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Dynamic model of temperature impact on cell viability and major product formation during fed-batch and continuous ethanolic fermentation in *Saccharomyces cerevisiae*

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ABSTRACT

The impact of the temperature on an industrial yeast strain was investigated in very high ethanol performance fermentation fed-batch process within the range of 30–47 °C. As previously observed with a lab strain, decoupling between growth and glycerol formation occurred at temperature of 36 °C and higher. A dynamic model was proposed to describe the impact of the temperature on the total and viable biomass, ethanol and glycerol production. The model validation was implemented with experimental data sets from independent cultures under different temperatures, temperature variation profiles and cultivation modes. The proposed model fitted accurately the dynamic evolutions for products and biomass concentrations over a wide range of temperature profiles. R^2 values were above 0.96 for ethanol and glycerol in most experiments. The best results were obtained at 37 °C in fed-batch and chemostat cultures. This dynamic model could be further used for optimizing and monitoring the ethanol fermentation at larger scale.

1. Introduction

Alternative energy sources must be developed to cope with fossil energy depletion and to reduce greenhouse gas emissions and global warming. Biofuels, derived from renewable resources are realistic substitutes to fossil fuels. Bio-ethanol, the main biofuel produced by fermentation of several feedstocks, constitutes a rapid and significant answer to these problems (Sánchez and Cardona, 2008).

Temperature is one of the main technological factors known to impact both the metabolism and the activity of *Saccharomyces cerevisiae (S. cerevisiae)* at industrial scale due to inhomogeneities in large scale bioreactor (Torija et al., 2003). An optimal temperature, different for growth and ethanol production, exists for each yeast species, and a suboptimal temperature can decrease production kinetics and yields (Aldiguier et al., 2004; Torija et al., 2003). Moreover, a temperature raise alters the cell viability and decreases the ethanol tolerance (Aldiguier et al., 2004; Torija et al., 2003). Besides, the rate of temperature variation significantly impacts the viability, thermal shocks being much more drastic than weak variations (Beney et al., 2000; Gervais and Martínez De Marañón, 1995; Guyot et al., 2005; Marechal et al., 1999; Martínez De Marañón et al., 1999).

Besides ethanol and CO_2, glycerol is the main by-product of the alcoholic fermentation and may account for up to 5% of the carbon in some industrial processes (Oura, 1977). The production of glycerol was reported to be coupled to an increase of the fermentation temperature (Aldiguier et al., 2004; Berovic et al., 2007; Omori et al., 1996; Torija et al., 2003). Moreover, on different *S. cerevisiae* strains a 10–20% higher production was reached when the temperature was shifted for 10 min from 27 to 45 °C or 50 °C (Omori et al., 1996). The glycerol on glucose yields obtained from cultures regulated at 36 and 39 °C were found 4 to 6-fold higher than those obtained at 30 °C (Aldiguier et al., 2004). In a range between 27 and 33 °C a coupling phenomenon was reported between growth and glycerol production in *S. cerevisiae*. Above 36 °C, a decoupling phenomenon was shown (Aldiguier et al., 2004) *i.e.* glycerol was still produced in absence of growth. It is reported that temperature leads to protein unfolding and then in a loss of enzyme functionality, and that glycerol limits heat damages. This metabolite, *in vitro*, was shown to stabilise and renature inorganic pyrophosphatases, involved in lipid anabolism and DNA synthesis (Zancan and Sola-Penna, 2005).

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Nomenclature

a	Ratkowsky parameter in μ_{max} expression ($°C^{-1}\ h^{-0.5}$)
a^2	Ratkowsky parameter in P_{mx} expression ($g\ l^{-1}$)
A_{G1}	activation energy for glycerol production divided by the gas constant (R) (°C)
A_{G2}	inactivation energy for glycerol production divided by the gas constant (R) (°C)
A_{P1}	activation energy for ethanol production divided by the gas constant (R) (°C)
A_{P2}	inactivation energy for ethanol production divided by the gas constant (R) (°C)
b	Ratkowsky parameter in μ_{max} expression ($°C^{-1}$)
b_2	Ratkowsky parameter in expression ($°C^{-1}$)
D	dilution rate based on reactor volume (h^{-1})
$\frac{d}{dt}$	derivative with respect to time (t)
G	glycerol concentration ($g\ l^{-1}$)
J	objective function (cost function)
K_d	specific rate of cell death (h^{-1})
$K_d b$	basal specific rate of cell death (h^{-1})
$K_d t$	maximum value of specific rate of cell death due to temperature (h^{-1})
K_{sx}	glucose saturation constant for the specific growth rate ($g\ l^{-1}$)
N	number of measurements
P	ethanol concentration ($g\ l^{-1}$)
P_{mx}	ethanol concentration inhibiting growth at which $\mu = 0$ ($g\ l^{-1}$)
Q_{in}	feed flow rate ($l\ h^{-1}$)
Q_{out}	outlet flow rate ($l\ h^{-1}$)
q_S	specific consumption rate of substrate ($g\ g_X^{-1}\ h^{-1}$)
$R_{G/S}$	glycerol yield based on sugar consumed ($g\ g^{-1}$)
$R_{P/S}$	ethanol yield based on sugar consumed ($g\ g^{-1}$)
$R_{X/S}$	biomass yield based on sugar consumed ($g\ g^{-1}$)
S	glucose concentration ($g\ l^{-1}$)
S_{in}	glucose feed concentration ($g\ l^{-1}$)
t	time
T	temperature of the reactor (°C)
V	reactor volume (l)
X_t	total cell concentration ($g\ l^{-1}$)
X_v	viable cell concentration ($g\ l^{-1}$)
$Y_{G/S}$	glycerol theoretical yield based on sugar consumed ($g\ g^{-1}$)
$Y_{P/S}$	ethanol theoretical yield based on sugar consumed ($g\ g^{-1}$)
$Y_{X/S}$	biomass theoretical yield based on sugar consumed ($g\ g^{-1}$)

Greek symbols

α_P	parameter "associated" with growth between μ and v_P
α_G	parameter "associated" with growth between μ and v_G
β_G	decoupling factor: glycerol specific production rate when $\mu = 0$ ($g\ g_X^{-1}\ h^{-1}$)
β_P	decoupling factor: ethanol specific production rate when $\mu = 0$ ($g\ g_X^{-1}\ h^{-1}$)
χ	parameters to be determined
γ_1	constant of decoupling factor for ethanol production $g\ g_X^{-1}\ h^{-1}$
γ_2	constant of decoupling factor for ethanol production ($g\ g_X^{-1}\ h^{-1}$)
γ_3	constant of decoupling factor for glycerol production ($g\ g_X^{-1}\ h^{-1}$)
γ_4	constant of decoupling factor for glycerol production ($g\ g_X^{-1}\ h^{-1}$)
μ	specific growth rate (h^{-1})
μ_{max}	maximum value of the specific growth rate (h^{-1})
v_{exp}	experimental data
v_G	specific production rate of glycerol ($g\ g_X^{-1}\ h^{-1}$)
v_{sim}	model predictive outputs
v_P	specific production rate of ethanol ($g\ g_X^{-1}\ h^{-1}$)
$v_{P_{max}}$	maximum value of the ethanol specific production rate ($g\ g_X^{-1}\ h^{-1}$)
θ_1	constant in μ_{max} expression (°C)
θ_2	constant in μ_{max} expression (°C)
θ_3	constant in P_{mx} expression (°C)
θ_4	temperature at the inflection point of the $K_d = f(T)$ sigmoid curve (°C)
θ_5	slope of the $K_d = f(T)$ sigmoid curve ($°C^{-1}\ h^{-1}$)

Moreover, glycerol and polyols in general participate in the thermal protection of proteins against denaturation and cell death (Back et al., 1979; Henle et al., 1982).

Glycerol synthesis is mainly documented for its involvement in (i) maintaining cell oxydo–reductive balance in the case of a cytosolic NADH excess (Verduyn et al., 1990), (ii) providing the cell with the G3P intermediate required for glycerophospholipid and triacylglycerol biosynthesis (Athenstaedt and Daum, 1999; Wang et al., 2001) and (iii) protecting the yeast against osmotic stress (Blomberg and Adler, 1989; Nevoigt and Stahl, 1997). Glycerol is synthesised via a deviation of the glycolysis pathway from the glycolytic intermediate dihydroxyacetone phosphate (DHAP). DHAP is reduced to glycerol-3-phosphate (G3P) by the glycerol 3-phosphate dehydrogenases (GPDH: GPD1 and GPD2) and then dephosphorylated into glycerol by specific phosphatases (GPP1 and GPP2). The activity of GPDH was found to increase with the temperature (Omori et al., 1996). In *S. cerevisiae*, at 27 °C, GPDH activity was improved by 1.15 to 1.25-fold with a 10 min heat shock at 45 °C or 50 °C (Omori et al., 1996). However, this was a transient activity enhancement as it decreased through cell divisions (Omori et al., 1996).

In literature, only few studies investigated and modeled the impact of the temperature on ethanol fermentation kinetics (Andrade et al., 2007; Atala et al., 2001; Phisalaphong et al., 2006; Rivera et al., 2007, 2006). Some took into account the viable biomass but none considered the glycerol production.

In the present study, a dynamic model was developed in order to predict and quantify the major production kinetics including glycerol and both total biomass and viable biomass during different ethanol fermentation processes. Fed-batch experiments in isotherm and with temperature upshift were carried out and the experimental data were used in order to calibrate the model parameters. An optimal set of parameters was obtained from a global optimization algorithm. The kinetic model was then validated with experimental data from fed-batch and heat-stress continuous cultures and showed robustness over a wide range of experimental conditions.

2. Methods

2.1. Microorganism, media and growth conditions

The industrial *S. cerevisiae* strain was supplied from Fermentis (France). The strain was maintained on YPD (yeast extract 1% (w/v), bactopeptone 2% (w/v), glucose 2% (w/v) and NaCl 0.9% (w/v)) agar medium at 4 °C. Pre-culture of yeast cells was carried out in a 5 ml tube of YPD rich medium (2 ml) at 30 °C for 12 h on a rotary