MFA of *Clostridium acetobutylicum* pathway: the role of glucose and xylose on the acid formation/uptake .

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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 This contribution reports a Metabolic Flux Analysis (MFA) regarding Clostridium acetobutylicum DSM 792 fermentation adopting reference sugars (glucose and xylose) as carbon sources. The attention on these sugars is particularly relevant because they are the main components of hydrolyzed lignocellulosic biomass. The results have pointed out that the butyrate formation pathway plays a key role both in the accumulation of butyrate and in butyrate uptake without acetone formation.

Introduction

Over the last decade, the depletion of oil resources and concerns regarding both economic and environmental issues associated with petroleum-based fuels have renewed interests for the search of sustainable biofuel that are the product of 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 fermentations of some Clostridium ...ate operating conditions. These strains are able to metabolize a great variety ...ono-, di- and polysaccharides. Under batch conditions the ...ducing clostridium strains proceeds with the production of cells, ...id and butyric acid during the initial growth phase ...trations increase (pH decrease), the metabolism of cells shifts to **...is**) and acidogenic cells shift to the solventogenesis state with a ...

...e cells become endospores unable to reproduce themselves [2].

...n the most promising feedstock for the ABE fermentation is quite ...erature have pointed out that the fermentation performances [3,4].

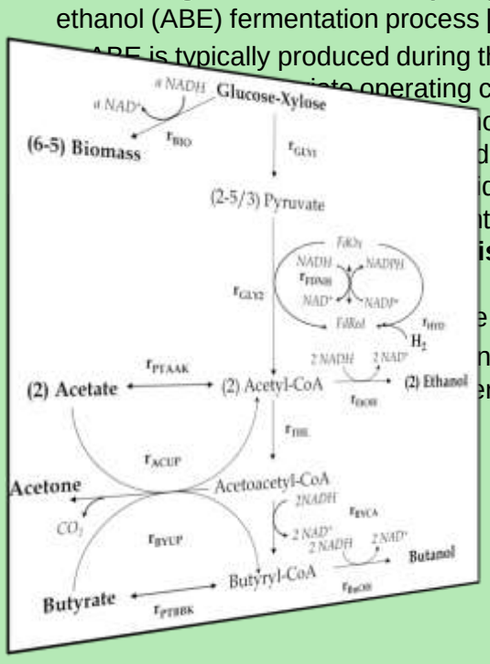

Fig. 1 – C. acetobutylicum metabolic pathway and relevant fluxes

Aim

In this study the MFA has been adopted to investigate the role of the main reaction steps of the *Clostridium acetobutylicum* metabolic pathway to convert reference sugars of hydrolyzed lignocellulosic biomass into butanol. Results of batch fermentation tests carried out using glucose and xylose as carbon source have been adopted for the flux assessment.

Materials & Methods

The assessment of in vivo fluxes has been carried out by processing the time-resolved concentration of substrates and products.

The analysis has been based on the known metabolic pathway (Fig. 1) translated into a set of reactions, e.g. a set of linear equations.

The reaction set regarding the glucose conversion is reported in Table 1 as Eq.s (T. 1.1) through (T.1.13).

The MFA of xylose fermentation has been proposed by substituting the reaction steps (T.1.1) and (T.1.2) with (T.1.14) and (T. 1.15).

The stoichiometric matrix (Fig2) of the model is characterized by a singularity that prevents the assessment of a unique set of fluxes of the primary metabolic activity.

The non linear constraint proposed by Desai et al. (1999) [5] relating the acetate and butyrate uptake fluxes has been adopted to solve the model equation set.

$$\frac{r_{BYUP}}{r_{ACUP}} = 0.315\,\frac{butyrate}{acetate}$$

The stoichiometric model of solventogenic clostridia has been restructured as a constrained minimization problem of the objective function:

$$\| A \cdot r - x \|^2 + \left(r_{BYUP} \frac{acetate}{\varepsilon + acetate} - 0.315 \frac{butyrate}{\varepsilon + butyrate} \right)^2$$

Table 1 – Stoichiometric matrix

Reaction Step	Flux	
$GLU \xrightarrow{6\cdot 12 \text{ ATP}, 6\cdot\gamma_{NADH}\text{ NADH}} 6BIO \quad a=\frac{6}{2}(\gamma_X-\gamma_{GLU})$	$+/-r_{BIO}$	(T.1.1)
$GLU \longrightarrow 2PYR + 2ATP + 2NADH$	$+r_{GLY1}$	(T.1.2)
$PYR \longrightarrow ACoA + CO_2 + FdRed$	$+r_{GLY2}$	(T.1.3)
$ACoA + 2NADH \longrightarrow E$	$+r_{EtOH}$	(T.1.4)
$ACoA \longrightarrow AA + ATP$	$+/-r_{PTAAK}$	(T.1.5)
$2ACoA \longrightarrow AACoA$	$+/-r_{THL}$	(T.1.6)
$AACoA + AA \longrightarrow A + ACoA + CO_2$	$+r_{ACUP}$	(T.1.7)
$AACoA + AB \longrightarrow BCoA + A + CO_2$	$+r_{BYUP}$	(T.1.8)
$AACoA + 2NADH \longrightarrow BCoA$	$+/-r_{BYCA}$	(T.1.9)
$BCoA \longrightarrow AB + ATP$	$+/-r_{PTBBK}$	(T.1.10)
$BCoA + 2NADH \rightarrow B$	$+r_{BuOH}$	(T.1.11)
$FdRed \rightarrow H_2$	$+r_{HYD}$	(T.1.12)
$FdRed \rightarrow NADH$	$+/-r_{FDNH}$	(T.1.13)
$XYL \xrightarrow{\frac{5\cdot 12}{v_X\cdot\gamma_{biom}} ATP - \frac{5}{2}\left\|\gamma_X-\gamma_{NADH}\right\| NADH} 5BIO$	$+/-r_{BIO}$	(T.1.14)
$XYL \longrightarrow \frac{5}{3}PYR + \frac{5}{3}ATP + \frac{5}{3}NADH$	$+r_{GLY1}$	(T.1.15)

$$\frac{d}{dt}\begin{bmatrix} GLU \\ BIO \\ PYR \\ ACoA \\ E \\ AA \\ AACoA \\ A \\ BCoA \\ AB \\ B \\ NADH \\ FdRed \end{bmatrix} = \mathbf{x} - \mathbf{A} \cdot \mathbf{r}$$

$$\mathbf{x} = \begin{bmatrix} -1 \\ 6 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ -\frac{6}{2}(\gamma_X - \gamma_{GLU}) \\ 0 \end{bmatrix}$$

$$\mathbf{A} = \begin{bmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 2 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & -1 & -2 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \end{bmatrix}$$

$$\mathbf{r} = \begin{bmatrix} r_{GLY1} \\ r_{GLY2} \\ r_{EtOH} \\ r_{PTAAK} \\ r_{THL} \\ r_{ACUP} \\ r_{BYUP} \\ r_{BYCA} \\ r_{PTBBK} \\ r_{BuOH} \\ r_{FDNH} \\ r_{HYD} \end{bmatrix}$$

Fig. 2 – Stoichiometric matrix

Results & Discussion

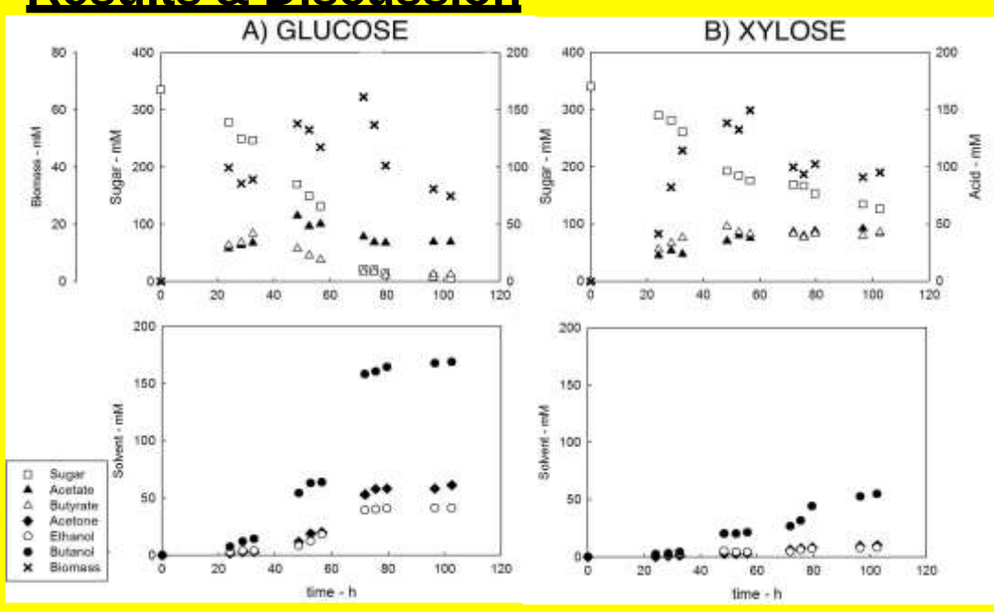

Fig. 3 – Time resolved concentration of metabolities

The concentration time series (Fig. 3) were processed to asses the specific net rates of acetate, butyrate, and acetone (Fig. 3 A-C) for both glucose and xylose fermentation.

The analysis of the **specific net rates** referred to glucose (Fig. 4A) pointed out that:
i)acetate and butyrate rates were maximum at the beginning of the fermentation and gradually decreased during the transition to the solventogenesis;
ii)acetate and butyrate rates were negative during the solventogenesis to point out the uptake of both acids;
iii)the rate of acetone increased at the solventogenesis threshold and it reached its maximum value late during the solventogenesis.

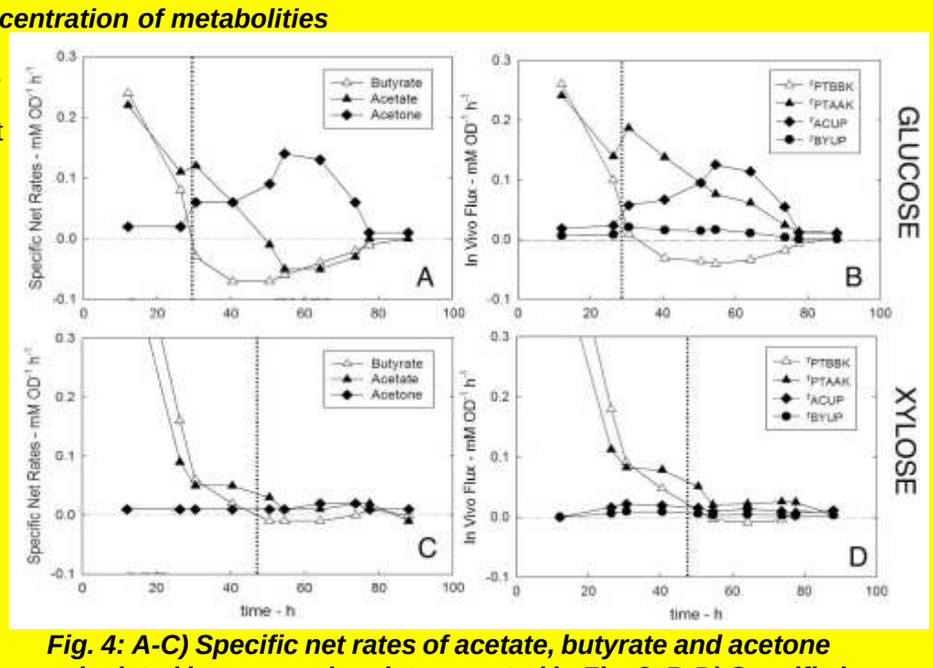

Fig. 4: A-C) Specific net rates of acetate, butyrate and acetone calculated by processing data reported in Fig. 2. B-D) Specific in vivo fluxes assessed by MFA.

The main dynamics observed for the test carried out adopting glucose as carbon source are still observed for xylose.
The main differences are: i) the butyrate specific net rate was definitively negative during the solventogenesis phase and the acetate uptake rate is nearby zero, that is only butyrate is definitively converted during solventogenesis; ii) the acetone production rate is quite low throughout the test.

Metabolic flux analysis has been adopted to assess the un-observable patterns of the metabolic activity (Fig. 4B-D). The rate r_{PTAAK} assessed for both glucose and xylose metabolisms decreased gradually throughout the fermentation and approached zero. The observed r_{PTAAK} vs. time profile is consistent with the acetate specific net rate vs. time profile.

Glucose – i) it has been assessed that the acetate is continuously produced (not consumed) according to the reaction (T.1.5) throughout the fermentation, indeed r_{PTAAK} is always positive during the fermentation (Fig. 4B); ii) it has been measured that the acetate is converted during the late solventogenesis phase (Fig. 4A). The analysis of results suggests that acetate uptake is via the acetone formation pathway, r_{ACUP}.

Xylose – i) it has been assessed that the acetate is continuously produced (not consumed) according the reaction (T.1.5) throughout the fermentation, indeed r_{PTAAK} is always positive during the fermentation (Fig. 4D); ii) the acetate conversion (r_{PTAAK}) vs. time (Fig. 4C-D) looks like the acetate specific net production rate. The analysis of results suggests that the acetone formation pathway, r_{ACUP}, is negligible.

The r_{PTBBK} assessed for both carbon sources decreases gradually since the beginning of the fermentation tests and it is a proof of that the butyrate is produced according to the butyrate formation pathway. As the solventogenesis starts, the butyrate formation pathway acts to uptake the butyrate.

As regards the role of the acetone formation pathway in the fate of the butyrate, the butyrate formation pathway appears to dominate the uptake with respect to the acetone formation pathway.

The analysis of reported results regarding the butyrate fate points out that **PTB and BK are still active during solventogenesis and they are responsible of the butanol production at a low acetone/butanol ratio**.

This scenario is still more marked for xylose fermentation.

Main Remarks

- **The analysis of the MFA has pointed out the role played by acid formation enzymes in the complex primary metabolism of solventogenic clostridia**.
- The effects of the carbon source on the relevance of each step of the acid formation/uptake pathway have been highlighted.
- The butyrate formation pathway plays a key role both in the production of butyrate and in butyrate uptake without acetone formation.

References

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