POLYTECHNIQUE MONTRÉAL

LE GÉNIE EN PREMIÈRE CLASSE

Development of high-speed and highly efficient butanol production systems from butyric acid with high density of living cells of Clostridium saccharoperbutylacetonicum

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- Contexte
- Paper discussion and results
- Main Result
- Secondary results
- Strengths & weaknesses
- Conclusion validity
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- Recommendations

Objective of the study

Developing of a highly efficient Butanol continuous production system from butyric acid, by recycling living cells of clostridium Saccharoperbutylacetonecum and activity regeneration.

Cadre de l'etude

Butanon porduction by ABE fermentation becomes more attractive (oil depletion, global warming ….)

ABE-producing pathways and metabolics well known

Advantages offered by the clostridium : ability to metabolize organic acids to produce butanol (lactic, acetic and butyric acids)

More interest on continuous production systems wich can overcome butanol production inhibition problem, best yields

Discussion and results

1- Living Cells in TYA-N vs Growing cells in TYA
@ D=0.75 h^{-1}, D= 0.85 h^{-1}

Result 1

2- Living Cells in TYA-N + Methyl Viologen
@ 0 mM, 0,01 mM et 0,1 mM of Methyl Viologen

Result 2

3- Living Cells in TYA-N + MV + activity regeneration
@ D= 0.85 h^{-1} , MV : 0,01 mM

Final Result

Discussion and results

Bacteria strain : clostridium saccharoperbutylacetonicum N1-4 (10% v/v, incubated @ 30C for 24h)

TYA medium		**TYA-N medium**	
Components	(g/l)	Compenents	(g/l)
Glucose	20 – 50 *	Glucose	20
Yeast Extract	2	Butyric acid	10
Tryptone	2	KH_2PO_4	0,5
CH_3COONH_4	3	$FeSO_4$’ $7H_2O$	0,01
KH_2PO_4	0,3		
$MgSO_4$’ $7H_2O$	0,5		
$FeSO_4$’ $7H_2O$	0,01		
Preculture , main culture		Continuous butanol production	

* : 20 g/l for preculture, and 50g/l for main culture.

Investigation 1

a- Living-Cells in TYA-N medium:

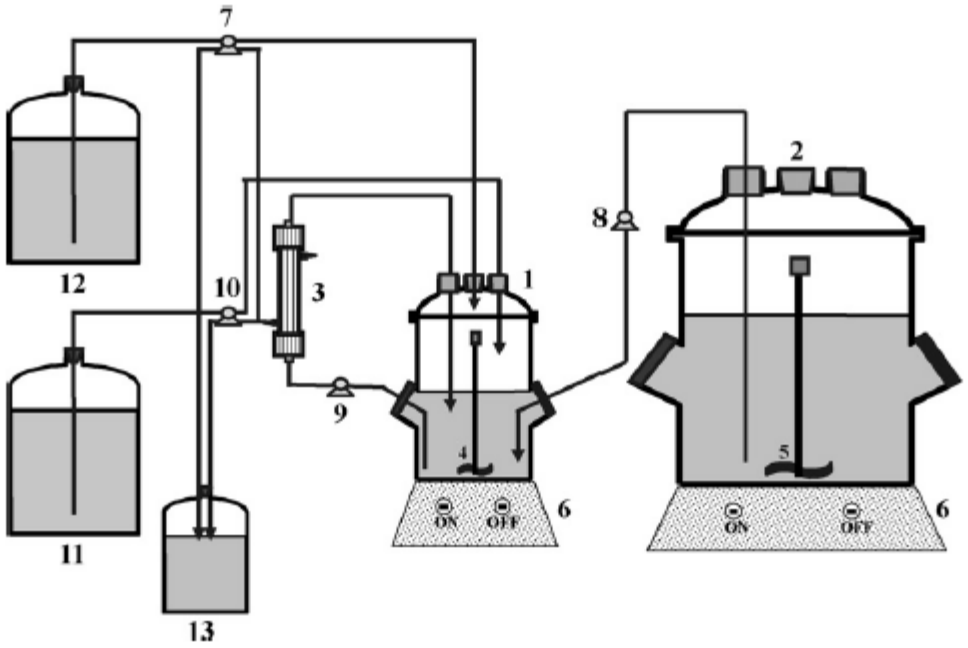
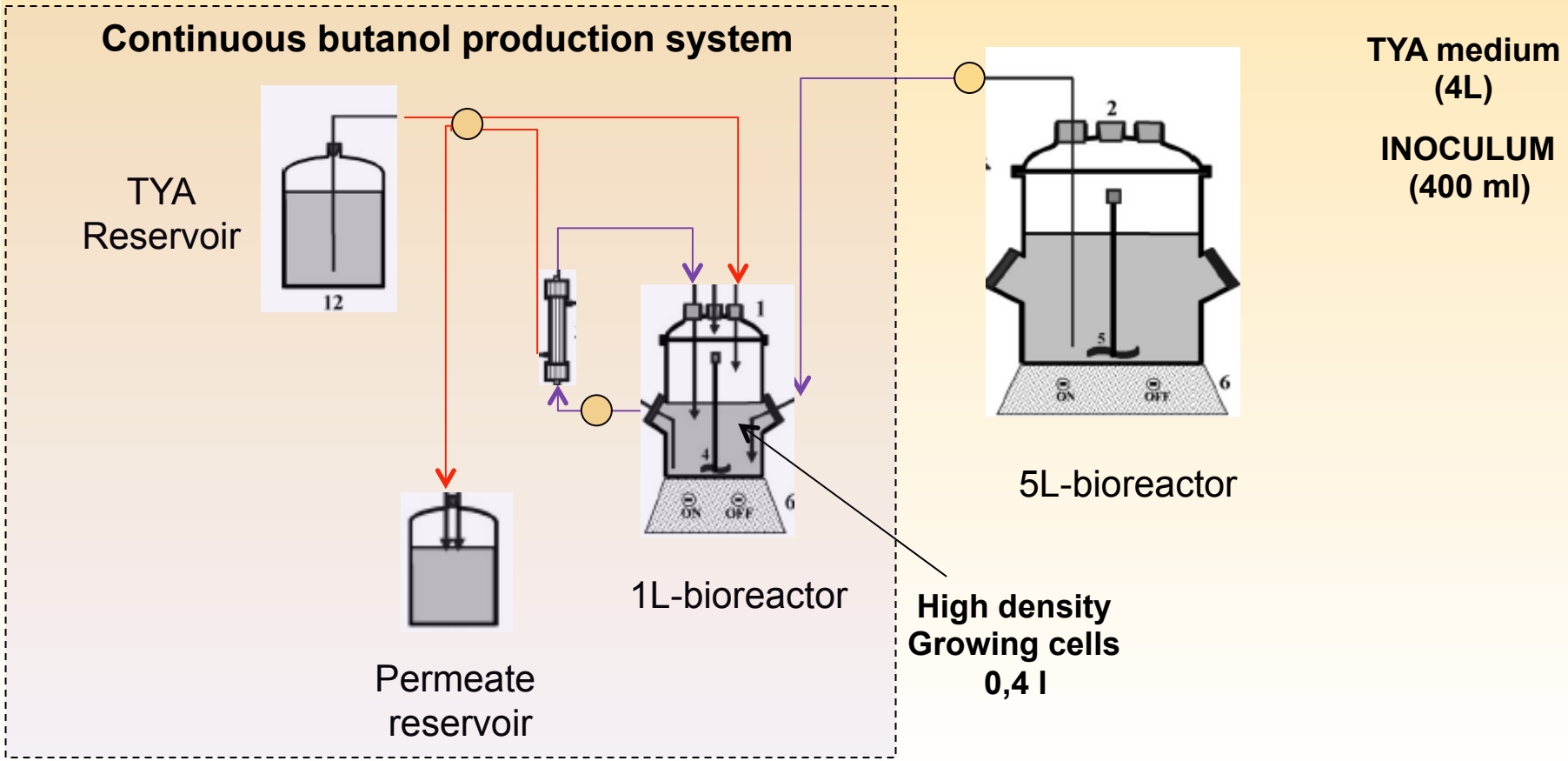


Fig. 2. Setup for continuous butanol production with high density of living cells with or without activity regeneration: (1) 1-l jar bioreactor; (2) 5-l jar bioreactor; (3) hollow fibre module; (4) agitator of 1-l jar bioreactor; (5) agitator of 5-l jar bioreactor; (6) magnetic stirrer; (7)–(10) peristaltic pump; (11) TYA–N source solution reservoir; (12) TYA medium reservoir; (13) permeate reservoir.

reservoir

Investigation 1

b- Growing cells in TYA :



Result 1

In living cells, the total cell concentrations (DCW) at both dilution rates of 0.75 and 0.85 h−1 were constantly maintained during continuous butanol production

In growing cells, DCW was more than 100 g l−1 at the end of continuous cultivation, leading to overflow of the broth…

Increase in production rates: butanol +45% and ABE +36%

Comparable results for butanol and ABE max concentration

BUT Lower byproduct concentrations

Result 1

C- living Cells with dillution of 0.75 and 0.85 h^{-1}

Both dilution rates showed comparable yields of butanol and ABE, but different butanol productivity (4.05 g l−1 h−1 at 0.75 h−1 and 5.68 g l−1 h−1 at 0.85 h−1)

Table 1
Kinetic parameters of continuous butanol production systems with high density of living cells, MV and activity regeneration.

System	D (h^{-1})	MV (mM)	Activity regeneration	Overall $C_{Butanol}$ ($g\,l^{-1}$)	Overall C_{ABE} ($g\,l^{-1}$)	Overall $P_{Butanol}$ ($g\,l^{-1}\,h^{-1}$)	Overall P_{ABE} ($g\,l^{-1}\,h^{-1}$)	Overall $Y_{Butanol}$ (C-mol/C-mol)	Overall Y_{ABE} (C-mol/C-mol)
1	0.75	0	–	5.40	6.77	4.05	5.08	0.541	0.669
2	0.85	0	–	6.69	8.56	5.68	7.28	0.528	0.663
3	0.85	0.01	–	7.16	9.10	6.09	7.70	0.591	0.740
4	0.85	0.1	–	5.87	7.25	4.99	6.16	0.518	0.625
5	0.85	0.01	+	9.40	11.5	7.99	9.73	0.686	0.831

TYA–N source solution contained 10 $g\,l^{-1}$ butyrate or MV of the indicated concentration at initial pH of 5.5. Continuous butanol productions were conducted at 30 °C for 25.5–26.5 h (Systems 1–4) and for 98.5 h (System 5) under the indicated conditions.

Result 1 (summary)

- Living cells offer constant cell mass concentration in broth than the growing cells and avoid its overflow and faoming

- Living cells offer an increase in production rates: butanol (+45%) and ABE (+36%)

-Living cells result in comparable results for butanol and ABE max concentration BUT Lower byproduct concentrations

- Dillution rate of 0,85 h^{-1} result in best ABE and butanol productivities.

1- Living Cells in TYA-N vs Growing cells in TYA
@ D=0.75 h^{-1}, D= 0.85 h^{-1}

Living cells @ , D= 0.85 h^{-1}

2- Living Cells in TYA-N @ D= 0.85 h^{-1} + Methyl Viologen
@ 0,01 mM and 0,1 mM of Methyl Viologen

Investigation 2

In this part of the study the use of Methyl Viologen was not clearly explained, why it was decided to do this experiment?

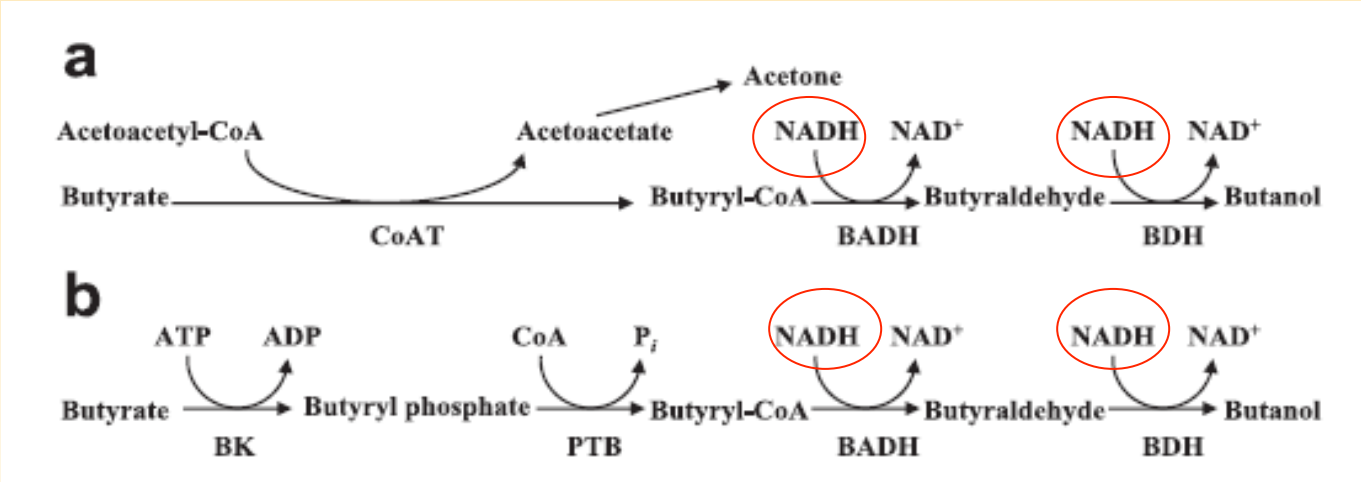
After analyzing a previous paper for the author, (Tashiro 2007) :

→ In the absence of glucose, butyrate will not be utilized by living cells, and little butanol production will be produced !!

→ Living cells require glucose metabolism for butanol production from butyrate.

Investigation 2

Butanol production from butyrate is considered to occur via 2 pathways



It was assumed that an electron carrier could compensate for glucose metabolism and can improve the yield of butanol to carbon source :

Result 2

Butanol concentration, productivity and yield were higher in the presence of 0,01mM of Methyl Viologen

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Kinetic parameters of continuous butanol production systems with high density of living cells, MV and activity regeneration.

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Discussions

Living cells lost their activities with incubation time

Lower butanol production by the end of the process

Large amount of substrate remaining in the broth

The system shows very low operational stability

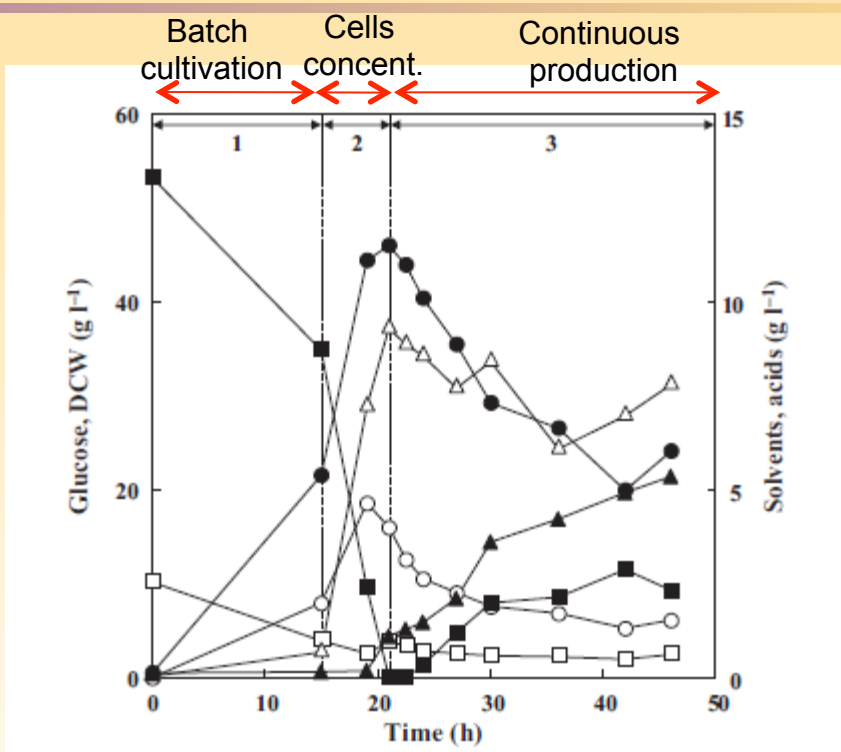

Fig. 3. Time course of continuous butanol production with high density of living cells and 0.01 mM of methyl viologen at dilution rate of 0.85 h^{-1}. Number 1, 2, and 3 double-arrowed lines indicate the respective periods of batch cultivation, operation for cell concentration and transient state, and continuous butanol production. ●, butanol; ○, acetone; ▲, butyric acid; △, dry cell weight; ■, glucose; □, acetate.

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Living cells @ , D= 0.85 h^{-1}

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0,1 mM of Methyl Viologen

3- Living Cells in TYA-N + MV + activity regeneration
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Final Result

Investigation 3

Activity regeneration investigation

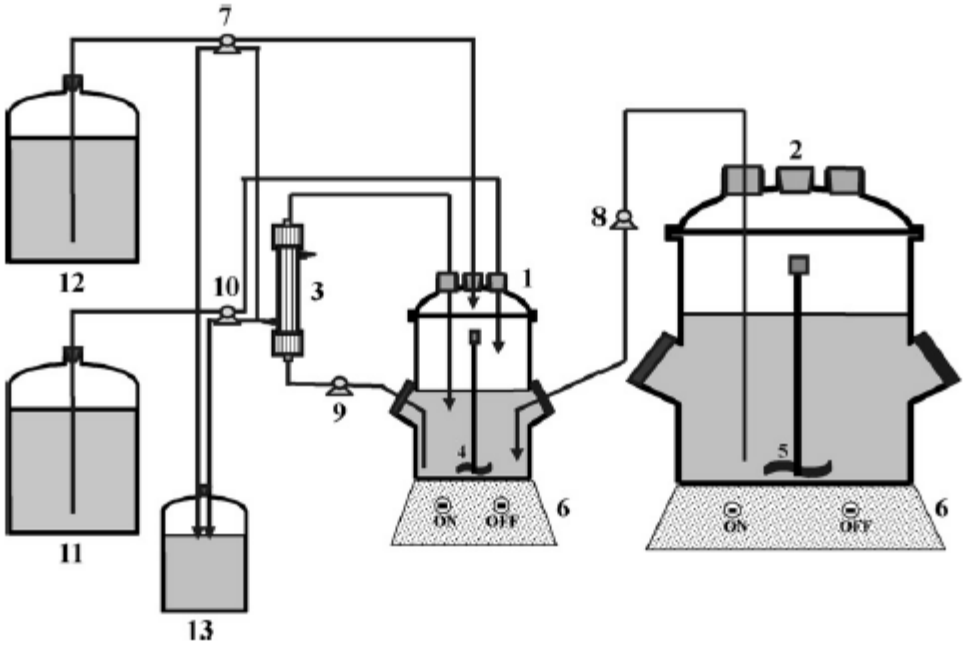
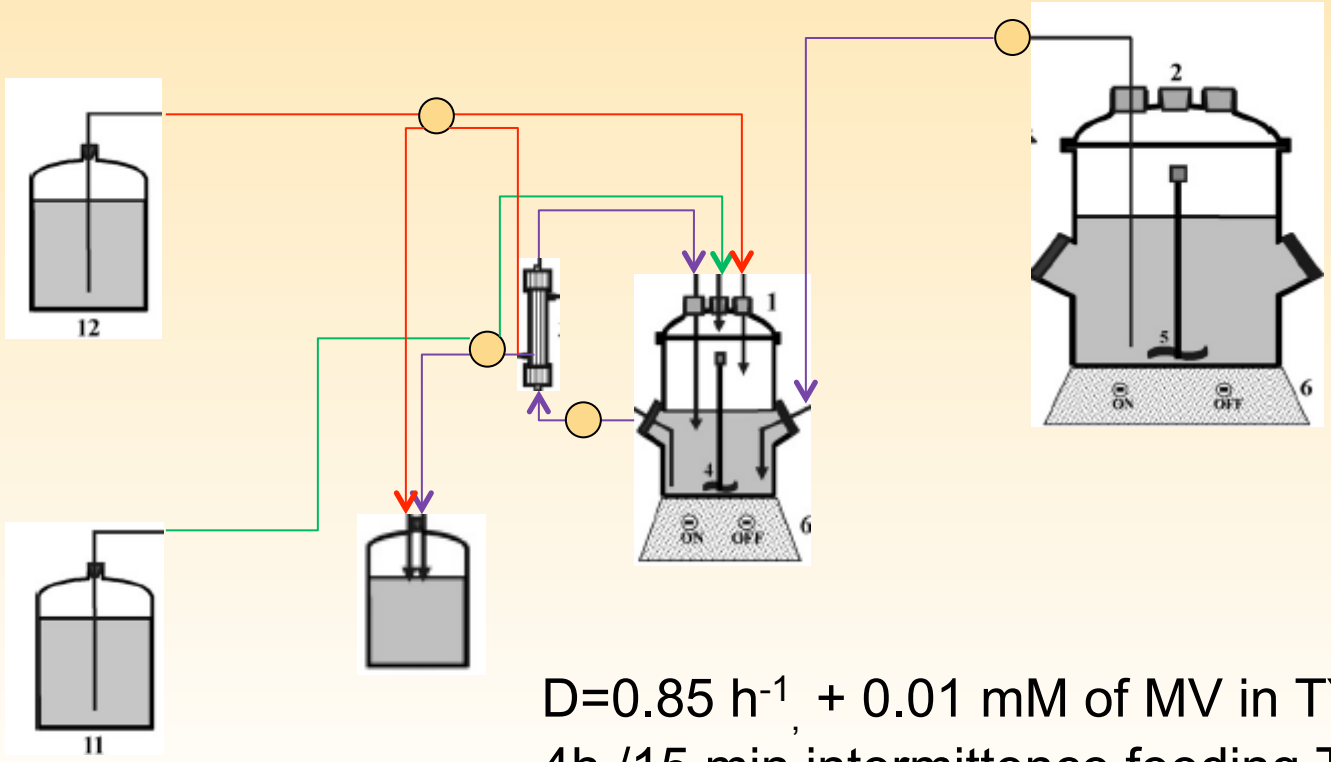


Fig. 2. Setup for continuous butanol production with high density of living cells with or without activity regeneration: (1) 1-l jar bioreactor; (2) 5-l jar bioreactor; (3) hollow fibre module; (4) agitator of 1-l jar bioreactor; (5) agitator of 5-l jar bioreactor; (6) magnetic stirrer; (7)–(10) peristaltic pump; (11) TYA–N source solution reservoir; (12) TYA medium reservoir; (13) permeate reservoir.

Resultat 3

Activity regeneration investigation



D=0.85 h^{-1}, + 0.01 mM of MV in TYA-N

4h /15 min intermittence feeding TYA-N/TYA

Resultat 3

Butanol concentration was maintained at a high level (>8g/l)
Residual substrate concentration was maintained at low level

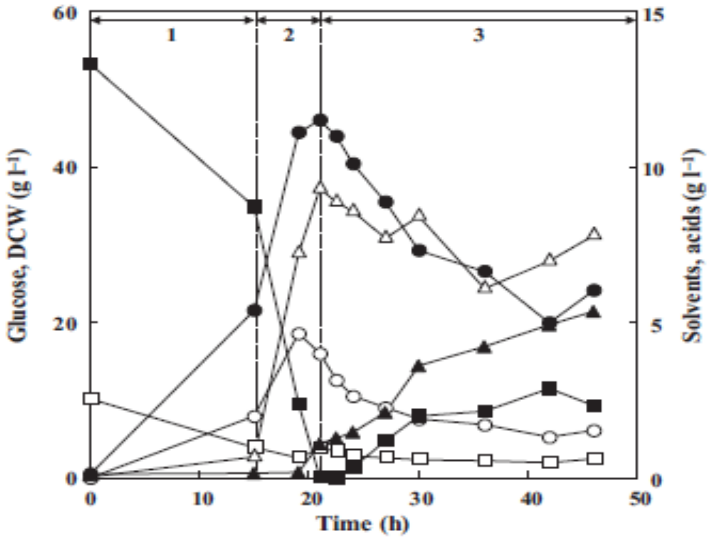


Fig. 3. Time course of continuous butanol production with high density of living cells and 0.01 mM of methyl viologen at dilution rate of 0.85 h^{-1}. Number 1, 2, and 3 double-arrowed lines indicate the respective periods of batch cultivation, operation for cell concentration and transient state, and continuous butanol production. ●, butanol; ○, acetone; ▲, butyric acid; △, dry cell weight; ■, glucose; □, acetate.

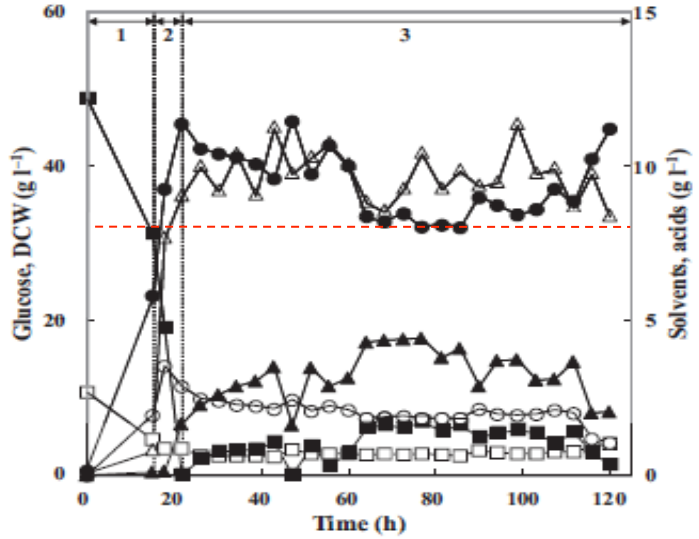


Fig. 4. Time course of continuous butanol production with high density of living cells and 0.01 mM of methyl viologen with activity regeneration. Periodical feedings of TYA–N source solution and TYA medium were carried out for 4 h and 15 min, respectively, at dilution rate of 0.85 h^{-1}. Symbols and double-arrowed lines indicate the same items as those in Fig. 3.

Resultat 3

Higher butanol to ABE ratio was obtained with activity regeneration thant without (table1)

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Kinetic parameters of continuous butanol production systems with high density of living cells, MV and activity regeneration.

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Main Result

The system gives the highest productivity and yield of butanol among the continuous butanol production systems with high density of living and growing cells reported to date in the available littérature (January 2011)

Table 2
A comparison of butanol production by different continuous production systems using high density of growing or living ABE-producing clostridia published to date.

Cells	Strains	Modes	Carbon sources	D (h^{-1})	$P_{Butanol}$ ($g\,l^{-1}$)	$Y_{Butanol}$ (C-mol/C-mol)	Reference
Living	*C. saccharoperbutylacetonicum* N1-4	Cell recycling	Butyrate+glucose	0.85	7.99	0.686[b]	This study
Living	*C. acetobutylicum* ATCC 824^T	Immobilization	Glucose	0.374	0.741	0.291[c]	Förberg and Häggström (1985)
Growing	*C. saccharoperbutylacetonicum* N1-4	Cell recycling	Glucose	0.85	7.34	0.365[c]	Tashiro et al. (2005)
Growing	*C. acetobutylicum* ATCC 824^T	Cell recycling	Glucose	0.327	2.79	0.303[c]	Pierrot et al. (1986)
Growing	*C. acetobutylicum* ATCC 824T	Cell recycling	Glucose	0.64	5.40[a]	ND[d]	Afschar et al. (1985)
Growing	*C. acetobutylicum* DSM 1731	Cell recycling	Glucose	0.4	4.10	0.323[c]	Schlote and Gottschalk (1986)
Growing	*C. saccharobutylicum* NCP 262^T	Immobilization	Lactose	0.97	2.00	0.336[e]	Friedl et al. (1991)
Growing	*C. saccharobutylicum* NCP 262^T	Immobilization	Lactose	1.0	4.10[a]	0.230[f]	Qureshi and Maddox (1987)
Growing	*C. saccharobutylicum* NCP 262^T	Immobilization	Lactose	1.1	2.09	0.366[e]	Qureshi and Maddox (1995)
Growing	*C. acetobutylicum* DSM 792	Immobilization	Lactose	0.97	4.43	0.389[e]	Napoli et al. (2010)
Growing	*C. beijerinckii* BA 101	Immobilization	Glucose	1.5	6.75	0.472[c]	Lienhardt et al. (2002)

Conclusion validity

Comparison with other system using only glucose as Carbone source is not very representative, because butyric acid feeding either as feed-stock component or from sub-production unit, will decrease dramatically the hole efficiency of the system !

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Secondary Results

Living cells have a positive effects on the butanol to ABE ratio and butanol&ABE yields

Dillution rate of 0.85 gives more productivities of butanol and ABE in the present system.

Methyl Viologen at 0,01 concentration can be used as an electron carrier to stimulate butyric bioconversion and improve the yield of butanol to carbon source

Higher butanol to ABE ratio can be obtained with activity regeneration.

Strengths

High density living-cells by recycling seems to be more efficient and practical than the immobilization one.

Activity Regeneration by intermittent flow (great positive effect on process stability with operation cost)

Optimizing the use of glucose in the conversion of butyric acid by introducing the electron carrier approach.

Optimizing the use of glucose by introducing the electron carrier approach.

Weaknesses

It was not be underlined that using TYA-N represents an inexpensive nutrient limited medium to produce enhanced butanol (big savings in OPEX)

In industrial scale, operation and maintenance of Cell filtration modules present a real challenge…and can substantially affect the OPEX.

Weaknesses

Bringing the process to an industrial scale :

Butyric acid market is the same as butanol one…..it is produced essentially from fossil oil, (same problems, same challenges)

- It can be supplied in feed-stocks (increase in OPEX), problems of using fossil-oil product…..
- or it can be produced loccally by bioconvesrion (more equipments, more maintenance, more operation expensive: increase both CAPEX and OPEX)

-In all cases the global efficiency of the process will dramatically deacrese

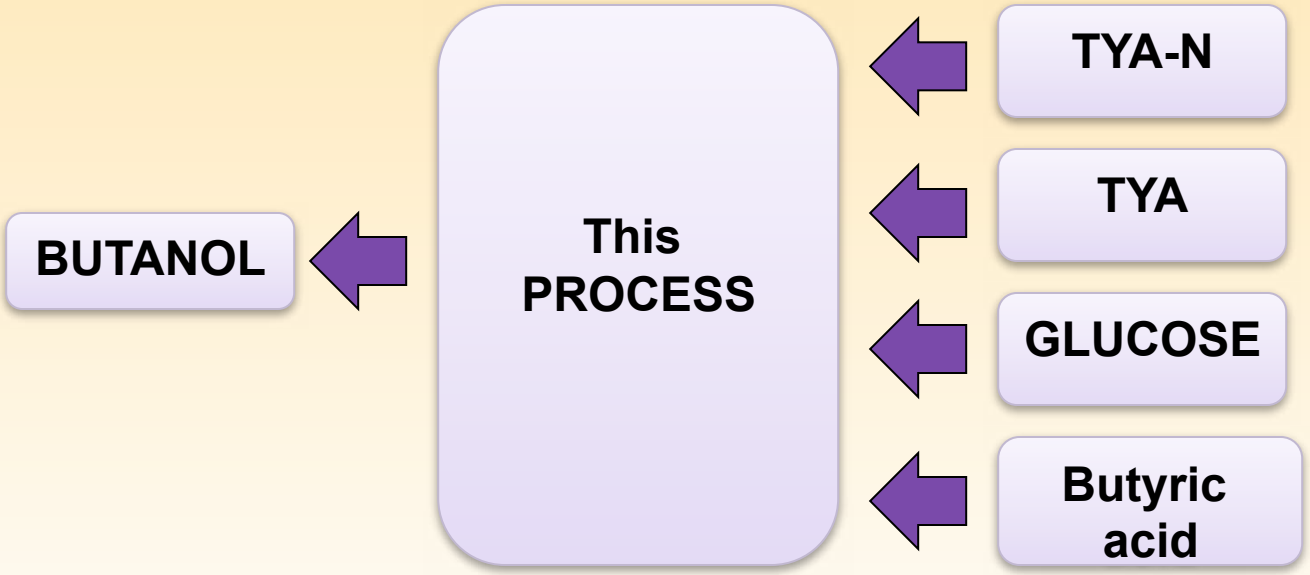
Inovative nature

The paper is based up-on many studies about :
- Living cells vs growing cells,
- production of butanol by cell recycling
- Using of Butyric acid as carbone source
- Adding an electron carrier to stimulate butyric conversion

Several studies were done about those topics since but It was a great idea to bringing to gather all these elements to develop a continuous production system and optimizing it.

Recomandations

Production system proposed in this paper



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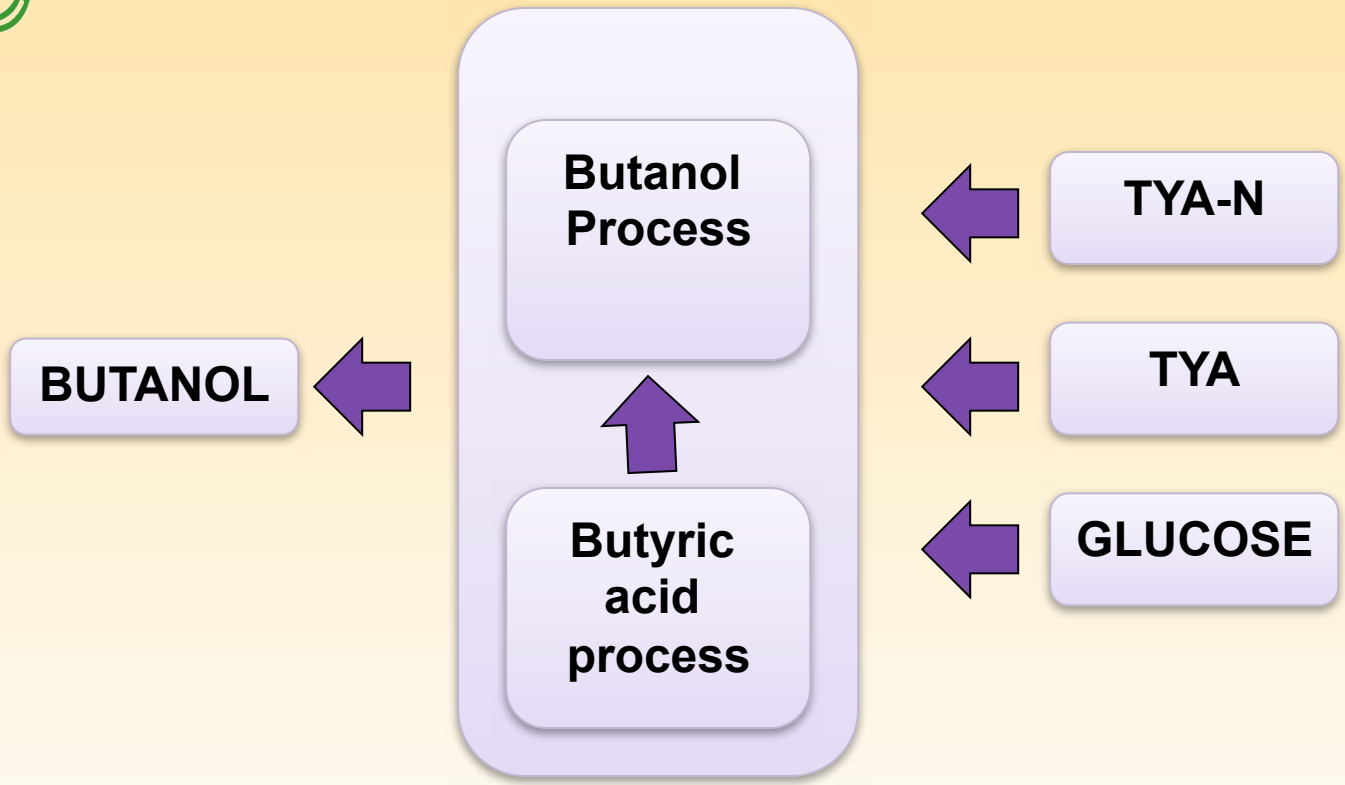

Compare global butanol yield and ABE productivities for this system to the other butanol continuous production systems reported to date.

Thank you