



Università degli Studi di Napoli *Federico II*

Butanol Production by *Clostridium acetobutylicum*: Assessment of Kinetics

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The economic scenario with the recent advances in fermentation system and downstream processing have contributed to improve ABE (Acetone-Butanol-Ethanol) fermentation feasibility and competitiveness

The selection of unconventional substrates is favored by the ability of *clostridia* strains to metabolize a wide range of carbohydrates like glucose, lactose etc..., typically present in food industry wastewater streams like cheese whey



***Clostridia* strains have been proven successful to produce ABE**

Information regarding the kinetics of substrate conversion, cell growth and butanol production are still lacking

The present study reports the preliminary results regarding the ABE production process by free *C. acetobutylicum* ATCC824:

- kinetics characterization**
- yields of the carbon source in cells, acids and solvents.**

Lactose solution was adopted as medium to emulate cheese whey.



Apparatus

Batch conditions



Continuous stirred tank reactor equipped with microfiltration unit





Diagnostics

The conversion process has been characterized in terms of:

- Cells
- Acids
- Solvents
- TOC (total organic carbon)
- pH

Material balance on carbon

$$(TOC_0 + X_0 \cdot \alpha_C) - (TOC_A + X_A \cdot \alpha_C) - 4 \cdot \frac{MW_C}{MW_L} (C_L^0 - C_{L,A}) = 0$$

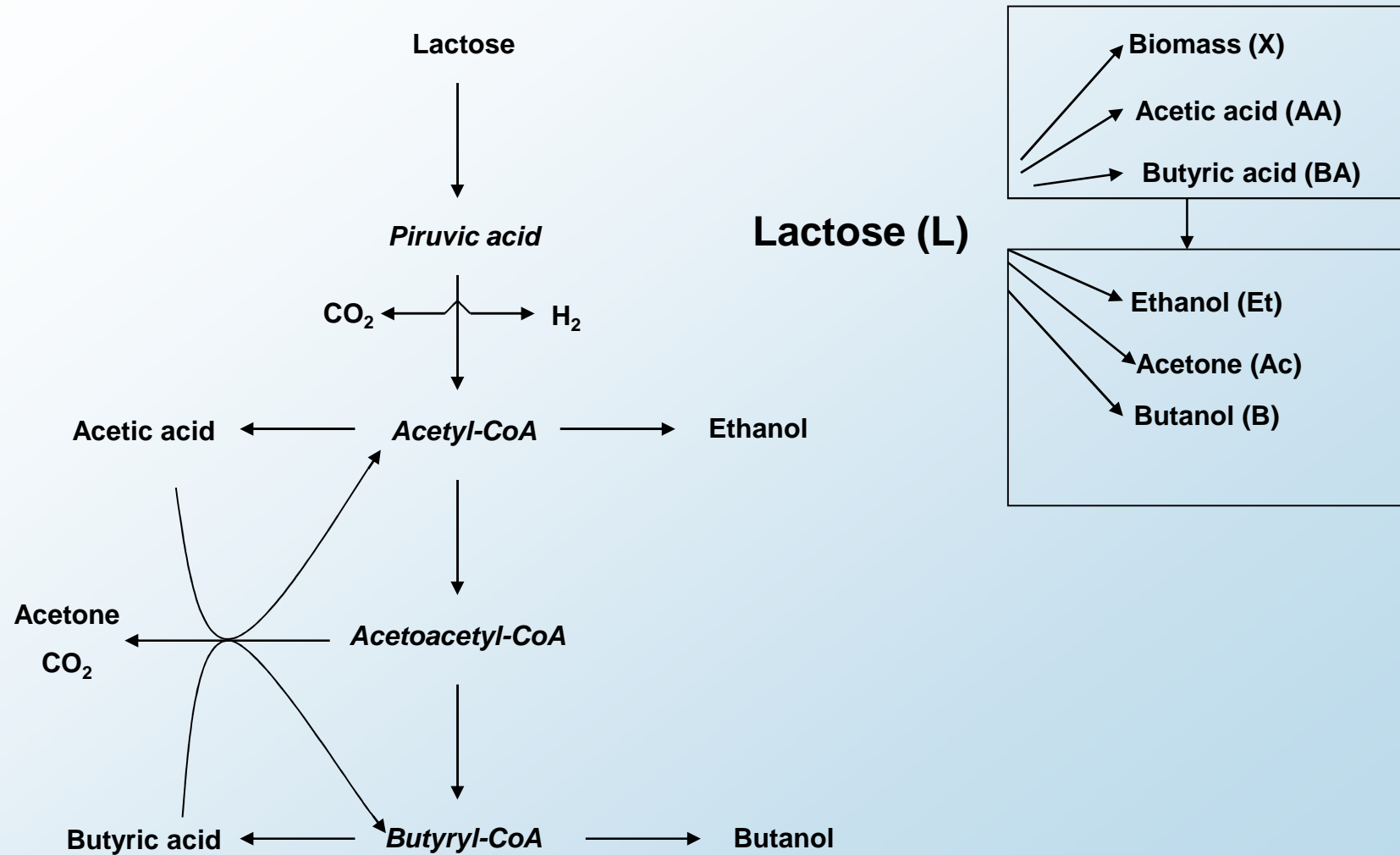
Organic carbon in liquid phase at t=0

Organic carbon in liquid phase at t=t_A

Carbon in CO₂ produced until t=t_A

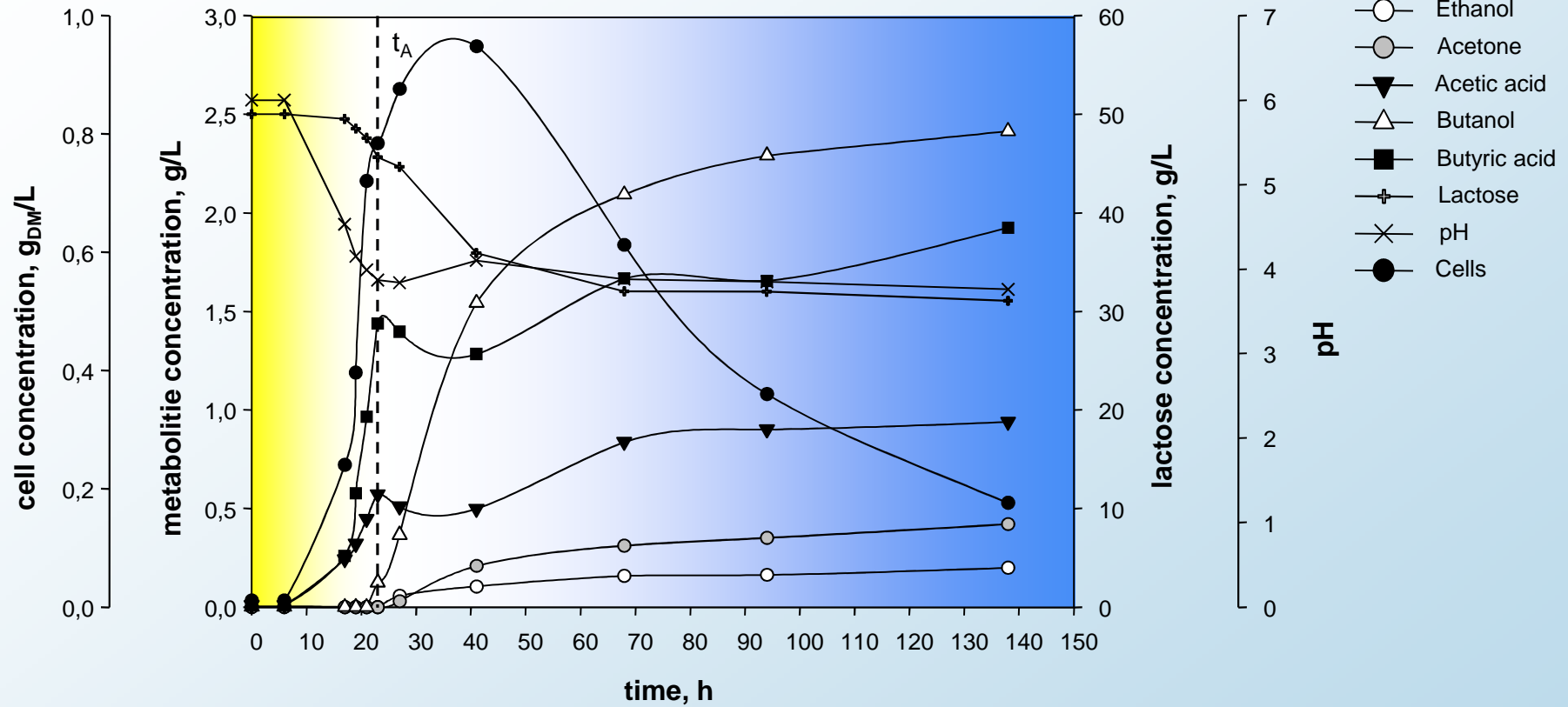


Clostridium acetobutylicum pathway



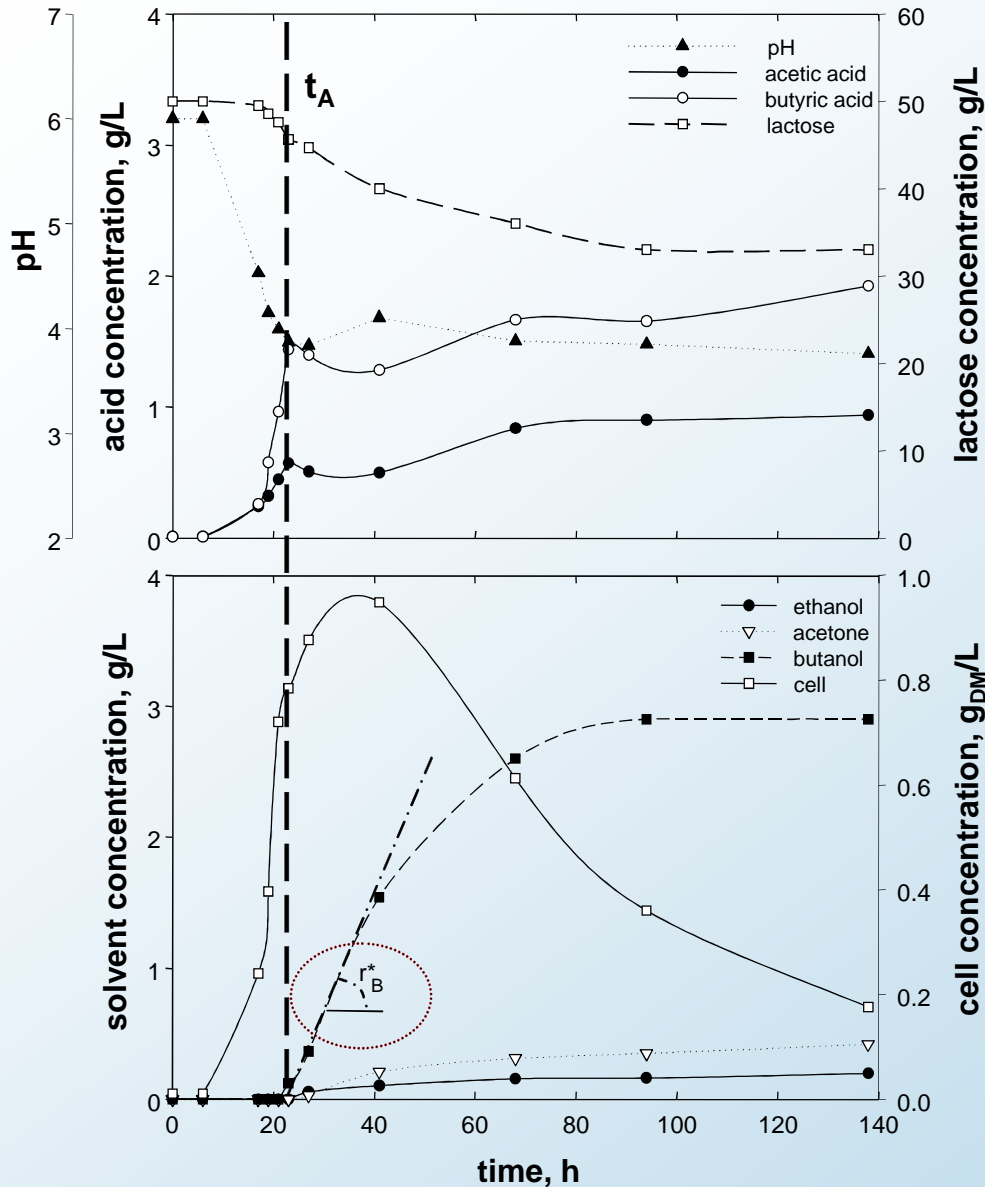


batch test



operating conditions

culture medium: 50 g/L Lactose, 5g/L Yeast Extract



Cultures were further characterized in terms of:

$$\mu = 0.29 \text{ h}^{-1}$$

$$(Y_{X/L})_{ac} = 0.18$$

$$(Y_{Sol/L})_{ov} = 0.20$$

$$(Y_{B/L})_{ov} = 0.17$$

$$\xi_L = 0.34$$

$$r_B^* = 80 \text{ mg/g}_{DM} \text{ h}$$

$$\delta_{ac} = 0.96$$

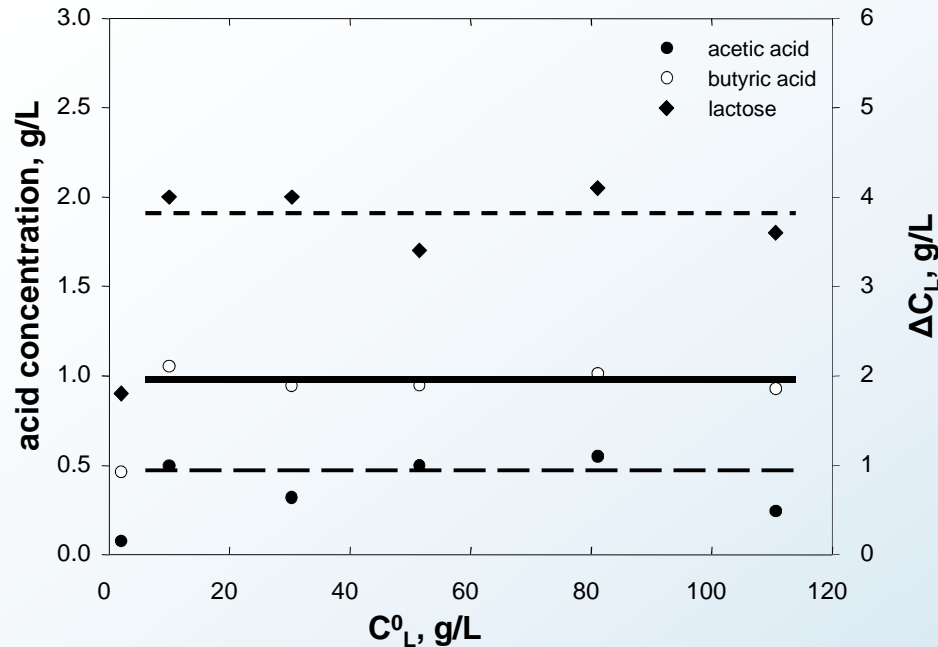
$$\delta_{ov} = 0.90$$

$$\frac{(TOC_A + X_A \cdot \alpha_c) + 4 \cdot \frac{MW_C}{MW_L} (C_L^0 - C_{L,A})}{(TOC_0 + X_0 \cdot \alpha_c)} \approx \delta_{ac}$$

$$\frac{(TOC + X_{max} \cdot \alpha_c) + 4 \cdot \frac{MW_C}{MW_L} (C_L^0 - C_L) + \frac{MW_C}{MW_{Ac}} \cdot C_{Ac}}{(TOC_0 + X_0 \cdot \alpha_c)} \approx \delta_{ov}$$



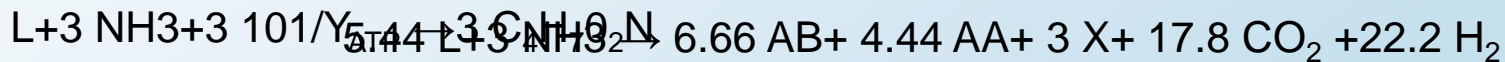
Acidogenesis phase



Acidogenesis phase is characterized by :

- ✓ cell specific growth rate, acid molar ratio and $(Y_{X/L})_{ac}$ constant for $C^0_L > 4$ g/L
- ✓ The amount of lactose converted is 4 g/L, if available
- ✓ The product of $(Y_{X/L})_{ac}$ and lactose converted at the threshold of the solventogenesis phase is nearby constant and equal to 0.7 g_{DM}/L

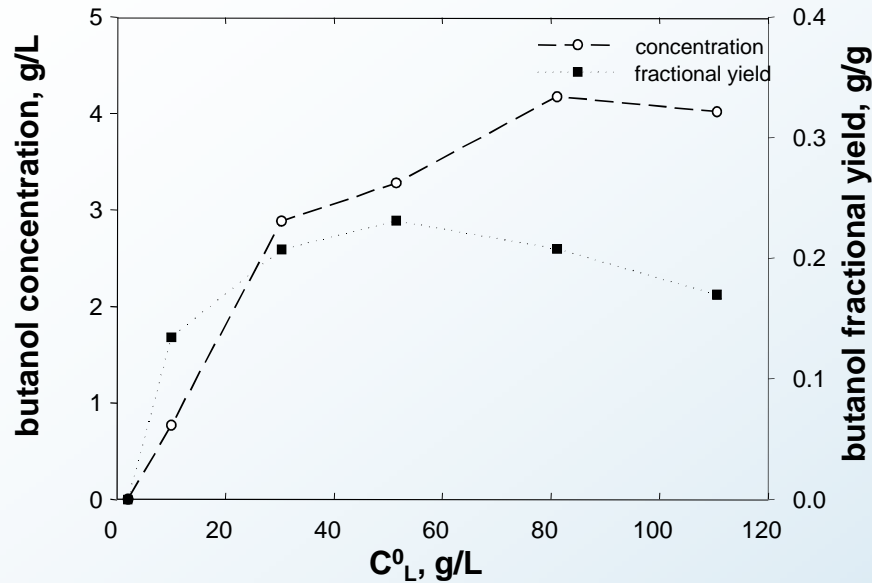
Theoretical yields: acidogenesis phase



Reaction	Experimental (g/g)	Theoretical (g/g)	Relative error (%)
$1/2 L \rightarrow 4 ATP + 2 AA + 2 CO_2 + 4 H_2$	0.18	0.16	12%
$1/2 L \rightarrow 3 ATP + BA + 2 H_2 + 2 CO_2$	0.28	0.29	3.5%
$Y_{ATP} = 10.5 \text{ g}_{DM}/\text{mol}$	0.13	0.14	7%

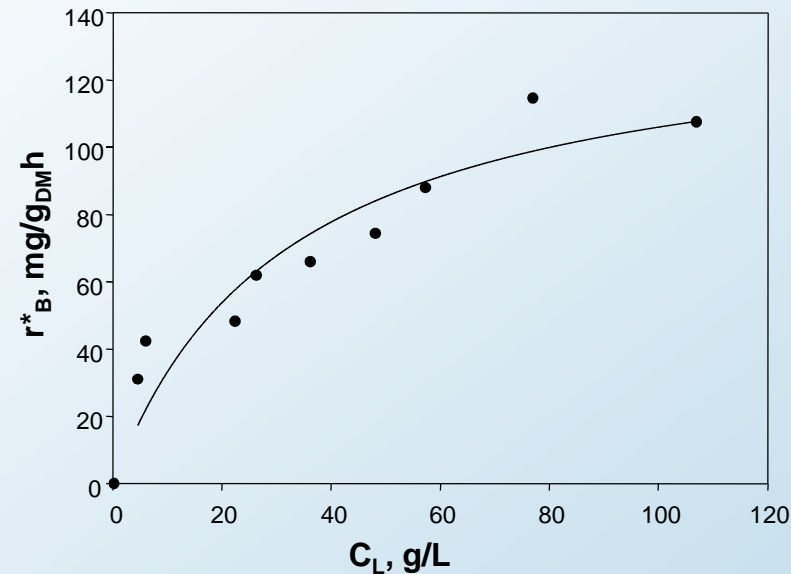


Solventogenesis phase



Solventogenesis phase is characterized by :

- ✓ The increase of butanol concentration with C_L^0
- ✓ The yield of lactose in butanol maximum at $C_L^0=50\text{g/L}$



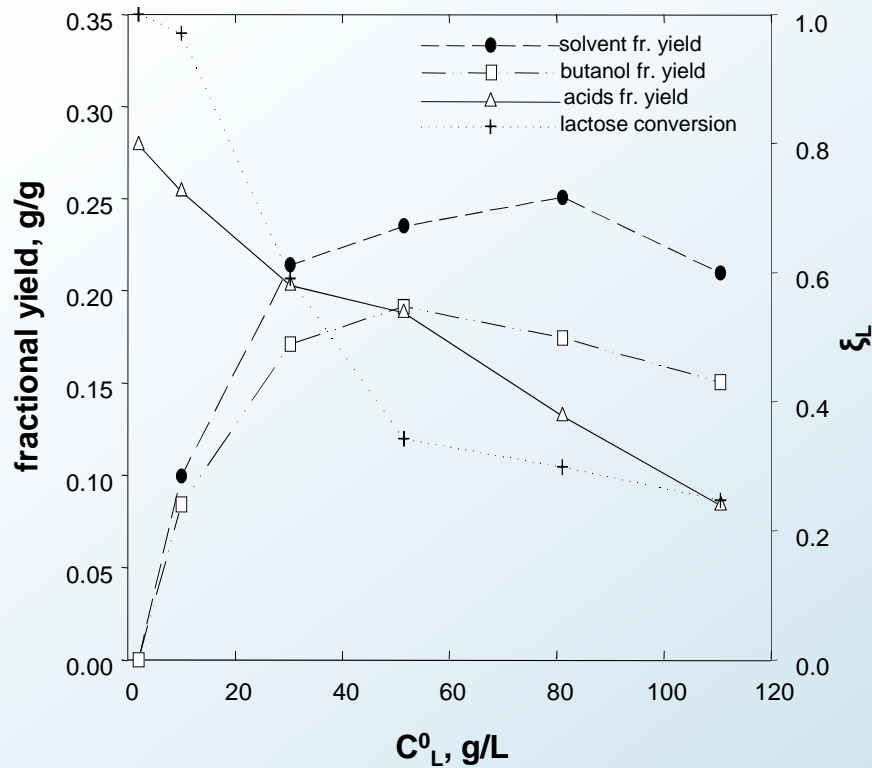
The specific rate of butanol :

- ✓ may be described by a Monod like relationship:

$$r_B = 130 \cdot \left(\frac{C_L}{C_L + 30} \right) \frac{\text{mg}_B}{\text{g}_{DM} \text{ h}}$$



Relevant data from batch tests as a function of initial lactose concentration C^0_L

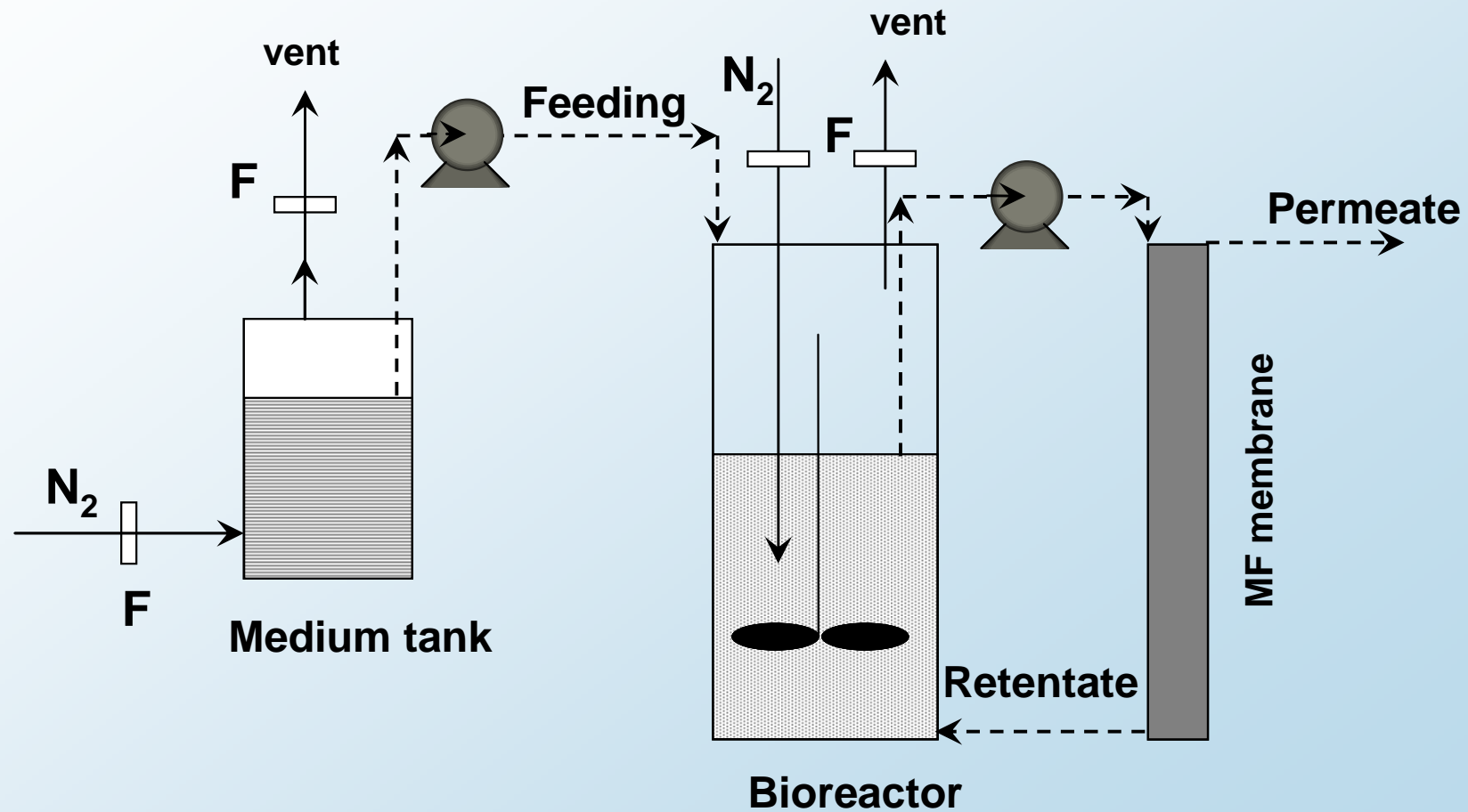


Overall conversion process is characterized by :

- ✓ maximum butanol yield of about $0.2 \text{ g}_B/\text{g}_L$ at $C^0_L=50\text{g/L}$, the half of theoretical maximum value ($0.4\text{g}_B/\text{g}_L$)
- ✓ the decrease of lactose conversion degree with C^0_L until 0.3
- ✓ the decrease of overall butanol selectivity ($Y_{B/L}/Y_{Sol/L}_{ov}$) with C^0_L until 0.65 (molar basis)

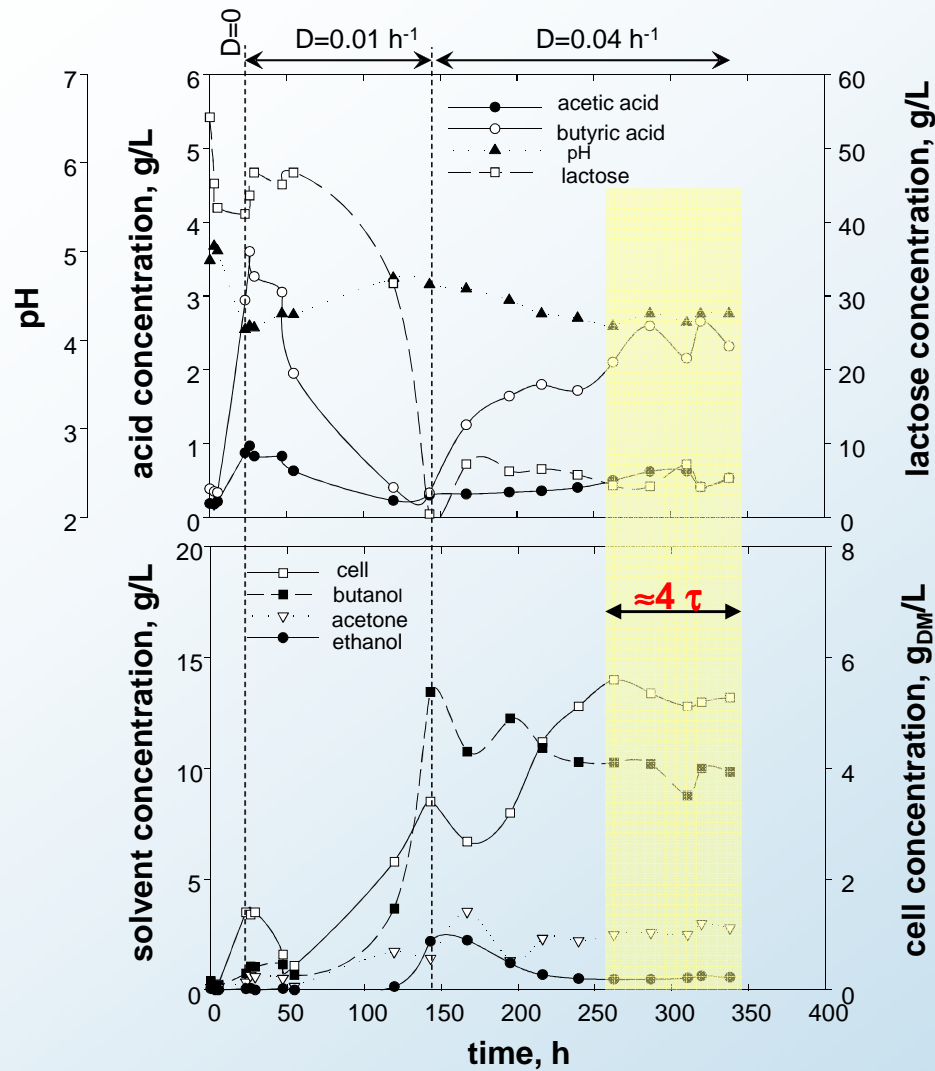


CSTR equipped with a microfiltration unit





Solventogenesis investigation: CSTR equipped with a microfiltration unit



$X, g_{DM}/L$	5.3	$\xi_L, -$	0.90
$C_L, g/L$	5.2	$-r_L, g_L/h g_{DM}$	0.34
$C_{AA}, g/L$	0.52	$r_{AA}, mg_{AA}/h g_{DM}$	3.9
$C_{BA}, g/L$	2.3	$r_{BA}, mg_{BA}/h g_{DM}$	17
$C_{Et}, g/L$	0.54	$r_{Et}, mg_{Et}/h g_{DM}$	4.1
$C_{AC}, g/L$	2.6	$r_{Ac}, mg_{Ac}/h g_{DM}$	20
$C_B, g/L$	9.9	$r_B, mg_B/h g_{DM}$	75
$Y_{B/Sol}, g_B/g_{Sol}$	0.22	selectivity ($Y_{B/L}/Y_{Sol/L}$), molar basis	0.70

reactor volume: 0.45 L culture medium: 50 g/L Lactose, 5g/L YE



MAIN REMARKS

- The ABE production by *Clostridium acetobutylicum* fermentation under batch conditions and continuous conditions was successful

Batch conditions

- The map of kinetic rates involved in the fermentation process under batch conditions has been assessed
- The butanol yield with respect to the initial lactose concentration is characterized by a maximum of about 0.10 at $C_L^0=30$ g/L
- The butanol productivity by fermentation increases with C_L^0 even though the pureness becomes progressively lower

Continuous conditions

- The conversion rate appears to be higher with respect to the value estimated under similar operating conditions in batch tests