Modeling the alcoholic fermentation of xylose by Pichia stipitis using a qualitative reasoning approach

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Abstract Qualitative Reasoning is a set of Artificial Intelligence theories, methods, and techniques that provide an answer to modeling problems in domains in which one can have a clear notion of how a system is functioning without being able to express it as classical mathematical equations, and where is posed the problem of using jointly quantitative and qualitative data, as well as processing a big amount of complex knowledge. SIMAO ('a System to Interpret Measurements And Observations') is an attempt to deal with such problems. Although primarily devised for heterogeneous data interpretation in hydroecology, it was thought possible to use SIMAO in a wider context, like biotechnological processes. Starting from specific problems posed by a batch fermentation, the D-xylose conversion into ethanol by the yeast Pichia stipitis, this paper describes how was built and used a SIMAO model aimed at predicting the fermentation issue from initial conditions, i.e. set-points values and substrate concentration.

1 Introduction

Because batch fermentations are discontinuous processes, one cannot perform actions on them once they have started. Initial conditions (i.e., oxygen supply, initial substrate level, pH and temperature) are therefore determinant on the fermentation issue. Thus, biotechnological engineers in the industry must carefully determine 'optimal' initial conditions before starting a new fermentation, according to the results they are expecting. This work is sometimes made difficult by at least the two following problems they are facing:

(1) Experiments are time-consuming (more than 200 hours), costly (experimental reactors are rather expansive and scale up is difficult from test results to higher capacities), and difficult to reproduce; it is thus anti-economic to proceed systematically by using a trial-error strategy.

(2) Technical and biological information capable to overcome these limitations is provided by many experimental results published in the specialized literature; however this information can be difficult to retrieve from the many titles and journal issues, and still necessitates a cautious analysis before becoming operational, due to the variability of experimental conditions (yeast strain, reactor and sensing apparatus characteristics, etc.). However, it would be interesting to be capable of implementing a new fermentation process without spending much time in determining operational conditions.

A possibility is to develop knowledge-based systems (i.e., computer softwares embodying expertise) capable of providing potential users with both compiled (shallow) operational knowledge derived from empirical know-how, and first principles (deep) knowledge necessary to support reliably tasks (such as situation assessment, prediction, diagnosis, explanations) that human operators must perform on the systems they are supervising. Qualitative Reasoning is a sub-field of Artificial Intelligence, aimed at dealing with complex systems when it is either impossible or inadequate to build 'classical' mathematical models [1, 2]. We believe it can bring new trade-offs to solve the above mentioned problems. In spite of its novelty, the qualitative approach is now getting consideration in biotechnological process modeling and supervision [3, 4]. The D-xylose batch fermentation by Pichia stipitis provides us with an example to illustrate this claim. The problem addressed here is to assess qualitatively the results that one can expect from a batch fermentation process, given its set-points values. For this we used the 'SIMAO' qualitative reasoning system that was first applied to data interpretation in hydroecology [5]; main features of SIMAO (an acronym standing for 'a System to Interpret Measurements And Observations') are described in [6].

2 The D-xylose batch fermentation process by Pichia stipitis

2.1 D-xylose as an alternative substrate for ethanol production

The decrease in petroleum resources with a subsequent tightening supply in a few countries and the problem of agricultural surpluses, lead to searching alternative sources of...
both materials and energy. One such alternative is the fermentative ethanol production from derived photosynthesis sources. Among these sources, the potential of lignocellulosic materials is frequently considered, because of their high availability and low cost. The last mentioned property has a favorable impact, since raw material cost makes up 55–75% of the final alcohol selling price. Hemicellulose carbohydrates comprise up to 55% of biomass-derived sugars. For the efficient bioconversion of lignocellulosic residues to ethanol, since fermentation of hexoses is well developed, the fermentation of hemicellulose derived sugars is necessary. D-xylose is the major sugar component of hemicelluloses, accounting for up to 60% of them [7]. The conversion of D-xylose to ethanol is limited to a small number of yeasts [8]. The discovery of yeast species capable to convert D-xylose into ethanol has enhanced the interest in the use of lignocellulose for ethanol production, since 70% of the raw material can be expected to be converted into ethanol. Among the xylose fermenting yeasts, Pichia stipitis belongs to the species which exhibit relative high ethanol yield and rate of fermentation [9, 10].

2.2 Main factors regulating the D-xylose fermentation by Pichia stipitis

2.2.1 Oxygen supply
D-xylose catabolism by yeasts leads to simultaneous productions of: (1) Cell biomass, through the tricarboxylic acid cycle, and (2) ethanol, through the fermentative pathway. The relative proportions of cell biomass and ethanol are dependent on the rate of oxygen transferred to the culture. This mechanism is similar to the 'Pasteur effect'. Under anaerobic conditions, yeast growth is severely restricted and xylose is preferentially converted into ethanol; in the meanwhile small amounts of xylitol are produced in relation to a NAD + cofactors deficiency. The first two reactions of the D-xylose catabolic chain in Pichia stipitis are the major limiting steps of the fermentation. In Pichia stipitis as in many other yeasts, xylose reductase is mainly NADPH-linked, whereas xylitol dehydrogenase is predominantly NAD-linked. Since the xylose catabolism does not provide a NAD + surplus, the resulting NADH accumulation leads to a redox imbalance under anaerobic conditions, which delays the reaction. This phenomenon, in turn, frequently results in the excretion of xylitol and concomitant low ethanol yields at low production rates [11]. The presence of exogenous hydrogen acceptors, like oxygen, is one of the keys of the xylose catabolism in these yeasts. This regulatory mechanism is referred to as the Kluver effect [12]. A low transfer rate of oxygen permits circumventing the imbalance of NAD + /NADH that occurs in anaerobic conditions. In oxygen limited conditions, ethanol production by Pichia stipitis is consequently stimulated and xylitol excretion is reduced [13]. In contrast, increasing the oxygen transfer rate tends to favor cell production and is detrimental to ethanol production. At high oxygen transfer rates, according to the Pasteur effect the carbon flows preferentially through the tricarboxylic acid cycle. In these conditions both the yield and the specific production rate of cells are enhanced, thus reducing the yield and rate of ethanol production. No ethanol is produced under strictly aerobic conditions.

As a conclusion it is clear that micro-aerobiosis is favorable to ethanol production, whereas both anaerobiosis and strict aerobiosis are detrimental.

2.2.2 Substrate concentration
Substrate tolerance of Pichia stipitis grown on D-xylose is enhanced in presence of oxygen [13]. Below 20 g/l, an increase in the initial substrate concentration tends to increase the ethanol production. This phenomenon is referred to as the Grabtree effect [14]. Within the range 20–30 g/l, cell and ethanol productions decline proportionally to the increase in the initial substrate concentration. This is owed to negative influences exerted on the yeasts both by the substrate itself and the ethanol produced, this latter effect being predominant [15].

2.2.3 Temperature and pH
Maximum rates of D-xylose fermentation and growth of Pichia stipitis occur at 30 °C, whereas the optimal pH lies between 4.5–5.5 pH units [16]. Temperature and pH values have separate influences on the fermentation parameters and affect both the yield and rate of xylose conversion.

2.3 The D-xylose batch fermentation process

2.3.1 Initial conditions
A batch fermentation is a discontinuous process (as opposed to a fed-batch process) in which all the input elements, i.e., the micro-organisms innoculum and the substrate, are filled in the reactor before starting the fermentation. Thus, apart of the strain of micro-organisms the substrate concentration in the culture medium is determinant. Before starting the process, set-points are to be set for regulated variables to provide the culture with appropriate environmental conditions, that are:

a) The stirring speed (expressed as rotation per minute, rpm) and the air inflow (expressed as air volume/reactor volume/minute,vvm), both determining the oxygen transfer rate (expressed as mmolar/l/hour) within the culture medium, which is a reliable indicator of oxygen uptake by the yeast. A low oxygen transfer rate denotes a low oxygen level, conversely a high oxygen transfer rate corresponds to high aerobic conditions.

b) pH and temperature that are regulated throughout the fermentation process by appropriate controllers.

Regulated variables are the sole variables it can eventually be acted upon during the fermentation process.

2.3.2 Fermentation parameters
They are strategic indicators of the fermentation process itself related to its major components, i.e. the yeast biomass and two co-products that are ethanol and xylitol. They are expressed as:

a) Substrate-related indicators: Ethanol production rate (g ethanol production/g substrate uptake), xylitol accumulation rate (g xylitol production/g substrate uptake); biomass production rate (g biomass production/g substrate uptake).

b) Kinetic indicators: Maximum specific ethanol productivity