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Evaluation of fermentation kinetics of xylose to ethanol fermentation in the presence of acetic acid by *Pichia stipitis:* Modeling and experimental data comparison

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Acetic acid is one of the main inhibitor that shows negative impact on kinetics of sugar fermentation in the presence of *Pichia stipitis*. Unstructured kinetic model has been formulated that describes cell mass growth and ethanol production as a function of ethanol, oxygen, xylose and acetic acid concentration. Experiments have been carried out in batch mode with the acetic acid concentration varying from 3 to 12 gL^{-1}. Kinetic parameters are estimated for *Pichia stipitis* with various operating conditions of fermentation. Among all the kinetic parameters a great reduction in μ_{MAX} and increase in $Y_{P/X}$ has been observed, which strongly affect the fermentation kinetics. Acetic acid presence in the fermentation lead to significant reduction in the maximum cell biomass concentration, reduction in xylose consumption rate, improvement in ethanol metabolic and reduction in ethanol production rate. This model describe physiological properties of PSA30 strain of *Pichia stipitis* and proposed models can be used to predict the influence of xylose, ethanol and acetic acid on cell growth and ethanol productivity in industrial fermentation.

Keywords: Acetic acid, Growth model, Kinetic parameter estimation, *Pichia stipitis*

Bio-ethanol from the fermentation process is considered as a potential alternative fuel. Being a renewable energy source, bio-ethanol has important advantages when compared to gasoline. Being an oxygen rich fuel, the emission of green house gases and particulate materials from ethanol combustion is lower[1]. For ethanol fermentation, generally streams coming from agricultural products such as corn, sugarcane, sweet sorghum are used. In recent years, agriculture residues such as byproducts of corn, sugarcane and wood industry have also been identified as potential sources for ethanol production[2]. Most of these agricultural feedstock contains both hexose and pentose sugars. Though hexose sugars are used today for ethanol production, it is also possible to utilize xylose (pentose) for the ethanol fermentation, as xylose is the second major product of saccharification of lignocellulosic feedstocks[3].

In the common bio-ethanol fermentation processes *Saccharomyces cerevisiae* yeast is widely used for ethanol production[4]. However, *S. cerevisiae* is only able to ferment glucose sugars, and cannot ferment pentose sugars like xylose[5]. Successful utilization of xylose would help in driving the process economics of lignocellulosic biomass to ethanol fermentation favorably. In several reports, yeast *Pichia stipitis* has been identified for efficient conversion of xylose to ethanol under micro-aerobic conditions[6].

Lignocellulosic hydrolysate generated from the agricultural residues contains high concentration of inhibitors that negatively affects microbial growth. Inhibitors of xylose fermentation in lignocellulosic hydrolysate include weak organic acids, sugar derived compounds like furfural and hydroxymethyl furfural and lignin degradation products. Formation of inhibitors in the pretreatment step of xylan hydrolysis is the main hurdle in the fermentation of xylose[7].

Inhibitor formation in the pretreatment varies with the type of feedstock, pretreatment. If the pretreatment is at high severity then more and strong inhibitors are generated. Acetyl groups from the biomass are converted into acetic acid and hemicelluloses is converted into furfural as inhibitors. Another inhibitor is 5-hydroxymethyl furfural, which is generated from the glucose. These inhibitors have been shown to negatively impact the fermentative performance (cell growth, ethanol yield, productivity and sugar

consumption rate) of *S. cerevisiae,* which is used for fermentation of mixed sugar of glucose and xylose. It has been observed that presence of low concentration weak acid can increase ethanol yield but at high acid concentration it reduces the cell mass growth and hence the ethanol productivity[8,9].

Acetic acid is a weak acid generated from the deacetylation of hemicelluloses during pretreatment. Acetic acid present in varying concentrations in all types of biomass. In hydrolysate acetic acid concentration varies in the range of 3 to 10 g L^{-1}, depends upon the feedstock and type of pretreatment. Elimination of acetic acid from the hydrolysate would increase the conversion cost of biomass to ethanol. Therefore, effect of these inhibitors on ethanol yield and productivity must be studied for commercialization of xylose fermentation. *S. cerevisiae*, which also has inhibition effect of acetic acid and it depends upon the *p*H of hydrolysate. Acetic acid is in un-dissociated form at low *p*H, which has a more impact on growth. Other toxins like HMF and furfural generated are low in concentrations and same can be metabolized by *S. cerevisiae*, due to which inhibitory effect can by nullified by these components[10-13].

Cell mass generated during the fermentation also get hamper due to the acetic acid. Acetic acid in un-dissociated form in fermentation broth is diffused through the cellular membrane after that it penetrates the cytoplasm and acidify it. To maintain the internal *p*H, ATP is utilized by the cell to pump H^+ protons out of the membrane so part of ATP is diverted to cell maintenance which leads in to lower cell mass yield. Increase in fermentation time is due to the yeasts pumping H^+ protons to reach the minimum *p*H for cell growth[14-16]. The inhibiting effect of acetic acid on the kinetics of the alcoholic fermentation by xylose-isomerase based *S. cerevisiae* has been presented by the Bellissimi *et al.*[17] and Casey *et al.*[18]. Acetic acid toxicity has been studied in xylose-fermenting *S. cerevisiae*. Acetic acid inhibition is *p*H dependent and undissociated form of acetic acid is the main inhibitor in glucose-xylose mixture hydrolysate[17,18].

Xylose to ethanol fermentation process could be further optimized by the development of realistic growth and fermentation models. In aerobic fermentation process oxygen should be continuously supplied in order to achieve higher productivities, as the role of oxygen in microorganism growth and metabolism is vital. Ethanol yield maximization is a challenging task as oxygen supply increases the ethanol productivity but if it exceeds the limit then it will reduce the ethanol yield due to respiration action by cell mass[19]. The inhibiting effect of acetic acid on the kinetics of the alcoholic fermentation is presented by the Andrade *et al.*[20]. A term of acetic acid is added in the kinetic model to consider the inhibitory effect in the fermentation of hydrolysate from biomass.

Kinetics of ethanol production by *Scheffersomyces stipitis* on xylose with the development of mathematical model is given by Daniele Farias *et al.*[21]. Inhibition effects of substrate, product on cell mass and ethanol production is included in the kinetic model. Kinetic model for *Pichia stipitis* proposed by Slininger *et al.*[22] consists of a four equation system of differential equations including xylose, ethanol, cell mass and oxygen concentration. The model was validated for various growth conditions. Inhibitory effects were not considered in this above mentioned models for fermentation of xylose by *Pichia stipitis*. In commercial scale fermentation, hydrolysate generated in the pretreatment of xylan hydrolysis consists of inhibitors such as acetic acid, furfural etc. So, extensive model needs to be developed for PSA30 *Pichia stipitis*, which can also take care of inhibitor effect in the kinetic model.

This is the first report for development of mathematical model which considers acetic acid inhibition and oxygen concentration in the model for xylose to ethanol fermentation by *Pichia stipitis*. The focus of this work is to estimate the effect of xylose, ethanol, oxygen and acetic acid concentration on cell growth and ethanol production. Kinetic model consisting of linear differential equation has been developed that describes cell mass growth, ethanol production, xylose consumption, oxygen concentration and acetic acid consumption. Kinetic parameters are dependent upon the operating conditions such as temperature, *p*H, media and nutrients. Re-estimation of kinetic parameters is required to get accurate kinetic profile of fermentation in presence of acetic acid when there is a change in operational conditions. In this work, inhibition term of acetic acid was added to previous model. Related kinetic parameters were re-estimated to describe the kinetics in the presence of acetic acid. The factors that are affecting the commercial xylose fermentative process are substrate inhibition, ethanol inhibition, aeration and acetic acid inhibition. In the literature, no proposed model is available which can accommodate all these factors into consideration for

xylose fermentation. Main objective of this work is to develop a mathematical model which can describe cell mass, substrate, oxygen, ethanol and acetic acid concentrations during fermentation process.

Experimental Section

Microorganism

Pichia stipitis ATCC 58784 was adapted by serial propagation in xylose hydrolysate. The adapted strain was designated as *Pichia stipitis* PSA30. It was maintained at -80°C in the form of glycerol stock in xylose rich hydrolysate.

Media and bioreactor assembly

Aerobic batch fermentations were carried out in 1-L New Brunswick Bio Flow fermenters equipped with *p*H control. Oxygen supply in the fermenter was maintained by sparging air into fermenter broth. All trials were conducted at constant aeration of 0.2 vvm. The inoculum for the fermenter was prepared by growing *Pichia stipitis* strain aerobically in a shaker set at 32°C in 500 mL flask containing 250 mL YPX media (1% yeast extract, 2% peptone and 3% xylose). Two stage inoculations were done to prepare inoculum for main fermentation. The inoculated flasks were incubated at 32°C in a rotary shaker at 250 rpm for 24 h. After incubation, a sample was analyzed for the cell mass concentration. This culture was used as inoculum for main fermentation of 1 L working volume in YPX media (1% yeast extract, 2% peptone and 5% xylose) with synthetic medium. Inoculum volume added was 10% of the main fermenter volume.

Xylose concentration in the starting fermentation media was maintained at 53 g L^{-1}. The acetic acid concentrations examined were 3, 7, 12 g L^{-1} and 0 (for control). Acetic acid concentrations selected above are to be expected in hydrolysate after lignocellulosic biomass pretreatment. Acetic acid was added at a concentration as mentioned above and the *p*H was readjusted before inoculation. As per requirement, the *p*H of the hydrolysate was adjusted to the value of 5.5.

Cell culture developed in shake flask was inoculated after *p*H maintenance in fermentation media. The fermentation cultures were stirred at 200 r.p.m. at 32°C temperature and aeration rate is maintained at 0.2 vvm. Dissolved oxygen was monitored with O_2 electrode. In case of constant *p*H requirement, the media *p*H was continuously controlled within ±0.2 from the desired value using *p*H control system with the BioFlow fermenter.

Analytical methods

Sugars (cellobiose, glucose, xylose), fermentation products (ethanol, xylitol, glycerol) and inhibitors (formic acid, acetic acid) were analyzed by high performance liquid chromatography (HPLC) using an Agilent 1100 system, refractive index detector and Bio-Rad Aminex HPX-87H column (300×7.8 mm I.D.) for separation of compounds at 5°C. Sulfuric acid (0.005 M) was used as the mobile phase at flow rate of 0.6 mL/min. Culture samples at selected time intervals were filtered at high speed centrifuge (8000 r.p.m.) and filtrate were removed. Dry cell weights were measured by keeping samples overnight in vacuum oven at 60°C. Cell growth was also measured by using spectrophotometer, in terms of optical density at A_{600nm}.

Data processing-model fit

The performance of the models was evaluated by using the residual standard deviation (RSD). To characterize the quality of model fitting, a percentage of the average of the experimental values measurement is used.

$$\text{RSD}\ (\%) = \left(\frac{\sqrt{\text{RSD}}}{\text{dp}'} \right)^{*} 100 \qquad \ldots(1)$$

where,

$$\text{RSD} = \frac{1}{np} \Sigma_{\text{p=1}}^{np} (\text{dp} - \text{Xp})^2$$

Model is accurate when value of RSD is small.

Mathematical modeling

In fermentation process cell mass production, substrate consumption, ethanol production and oxygen concentration is described by differential equations. The specific growth rate follows dissolved oxygen limited Monod growth kinetics as mentioned in below equations. In these experimentation combined model is utilized which expresses the cell mass growth as a function of substrate, cell mass, product concentration. Additional term of oxygen concentration is added to this equation to accommodate the effect on cell mass concentration as it is proved that oxygen concentration plays major role in cell mass concentration.

$$\frac{dx}{dt} = \mu * X \quad \ldots (2)$$

As discussed μ i.e. cell mass specific growth rate which is dependent on oxygen, substrate and product concentration, so following terms are added.

$$\mu = \mu_{max} * \left(\frac{C_{ox}}{K_{ox} + C_{ox}}\right)$$

where, μ_{max} is dependent on substrate and product concentration

Growth rate of cell mass is influenced by substrate and product inhibition. Slininger model for dependence of maximum specific growth rate on product and substrate concentration given by following equation.

Kinetic model mentioned by Slininger *et al.* does not account inhibition generated due to acetic acid. A new term is proposed in this work and added in the original kinetic model of Slininger *et al.* to express the model for our strain. Revised growth model equation is mentioned as below

$$\frac{dx}{dt} = \mu_{MAX} * \left(\frac{S}{K_S + S} - \frac{K_I}{K_I + Sm - S}\right) * \left(1 - \left(\frac{P}{P_{max}}\right)^n\right) * \left(\frac{C_{ox}}{K_{ox} + C_{ox}}\right) * \left(1 - \frac{CA}{CA_{max}}\right)^m * X \quad \ldots (3)$$

In above equation, the new term m and CA_{max} describes inhibition by acetic acid on cell mass growth.

The ethanol production rate of *Pichia stipitis* is related to growth associated & non-growth associated product mode. Product formation rate is given by Luedeking and Piret and represented by following differential equation.

$$\frac{dP}{dt} = Y_{P/X} * \frac{dx}{dt} + m_e * X \quad \ldots (4)$$

Substrate consumption rate will be depending upon product formation rate.

$$\frac{dS}{dt} = -\frac{1}{Y_{P/S}} * \frac{dP}{dt} \quad \ldots (5)$$

$$\frac{dS}{dt} = -\frac{X}{Y_{P/S}} * Y_{P/X} * \mu - \frac{me * X}{Y_{P/S}} \quad \ldots (6)$$

The dissolved oxygen concentration in the medium is depending upon oxygen uptake rate and oxygen transfer rate.

$$\frac{dCox}{dt} = K_L a * (C^*_{ox} - C_{ox}) - q_{O2} * X \quad \ldots (7)$$

OUR is related to biomass concentration in broth i.e. oxygen required for biomass production rate

$$OUR = q_{O2} * X = \frac{1}{Y_{X/OX}} * \frac{dx}{dt} \quad \ldots (8)$$

Substitute equation (8) in equation (7) we will get

$$\frac{dCox}{dt} = K_L a * (C^*_{ox} - C_{ox}) - \frac{1}{Y_{X/OX}} * \frac{dx}{dt} \quad \ldots (9)$$

In experimental details, it has been observed that acetic acid got consumed with fermentation of xylose fermentation. For the incorporation of acetic acid term in the kinetics, rate of acetic acid needs to be find out throughout the fermentation time. During fermentation, acetic acid is consumed and same has been converted to biomass through TCA cycle. Therefore rate of acetic acid consumption is given by:

$$\frac{dCA}{dt} = -\frac{1}{Y_{X/CA}} * \mu * X \quad \ldots (10)$$

Following equations from Slininger model is modified to estimate the dependency of yield of ethanol per xylose consumed for *Pichia stipitis PSA30* strain.

$$Y_{P/S} = 0.421 - 0.343 * \mu \quad \ldots (11)$$

$$me = ME_{max} * (\exp(-S/K_I') - \exp(-S/K_S')) * (1 - P/p_{max}')^{n'}) \quad \ldots (12)$$

$$Y_{X/CA} = 1.4 - 0.1 * \mu \quad \ldots (13)$$

Results and Discussion

The latest model developed by Slininger *et al.*[22] for xylose fermentation which includes substrate, product inhibition, and oxygen concentration was simulated for the experiment in presence of acetic acid. The results of the simulation were compared with experimental data as shown in Fig. 1.

As can be seen in Fig. 1, the original model developed by Slininger *et al.*[22] was not capable of describing the kinetics of xylose fermentation when acetic acid is present in the fermentation broth. The experimental data obtained in the presence of acetic acid suggests a reduction in the rate of substrate consumption, which leads to an increase in fermentation time and to lower productivity compared with the fermentation that is conducted without acetic acid. Thus, a new evaluation of the kinetics of xylose fermentation, it is necessary to develop reliable models for fermentation in the presence of acetic acid.

Kinetic models developed up till now, not able to account inhibition generated due to acetic acid. Though other inhibitors are also present like HMF, Furfural etc., but in our xylan hydrolysis, inhibitory level of furfural and HMF are lower. So, was eliminated inhibition term of furfural and HMF. Only acetic acid is considered for inhibition. Thus acetic acid generated from the acid hydrolysis is considered as a growth inhibitor. A new term is proposed in this work and added in the original kinetic model of Slininger *et al.* to express the model for our strain. Revised growth model equations are used which are mentioned above.

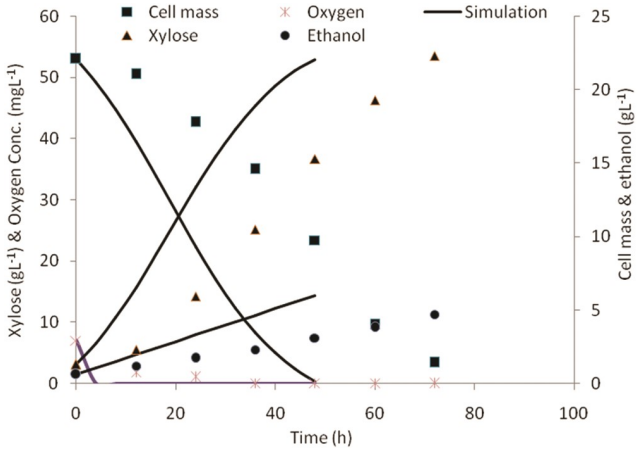

Fig. 1 — Experimental and simulated data by available model for xylose fermentation

The experiments were performed with addition of acetic acid in the medium of synthetic xylose to study the effect of acetic acid inhibition on fermentation kinetics. Xylose concentration was maintained constant for all experiments at 53 g/L. Fermentation was carried out at 30°C and at initial *p*H of 5.5. Samplings were done at specified intervals regularly. The fermentation experimental data of acetic acid inhibition study is shown in Table 1 and re-estimated kinetic parameters are shown in Table 2. Concentration profiles of experimental and model predicted values are shown in Fig. 2.

Model discussed earlier in this work was not capable to evaluate the fermentation kinetics consisting of acetic acid inhibition. To predict the fermentation kinetics accurately, new term was added in earlier model to reformulate it. In the new model, represented by equation (2) - (13), eight parameters are estimated: $Y_{P/X}$, μ_{MAX}, P_{max}, P_{max}', ME_{max}, n, m, CA_{max}. These parameters vary with the experimental conditions like agitation and acetic acid concentration. Parameters that don't have higher influence were kept constant. i.e. Xm= 260 g L^{-1}, K_{ox}= 0.1 mgL^{-1}, Y_{xox}=0.003 $g.mg^{-1}$, K_s=0.1, n' =1.7, K_s'=45.9, K_I=63, K_I'=65, K_La=3.5 h^{-1}. Slininger *et al.*[22] have shown that the fixed parameters are statically non-significant for the mathematical model.

Table 2 — Kinetic parameters comparison in presence of acetic acid

Kinetic parameter	Unit	Estimated parameter from Slininger *et al.* model	Re-estimated kinetic parameter considering acetic acid inhibition
$Y_{P/X}$	g/g	1.4	2.3
MEmax	g/g/h	1.75	0.8
μ_{MAX}	h^{-1}	0.8	0.7
n	-	1.4	7.8
P_{max}	g/L	41	39
Pmax'	g/L	43	38
CA_{max}		--	15
m		--	0.05

Table 1 — Experimental data at various acetic acid concentrations

Initial acetic acid concentration (gL^{-1})	Fermentation time (h)	Final acetic acid concentration (gL^{-1})	Maximum cell mass concentration (gL^{-1})	Maximum ethanol concentration (gL^{-1})
3	84	0.1	5.3	22.4
7	108	3.5	5.1	22.5
10	148	6.9	4.2	21.9
12	240	9.5	3.2	21.9

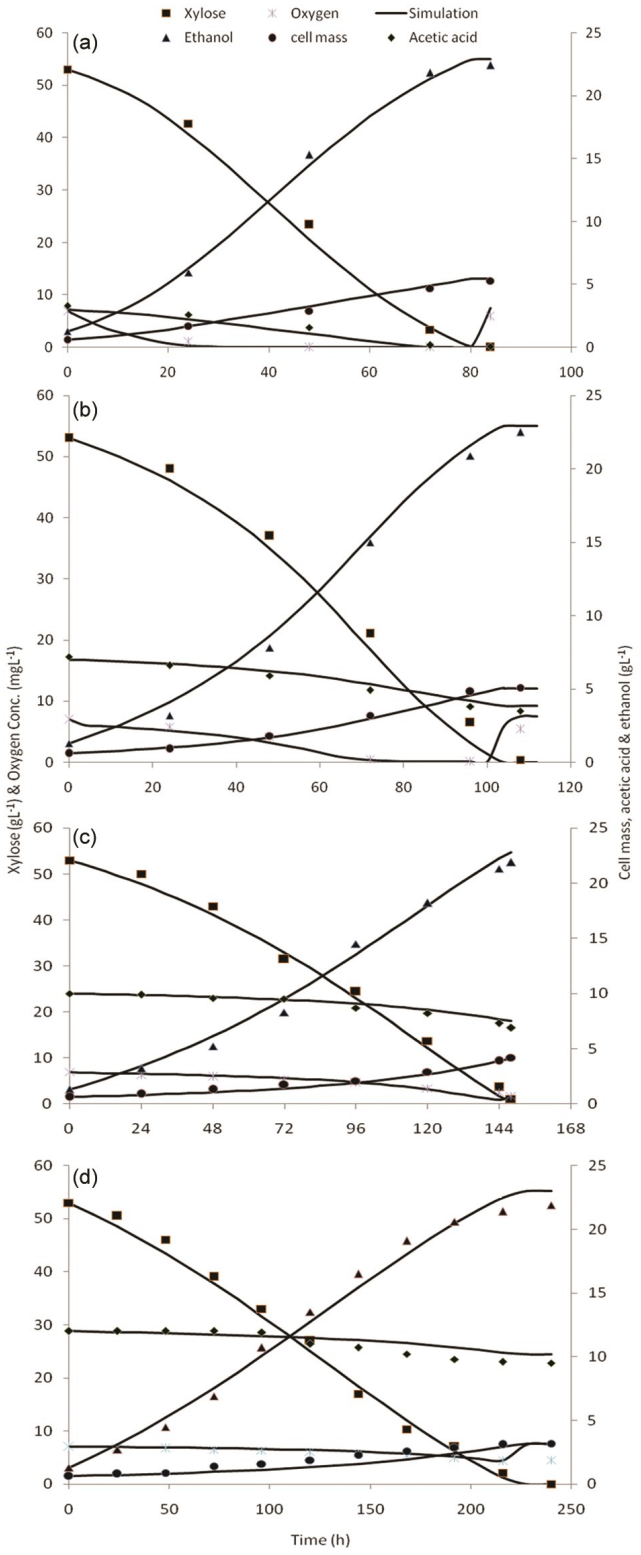

Fig. 2 — Experimental and simulated data by modified model at various acetic acid concentrations a) 3 g L^{-1} b) 7 g L^{-1} c) 10 g L^{-1} d) 12 g L^{-1}

For simultaneous estimation of kinetic parameters, MATLAB ODE solver has been used with minimization operation for error reduction. For the batch mode experimentation as mentioned above, the kinetic parameter estimation was performed to describe the concentration profiles and kinetic parameters were estimated to minimize the object function in MATLAB. The values of kinetic parameter are compared with those obtained by original Slininger et al. model for *Pichia stipitis PSA30* strain. The parameter CA_{max} and m are added to the original model used by Sliniger *et al.*[22] in order to represent the kinetics of fermentation in presence of acetic acid. The mode of inhibition followed a hyperbolic-type function. After the re-estimation procedure, the new kinetic parameters that minimize the objective function are as follows. $Y_{P/X}$= 2.3,μ_{MAX}= 0.7 h^{-1}, n=7.8, P_{max} = 39 g/L, P_{max}'= 38, ME_{max} = 0.8, m=0.04, CA_{max}=15. The parameters re-estimated in Table 2, shows reduction in value of μ_{MAX} i.e. reduction of growth rate of *Pichia stipitis*, when compared with fermentation without acetic acid. Re-estimated μ_{MAX} value is 0.7 h^{-1} as compare to 0.8 h^{-1} for model without considering acetic acid effect. It suggests that there is increase in toxicity due to acetic acid.

The values of yield of ethanol per biomass formed ($Y_{P/X}$) were higher than the obtained by Slininger *et al.* This can be explained by an increase in ethanol formation with the relation to biomass produced in presence of acetic acid in media. Similar observation also explained by Maiorella *et al.*[16] and Andrade *et al.*[20] There is increase in ethanol formation with relative to cell mass generation due to presence of acetic acid. In presence of acetic acid, when intracellular *p*H is low, cells require additional ATP to pump out the excess protons. Because of this additional ATP, cell growth is decreased and higher energy for maintenance is required. Proposed model simulation and experimental data for the fermentation in presence of acetic acid is shown in Fig. 2. The additional inhibitory term, which takes into account the impact of acetic acid on the kinetics, results in accurate description of the fermentation profile.

Accuracy of model fitting is checked by RSD (%) and cumulative data is shown in Table 3. The concentrations of cell mass, xylose, ethanol, acetic acid and oxygencalculated using the model after parameter estimation presented deviations in the range 3.6-25% from the experimental data, while the RSDs without parameter updating and using the

Table 3 — Residual standard deviations for experimental and model data

Acetic acid concentration (g/L)	Variables				
	Cell mass	Xylose	Ethanol	Oxygen Conc.	Acetic acid
3	6.2	5.8	3.4	25.3	9.1
7	5.5	7.3	5.3	25.6	5.9
10	8.2	5.6	6.8	13.6	3.6
12	9.3	5.7	6.1	16.1	5.1

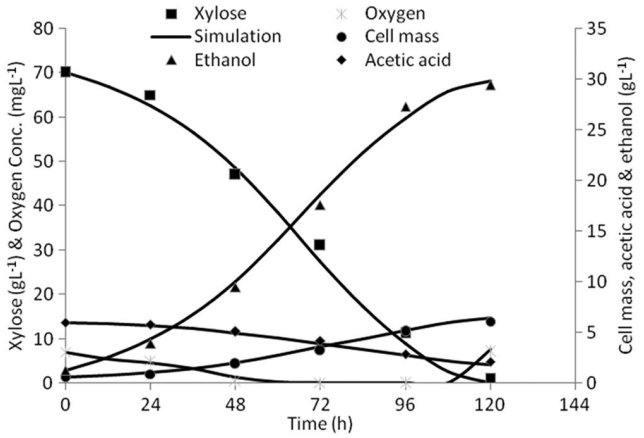

Fig. 3 — Validation of the model with experimental values (symbol) and model predicted values at acetic acid concentration 6 g L^{-1}

Slininger *et al.*[22] reached 87%, which are too inaccurate to describe the experimental data in presence of acetic acid. For model validation, another experiment was done with initial xylose concentration of 70 g L^{-1} and acetic acid of 6 g L^{-1}. Model predicted and experimental data comparison is shown in Fig. 3. The RSDs (%) between the model and experimental data were 7.7, 5.7, 4.8, 14.4 and 4.5 for X, S, P, C_{ox} and C_A.

The fermentation ability of *Pichia stipitis* for xylose fermentation in the presence of acetic acid was evaluated. The kinetics was evaluated based on an existing and well validated model. New expression have developed for cell mass growth taking into account the inhibition by acetic acid, which is one of the main inhibitor in fermentation of lingo cellulosic feedstock. For this purpose, batch fermentation was carried out at various concentrations of acetic acid and the experimental data used to re-estimate the kinetic parameters.

Main objective of this work was to define the fermentation kinetics in the presence of acetic acid. The model including acetic acid inhibition and re-estimating the kinetic parameters were shown to predict the fermentation profile of xylose to ethanol fermentation with high accuracy. Existing model in the literature and parameters are not suitable to represent kinetics of the xylose fermentation in the presence of acetic acid.

Fermentation in presence of acetic acid had a low value of μ_{MAX}= 0.7 h^{-1} compared to 0.8 h^{-1} for experimentation without acetic acid. It suggests that growth rate is lower in presence of acetic acid. Same observation was recorded by Narendranath *et al.*[13] and Andrade *et al.*[20]. These model shows that presence of acetic acid decreases the μ from 0.35 to 0.1 h^{-1} for *Saccharomyces cerevisiae*. Growth rate mainly used to evaluate the microbes performance to the medium and it affects the fermentation time as fermentation mainly depends upon the growth rate.

It has been observed that in presence of acetic acid, ethanol volumetric productivity decreases. In case of lower acetic acid inhibition of 3 and 7 g L^{-1}, the ethanol productivity reduced to 0.27 and 0.21 g ethanol L^{-1} h^{-1} respectively as compare to 0.53 g ethanol L^{-1} h^{-1} for without acetic acid. This is due to the increased in lag phase as acetic acid inhibits the cell mass growth. For higher acetic acid, concentration of 10 and 12 g L^{-1}, the ethanol productivity reduced to 0.15 and 0.09 g ethanol L^{-1} h^{-1} respectively. Other inhibitions like HMF and furfural were not considered in this work as these are present at lower concentration and below the inhibition level. Additional kinetic parameter CA_{max} was used which is the maximum acetic acid concentration at which the cell mass growth ceases. The other kinetic parameter m is exponent governing acetic acid inhibition of growth, which was found to be 0.05. This is lower than one, so acetic acid inhibition is hyperbolic inhibition.

Slininger *et al.*[22], Du Prezz *et al.*[23] and D. Farias *et al.*[21] show that the xylose fermentation process through *Pichia stipitis* is inhibited by ethanol concentration from 42 to 55 g L^{-1}. In our work, the final ethanol concentration reached up to 41 g L^{-1}. However, the fermentation time (176 h) was quite less as compared to published literature values of 240 h by D. Farias *et al.*[21].

Though the inhibitory effect of acetic acid is on cell mass growth and fermentation time, the ethanol yields are not decrease marginally as without acetic acid medium (with reference to substrate consumption). Ethanol yield are only reduced by 4% only for higher acetic acid. Quality of the data fitting

was checked by the standard residual deviations (RSD), which describes the average percentage deviations of predicted values from the experimental values. It had been observed that, after parameter re-estimation deviations are in the range of 4% - 25% for cell mass, xylose, ethanol, acetic acid and oxygen concentration. In case of oxygen concentration, the deviations are higher; this may be due to measuring inaccuracy of experimental values by DO probe. Relatively low value of RSD (%) shows good quality of data fitting and more predictive model.

Model validation was done with batch experimentation at 70 g L^{-1} initial xylose concentration and acetic acid concentration of 6 g L^{-1}. Experimental and model predicted RSD values for model validation found lower. Experimental and model predicted fermentation profile is shown in Fig. 3. It shows that model selected describes the kinetic of fermentation in presence of acetic acid accurately.

Conclusion

Kinetic parameters estimated for acetic acid inhibition model is differed than those estimated using earlier model. Thus the re-estimation was necessary to study the acetic acid inhibition effect on xylose fermentation. In commercial scale, acetic acid will be the main inhibitor as it is generated in the xylan hydrolysis. A new term of acetic acid inhibition must be added to include the effect of fermentation time on reactor design. The addition of acetic acid strongly affected μ_{MAX},$Y_{P/X}$ parameters. The proposed model can contribute to the development of an optimal and cost effective process for ethanol production from xylose by *Pichia stipitis*. Approach described in this work can be used in process optimization, design and control, simulation and optimization of the process. Findings from this work can be helpful for efficient xylose utilization and high ethanol productivity and yield, which can lead to reduction in production cost of industrial scale.

Abbreviations

C_{ox} dissolved oxygen concentration (mg L^{-1})
C_{ox} * critical dissolved oxygen concentration (mg L^{-1})
CA acetic acid concentration (g L^{-1})
CA_{max} maximum concentration of acetic acid at which cell growth ceases (g L^{-1})
dp experimental value
dp' average of experimental values
h time (h)
K_I Substrate inhibition constant for growth (g L^{-1})
K_I' Parameter governing substrate inhibition of fermentation (g L^{-1})
K_L mass transfer coefficient (m s^{-1})
K_La volumetric mass transfer coefficient (s^{-1})
K_{ox} saturation constant for oxygen limited growth (mg L^{-1})
K_S Saturation constant governing xylose limited growth (g L^{-1})
K_S' Saturation constant governing xylose limited fermentation (g L^{-1})
m_e specific ethanol productivity for maintenance (g g^{-1} h^{-1})
ME_{max} maximum specific maintenance productivity (g g^{-1} h^{-1})
n exponents governing ethanol inhibition of growth
np experimental value
n' exponents governing ethanol inhibition of fermentation
P ethanol concentration (g L^{-1})
P_{max} maximum ethanol concentration allowing growth (g L^{-1})
P_{max}' maximum ethanol concentration allowing fermentation (g L^{-1})
S xylose concentration (g L^{-1})
S_m maximum xylose concentration allowing growth (g L^{-1})
q_{o2} specific oxygen uptake rate (mol O_2 kg^{-1} s^{-1})
X cell mass concentration (g L^{-1})
Xp value predicted by mathematical model
$Y_{P/X}$ yield of ethanol per biomass formed (g g^{-1})
$Y_{P/S}$ ethanol yield per xylose consumed (g g^{-1})
$Y_{X/OX}$ yield of biomass per oxygen consumed (g mg^{-1})
$Y_{X/CA}$ yield of biomass per acetic acid consumed (g g^{-1})
μ specific growth rate (h^{-1})
μ_{max} maximum specific growth rate (h^{-1})
μ_{MAX} maximum specific growth rate at substrate saturation if S & P are notInhibitory (h^{-1})

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