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Enzymatic hydrolysis of market vegetable waste and subsequent ethanol fermentation-Kinetic evaluation

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Abstract

In this work, kinetic properties evaluation was made for bioethanol production from sugar hydrolysate of vegetable waste. The saccharified sugars were fermented by using *Saccharomyces cerevisiae.* The effect of various saccharification factors on sugars release were studied and observed that the optimized conditions contributed to 14.4 $gL^{-1}$of fermentable sugars production. The produced sugars were subjected to batch fermentation by *Saccharomyces cerevisiae* at pH 4.5 and the kinetic parameters of fermentation were estimated by fitting the experimental data in modified logistic equations. The data revealed product (ethanol) yield ($Y_{P/S}$) of 0.39g/g of reducing sugars. Maximum specific growth rate (μ_{max}), the yield of ethanol on biomass ($Y_{P/X}$) and the yield of biomass on sugars utilization ($Y_{X/S}$) were determined to be 0.18 h^{-1}, 1.097 g/g and 0.313 g/g, respectively. The process yielded 4.13 gL^{-1} of ethanol by consumption of 10.6 gL^{-1} sugars with a volumetric production rate of 0.0861±0.002 gL^{-1} h^{-1}.

Keywords: Vegetable waste; Holocellulolytic enzymes; Ethanol; *Saccharomyces cerevisiae*; Fermentation; kinetics

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Introduction

With an ever increasing world population, cumulative number of motor vehicles, consequent dwindling of fossil fuel reserves and volatility in the price of oil is demanding for an alternative realistic and sustainable solution for future energy requirement. Globally there is a potential concern for the development of ecologically sustainable biofuels from an amalgamated lignocellulosic biomass through commercially viable bioprocesses (Kiyohiko et al. 2011). Bioethanol is considered as a substantial alternative potential biofuel to petrol due to its clean burning and non-petroleum liquid nature (Ganapathy et al. 2010). Lignocellulose exemplifies a huge quantum of energy on the earth which can meet with the future provisions of bioethanol. In order to mitigate food-feed-fuel tradeoffs, it is necessary to integrate all sorts of cellulosic biomasses into an agronomic-biobased economy (Mahro and Timm. 2007). These days, bio-based fuels derived from the crops other than high-albedo ones include corn, sugarcane and switch grass seemed to be the cusp of commercialization due to the current global rise in food-fuel prices.

Lignocellulose is replenishable (Soltes 1980) and a complex hetero-polymeric constituent (30-50% of cellulose, 15-35% of hemicellulose and 10-20% of lignin) (Pettersen. 1984; Badger. 2000; Girio et al. 2010; Kumar et al. 2008) that plays a significant role in maintaining the structural integrity of plant cell wall, in which cellulose forms skeleton entangled by hemicellulose and lignin, where lignin acts as a cement barrier by forming covalent linkages with hemicellulose and non-covalent linkages with cellulose (Kumar et al. 2008). Lignocellulosic-based feedstock generated from agriculture and food processing industries has been given a prodigious deliberation for the intention of its value added bio-based chemicals and bioenergy (Pandey et al. 2000; Himanish and Sudhir 2004; Foyle et al. 2007). Investigation into new lignocellulosic sources among various agricultural and industrial residues for use in biofuel production is very crucial and aids in developing a sustainable strategy for the development of renewable biofuels. India produces about 127 million tons of fruits and vegetables and 198 million tons of food grains resulting in ~ 200 million tons of agricultural waste annually (Himanish and Sudhir 2004). The unutilized post-harvest left over of fruits accounts to 20-30% of the produce

with an annual loss of Rs. 3,000 crores (Ashok 1998; Food Digest 1995) while in US the annual wastage of fruits and vegetables is reported to be 23 and 25%, respectively (Food Digest 1997). Recent reports on the utilization kitchen and house hold food wastes showed their potency for bioethanol production (Deniz and Oya 2013; Matsakas et al., 2014).

Enzymatic conversion of polymeric feedstock to monomers is an essential step for the production of bioethanol with high yields and low production cost (Galbe and Zacchi 2002). At the same time it is one of the bottle necks due to the indigenous complex crystalline-cellulose polymer of lignocellulose which necessitates the use of high enzyme loadings due to nonproductive binding of enzymes with lignin (if exists) and other portions of the solid (Excoffier et al. 1991). Holocellulolytic enzymes are well-known for industrial applications and are indispensable for bioethanol production from lignocellulosic materials. They contribute around 60% of the total cost of lignocellulosic ethanol production when procured from external sources (Himmel et al. 2007), but the cost is significantly reduced once the in-house produced enzymes are employed for saccharification process.

The kinetic parameter evaluation of fermentation process signifies the relationship among the principle state variables to provide the assessable information on the performance of the system (Garden 2000). The information is crucial for analysing the overall process with respect to biological prominence of each parameter with statistical reliability and to design and operate a bioreactor for optimal level (Boulton 1996). In our previous report (Chiranjeevi et al. 2013), sodium chloride pretreatment was developed for treating vegetable biomass, while in the present work, as a perpetuation, an investigation was executed on the effect of enzyme dosage, substrate loading with respect to incubation time on saccharification followed by the evaluation of kinetic parameters for ethanol fermentation (using *Saccharomyces cerevisiae*) which include substrate (sugars) consumption, formation of bio-products (biomass and ethanol), maximum specific growth rate, ethanol yield coefficients, maintenance coefficient etc.,

Materials and methods

Chemicals

Laboratory grade D- (+) Glucose, D- (+) Xylose, bovine serum albumin (BSA), Di-nitro salicylic acid (DNS), sodium potassium tartarate (SPT), citric acid, potato dextrose broth, yeast extract, agar–agar type-I, birch wood xylan, peptone, KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $MnSO_4$, $CaCl_2.2H_2O$, NH_4SO_4, $NaClO_2$ were obtained from HIMEDIA, Mumbai, India and used in the present study. The market vegetable waste (MVW i.e. cabbage, cauliflower and spinach wastes) was procured from the local vegetable market, Hyderabad, India.

Microorganisms and maintenance

For enzymes production, *Cladosporium cladosporioides* [ID No.7530] was cultivated on Potato Dextrose Agar (PDA) slants at 28°C for 3 days and stored at 4°C. For fermentation, *Saccharomyces cerevisiae* was grown on Yeast Extract Peptone Dextrose Agar (YEPDA) slants at 28°C, maintained at 4°C. Both the organisms were maintained by sub-culturing for every three months.

Production of enzymes for saccharification

The enzyme fermentation experiments were accomplished according to (Chiranjeevi et al. 2012). In brief, the production was carried out in 250 ml Erlenmeyer flasks comprising 100 ml of production medium with the pre-optimized medium components. Aliquots were collected at specific intervals (for every 24h) and centrifuged at 10,000 rpm, 4ºC for 20 min (3500, Kubota centrifuge, Japan) to remove the suspended spores and partly utilized rice straw. The clear supernatant obtained was analysed for xylanase, endo-cellulase (CMCase) and exo-cellulase (FPase) activities using substrates, 2% (w/v) of birch wood xylan, 1% (w/v) of carboxymethyl cellulose (CMC) and 0.5 mg of filter paper respectively (Ghose 1987). One unit of enzyme activity is defined as the amount of enzyme which produces 1µmol of reducing sugar in the reaction mixture per minute under the specified conditions. The reducing sugars were estimated by standard DNS method (Miller 1959).

Pretreatment and delignification of MVW

In order to make MVW dust free, it was subjected to water washes and chopped into pieces to a size of 10 mm and then dried in hot air oven (CASSIA) for overnight at 50°C to acquire a constant weight. The dried material was shredded and then sieved to obtain uniform particle size of 0.5 mm. The processed biomass was stored at room temperature in a Ziploc bag until further usage. Sodium chloride pretreatment (Chiranjeevi et al. 2013) followed by acidified delignification (Sengupta et al. 1958) was performed for MVW. The treated solid material was subjected to neutralization with extensive hot water washes.

Enzymatic hydrolysis of MVW

Hydrolysis experiments for delignified MVW were conducted in 250ml Erlenmeyer flasks in water bath shaker (Rotek, India) at 50°C using 50mM sodium citrate buffer of pH 5 (for xylanases) and pH 3.5 (for cellulases) with shaking at 90 rpm. With the intention of preeminent biomass conversion, enzymatic hydrolysis was executed in two successive stages, which also enabled to observe the efficiency of each enzyme. The first stage involves the digestion of hemicellulose portion by means of xylanases followed by cellulose digestion with cellulases in the second stage. The hydrolysis was also performed at different enzyme loads per gram of biomass (5-25 IU) to evaluate the optimum dosage of enzyme required for efficient holocellulose conversion. Furthermore, the effect of substrate concentration (5-20% w/v) with respect to incubation time on enzymatic hydrolysis was evaluated. Aliquots were collected in specific intervals (hourly) and the released total reducing sugars (TRS) were estimated by DNS method (Miller 1959). The sugar hydrolysate was clarified by centrifugation (Sorval Cooling centrifuge) at 10,000 rpm, 4°C for 30 min and filtered through a 0.22µm PVC membrane filter. This clear hydrolysate with sugar concentration of 14 gL^{-1} was used for ethanol fermentation. The rate of enzymatic hydrolysis was determined by using following equation:

$$\text{Enzymatic hydrolysis (\%)} = \left[\frac{\text{Total reducing sugars} \times 0.9}{\text{Sample weight} \times \text{Holocellulose content}}\right] \times 100$$

Ethanol fermentation in batch culture

Ethanol fermentation experiments were carried out in 250 ml shake flasks comprising 100 ml of Yeast Extract Peptone (YEP) medium (composition: Yeast extract 1 gL^{-1}, Peptone 1 gL^{-1}, NH_4SO_4 1 gL^{-1}, KH_2PO_4 1 gL^{-1}, $MgSO_4$ 0.5 gL^{-1}, and

$MnSO_4$ 0.5 gL^{-1}), supplemented with concentrated sugar hydrolysate of MVW at the initial medium pH of 4.5. The sterilized flasks were inoculated with 5% (v/v) inoculum of *Saccharomyces cerevisiae* and incubated in an orbital shaker at 110 rpm, 28°C for 48h. For analysis, aliquots were withdrawn at every 12h for the estimation of pH, sugar consumption, biomass generation and ethanol production.

Analytical methods

Cell growth (biomass) was assessed by measuring the optical density of aliquots collected, using a UV-Visible spectrophotometer (UV-2450, Shimadzu) at A660 nm, and the amount of biomass produced (gL^{-1}) was evaluated by a calibration curve of biomass dry weight (gL^{-1}) versus optical density at 660nm. During fermentation, the concentration of total reducing sugars (TRS) were determined spectrophotometrically using DNS as well as by HPLC (Agilent, USA) using amino column (Luna 5µ NH_2 100A, 250 x 4.6 mm, Phenomenex) connected to ELSD (Evaporate Light Scattering Detector). The conditions employed were, water: acetonitrile (25:75), mobile phase; 0.8 ml/min., flow rate; 30°C, column temperature; 45°C, detector temperature. The ethanol concentration was determined by Gas Chromatography (Agilent, USA) using HP-5-5% phenyl methyl siloxane column at 180°C, FID (Flame Ionization Detector) detector at 180°C and Nitrogen gas as a carrier.

Kinetics of substrate (sugars) utilization, biomass production and product (ethanol) formation

Monod or logistic equation was used to explain the growth kinetics of the microbe. The following logistic equation was employed to evaluate the growth of *Saccharomyces cerevisiae* during ethanol fermentation.

$$\frac{dX}{dt} = \mu_{\max}\left[1 - \frac{X}{X_{\max}}\right] \text{eq. (1)}$$

Where, μ_{max} is the maximum specific growth rate with respect to the fermentation conditions. At the time of initial fermentation process, (t=0), the inoculum added is considered as initial biomass, X=X_0. By integrating eq. (1) of the kinetic model, a modified logistic equation can be formulated for calculating the rate of yields of biomass production and it follows as:

$$X = \frac{X_0 X_{\max} e^{\mu \max t}}{X_{\max} - X_0 + X_0 e^{\mu \max t}} \text{eq. (2)}$$

This equation signifies the relationship between the fermentation time and biomass and was used to fit the experimentation data using MATLAB® software (The MathWorks), which enabled to estimate μ_{max} and X_{max}. There is a significant relationship between the rate of ethanol production dP/dt and specific growth rate μ ($dX/(Xdt)$. The lag time of ethanol production (Δt) was introduced in the equation and hence the rate of ethanol production was:

$$\frac{dP}{dx} = Y_{p/X}\frac{dX}{d(t-\Delta t)} \text{eq. (3)}$$

The equation (3) can be integrated with μ_{max} and X_{max} which are determined from equation (2) and the resultant model is defined by the equation (4) and the two parameters such as yield coefficient of ethanol on biomass ($Y_{P/X}$) and production lag time (Δt) were estimated by fitting the experimental data in the equation (4).

$$P = Y_{P/X}\left[\frac{X_0 X_{\max} e^{\mu_{\max}(t-\Delta t)}}{X_{\max} - X_0 + X_0 e^{\mu_{\max}(t-\Delta t)}} - \frac{X_0 X_{\max} e^{-\mu_{\max}\Delta t}}{X_{\max} - X_0 + X_0 e^{-\mu_{\max}\Delta t}}\right] \text{eq. (4)}$$

For ethanol fermentation process, the equation relates the sugars utilization in terms of biomass production and maintenance coefficient of biomass produced (Sonali and Banwari 2007). The rate of sugars utilization was determined as below:

$$-\frac{dS}{dt} = \frac{1}{Y_{\frac{X}{S}}} \times \frac{dX}{dt} + m(X) \text{eq. (5)}$$

By combining the eqns. (1) and (3) and the determined parameters, the eq. (5) can be integrated and the rate of sugars utilization was obtained by eq. (6) and the yield coefficient of biomass on sugars ($Y_{X/S}$) and maintenance coefficient (m) were estimated.

$$S = S_0 - \frac{1}{Y_{\frac{X}{S}}}\left[\frac{X_0 X_{\max} e^{\mu_{\max} t}}{X_{\max} - X_0 + X_0 e^{\mu_{\max} t}} - X_0\right] - \frac{X_{\max} m}{\mu_{\max}} \ln \frac{X_{\max} - X_0 + X_0 e^{\mu_{\max} t}}{X_{\max}} \text{eq. (6)}$$

Results and discussion

Preparation of MVW substrate for enzymatic digestion process

In our previous report (Chiranjeevi et al. 2013), the contents of vegetable waste were evaluated for ash, cellulose, hemicellulose, lignin and other extractives, and determined to be 3.9±0.2%, 41.3±1.5%, 28±1.0%, 11.6±1.5% and 16.7±0.3% (w/w) respectively. Patle and Lal (Sonali and Banwari 2007) were able to estimate the contents of total solids and reducing sugars for fruit and vegetable wastes and noticed the similar tendency however the percentage of contents were found to be varied from one substrate to other. Lignin is the major obstacle for cellulo/hemicellulolytic enzymes attack on structural polysaccharides existing in lignocellulosic biomass (Excoffier et al. 1991; Zheng et al. 2007) and this can be improved by subjecting the material to pretreatment in order to disrupt the lignin-carbohydrate linkages (He et al. 2008). Keeping this in view, the selected MVW was subjected to sodium chloride pretreatment followed by acidified delignification which resulted in 63±1.1 (w/w) of holocellulose (cellulose + hemicellulose) with loss of residual soluble sugars (6.2±0.1% w/w). This method facilitates the complete dislocation of lignin polymer linked with cell wall structural carbohydrates of plant cell by oxidation reactions (Sengupta et al. 1958). In fact Bassem et al (2009) reported 69% of holocellulose from biomass of a shrub, *Buddleja davidii* (butterfly bush) by using acidified delignification. It is moreover worth mentioning that the generation of toxicity compounds such as formic, acetic and levulinic acids, and sugar degraded compounds like furfurals, HMF together with monomeric phenols produced during lignin degradation (Ulbricht et al. 1984, Bardet et al. 1985) which are known to inhibit the employed yeast cell growth during ethanol fermentation (Palmqvist et al. 2000). The treatment method for MVW used for present investigation implicates a mild treatment environment which encounter for less toxins formation with high purity of holocellulose (Chiranjeevi et al. 2013) that could be bio-converted to fermentable sugars by cellulases and hemicellulases.

Effect of enzyme supplementation and sugar yields

In-house produced holocellulolytic enzymes were used for saccharification of biomass. Enzymes production was carried

out by *Cladosporium cladosporioides* using rice straw as a carbon source in order to reduce the cost of enzymes required for saccharification of vegetable waste. The effect of enzymes dosage on hydrolysis of pretreated vegetable waste was investigated at 50°C with various enzyme loads (5-25 IU/g of substrate) and the reaction was performed for the duration of 6 hours. The release of sugars increased with increase in enzyme dosage up to 25 IU/g and reached maximum concentration of 15.1 gL^{-1}. Since no significant difference in sugar release was found between the enzyme loadings of 20 IU/g and 25 IU/g, further experiments were aimed using enzyme loadings of 20 IU/g and was considered as an optimum enzyme concentration required for pretreated MVW saccharification (Fig. 1). After optimizing the dosage of cellulases (extracted on 3rd of production) and xylanases (extracted on 8th day of production) (Fig. 2), again the hydrolysis was carried out with cocktail of cellulases and xylanases at a fixed dose of each 20 IU/g of substrate. As enzymatic saccharification requires synergy between the cellulases (endo+exo) and xylanases (β-xylosidases) to accomplish proper hydrolysis of the substrate, the influence of enzymes synergy on saccharification of pretreated MVW was investigated. The synergy of enzymes in the release of fermentable sugars was shown in Figure 3. The highest sugar yield, 14.8 gL^{-1} was observed in the case of enzymes employed together. The saccharified sugar yield was comparable with Takagi et al. (Toshiyuki et al. 2012) where the authors observed 14.4 gL^{-1} of reducing sugars from acid hydrolysed water hyacinth powder with 400 IU of enzyme in 24h of reaction duration. Similar trend has been reported by Leonardo et al. (2011) where 15.3 gL^{-1} of reducing sugars were observed for 6h hydrolysis of steam pretreated sugarcane bagasse by enzyme cocktail of

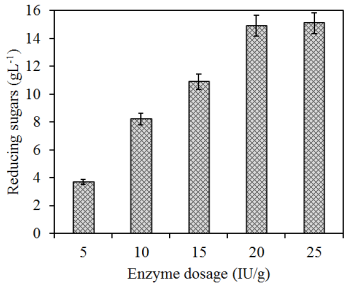

Figure 1. Effect of enzyme dosage on release of reducing sugars from pretreated MVW; the dosage of enzyme employed was 5-25 IU/g of material.

Trichoderma reesei RUT C30 and *Aspergillus awamori* 2B.361 U2/1 strains. Enzymatic hydrolysis of carrot waste resulted in 80 kgm^{-3} of the total reducing sugars (He et al. 2008). However it is interesting to note that Takagi et al. (Toshiyuki et al. 2012) used a very high dosage of enzyme 400 IU and 24h of incubation time while in the present study 200 fold less enzyme was used for saccharification still similar yield was observed. This further suggests that the presently used *C. cladosporioides* secretome is more effective compared to reported by Takagi et al. (Toshiyuki et al. 2012). The sugar yields gave an assessment on the amount bound sugars that can be liberated during the enzymatic hydrolysis. Several studies showed that the action of endo cellulases and β-xylosidases produce disaccharides of cellulose (cellobiose) and xylan (xylo-oligosaccharides), which are well recognized to strongly inhibit the action of respective enzymes (Ghosh et al. 1982; Gusakov et al. 1985; Oh et al. 2000). In view of effective saccharification of *C. cladosporioides* solution, it may be assumed that the presently employed strain may produce synergic ratio of biomass hydrolysing enzymes compared to others reported strains (Toshiyuki et al. 2012; Leonardo et al. 2011).

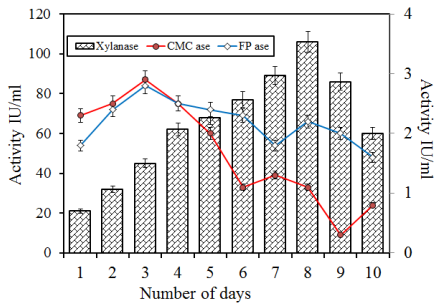

Figure 2. Holocellulolytic enzymes production profile by *Cladosporium cladosporioides*.

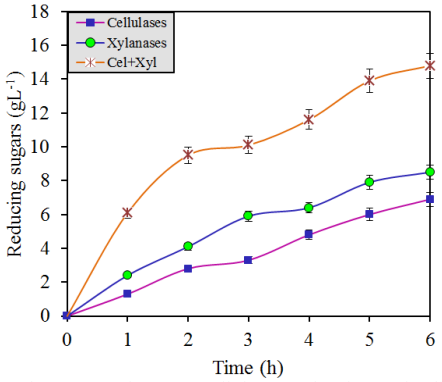

Figure 3. The synergy between cellulases and xylanase loadings, the load of enzymes used was 20 IU/g of material.

Effects of substrate concentration and incubation time on enzymatic hydrolysis

The effect of substrate concentration on yields of enzymatic hydrolysis was investigated by considering various substrate loads (5%, 10% and 15% w/v), keeping the load of enzyme constant (20 IU/g of substrate). The hydrolysis was performed for about 6h and the profile of enzymatic hydrolysis was shown in Fig. 4. At initial stages, the rate of enzymatic hydrolysis proceeded rapidly and after 6h of incubation 23.4% of hydrolysis improvement was attained at a load of 10% substrate. Although the initial content of reducing sugars

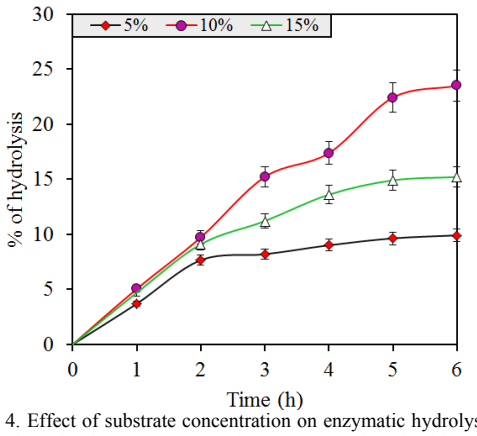

Figure 4. Effect of substrate concentration on enzymatic hydrolysis, 5-15% (w/v) of substrate concentrations were employed with enzyme dose of 20 IU/g.

liberated was enhanced with boost in substrate concentration, the final sugar content obtained in 10% of substrate load was

more than the other loads. The effect of substrate concentration on hydrolysis of biomass is different to different substrates (Kerstin et al. 2000). The observed drop in enzymatic hydrolysis with rise in substrate load might be due to the lack of appropriate enzyme accessibility to the surface of carbohydrate polymers present in vegetable biomass. Similar trend of hydrolysis reduction with increase in substrate load was observed for steam exploded sunflower stocks by Sharma et al (Sanjeev et al. 2002).

Ethanol batch fermentation-kinetics of biomass and ethanol production, and sugars utilization

The kinetic study is very crucial for the interpretation of a potential fermentation process and it entails basic information on the relationship among the pattern of substrate utilization, microbial growth and product formation (metabolite). A logistic equation permits to calculate the fermentation parameters of biological significance employing sigmoid profiles. A batch fermentation of hydrolysate (sugar concentration 14 gL^{-1}) obtained from enzymatic saccharification was executed at 28°C, pH 4.5, and 110 rpm. The profile of ethanol production and utilization of total reducing sugars

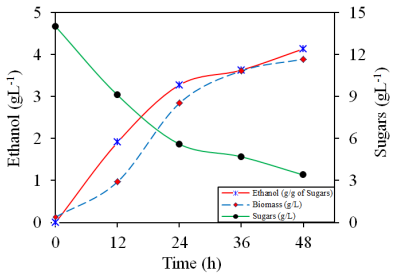

Figure 5. Ethanol production (*circles*), biomass production (*crosses*) and sugars consumption (*diamonds*), fermentation was carried for 48h at 28ºC in shaking flasks.

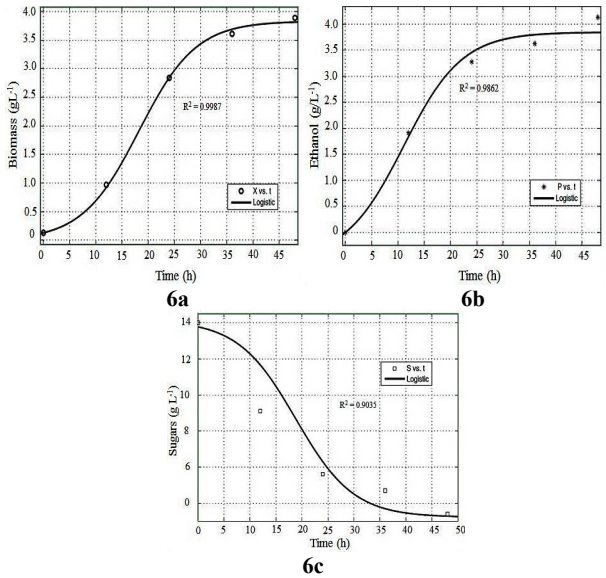

Figure 6.(a) Kinetics of biomass growth (*circles*), (b) ethanol production (*stars*) and (c) sugars consumption (*squares*) by *Saccharomyces cerevisiae*. The curves represent the fitted experimental data using modified logistic equations with corresponding correlation co-efficients.

(TRS) in the fermentation (see supplementary data for sugar analysis by HPLC) was illustrated in Fig. 5. The low pH was selected to overcome the bacterial contamination.

TRS utilization started with in 12h and 75.7% of TRS were utilized in the duration of 48h of fermentation. The ethanol concentration continued to increase until 48h (see supplementary data for GC analysis) and the concentration of 4.31 g L^{-1} (calculated based on utilized sugars determined after 48h of fermentation) was accomplished corresponding to a yield of 0.39 g ethanol /g of TRS. It can be evident from Figure 5 that the final biomass concentration produced was 3.89 g L^{-1} with a yield of 0.36 g/g of TRS (calculated based on sum of sugars utilized). Based on the experimental data of sugars utilized and initial biomass concentration, the kinetic parameters such as maximum specific growth rate (μ_{max}), maximum biomass concentration (X_{max}), the yield coefficients with respect to biomass ($Y_{P/X}$) and substrate ($Y_{X/S}$), lag time (Δt) and maintenance coefficient (m) were estimated. The concentrations of biomass, ethanol and TRS determined at various time intervals were fitted using eqns. (2), (4) and (6) respectively. The estimated kinetic parameters were furnished in Table 1. Figures 6a, 6b, 6c exemplifies that the model fits well to the results obtained in the experiment with correlation coefficients (R^2) of 0.9987, 0.9862 and 0.9035 for biomass generation, ethanol production and sugars utilization respectively.

Table 1. Kinetic parameters estimated from experimental data on fermentation of sugars of vegetable waste produced by enzymatic hydrolysis.

Kinetic parameter	Sugars (MVW)
μ_{max} (h^{-1})	0.18
X_{max} (gL^{-1})	3.832
$Y_{P/X}$ (g/g)	1.097
$Y_{X/S}$ (g/g)	0.313
$Y_{P/S}$ (g/g)	0.39
Δt (h)	7.726
m (h^{-1})	0.02
Note: For ethanol production; SSE: 0.1528 & RMSE: 0.2257; For sugars consumption; SSE: 7.045 & RMSE: 1.532	

The maximum specific growth rate (μ_{max}) and maximum biomass concentration (X_{max}) estimated were 0.18h^{-1} and 3.832 gL^{-1}. The determined value of μ_{max} is comparable with the earlier reports in the literature for yeasts (Maria et al. 2011; Wang et al. 2004). The yield coefficients of biomass ($Y_{X/S}$) and ethanol ($Y_{P/S}$) on sugars were determined to be 0.313 g/g and 0.39 g/g and whereas the estimated yield coefficient of ethanol on biomass ($Y_{P/X}$) was 1.097 g/g. The experimental data and those predicted by the kinetic model for ethanol production and TRS utilization were shown in Figures 6b and 6c. There was a little difference between the predicted data and the experimental results, which were reasonable with 0.1528 SSE, 0.2257 RMSE for ethanol production, and 7.045 SSE, 1.532 RMSE for sugars consumption. In general, it is established that the sugar uptake process signifies the major control mechanism for the rate of glycolytic flux under anaerobic circumstances (Pretorius et al. 2003) and rate of sugars uptake by the yeast cells is a result of the kinetics of the substrate inhibition and the process of transportation (Bisson 1999). Ethanol formation results from sugar metabolism and hence ethanol is a yeast cell growth-associated bioproduct (Luedeking and Piret 1959), the lag time (Δt) parameter of ethanol production to growth of *S. cerevisiae* was deliberated in the equation (4) and it was determined as 7.726h for the employed sugars of MVW and it reflected the fast utilization of the employed sugars for the production of ethanol.

Conclusions

Enzymatic saccharification was performed for sodium chloride pretreated MVW employing in-house produced holocellulolytic enzymes. The hydrolytic reaction conditions in terms of enzyme dosage, substrate loadings and incubation time were studied. A modified logistic kinetic model was employed for studying the flask-scale fermentation of sugars produced by saccharification of MVW using *Saccharomyces cerevisiae.* Based on the logistic equations the growth of yeast, growth associated production of ethanol including lag time, and utilization of sugars for biomass production and maintenance were estimated using mathematical software. The kinetic parameters found, such as X_{max}, μ_{max}, $Y_{P/S}$, $Y_{X/S}$ and m could be employed as indicators to address the vegetable waste fermentation issues.

Nomenclature

MVW	Market vegetable waste
μ	Specific growth rate
μ_{max}	Maximum specific growth rate (h^{-1})
X	Biomass concentration (g L^{-1})
X_{max}	Maximum biomass concentration (g L^{-1})
X_0	Initial biomass concentration (g L^{-1})
M	Maintenance coefficient (h^{-1})
t	Time (h)
P	Product (ethanol) concentration (g L^{-1})
S_0	Initial fermentable sugar concentration (g L^{-1})
$Y_{p/s}$	Yield coefficient of ethanol on sugar (g ethanol/ g sugar)
$Y_{p/x}$	Yield coefficient of ethanol on biomass (g ethanol/g biomass)
$Y_{x/s}$	Yield coefficient of biomass on sugar (g biomass/ g sugar)
Δt	Lag time in ethanol production (h)
RMSE	Root mean square error
SSE	Some of squares of error

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