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Probing the bioethanol production potential of *Scheffersomyces* (*Pichia*) *stipitis* using validated genome-scale model

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Abstract A detailed in silico analysis of different strategies for enhancement of bioethanol production by *Scheffersomyces stipitis*, = *Pichia stipitis*, using validated genome-scale metabolic model is presented. Glucose inhibition on xylose uptake is dominant in *S. stipitis* which makes fed-batch fermentation more effective for higher sugar concentrations. Bioethanol production potential of *S. stipitis* can be improved by growth media modification by introducing certain amino acids in small quantities. Slower sugar uptake by *S. stipitis* can be overcome by community-culture with recombinant *Escherichia coli* strain ZSC113, which has a higher xylose uptake rate. Ethanol yield and productivity of community-culture can be further enhanced by genetic modification of *E. coli* strain ZSC113.

Keywords Community-culture · Dynamic flux balance modelling · Ethanol production · *Pichia stipitis* · *Scheffersomyces stipitis* · Xylose uptake

List of symbols

A	Matrix of stoichiometric coefficients
a	Specific interfacial air–liquid area, m^{-1}
E	Ethanol concentration g l^{-1}
e	Ethanol
EC	*Escherichia coli*
F	Feed rate l h^{-1}
G	Glucose concentration g l^{-1}
G_f	Glucose concentration in the feed g l^{-1}
g	Glucose
K	Half-saturation constant for substrate uptake g l^{-1}
K_{ie}	Ethanol inhibition constant for substrate uptake g l^{-1}
K_{igz}	Glucose inhibition constant for xylose uptake g l^{-1}
k_L	O_2 mass transfer coefficient m h^{-1}
O	Dissolved O_2 concentration g l^{-1}
o	O_2
SS	*Scheffersomyces stipitis*
V	Volume l
v	Vector of reaction and exchange fluxes
w	Vector of weights with contribution of flux to cell mass formation
X	Cell mass concentration g l^{-1}
Z	Xylose concentration g l^{-1}
z	Xylose
Z_f	Xylose concentration in the feed g l^{-1}
μ	Specific growth rate h^{-1}

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Introduction

Efficient utilization of both glucose and xylose is essential for economic production of bioethanol from lignocellulosic biomasses. *Scheffersomyces stipitis*, previously known as *Pichia stipitis,* is a native xylose-fermenting yeast with potential for bioethanol production from lignocelluloses. It is a respiratory yeast and therefore O_2 supply to the cell has significant role in the production of cell mass and ethanol. High aeration rate results in the production of cell mass, while ethanol fermentation demands a microaerobic condition for maintaining cell viability and NADH balance. During fermentation of glucose/xylose mixtures, glucose is the preferred sugar and inhibition of xylose uptake occurs by catabolite repression in the presence of high glucose concentration (Grootjen et al. 1991).

Flux balance analysis (FBA) is an efficient constraint-based approach to analyse a genome-scale metabolic network reconstruction, which represents the cell biochemical network as a set of underdetermined constrained mass balances (Orth et al. 2010). FBA methods assume time-invariant extracellular conditions and have been shown to generate steady-state predictions consistent with continuous culture. Dynamic flux balance analysis (dFBA) is an extension of classical FBA for accounting the dynamic situations. The dFBA models are obtained by combining stoichiometric equations for intracellular metabolism with dynamic mass balances on key extracellular substrates and products under the assumption of fast intracellular dynamics (Mahadevan et al. 2002). Dynamic FBA models have been used for analysis of bioethanol production by co-culture fermentation of *Saccharomyces cerevisiae* and *Escherichia coli* strain ZSC113 (Hanly et al. 2012; Lisha and Sarkar 2014). Hanly and Henson (2013) investigated the bioethanol production potential of mono-culture of *S. stipitis* and co-culture of *S. stipitis* with respiratory deficient *S. cerevisiae* during batch fermentation of glucose/xylose mixtures.

In this study, we present an in silico analysis of different strategies for enhancement of bioethanol production by *S. stipitis* using validated genome-scale metabolic model. The strategies involve both process systems engineering and genetic engineering based approaches. The in silico bioethanol production potential of *S. stipitis* are investigated for both batch and fed-batch fermentation. The impacts of the presence of different amino acids in growth media on bioethanol production by *S. stipitis* have been analysed. The effect of community-culture on bioethanol productivity are investigated by considering a sequential fed-batch fermentation of first recombinant *E. coli* strain ZSC113 and then wild-type *S. stipitis*, which attempts to overcome the problem of slower uptake of xylose by *S. stipitis* and ethanol inhibition on xylose uptake. The effects of a set of genetic modifications which redirects carbon flux from various by-products to ethanol in ZSC113 are also analysed for such a sequential-culture bioethanol production.

Materials and methods

Dynamic flux balance model (dFBA) for community-culture

The dFBA model is developed by coupling intracellular steady state flux balances to dynamic extracellular mass balances through expressions for substrate uptake kinetics of glucose, xylose and O_2. The community-culture stoichiometric models used in the present study are adapted from *i*BB814 *S. stipitis* (Balagurunathan et al. 2012) and *i*AF1260 *E. coli* (Feist et al. 2007) genome-scale metabolic reconstructions. The xylose-selective recombinant *E. coli* strain ZSC113 is formulated from the genome-scale metabolic model *i*AF1260 by constraining the glucose exchange and glucokinase fluxes to zero. The assumption in modelling the community-culture of *S. stipitis* and ZSC113 is that the microbes compete for the available substrates and each species tries to maximize its own growth rate. The model for the community-culture system can thus be developed by combining the dFBA models for individual species. The linear program to solve the underdetermined flux balance model of community-culture of two microbial species *S. stipitis* (SS) with *E. coli* (EC) can thus be formulated as follows:

$$\begin{aligned} &\underset{v_{SS},v_{EC}}{\text{Maximize}}\ \mu = \mu_{SS} + \mu_{EC} = w_{SS}^{T} v_{SS} + w_{EC}^{T} v_{EC} \\ &\text{Subject to}: \begin{pmatrix} A_{SS} & 0 \\ 0 & A_{EC} \end{pmatrix} \begin{pmatrix} v_{SS} \\ v_{EC} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix} \\ &\begin{pmatrix} v_{SS,\min} \\ v_{EC,\min} \end{pmatrix} \le \begin{pmatrix} v_{SS} \\ v_{EC} \end{pmatrix} \le \begin{pmatrix} v_{SS,\max} \\ v_{EC,\max} \end{pmatrix} \end{aligned} \quad (1)$$

where v_{min} and v_{max} are vectors for lower and upper bounds of the fluxes.

The substrate uptake kinetics for the microorganism are modelled as Michaelis–Menten kinetics with additional regulatory term to account for growth rate suppression at high ethanol concentration:

$$v_{g,SS} = v_{g,\max,SS}\frac{G}{K_{g,SS}+G}\cdot\frac{1}{1+\frac{E}{K_{ieg,SS}}} \quad (2)$$

$$v_{z,SS} = v_{z,\max,SS}\frac{Z}{K_{z,SS}+Z}\cdot\frac{1}{1+\frac{E}{K_{iez,SS}}}\cdot\frac{1}{1+\frac{G}{K_{igz,SS}}} \quad (3)$$

$$v_{o,SS} = v_{o,\max,SS}\frac{O}{K_{o,SS}+O} \quad (4)$$

$$v_{z,EC} = v_{z,\max,EC}\frac{Z}{K_{z,EC}+Z}\cdot\frac{1}{1+\frac{E}{K_{iez,EC}}} \quad (5)$$

$$v_{o,EC} = v_{o,\max,EC}\frac{O}{K_{o,EC}+O} \quad (6)$$

where v_g, v_z and v_o are the glucose, xylose and O_2 uptake rates, respectively.

The dynamic mass balances for the extracellular environment are described by the usual ordinary differential equations:

$$\frac{dV}{dt} = F \quad (7)$$

$$\frac{d(VX_{SS})}{dt} = \mu_{SS}VX_{SS} \quad (8)$$

$$\frac{d(VX_{EC})}{dt} = \mu_{EC}VX_{EC} \quad (9)$$

$$\frac{d(VG)}{dt} = FG_f - v_{g,SS}VX_{SS} \quad (10)$$

$$\frac{d(VZ)}{dt} = FZ_f - v_{z,SS}VX_{SS} - v_{z,EC}VX_{EC} \quad (11)$$

$$\frac{d(VO)}{dt} = -v_{o,SS}VX_{SS} - v_{o,EC}VX_{EC} + k_La V(O_{sat} - O) \quad (12)$$

$$\frac{d(VE)}{dt} = v_{e,SS}VX_{SS} + v_{e,EC}VX_{EC} \quad (13)$$

where k_La and O_{sat} (O_2 saturation concentration) values are taken as 10.1 h^{-1} and 0.24 mM, respectively.

Table 1 Substrate uptake parameters for mono-culture and community-culture fermentation of glucose/xylose mixtures (Hanly and Henson, 2013; Hanly et al. 2012)

Parameter	*S. stipitis*	*E. coli* (ZSC113)
$v_{g,\max}$ (mmol g DW^{-1} h^{-1})	6.5	–
$v_{z,\max}$ (mmol g DW^{-1} h^{-1})	5.5	12
$v_{o,\max}$ (mmol g DW^{-1} h^{-1})	11	20
K_g (g l^{-1})	1	–
K_z (g l^{-1})	0.25	0.25
K_o (mM)	0.0125	0.024
$K_{i,e.g.}$ (g l^{-1})	10	–
$K_{i,ez}$ (g l^{-1})	4.5	20
$K_{i,gz}$ (g l^{-1})	0.5	–

The specific growth rate (μ) and ethanol exchange flux (v_e) are resolved by the solution of inner flux balance model. All the computations are performed in MATLAB environment using ode15 s to integrate the extracellular dynamic mass balance equations and the COBRA Toolbox (Becker et al. 2007) with MATLAB interface to the GNU linear program code *glpk* to solve the inner linear program. Dynamic FBA model for the mono-culture of *S. stipitis* is developed following the same procedure. The substrate uptake parameters used for all the dynamic simulations are listed in Table 1. The uptake parameters for *S. stipitis* and ZSC113 are taken from Hanly and Henson (2013) and Hanly et al. (2012), respectively.

Results and discussion

Dynamic FBA (dFBA) model validation

In order to have confidence in predictions of dFBA model, we first validated the developed dFBA model by comparing its predictions for measurable state variables against the data reported in literature. Kinetic parameters used for dFBA modelling are collected from Hanly and Henson (2013) and Weierstall et al. (1999). The comparison of experimental data and dFBA model predictions for bioethanol production by *S. stipitis* using glucose and xylose as the sole carbon source is shown in Fig. 1a and Fig. 1b. The experimental data are collected from Agbogbo et al. (2006). (It may be noted that the model

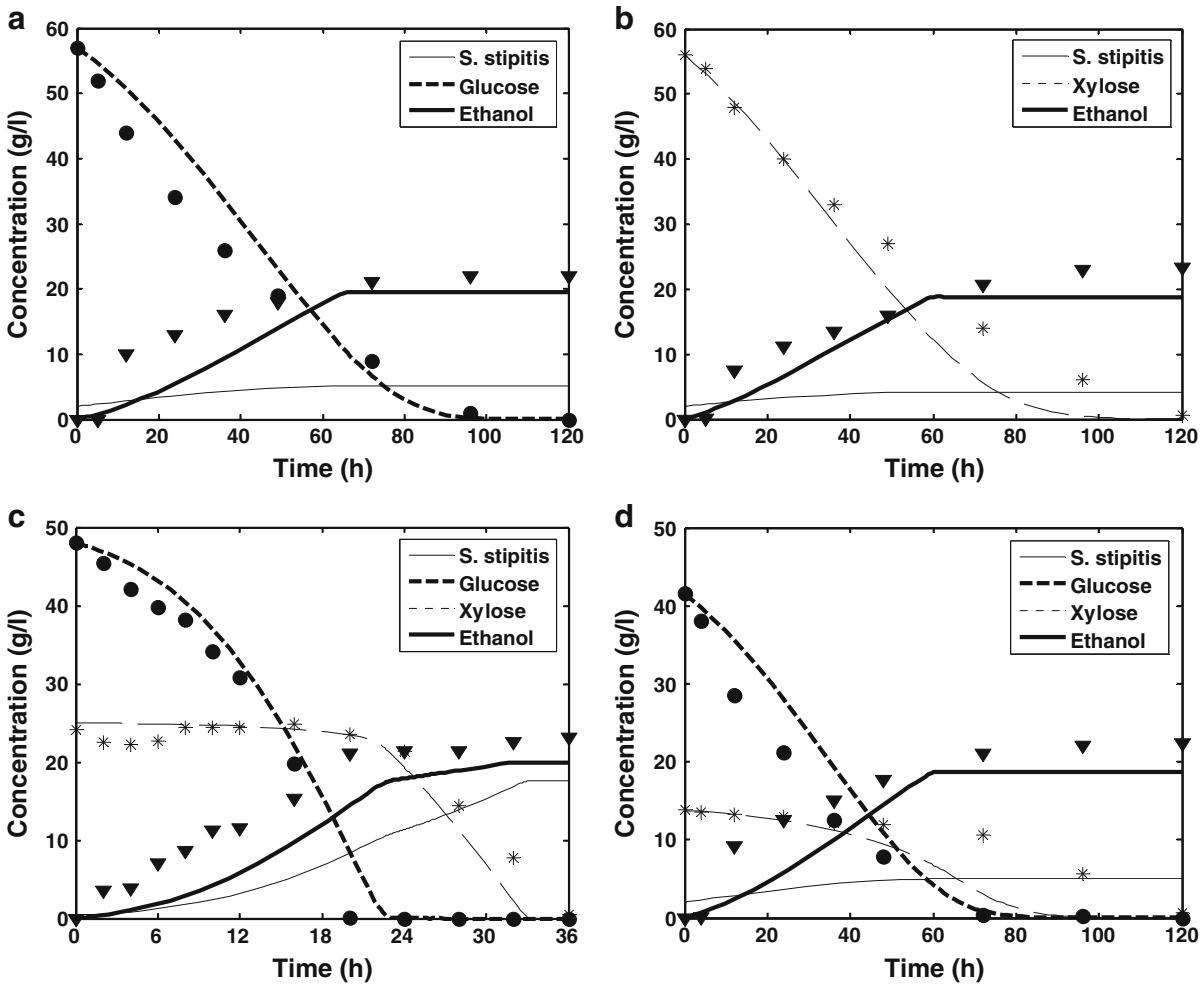

Fig. 1 Comparison of model predictions and experimental data for *S. stipitis* bioethanol production from **a** glucose (Agbogbo et al. 2006), **b** xylose (Agbogbo et al. 2006), **c** 2:1 glucose/xylose mixture (Taniguchi et al. 1997) and **d** 3:1 glucose/xylose mixture (Agbogbo et al. 2006). Experimental data are indicated by symbols

predictions represent experimental data reasonably well for both the cases.) The comparison of dFBA model predictions and experimental data for 2:1 and 3:1 glucose/xylose mixtures are shown in Fig. 1c and Fig. 1d, respectively. The experimental data are collected from Taniguchi et al. (1997) for Fig. 1c and from Agbogbo et al. (2006) for Fig. 1d. A reasonably good fit between model predictions and experimental data are observed for glucose consumption, xylose consumption and ethanol production for both the cases. The model predicts well the experimentally observed sequential uptake of glucose and xylose by *S. stipitis*.

Effect of media modification on bioethanol production by mono-culture of *S. stipitis*

Nutrients in fermentation media play an important role in bioethanol production by *S. stipitis*. The effect of various amino acids in the media on batch ethanol production by *S. stipitis* was investigated by a series of simulations with the substrate uptake parameters as given in Table 1. Table 2 and Supplementary Fig. 1 summarize the results of this study.

The batch culture of *S. stipitis* using 16 g glucose l^{-1} and 8 g xylose l^{-1} with 0.4 g inoculum l^{-1} produced 9.6 g ethanol l^{-1} in 24 h, with an ethanol

Table 2 Effect of amino acids in the fermentation media on batch ethanol yield by *S. stipitis* mono-culture

Amino acid used	Ethanol yield Y_{eth} (g g^{-1})	Increase in Y_{eth} (%) with respect to the standard medium
L-alanine	0.45	12.5
L-arginine	0.42	5
L-asparagine	0.45	12.5
L-aspartate	0.44	10
L-glutamate	0.43	7.5
L-glutamine	0.43	7.5
Glycine	0.42	5
L-proline	0.44	10
L-serine	0.42	5
Standard medium	0.4	

yield (Y_{eth}) of 0.4 g g^{-1}. The effect of amino acids in the fermentation media were simulated by constraining the amino acid fluxes to a specific value. The effects of 20 amino acids were tested individually for a flux value of 0.5 mmol g DW^{-1} h^{-1}. The presence of nine amino acids (L-alanine, L-arginine, L-asparagine, L-aspartate, L-glutamate, L-glutamine, glycine, L-proline and L-serine) in the media enhanced bioethanol production with respect to the standard fermentation medium (Table 2). These amino acids enhanced both cell mass and ethanol production and the enhancement in ethanol production was in the range of 5.5–11.4 %. The highest enhancement in ethanol production was obtained with L-asparagine. Simulations are also carried out with amino acid flux values of 1, 2 and 5 mmol g DW^{-1} h^{-1} and these enhanced ethanol production by 1 to 5 % over that obtained using 0.5 mmol g DW^{-1} h^{-1}. Presence of the remaining 11 amino acids results in negligible changes in ethanol production (9.6 ± 0.04 g ethanol l^{-1}) with respect to the standard medium.

Effect of amino acids in the growth media on the conversion of D-xylose to ethanol by the yeast *P. stipitis* NRRL Y-7124 has been investigated by Slininger et al. (2006): certain a mino acids (L-alanine, L-asparagine, L-aspartate, L-glutamate, glycine, L-histidine, L-leucine and L-tyrosine) when added to the media enhanced ethanol accumulation and this might be due to the requirement of a nitrogen source to provide an optimum carbon to nitrogen ratio for supporting non-growth associated ethanol production.

Comparison of batch and fed-batch fermentation

During batch fermentation, substrate inhibition and catabolite repression may result in reduced production of desired metabolites and fed-batch fermentation as an alternative mode of operation to overcome these undesired effects. Substrate inhibition and catabolite repression are highly active in *S. stipitis* lignocellulosic fermentation. In order to compare batch and fed-batch bioethanol production by *S. stipitis,* four different substrate/feed compositions were selected such that the total amount of sugar being fermented was the same. Table 3 summarizes the results of the study. As sugar concentration in the medium increases, production of bioethanol decreases during batch fermentation. But fed-batch operation is clearly superior to batch fermentation for high sugar concentration in the medium. The enhancement in fed-batch Y_{eth} is 5 % for 40 g sugar to 37.5 % for 100 g sugar.

Unrean and Nguyen (2013) compared the performance of *S. stipitis* batch and fed-batch culture for the same amount of total sugar (100 g l^{-1}) treated and reported an Y_{eth} of 0.4 g g^{-1} from fed-batch fermentation, which is 29 % higher compared to batch fermentation. Gutierrez-Rivera et al. (2012) have reported the bioethanol production potential of *P. stipitis* NRRL Y-7124 from various glucose/xylose mixtures during batch fermentation; the various glucose/xylose compositions investigated were: 25/10, 50/20, 75/30 and 100/40 g l^{-1}, respectively. The reported Y_{eth} are 0.29, 0.34, 0.32 and 0.32 g g^{-1}, respectively. The experimental Y_{eth} for 75/30 mixture agrees very well with our simulated Y_{eth} for 75/25 mixture (0.32 g g^{-1}, Table 3). Also Unrean and Nguyen (2013) reported an Y_{eth} of 0.31 g g^{-1} for 75/25 mixture.

Fed-batch community-culture with recombinant *E. coli* strain ZSC113

The fed-batch community-culture of *S. stipitis* with xylose-selective *E. coli* strain ZSC113 was studied by simulations of a five litre bioreactor. The model parameter values and the operating conditions used for the fermentation of 260 g total sugar (195 g glucose + 65 g xylose) with 1 g inoculum l^{-1} are shown in Table 1 and Table 4. For comparison, mono-culture of *S. stipitis* was simulated using the same operating conditions. Supplementary Fig. 2

Table 3 Operating conditions and results for batch and fed-batch *S. stipitis* fermentation

Total sugar (g) (Glucose + Xylose)	Inoculum (g)	Final volume (l)	Feed rate ($l\ h^{-1}$)	Ethanol yield, Y_{eth} ($g\ g^{-1}$)		Increase of fed-batch Y_{eth} (%)
				Batch	Fed-batch	
40 (30 + 10)	0.2	1.5	0.016	0.4	0.42	5
60 (45 + 15)	0.3	1.67	0.015	0.39	0.43	10.2
80 (60 + 20)	0.4	1.75	0.013	0.33	0.44	33.3
100 (75 + 25)	0.5	1.8	0.011	0.32	0.44	37.5

Table 4 Operating conditions for fed-batch mono-culture and community-culture bioethanol production

Operating conditions	Value
Initial volume (l)	1
Final volume (l)	5
Initial glucose concentration ($g\ l^{-1}$)	15
Initial xylose concentration ($g\ l^{-1}$)	5
Glucose feed concentration ($g\ l^{-1}$)	45
Xylose feed concentration ($g\ l^{-1}$)	15
Inoculum concentration ($g\ l^{-1}$)	
Mono-culture	1
Community-culture	0.1 (ZSC113) + 0.9 (*S. stipitis*)
Fermentation time (h)	
Mono-culture	120
Community-culture	60
Feed rate ($l\ h^{-1}$)	
Mono-culture	0.033
Community-culture	0.067

shows schematically the modes of operation for fed-batch mono-culture and community-culture fermentations. The results of the simulation for fed-batch mono-culture of *S. stipitis* with a constant feed flow rate ($F = 0.033\ l\ h^{-1}$) are shown in Fig. 2a. This fed-batch fermentation for a fermentation time of 120 h produced 114 g ethanol ($23\ g\ l^{-1}$). The corresponding Y_{eth} and ethanol productivity (Pr_{eth}, defined as the overall rate of ethanol production) were 0.46 g g^{-1} and 0.95 g h^{-1}, respectively. The simulated Y_{eth} was 90 % of the maximum theoretical value. From Fig. 2a, it is clear that glucose consumption is very fast and almost 99 % of glucose was consumed in the initial 20 h. Xylose uptake was very slow and this reduced Pr_{eth}.

Ethanol production potential of recombinant *E. coli* strain ZSC113 (xylose selective microorganism) during co-culture fermentation with *S. cerevisiae* has been reported by various research groups (Hanly et al. 2012; Lisha and Sarkar 2014). To find a suitable strategy for enhancing ethanol productivity (Pr_{eth}), a community-culture of *S. stipitis* with *E. coli* strain ZSC113 is investigated here. The kinetic parameters used for dFBA modelling are taken from Hanly et al. (2012). To produce ethanol, *E. coli* requires anaerobic conditions, while *S. stipitis* needs microaerobic conditions. So a sequential fed-batch community-culture is proposed as shown in Supplementary Fig. 2b. In such a sequential operation, the fermentation was started with ZSC113 under anaerobic conditions and then switched over to a microaerobic condition when *S. stipitis* was added to the system. The consumption of xylose by ZSC113 was fast compared to *S. stipitis* and, thus when *S. stipitis* was added later, it essentially consumes glucose and produces the major part of the total ethanol. This avoids the ethanol's inhibition by ethanol of xylose uptake by *S. stipitis* and results in higher ethanol productivity. Ethanol is produced mainly from glucose and, therefore, if the sequential-culture starts with *S. stipitis* instead of ZSC113, the ethanol produced from glucose will inhibit the xylose uptake of both the strains. The operating conditions, such as total amount of sugar and total inoculum, were kept same as that of mono-culture simulation, and the operating parameters are shown in Table 4. The inoculum ratio, switching time and fermentation time were determined by systematic sensitivity analysis.

Figure 2b shows the simulation result for community-culture of *S. stipitis* with *E. coli* strain ZSC113 with an inoculum ratio of 9:1 (*S. stipitis*: *E. coli*) and constant feed rate of $0.067\ l\ h^{-1}$. For a fermentation time of 60 h and with anaerobic to microaerobic switch at 5 h, 95.6 g ethanol ($19.1\ g\ l^{-1}$) was produced. The corresponding Y_{eth} and Pr_{eth} were 0.37 g g^{-1} and 1.59 g h^{-1}, respectively. This

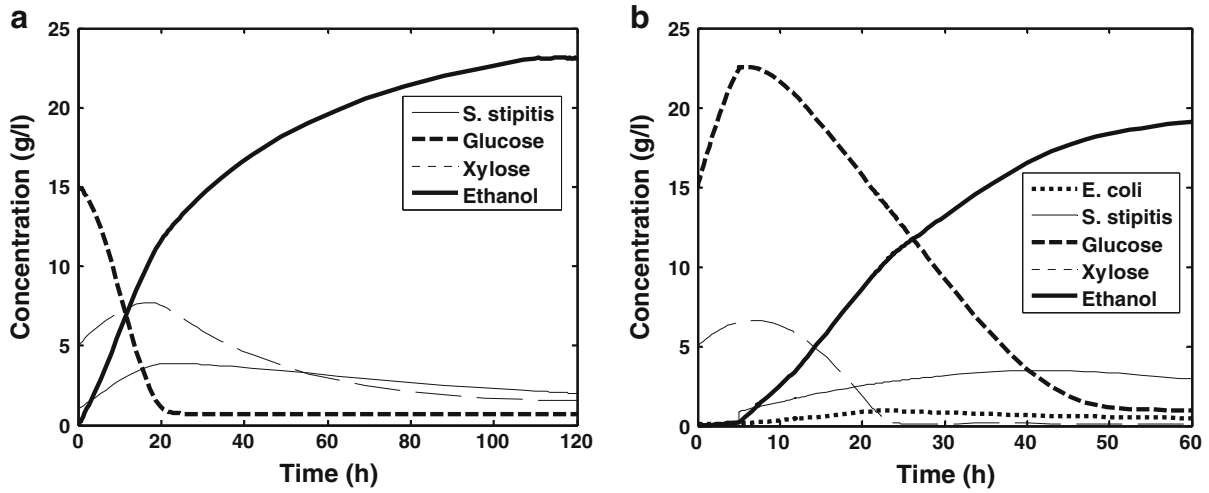

Fig. 2 Fed-batch bioethanol production from glucose/xylose mixtures **a** mono-culture of *S. stipitis* and **b** community-culture of *S. stipitis* and *E. coli* strain ZSC113

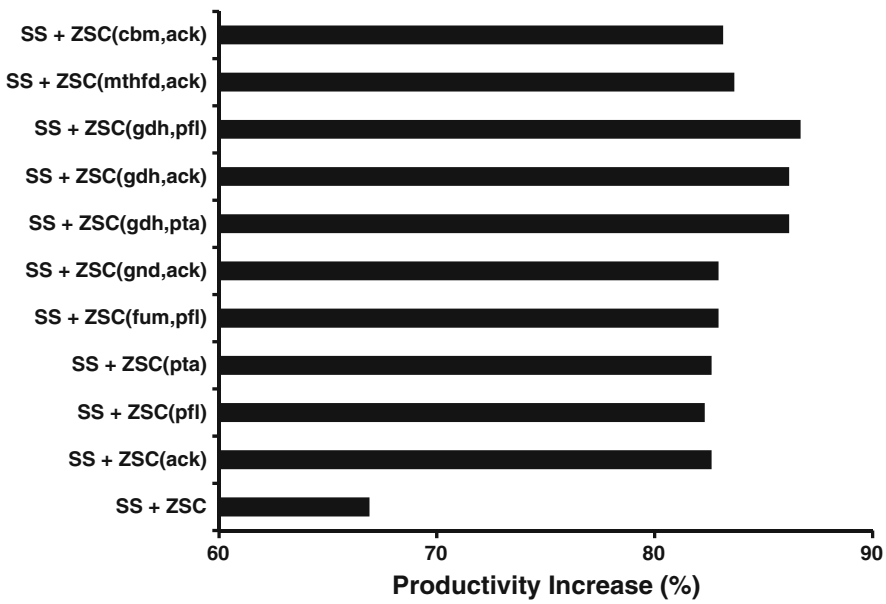

Fig. 3 Productivity increase of fed-batch community-culture of *S. stipitis* and *E. coli* strain ZSC113 (with and without genetic modification) with respect to the mono-culture of *S. stipitis* (SS: *S. stipitis*, ZSC: *E. coli* strain ZSC113 and genes deleted from *E. coli* strain ZSC113 are shown in brackets. The deleted genes are *ack* (acetate kinase), *pfl* (pyruvate formate lyase), *pta* (phosphotransacetylase), *fum* (fumarase), *gnd* (phosphogluconate dehydrogenase), *gdh* (glutamate dehydrogenase), *mthfd* (methylenetetrahydrofolate dehydrogenase) and *cbm* (carbamate kinase))

sequential scheme resulted in enhanced Pr_{eth} (67.3 % enhancement) but reduced Y_{eth} (19.5 % reduction) with respect to mono-culture of *S. stipitis*. In the community-culture, the contribution of ethanol from *S. stipitis* and ZSC113 are 18.1 and 1.01 g l^{-1}, respectively. Acetate (1.51 g l^{-1}), formate (2.24 g l^{-1}) and succinate (0.001 g l^{-1}) were also produced as by-products from ZSC113 (Supplementary Fig. 3). Distribution of carbon flux to other by-products, such as acetate and formate, in ZSC113 might be the reason for lower Y_{eth} of community-culture compared to mono-culture.

Enhancement in community-culture bioethanol production by genetic modification of ZSC113

Kim and Reed (2010) reported a number of genetic engineering strategies for enhanced ethanol production by wild-type *E. coli* from glucose. We tested a set of such gene deletion strategies (Supplementary Table 1) on ZSC113 and investigated their potential for enhanced ethanol production from community-culture fermentation of ZSC113 with *S. stipitis*. The results are shown in Supplementary Table 2. Gene deletions were simulated by constraining both bounds of the specific reaction to zero in the genome-scale metabolic model of ZSC113. All the in silico genetic modifications resulted in enhanced Y_{eth} and Pr_{eth} compared to the base case (*S. stipitis* and ZSC113 without genetic modification). The double-gene deletion involving glutamate dehydrogenase and acetate kinase ($\Delta gdh + \Delta ack$) produces 106 g ethanol (21.2 g l^{-1}) with a Y_{eth} and Pr_{eth} of 0.42 g g^{-1} and 1.78 g h^{-1}, respectively. The amounts of ethanol produced by *S. stipitis* and ZSC113 were 17.83 and 3.51 g l^{-1}, respectively.

After genetic manipulation, ethanol production by ZSC113 increased from 1.01 to 3.51 g l^{-1}. The enhancement in Y_{eth} and Pr_{eth} of the genetically-modified system was 13.5 and 11.9 %, respectively, with respect to the base case. The strategy $\Delta gdh + \Delta ack$ reduced the formation of acetate and formate as by-products significantly (0.015 and 0.18 g l^{-1}, respectively). The reduction in by-products is responsible for enhanced production of ethanol as the carbon flux is now redirected from these by-products to ethanol. The other manipulations also result in enhanced production of ethanol (Supplementary Table 2) and corresponding reduction in by-products formation (Supplementary Fig. 3).

Figure 3 shows the enhancement in Pr_{eth} of community-culture of *S. stipitis* and *E. coli* strain ZSC113 (with and without genetic manipulation) with respect to the mono-culture of *S. stipitis*. The enhancement in Pr_{eth} of community-culture (ZSC113 without genetic manipulation) was 66.9 % compared to mono-culture. A particular genetic manipulation ($\Delta gdh + \Delta pfl$) on ZSC113 predicts 86.7 % enhancement in Pr_{eth}. All other manipulations also predict Pr_{eth} enhancement in the range of 82 % to 87 %. Similarly, the Y_{eth} was also improved by genetic manipulations on ZSC113 and an Y_{eth} of 89.1–91.3 % of mono-culture is now predicted.

In conclusion, this study demonstrates the potential of genome-scale metabolic reconstructions as a highly valuable tool for screening and designing of strains with desirable properties.

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