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Multi-objective optimization of simultaneous saccharification and fermentation for cellulosic ethanol production

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ABSTRACT

A multi-objective optimization of simultaneous saccharification and fermentation process for cellulosic ethanol production was carried out to simultaneously maximize the ethanol yield/cellulose conversion and minimize the enzyme consumption by manipulating the initial sugar concentrations, and cellulose and enzyme loadings. The study was based on an experimentally verified kinetic model. Several bi-objective optimization problems with different combinations of objectives and constraints were solved by a controlled elitist genetic algorithm, a variant of the non-dominated sorting genetic algorithm II (NSGA-II). The optimum operating conditions were verified by experiments. There was significant performance improvement in terms of ethanol yield, cellulose conversion and enzyme loading. An overall 40% reduction of enzyme consumption per ethanol produced was attained at the same ethanol yield (32%) of a non-optimized process. However, the optimum conditions are highly sensitive to the selected kinetic model and associated kinetic parameters therefore, selection of the appropriate kinetic model is critical.

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1. Introduction

Second-generation bioethanol produced from lignocellulosic biomass, i.e., waste plant matter from forestry or agriculture is a potential alternative to fossil fuels due to its renewable nature and availability [1,2]. The bioconversion of nonedible polysaccharides (cellulose and hemicellulose) in agricultural residues to ethanol is commercially viable. However, bioconversion of woody biomass, from forestry residues, to ethanol has not yet been translated from demonstration scale due to high capital and operating costs [3,4]. In addition to the issues associated with removing the lignin, there are several technical challenges, e.g., low depolymerization efficiency of cellulolytic enzymes, high end-product inhibition and insufficient mixing at high substrate concentration which need to be overcome to make the cellulosic ethanol more competitive with fossil-based transportation fuels.

Although simultaneous saccharification and fermentation (SSF) helps to mitigate the inhibitory effect of converted sugars by in-situ ethanol fermentation [5,6], the performance (reaction conversion, final ethanol yield and concentration) is highly dependent on the type of lignocellulosic feedstock, the substrate concentration, the type and amount of cellulolytic enzymes and microorganisms, solution pH and reaction temperature among others [7]. Moreover, SSF is usually requires a high substrate loading to achieve a high enough ethanol concentration to make the process economically viable but high substrate loading limits mixing and mass transfer of the hydrolysis fermentation system and subsequently the overall performance of the process. Simultaneously optimizing substrate concentration and enzyme/microorganism loading could potentially minimize these transport phenomena limitations while maximizing ethanol formation [8–10].

Optimization of SSF process based on statistically designed experiments has been widely studied [11–16]. Benjamin et al. applied a central composite design (CCD) under response surface methodology to maximize the combined sugar yield and ethanol concentration for batch and fed-batch SSF of sugarcane [13]. A three-factor-three-level Box-Behnken design was employed to predict the optimum substrate concentration, enzyme loading, and inoculum size for maximum ethanol yield from cassava peel [14]. Cavalaglio and co-workers [15] identified the optimal water-insoluble substrate amount, optimal liquid fraction and enzyme loading of a SSF bioconversion of *Phragmites australis* through CCD. A Taguchi orthogonal array design was implemented by Das et al. to find the optimum operation conditions (cellulose and

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hemicellulose loading, yeast amount, solution pH and temperature) for ethanol production from *Eichhornia crassipes* [16]. These studies outline the main impacts and interaction of the key operating parameters of SSF process. However, the accuracy of optimization results relies strongly on the design of the set of experiments and therefore difficult to compare or draw major trends from. Furthermore, response surfaces are valid only in range of parameters studied and therefore cannot be applied to wider ranges directly. Systematic optimization of SSF process based on mechanistic mathematical model is more attractive as it provides more reliable and accurate predictions of system performance.

In contrast to the many studies using response surface methodology, optimization using mechanistic kinetic and reactor models is less well studied. Wang et al. assessed the effects of substrate, enzyme and cell feeding strategies on fed-batch simultaneous saccharification and co-fermentation (SSCF) of SO_2-catalyzed steam pre-treated wheat straw [17]. With the model-based multi-feed strategy, SSCF is capable of handling high levels of water insoluble solids (WIS), up to 22% (w/w), producing a final ethanol concentration of 57.3 g/L [17]. Unrean and co-workers developed a model to quantitatively characterize the dynamic responses of yeast cell growth, hydrolysis and fermentation kinetics in a fed-batch SSF process [10] and achieves a final ethanol concentration of 65.0 g/L and ethanol yield over 60%. Liu et al. optimized the reaction temperature of a SSF process for the maximum ethanol production by incorporating a temperature-dependent kinetic model [18]. A maximum ethanol concentration of 89.3 g/L was achieved by initiating the SSF experiment at a low temperature of 26 °C and holding for 30 h, followed by a rapid temperature increase to 31.9 °C, and a slow decrease to 18 °C after 65 h [18]. All these optimization studies for bioethanol production involved a single objective function (ethanol concentration or ethanol yield) without considering the costs associated with enzyme consumption. Maximum ethanol yield and minimum enzyme loading can't be achieved simultaneously. Optimization studies incorporating these conflicting objectives would be invaluable to process engineers and decision makers.

In the current work, multi-objective optimization (MOO) of SSF to maximize the cellulose conversion/ethanol yield and to minimize enzyme loading was carried out based on enzymatic hydrolysis kinetics and a dynamic metabolic model of yeast cell. To the best of our knowledge, the present work is the first attempt to investigate the improvement of SSF performance by systematic multi-objective optimization using a validated kinetic model. After the careful assessment of the interactions between of substrate concentration and enzyme loading, several bi-objective optimization problems were defined and solved by a controlled elitist genetic algorithm for Pareto optimal solutions. Fast convergence to the true Pareto optimal fronts and well-distributed solutions were obtained in three case studies with different combinations of objectives and constraints. The reliability and accuracy of the bi-objective optimization of SSF were verified by comparing the experimental results with the model predictions. This optimization study not only gives us deeper insight into interactions of key operating parameters of SSF process, but also provides a methodology to balance these interactions into an optimized process.

2. Multi-objective optimization of SSF

2.1. Kinetic modeling of SSF process

A model to predict the ethanol production in SSF is prerequisite for the optimization investigation. An integrative model combining the kinetic of enzymatic hydrolysis and dynamic fermentative metabolism model developed by Philippidis et al. [19–21] and Shadbahr et al. [22] was employed in this study. This model considers the fermentation of glucose and mannose alongside simultaneous enzymatic hydrolysis of cellulose to glucose, and is capable of predicting the dynamic profiles of released sugars and ethanol over wide range of operating conditions [21]. Detailed kinetic and reactor model as well as the methodology used for determination of the reaction kinetic parameters were presented in our previous study [22]. The model and kinetic parameters used here are included in the Appendix for brevity.

2.2. Formulation of optimization problems

The SSF process involves the interaction between the enzyme which hydrolyses solid substrate to sugars and yeast which utilizes the sugars for growth and fermentation [23]. Optimal operation of SSF relies on balancing the rates of hydrolysis and fermentation, which can be achieved by proper selection of the initial substrate loading, the enzyme dosage and inoculum size. The conversion of cellulose (X), the final ethanol yield (Y) and/or ethanol concentration ($[E]_f$) are the key performance parameters of SSF process. The operating cost of SSF is impacted most dramatically by the enzyme loading. As such, maximization of ethanol yield is the main objective function for the optimization of SSF process. This objective can be further specified to maximization of cellulose conversion, an important indicator of the depolymerization efficiency of cellulolytic enzymes. Minimization of enzyme consumption, which is essentially in conflict with the first objective, can be considered as another objective function. Several combinations of the two objective functions are outlined in Table 1. Definitions of the objective functions considered in this study are listed below.

Cellulose conversion: $X = \frac{[C]_0 - [C]_f}{[C]_0} \times 100\%$

Ethanol yield: $Y = \frac{[E]_f - [E]_0}{0.511 \times ([G]_0 + [M]_0 + 1.111[C]_0)} \times 100\%$

Enzyme consumption per 1 g/L ethanol produced: $Z = [enzc] \big/ [E]_f$

Where $[C]_0$ (w/v) is the cellulose loading; $[G]_0$, $[M]_0$ and $[E]_0$ (g/L) are the initial concentration of glucose, mannose and ethanol; $[C]_f$ and $[E]_f$ (g/L) are the final concentration of cellulose and ethanol; and $[enzc]$ is the enzyme loading (FPU/g cellulose).

Important operating parameters in SSF process include initial concentration of fermentable sugars, cellulose loading, dosage of the enzymes, and the loading of yeast strain. Earlier experiments indicate that SSF performance was not significantly influenced by the dosage of β-glucosidase and the loading of yeast strain [19,22,24]. Therefore, in this multi-objective optimization study, three variables (initial concentration of fermentable sugars, cellulose loading, and dosage of cellulase) were used as decision variables due to the significant impact on the performance of SSF. The lower and upper bounds of the decision variables used for the optimization are summarized in Table 1 and are based on the

Table 1
Optimization problem formulations for SSF of cellulose.

Case	Objectives	Constraints	Decision variables
I	Max $I_1(u) = X$ Min $I_2(u) = Z$	$[E]_f \geq 12$ g/L	$5.0 \leq [G]_0 \leq 10.0$ (g/L) $[M]_0 = 0.9\ [G]_0$ $5.0 \leq [C]_0 \leq 8.0\%$ (w/v) $10.0 \leq [enzc] \leq 20.0$ (FPU/g cellulose)
II	Max $I_1(u) = Y$ Min $I_2(u) = Z$	$[E]_f \geq 12$ g/L	Same as Case I
III	Max $I_1(u) = Y$ Min $I_2(u) = [enzc]$	$Y \geq 30\%$ or $X \geq 20\%$	Same as Case I

experimental stability and process economy of SSF process reported in open literature [20,22,24]. To achieve a better and fast convergence of Pareto optimal solutions, a constraint was defined in each bi-objective optimization problem. The optimization problem formulation is summarized in Table 1.

2.3. Controlled elitist multi-objective genetic algorithm

The genetic algorithm (GA), an adaptive heuristic search method based on population genetics, has been proved to be one of the most robust optimizers for MOO problems [25–28]. GA mimics the principles of natural genetics and natural selection in solving optimization problems through four basic operators, namely inheritance, cross-over, reproduction and mutation [29,30]. However, GA sometimes fails to address complex high dimensional multimodal problems where fitness function evaluation becomes computationally complex [31]. In this study, a controlled elitist GA, which not only improves the chance of finding global optimal solutions but also increases the diversity of the population is employed to solve bi-objective optimization problems. Bi-objective optimization problems listed in Table 1 were performed by implementing "gamultiobj" tool (controlled elitist GA) provided by MATLAB R2016b.

3. Experimental method

Experimental investigations of batch SSF process were carried out to verify the optimization results. The experimental methods with respect to feedstock and enzyme compositions, yeast preparation, SSF experiments, and analytical method are explained elsewhere [22] and not included here for brevity. The SSF experiments were performed at 37 °C in 250 mL jacketed flask with 100 mL active volume and the solution pH maintained at 5.0 over the 96 h reaction time. Other operating conditions are summarized in Table 2. Exps. #1 and #2 in Table 2 were repeated to ensure reproducibility of the experiments. The results of Exps. #1 and #2 are the average values from two runs.

4. Results and discussion

The solutions of bi-objective problems listed in Table 1 give rise to Pareto-optimal sets and the range of trade-offs between the competing objectives. The predicted enhancement of SSF performance by bi-objective optimization was verified by experiment using optimal operating conditions.

4.1. Case I: maximization of cellulose conversion and minimization of enzyme consumption per ethanol produced

Fig. 1a presents the Pareto optimal solutions for simultaneous maximization of cellulose conversion and minimization of enzyme consumption per 1 g/L ethanol produced (hereinafter referred to unit enzyme consumption). Not surprisingly, the objectives are in conflict – i.e., cellulose conversion increases at increased unit enzyme consumption. Moreover, there is an upper bound of cellulose conversion (roughly 30%) which cannot be further improved by increasing the enzyme loading and/or varying the feedstock conditions.

Each point on the Pareto optimal front corresponds to a set of decision variables, which are plotted in Figs. 1b-d. Fig. 1b illustrates that higher cellulose loading resulted in reduced cellulose conversion even though higher enzyme loading was used. The effect of cellulose loading on cellulose conversion is mainly caused by two factors. Firstly, inhibition of cellulose hydrolysis by cellobiose, glucose and ethanol is more severe as cellulose loading increases, leading to a reduced conversion of cellulose. Secondly, cellulase activity is profoundly influenced by the direct physical contact between cellulolytic enzyme and substrate. Higher solid loading limits the cellulose accessibility to cellulase, which limits the effectiveness of cellulase. Therefore, to maximize cellulose conversion, lower cellulose loading with relatively higher enzyme loading would be an option. A similar impact of initial sugar concentrations (glucose and mannose) on cellulose conversion is also observed (Fig. 1c). At higher initial concentrations of sugars there is a resulting increase in inhibition of hydrolysis by the end product. Fig. 1d summarizes the optimum enzyme loading as a function of cellulose conversions. In general, increasing enzyme loading (ignoring sugars concentration and cellulose loading) improves the hydrolysis rate, particularly at lower initial sugar concentrations. From Fig. 1e, it is clear operating conditions leading to highest final ethanol concentration were different with those for maximum cellulose conversion.

In order to confirm that the obtained Pareto solutions of Case I are the true global optimal solutions, optimization was also carried out on divided domains of sugar concentrations and cellulose loading. That is, optimization was performed at low sugar (5.0–7.5 g/L glucose and 4.5–6.75 g/L mannose) and high sugar concentrations (7.5–10.0 g/L glucose and 6.75–9.0 g/L mannose), low cellulose (5.0–6.5% w/v) and high cellulose loading (6.5–8.0% w/v) separately while keeping the same lower and upper limits for the other two variables. Fig. 2 compares the results of five different optimization scenarios. The converged Pareto optimal solutions of Case I are the global optimums with respect to the two objectives in the defined searching domain (Fig. 2).

Batch SSF experiment (Exp. #1) under the calculated optimal operating conditions was carried out to validate the optimization results. The maximum optimized final ethanol concentration was 14.11 g/L and ethanol yield was 25.60%. The experimental average final ethanol concentration and ethanol yield (Exp. #1) are 13.8 g/L and 25.03%, very close to the optimization predictions. This confirms the approach of using systematic multi-objective optimization in SSF analysis.

4.2. Case II: maximization of ethanol yield and minimization of enzyme consumption per ethanol produced

With our validated model, the next step is to determine conditions where ethanol yield is maximized and the unit enzyme consumption minimized. The same constraint and ranges of decision variables as those of Case I were used. Well-distributed Pareto optimal solutions and the corresponding optimal sets of decision variables are illustrated in Fig. 3. A trade-off exists between the two objectives; it is challenging to reduce the unit enzyme consumption without sacrificing the ethanol yield. Similar to Case I, ethanol yield cannot be continuously increased by optimizing the operating conditions and there is an upper limit of ethanol yield for batch SSF.

As seen from Fig. 3b, optimal solutions for initial glucose concentration converge to the higher bound (logically higher initial sugar concentrations tend to increase ethanol yield). Maximizing ethanol yield as an objective forces the optimizer to restrict the

Table 2
Optimal operating conditions of SSF process for experimental validations.

Exp. #	$[G]_0$ (g/L)	$[M]_0$ (g/L)	$[C]_0$% (w/v)	[*enzc*] (FPU/g cellulose)
1	10.0	9.0	8.0	10.0
2	10.0	9.0	5.32	12.23
3	6.88	6.19	5.07	10.0

* The activity for the β-glucosidase and yeast loading were fixed at 30 U/g cellulose and 5.0 g dry cell/L for all experiments.

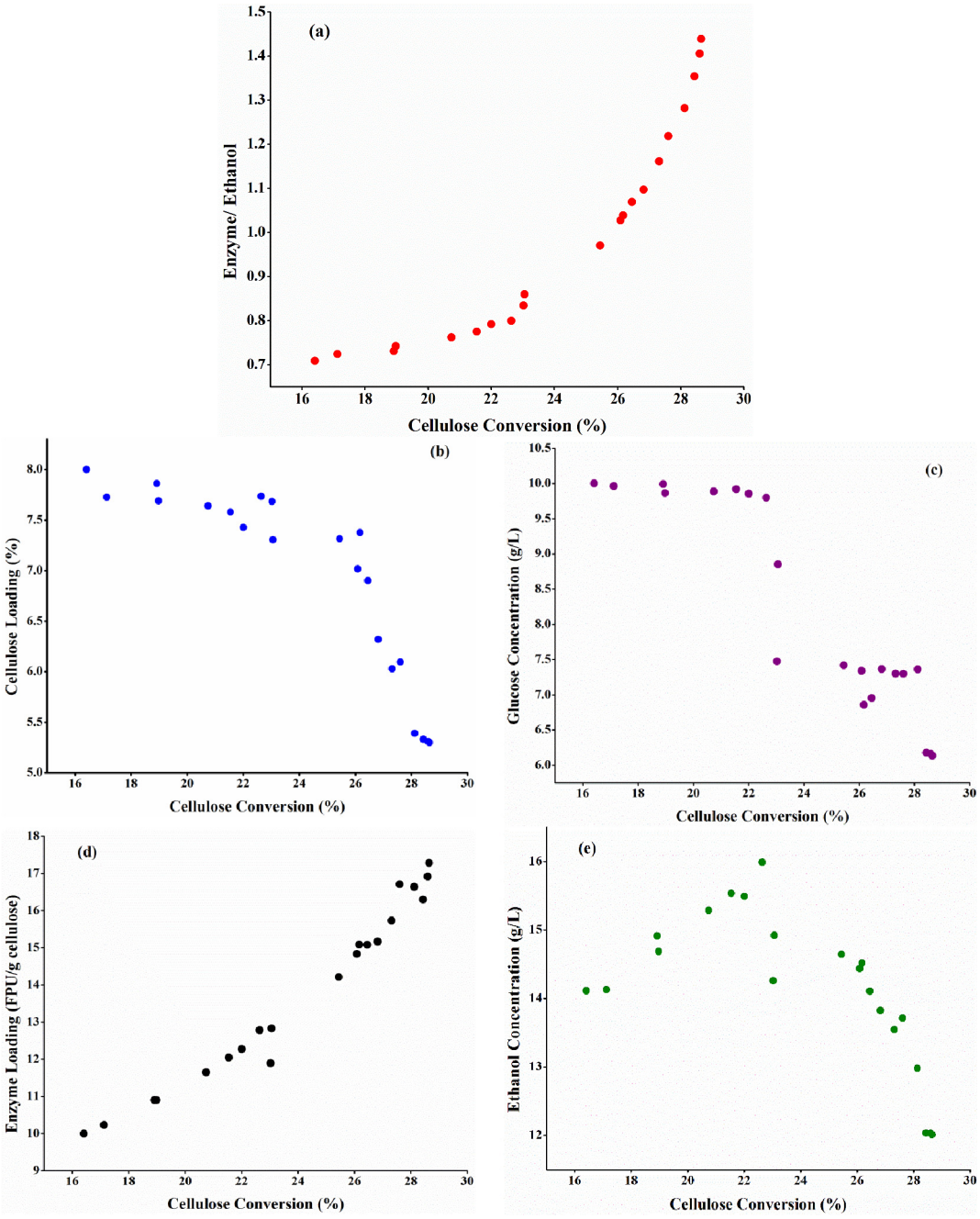

Fig. 1. Pareto optimal solutions and corresponding decision variables for Case I, (a) objectives trade-offs, cellulose conversion vs (b) cellulose loading, (c) glucose concentration, (d) enzyme loading, and (e) ethanol concentration.

sugar concentration to a narrow range. A significant scatter in other two decision variables, $[C]_0$ and [*enz*] accompanied the convergence of Pareto solutions. As illustrated from Figs. 3c and d, different combinations of cellulose loading and enzyme loading are able to generate the same or very close objective values (points A, B and C in Fig. 3a), the set of solutions ultimately chosen (by the optimizer) for generating the Pareto optimal points is determined by parameters randomly generated and convergence by the optimization algorithm. Fig. 3 demonstrates the impact of the variables on the targeted objectives. For example, when low cellulose loading and high concentration of fermentable sugars (glucose and mannose) are selected, increasing enzyme loading results in higher ethanol yield and/or lower unit enzyme consumption. However, if high cellulose loading and sugar concentrations are used, increasing enzyme loading has little impact on ethanol yield due to the severe product inhibition.

The optimized variables from Case II were also verified by batch SSF process under the operating conditions of Exp. #2 (Table 2). This time, small deviations from the calculated optimum objective values were obtained. The predicted ethanol yield and unit enzyme consumptions are 34.06% and 0.89 FPU L/g-g, comparable to the average experimental values of 33.78% and 0.90 FPU L/g-g respectively. The enhancement of SSF performance through optimization (Fig. 4) was assessed by comparing ethanol yields and unit enzyme consumptions from three non-optimized experiments [22]. Experimental point D is certainly better than points E and F in

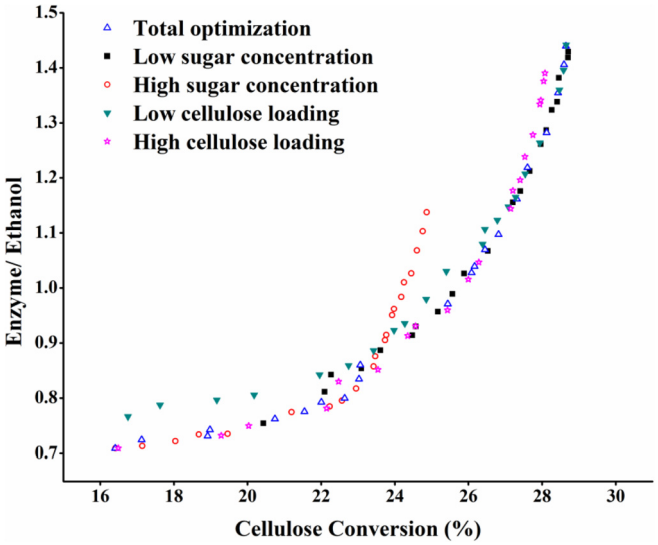

Fig. 2. Comparison of optimization results at different ranges of sugar and cellulose concentrations.

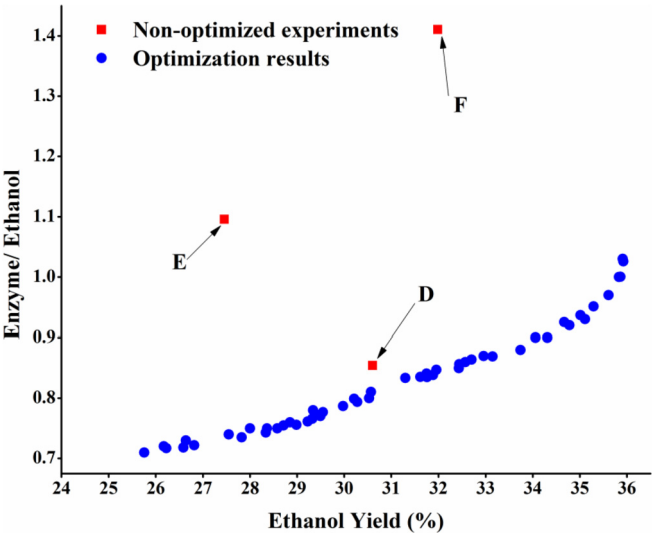

Fig. 4. Enhancement of SSF performance by optimization in Case II.

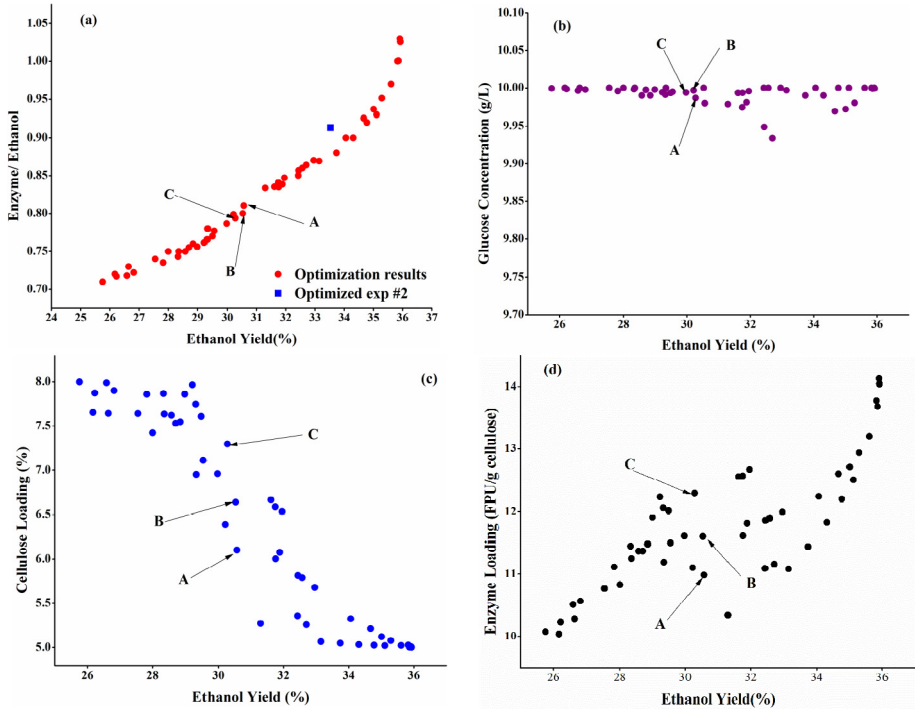

Fig. 3. Pareto optimal solutions and corresponding decision variables for Case II with experimental validation. (a) objectives trade-offs, ethanol yield vs (b) glucose concentration, (c) cellulose loading, and (d) enzyme loading.

terms of the two objectives. However, all the points on the Pareto set are better than the experimental data, leading to increase of ethanol yield or remarkable reduction in unit enzyme consumption.

4.3. Case III: maximization of ethanol yield and minimization of enzyme loading

The total amount of enzyme consumption is one of the key factors impacting the overall cost of bioethanol production by SSF. To study this in a third case, maximization of ethanol yield and minimization of enzyme loading were investigated under two scenarios with different constraints: (I) ethanol yield not less than 30% ($Y \geq 30\%$); and (II) cellulose conversion not lower than 20% ($X \geq 20\%$). Comparison of the optimization results for the two scenarios is presented in Fig. 5.

Fig. 5a show the converged Pareto optimal solutions from the two scenarios differ slightly. For constrained optimization problems, constraints can be considered as high-priority (hard) objectives which must be satisfied before the optimization of the remaining soft objectives (ethanol yield and enzyme loading in this case) takes place [32]. As such, as one varies the high priority

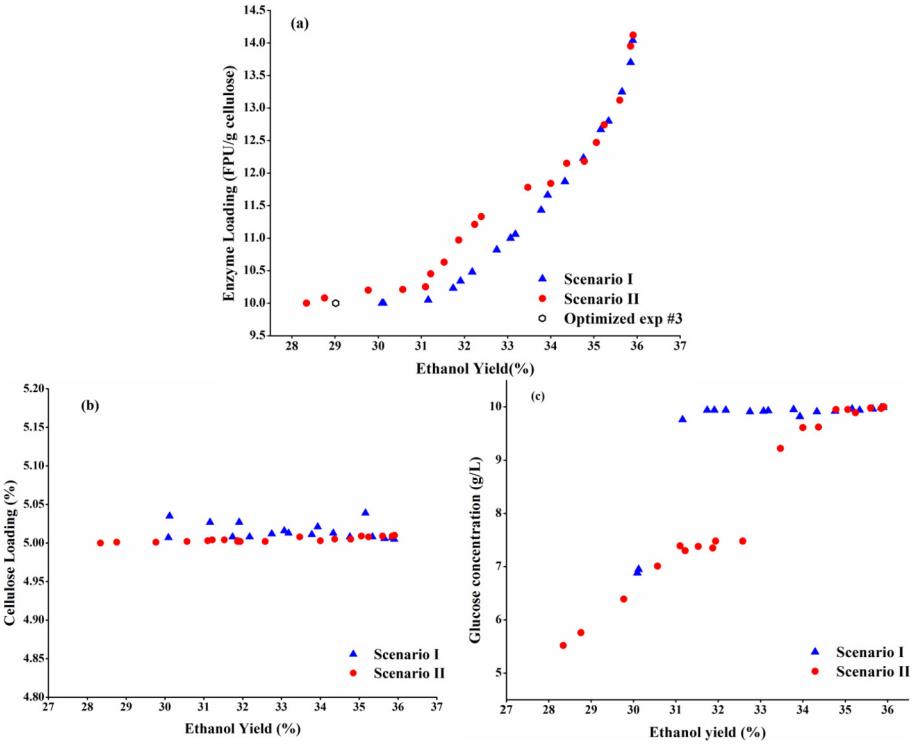

Fig. 5. Pareto optimal solutions and corresponding decision variables of Case III. (a) objectives trade-offs, ethanol yield vs (b) cellulose loading, and (c) glucose concentration.

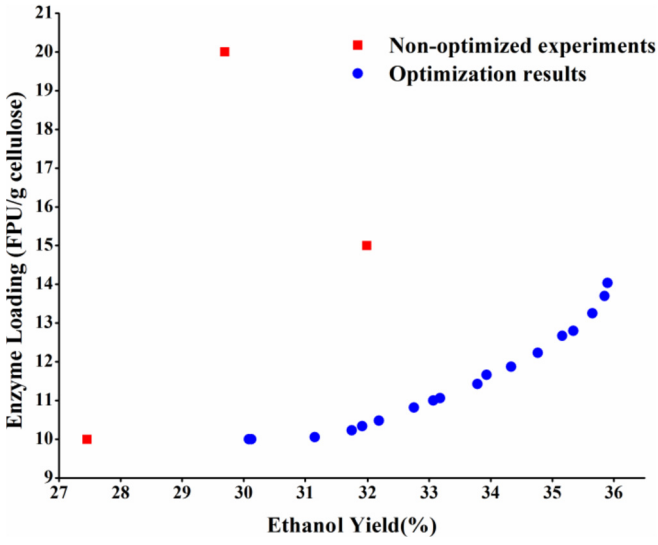

Fig. 6. Enhancement of SSF performance by optimization in Case III.

objectives from $Y \geq 30\%$ to $X \geq 20\%$ the solution changes. For instance, when lower enzyme loadings ($\leq$12.0 FPU/g) are selected, the constraint of $Y \geq 30\%$ forces higher sugar concentrations and lower cellulose loading to achieve maximum ethanol yield, whereas $X \geq 20\%$ forces the optimizer to select lower sugar and cellulose concentrations. This reveals that increasing sugar concentrations in the feedstock are favorable to the attainment of maximum ethanol yield at low enzyme loading. However, when enzyme loading is higher than 12.0 FPU/g both scenarios converged to the same Pareto front.

Enzyme loading converged to values lower than 14.5 FPU/g and cellulose loading converged to lower bound in both scenarios, indicating that maximum ethanol yield or cellulose conversion cannot be achieved by purely increasing the enzyme loading. Balanced rates of hydrolysis and fermentation rates as a result of proper combination of feedstock condition and enzyme loading are essential for the optimal operation of batch SSF. This observation is in agreement with the conclusions of experimental investigators [8,33,34].

Experimental validation of optimization results for Case III was also performed. Under the same operating conditions, predicted ethanol concentration by optimization is 10.56 g/L and corresponding to ethanol yield of 30.09%, again in good agreement with experimental values from Exp. #3 of 10.29 g/L and 29.32%. Enhancement of SSF performance with respect to maximizing ethanol yield and minimizing enzyme loading by optimization can be shown by comparison of our previous (non-optimized) experimental results (Fig. 6) [22]. To achieve the same ethanol yield, total enzyme consumption can be reduced more than 50% by optimization.

5. Conclusion

Multi-objective optimization of SSF for bioethanol production was investigated in this study by employing the controlled elitist GA. In Case I, the objective was to maximize cellulose conversion while simultaneously minimizing enzyme consumption per ethanol produced. Results indicate that cellulose conversion increases as the unit enzyme consumption increases, whereas conversion decreases at increasing cellulose loading. In Case II, optimization were performed to maximize ethanol yield and minimize enzyme consumption per ethanol produced. A reduction in the unit enzyme consumption cannot be achieved without sacrificing the ethanol yield. Under high cellulose loading conditions, increasing enzyme loading has little impact on ethanol yield. In Case III, the optimum condition(s) where ethanol yield was maximized and enzyme loading minimized were determined. The data indicate a combination of low cellulose loading and high initial sugar concentration favours high ethanol yield at low enzyme loading.

Batch SSF experiments conducted under the predicted optimal operating conditions were used to verify the optimization results. Good agreement between the experimental measurements and optimization predictions was obtained, indicating that performance enhancement of SSF is attainable by systematic optimization based on reliable and robust kinetic models. The results and findings of this study can further be applied in a pilot plant to evaluate the performance of process in a larger scale.

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Appendix

The reaction network of the studied SSF process follows the route below:

$$Cellulose(C) \xrightarrow{r_1} Cellobiose(B) \xrightarrow{r_2} Glu\cos e(G) \xrightarrow{r_X, r_G} Ethanol(E) \& Cellmass(X)$$

$$Mannose(M) \xrightarrow{r_X, r_M} Ethanol(E) \& Cellmass(X)$$

Scheme A.1. Reaction network of SSF process.

The rate equations of the listed reactions in Scheme A.1 are as follows:

$$r_1 = \frac{k_1'[C]e^{-\lambda t}}{1 + [B]/K_{1B} + [G]/K_{1G}} \left(\frac{K_{1E}}{K_{1E} + [E]}\right) \text{ where } k_1' = \frac{k_1[enzc]}{K_{eq} + [enzc]} \tag{A.1}$$

$$r_2 = \frac{k_2[enzg][B]}{K_M(1 + [G]/K_{2G}) + [B]}(1 - K_L[L]) \tag{A.2}$$

$$r_X = \mu_m[X]\left(\frac{[G] + [M]}{K_G + [G] + [M]}\right)\left(\frac{K_E}{K_E + [E]}\right) \tag{A.3}$$

$$r_G = \frac{[G]}{[G] + [M]}\left(\frac{r_X}{Y_{XG}} + m_s[X]\right) \tag{A.4}$$

$$r_M = \frac{[M]}{[G] + [M]}\left(\frac{r_X}{Y_{XG}} + m_s[X]\right) \tag{A.5}$$

Kinetic parameters used in the optimization studies can be found in Table A.1. The mass balance equations of major components in the SSF process are listed below:

$$\frac{d[C]}{dt} = -r_1 \tag{A.6}$$

$$\frac{d[B]}{dt} = 1.056r_1 - r_2 \tag{A.7}$$

$$\frac{d[G]}{dt} = 1.053r_2 - r_G \tag{A.8}$$

$$\frac{d[M]}{dt} = -r_M \tag{A.9}$$

$$\frac{d[X]}{dt} = r_X \tag{A.10}$$

$$\frac{d[E]}{dt} = 0.511(r_G + r_M) \tag{A.11}$$

Table A.1
Kinetic parameters of the SSF process

Kinetic Parameters		Low sugar level	High sugar level
k_1	h^{-1}	0.165 −0.256	0.043–0.074
λ	h^{-1}	0.058 −0.064	0.019–0.039
K_{eq}	FPU/g	117.90	117.81
k_2	g/U·h	0.24 −0.33	0.19–0.20
$\mu_{m,}$	h^{-1}	0.18 −0.21	0.39–0.40

Other Parameters					
K_{1B}	g/L	5.85	K_M	g/L	10.56
K_{1E}	g/L	50.35	K_G	g/L	3.73×10^{-5}
K_{1G}	g/L	53.16	K_L	L/g	0.0053
K_{2G}	g/L	0.62	m_s		0
K_E	g/L	50	Y_{XG}	g/g	0.113

Nomenclature

$[B]$	concentration of cellobiose (g/L)
$[C]$	concentration of cellulose (g/L)
$[E]$	concentration of ethanol (g/L)
$[enzc]$	cellulase activity concentration (FPU/g cellulose)
$[enzg]$	β-glucosidase activity concentration (U/g cellulose)
$[G]$	concentration of glucose (g/L)
k_1	maximum specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_1'	lumped specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_2	specific rate of cellobiose hydrolysis to glucose (g/U·h)
K_{eq}	cellulase adsorption saturation constant (FPU/g cellulose)
K_E	ethanol inhibition constant for the microorganism (g/L)
K_G	glucose saturation constant for the microorganism (g/L)
K_L	constant for β-glucosidase adsorption to lignin (L/g)
K_m	cellobiose saturation constant for β-glucosidase (g/L)
K_{1B}	inhibition constant of cellulase by cellobiose (g/L)
K_{1E}	inhibition constant of cellulase by ethanol (g/L)
K_{1G}	inhibition constant of cellulase by glucose (g/L)
K_{2G}	inhibition constant of β-glucosidase by glucose (g/L)
$[L]$	concentration of lignin (g/L)
$[M]$	concentration of mannose (g/L)
m_s	specific rate of substrate consumption for maintenance requirements (h^{-1})
r_G	volumetric rate of glucose consumption (g/L·h)
r_M	volumetric rate of mannose consumption (g/L·h)
r_X	volumetric rate of cell mass production (g/L·h)
r_1	volumetric rate of cellulose hydrolysis to cellobiose (g/L·h)
r_2	volumetric rate of cellobiose hydrolysis to glucose (g/L·h)
t	time (h)
$[X]$	concentration of cell mass (g/L)
Y_{XG}	yield coefficient of cell mass from glucose (g/g)
λ	rate of decrease in cellulose specific surface area (h^{-1})
μ_m	maximum specific growth rate of the microorganism (h^{-1})

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