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NEW RESULTS ON THE FIELD OF "WHITE BIOTECHNOLOGY"

A. NEMETH✉, G. NAGY, B. SEVELLA

Budapest University of Technology and Econpomics, Department of Applied Biotechnology and Food Science
H–1111 Budapest Műegyetem rkp. 3., HUNGARY
✉E-mail: naron@f-labor.mkt.bme.hu

"White biotechnology" term is used to describe the production of chemical compounds by enzymatic or microbial (biotechnological) methods. Our research group focuses on the field of glycerol utilization and lactic acid production, and in this work we present a new kinetic model based on our laboratory lactic acid experiments, and used for planning continuous fermentation with high productivity.

Keywords: Lactic acid, kinetic model, continuous fermentation

Introduction

"White Biotechnology" was defined by Karl-Erich Jaeger [1] as an expression describing the biotechnological production of compounds with the help of enzymes and/or microorganisms.

The work in our research group has been focusing on this field since many years, and the main topics became glycerol and lactic acid platforms. In this report we present the results of our developments on the field of fermentative lactic acid production.

Lactic acid (LA) is a chiral carbon acid, known since more than a century, and used over several decades mostly for food industry. Recently its application field was significantly expanded (pharmaceutical and polymer industry) as well as its production volume, thus it came again into the focus of researches. Although it can be produced chemically as well as biologically, in the former case racemic mixture is formed, in the latter case – depending on the producer strain – optically pure (L- or D-lactic acid) arises. Most probably this is the reason, why it is mainly biologically produced via microbial fermentation.

The fermentation ability of microorganism admit of biological production of lactic acid on glucose (Glu) substrate resulting in either lactic acid alone as product (homofermentatives, using Embden-Meyerhof-Parnas metabolic route) or lactic acid together with further products such as acetic acid, ethanol, CO_2 (hetero-fermentatives, pentose-phosphate route). There are also some strains producing solely lactic acid on glycose, or together with by-products on C_5 sugars. They are usually called as facultative homofermentatives. While from the point of view of white biotechnology certainly homofermentatives are of most important, for the food industry heterofermentatives are also in the focus of interest. The reason is that in the former case the goal is to convert as much substrate into product as much is possible, while in the latter case, the given ratio of the various fermentation products serves as aroma and flavour compounds.

The efficiency of lactic acid fermentation is usually given with volumetric productivity (g lactic acid/L broth/hour). In this term the published data are in a very wide range (1.5–35 $g \cdot L^{-1} \cdot h^{-1}$) [2] depending on the applied strain, fermentation technique, and media. However, the known industrial processes with batch operation resultin a productivity range of 2.5–3 $g \cdot L^{-1} \cdot h^{-1}$.

We already presented [3-4] that our homofermentative microorganism belonging to lactobacilli genus makes a competitive lactic acid production possible. In this report we present a kinetic model built up on the basis of several batch lactic acid fermentations. This model was applied in simulation studies to plan continuous fermentation resulting in higher volumetric productivity.

Material and Methods

Lactobacillus MKT878 was chosed on the basis of an earlier screening program run at our laboratory [3] and was deposited at National Collection of Agricultural and Industrial Microorganism with reference number NCAIM-B02375. Batch fermentation were carried out on the media optimized for this strains previously as follows: 120 $g \cdot L^{-1}$ glucose, 30 $g \cdot L^{-1}$ cornstep-liquor (Hungrana, Roquette), 6 $g \cdot L^{-1}$ yeast extract (YE), 0.5 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.3 $g \cdot L^{-1}$ $FeSO_4 \cdot {}_7H_2O$, 0.01 $g \cdot L^{-1}$ $MnSO_4$.

Fermentations were carried out in Biostat Q bench top fermenter (BBraun) at pH = 5.8 (controlling with 20% NaOH and 25% H_2SO_4), 37 °C and 700 rpm stirring

rate. 3 agar slants served as inoculums after suspension of cells in sterile water. The process was followed due sampling, and OD_{600} was measured after 20x dilution to determine cell density (dry weight ($g \cdot L^{-1}$) = $0.5 \cdot OD_{600}$). The filtered (through 0,2 μm pore size filter)) supernatant of the sample was analysed with Waters Breeze HPLC system (0,5 $ml \cdot min^{-1}$ 5 mM H_2SO_4 as eluant on BioRad Aminex HPX87H column at 65 °C with RI detection at 40 °C) for glucose and lactic acid. Since the rather rare sampling there were not enough measured data for kinetic evaluation, further dry weight, glucose and lactic acid data was calculated on the basis of base consumption (of pH control) which is proportional to the cell and product formation, and these data series were used for fitting the kinetic equations with *Berkeley Madonna 8*. software. For the calculation of dry weight, glucose and lactic acid the following factors were applied:

$OD_{calculated} = 1.26 \cdot$base consumption,
$DW_{calculated} = 0.5 \cdot OD_{calculated}$
$LA_{calculated} = 0.52 \cdot$base consumption,
$Glu_{calculated} = Glu_0 - (DW_{calculated} - LAc_{alculated}) \cdot 1.2$

Results

In *Fig. 1* a tipical batch fermentation is shown with the measured and calculated data points, the latter was enabling the kinetic studies.

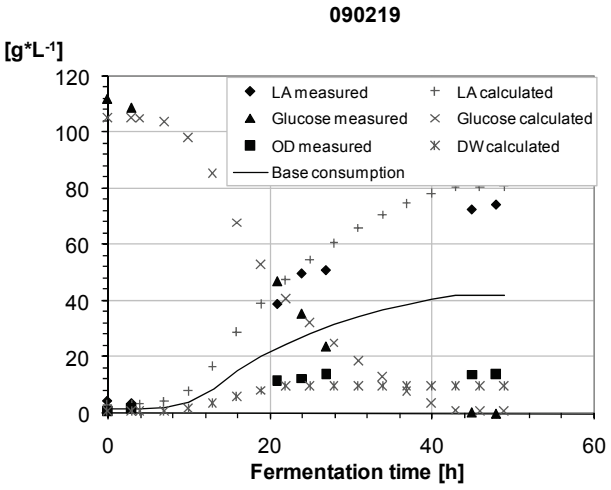

Figure 1: Batch LA fermetnation

As basis of our fermentation model the Monod equation was applied (Eq. 1) completed with the product formation model of Luedeking-Piret (Eq. 2). While the Monod-model can calculate the changes in biomass concentration, L-P model is able to predict the changes in product concentration. The substrate consumption was calculated with the overall yield ($Y_{x/s}$) from the growth rate (Eq. 3).

The applied initial conditions were as follows: $S_0 = 105.3$ $g \cdot L^{-1}$, $x_0 = 0.67$ $g \cdot L^{-1}$ and $P_0 = 3.3$ $g \cdot L^{-1}$.

$$\frac{dx}{dt} = \mu \cdot x, \ \mu = \mu_{\max} \cdot \frac{S}{K_S + S} \quad (1)$$

$$\frac{dP}{dt} = a \cdot \mu \cdot x + b \cdot x \quad (2)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}} \cdot \frac{dx}{dt} \quad (3)$$

It can be seen on Fig. 1 biomass reaches its maximum (plateau) much earlier than the product concentration. The 1-3. equation system is not able to handle this situation, since through the overall yield the biomass is connected directly to the substrate. Thus, when the culture reaches its plateau, the substrate has already zero value, although according to the measurements, there is a continuing product formation from substrate. To solve this problem, the model had to be reconstructed as follows: the growth-independent part of the product formation had to be converted into maintenance term (Eq. 4) which appeared also in the substrate equation (Eq. 5)

$$\frac{dP}{dt} = a \cdot \mu \cdot x + m \cdot x \quad (4)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}} \cdot \frac{dx}{dt} - m \cdot x \quad (5)$$

The value of the specific maintenance coefficient ('m') was determined from the slope of the substrate consumption, after the biomass reached its plateau. In the case of the presented fermentation (Fig. 1) m = 0.222 h^{-1} was obtained. Finally 3 variables had to be fitted to 3 data series, meanwhile 3 parameter had to be determined (yield, K_s, $\mu_{\max}$) For the flexibility of the model the duration of the lagphase and the time point of cell growth stop had to be determined either by experiments or simulations. The measured and simulated data of the presented (Fig. 1) batch fermentation can be seen on *Fig. 2*.

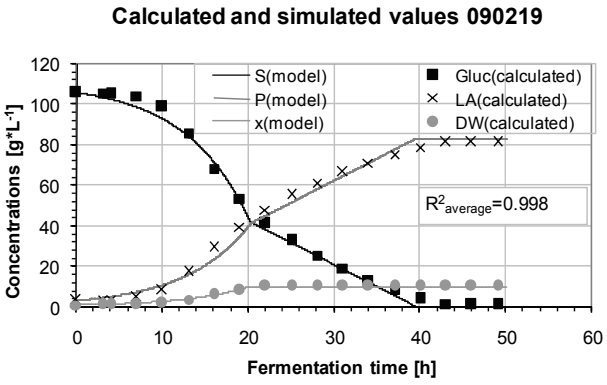

Figure 2: Fitting model to calculated dataseries

Model fitting resulted in an adequate model with the following parameters: $\mu_{\max} = 0.134$ h^{-1}, $K_s = 0.268$ $g \cdot L^{-1}$, $Y_{x/s} = 0.143$ $g \cdot g^{-1}$, $t_{lag} = 0.132$ h, $t_{stop} = 20.22$ h, $a = Y_p/Y_{x/s} = 4.18$.

Although, the fitting results showed excellent agreement with the experimental data further fermentation was used for model verification (*Fig. 3*).

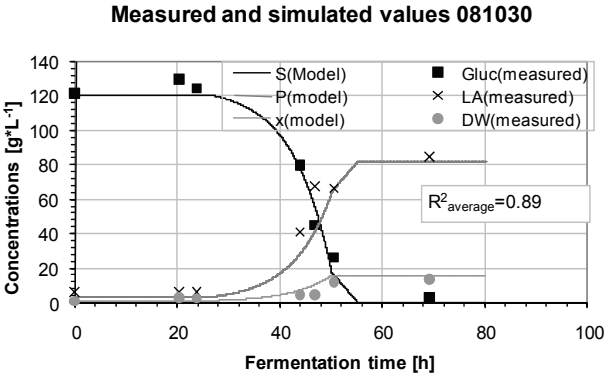

Figure 3: Model verification with further fementation, t_{lag} = 26,5 h, t_{stop} = 46 h

Since the correlation in this case was also appropriate after setting up the individual parameters (i.e. t_{lag} and t_{stop}), we used this model to predict the behaviour of a continuous system.

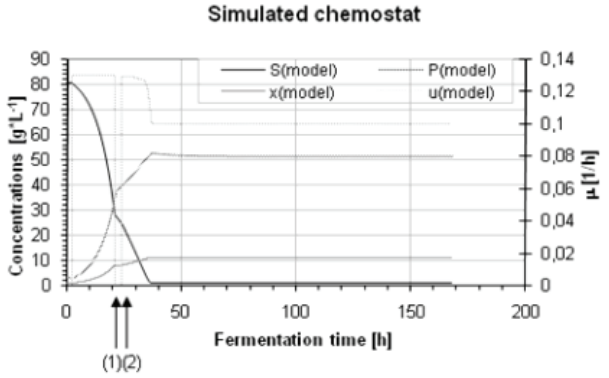

Figure 4: Modelling continuous operation
(1) cell growth stop (t_{stop})
(2) Feed start with S_f = 80 g·L^{-1} substrate concentration

The aim of our simulation was to reach high volumetric productivity beside high (industrially preparable, cost effective) product concentration. According to simulation results (*Fig. 4*) beside D = 0.1 h^{-1} dilution rate 51.6 g·L LA concentration can be reached, which resulted in J_p = 5.2 g·L·h^{-1} volumetric productivity, that is nearly two fold of the original batch process's value.

Since the presented product concentration is really high, before using the model in further simulation studies we want to try experimentally to verify the continuous operation.

Summary

During the development of a fermentation technology of the more and more promisable and platform forming lactic acid we build up a kinetic model, which is able to describe the two steps of the fermentation: 1. cell growth, and 2. product formation as a "byproduct" of energy production of cells for maintenance. This model predict results beeing very closely to the measured data, thus we used it for examining the continuous operation of LA fermentations.

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