Biotechnology Reports 24 (2019) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

# Bioethanol production from sugarcane leaf waste: Effect of various optimized pretreatments and fermentation conditions on process kinetics

Preshanthan Moodley, E.B. Gueguim Kana*

*University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg, South Africa*

ARTICLE INFO

*Article history:*
Received 29 September 2018
Received in revised form 3 January 2019
Accepted 21 March 2019

*Keywords:*
Lignocellulosic bioethanol
Microwave pretreatment
Sugarcane
Fermentation kinetics
Inorganic salt pretreatment

ABSTRACT

This study examines the kinetics of *S. cerevisiae* BY4743 growth and bioethanol production from sugarcane leaf waste (SLW), utilizing two different optimized pretreatment regimes; under two fermentation modes: steam salt-alkali filtered enzymatic hydrolysate (SSA-F), steam salt-alkali unfiltered (SSA-U), microwave salt-alkali filtered (MSA-F) and microwave salt-alkali unfiltered (MSA-U). The kinetic coefficients were determined by fitting the Monod, modified Gompertz and logistic models to the experimental data with high coefficients of determination $R^2 > 0.97$. A maximum specific growth rate ($\mu_{max}$) of 0.153 $h^{-1}$ was obtained under SSA-F and SSA-U whereas, 0.150 $h^{-1}$ was observed with MSA-F and MSA-U. SSA-U gave a potential maximum bioethanol concentration ($P_m$) of 31.06 g/L compared to 30.49, 23.26 and 21.79 g/L for SSA-F, MSA-F and MSA-U respectively. An insignificant difference was observed in the $\mu_{max}$ and $P_m$ for the filtered and unfiltered enzymatic hydrolysate for both SSA and MSA pretreatments, thus potentially reducing a unit operation. These findings provide significant insights for process scale up.
© 2019 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

## 1. Introduction

Global energy demand is currently met by fossil fuels with more than 80% of the total energy market comprising of these conventional sources. The transport sector alone accounts for 60% of the total usage [1]. However, the finite supply of these fossil fuels and their contribution to greenhouse gas emission upon combustion are major challenges. It is therefore necessary to obtain an alternative source of energy [2].

Lignocellulosic biomass is considered an important feedstock for biofuel production in mitigating fossil fuel dependence and its related greenhouse gas emissions ([3,4]). Agricultural wastes, such as sugarcane leaves are currently a major problem for agriculture from an environmental standpoint, thus its conversion to biofuels is highly advantageous [5]. Second generation bioethanol is one such fuel and is considered clean, affordable and sustainable with the inherent capacity to replace conventional fuel [6]. In contrast, first generation bioethanol utilizes edible feedstocks thereby contributing to the food versus fuel debate [7].

Microorganisms such as *Saccharomyces cerevisiae* are often employed in the production of bioethanol, thus playing a key role in the fermentation process. However, due to the recalcitrant properties of lignocellulosic biomass, these microorganisms are unable to hydrolyse or access the glucose polymer, cellulose. Furthermore, enzymatic hydrolysis is also hampered due to the complexities in the lignocellulosic structure [1]. For this reason, the biomass has to undergo an effective pretreatment prior to fermentation [8]. Our previous work established a steam and microwave-assisted sequential salt-alkali pretreatment (SSA and MSA respectively) which effectively enhanced enzymatic hydrolysis [9]. However, the effect of steam and microwave pretreatment could significantly impact on the process kinetics and ultimately the scale-up efficiency and productivity. Currently, there is a scarcity of studies comparing the effect of steam and microwave pretreatment of sugarcane leaves on bioethanol production kinetics using *Saccharomyces cerevisiae* BY4743.

Two main fermentation modes have been frequently reported for bioethanol production, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) [10]. In the SHF process, the pretreated material is hydrolysed to simple sugars and subsequently undergoes fermentation. A major advantage of this process is it allows independent optimization of the enzymatic and fermentation phase to maximize sugar and

* Corresponding author.
*E-mail address:* kanag@ukzn.ac.za (E.B. Gueguim Kana).

https://doi.org/10.1016/j.btre.2019.e00329
2215-017X/© 2019 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**Nomenclature**

SSA-F Steam saltsalt-alkali filtered enzymatic hydrolysate
SSA-U Steam saltsalt-alkali unfiltered enzymatic hydrolysate
MSA-F Microwave saltsalt-alkali filtered enzymatic hydrolysate
MSA-U Microwave saltsalt-alkali unfiltered enzymatic hydrolysate
$X$ Cellconcentration, g/L
$X_0$ Initialcell concentration, g/L
$X_{max}$ Maximumcell concentration, g/L
$\mu_{max}$ Maximumspecific growth rate
$P$ Ethanolconcentration, g/L
$P_{max}$ Maximumpotential ethanol concentration, g/L
$r_{p,m}$ Maximumethanol production rate, g/L.h
$t$ Fermentationtime, h
$t_L$ Lagphase, h
$S$ Substrateconcentration, g/L
$K_S$ Monodconstant, g/L

ethanol yield respectively [10]. However, there is high cost to separating the solid and liquid fractions of the hydrolysate, particularly at large scale [11]. Several studies have highlighted the SSF system as a potential solution. In this strategy, the hydrolysis and fermentation occur in the same reactor, thus negating the need for a separation stage. This technique also has significant drawbacks such as specialised equipment requirements, high concentration of inhibitor formation and non-reusability of the yeast due to lignin separation [10]. However, the main drawback is the different optimum temperatures required for the enzyme (usually cellulase) and the fermenting microorganism, usually 50 and 30 °C respectively [12]. Ultimately, preference is given to the microorganism resulting in a sub-optimal saccharification process. Another potential solution, is to remove the separation stage from the SHF process. There is a dearth of knowledge on the effect of filtered and unfiltered enzymatic hydrolysate on fermentation process kinetics. Moreover, the solid waste residue from the SHF process effluent could be an attractive additional revenue stream for animal feed since the plant material has been delignified to enhance digestibility. Furthermore, there could be an increase in protein content due to the yeast cell biomass [13].

With increasing interest in the commercial applications of batch bioethanol processes, several kinetics models have been proposed which describe microbial growth, product formation and substrate consumption [14]. These models are extremely useful in the process development of bioethanol production, since they assist in predicting fermentation performance in response to changes in various factors [15]. This study employs the Monod, logistic and modified Gompertz models to comparatively describe the microbial growth and bioethanol production from pretreated SLW.

The aim of this study was to therefore examine the kinetics of SHF bioethanol fermentation from two previously optimized pretreatment techniques of sugarcane leaf waste, under two fermentation modes using *Saccharomyces cerevisiae* BY4743. These include SSA filtered enzymatic hydrolysate (SSA-F), SSA unfiltered enzymatic hydrolysate (SSA-U), MSA filtered enzymatic hydrolysate (MSA-F) and MSA unfiltered enzymatic hydrolysate (MSA-U). In addition, the potential of the fermentation effluent as animal feed was also explored.

## 2. Methods

### 2.1. Feedstock and pretreatment

Sugarcane leaf waste (SLW) employed in this study was harvested from a sugarcane plantation located in the North Coast of South Africa. Prior to pretreatment, the leaves were dried at 60 °C for 72 h and milled to $\leq$ 1 mm. The substrate pretreatment protocols have been described in our previous study [9]. For the steam salt-alkali method (SSA), SLW was first treated with 1.73 M $ZnCl_2$ for 30 min at 121 °C followed by 1.36 M NaOH at 121 °C for 30 min. For the microwave-assisted salt-alkali (MSA), SLW was pretreated with 1.67 M $ZnCl_2$ at 400 W for 5 min in the first stage followed by 1.52 M NaOH in the second stage. All pretreated samples were washed thoroughly with deionized water until a neutral pH was obtained. Briefly, the SSA and MSA pretreatment exhibited a lignin removal of 80.5 and 73% and hemicellulose removal of 51.9 and 62% respectively. Similar cellulose recoveries of 88 and 87% were observed for the SSA and MSA pretreatments respectively [9]. Enzymatic hydrolysis was performed in sodium citrate buffer (pH 4.8, 0.05 M) with a solid and enzyme loading of 10% (w/v) and 10 FPU/g respectively. The commercial cellulase enzyme preparation, Cellic CTec 2, was kindly supplied by Novozymes (Novozymes A/S, Denmark). Saccharification was achieved at 50 °C for 72 h at 120 rpm in a shaking incubator.

### 2.2. Microorganism and inoculum development

This study employed *Saccharomyces cerevisiae* BY4743 and was supplied by the Department of Genetics, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The culture was aseptically maintained on a double strength YPD slant (20 g/L yeast extract, 40 g/L peptone and 40 g/L dextrose). Prior to fermentation, the stock culture was streaked onto YPD media (10 g/L yeast extract, 20 g/L peptone and 20 g/L dextrose) and incubated at 30 °C for 24 h thereafter a single colony was inoculated into YPD broth and incubated at 30 °C overnight in a shaking incubator at 120 rpm.

### 2.3. Batch fermentation

#### 2.3.1. Fermentation medium

Bioethanol production was investigated with two pretreatment types under two different fermentation modes. These included: steam salt-alkali filtered enzymatic hydrolysate (SSA-F), steam salt-alkali unfiltered enzymatic hydrolysate (SSA-U), microwave salt-alkali filtered enzymatic hydrolysate (MSA-F) and microwave salt-alkali unfiltered enzymatic hydrolysate (MSA-U). For experiments examining the effects of unfiltered enzymatic hydrolysate on process kinetics, the enzymatic hydrolysate did not undergo a filtering process to remove the solid residues. Pretreated SLW was added to give an initial glucose concentration of 60 g/L in the fermentation media. Additional nutrients consisted of yeast extract 5 g/L, peptone 5 g/L, $KH_2PO_4$ 2 g/L, $MgSO_4{\cdot}7H_2O$ 1 g/L and $(NH_4)_2SO_4$ 1 g/L.

#### 2.3.2. Fermentation conditions

Bioethanol fermentation was performed in 100 ml Erlenmeyer flasks. A 10% (v/v) inoculation was used with an initial cell count of between $10^6$ and $10^8$ cells/ml. The pH of the fermentation medium was adjusted to 4.5 and fermentation was carried out at 30 °C with an agitation of 120 rpm for approximately 24 h or until ethanol production ceased. Aliquots were withdrawn for sugar, ethanol and biomass analysis every 2 h.

### 2.4. Analytical methods

Total reducing sugar was quantified with the 3,5-dinitrosalicylic acid method [16] and glucose was measured using a glucose kit assay (Megazyme). The yeast biomass concentration in the fermentation broth was determined using a pre-established correlation dependence on biomass dry weight as a function of cell count [14]. The concentration of bioethanol was determined using a Vernier Ethanol sensor interfaced with the Vernier LabQuest monitor (ETH-BTA, Vernier Software and Technology, USA). The sensor employs a metal oxide semiconductor to detect ethanol. In the measuring principle, ethanol is consumed in a combustion reaction with the metal oxide, thus reducing the internal resistance of the sensor element. The change in resistance is converted to a response voltage corresponding to ethanol concentration. The sensor was calibrated and tested with known concentrations of ethanol prior to analysis. Crude protein, ash and fat content in the fermentation effluent was analysed using previous established protocols [17,18].

### 2.5. Kinetic models and calculation of kinetic parameters

The kinetic studies of the four fermentation types (SSA-F, SSA-U, MSA-F and MSA-U) were investigated. The growth kinetics was described using Monod's equation with the following conventions: (a) the broth culture in the flask was homogenous, (b) yeast cells were viable and (c) the mixing speed of 120 rpm was in excess of the needs for the fermentation process to provide adequate mass transfer. The Monod equation describes the relationship between cell growth rate and substrate concentration. To obtain the Monod kinetic parameters $K_S$ and $\mu_{max}$, five experiments with varying initial glucose concentration (10, 20, 40, 50, 70 g/L) were conducted in duplicate. The processes were sampled every 2 h, and sugar consumption and cell growth were monitored. The specific growth rates ($\mu$) were estimated using experimental data obtained during the exponential phase by linear regression from the slope of natural log of biomass vs time (Eq. (1)):

$$\mu = \frac{\ln(X_2 - X_1)}{t_2 - t_1} \tag{1}$$

The maximum specific growth rate ($\mu_{max}$) and Monod constant ($K_S$) were subsequently estimated using the non-linear least squares method [19].

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{2}$$

In addition to the Monod kinetic model, the logistic model is increasingly being used to describe microbial growth systems. A term considering inhibition of growth by ethanol concentration was not included, since the maximum ethanol concentration obtained in this study is far below the 15% threshold which inhibits yeast cells [20]. The differential form of the logistic Eq. (3) is shown below:

$$\frac{dX}{dt} = \mu_{max} \cdot \left(1 - \frac{X}{X_{max}}\right) \cdot X \tag{3}$$

where $X_{max}$ is the maximum yeast cell concentration (g/L) and $\mu_{max}$ is the maximum specific cell growth rate ($h^{-1}$). With the following boundary conditions: $t = 0$, $\therefore X = X_0$, a sigmoidal variation of $X$ is given as a function of $t$. Eq. (3) can then be integrated to give the logistic Eq. (4) which describes the exponential and stationary phase. The experimental data was used to fit this equation.

$$X = \frac{X_0 \cdot \exp(\mu_{max} \cdot t)}{1 - (X_0/X_{max}) \cdot (1 - \exp(\mu_{max} \cdot t))} \tag{4}$$

The above logistic model does not predict the death phase of microorganisms after the stationary phase [14].

The modified Gompertz model was adopted to describe the kinetics of bioethanol formation (Eq. (5)). This model defines the change in ethanol concentration during the course of fermentation. Experimental data was used to fit the modified Gompertz equation using the least squares method (CurveExpert V1.5.5):

$$P = P_m \cdot \exp\left\{-\exp\left[\frac{r_{p,m} \cdot \exp(1)}{P_m}\right] \cdot (t_L - t) + 1\right\} \tag{5}$$

where P is the bioethanol concentration (g/L), $P_m$ is the potential maximum bioethanol concentration (g/L), $r_{p,m}$ is the maximum bioethanol production rate (g/L h) and $t_L$ is the lag phase (h).

The sugar utilization, ethanol (EtOH) productivity and fermentation efficiency were calculated using Eqs. (6)–(8) respectively:

$$Sugar\ utilization\ (\%) = \frac{Amount\ of\ Initial\ sugar - final\ sugar}{Amount\ of\ initial\ sugar} \times 100 \tag{6}$$

$$Ethanol\ productivity\ (g/L.h) = \frac{Maximum\ ethanol\ concentration\ (g/L)}{Fermentation\ time\ (h)} \tag{7}$$

$$Fermentation\ efficiency\ (\%) = \frac{Experimental\ ethanol\ yield\ (g/L)}{Theoretical\ ethanol\ yield\ (g/L)} \times 100 \tag{8}$$

## 3. Results and discussion

### 3.1. Growth kinetics of S. Cerevisiae BY4743 on pretreated SLW

The change in biomass concentration over time under the four different fermentation conditions is shown in Fig. 1. A shorter lag time of 4 h was observed under SSA-F and SSA-U whereas, slightly longer lag times of 6 h were observed under MSA-F and MSA-U. This can be attributed to the presence of trace amounts of certain inhibitory products in the MSA enzymatic hydrolysate. Microwave-assisted pretreatment has been reported to have a higher severity factor; thus producing a higher concentration of

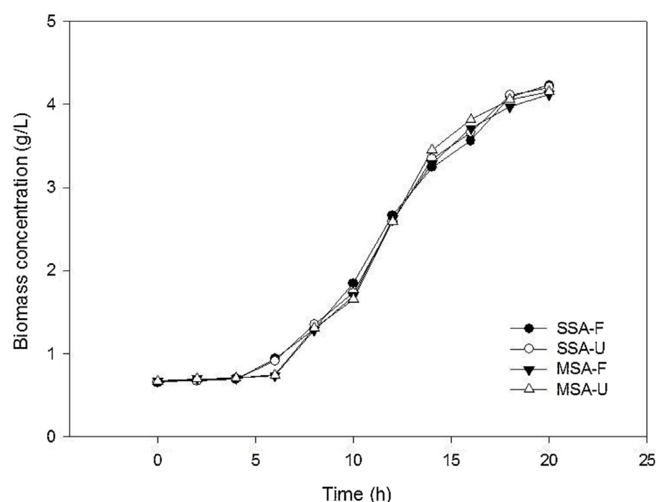


**Fig. 1.** Time course of biomass concentration under the four examined fermentation conditions.

inhibitory compounds compared to steam pretreatment [21]. In addition, there may be production of inhibitors during enzymatic hydrolysis due to the hydrothermal breakdown of the lignocellulosic components owing to the process temperature and duration. Some of the reported inhibitors from microwave-assisted metal chloride pretreatment of SLW include acetic acid, furfural and hydroxymethyl furfural (HMF) [22]. Acetic acid increases the intracellular pH of *S. cerevisiae* leading to an increase in the lag phase and decrease in the growth rate [23]. Similarly, furfural and HMF can synergistically affect the growth rate of *S. cerevisiae* by affecting glycolytic activity, causing oxidative stress and reducing the activity of various dehydrogenases [24].

The logistic models were in agreement with the experimental values, showing high coefficients of determination ($R^2 > 0.97$). The estimated values for the kinetic parameters were found to be in close range with the empirical values. These values and the developed models for each fermentation type are summarized in Table 1. Slightly higher $X_{max}$ values were obtained under SSA-F and SSA-U fermentation conditions (4.70 and 4.56 g/L respectively) compared to 4.36 and 4.35 g/L for MSA-F and MSA-U respectively, further suggesting the steam pretreated substrate was more favourable and promoted cell growth. Phukoetphim et al. [14] reported a similar maximum cell concentration ($X_{max}$) of 5.145 g/L from sweet sorghum juice whereas, Dodic et al. [25] reported a value of 8.381 g/L from sugar beet juice. These varying $X_{max}$ values could be accounted for by differences in yeast strain, substrate and working volume. The maximum specific growth rate ($\mu_{max}$) obtained from the logistic model under SSA-F and SSA-U conditions were 0.24 and 0.26 $h^{-1}$ respectively whereas, MSA-F and MSA-U were 0.28 and 0.29 $h^{-1}$ respectively. This was an indication that filtered and unfiltered enzymatic hydrolysate had a negligible effect on the maximum specific growth rate. This further implies the non-requirement of a separation stage, thus enhancing process economics and productivity at a large scale. However, the $\mu_{max}$ values obtained from the Monod model were 0.153 $h^{-1}$ for SSA-F and SSA-U, and 0.150 $h^{-1}$ for MSA-F and MSA-U. The difference in the $\mu_{max}$ values between model types (logistic and Monod) can be ascribed to the intrinsic parameters and boundaries employed by each model. For instance, the logistic model considers the biomass concentration from the lag phase to stationary phase, disregarding the substrate utilization whereas, Monod considers both the biomass concentration (however only in exponential phase) and the rate limiting substrate [26]. Differences in $\mu_{max}$ values from the logistic and Monod models have been previously reported. Manikandan and Viruthagiri [27] observed a $\mu_{max}$ of 0.307 and 0.095 $h^{-1}$ using the Monod and logistic model respectively for ethanol production from wheat flour. Likewise, the Monod and logistic model gave $\mu_{max}$ values of 0.65 and 0.45 $h^{-1}$ using glucose for ethanol production [28]. The maximum specific growth rates obtained in the present study are within range of previous studies. Srimachai et al. [29] reported a $\mu_{max}$ of 0.15 $h^{-1}$ from oil palm frond juice and a $\mu_{max}$ of 0.27 $h^{-1}$ was reported from sweet sorghum juice [14]. The obtained $\mu_{max}$ values are highly desirable, particularly for commercial scale up since growth rates > 0.025 $h^{-1}$ have been shown to linearly increase the fermentative capacity of *Saccharomyces* species. Furthermore, higher growth rates may trigger respirofermentative metabolism, thus resulting in an increase in fermentative capacity [30]. Moreover, the $\mu_{max}$ values are within range of previous pilot scale studies. For example, a $\mu_{max}$ of 0.34 $h^{-1}$ was reported in the production of ethanol from molasses at 300 000 L [31].

**Table 2**
The effect of different fermentation conditions and substrate type on Monod kinetic parameters.

| Substrate | Kinetic parameter | | Reference |
|---|---|---|---|
| | $\mu_{max}$ ($h^{-1}$) | Ks (g/L) | |
| SLW (SSA-F and SSA-U) | 0.153 | 4.19 | This study |
| SLW (MSA-F and MSA-U) | 0.150 | 5.61 | This study |
| Sorghum leaves | 0.176 | 10.11 | [33] |
| Sweet sorghum juice | 0.119 | 2.08 | [37] |
| Sweet sorghum juice | 0.313 | 47.51 | [32] |
| Glucose | 0.133 | 3.7 | [38] |
| Oil palm frond juice | 0.15 | 10.21 | [29] |

Monod constants ($K_S$) of 4.91 g/L for SSA-F and SSA-U, and 5.61 g/L for MSA-F and MSA-U were obtained (Table 2). A lower $K_S$ value indicates the microorganism's inherent affinity to the substrate since its reciprocal describes the cells affinity to the substrate type. The higher $K_S$ value obtained under MSA-F and MSA-U conditions could be explained by the presumptive presence of inhibitory compounds in the fermentation medium. Overall, *S. cerevisiae* showed a higher substrate affinity with the steam (0.20 g/$L^{-1}$) and microwave (0.17 g/$L^{-1}$) pretreated SLW compared to previous studies on sweet sorghum juice (0.021 g/$L^{-1}$, [32]) and sorghum leaves (0.10 g/$L^{-1}$, [33]). The difference in $K_S$ is affected by substrate type and concentration, and yeast strain and concentration [34].

### 3.2. Kinetics of bioethanol fermentation from pretreated SLW

The experimental profiles for bioethanol production and glucose consumption with *S. cerevisiae* from SSA-F and MSA-F are shown in Fig. 2. Ethanol production commenced almost immediately from the initial hours of fermentation and increased gradually until it peaked at 18 h into the process. Under SSA-F conditions, a considerably higher ethanol concentration (28.47 g/L) was achieved compared to MSA (23.01 g/L). This higher ethanol production under SSA-F compared to MSA-F conditions is substantiated by the higher maximum specific growth rate and substrate affinity observed in the Monod models. Li et al. [35] reported a significantly lower ethanol concentration (17.5 g/L) from acid pretreated corn leaves. Similarly, a lower ethanol concentration (4.71 g/L) from acid pretreated sugarcane leaves was reported by Jutakanoke et al. [36]. Under SSA-F conditions, *S. cerevisiae*

**Table 1**
The logistic models describing cell growth under different fermentation condition.

| Fermentation conditions | $X_o$ (g/L) | | $X_{max}$ (g/L) | | $\mu_{max}$ ($h^{-1}$) | | Logistic equation | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| | Pred | Exp | Pred | Exp | Pred | Exp | | |
| SSA-F | 0.27 | 0.26 | 4.70 | 4.41 | 0.24 | 0.24 | $X = \frac{0.27 \cdot \exp(0.24 \cdot t)}{1-(0.27/4.70) \cdot (1-\exp(0.24 \cdot t))}$ | 0.98 |
| SSA-U | 0.23 | 0.20 | 4.56 | 4.54 | 0.26 | 0.24 | $X = \frac{0.23 \cdot \exp(0.26 \cdot t)}{1-(0.23/4.56) \cdot (1-\exp(0.26 \cdot t))}$ | 0.98 |
| MSA-F | 0.20 | 0.16 | 4.36 | 4.27 | 0.28 | 0.26 | $X = \frac{0.20 \cdot \exp(0.28 \cdot t)}{1-(0.20/4.36) \cdot (1-\exp(0.28 \cdot t))}$ | 0.98 |
| MSA-U | 0.18 | 0.14 | 4.35 | 3.15 | 0.29 | 0.27 | $X = \frac{0.18 \cdot \exp(0.29 \cdot t)}{1-(0.18/4.35) \cdot (1-\exp(0.29 \cdot t))}$ | 0.97 |

Pred – Predicted.
Exp – Experimental.

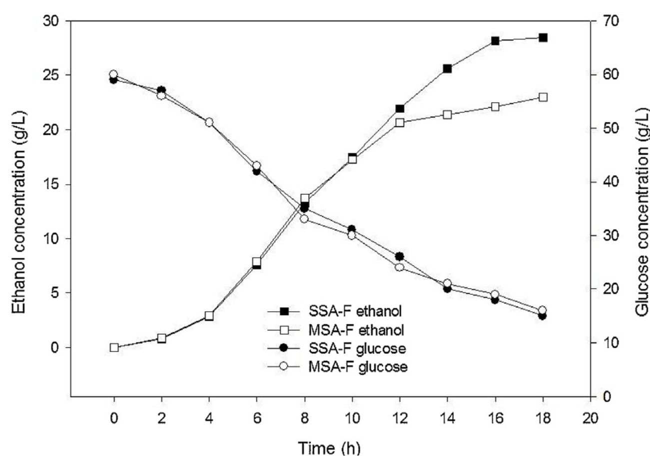


**Fig. 2.** Times course of bioethanol production and glucose consumption from SLW under SSA-F and MSA-F fermentation conditions.

showed a higher glucose consumption rate of 4.5 g/L.h from 0 to 6 h into the fermentation whereas a lower glucose consumption rate of 4.0 g/L.h was observed with MSA-F between 0 and 8 h of fermentation.

The profile of ethanol production and glucose consumption from SLW with SSA-U and MSA-U is shown in Fig. 3. The ethanol production and sugar consumption trends, showed similarities to the SSA-F and MSA-F conditions presented in Fig. 2. The ethanol production commenced 2 h into the fermentation and peaked at 18 h with 28.81 g/L (SSA-U) and 16 h with 22.72 g/L (MSA-U). Similarly, *S. cerevisiae* reached a maximum glucose consumption rate of 5 g/L.h during the first 6 h of fermentation with SSA-U whereas, a glucose consumption rate of 4.5 g/L.h was obtained during the first 7 h of fermentation with MSA-U. The lower ethanol concentration observed under MSA-U conditions could be ascribed to the decline in pH from 4.54 to 3.90 compared to the SSA-U process with a relatively stable pH slightly decreasing from 4.55 to 4.23 (Fig. 4). The decline in pH could be attributed to the generation of acetic acid from the hydrothermal breakdown of the hemicellulosic acetyl groups present in the pretreated sugarcane leaf waste biomass [39]. Furthermore, the severity of microwave pretreatment would infer a higher concentration of acetic acid release compared to steam pretreatment. In addition, the presumptive presence of furfural in the enzymatic hydrolysate could be a contributor to the decline in pH since *S. cerevisiae* has been shown to metabolize furfural compounds into furoic acid and

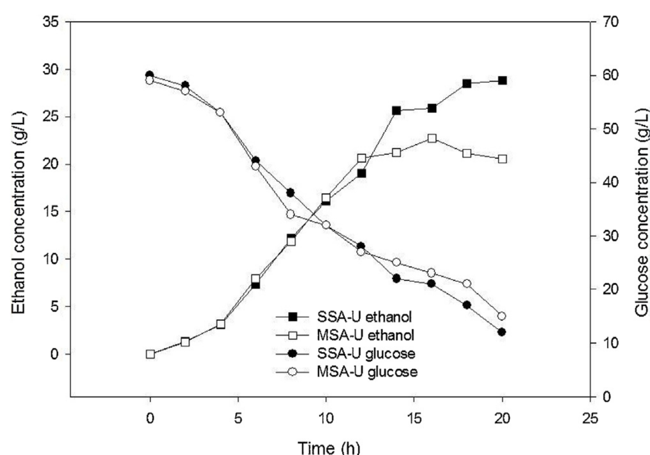


**Fig. 3.** Times course of bioethanol production and glucose consumption from SLW under SSA-U and MSA-U fermentation conditions.

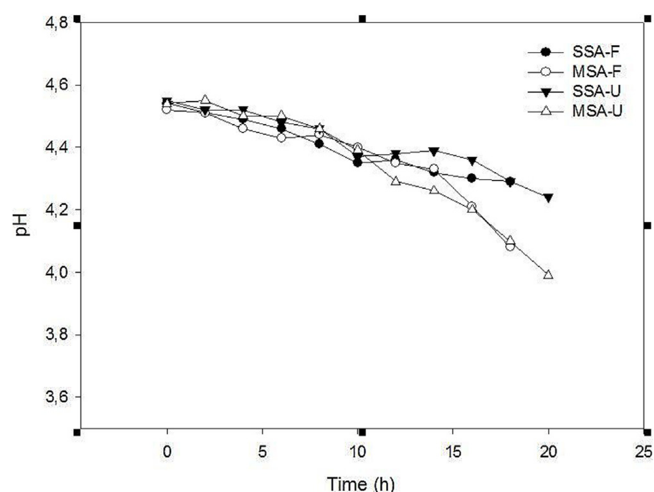


**Fig. 4.** Time course of pH evolution during ethanol fermentation from SLW under SSA-F, SSA-U, MSA-F and MSA-U conditions.

furfuryl alcohol [40]. The decline in pH from the 11$^{th}$ h of fermentation (MSA-U) also coincided with the decrease in glucose consumption and ethanol production rates from 2.5 to 1 g/L.h and 2.3 to 0 g/L.h respectively. Furthermore, *S. cerevisiae* is known to produce ethanol optimally at pH of 4.5. A pH beyond this range affects the activity of plasma membrane-bound proteins and includes both enzymes and transport proteins [41]. The pH of the SSA-F and SSA-U experiments showed a relatively slower drift remaining close to the optimum value of 4.5 compared to MSA-F and MSA-U with the final pH values below 4. This could account for the lower ethanol concentration and glucose consumption.

The modified Gompertz model fit the experimental data well under the four fermentation conditions (SSA-F, SSA-U, MSA-F, MSA-U) with high coefficients of determination ($R^2$) > 0.99 (Table 3). The potential maximum bioethanol concentration ($P_m$) ranged from 31.06 g/L (SSA-U) to 21.79 g/L (MSA-U). The high $P_m$ value obtained under SSA-U can be attributed to its higher $\mu_{max}$ and $1/K_S$ values compared to MSA-U thus inferring the *S. cerevisiae* cells had a higher affinity for the steam pretreated substrate. In addition, taking into account the aforementioned factors that contributed to the decline in pH, the higher $P_m$ obtained for SSA-U was expected. The undissociated form of weak lipophilic acids such as acetic acid induces acidification of the cell cytoplasm by accumulating inside the cells. This leads to a decrease in cell metabolic activity [42].

Yan et al. [43] reported a higher $P_m$ (104 g/L) from food waste using *S. cerevisiae* HO58 whereas, a lower $P_m$ (17.15 g/L) was recorded from sorghum leaves using *S. cerevisiae* BY4743 [33]. The differences in $P_m$ can be attributed to different sugar concentrations and yeast strains employed. Compared to the reported

**Table 3**
Comparison of the kinetic values in the modified Gompertz model from SLW and other lignocellulosic biomass.

| Substrate | Modified Gompertz model | | | | References |
|---|---|---|---|---|---|
| | $P_m$ (g/L) | $r_{p,m}$ (g/L.hr) | $t_L$ (h) | $R^2$ | |
| SLW (SSA-F) | 30.49 | 2.81 | 3.39 | 0.99 | This study |
| SLW (SSA-U) | 31.06 | 2.44 | 3.14 | 0.99 | This study |
| SLW (MSA-F) | 23.26 | 2.85 | 3.17 | 0.99 | This study |
| SLW (MSA-U) | 21.79 | 2.79 | 3.22 | 0.99 | This study |
| Sorghum | 17.15 | 0.52 | 6.31 | 0.98 | [33] |
| Sugar beet raw juice | 73.31 | 4.39 | 1.04 | 0.99 | [25] |
| Sweet sorghum juice | 60.04 | 2.09 | 3.07 | 0.99 | [14] |
| Food waste | 104 | 2.22 | 6.41 | 0.99 | [43] |
| Oil palm frond juice | 3.79 | 0.08 | 0.77 | – | [29] |

maximum ethanol production rate ($r_{p,m}$) of 2.09 g/L.hr obtained from sweet sorghum juice and 0.08 g/L.hr reported from oil palm frond [29], the $r_{p,m}$ obtained in this study between 2.44 and 2.85 g/L.h, are desirable since higher production rates are preferred at large scale. Likewise, a shorter lag time is favoured thereby implying the yeast cells have acclimated to the fermentation conditions. The lag time ($t_L$) for bioethanol production in this study ranged from 3.14 to 3.39 h thereby indicating no significant variation between the SSA-F, SSA-U, MSA-F and MSA-U experiments. Higher lag times have been reported by Rorke and Gueguim Kana [33] from sorghum leaves (6.31 h) and Yan et al. [43] from enzymatically pretreated food waste (6.41 h). A similar lag time of 3.07 h was observed by Phukoetphim et al. [14] from sweet sorghum juice whereas a low lag time (1.04 h) was reported from sugar beet raw juice [25]. Lag time can be affected by factors such as working volume, inoculum type and size, and substrate type and concentrations.

The SSA-F and SSA-U experiments gave a similar fermentation efficiency (92.86 and 93.97% respectively) and ethanol productivity (1.095 and 1.11 g/L h respectively), as shown in Table 4. Sugar utilization followed a similar trend with 86.67% and 83.33% for SSA-F and SSA-U respectively. MSA-F and MSA-U gave lower fermentation efficiencies of 75.05 and 74.10% respectively and ethanol productivities of 0.885 and 0.874 g/L h respectively. Therefore, no significant difference was observed between filtered and unfiltered enzymatic hydrolysate, indicating that the presence of sugarcane leaf biomass did not hinder bioethanol production. In fact, under SSA-U conditions, ethanol production was slightly higher. Reported ethanol production from oil palm frond juice and sugarcane juice showed a lower fermentation efficiency compared to SSA but a higher efficiency compared to MSA (Table 4; [29,44]).

The unfiltered enzymatic hydrolysate results (SSA-U and MSA-U) are comparable to previous studies where the enzymatic hydrolysate was filtered prior to fermentation. For instance, Mishra et al. [46] observed an ethanol concentration of 29 g/L from filtered enzymatic hydrolysate of acid pretreated rice straw. A maximum ethanol concentration of 2.95 g/L was reported from the filtered enzymatic hydrolysate of alkali pretreated hazelnut shells [47]. This is an indication that unfiltered enzymatic hydrolysate gave similar ethanol concentrations to filtered enzymatic hydrolysate in a separate hydrolysis and fermentation (SHF) system. The separation of the solid biomass requires an additional unit operation that can contribute to about 5% of the annual operating costs; thus impacting on the process economics at large scale [11]. In addition, the process time for centrifugation or filtering reduces the productivity of the process and impacts the number of batch runs annually. Some studies have employed the simultaneous saccharification and fermentation (SSF) system to circumvent the need for a filtering stage however, previous reports on SSF have given significantly lower ethanol concentrations compared to the concentrations obtained in this study. For example, a SSF system using steam exploded acorn produced 1.97 g/L ethanol [48] while acid pretreated *Saccharina japonica* gave 6.65 g/L ethanol [49]. A slight higher ethanol concentration of 13.6 g/L was reported from *Arundo donax* [50]. Although these lower yields could be attributed to many factors such as yeast strain and substrate, the SHF system does offer some attractive features. This includes the ability to optimize the saccharification and fermentation process separately, thereby improving the respective product yields.

### 3.3. Feed analysis

Effluent from the SSA-U process (ethanol concentration of 28.81 g/L and final biomass concentration of 4.56 g/L) underwent compositional and nutritional analysis to determine its potential as animal feed (Table 5). The solid biomass from the SSA-U effluent was shown to contain 6.0% crude protein. Protein content values of between 1.6 and 26% are commonly reported in feedstock compositions and therefore the obtained protein content of 6.0% fell within this range [51]. Other common animal feed such as wheat and corn cobs have reported a protein content of 4.8 and 3.0% respectively [51]. The high protein content from the SSA-U biomass can be accounted for by the nitrogen rich yeast biomass. Furthermore, the SSA-U process gave a fat content of 2.57%, which was well within the reported range of 0.1 to 19.3% from other lignocellulosic biomass [51]. Cotton seeds and wheat have previously been reported to contain a similar fat content, 2.5 and 1.9% respectively. Since fat provides more than twice the energy compared to carbohydrates and proteins, it is an essential component in animal feed [52]. A major bottleneck with many animal feeds is the low digestibility due to the high lignin content [13]. The SSA pretreatment of SLW caused significant (80.5%)

**Table 4**
Comparison of bioethanol production from SLW and other reported lignocellulosic biomass.

| Substrate | Sugar utilization (%) | Max ethanol production (g/L) | Ethanol productivity (g/L hr) | Fermentation efficiency (%) | Reference |
|---|---|---|---|---|---|
| SLW (SSA-F) | 86.67 | 28.47 | 1.095 | 92.86 | This study |
| SLW (SSA-U) | 83.33 | 28.81 | 1.11 | 93.97 | This study |
| SLW (MSA-F) | 78.33 | 23.01 | 0.885 | 75.05 | This study |
| SLW (MSA-U) | 76.27 | 22.72 | 0.874 | 74.10 | This study |
| Oil palm frond juice | 94.05 | 11.50 | 0.12 | 76.52 | [29] |
| Sugarcane juice | 98.00 | 67.00 | 0.93 | 78.43 | [44] |
| Sweet sorghum juice | 100 | 72.43 | 1.01 | 94.60 | [45] |

**Table 5**
Comparison of the feed analysis for the SSA-U solid residues and other common animal feed.

| **Substrate** | Ash % | Fat | CP | Ca | Mg | K | Na | P | Zn mg/kg | Cu | Mn | Fe | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SLW (SSA-U) | 6.27 | 2.57 | 6.0 | 0.11 | 0.09 | 0.33 | 0.60 | 0.29 | 33 | 4 | 30 | 132 | This study |
| Wheat | 7.6 | 1.9 | 4.8 | 0.31 | 0.14 | 1.55 | 0.12 | 0.10 | ND | ND | ND | ND | [51] |
| Corn cob | 2.2 | 0.6 | 3.0 | 0.10 | 0.06 | 0.90 | 0.04 | 0.06 | ND | ND | ND | ND | [51] |
| Cotton seeds | 2.8 | 2.5 | 6.2 | 0.18 | 0.17 | 1.16 | 0.02 | 0.12 | ND | ND | ND | ND | [51] |

CP- Crude protein.
ND – Not determined.

delignification, thereby enhancing the digestibility [9]. The effluent can be supplemented with additional nutrients, depending on the specific requirements. Developing a suitable methodology for the use of this waste-stream for animal feeding could enhance the environmental and economic outlook of this process since no waste treatment and disposal will be required.

## 4. Conclusion

In this study, three empirical models, i.e. Monod, logistic and modified Gompertz, were employed to describe *S. cerevisiae* BY4743 growth and ethanol production from pretreated SLW under SSA-F, SSA-U, MSA-F and MSA-U fermentation conditions. All models fit the experimental data well with high coefficients of determination $R^2 > 0.98$, indicating their potential application for large scale operations. Steam salt-alkali pretreated SLW produced 25% more bioethanol compared to microwave salt-alkali. Furthermore, no difference was observed between filtered and unfiltered enzymatic hydrolysate experiments for both pretreatments. These findings provide crucial insights into enhancing the cost, productivity and environmental outlook for scale up processes.

## Conflict of interest

None.

## Acknowledgements

The financial assistance of the National Research Foundation of South Africa (NRF) [grant number 101275] towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily attributed to the NRF.

## References

[1] H. Zabed, J.N. Sahu, A.N. Boyce, G. Faruq, Fuel ethanol production from lignocellulosic biomass: an overview on feedstocks and technological approaches, Renew. Sustain. Energy Rev. 66 (2016) 751–774.
[2] L.M. Chng, K.T. Lee, D.J.C. Chan, Synergistic effect of pretreatment and fermentation process on carbohydrate-rich *Scenedesmus dimorphus* for bioethanol production, Energy Convers. Manage. 141 (2017) 410–419.
[3] N. Akhtar, D. Goyal, A. Goyal, Characterization of microwave-alkali-acid pre-treated rice straw for optimization of ethanol production via simultaneous saccharification and fermentation (SSF), Energy Convers. Manage. (2016), doi: http://dx.doi.org/10.1016/j.enconman.2016.06.081.
[4] B. Franko, M. Galbe, O. Wallberg, Bioethanol production from forestry residues: a comparative techno-economic analysis, Appl. Energy 184 (2016) 727–736.
[5] A.R. Dominguez-Bocanegra, J.A. Torres-Munoz, R.A. Lopez, Production of bioethanol from agro-industrial wastes, Fuel 149 (2015) 85–89.
[6] A. Mansouri, R. Rihani, A.N. Laoufi, M. Ozkan, Production of bioethanol from a mixture of agricultural feedstocks: biofuels characterization, Fuel 185 (2016) 612–621.
[7] S.K. Bhatia, S.-H. Kim, J.-J. Yoon, Y.-H. Yang, Current status and strategies for second generation biofuel production using microbial systems, Energy Convers. Manage. 148 (2017) 1142–1156.
[8] K. Sakimoto, M. Kanna, Y. Matsumura, Kinetic model of cellulose degradation using simultaneous saccharification and fermentation, Biomass Bioenergy 99 (2017) 116–121.
[9] P. Moodley, E.B. Gueguim Kana, Development of a steam or microwave-assisted sequential salt-alkali pretreatment for lignocellulosic waste: effect on delignification and enzymatic hydrolysis, Energy Convers. Manage. 148 (2017) 801–808.
[10] D. Carrillo-Nieves, H.A. Ruiz, C.N. Aguilar, A. Illina, R. Parra-Saldivar, J.A. Torres, J.L.M. Hernandez, Process alternatives for bioethanol production from mango stem bark residues, Bioresour. Technol. 239 (2017) 430–436.
[11] A. Aden, T. Foust, Technoeconomic analysis of the dilute sulfuric acid and enzymatic hydrolysis process for the conversion of corn stover to ethanol, Cellulose 16 (2009) 535–545.
[12] K. Olofsson, M. Bertilsson, G. Liden, A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks, Biotechnol. Biofuels (2008), doi:http://dx.doi.org/10.1186/1754-6834-1-7.
[13] F. Zadrazil, A.K. Puniya, Studies on the effect of particle size on solid-state fermentation of sugarcane bagasse into animal feed using white-rot Fungi, Bioresour. Technol. 54 (1995) 85–87.
[14] N. Phukoetphim, A. Salakkam, P. Laopaiboon, L. Laopaiboon, Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: logistic and modified Gompertz models, J. Biotechnol. 243 (2017) 69–75.
[15] K. Manikandan, V. Saravanan, T. Viruthagiri, Kinetics studies on ethanol production from banana peel waste using mutant strain of Saccharomyces cerevisiae, Indian J. Biotechnol. 17 (2008) 83–88.
[16] G.M. Miller, Use of Dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.
[17] J.R. Whitaker, P.E. Granum, An absolute method for protein determination based on difference in absorbance at 235 and 280 nm, Anal. Biochem. 109 (1980) 156–159.
[18] W. Horwitz, Official Methods of Analysis of the AOAC, 13th ed, The Association of Official Analaytical Chemists, Arlington, VA, 1980.
[19] P. Englezos, N. Kalogerakus, Applied Parameter Estimation for Chemical Engineers, Marcel Dekker, New York, 2001.
[20] F.W. Bai, W.A. Anderson, M. Moo-Young, Ethanol fermentation technologies from sugar and starch feedstocks, Biotechnol. Adv. 26 (2008) 89–105.
[21] A. Aguilar-Reynosa, A. Romani, R.M. Rodriguez-Jasso, C.N. Aguilar, G. Garrote, H.A. Ruiz, Microwave heating processing as alternative of pretreatment in second-generation biorefinery: an overview, Energy Convers. Manage. 136 (2017) 50–65.
[22] P. Moodley, E.B. Gueguim Kana, Microwave-assisted inorganic salt pretreatment of sugarcane leaf waste: effect on physiochemical structure and enzymatic structure, Bioresour. Technol. 235 (2017) 35–42.
[23] M.E. Pampulha, M.C. Loureiro-Dias, Energetics of the effect of acetic acid on growth of Saccharomyces cerevisiae, FEMS Microbiol. Lett. 184 (2000) 69–72.
[24] A. Iwaki, T. Kawai, Y. Yamamoto, S. Izawa, Biomass conversion inhibitors furfural and 5-Hydroxymethylfurfural induce formation of messenger RNP granules and attenuate translation activity in Saccharomyces cerevisiae, Appl. Environ. Microbiol. 79 (2013) 1661–1667.
[25] J.M. Dodic, D.G. Vucrovic, S.N. Dodic, J.A. Grahovac, S.D. Popov, N.M. Nedeljikovic, Kinetic modelling of batch ethanol production from sugar beet raw juice, Appl. Energy 99 (2012) 192–197.
[26] F. Kargi, Re-interpretation of the logistic equation for bath microbial growth in relation to Monod kinetics, Lett. Appl. Microbiol. 48 (2008) 398–401.
[27] K. Manikandan, T. Viruthagiri, Simultaneous saccharification and fermentation of wheat bran flour into ethanol using coculture of amylotic *Aspergillus niger* and thermotolerant *Kluyveromyces marxianus*, Front. Chem. Eng. China 3 (2009) 240–249.
[28] H. Shafaghat, G.D. Najafppour, P.S. Rezaei, M. Sharifzadeh, Growth kinetics and ethanol productivity of *Saccharomyces cerevisiae* PTCC 24860 on various carbon sources, World Appl. Sci. J. 7 (2009) 140–144.
[29] T. Srimachai, K. Nuithitkul, S. O-thong, P. Kongjan, K. Panpong, Optimization and kinetic modeling of ethanol production from Oil Palm Frond Juice in batch fermentation, Energy Procedia 79 (2015) 111–118.
[30] P.V. Hoek, J.P.V. Dijken, J.T. Pronk, Effect of specific growth rate on fermentative capacity of Baker's yeast, Appl. Environ. Microbiol. 64 (1998) 4226–4233.
[31] M. Arshad, T. Hussain, M. Iqbal, M. Abbas, Enhanced ethanol production at commercial scale from molasses using high gravity technology by mutant S. Cerevisiae, Braz. J. Microbiol. (2017), doi:http://dx.doi.org/10.1016/j.bjm.2017.02.003.
[32] P. Ariyajaroenwong, P. Laopaiboon, A. Salakkam, P. Srinophakun, L. Laopaiboon, Kinetic models for batch and continuous ethanol fermentation from sweet sorghum juice by yeast immobilized on sweet sorghum stalks, J. Taiwan Institute Chem. Eng. 66 (2016) 210–216.
[33] D.C.S. Rorke, E.B. Gueguim Kana, Kinetics of bioethanol production from waste Sorghum Leaves using *Saccharomyces cerevisiae* BY4743, Fermentation 3 (2017) 19.
[34] E. Felix, O. Clara, A.O. Vincent, A kinetic study of the fermentation of cane sugar using Saccharomyces cerevisiae, Open J. Phys. Chem. 4 (2014) 26–31.
[35] P. Li, D. Cai, Z. Luo, P. Qin, C. Chen, Y. Wang, C. Zhang, Z. Wang, T. Tan, Effect of acid pretreatment on different parts of the corn stalk for second generation ethanol production, Bioresour. Technol. 206 (2016) 86–92.
[36] R. Jutakanoke, N. Leepipatpiboon, V. Tolieng, V. Kitpreechavanich, T. Srinorakutara, A. Akaracharanya, Sugarcane leaves: pretreatment and ethanol fermentation by Saccharomyces cerevisiae, Biomass Bioenergy 283 (2012) 283–289.
[37] P. Thangprompan, A. Thanapimmetha, M. Saisriyoot, L. Laopaiboon, P. Srinophakun, Production of ethanol from sweet sorghum juice using VHG technology: a simulation case study, Appl. Biochem. Biotechnol. 171 (2013) 294–314.
[38] J. Singh, R. Sharma, Growth kinetic and modelling of ethanol production by wild and mutant *Saccharomyces cerevisiae* MTCC170, Eur. J. Exp. Biol. 5 (2015) 1–6.
[39] L.J. Jonsson, C. Martin, Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects, Bioresour. Technol. 199 (2016) 103–112.
[40] I.S. Horvath, C.J. Franzen, M.J. Taherzadeh, C. Niklasson, G. Liden, Effects of furfural on the respiratory metabolism of *Saccharomyces cerevisiae* in Glucose-Limited Chemostats, Appl. Environ. Microbiol. 69 (2003) 4076–4086.
[41] N.V. Narendranath, R. Power, Relationship between pH and medium dissolved solids in terms of growth and metabolism of lactobacilli and *Saccharomyces cerevisiae* during ethanol production, Appl. Environ. Microbiol. 71 (2005) 2239–2243.

[42] M.E. Pampulha, M.C. Loureiro-Dias, Combined effect of acetic acid, pH and ethanol on intracellular pH of fermenting yeast, Appl. Microbiol. Biotechnol. 31 (1989) 547–550.
[43] S. Yan, X. Chen, J. Wu, P. Wang, Pilot-scale production of fuel ethanol from concentrated food waste hydrolysates using *Saccharomyces cerevisiae* H058, Bioprocess Biosyst. Eng. 36 (2013) 937–946.
[44] C.L. Ramos, W.F. Duarte, A.L. Freire, D.R. Dias, E.C.A. Eleutherio, Evaluation of stress tolerance and fermentative behavior of indigenous Saccharomyces cerevisiae, Braz. J. Microbiol. 44 (2013) 935–944.
[45] X. Wu, S. Staggenborg, J.L. Propheter, W.L. Rooney, J. Yu, D. Wang, Features of sweet sorghum juice and their performance in ethanol fermentation, Ind. Crop. Prod. 31 (2010) 164–170.
[46] A. Mishra, A.K. Sharma, S. Sharma, R. Bagai, A.S. Mathur, R.P. Gupta, D.K. Tuli, Lignocellulosic ethanol production employing immobilized *Saccharomyces cerevisiae* in a packed bed reactor, Renew. Energy 98 (2016) 63–67.
[47] E.F. Hosgun, D. Berikten, M. Kivanc, B. Bozan, Ethanol production from hazelnut shells through enzymatic saccharification and fermentation by low-temperature alkali pretreatment, Fuel 196 (2017) 280–287.
[48] C. Sasaki, Y. Kushiki, C. Asada, Y. Nakamura, Acetone–butanol–ethanol production by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) methods using acorns and wood chips of *Quercus acutissima* as a carbon source, Ind. Crop. Prod. 62 (2014) 286–292.
[49] J. Lee, P. Li, J. Lee, H.J. Ryu, K.K. Oh, Ethanol production from *Saccharina japonica* using an optimized extremely low acid pretreatment followed by simultaneous saccharification and fermentation, Bioresour. Technol. 127 (2013) 119–125.
[50] S. Mutturi, G. Liden, Model-based estimation of optimal temperature profile during simultaneous saccharification and fermentation of Arundo donax, Biotechnol. Bioeng. 111 (2013) 866–875.
[51] NRC, Table of Feed Compositions Accessed from [https://kb.wisc.edu/images/group256/shared/03-Feed-Composition/NRC-Feed-Composition-Tables.xls] on 11-09-2017, (2001) .
[52] M.I. Gurr, The chemistry and biochemistry of plant fats and their nutritional importance, in: J. Wiseman (Ed.), Fats in Animal Nutrition, Butterworths, Essex, 1984, pp. 8.