KINETIC MODELING OF ETHANOL FERMENTATION BY YEAST *KLUYVEROMYCES MARXIANUS* FROM LACTOSE- AND INULIN- CONTAINING SUBSTRATES

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Abstract. An unstructured kinetic model was developed in this study for the batch production of bioethanol by the yeast *Kluyveromyces marxianus* DSM 5422 from the renewable sources of agricultural and food processing origin, such as whey permeate or inulin, which include the terms of both substrate and product inhibitions. Experimental data collected from multiple fermentations in bioreactors with three different initial concentrations for each substrate were used to estimate the parameters and to validate the proposed model. The growth of *K.marxianus* can be expressed by the Haldane-type extended Monod model in combination with the Jerusalimsky term for the non-competitive product inhibition and the Luedeking–Piret equation was adequate to describe the growth-associated formation of ethanol as the target product. The parameters in the models were estimated by minimizing mean-squared errors between the predictions of the models and the experimental data using the differential evolution (DE) algorithm and the L-BFGS-B nonlinear optimization code. In all cases, the model simulation matched well with the fermentation data being confirmed by the high R-squared values (0.984, 0.992 and 0.965 for WP, lactose and inulin, respectively). The kinetic models proposed here can be employed for the development and optimization of the bioethanol production processes from renewable resources.

Keywords: bioethanol, kinetic model, whey permeate, inulin, *Klyuveromyces marxianus*.

Introduction

In recent years the yeast *Kluyveromyces marxianus* has attracted an increasing attention due to versatile biotechnological applications. It can be used as an efficient producer for valuable microbial products including a number of enzymes, flavor and fragrance compounds as well as bioethanol, particularly from renewable resources [1-3].

The ability to utilize a variety of carbon sources, an enhanced thermotolerance, a rapid growth and a strong Crabtree-negative character of cells are the advantageous traits, which promote the use of *K. marxianus* for industrial bioprocesses [1; 3]. Although, these non-conventional food-grade yeasts have been subjected to still insufficient investigation efforts and quantitative studies of technologically important processes are rarely reported [4; 5]. Such in-depth studies are particularly needed because significantly different growth parameters have been reported not only for different strains of *K. marxianus* but also for the same strain when investigated in different laboratories [2; 6]. Such a metabolic diversity makes it difficult to generalize the knowledge about these yeasts and therefore encourages researchers to focus at least initially, on the reduced number of strains chosen from key culture collections [2].

A substantial phenotypic variation in the growth parameters can be observed in the production of bioethanol from lactose- or inulin-containing substrates, which are provided by the operation of β-galactosidase or endo- and exo-inulinases in *K. marxianus* cells [2; 3; 7-9]. Within the foregoing, particularly the proposed need [2] to study a limited number of strains, a certain attention should be given to the *K.marxianus* DSM 5422. This strain has been proposed as an efficient producer of ethyl acetate and appears relatively well-studied in this context [10, 11]. Although, it has been also used for the production of bioethanol from the renewable lactose-containing substrate such as cheese whey [12-14]. There are several reports on the ethanol production from another technologically promising substrate such as inulin and inulin - containing raw materials by a variety of *K. marxianus* strains [15-21] although DSM 5422 remains unrepresented among them.

Therefore, a comparative analysis for the above two substrates could give a fuller insight into the potential of *K. marxianus* DSM 5422 for production of bioethanol from the renewable, inexpensive and abundant raw materials [8; 22]. It is well known that the behavior of microbial systems can be evaluated by the growth kinetic parameters, which constitute appropriate mathematical models [23-25]. Even relatively simple kinetic models could be indispensable for the design and successful operation of industrial bioprocesses and for obtaining quantitative information about the function of microbial cells [23]. Thus, relevant parameters of Monod kinetics such as the maximum specific growth rate (μ_{max}), the saturation constant (K_s) and the yield of biomass ($Y_{x/s}$) can be considered as

passport data for a particular organism [26]. Several kinetic models have been developed, which describe the ethanol production by different *K. marxianus* strains on lactose – containing substrates [27-30], including the strain DSM 5422 [12]. However, these models appear as differently structured, may contain quite distinctive parameters, as well as often disregard the possible effects of product and/or substrate inhibition [12; 27] and remain restricted by too narrow range of substrate concentrations. The kinetics of ethanol production by *K. marxianus* on the inulin – containing substrates has been only partly described [15; 16], and the full kinetic model is still not developed, which would allow to predict the concentration profiles of substrate, product and biomass during alcoholic fermentation.

The objective of the present study is to develop a kinetic model based on the time-course measurements of substrate, product and biomass changes during the ethanol production by the yeast *K. marxianus* DSM 5422 at varied initial concentrations of lactose- and inulin – containing substrates.

Materials and methods

Organism and cultivation conditions

The yeast *Kluyveromyces marxianus* DSM 5422 was obtained from the Leibniz-Institute DSMZ (German Collection of Microorganisms and Cell Cultures), and maintained on YPD agar. YPD contained (per liter of distilled water) 10 g of yeast extract (Biolife), 20 g of peptone (Biolife), 20 $g \cdot l^{-1}$ of glucose (Sigma) and 20 $g \cdot l^{-1}$ of agar (Biolife).

For the preculture 5 mL of liquid semi-synthetic medium with lactose as carbon source 50 $g \cdot l^{-1}$, yeast extract 5 $g \cdot l^{-1}$ (Biolife), $MgSO_4$ 0.7 $g \cdot l^{-1}$ (Sigma), KH_2PO_4 1 $g \cdot l^{-1}$ (Sigma), K_2HPO_4 0.1 $g \cdot l^{-1}$ (Sigma), $(NH_4)_2SO_4$ 5 $g \cdot l^{-1}$ (Sigma) in 25 mL test tube was inoculated with a single colony from YPD agar plate. Preculture were cultivated at 30 ºC with an agitation speed of 180 rpm in orbital Shaker – Incubator ES-20 (bioSan).

For cultivation in bioreactors, the first preculture was grown in liquid semi-synthetic medium with lactose as carbon source. For the second preculture, 1 l of defined medium in a 2 l flask with cotton stopper was inoculated with 0.05 % (v/v) of the first preculture. Cultivations were carried out at 30°C with agitation speed of 180 rpm.

For bioreactor experiments the second preculture and main culture were grown on whey permeate (WP) (lactose concentrations 120 $g \cdot l^{-1}$, 135 $g \cdot l^{-1}$ and 150 $g \cdot l^{-1}$) obtained from Smiltene milk factory or liquid semi-synthetic medium containing: lactose (Sigma) (120$g \cdot l^{-1}$, 135$g \cdot l^{-1}$ and 150$g \cdot l^{-1}$) or inulin (Dion-Bioline) (80 $g \cdot l^{-1}$, 150 $g \cdot l^{-1}$ and 200 $g \cdot l^{-1}$) as a carbon source, yeast extract 5 $g \cdot l^{-1}$, $MgSO_4$ 0.7 $g \cdot l^{-1}$, KH_2PO_4 1 $g \cdot l^{-1}$, K_2HPO_4 0.1 $g \cdot l^{-1}$, $(NH_4)_2SO_4$ 5 $g \cdot l^{-1}$. Cultures in shake flasks were cultivated at 30 ºC with an agitation speed of 180 rpm.

The main cultivations were carried out in a bioreactor BIOSTAT Q PLUS (Sartorius Stedim Biotech GMbH, Goettingen, Germany) with a working volume 0.4 L, at 30 ºC and 40 ºC, the stirring speed 400 rpm and airflow rate (0.2, 0.8 and 1.4 1 $l \cdot l^{-1} \cdot min^{-1}$) were used. The fermentation medium pH 5.0 was controlled by adding 10 % KOH.

Analytical methods

The yeast growth was monitored spectrophotometrically at the OD_{600}, according to the calibration curve: Biomass dry weight ($g \cdot l^{-1}$) = $0.33 \cdot OD_{600}$. To determine the biomass dry weight, exponentially growing cells were washed three times with distilled water and dried at 104°C until a constant weight.

To estimate the inulin concentration a sample was first incubated (10 µl for 1 ml of sample at 60 ºC for 3 h) with a commercial preparation of inulinases Fructozyme L (Novozymes A/S, Denmark). After inulin hydrolysis by Fructozyme L glucose and fructose were determined enzymatically using the K-SUFRG assay kit (Megazyme, Ireland).

The ethanol and lactose concentrations were determined by HPLC (Agilent 1100 Series), using column Aminex HPX-87H (length 300 mm, i.d. 7.8 mm) with a refractive index detector. The column temperature was 45°C, mobile phase 0.005 $mol \cdot l^{-1}$ H_2SO_4, flow rate 0.6 $ml \cdot min^{-1}$ and sample volume 20 µl.

All analytical measurements were performed at least in duplicate.

Model formulation

Equation 1 describes the kinetics of biomass formation featuring the Monod-type substrate limitation in combination with the Haldane substrate inhibition model [31; 32] and the Jerusalimsky term for the non-competitive product inhibition [33]. It is also known as the Haldane-Boulton model [34].

The system of Ordinary Differential Equations (ODE) summarized below (eq. 2, 3, 4) represents a general mathematical model capable of describing the batch kinetics of ethanol fermentation by *K. marxianus* from lactose- and inulin- containing substrates as mentioned above:

$$\mu = \frac{\mu_{\max} \cdot S}{K_S + S + \frac{S^2}{K_{I,S}}} \cdot \left(\frac{K_{I,P}}{K_{I,P} + P} \right). \quad (1)$$

$$\frac{dX}{dt} = \mu \cdot X , \quad (2)$$

$$\frac{dS}{dt} = -1 \left(\frac{\mu}{Y_{\frac{X}{S}}} \right) \cdot X + m_S \cdot X , \quad (3)$$

$$\frac{dP}{dt} = \frac{\alpha \cdot dX}{dt} + \beta \cdot X . \quad (4)$$

Here S, X, P are product, biomass and product concentrations ($g \cdot l^{-1}$), respectively, μ denotes the specific growth rate (h^{-1}), μ_{max} denotes the maximum specific growth rate (h^{-1}), K_S is the substrate limitation constant ($g \cdot l^{-1}$), $K_{I,S}$ is the substrate inhibition constant ($g \cdot l^{-1}$), $K_{I,P}$ is the product inhibition constant ($g \cdot l^{-1}$). $Y_{X/S}$ is the yield coefficient for cells on substrate ($gX \cdot gS^{-1}$), m_S denotes the maintenance coefficient for cells ($gS \cdot gX^{-1} \cdot h^{-1}$), α and β are growth- and non-growth-associated terms, respectively.

Therefore, the impact of both inhibition constants (K_{IS}, K_{IP}) appears not only in the expression of the specific growth rate (eq. 1), but also is carried over the whole ODE system (eqns. 2, 3, 4) containing this essential process variable. Thus, equation 2 represents the generalized population growth model where the rate constant μ values are determined by equation 1. As well as in equations 3 and 4 describing the rates of substrate consumption [27; 33] and product formation [35], respectively. The whole system of ODE describing the batch kinetics of fermentation by *K. marxianus* was solved using the Real-valued Variable-coefficient ODE solver, with the fixed-leading-coefficient implementation.

Computational methods

Simulations were done using *Python* on a laptop computer with Intel *i*5 processor and 6GB of RAM. Differential equations used in the models were integrated using *SciPy integrate.odeint* function from *SciPy* [36]. The experimental data were stored and maintained in *pandas*. *DataFrame* [37]. The processing and visualization of the simulation results was done using the *Matplotlib* [38] and *Statgraphics Centurion* (Manugistics,Inc, USA) .

To standardize the range of variables the feature scaling was employed (eq. 5) before calculation the sum of square errors between the values of the observed and predicted concentrations.

$$x' = \frac{x - \min(x)}{\max(x) - \min(x)} \quad (5)$$

The model parameters were estimated by the Differential Evolution (DE) algorithm [39] from *Sci Py* library by minimizing the sum of square errors calculated between the measured and model prediction for biomass, substrate and ethanol. The population size for the Differential Evolution algorithm was set to 100, which is sufficient taking into account the number of the model parameters.

At the end of the parameter estimation the L-BFGS-B method is used to polish the best population member.

The leave-one-out cross validation (LOOCV) procedure [40] was employed to validate the kinetic models and the linear plots of the actual data against those predicted by models used to assess the goodness-of-fit for them according to adjusted R^2 values.

Results and discussion

A set of conventional batch fermentation experiments was carried out at varied concentrations of distinctive substrates (Table 1) in order to perform the parameter estimation for the proposed model (equations 1-4) using the obtained data of biomass, substrate and ethanol concentration changes. During these procedures the ordinary differential equations (ODE) were integrated numerically by means of the Differential Evolution (DE) algorithm [39] as described above.

Table 1

Parameters and indices of the goodness -of-fit statistics of kinetic models describing the ethanol fermentation of whey permeate, lactose and inulin by the yeast *Kluyveromyces marxianus* DSM 5422

Parameter / Statistical Index	Description	Unit	Medium A whey permeate as the lactose (120-150 g·l^{-1}) source	Medium B lactose (120-150 g·l^{-1})	Medium C inulin (80-200 g·l^{-1})
μ_{max}	Maximum specific growth rate of biomass	h^{-1}	0.7500	0.6567	0.7500
$Y_{X/S}$	Yield coefficient for biomass (X) on substrate (S)	GX·gS^{-1}	0.0394	0.0673	0.0785
ms	Maintenance coefficient	GS·gX^{-1}·h^{-1}	0.4287	0.0620	0.0000
K_S	Half-saturation constant	g·l^{-1}	0.1000	26.4858	2.0833
$K_{I,S}$	Substrate inhibition constant	g·l^{-1}	13.9648	589.2454	584.7218
$K_{I,P}$	Product inhibition constant	g·l^{-1}	21.2391	13.9478	7.4423
α	growth-associated term [a]	-	5.0000	5.0000	4.1232
β	non growth-associated term [a]	-	0.4185	0.0686	0.0033
RSME	Root-Mean-Square-Error of the model	-	0.0398	0.0201	0.0397
R^2	R-square (coefficient of determination) of the model	%	98.40	99.21	96.34
R^2	Leave-one-out-cross-validated (LOOCV) R-square	%	97.76	98.77	93.95

a - term of the Luedeking-Piret equation [35]

Table 1 also demonstrates that the different substrates for the ethanol fermentation by *Kluyveromyces marxianus* do not affect the structure of the model, which follows from the identical parameter sets being eligible for the system of relevant ODE. Although, the numerical values of the parameters are noticeably affected. Thus, a substantially reduced *Ks* value corresponding to whey permeate (WP) fermentations at a whole range of concentrations indicates a high affinity of *K. marxianus* with respect of this substrate [26; 33]. At the same time the WP exhibits a much more pronounced non-competitive substrate inhibition when compared (Table 1) with the other two carbon

sources (lactose, inulin), presumably due to its complex composition containing a wide range of osmolytes [41]. Such WP specificities could also cause the apparent increase in the maintenance coefficient (*ms*) value reflecting the impact of metabolic costs for osmotic adjustment and, as a consequence, a relatively reduced yield coefficient ($Y_{x/s}$) for biomass on substrate (Table 1). It is essential that despite noticeable differences in parameter values the maximum growth rates (μ_{max}; equation 1) remain at rather high level and are almost unaffected for all three substrates under study. This is well in line with the notions on *K.marxianus* as the fastest growing eukaryote on Earth [4]. Besides, in all cases the formation of ethanol can be described (equation 4) according the Luedeking-Piret kinetics [35] as the almost solely growth-associated process where the specific rate of product formation is proportional to the specific growth rate of the yeast *K. marxianus*. This is indicated by significantly higher values for the growth associated (α) parameters when compared (Table 1) with those non-growth-associated (β) terms [35]. However, for the WP fermentation also partially mixed-growth-associated ethanol formation could occur as indicated by a slightly elevated β value (Table 1).

The matching quality of the proposed kinetic models was evaluated by the linear plots (Fig.1) of the actual experimental data against those predicted by the models. The highly significant R-square values (coefficient of determination) indicate that the model adequately describes the actual changes of biomass, substrate and product concentrations during ethanol fermentation of whey permeate, lactose or inulin by the yeast *K. marxianus* since only a relatively small proportion (0.79-3.66 %) of the total variance remains unexplained (Table 1). This is also confirmed by the relatively low RMSE (Root-Mean-Square-Error) values of the model (Table 1).

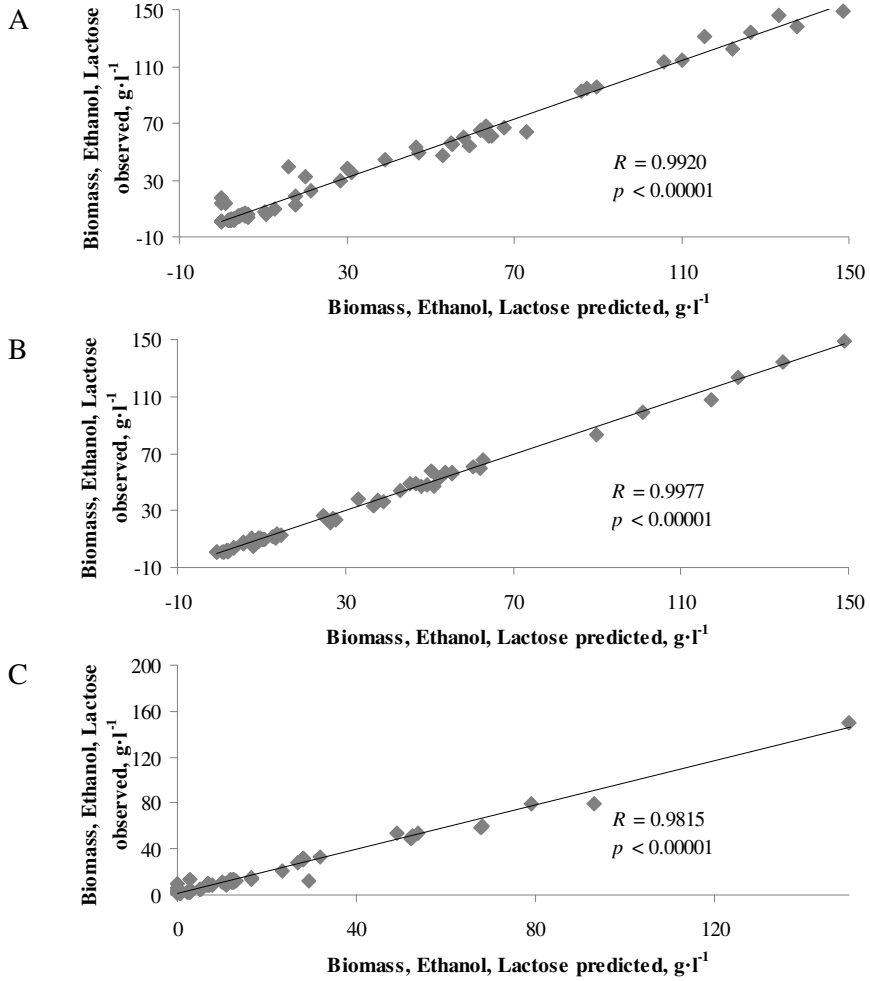

Fig. 1. **Linear plots of the actual concentrations of biomass, substrate /lactose or inulin/ and ethanol against those predicted by unstructured kinetic models (equations 1-4) for the yeast *Kluyveromyces marxianus* DSM 5422.** The observed versus predicted plots (A,B,C) for the estimates obtained during the ethanolic fermentation of Whey Permeate (WP), lactose or inulin, respectively, as specified in Table 1

In addition, the validation of the model using the leave-one- out cross-validation [40] procedure (LOOCV) resulted in slightly reduced R-square values (Table 1), which still remain within the limits of high ($p < 0.00001$) statistical significance.

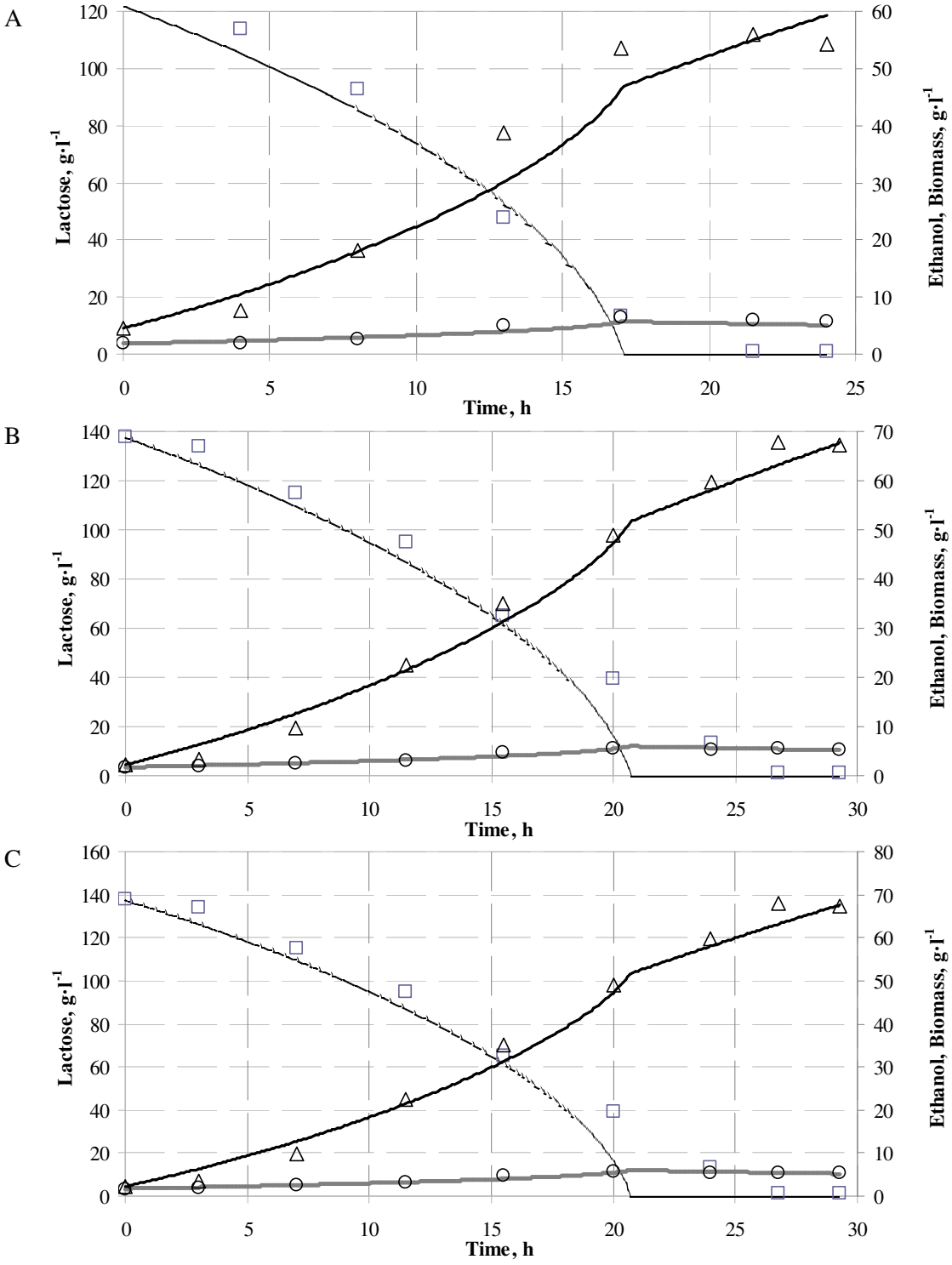

Fig. 2. **Experimental (symbols) and predicted (lines) profiles for biomass (Δ), ethanol (○) and lactose (□) concentrations in batch fermentations of the yeast *Kluyveromyces marxianus* DSM 5422 in the Whey Permeate (WP) medium containing lactose 120 g·l^{-1} (A), 135 g·l^{-1} (B) and 150 g·l^{-1} (C)**

The batch kinetics of biomass and ethanol production was studied at different initial substrate concentrations (S_0) of distinctive substrates (Table 1). Figure 2 shows the time course profiles of batch

fermentations of whey permeate to ethanol by *K. marxianus* at different initial lactose concentrations (S_0 = 120, 135 and 150 g·l^{-1}).

According to the model, the most of initial lactose can be metabolized by the yeast within 23 h or even earlier (fig. 2A) and the ethanol concentration and cell mass achieved 72.85 g·l^{-1}and 6.37 g·l^{-1}, respectively, at a maximum lactose concentration osf 150 g·l^{-1} (Fig. 2C). Besides, both the ethanol concentration and the cell mass increase in proportion to growing initial concentrations of lactose in the fermentation medium (Table 1, Fig.2 A-C). In cases with two other fermentation media, containing pure lactose or inulin as the carbon sources, the cell growth, substrate consumption and ethanol production profiles (data not shown) appeared as rather similar. In these fermentations the growing initial substrate concentrations also are followed by the proportionally increased ethanol and biomass concentrations. Although, there are substantial differences in both ethanol and biomass concentrations that can be achieved when using fermentation media with such carbon sources. Thus, the media containing pure lactose or inulin especially promote the formation of biomass, which concentration, for example, for inulin, can be achieved up to 16.41 g·l^{-1} (at S_0 = 200 g·l^{-1}). In turn, for pure lactose the biomass concentration may be lower, reaching 10.74 g·l^{-1} (at S_0 = 150 g·l^{-1}), however, significantly above that observed in the whey permeate medium. In turn, the concentration of ethanol, which can be achieved using these substrates, appears lower when compared to the whey permeate - containing medium. Although, even in these cases, at high initial substrate concentrations (S_0 = 150 g·l^{-1} or above) the ethanol concentration may exceed 60 g·l^{-1}.

Several technologically relevant parameters, obtained at the same initial concentration of the carbon source, are represented in Table 2, thus enabling an assessment of different substrate impacts on ethanol production and biomass formation by *K. marxianus* DSM 5422. It is obvious that the fermentation medium containing pure lactose or inulin as the carbon source provides a much higher biomass productivity (Q_x) and yield ($Y_{x/s}$) per unit of the substrate consumed. This could be explained by differences in the substrate composition, for instance, by the presence of osmolytes in the whey permeate as mentioned above [41] and perhaps even more by a possible nitrogen deficiency in this source unlike the pure lactose- and inulin-containing media containing the yeast extract supplement [42]. At the same time the differences in the volumetric ethanol productivity (Q_p) and the specific rate of product formation (*qp*) for the whey permeate medium are relatively less pronounced when compared with the other two media (Table 2). Of particular note is the fact that the whey permeate-containing medium turns out to be the most appropriate to achieve the highest yield of ethanol per unit of substrate consumed ($Y_{p/s}$ = 0.460 gg^{-1}), which accounts for 90.2 % of theoretical, which is substantially higher than 79.2 % and 64.5 % for the pure lactose- and inulin-containing medium, respectively (Table 2). This fact in conjunction with the highest achievable ethanol concentration mentioned above indicates that the whey permeate - containing medium and the yeast *K. marxianus* DSM 5422 could find technological applications to produce ethanol from this renewable source.

Table 2

Summary of parameters for ethanol fermentation of whey permeate, lactose and inulin by the yeast *Kluyveromyces marxianus* DSM 5422

Parameter	**Carbon source**		
	Whey permeate (medium A)	Lactose (medium B)	Inulin (medium C)
Substrate consumption $S_0 - SI$, g·l^{-1}	148.74	148.71	149.99
Ethanol volumetric productivity *Qp*, g·(lh)$^{-1}$	2.506	4.056	3.167
Ethanol yield *Yp/s*, g·g^{-1}	0.460	0.404	0.329
Biomass volumetric productivity *Qx*, g ·(L·h)$^{-1}$	0.179	0.742	0.764
Biomass yield *Yx/s*, g·g^{-1}	0.0293	0.0654	0.0785
Specific rate of ethanol formation *Qp*, g· (g·h)$^{-1}$	0.394	0.434	0.296
Specific rate of substrate consumption *qs*, g ·(g·h)$^{-1}$	1.443	1.214	0.909

Conclusions

A simple unsegregated and unstructured kinetic model has been developed for the batch production of bioethanol by the yeast *Kluyveromyces marxianus* DSM 5422 from the renewable sources of agricultural and food processing origin, such as whey permeate or inulin, which includes the terms of both substrate and product inhibition. *Kluyveromyces marxianus* shows the highest yield of ethanol (90.2 % of theoretical) on whey permeate as substrate, while ethanol and biomass productivity was lower as compared with semi-synthetic lactose or inulin medium due to nitrogen deficiency in whey permeate. It can be concluded that whey permeate is a suitable raw material for bioethanol fermentation by *Kluyveromyces marxianus*. There is reverse correlation between the biomass yield and ethanol yield on all three substrates. In all cases, the model simulation matched well with the whey permeate, inulin and lactose fermentation data of biomass growth, ethanol production and substrate consumption being confirmed by the high R^2 values.

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References

1. Morrissey J.P., Etschmann M.M.W, Schrader J., de Billerbeck G.M. Cell factory applications of the yeast *Kluyveromyces marxianus* for the biotechnological production of natural flavour and fragrance molecules. Yeast, vol.32,Nr.1, 2015,pp. 3-16.
2. Fonseca G.G., Heinzle E., Wittmann Ch., Gombert,A.K. The yeast *Kluyveromyces marxianus* and its biotechnological potential. Appl. Microbiol. Biotechnol vol. 79, Nr.3, 2008, pp. 339-354.
3. Lane M.M., Burke N., Karreman R., Wolfe K.H., O'Byrne C. P., Morrisey J.P. Physiological and metabolic diversity in the yeast *Kluyveromyces marxianus*. Antonie van Leeuwenhoek, vol. 100, Nr.4,2011,pp. 507-519.
4. Rocha S.N., Abrahão-Neto J., Cerdán M.E., María I González-Siso,M.I.and Gombert,A.K. Heterologous expression of glucose oxidase in the yeast *Kluyveromyces marxianus*. Microbial Cell Factories, vol. 9:4, 2010.
5. Fonseca G.G., Barbosa de Carvalho N.M., Gombert A.K. Growth of the yeast *Kluyveromyces marxianus* CBS 6556 on different sugar combinations as sole carbon and energy source. Appl. Microbiol.Biotechnol.,vol .97, Nr.11,2013, pp. 5055-5067.
6. Fonseca G.G., Gombert A.K., Heinzle E., Wittmann Ch. Physiology of the yeast *Kluyveromyces marxianus* during batch and chemostat cultures with glucose as the sole carbon source. FEMS Yeast Res.,vol. 7, Nr.3,2007, pp. 422-435.
7. Vincenzi A., Maciel M.J., Burlani E.,L., Oliveira E.C., Volpato G., Lehn D.N., Volken de Souza C.F. Ethanol bio-production from ricotta cheese whey by several strains of the yeast *Kluyveromyces*. Am.J. Food Technol.vol. 9 Nr.6, pp.281-291.
8. Guimarães P.M.R.,. Teixeira J.A., Domingues L. Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey. Biotechnol. Advances, vol. 28,Nr.3, 2010, pp. 375-384.
9. Gao J., Yuan W., Li Y., Xiang R., Hou Sh., Zhong Sh., Bai F. Transcriptional analysis of *Kluyveromyces marxianus* for ethanol production from inulin using consolidated bioprocessing technology. Biotechnol. Biofuels vol.8:115, 2015.
10. Löser Ch., Urit Th,, Bley Th. Perspectives for the biotechnological production of ethyl acetate by yeasts. Appl. Microbiol. Biotechnol., vol. 98, Nr.12,2014, pp. 5397-5415.
11. Löser Ch., Urit Th., Gruner E., Bley Th. Efficient growth of *Kluyveromyces marxianus* biomass used as a biocatalyst in the sustainable production of ethyl acetate. Energ. Sustain. Soc., vol. 5:2, 2015.
12. Sofia D., Joshi Y.A., Poletto M. Kinetics of bioethanol production from lactose converted by *Kluyveromyces marxianus*. Chem.Eng.Trans.,vol.32,2013, pp. 1135-1140.

13. Lukjanenko J., Kovtuna K., Scherbaka R., Vigants A. Bioethanol and biomass production by *Kluyveromyces marxianus* during lactose fermentation at different salts and substrate concentrations. J.Biotechnol., vol.185S:122S,2014.
14. Vigants A., Lukjanenko J., Grube M., Liepins J. The influence of fermentation conditions on biomass composition during ethanol biosynthesis from cheese whey lactose concentrate by *Kluyveromyces marxianus*. J.Biotechnol., vol.185S:122S,2014.
15. Margaritis A., Bajpai P. Effect of sugar concentration in Jerusalem artichoke extract on *Kluyveromyces marxianus* growth and ethanol production. Appl. Environ. Microbiol.,vol. 45, Nr. 2,1983, pp. 723-725.
16. Bajpai P., Margaritis A. Ethanol inhibition kinetics of *Kluyveromyces marxianus* grown on Jerusalem artichoke juice. . Appl. Environ. Microbiol.,vol. 44, Nr. 6,1982, pp. 1325-1329.
17. Yuan W.J, Zhao X., Chen L., Bai F.W. Improved ethanol production in Jerusalem artichoke tubers by overexpression of inulinase gene in *Kluyveromyces marxianus*. Biotechnol.Bioproc.Eng.,vol.18, Nr.4,2013, pp. 721-727.
18. Yuan W.J., Chang B.L., Ren J.G., Liu J.P., Bai F.W., Li Y.Y. Consolidated bioprocessing strategy for ethanol production from Jerusalem artichoke tubers by *Kluyveromyces marxianus* under high gravity conditions.J.Appl.Microbiol.,vol.112,Nr.1, 2012, pp. 38-44.
19. Yuan W.J., Zhao X.Q, Ge X.M., Bai F.W. Ethanol fermentation with *Kluyveromyces marxianus* from Jerusalem artichoke grown in salina and irrigated with a mixture of seawater and freshwater. J.Appl.Microbiol.,vol.105,Nr.6, 2008.
20. Gao J.O., Chen L.J., Yuan W.J. Effects of carbon sources, oxygenation and ethanol on the production of inulinase by *Kluyveromyces marxianus* YX01.J.BioSci.Biotech.,vol.1,Nr.2,2012, pp.155-161.
21. Yang L., He Q.S., Corscadden K., Chibuike C., Udenigwe Ch.C. The prospects of Jerusalem artichoke in functional food ingredients and bioenergy production. Biotechnol.Reports, vol.5,2015, pp.77-88.
22. Chi Z.M., Zhang T., Cao T.S., Liu X.Y., Cui W., Zhao Ch.H. Biotechnological potential of inulin for bioprocesses. Bioresource Technol.,vol.102,Nr.6,2011, pp. 4295-4303.
23. Nielsen J. Fermentation kinetics: Central and modern concepts. In: Fermentation Microbiology and Biotechnology, El-Mansi, E.M.T. et al.,Eds,Third edition,2011, CRC Press, pp. 37-76.
24. Vinayagam R., Vytla R.M., Chandrasekaran, M. Development of a simple kinetic model and parameter estimation for biomass and nattokinase production by *Bacillus subtilis* 1A752. Austin J. Biotechnol. Bioeng., vol.2,Nr.1,pp. 1036-1040.
25. Almquist J., Cvijovic M., Hatzimanikatis V., Nielsen J., Jirstrand M. Kinetic models in industrial biotechnology – Improving cell factory performance. Metabol.Eng.,vol.24,,2014, pp.38-60.
26. Panikov N. Kinetics, Microbial Growth.In: Encyclopedia of Bioprocess Technology,Wiley Online Library,2002, pp. 1513-1540.
27. Zafar S., Owais M., Mohammed Saleemuddin,M. and Husain,S. Batch kinetics and modelling of ethanolic fermentation of whey. Int.J.FoodSci.Technol.,vol.,40,Nr.6,2005,pp.597-604.
28. Ariyanti D., Hadiyanto H. Ethanol production from whey by *Kluyveromyces marxianus* in batch fermentation system: kinetics parameters estimation. Bull.Chem.Reaction Eng.Catalysis, vol.7, Nr.3,2013, pp.179-184.
29. Parrondo,J., García,L.A. and Díaz,M. Nutrient balance and metabolic analysis In a *Kluyveromyces marxianus* fermentation with lactose-added whey. Brazilian J. Chem.Eng.,vol. 26, Nr. 3, 2009, pp. 445-456.
30. Longhi L.G.S., Luvizetto D.J., Ferreira L.S., Rech R., Ayub M.A.Z., Secchi A.R. A growth kinetic model of *Kluyveromyces marxianus* culture on cheese whey as substrate. J. Ind. Microbiol. Biotechnol., vol. 31, Nr.1,2004, pp. 35-40.
31. Andrews J.F. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates. Biotechnol. Bioeng., vol.10, Nr6,1968, pp. 707-723.
32. Guisasola A., Baeza J.A., Carrera J., Sin G., Vanrolleghem P.A., Lafuente J. The influence of experimental data quality and quantity on parameter estimation accuracy: Andrews inhibition model as a case study. Education Chem.Engineers, vol.1,2006, pp.139-145.
33. Moser A. Bioprocess Technology: Kinetics and Reactors, Springer-Verlag, New York Inc.,1988, 480 p.

34. Arellano-Plaza M., Herrera-López E.J., Díaz-Montaño D.M., Moran A., Ramírez-Córdova J.J. Unstructured kinetic model for tequila batch fermentation. Int.J. Math.Comput. Simul.,vol.1,Nr.1,2007, pp.1-6.
35. Luedeking R. Piret E.L. A kinetic study of the lactic acid fermentation. Batch process at controlled pH. Biotechnol. Bioeng., vol.67,Nr. 6,2000,pp. 636-644.
36. Jones E., Oliphant T., Peterson P. SciPy: Open source scientific tools for Python, 2001. URL http://www. scipy. org, 73,2015, 86.
37. McKinney W. Data Structures for Statistical Computing in Python. In: van der Walt,S. and Millman J. (Eds.), Proceedings of the 9th Python in Science Conference, 2010, pp. 51-56
38. Hunter J.D. Matplotlib. A 2D graphics environment. Comput. Sci. Eng., vol.9, Nr. 3, 2007, pp. 99-104.
39. Storn R., Price K. Differential evolution – A simple and efficient heuristic for global optimization over continuous spaces. J. Global Optim.,vol. 11,Nr. 4,1997, pp. 341-359.
40. Arlot S., Celisse A.A survey of cross-validation procedures for model selection. Stat.Surv.,vol.4,Nr.1,2010, pp. 40-79.
41. Dale M.C., Eagger A. ,Okos M.R. Osmotic inhibition of free and immobilized *K.marxianus* anaerobic growth and ethanol productivity in whey permeate concentrate. Proc.Biochem.,vol.29,Nr.7,1994, pp. 535-544.
42. Moreira N.L., dos Santos L.F., Soccol C.R., Suguimoto H.H. Dynamics of ethanol production from deproteinized whey by *Klyuveromyces marxianus*: an analysis about buffering capacity, thermal and nitrogen tolerance. Braz.Arch.Biol.Technol.,vol.58,Nr.3,2015, pp. 454-461.