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Dynamic Parameter Estimation Problem for Ethanol Production from Seaweed

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Ethanol has been studied extensively as one of the current and probably future energy vectors. Fermentation of hydrolysed oligosaccharides from macroalgae biomass to ethanol has been certified, and several processing options have been proposed. In the present work, we model the production of ethanol based on *Laminaria*, a seaweed genus that belongs to the so-called "brown algae" group, as the carbon source. In brown algae, the most relevant sugars that can be used as substrate for fermentation are mannitol -the alcohol form of the sugar mannose- and laminaran, a linear polysaccharide of(1,3)-β-D-glucopyranose. We consider the yeast *Pichia angophorae* as the fermenting microorganism. The model includes dynamic mass balances for biomass, ethanol, mannitol and laminaran. Growth is controlled via limiting functions that modify the biomass equation for temperature and oxygen transfer rate (OTR). It is also modified by including a term that considers inhibition by ethanol. Based on the proposed model, a dynamic parameter estimation problem is formulated, the objective function being weighted least-squares fit to data, subject to the mass balance equations. The data set for parameter estimation was obtained in batch liquid cultures, with experiments performed over 40 hours. Numerical results provide useful insights on ethanol production using macroalgae biomass as carbon source.

1. Introduction

Macroalgae (colloquially known as seaweed) is a grouping of organisms that do not share a common ancestor, but share some common characteristics like being multicellular, visible to the naked eye, and growing in different zones of the sea, especially in the benthic zone. They are usually divided into three large informal groups according to their pigmentation (brown, red and green), which are indicators of their different biochemical characteristics and within each group a large diversity of physiology and modes of reproduction exist. Viability of ethanol production by fermentation of macroalgal biomass has been discussed in the context of renewable liquid fuels (Kumar et al., 2013). At the moment, some pilot plants around the world are either being planned or constructed, and special interest has been attracted by genetically engineered microorganisms specially designed for the consolidated bio-processing of macroalgae biomass (Wargacki et al., 2012). However, regarding economic evaluation, it is still unclear as to which is the real potential of macroalgal biomass ethanol to replace liquid fuels, with even one of the pilot plants planned facing uncertain future due to the consideration of the opportunity cost of selling the unprocessed algae. The same issue arises given that macroalgae are sources of high value products, mainly, agar, carrageenan and alginate. However, this can be seen both as an unfavourable comparison for ethanol production (considered to be a low-price commodity), but also as a possibility, since the integration of ethanol production with the production of some of these high value products is possible (Khambhaty et al., 2012), possibly allowing for all or some of the energetic provision of these plants, reducing their fossil fuel consumption. This changes the angle of the analysis from an objective like specially growing macroalgae for liquid fuel production as commodity, which appears to be un-economical, to designing an energy production process to be attached to existing high value products production processes. Therefore, there is still need for more research and modeling on the hydrolysis and

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fermentation processes that take place based on seaweed as carbon source. Several ways of extracting energy from macroalgae have been devised (Roesijadi et al. 2010), including gasification, fermentation to alcohols and anaerobic digestion among others. In this work, the possibility of using macroalgae biomass as substrate for ethanol production by fermentation is analyzed. The ethanol production process is based on extraction and hydrolysis of the cell wall and/or storage of polysaccharides, followed by fermentation to ethanol.

Within the aforementioned brown macroalgae, there are three main sources of molecules capable of being transformed into ethanol. They are: a) Alginate, a co-polymer of alpha-L-guluronate and its C5 epimer beta-D-mannuronate, which requires a certain pre-treatment to be utilized, but is also a possible high value co-product of a brown macroalgae bio-refinery, with use in the food industry as a water-retainer, and a suspending and emulsifying agent in rubber and paint industries; b) Laminaran, a β-(1-->3) linked glucan, with some branching occuring in a β-(1-->6) fashion, which can be fermented without prior treatment provided the chosen microorganism has β-(1-->3) glucanases; c) Mannitol, a sugar alcohol that is only fermented aerobically due to it being firstly oxidized to fructose by a mannitol deshydrogenase enzyme. In this reaction, NADH is generated, so in order to regenerate NAD+, oxygen or transhydrogenase is required (Horn et al., 2000).

The objective of this work is to propose a suitable model for the fermentation stage, for later integration to the remaining stages of the process into a superstructure that allows entire process optimization.

2. Process description

The system under study is the ethanol production stage via fermentation of seaweed biomass, specifically the brown macroalgae *Laminaria hyperborea*, in batch liquid cultureconsidering laminaran and mannitol as substrates, which correspond to the 25 and 30 % of *Laminaria´*s dry weight, respectively (Horn et al., 2000).The fermenting yeast is *Pichia angophorae*, which can produce ethanol directly from mannitol and laminaran. A dynamic model is proposed and its parameters are estimated for batch fermentation under oxygen limited conditions. The experimental data is taken from (Horn et al., 2000).

3. Mathematical model

We formulate mass balances for external metabolites and biomass during *P. angophorae* growth and fermentation by substrate consumption and biomass and product accumulation. The resulting Differential Algebraic Equation (DAE) system is as follows:

$$dX/dt = \mu X - kd\,X \quad (1)$$

$$\mu = (\mu_m f(O_2) + \mu_l) f(E) \quad (2)$$

$$f(O_2) = \left(OTR/OTR_{opt}\right) * exp\left(1 - \left(OTR/OTR_{opt}\right)\right) \quad (3)$$

$$f(E) = 1/(1 + C_E/K_i) \quad (4)$$

$$\mu_m = \mu_{max,m} C_m / \left(K_{s,m} + C_m + a_m C_m^{\lambda_m} + b_m C_l^{v_m}\right) \quad (5)$$

$$\mu_l = \mu_{max,l} C_l / \left(K_{s,l} + C_l + a_l C_l^{\lambda_l} + b_l C_m^{v_l}\right) \quad (6)$$

$$dC_m/dt = Y_{xm}\mu\,X \quad (7)$$

$$dC_l/dt = Y_{xl}\mu\,X \quad (8)$$

$$dC_{O_2}/dt = OTR - OUR \quad (9)$$

$$OTR = K_L a\left(C_s - C_{O_2}\right) \quad (10)$$

$$OUR = q_o X \quad (11)$$

$$dC_E/dt = Y_{xE}\mu\,X \quad (12)$$

The growth rate of *P. angophorae* is represented by the sum of the specific growth rate over the two considered substrates, mannitol (μ_m) and laminaran (μ_l). As indicated in the previous section, mannitol metabolism depends on oxygen concentrations, so we included a limiting function$f(O_2)$ which can vary between 0 and 1 affecting the specific growth rate on mannitol. The net growth rate (μ) is inhibited by substrate. The toxic effect of high ethanol concentrations is modelled by the limiting function $f(E)$ (Laiglecia et al., 2013).The model includes a mass balance for dissolved oxygen due to the importance of

this variable for *Pichia* growth on mannitol. Table 1 shows the nomenclature and units for the model variables.

Table 1: Model Variables

Notation	Description	Units
X	Biomass concentration	g/L
C_E	Ethanol concentration	g/L
C_m	Mannitol concentration	g/L
C_l	Laminaran concentration	g/L
C_{O_2}	Dissolved oxygen concentration	g/L
μ	Growth rate	1/h
μ_m	Growth rate on mannitol	1/h
μ_l	Growth rate on laminaran	1/h
OTR	Oxygen transfer rate	g/Lh
OUR	Oxygen uptake rate	g/Lh
$f(O_2)$	Oxygen limiting function	-
$f(E)$	Ethanol inhibition function	-

4. Parameter estimation

We formulate a parameter estimation problem in an equation oriented control vector parameterization environment with a maximum likelihood objective function. We implement the parameter estimation problem in gPROMS (g-PROMS 2011), which allows selecting a variance model for the experimental data set. The optimization algorithm determines both the values of dynamic model parameters and variance model parameters.

Assuming that measurement errors are independent and normally distributed, with zero average and standard deviation σ_{ij}, the objective function (Eq. 13) is:

$$\emptyset = \frac{N}{2} ln(2\pi) + \frac{1}{2} min_p \sum_{i=1}^{NM} \sum_{j=1}^{NT} \left\{ \left[ln\left(\sigma_{ijk}^2\right) + \frac{\left(C_{ij}^M - C_{ij}\right)^2}{\sigma_{ijk}^2} \right] \right\} \tag{13}$$

where the summation is over NM measured state variables (C_{ij}) and *NT* data points for each measured variable; σ_{ij} is the variance of the *jth* measurement of variable *i*, which is determined by the measured variable variance model. *N* is the total number of measurements and vector ***p*** corresponds to estimated parameters vector.
The parameter estimation problem is then formulated as:

$$\begin{aligned} &\min \emptyset \\ &\text{s.t.} \\ &DAE\ model \\ &C_i(O)=C_i^o \end{aligned} \tag{14}$$

The model performance for calibration purposes has been tested quantitatively by two diagnostic measures based on average values of the main state variables. The mean error (ME) (Eq15) is a measure of the model bias, which gives information on the model's trend to over- or underestimates a variable. The relative error (RE) (Eq. 16), characterizes the model accuracy.

$$ME = \frac{\sum_{i=1}^{NT}\left(C_i - \widehat{C}_\iota\right)}{NT} \tag{15}$$

$$RE = \frac{\sum_{i=1}^{NT}\left|C_i - \widehat{C}_\iota\right|}{\sum_{i=1}^{NT} C_i} \tag{16}$$

where C_i and $\hat{C}_i$ are the observed and predicted values of the main state variables, respectively; *NT* is the number of observations of each state variable.

5. Results and discussion

We address the dynamic parameter estimation problem with gPROMS for a batch ethanol production system for *P. angophorae* growing on *L. hyperborea* extract. Optimal parameter values obtained and fixed values parameters are shown in Table 2. Figure 1 shows observed data from (Horn et al., 2000) and concentration profiles with the estimated parameters. Table 3 presents the goodness-of-fit statistics for the model state variables based on averages values. The medium error (ME) and the relative error (RE) show that the concentrations achieved a good agreement between model simulation and experimental data.

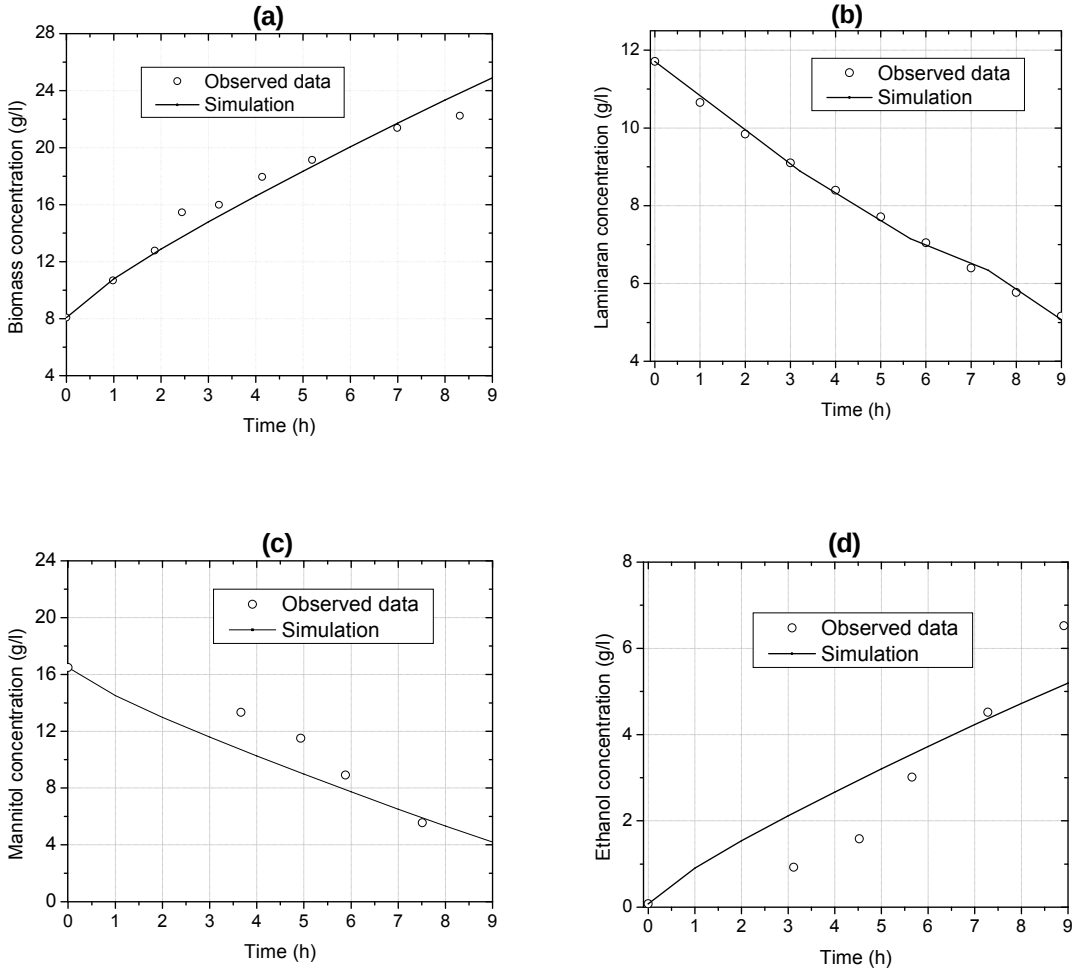

Figure 1: Observed data and simulation profiles for biomass (a), laminaran (b), mannitol (c) and ethanol (d) concentrations

Table 2: Model parameters. (*) Parameters estimated in this work. Remaining parameters taken from (Horn et al., 2000; Nakamura et al., 2001; Acik, 2009)

Parameter	Description	Value	Unit
$\mu_{max,m}$	Maximum growth with mannitol	0.141 (*)	1/h
$\mu_{max,l}$	Maximum growth with laminaran	0.795 (*)	1/h
$K_{s,m}$	Half saturation constant for mannitol uptake	$1.0x10^{-5}$(*)	g/L
$K_{s,l}$	Half saturation constant for laminaran uptake	2.499 (*)	g/L
K_i	Ethanol inhibition constant	0.475 (*)	g/L
k_d	Mortality rate	$1.0x10^{-5}$	1/h
OTR_{opt}	Optimal oxygen transfer rate	769.7	g/L/h
a_m	Inhibition parameter for high mannitol concentration	$1.1x10^{-3}$	--
a_l	Inhibition parameter for high mannitol concentration	$7.8x10^{-4}$	--
λ_m	Inhibition parameter for high mannitol concentration	2.5	--
λ_l	Inhibition parameter for high mannitol concentration	2.7	--
b_m	Inhibition parameter for high laminaran concentration	$7.2x10^{-4}$	--
b_l	Inhibition parameter for high laminaran concentration	$1.5x10^{-4}$	--
v_m	Inhibition parameter for high laminaran concentration	2.4	--
v_l	Inhibition parameter for high laminaran concentration	1.5	--
q_o	Oxygen uptake rate	2.475 (*)	1/h
$K_l a$	Oxygen transfer coefficient	800.0	1/h
C_s	Saturated dissolved oxygen concentration	$5.59x10^{-3}$	g/L
Y_{mx}	Biomass yield for mannitol	0.739	$g_X/g_{mannitol}$
Y_{lx}	Biomass yield for laminaran	0.391	$g_X/g_{laminaran}$
Y_{xE}	Ethanol yield	0.303	$g_{Ethanol}/g_X$

Table 3. Statistical measurements

	$C_{ethanol}$	$C_{laminaran}$	$C_{mannitol}$	$C_{biomass}$
ME	-0.237	-0.016	0.454	0.416
RE	0.262	0.015	0.158	0.054

6. Conclusions

In this work we have formulated a dynamic mathematical model for the ethanol fermentation process of *Laminaria hyperborea* extract by the yeast *Pichia angophorae*. The model has been calibrated with experimental data from Horn *et al* (Horn et al., 2000). The model allows for a preliminary study on the bioconversion of brown seaweed to ethanol, based only on laminaran and mannitol. This is in line with the possibility to obtain energy out of residues of currently working alginate production processes. The present model has been formulated within an optimal control problem framework. The proposed model can serve as a guidance tool for the dynamic optimization of the fermentation step of this process, which will improve the yield towards the product in the context of generating renewable energy.

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