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KINETICS OF BIOETHANOL PRODUCTION USING SACCHAROMYCES CEREVISIAE STRAIN Y-35

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ABSTRACT

In the present work, an attempt was made to explore the potential of *S. cerevisiae* Y-35 for fermentation of glucose to produce ethanol in batch culture. Effects of parameters, such as initial inoculum loading in the range of 5-40 gL^{-1} dry cell weight (DCW) and glucose concentration (in the range of 5-26% by weight) were investigated. Maximum ethanol yield and volumetric productivity were obtained at inoculum loading of 20 gL^{-1} DCW and increased marginally at 40 gL^{-1} DCW. With increased initial sugar concentration, volumetric productivity was increased and the maximum productivity of 10.46 $gL^{-1}.h^{-1}$ was obtained with 13% sugar concentration at 4 h, corresponding to 94% of the maximum theoretical possible conversion. At high sugar concentrations, high productivity was obtained up to 10 h, corresponding to 6.9 and 5.9 $gL^{-1}h^{-1}$ at 20% and 26% initial glucose concentrations, respectively. The high productivities obtained with the yeast, even at 20-26% sugar concentrations, implies the robustness of the yeast strain and potential for its industrial use. Furthermore, in order to understand the kinetic behavior, the experimental data was fitted into a kinetic model based on modified Monod equation to predict the inhibitory effects of ethanol and glucose on fermentation performance. A MATLAB® program was employed to estimate the kinetic parameters in the model. High R^2 and low RMSE values supported good agreements between experimental data and model predictions.

KEYWORDS: Bioethanol, Dry Cell Weight, Fermentation, Kinetics, Monod Equation, *Saccharomyces Cerevisiae* Y-35

INTRODUCTION

In the last few decades, fossil fuel reserves are fast depleting due to the increased usage of transportation fuel, which is also raising environmental concerns due to the increased particulate and greenhouse gases emissions. This has led to intensive research for green alternative fuels such as bioethanol and biodiesel. It has been estimated that U.S. could produce 284 billion liters of cellulosic ethanol per year by 2030, more than half of today's U.S. gasoline demand (RFA, 2015). The most economical and widely used method for the production of ethanol involves fermentation of sugars by yeast, *Saccharomyces cerevisiae*. It is the choice organism for sucrose and starch based ethanol industries. In order to efficiently produce ethanol, specific growth rate, sugar consumption rate, volumetric productivity, ethanol yield, and ethanol tolerance must be on higher side for a microorganism (Zabed et al., 2014). However, the selection of a particular industrial strain is usually based on historical grounds, rather than scientific and hence suboptimal for their purposes (Steensels et al., 2014). Industrial processes rarely use the best performing strain. Therefore, there is still a lot of scope to

exploit the fermentation performance of the unexplored natural yeast diversity (Wang et al., 2012). Yeast *S. cerevisiae* was chosen in present study as it is the best known microorganism for ethanol production from glucose offering high ethanol yields (95–99% of the theoretical) and high ethanol tolerance up to 10% (w/v) in fermentation medium (Talebnia et al., 2010).

To develop a fermentation process at an industrial scale, information on kinetics is significantly valuable for the better process control, reduction in process cost, and improvement of product quality (Olaoye et al., 2013). Various kinetic models have been proposed to quantitatively describe the dynamic behavior of fermentation systems (Huang et al., 2010; Sansonetti et al., 2011). Most of the models describing microbial growth during ethanol fermentation are empirical and based on either Monod's equation or on its various modifications which take into account the inhibition of microbial growth by a high concentration of product and/or substrate.

The present investigation is aimed at the evaluation of fermentative performance of *S. cerevisiae* Y-35 for D-glucose at different initial inoculum loading and sugar concentrations. In this study, a kinetic model incorporated with the effects of both substrate and product inhibition is utilized. To the best of author's knowledge, no data has been reported on the fermentation kinetics of *S. cerevisiae* Y-35. At different initial sugar concentrations, data obtained from the experimental observations was processed by a MATLAB® program to predict substrate utilization and product formation and to determine the kinetic parameters.

MATERIALS AND METHODS

Materials

S. cerevisiae NRRL Y-35 was obtained from ARS culture collection, USDA, Peoria, IL, USA. Cell pellets were grown and maintained on YPD agar media consisting of per liter: 10 g yeast extract, 20 g peptone, 20 g glucose, and 20 g agar. All chemicals were of pure analytical grade unless otherwise specified.

Inoculums Development

The medium used for the inoculums preparation consisted of (per liter): 10 g yeast extract, 20 g peptone, and 20 g glucose. Medium was autoclaved at 121°C for 15 minutes and glucose was added separately with a 0.22 µm syringe filter. Inoculums was prepared by transferring a loopful of an isolated colony from an agar plate to a 500 ml Erlenmeyer flask containing 200 ml of inoculums media, cultivated in the incubator shaker at 30°C, 150 rpm for 24h.

Fermentation Conditions

Fermentation experiments were performed in 100 ml Erlenmeyer flasks with rubber septa closures, containing 50 ml sterilized fermentation medium. The composition of medium was (per liter): 3 g yeast extract, 6 g KH_2PO_4, 2 g $(NH_4)_2SO_2$, and 0.4 g $MgSO_4$. The inoculum loading was in the range of the 5 to 40 gL^{-1} DCW. The experiments were carried out in an incubator shaker at 30°C and 120 rpm. Samples were withdrawn with the help of a needle syringe periodically for analyses of residual glucose and ethanol. Experiments were performed in duplicates and average values were reported.

Analytical Methods

Dry cell weight was determined by harvesting cells by centrifugation and washing with 1% (w/v) NaCl, followed by drying at 105°C for 2-3 h till constant cell mass was observed. Glucose concentration was determined by a high

performance liquid chromatography (Agilent 1200 series, Agilent Technologies) equipped with a RI detector (Agilent 1200 series) and a 300 mm X 6.5 mm Sugar-PAK I column (Waters, Division of Millipore) with a suitable guard column. Separation was achieved at 65°C with Millipore water as eluent at 0.25 ml min^{-1} flow rate and 5 µl injection volume. All samples were filtered through a 0.22 µm syringe filter prior to analysis. Ethanol was analyzed by a gas chromatograph (Agilent 7890A series) equipped with a Stabil Wax column (30m× 250 µm× 0.5 µm; Restek Corp., USA) at 250°C, FID detector at 300°C, nitrogen at 23 psi pressure, and helium as a carrier gas.

Kinetics of Fermentation

The kinetic model used in this study is based on modified Monod expressions and has been used previously (Krishnan et al., 1999; Dhabhai et al., 2012; Dhabhai et al., 2013). This model incorporates substrate and product inhibition functions. A MATLAB® programme was used to determine the kinetic parameters values (µ, μ_m, K_S, K_S', K_I, K_I', V_m, β, and γ). Model parameter estimation was carried out using fermentation results obtained from different sets of sugar concentrations with low (50.4 gL^{-1} and 100.8 gL^{-1}), moderate (125.5 gL^{-1} and 151.2 gL^{-1}), and high values (198.4 gL^{-1} and 264.2 gL^{-1}). The average values obtained from the fermentation at different sugar concentration were used for parameter estimation. Predicted values of substrate utilization and product formation were obtained using the programme. The model equations are as follows:

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_m S}{K_S + S + S^2/K_i}\left\{1 - \left(\frac{P}{P_m}\right)^{\beta}\right\} \quad (1)$$

$$v = \frac{1}{X}\frac{dP}{dt} = \frac{v_m S}{K_S' + S + \frac{S^2}{K_i'}}\left\{1 - \left(\frac{P}{P_m'}\right)^{\gamma}\right\} \quad (2)$$

$$-\frac{dS}{dt} = \frac{1}{Y_{P/S}}\frac{dP}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + mX \quad (3)$$

where: K_s, Monod constant for microbial growth (gL^{-1}); K_s', Monod constant, for product formation (gL^{-1}); K_i, Inhibition constant for microbial growth (gL^{-1}); *Ki'*, Inhibition constant for product formation (gL^{-1}); *P*, ethanol concentration (gL^{-1}); P_m, ethanol concentration above which cells do not grow (gL^{-1}), *Pm'*, ethanol concentration above which cells do not produce ethanol (gL^{-1}); *S*, substrate concentration (gL^{-1}); *X*, cell dry weight (gL^{-1}); $Y_{P/S}$, product yield (g product g^{-1} substrate); $Y_{X/S}$, cell yield constant from glucose (g cells g^{-1} substrate); µ, specific growth rate (h^{-1}); V, specific rate of product formation (h^{-1}); μ_m, maximum specific growth rate (h^{-1}); V_m, maximum specific rate of product formation (h^{-1}).

RESULTSS AND DISCUSSIONS

Effect of Inoculum Loading

Batch experiments were conducted at four different initial inoculum loading (5, 10, 20, and 40 gL^{-1} dry cell weight) The initial glucose concentration was 5% (wv^{-1}) and the process conditions were kept constant at agitation speed and temperature of 120 rpm and 30°C, respectively. Figure 1 presents the trend of ethanol production and glucose consumption (gL^{-1}) at different initial inoculum loadings.

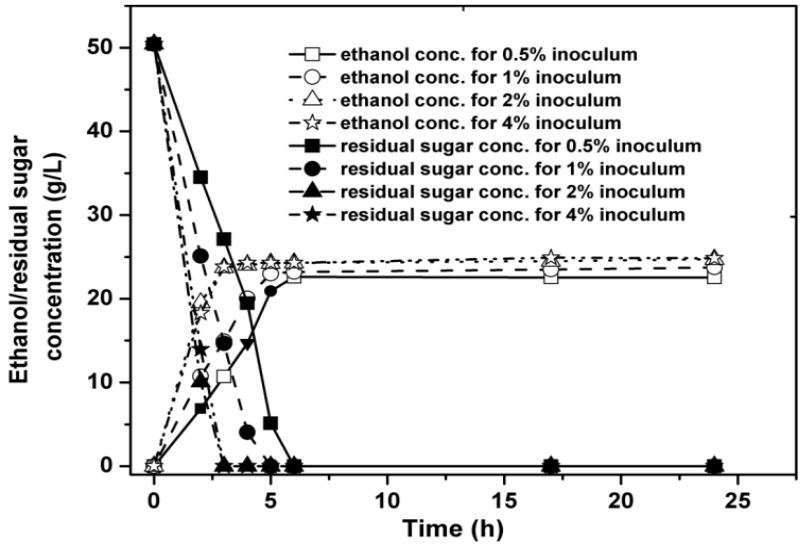

Figure 1: Ethanol Production and Residual Sugar Concentration with Respect to Fermentation Time at Initial Inoculum Loading of 5, 10, 20, And 40 Gl^{-1} DCW Conditions: Initial Substrate Concentration: 5% Wv^{-1}, Agitation Speed: 120 Rpm, and Temperature: $30^{o}c$

The maximum ethanol productivity of 7.9 $gL^{-1}h^{-1}$ was obtained at 20 gL^{-1} DCW inoculum loading, which produced 23.7 gL^{-1} ethanol in just 3 h, corresponding to 92.2% of the maximum theoretical conversion Further, increasing the inoculum concentration to 40 gL^{-1}, no significant increase in final ethanol concentration, yield, and productivity was obtained. Therefore, among four different loadings, inoculum concentration of 20 gL^{-1} DCW was chosen to be the optimum inoculum loading on the basis of ethanol yield, glucose consumption rate, and productivity. Zabed et al. (2014) have also reported that initial inoculum loading significantly affects sugar consumption rate and ethanol productivity. Dada et al. (2012) and Powchinda et al. (1997) have also stated that higher inoculum size may negatively affect ethanol production and lower yield and productivity may be obtained due to decreased viability of yeast population.

Effect of Sugar Loading

Initial sugar concentration is an important influencing parameter; as it directly affects both biomass and ethanol production. Generally, the rate of ethanol formation is increased with increased sugar loading up to a certain level, afterwards it may exceed the sugar consuming capacity of yeast due to substrate/product inhibition. High sugar concentration works as an inhibitor for yeast as it may cause osmotic shock of the cells and slow down their mass and heat transfer (Nikolic et al., 2010). A wide range of sugar concentration was chosen to see the effect of initial sugar concentration on fermentation. The summary of fermentation results is presented in Table 1.

Table 1: Fermentation Results with Different Initial Sugar Concentrations

Initial Sugar Concentration (Gl^{-1})	Time to Obtain Final Ethanol Concentration (H)	Final Ethanol Concentration (Gl^{-1})	Ethanol Yield ($G\ G^{-1}$)	Volumetric Ethanol Productivity ($Gl^{-1}h^{-1}$)	Consumed Sugar Concentration (Gl^{-1})	% of Max Theoretical Possible Conversion
50.4	4	24.1	0.48	6.01	50.4	93.5
100.8	6	45.9	0.49	7.66	100.8	97.7
125.5	8	56.1	0.45	7.01	125.5	87.7
151.2	8	63.1	0.42	7.89	151.2	81.9
198.4	24	77.0	0.39	3.20	198.4	76.1
264.2	48	107.2	0.40	2.23	46.8	79.6

With increased sugar concentration, final ethanol concentration was increased but the productivity decreased above 151.2 gL^{-1} sugar concentration. Ethanol productivity of 7.9 $gL^{-1}h^{-1}$ was obtained at 151.2 gL^{-1} in 8 h, while it slightly decreased to 6.1 $gL^{-1}h^{-1}$ at increased sugar concentration of 198.4g L^{-1}. The final productivity was found to be 3.2 $gL^{-1}h^{-1}$ in 24 h, at the point of complete consumption of sugar Najafpour et al. (2004) reported a study of batch fermentation of sugar by *S. cerevisiae* ATCC 24860. At an initial sugar concentration of 50 gL^{-1}, sugar consumption and ethanol production were obtained as 99.6% and 12.5% vv^{-1}, respectively, after 27 h. While in the present study, at an initial sugar concentration of 50.4gL^{-1}, 100% sugar was consumed in only 4 hours.

High sugar concentration, especially in batch fermentations, tends to inhibit fermenting organism, due to: (i) inherent limitation on sugar transport inside microorganism, (ii) extent of sugar metabolism, and (iii) high sugar concentration may lead to non-homogeneous concentration profiles generated by insufficient mixing due to increased viscosity of the solution (Koppram et al., 2014). All these factors may contribute to lower productivity and/or lower yield at high sugar concentrations. In general, initial glucose concentration in the range of 150-175 gL^{-1} has been reported to be inhibitory for the growth of most yeast species (Nikolic et al., 2010; Ozmihci and Kargi, 2007; Chandel et al., 2009).

Tang et al. (2010) studied ethanol fermentation in a continuous stirred tank reactor (CSTR) by a flocculating yeast strain at a dilution rate of 0.083 h^{-1}, achieved an ethanol concentration of 80 gL^{-1} with an ethanol productivity of 6.6 $gL^{-1}h^{-1}$. Zhang et al. (2011) investigated bioethanol production by simultaneous saccharification and fermentation (SFF) from raw sweet potato with *S. cerevisiae* strain CCTCC M206111 and obtained maximum ethanol concentration of 128.51 gL^{-1} with an ethanol productivity of 4.76 $gL^{-1}h^{-1}$. Ethanol productivity obtained in the present batch study with the use of *S. Cerevisiae* Y-35 was high as compared to the productivities reported in the CSTR and SSF studies. This implies the industrial potential of this yeast strain.

Kinetics of Fermentation

Model parameter estimation may not be accurate in case of inhibitory initial sugar concentration as the microorganism is performing at a sub-optimal level (Dhabhai et al. 2013). Furthermore, according to model equations, high values of inhibition constants K_I and K_I` and low values of reaction constants $K_S$ and $K_S$` would give high value of μ and V respectively, which generally result in higher product yield and volumetric productivity (Dhabhai et al. 2013).

The estimated values of model parameters are presented in Table 2. The experimental and predicted values of product and substrate concentrations with fermentation time are presented in Figure 2 and Figure 3. The values of R^2 were found to be 0.99 in all cases for both ethanol formation and glucose utilization, while low RMSE values were obtained, which indicates the goodness of model fitting.

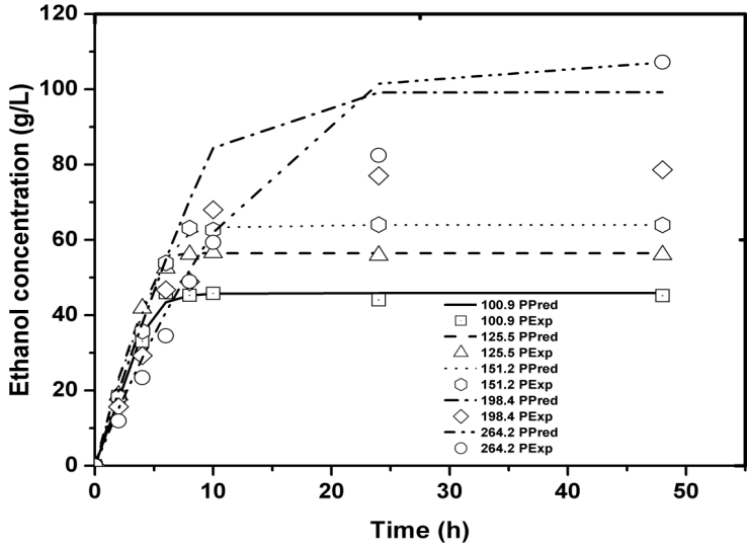

Figure 2: Comparison between Experimental and Predicted Data for Ethanol Production at Different Initial Glucose Concentration Pred: Predicted Trend; Exp: Experimental Trend. P1, P2, P3, P4, P5, and P6 Are The Ethanol Concentrations at Glucose Concentrations: 50.4gl^{-1}, 100.8gl^{-1}, 125.5gl^{-1}, 151.2gl^{-1}, 198.4gl^{-1}, and 264.2gl^{-1}, Respectively

In the case of low initial substrate concentration, i.e. in the range of 5-10%, the average values of μ_m and V_m were found to be 1.6 h^{-1} and 1.01 h^{-1}, respectively. For moderate substrate concentration (13-16%), the values of μ_m and V_m were found to be 0.56 h^{-1} and 23.1 h^{-1},, while at high initial substrate concentrations, i.e. 20 and 26%, the values were obtained 8.5 h^{-1}, and 9.8 h^{-1},, respectively. The highest value of V_m was obtained with moderate substrate concentration, indicating 13-16% concentration range to be the optimum for high ethanol yield and productivity.

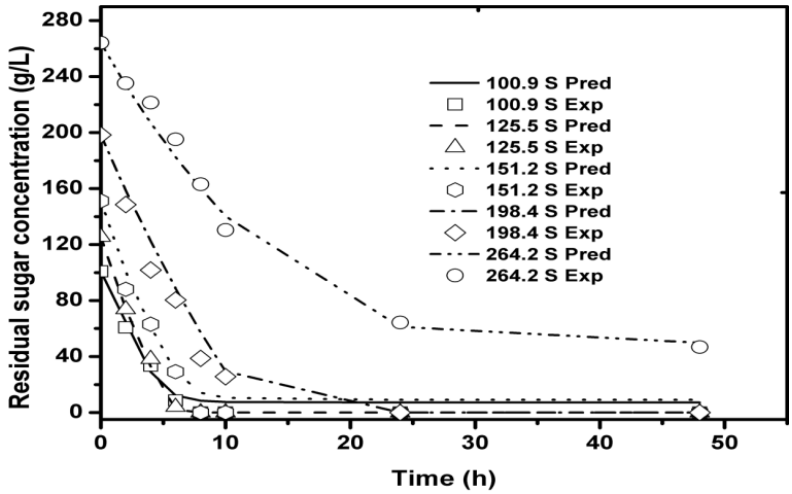

Figure 3: Comparison between Experimental and Predicted Data for Residual Sugar Concentration at Different Initial Glucose Concentration Pred: Predicted Trend; Exp: Experimental Trend. S1, S2, S3, S4, S5, and S6 Are the Residual Glucose Concentrations At 50.4g L-1, 100.8g L-1, 125.5g L-1, 151.2g L-1, 198.4g L-1, and 264.2 Gl-1, Respectively

At low (5-10%) initial sugar concentration, the values of K_s, K_s`, $K_I$ and $K_I$` were obtained as 57.41 gL^{-1}, 0.59gL^{-1}, 41.72 gL^{-1}, and 203.34 gL^{-1}, respectively, while at moderate (13-16%) range, the values of reaction rate constants K_s and K_s` were low (6.97 and 0.76 gL$^{-1}$, respectively) and inhibition constants $K_I$ and $K_I$` were high (168.11 and 9959.10 gL^{-1}, respectively), indicating high growth rate and less inhibition effects on glucose fermentation with *S. cerevisiae*. At high

sugar concentration (20-26%), the pronounced inhibition effect is evidenced by the low values of inhibition constants K_I and K_I` (0.38 and 12.08 $gL^{-1}$) and the high values of reaction rate constants Ks and Ks` (252.20 and 43.58 gL^{-1}) as compared to the values obtained at moderate substrate concentration.

Table 2: Estimated Values of Model Parameters for Glucose Fermentation at Different Initial Sugar Concentrations

Parameter	Initial Sugar Concentration		
	Low	Moderate	High
R^2 for S	0.99 L1* 0.99 L2	0.99 M1 0.99 M2	0.99 H1 0.99 H2
RMSE for S	0.91 L1 16.83 L2	5.56 M1 25.31 M2	30.99 H1 22.59 H2
R^2 for P	0.99 L1 0.99 L2	0.99 M1 0.99 M2	0.99 H1 0.99 H2
RMSE for P	1.11 L1 4.37 L2	5.35 M1 6.73 M2	43.41 H1 17.62 H2
$\mu_m[h^{-1}]$	1.60	0.56	8.48
$V_m[h^{-1}]$	1.02	23.09	9.85
Ks $[gL^{-1}]$	57.41	6.97	252.20
Ks' $[gL^{-1}]$	0.59	0.76	43.58
K_I $[gL^{-1}]$	41.72	168.11	0.38
K_I` $[gL^{-1}]$	203.34	9959.10	12.08
β [dimensionless]	4.59	0.99	0.02
γ[dimensionless]	0.30	0.01	0.68

*Glucose concentrations L1-50.4 gL^{-1}, L2-100.8 gL^{-1}, M1-125.5 gL^{-1}, M2-151.2 gL^{-1}, H1-198.4 gL^{-1} and H2-264.2 g L^{-1}.

In a similar study by Tesfaw et al., (2014) when initial sugar concentration was increased in the range of 85–156 gL^{-1}, the average specific growth rate and average biomass yield were significantly inhibited whereas average specific substrate uptake, average specific ethanol productivity, and average ethanol yield were increased. Results obtained in the present study are in accordance with the findings of Tesfaw et al., 2014. Similarly, Birol et al. (1998) fermented glucose using immobilized *S. cerevisiae* ATCC 9763 and studied a variety of different kinetic models. At an initial glucose concentration of 2%, μ_m and K_s were 0.186 h^{-1} and 0.390 gL^{-1} respectively, while at a glucose concentration of 10%, μ_m and Ks were 0.758 h^{-1} and 362.65 gL^{-1}, respectively The values of constants in the present study were found to be more favorable for high yield and productivity. Thus, in the present study, *S. cerevisiae* Y-35 showed enhanced performance up to sugar concentration of 16% as compared to the strain ATCC 9763.

CONCLUSIONS

The effects of initial inoculum and sugar concentration on ethanol concentration, yield, and productivity were evaluated in this work with a view to determine the robustness and possible industrial application of *S. cerevisiae* Y-35. Inoculum loading of 20 gL^{-1} DCW gave maximum ethanol yield and concentration. High productivity of 7.9 $gL^{-1}h^{-1}$ was obtained at 151.2 gL^{-1} in 8 h In order to see the inhibition effects of initial sugar concentration, kinetic study using a modified Monod model was carried out. Model fitting seemed to be reliable as indicated by the high R^2 and low RMSE values. The highest value of V_m was obtained at the moderate sugar concentration, whereas on increasing the sugar concentration to 198.4 gL^{-1} and 264.2 gL^{-1}, the pronounced inhibition effect was evident. Favorable values of constants (μ_m and K_i) and low K_s implies that this yeast performs much better even at sub-inhibitory concentration, which shows great

potential for its use for large scale fermentation operation.

NOMENCLATURES

K_s, Monod constant for microbial growth (gL^{-1}); K_s', Monod constant, for product formation (gL^{-1}); K_i, Inhibition constant for microbial growth (gL^{-1}); *Ki'*, Inhibition constant for product formation (gL^{-1}); *P*, ethanol concentration (gL^{-1}); P_m, ethanol concentration above which cells do not grow (gL^{-1}), *Pm'*, ethanol concentration above which cells do not produce ethanol (gL^{-1}); *S*, substrate concentration (gL^{-1}); *X*, cell dry weight (gL^{-1}); $Y_{P/S}$, product yield (g product g^{-1} substrate); $Y_{X/S}$, cell yield constant from glucose (g cells g^{-1} substrate); μ, specific growth rate (h^{-1}); V, specific rate of product formation (h^{-1}); μ_m, maximum specific growth rate (h^{-1}); V_m, maximum specific rate of product formation (h^{-1}); DCW, dry cell weight (gL^{-1}).

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REFERENCES

1. Birol G, Doruker P., Kirdar B., Onsan Z. İ., and Ulgen K. (1998). Mathematical description of ethanol fermentation by immobilized Saccharomyces cerevisiae, Process Biochem., 33(7), 763–771.
2. Chandel A. K., Kapoor R. K., Singh A., and Kuhad R.C. (2007). Detoxification of sugarcane bagasse hydrolysates improves ethanol production by Candida shehatae NCIM 3501, Bioresour. Technol., 98, 1947-1950.
3. Dada O., Kalil M. S. and Yusoff W. M. W. (2012). Effects of inoculum and substrate concentrations in anaerobic fermentation of treated rice bran to acetone, butanol and ethanol, Bacteriology Journal, 2, 79-89.
4. Dhabhai R., Chaurasia S. P., and Dalai A. K. (2012). Bioconversion of wheat straw lignocellulosic sugars to ethanol by recombinant Escherichia coli, J. Renewable Sustainable Energy, 4, 053127.
5. Dhabhai R., Chaurasia S. P., Singh K., and Dalai A. K. (2013). Kinetics of bioethanol production employing mono- and co-cultures of Saccharomyces cerevisiae and Pichia stipitis, Chem. Eng. Technol., 36, 1651–1657.
6. Huang W.H., and Wang F. S. (2010). Kinetic modeling of batch fermentation for mixed-sugar to ethanol production, J. Taiwan Inst. Chem. Eng., 41, 434–439.
7. Koppram R. and Olsson L. (2014). Combined substrate, enzyme and yeast feed in simultaneous saccharification and fermentation allows bioethanol production from pretreated spruce biomass at high solids loadings, Biotechnology Biofuels., 7, 54.
8. Krishnan M. S., Ho N. W. Y., and Tsao G. T. (1999). Fermentation kinetics of ethanol production from glucose and xylose by recombinant Saccharomyces 1400 (pLNH33), Appl. Biochem. Biotechnol. 77–79, 373–388.
9. Najafpour G., Younesi H., and Ismail K. S. K. (2004). Ethanol fermentation in an immobilized cell reactor using Saccharomyces cerevisiae, Bioresour. Technol., 92 (3), 251–260.

10. Nikolíc S., Mojovíc L., Pejin D., Rakin M., and Vukasinovic M. (2010). Production of bioethanol from corn meal hydrolysates by free and immobilized cells of Saccharomyces cerevisiae var. ellipsoid us, Biomass and Bioenergy, 85, 1750-1755.
11. Olaoye O. S. and Kolawole O. S. (2013). Modeling of the Kinetics ethanol fermentation from glucose biomass in batch culture with a non-structured model, International Journal of Engineering Research and Applications, 3(4), 562-565.
12. Ozmihci S. and Kargi F. (2007). Kinetics of batch ethanol fermentation of cheese-whey powder (CWP) solution as function of substrate and yeast concentrations, Bioresour. Technol., 98, 2978–2984.
13. Powchinda O., Delia-Dupuy M. L., and Strehaiano P. (1997). Alcoholic fermentation from sweet sorghum: some operating problems, 9th Annual Meeting of the Thai Society for Biotechnology, Bangkok, Thailand, Available at: http://www.thaiscience.info/, accessed on 24 August, 2015.
14. Renewable fuels associations, (2015). Cited from: http://www.ethanolrfa.org/pages/advanced-ethanol, accessed on 24 August, 2015.
15. Sansonetti S, Hobley T. J, Calabrò V, Villadsen J, and Sin G. (2011). A biochemically structured model for ethanol fermentation by Kluyveromyces marxianus: a batch fermentation and kinetic study, Bioresour. Technol., 102, 7513–7520.
16. Steensels J, Snoek T., Meersman E., Nicolino M. P., Voordeckers K, and Verstrepen K. J. (2014). Improving industrial yeast strains: exploiting natural and artificial diversity, FEMS Microbiol. Rev., 38, 947–995.
17. Talebnia F., Karakashev D., and Angelidaki I. (2010). Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation, Bioresour. Technol., 101, 4744-4753.
18. Tang Y. Q., An M. Z., Zhong Y. L., Shigeru M., Wu X. L., and Kida K. (2010), Continuous ethanol fermentation from non-sulfuric acid-washed molasses using traditional stirred tank reactors and the flocculating yeast strain KF-7, J. Biosci. Bioeng, 109(1), 41-46.
19. Tesfaw A, and Assefa F. (2014). Current Trends in Bioethanol Production by Saccharomyces cerevisiae: Substrate, Inhibitor Reduction, Growth Variables, Coculture, and Immobilization, Int. Scholarly Res. Not, 532852, 1- 11.
20. Wang Q. M, Liu W. Q, Liti G, Wang S. A and Bai F.Y. (2012). Surprisingly diverged populations of Saccharomyces cerevisiae in natural environments remote from human activity, Mol. Ecol, 21, 5404 – 5417.
21. Zabed H, Faruq G, Sahu J. N, Azirun M. S, Hashim R, and Boyce A. N. (2014). Bioethanol Production from Fermentable Sugar Juice, Sci. World J, 957102, 1-11.
22. Zhang L, Zhao H, Gan M, Jin Y, Gao X, Chen Q, Guan J, and Wang Z. (2011). Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales, Bioresour. Technol, 102(6), 4573-4579.