Butanol production by fermentation of *Clostridium acetobutylicum*: solventogenic kinetics

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Acetone-Butanol-Etanol is typically produced during the second stage of batch fermentations of some Clostridium strains under selected operating conditions: acids are consumed along with the carbon source and pH increase The objective of this study was to establish a continuous butanol production system by means of Clostridium acetobutylicum, lactose as the sole carbon source, and cell recycling (CSTR equipped with a microfiltration unit). Continuous cultures were carried out under a wide interval of operating conditions (diluition rate and recycle flux) in order to characterize the fermentation process under solventogenesis phase.

Introduction

Depletion of oil resources and concerns regarding both economic and environmental issues have renewed interests for the search of sustainable biofuel produced from renewable resources known as biomass.
The spectrum of biofuels includes the butanol, a simple four carbon alcohol characterized by interesting features: low vapour pressure, blending with either gasoline or diesel at any fraction, energy content close to that of the gasoline. Butanol may be produced from renewable resources by the acetone butanol ethanol (ABE) fermentation process [1].
ABE is typically produced during the later stage of batch
ntations of some Clostridium strains under appropriate operating conditions. These strains are able to
of substrates, pentoses, hexoses, mono-, di- and polysaccharides. Under batch

m strains proceeds with the production of cells, hydrogen, carbon

n phase (**acidogenesis**). As the acid concentrations increases (pH
fts to solvent production
ls shift to the solventogenesis state with a morphological change.

e endospores unable to reproduce themselves [2].

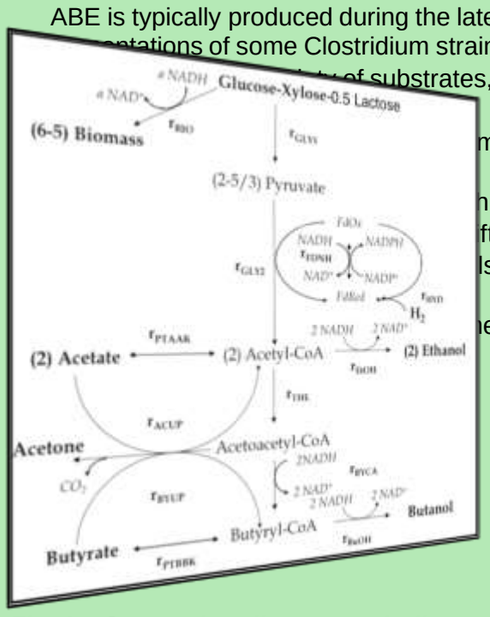


Fig. 1 – C. acetobutylicum metabolic pathway and relevant fluxes

The design and the optimisation of continuous bioreactors require to know the kinetics of both phases : acidogenesis and solventogenesis phases [3].

Aim

This study is about the continuous butanol production by means of *Clostridium acetobutylicum* adopting lactose as carbon source. A cell recycling reactor system was adopted: CSTR equipped with a microfiltration unit.
Continuous cultures were carried out under a wide interval of operating conditions in order to characterize the fermentation process under solventogenesis phase.

Materials & Methods

Continuous fermentations were carried out in the apparatus sketched in Fig. 1 consisting of: a fermenter, a pH controller and a microfiltration unit.

The system was described bte the equation set:

$$\begin{cases} D = d_{OUT} + d_P \\ d_{OUT} = \dfrac{Q_{OUT}}{V} \\ d_P = \dfrac{Q_P}{V} \\ W_X = D_{OUT} \cdot X_T \end{cases}$$

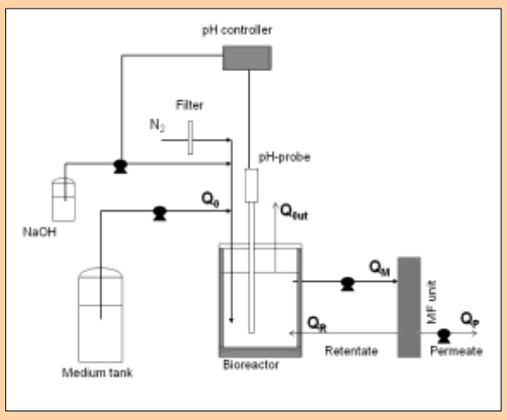
where W_X is the productivity of biomass and X_T the total biomass.

The cell recycle was characterized in terms of recycle ratio R [4]:

$$R = \frac{Q_P}{Q_0}$$

Tests were carried out at :

Fig. 1. Outline of the apparatus adopted for continuous tests.



REACTIONS SET

$AA)\ L \rightarrow 8ATP+4AA+4CO_2+8H_2-4H_2O$ $BA)\ L \rightarrow 6ATP+2BA+4CO_2+4H_2$

$Et\ \&\ Ac)\ L+2AA \rightarrow 4ATP+2Ac+6CO_2+4H_2+2Et$

$B\&\ Ac)\ L+2AA \rightarrow 4ATP+2Ac+6CO_2+4H_2+B+H_2O$ $B\&\ Ac)\ L+2AB \rightarrow 4ATP+2Ac+6CO_2+4H_2+B$

$B)\ L \rightarrow 4ATP+4CO_2+B+2H_2O$ $Et)\ L \rightarrow 4ATP+4CO_2+4Et$

$X_A)\ L+3NH_3+3(101/Y_{ATP})ATP \rightarrow 3X_A$

BIOMASS BALANCE

Under continuous solventogenic cultures, the cells may be classified in three groups: acidogenic cells (X_A), solventogenic cells (X_S), and spore (X_D).

$$\begin{cases} W_X = W_X^A + W_X^S + W_X^D \\ W_X^A = \mu X_A - \mu_S X_A \\ W_X^S = \mu_S X_A - \mu_D X_S \end{cases}$$

CELLS TRANSFORMATION PATHS

$$\begin{cases} L \xrightarrow{\mu} X_A \\ X_A \xrightarrow{\mu_S} X_S \\ X_S \xrightarrow{\mu_D} X_D \\ X_A, X_S, X_D \xrightarrow{\mu_{lysis}} cell\ lysis \end{cases}$$

Where μ_S, μ_D, μ_{lyses} are the specific rate of solventogenic cells formation, spores formation and cell lyses, respectively.

Results & Discussion

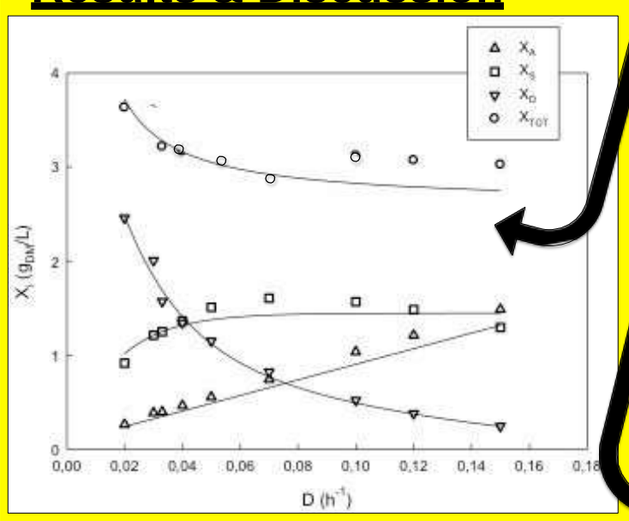


Fig. 3 – Steady state continuous fermentation. Acidogenic cells, solventogenic cells, spore and total biomass vs. dilution rate. R = 0.88.

The concentration of acidogenic cells and solventogenic cells increased progressively with D. On the contrary the concentration of solventogenic cells decrease.

$D < 0.1 h^{-1}$: μ and μ_S increase with D

$D > 0.1\ h^{-1}$: μ_S decreases and μ increases with D

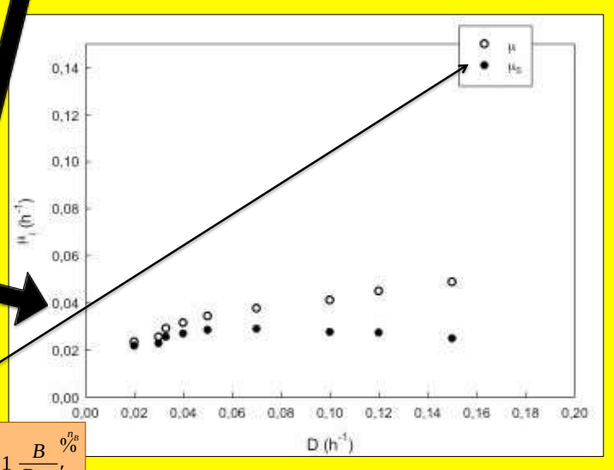


Fig. 4. μ and μ_S vs. dilution rate.

$$\mu = \mu_{max} \cdot \frac{L}{L+K_L} \cdot \prod_{i=1}^{n} \left(1 - \frac{A_i}{A_{i,max}}\right)^{n_i}$$

Specific growth rate (μ) of acidogenic cells assessed according to Napoli et al. 2011 [5]

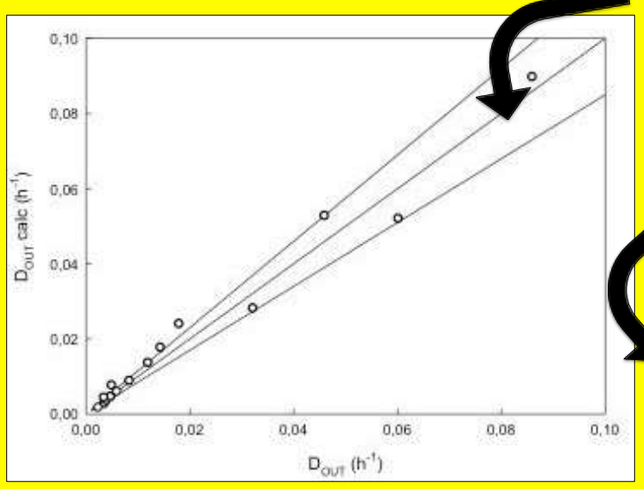


Fig. 5. Calculated vs. measured D_{OUT} . Crosshair : ±10% error

Fig. 5: a parity plot of predicted D_{OUT} vs. experimental D_{OUT}. The analysis points out that the model results are in good agreement with the experimental data points.

The productivities of butanol and ABE increased progressively with D.
At D = 0.15 1/h and R = 0.88 the ABE production is 0.53 g/L h.

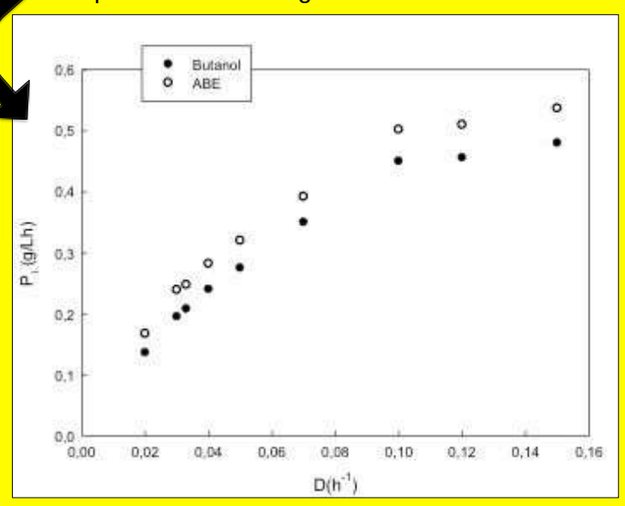


Fig. 6. Productivity vs. dilution rate

Total inhibition equation

$$r_B = r_B^{MAX} \cdot \frac{L}{L+K_B} \cdot \prod_{\#} \left(1 - \frac{B}{B_{max}}\right)^{n_B}$$

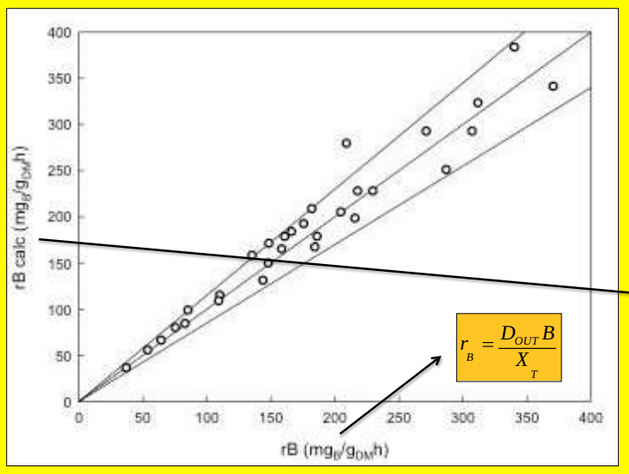


Fig. 7. r calculated vs. r measured. Crosshair : ±10% error.

$r_B^{MAX} = 300 mg/g_{DM}h$
$K_B = 6.3 g/L\ B^{MAX} = 7.045 g/L$
$n_B = 0.5395$

The complete set of model parameters: r_B^{MAX}, K_B, B^{MAX}, n_B was determined by a parametric inference procedure applied to the total inhibition equation matched against data obtained in experiments carried out in continuous tests.

Acknowledgements

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Main Remarks

- **The characterization of *C. acetobutylicum* DSM 792 metabolism on lactose during solventogenic phase was carried out.**
- The results have been worked out to assess the concentration of acidogenic cells (X_A), solventogenic cells (X_S), spore (X_D) and the butanol and solvent production.
- The specific butanol production rate (r_B) at different D was obtained. The experimental results were successfully correlated by a product-inhibition model.

References

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