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Modelling of the pH dynamic and its effect over the Isopropanol-Butanol-Ethanol fermentation by *Clostridium acetobutylicum* pIPA3-Cm2

Hugo I. Velázquez-Sánchez, Alma R. Dominguez-Bocanegra, Ricardo Aguilar-López*

Centro de Investigación y de Estudios Avanzados del IPN, Biotechnology and Bioengineering Department, Av. Instituto Politécnico Nacional 2508 Col. San Pedro Zacatenco, 07360 Ciudad de México, Mexico

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ABSTRACT

This work makes use of the so-called phenomenological non-structured modelling approach to propose a novel mathematical structure for the description and prediction of the *pH* effect over an Isopropanol-Butanol-Ethanol (IBE) fermentation system by *Clostridium acetobutylicum* pIPA3-Cm2, which includes both an in-line approximation of the *pH* of the culture medium and also novel expressions to reflect its real time effect over the biomass growth and metabolic response in an attempt to generate a base for the development of process intensification strategies for said fermentation system for biofuel production purposes.

The proposed model attained a correlation index $R^2 = 0.9931$ and a *p-value* < 0.001 versus experimental data reported by Lee at al. (2012), predicting a total of 20.1617 $kg\,m^{-3}$ of IBEs with a yield of 0.3341 $kg_{IBE}\,kg_{Sg}^{-1}$ after 42 h of batch fermentation. Additionally, both the proposed parameters for critical and optimal *pH* ($pH_c = 3.4977$; $pH_{op} = 6.5$) and simulation results based on the equation proposed for the modelling of the *pH* dynamic were consistent with experimental reports for both ABE and IBE fermentation systems under a wide array of operational conditions.

1. Introduction

Biofuels, which are all compounds of organic nature derived from living beings and their metabolism that can potentially be utilized as fuels, lead a group of alternative energy sources aimed to provide solution to the environmental issues caused by the overexploitation of fossil ones [18]. One of the most studied biofuels in the last decade is butanol, considering that it can offer better properties regarding fuel mileage yield, lower gaseous emissions, higher energy content and lower hygroscopicity versus the currently developed processes for the production of ethanol and biodiesel [6].

Traditionally, the methodology to obtain butanol via fermentation is based on the transformation of various sugars such as glucose or sucrose by Gram-positive bacteria of the genus *Clostridium*, via a metabolic pathway called ABE (acetone-butanol-ethanol) [12]. ABE fermentation presents inherent restrictions that have prevented its consolidation as a mature technology such as low production yields, inhibitory effect over the culture's growth due both solvents and glucose accumulation and mechanisms of metabolic regulation own of Gram-positive bacilli such as the sporulation process [19]. Additionally, there exists the limitation imposed by the outlet stream composition, where the low concentration of the main product (less than 3% w/v) combined with the presence of acetone, which is deemed as undesirable if the main goal of the fermentation is to produce a biofuel capable blend, make the design and operation of *downstream* processes either too energetic demanding (via distillation) or expensive to implement (via membrane technologies) [28].

Form the process engineering standpoint, in the last five years there had been advances regarding the design and implementation of alternative recovery processes such as the use of gas stripping [25], adsorption either outside the fermenter [8] or *in situ* [26] or via pervaporation [27]. And while those recovery procedures do indeed improve butanol concentration into the fermenter outlet up to 5.5 times compared with traditional batch processes those technologies must still deal with the presence of acetone in the mixture in various degrees.

As a response for such persisting inconvenient novel genetic manipulation strategies had been developed to either reduce significantly the acetone production of the Clostridial cultures [11] or transform it into a more suitable molecule for fuel purposes [21]. In this regard, Isopropanol-Butanol-Ethanol fermentation is a novel bioprocess derived from the genetic manipulation of the classic ABE-producing bacteria *C. acetobutylicum* by the introduction of the $adh_{B\text{-}593}$ alcohol dehydrogenase from *C. beijerinckii*, which transforms acetone from the culture broth into isopropanol. This strategy was developed because it

* Corresponding author.
E-mail address: raguilar@cinvestav.mx (R. Aguilar-López).

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Nomenclature

D	reactor dilution rate, h^{-1}
k_{but}	butanol growth inhibition constant, $g\,L^{-1}$
k_{IsoA}	isopropanol-acetic acid affinity constant, $g\,L^{-1}$
k_{Sb}	butanol-butyric acid affinity constant, $g\,L^{-1}$
k_{Sg}	glucose affinity constant, $g\,L^{-1}$
k_{SgAct}	glucose-acetic acid affinity constant, $g\,L^{-1}$
k_{SgEt}	glucose-ethanol affinity constant, $g\,L^{-1}$
k_{SgSb}	butyric acid-glucose affinity constant, $g\,L^{-1}$
k_{Si}	glucose growth inhibition constant, $g\,L^{-1}$
pH_{Act}	acidity adjustment constant, –
pH_c	critical pH for solventogenesis, –
pH_{op}	optimal growth pH, –
Sg_a	glucose concentration in the feed stream, $g\,L^{-1}$
Y_{ButSb}	butanol per butyric acid mass yield, $g\,g^{-1}$
Y_{IsoAct}	isopropanol per acetic acid mass yield, $g\,g^{-1}$
Y_{XSg}	biomass per glucose mass yield, $g\,g^{-1}$

Greek symbols

ν_{maxAct}	maximum specific acetic acid production rate, h^{-1}
ν_{maxBut}	maximum specific butanol production rate, h^{-1}
ν_{maxEt}	maximum specific ethanol production rate, h^{-1}
ν_{maxIso}	maximum specific isopropanol production rate, h^{-1}
ν_{maxSb}	maximum specific butyric acid production rate, h^{-1}
μ_{maxX}	maximum specific cell growth rate, h^{-1}

does increase the viability of the alcohol mixture as biofuel or fuel extender by removing the need to recover and segregate acetone from the reactor outlet, reducing the theoretical cost of *downstream* operations [10].

The implementation of fermentation technologies based on this novel IBE process requires the analysis and establishment of the proper operating conditions for the culture to express its maximum production potential to take advantage of the facilitated recovery operations. One of the most common strategies involves the selection of the adequate carbon source to maximise solvent production and growth rate of the culture [2].

Nonetheless, *Clostridium* metabolism is not only regulated at macroscopic scale only by the selection of the available carbon source, but also by the *pH* dynamic of its surroundings. *Clostridium* genus bacteria present at least two growth stages perfectly characterised under its life cycle. First there is the so-called acidogenic stage, where the cells partially oxidize sugar-based carbon sources to grow and to generate organic acids including butyric, acetic and lactic ones, which produce a drop on the *pH* medium, which after a certain threshold induces the switch to the so-called solventogenic stage, where the produced acids are reincorporated by the cells to initiate the sporulation process and then transformed into butanol and acetone in wild type strains [31].

Currently, the study of the effect of the *pH* dynamic into the culture medium in *Clostridium* based fermentations is primarily made via an experimental approach existing a plethora of reports into literature [9,20,1,24], where either the culture is growth under different initial *pH* conditions and there is a monitoring of the resulting solvent production and growth behaviour or there are attempts to manipulate the *pH* of the culture towards an "optimal" pattern that could maximise the system performance via the implementation of empirical closed-loop systems at varying pH set-points and starting time politics.

Despite these experimental strategies had helped to create a framework to understand and propose operational politics for the manipulation of the *pH* into IBE fermentation processes there is the downside that some results are not fully reproducible or applicable due to differences over strains, reactor configurations or medium compositions across systems, which in turn would make necessary to repeat essays to readjust the conditions to match the requirements of the new system.

To try to solve such issue, mathematical modelling and numerical simulation techniques can be used to reduce the need to conduct experimental work and then serve as a basis for the application of more consistent and objective optimization and process intensification methodologies that could lead to the development of novel fermentation processes with better performance than the current established ones.

Currently there exist little previous evidence into literature of the inclusion of pH related terms for the description of metabolism changes in *Clostridium* cultures. The most recent advance in the matter are a series of works reported by Thorn, King and Sabari [22] and Millat et al. [13], where they proposed a sigmoid-like function to act over key enzymatic kinetic rates in an attempt to simulate the switch from

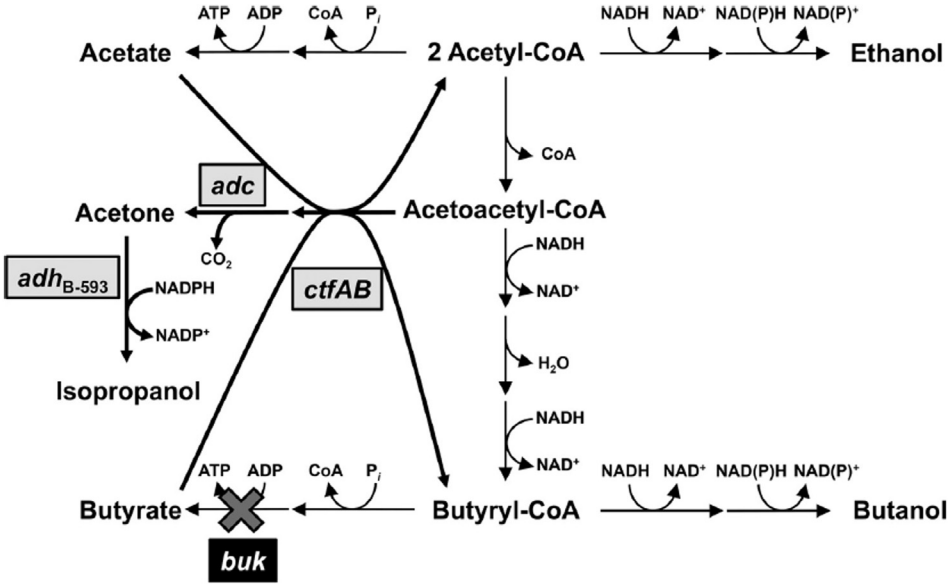

Fig. 1. Simplified diagram of the modified IBE metabolic pathway of *Clostridium acetobutylicum* pIPA3-Cm2 reported by Lee et al. [10].