

Table 1: Experimental setup and outcome of the Simplex lattice mixture design

Exp't No. *	Coded volumetric levels			Actual RSE (mg/mL)	Predicted RSE (mg/mL)	Residual (mg/mL)
	Celluclast (x)	Hemicellulase (y)	Laccase (z)			
1	0.000000	0.000000	1.000000	1.229	1.364	-0.136
2	1.000000	0.000000	0.000000	2.037	1.954	0.082
3	0.000000	0.500000	0.500000	1.935	1.965	-0.031
4	0.000000	1.000000	0.000000	2.000	1.976	0.024
5	0.333333	0.333333	0.333333	2.252	2.486	-0.234
6	0.166667	0.166667	0.666667	2.191	2.220	-0.029
7	0.666667	0.166667	0.166667	2.370	1.925	0.445
8	0.166667	0.666667	0.166667	2.282	2.231	0.052
9	0.500000	0.500000	0.000000	1.602	1.670	-0.068
10	0.500000	0.000000	0.500000	1.554	1.659	-0.105
*The experimental design was randomized						

After model validation, a surface plot (**Figure 3**) was generated from the model to assess the dynamics of the data and to identify the optimum blend composition. From the plot, it is seen that the optimum blend composition exists around a point where equal proportions of Celluclast (x), Hemicellulase (y) and Laccase (z) form the enzyme mixture. The ratio was identified as 0.3333: 0.3333: 0.3333 of Celluclast, Hemicellulase and Laccase, respectively (**Table S3**). From **Table S3**, the predicted RSE for this mixture composition was about 2.486 mg/mL with a 95% confidence interval of 2.063<**x**<2.908 mg/mL. A

confirmatory test was run, which yielded about 2.215 mg/mL in conformity with the 95% confidence interval range. The predicted optimum composition was therefore used in subsequent experiments.

3.2 Optimum EPFB saccharification conditions

Table 2 shows the results obtained from the Central Composite Design. Approximately 0.593 and 2.385 mg/mL were the observed minimum and maximum RSE, respectively. A reduced quadratic model was fitted to the data, and the resulting model (R^2 = 0.81987) was used to predict the RSE. There were insignificant variations between actual and predicted RSE values. The ANOVA of the fitted quadratic CCD model is shown in **Table S4**. Herein, the *p*-values of almost all the terms were within the statistically significant range (*p*-value $\leq$ 0.05).

Table 2: Experimental setup and outcome of the Central Composite Design

Exp't No. *	Factor levels			Actual RSE (mg/mL)	Predicted RSE (mg/mL)	Residual (mg/mL)
	Temp. (° C)	pH	Time (hrs)			
1	50	5	12	2.068	1.852	0.217
2	50	7	48	0.819	0.754	0.065
3	60	5	48	2.244	2.280	-0.037
4	60	7	12	2.195	2.073	0.122
5	55	6	24	2.142	2.096	0.046
6	50	5	48	1.697	2.085	-0.388
7	50	7	12	2.274	2.371	-0.097
8	50	5	12	1.094	1.555	-0.460
9	60	7	48	0.593	0.949	-0.356
10	55	6	24	2.324	2.096	0.228
11	40	6	24	2.385	2.202	0.183
12	70	6	24	2.110	1.802	0.308
13	55	4	24	1.323	1.107	0.216
14	55	8	24	0.926	0.911	-0.015
15	55	6	6	2.219	2.319	-0.100
16	55	6	54	2.134	1.725	0.409
17	55	6	24	1.713	2.096	-0.383
*The experimental design was randomized						

The surface plots of the CCD design are shown in **Figure 4**. In **Figure 4(a)**, the plot indicates that (at a fixed time of 24 hrs) operating at a temperature between 40-55 ^{o}C and a pH around 6 could result in optimum sugar yield. In **Figure 4(b)**, varying saccharification time and temperature at a fixed pH (6) hinted that short time and low temperature favours high sugar yield. Lastly, in **Figure 4(c)**, varying pH and time at a fixed temperature of 40 ^{o}C shows a possible high RSE over all the ranges of pH and time. Referring to run 11 in **Table 2**, it is seen that the maximum sugar yield was obtained at 40 ^{o}C, pH 6 and at 24 hrs. It is hereon convincing to accept these parameters as the optimum EPFB saccharification conditions for the optimized blend of Celluclast, Hemicellulase and Laccase. A time-course study (**Figure 5**) of the optimized enzyme blend composition at the optimum saccharification conditions yielded about 2.431 mg/mL of reduced sugar within 24 hours, which is not far from the expected maximum. Interestingly, the RSE yields for 24 and 48 hours (2.431 and 2.418, respectively) were not very much

different from each other and, thus, operating at the later will save operation time and reduce process economics favourably.

4.0 Conclusion

We have demonstrated how to systematically and logically employ statistical experimental design techniques in optimizing enzyme cocktails. Results herein have revealed a volumetric blend ratio of 0.3333: 0.3333: 0.3333 of Celluclast (5%), Hemicellulase (5%) and Laccase (2%), respectively, as the optimum enzyme blend ratio for the saccharification of hydrothermally pretreated EPFB (10% solid loading). The corresponding optimum saccharification conditions were shown to be 40 ^{o}C, pH 6 and at 24 hrs. Increasing the time beyond 24 hrs did not show any relevant increase in RSE. The maximum RSE for the unblended Celluclast (5%) and Hemicellulase (5%) were 1.77 mg/mL and 1.67 mg/mL, respectively. However, the optimum RSE for the mixture (blended) and subsequent operating parameters optimization using CCD were 2.215 mg/mL and 2.431 mg/mL, respectively. The overall results exemplify the significance of additives (e.g., Hemicellulase and Laccase) and the associated synergism in lignocellulosic biomass saccharification.

Declarations

Acknowledgement

The authors acknowledge Autodisplay Biotech GmbH (Germany) for research grant GL00139.

Ethics declaration

Human and animal ethics approval

There is no human or animal ethical requirements in relation to this study.

Conflict of interest

The authors declare no conflict of interest.

References

1. Abdullah N, Sulaiman F (2013) The Oil Palm Wastes in Malaysia. In: Matovic MD (ed) Biomass Now - Sustainable Growth and Use. InTech,
2. Agrawal D, Tsang A, Chadha BS (2021) Economizing the lignocellulosic hydrolysis process using heterologously expressed auxiliary enzymes feruloyl esterase D (CE1) and β-xylosidase (GH43) derived from thermophilic fungi Scytalidium thermophilum. Bioresour Technol 339:125603. doi: 10.1016/j.biortech.2021.125603

3. Anyaoha KE, Sakrabani R, Patchigolla K, Mouazen AM (2018) Critical evaluation of oil palm fresh fruit bunch solid wastes as soil amendments: Prospects and challenges. Resour Conserv Recycl 136:399–409. doi: 10.1016/j.resconrec.2018.04.022
4. Arthur W, Diedericks D, Coetzee G, et al (2021) Kinetic modelling of cellulase recycling in paper sludge to ethanol fermentation. J Environ Chem Eng 9:105981. doi: 10.1016/j.jece.2021.105981
5. Badhan A, Wang Y, Gruninger R, et al (2014) Formulation of enzyme blends to maximize the hydrolysis of alkaline peroxide pretreated alfalfa hay and barley straw by rumen enzymes and commercial cellulases. BMC Biotechnol 14:31. doi: 10.1186/1472-6750-14-31
6. Baruah J, Nath BK, Sharma R, et al (2018) Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-Added Products. Front Energy Res. doi: 10.3389/fenrg.2018.00141
7. Bhagia S, Dhir R, Kumar R, Wyman CE (2018) Deactivation of Cellulase at the Air-Liquid Interface Is the Main Cause of Incomplete Cellulose Conversion at Low Enzyme Loadings. Sci Rep 8:1350. doi: 10.1038/s41598-018-19848-3
8. Box GEP, Hunter JS (1957) Multifactor experimental design for exploring response surfaces. Ann Math Stat 28:195–241.
9. Box GEP, Wilson KB (1951) On the Experimental Attainment of Optimum Conditions. J R Stat Soc Ser B 13:1–38. doi: 10.1111/j.2517-6161.1951.tb00067.x
10. Coteaux T (1998) Mixture designs. In: Massart DL, Vandeginste BGM, Buydens LMC, et al. (eds) Data Handling in Science and Technology- Handbook of Chemometrics and Qualimetrics: Part A. Elsevier, pp 739–769
11. Dahmen N, Lewandowski I, Zibek S, Weidtmann A (2018) Integrated lignocellulosic value chains in a growing bioeconomy: Status quo and perspectives. GCB Bioenergy 11:gcbb.12586. doi: 10.1111/gcbb.12586
12. Davis R, Bartling A, Tao L (2020) Biochemical Conversion of Lignocellulosic Biomass to Hydrocarbon Fuels and Products: 2019 State of Technology and Future Research.
13. Dungani R, Aditiawati P, Aprilia S, et al (2018) Biomaterial from Oil Palm Waste: Properties, Characterization and Applications. In: Palm Oil. InTech,
14. Gao D, Uppugundla N, Chundawat SP, et al (2011) Hemicellulases and auxiliary enzymes for improved conversion of lignocellulosic biomass to monosaccharides. Biotechnol Biofuels 4:5. doi: 10.1186/1754-6834-4-5
15. Gorman JW, Hinman JE (1962) Simplex Lattice Designs for Multicomponent Systems. Technometrics 4:463–487.
16. Gusakov A V., Salanovich TN, Antonov AI, et al (2007) Design of highly efficient cellulase mixtures for enzymatic hydrolysis of cellulose. Biotechnol Bioeng 97:1028–1038. doi: 10.1002/bit.21329
17. Hodge DB, Karim MN, Schell DJ, McMillan JD (2009) Model-Based Fed-Batch for High-Solids Enzymatic Cellulose Hydrolysis. Appl Biochem Biotechnol 152:88–107. doi: 10.1007/s12010-008-8217-0

18. Hsieh CC, Cannella D, Jørgensen H, et al (2014) Cellulase Inhibition by High Concentrations of Monosaccharides. J Agric Food Chem 62:3800–3805. doi: 10.1021/jf5012962
19. Intasit R, Cheirsilp B, Suyotha W, Boonsawang P (2021) Synergistic production of highly active enzymatic cocktails from lignocellulosic palm wastes by sequential solid state-submerged fermentation and co-cultivation of different filamentous fungi. Biochem Eng J 173:108086. doi: 10.1016/j.bej.2021.108086
20. Isikgor FH, Becer CR (2015) Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym Chem 6:4497–4559. doi: 10.1039/C5PY00263J
21. Jampana SR, Jia L, Ramarao B V., Kumar D (2021) Experimental investigation of the adsorption and desorption of cellulase enzymes on zeolite-β for enzyme recycling applications. Bioprocess Biosyst Eng 44:495–505. doi: 10.1007/s00449-020-02459-6
22. Kari J, Molina GA, Schaller KS, et al (2021) Physical constraints and functional plasticity of cellulases. Nat Commun 12:3847. doi: 10.1038/s41467-021-24075-y
23. Keskin Gündoğdu T, Deniz İ, Çalışkan G, et al (2016) Experimental design methods for bioengineering applications. Crit Rev Biotechnol 36:368–388. doi: 10.3109/07388551.2014.973014
24. King BC, Donnelly MK, Bergstrom GC, et al (2009) An Optimized Microplate Assay System for Quantitative Evaluation of Plant Cell Wall-Degrading Enzyme Activity of Fungal Culture Extracts. Biotechnol Bioeng 102:1033–1044. doi: 10.1002/bit.22151
25. McCann MC, Carpita NC (2015) Biomass recalcitrance: a multi-scale, multi-factor, and conversion-specific property. J Exp Bot 66:4109–4118. doi: 10.1093/jxb/erv267
26. Michelin M, Ruiz HA, Silva DP, et al (2014) Cellulose from Lignocellulosic Waste. In: Polysaccharides. Springer International Publishing, Cham, pp 1–33
27. Mosier N, Wyman C, Dale B, et al (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686. doi: 10.1016/j.biortech.2004.06.025
28. Obeng EM, Adam SNN, Budiman C, et al (2017) Lignocellulases: a review of emerging and developing enzymes, systems, and practices. Bioresour Bioprocess 4:16. doi: 10.1186/s40643-017-0146-8
29. Obeng EM, Brossette T, Ongkudon CM, et al (2018a) The workability of Escherichia coli BL21 (DE3) and Pseudomonas putida KT2440 expression platforms with autodisplayed cellulases: a comparison. Appl Microbiol Biotechnol 102:4829–4841. doi: 10.1007/s00253-018-8987-4
30. Obeng EM, Ongkudon CM, Budiman C, et al (2018b) An optimal blend of single autodisplayed cellulases for cellulose saccharification - a proof of concept. J Chem Technol Biotechnol 93:2719–2728. doi: 10.1002/jctb.5628
31. Pazhany AS, Henry RJ (2019) Genetic Modification of Biomass to Alter Lignin Content and Structure. Ind Eng Chem Res 58:16190–16203. doi: 10.1021/acs.iecr.9b01163
32. Rohrbach JC, Luterbacher JS (2021) Investigating the effects of substrate morphology and experimental conditions on the enzymatic hydrolysis of lignocellulosic biomass through modeling. Biotechnol Biofuels 14:103. doi: 10.1186/s13068-021-01920-2

33. Shen L, Su Y, Sun Y, et al (2021) Establishment of a highly efficient and low cost mixed cellulase system for bioconversion of corn stover by Trichoderma reesei and Aspergillus niger. Biocatal Agric Biotechnol 32:101849. doi: 10.1016/j.bcab.2020.101849
34. Sui W, Liu X, Sun H, et al (2021) Improved high-solid loading enzymatic hydrolysis of steam exploded corn stalk using rapid room temperature γ-valerolactone delignification. Ind Crops Prod 165:113389. doi: 10.1016/j.indcrop.2021.113389
35. Tozakidis IEP, Brossette T, Lenz F, et al (2016) Proof of concept for the simplified breakdown of cellulose by combining Pseudomonas putida strains with surface displayed thermophilic endocellulase, exocellulase and β-glucosidase. Microb Cell Fact 15:103–114. doi: 10.1186/s12934-016-0505-8
36. Tu M, Chandra RP, Saddler JN (2007) Evaluating the Distribution of Cellulases and the Recycling of Free Cellulases during the Hydrolysis of Lignocellulosic Substrates. Biotechnol Prog 23:398–406. doi: 10.1021/bp060354f
37. Vignesh N, Chandraraj K (2021) Improved high solids loading enzymatic hydrolysis and fermentation of cotton microdust by surfactant addition and optimization of pretreatment. Process Biochem 106:60–69. doi: 10.1016/j.procbio.2021.04.002
38. Voinovich D, Campisi B, Phan-Tan-Luu R (2009) Experimental Design for Mixture Studies. In: Comprehensive Chemometrics. Elsevier, pp 391–452
39. Wang Y, Fan C, Hu H, et al (2016) Genetic modification of plant cell walls to enhance biomass yield and biofuel production in bioenergy crops. Biotechnol Adv 34:997–1017. doi: 10.1016/j.biotechadv.2016.06.001
40. Ying W, Zhu J, Xu Y, Zhang J (2021) High solid loading enzymatic hydrolysis of acetic acid-peroxide/acetic acid pretreated poplar and cellulase recycling. Bioresour Technol 340:125624. doi: 10.1016/j.biortech.2021.125624
41. Zhang Y, Huang M, Su J, et al (2019) Overcoming biomass recalcitrance by synergistic pretreatment of mechanical activation and metal salt for enhancing enzymatic conversion of lignocellulose. Biotechnol Biofuels 12:12. doi: 10.1186/s13068-019-1354-6
42. Zhang Y, Liu Y-Y, Xu J-L, et al (2012) High solid and low enzyme loading based saccharification of agricultural biomass. BioResources 7:345–353.
43. Zhou J, Wang Y-H, Chu J, et al (2009) Optimization of cellulase mixture for efficient hydrolysis of steam-exploded corn stover by statistically designed experiments. Bioresour Technol 100:819–825. doi: 10.1016/j.biortech.2008.06.068

Figures

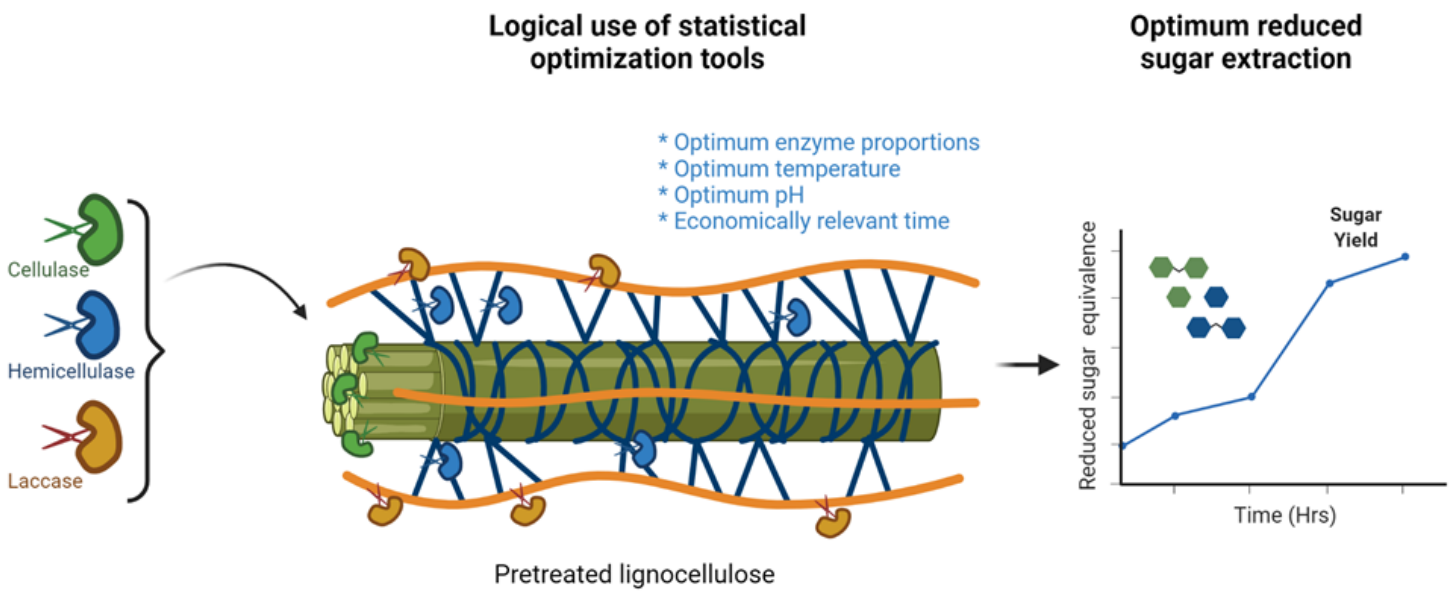

Figure 1

A systematic approach for optimizing cellulase cocktail

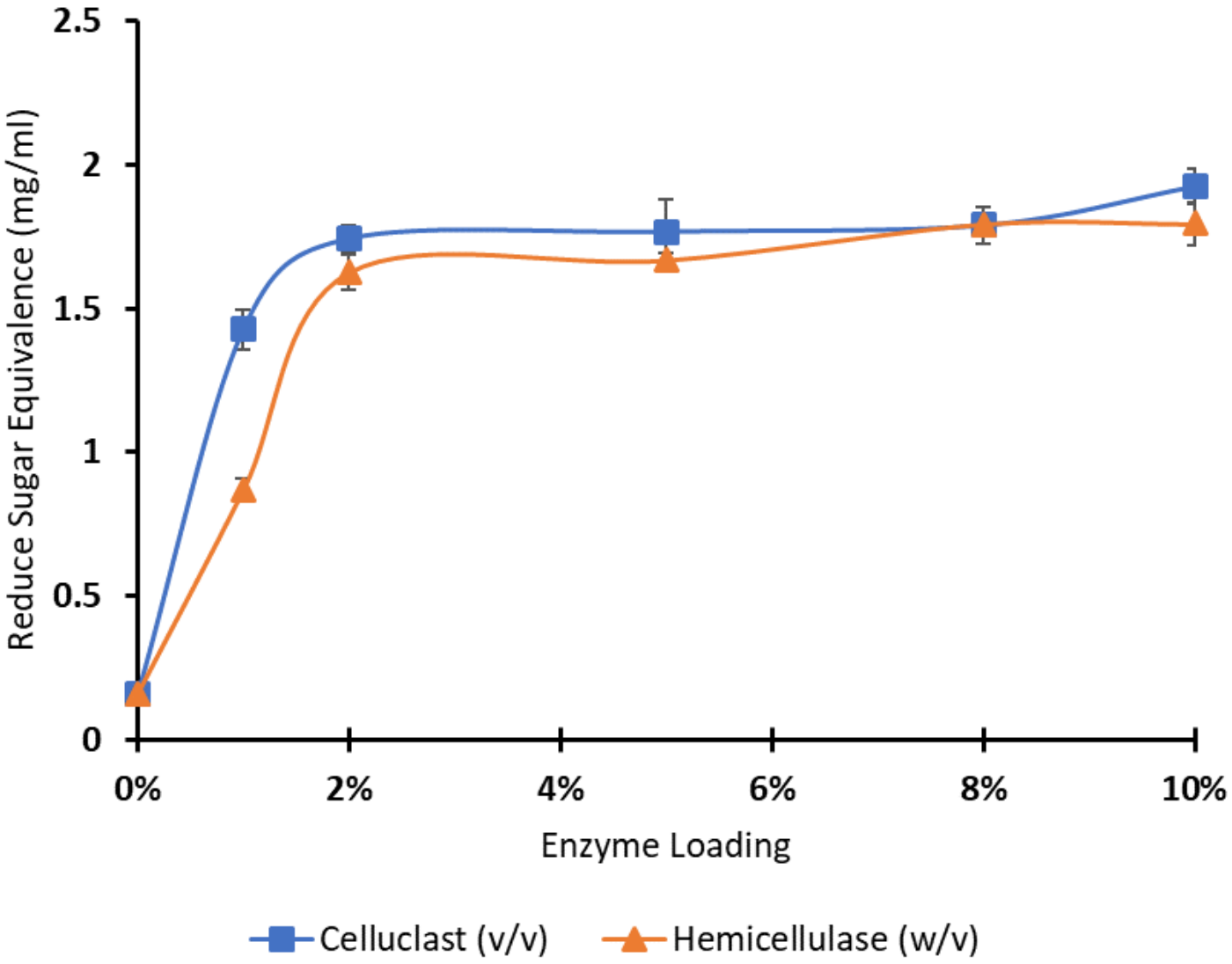

Figure 2

Reduced sugar profile for Celluclast and Hemicellulase

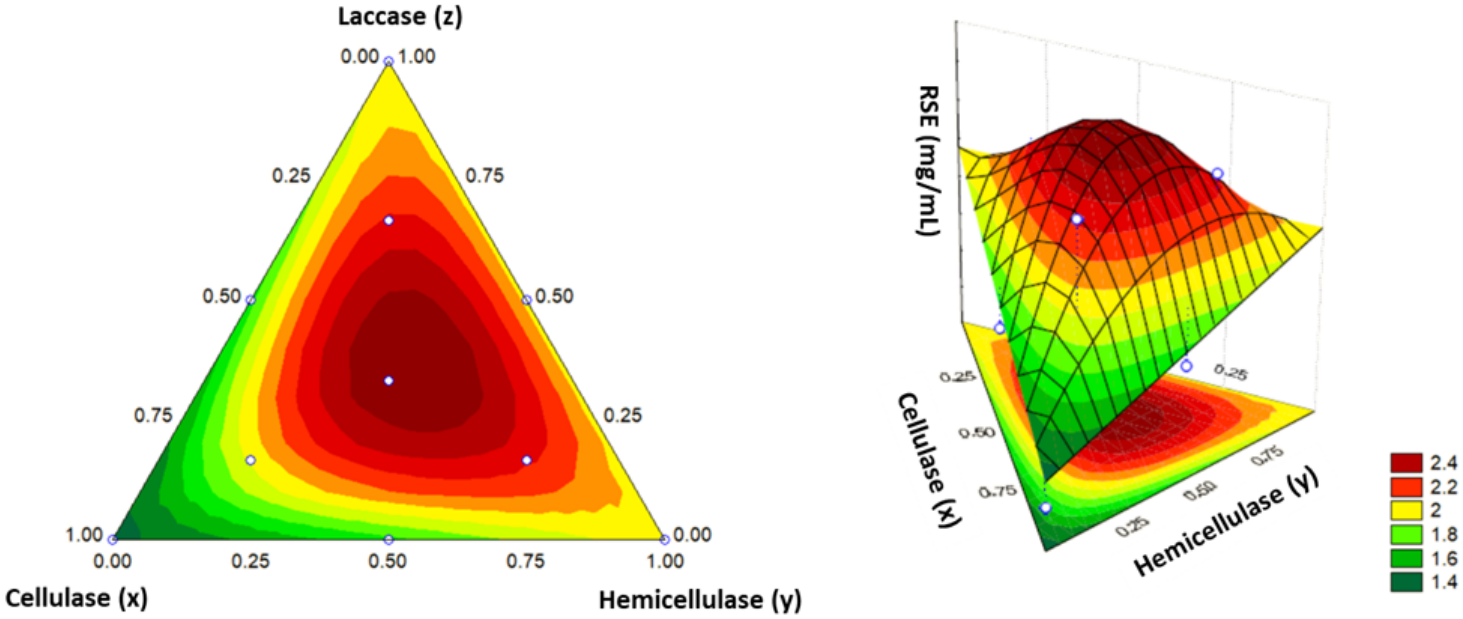

Figure 3

Ternary and surface plots for the Simplex lattice mixture design.

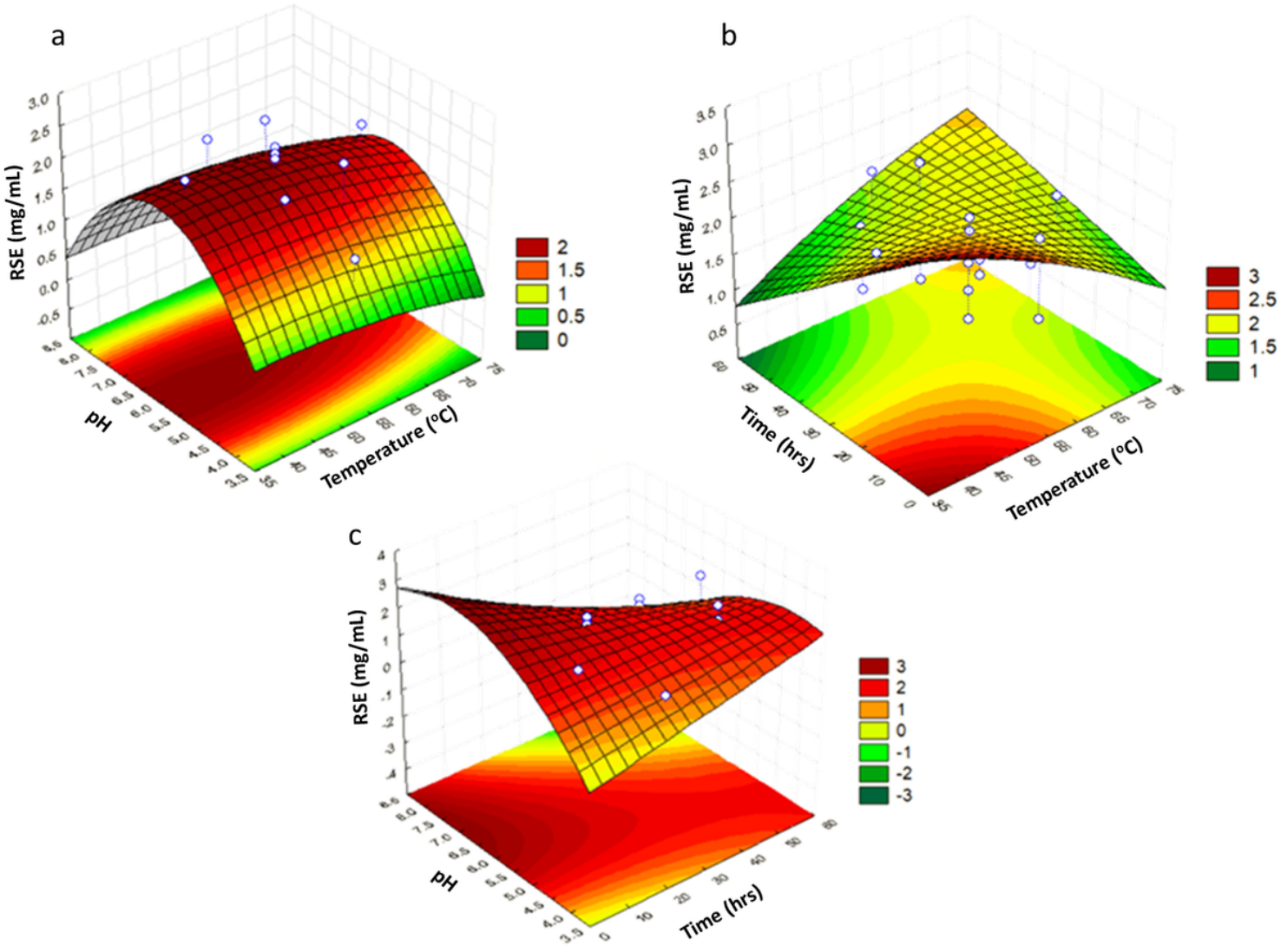

Figure 4

Surface plots for the reduced quadratic CCD. a: pH: Temp: RSE with time fixed at 24hrs; b: Time: Temp: RSE with pH fixed at 6; c: Time: pH: RSE with temperature fixed at 40 oC.

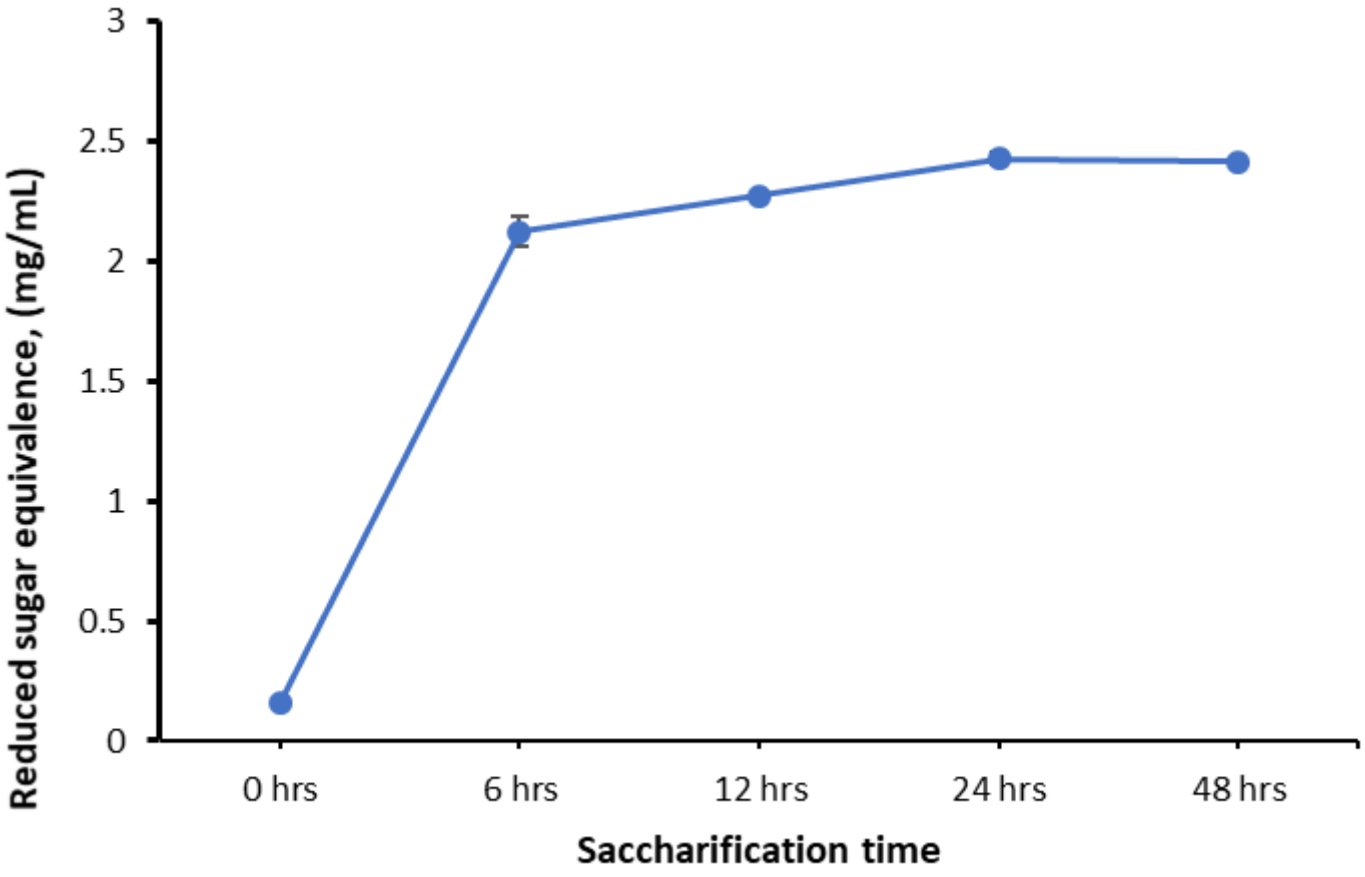

Figure 5

Time course study for the optimized enzyme blend. This data is based on technical replicates.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Obengetal2021SupplementaryInformation.docx
- Obengetal.2021Supplementarymaterial2.xlsx