

CONTINUOUS CULTURE OF FLOCCULENT YEAST FOR ETHANOL PRODUCTION

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➤ Brief Introduction



Bioethanol production became an important issue because:

- EU has established that 5.75% of transport fuels must be obtained from renewable sources (such as bioethanol) till 2010;
- Bioethanol production is a well known and developed technology but gains in productivity are still possible;
- The interest for the use of new raw materials (lignocellulosic based) was raised by the recent oil crisis.



How to increase productivity of the bioethanol production process?



Possible strategies:

- 1. use of high concentration of active microorganisms in the bioreactor.**
- 2. increase of dilution rate.**

$$r_p = P \cdot D = Y \cdot X \cdot D$$



How to increase concentration of active microorganisms in the bioreactor?

- By cells immobilisation;
- By natural settling techniques
 - using cells flocculation ability.



Answer: many yeast and *Zymomonas* strains have good flocculation ability.



Bioreactors using cells flocculation techniques could present some advantages:

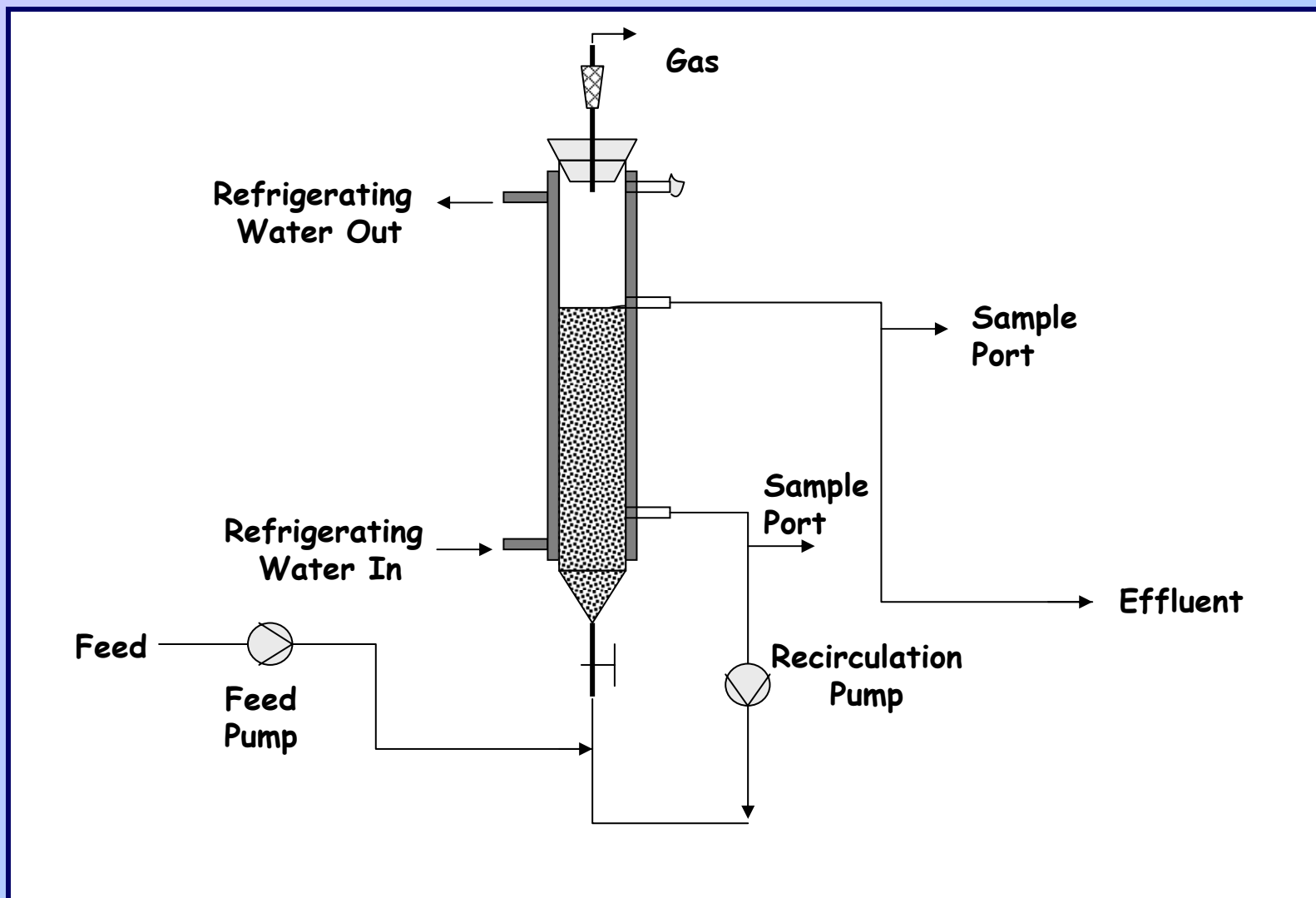
- Low associated capital and operational costs;
- Design simplicity.

➤ The importance of Bioreactor Configuration

Bioreactor Configuration		r_p (g _{EtOH} /L h)
Batch and Fed-Batch		2-5
Continuous Stirred Tank Reactor	Free Cells	6-8
	Immobilized Cells	10-16
	Entrapm. by Membranes	10-30
Packed Bed Reactor		16-40
Fluidized Bed Reactor	Free Cells	50-170
	Recycled Cells	190*

* Productivity achieved at $D=2,5 \text{ h}^{-1}$.

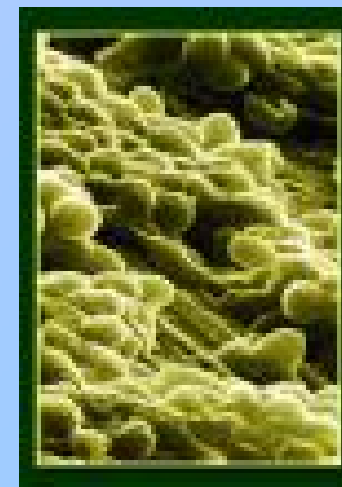
- **The Fluidized Bed Reactor (FBR)**
- **with Flocculated Cells Recycling**



➤ Strain Selection and Batch Tests

- *S. cerevisiae* strains {
 - CCMI 396
 - DER 24
 - DSMZ 2548
 - NCYC 1119
 - F

- Relevant parameters
 - Maximum specific growth rate (μ_m)
 - Ethanol yield ($Y_{P/S}$)
 - Ethanol tolerance



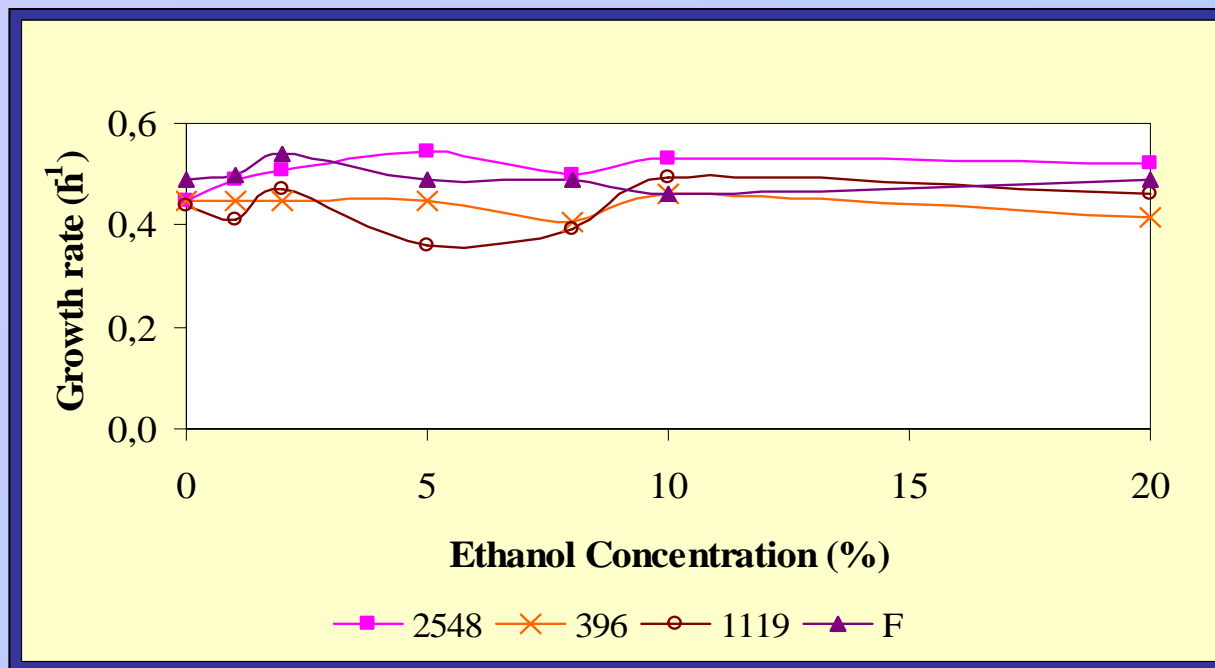
➤ Strain Selection and Batch Tests

Strain	μ_m (h ⁻¹)	X _f (g/L)	P _f (g/L)	Y _{P/S} (g/g)	<r _p > (g/Lh)
CCMI 396	0.366	9.87	31.50	0.26	1.31
DER 24	0.366	11.08	41.50	0.35	1.73
DSMZ 2548	0.396	13.60	46.60	0.23	1.94
NCYC 1119	0.440	nd	49.59	0.39	2.07
F	0.490	12.73	43.50	0.40	1.81

Substrate: sucrose 120 g/L

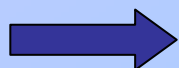
Temperature: 28°C

➤ Strain Selection and Batch Tests



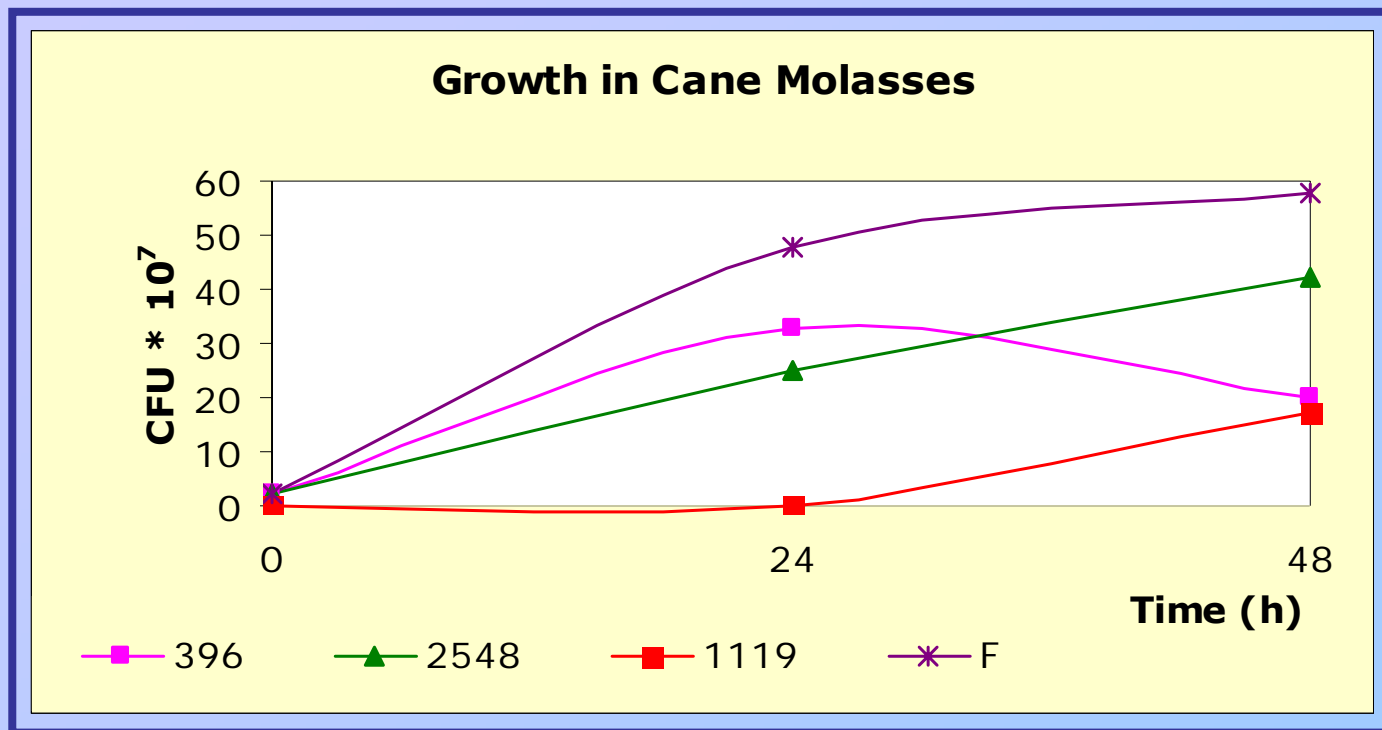
Batch Conditions:

- Ethanol addition in concentrations ranging from 0 to 20%
- Initial sucrose: 120 g/L
- Temperature: 28°C
- Shake flasks



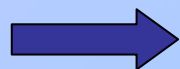
all strains showed good tolerance to ethanol.

➤ Strain Selection and Batch Tests



Batch Conditions:

- Initial sugar: 120 g/L
- Temperature: 28°C
- Shake flasks



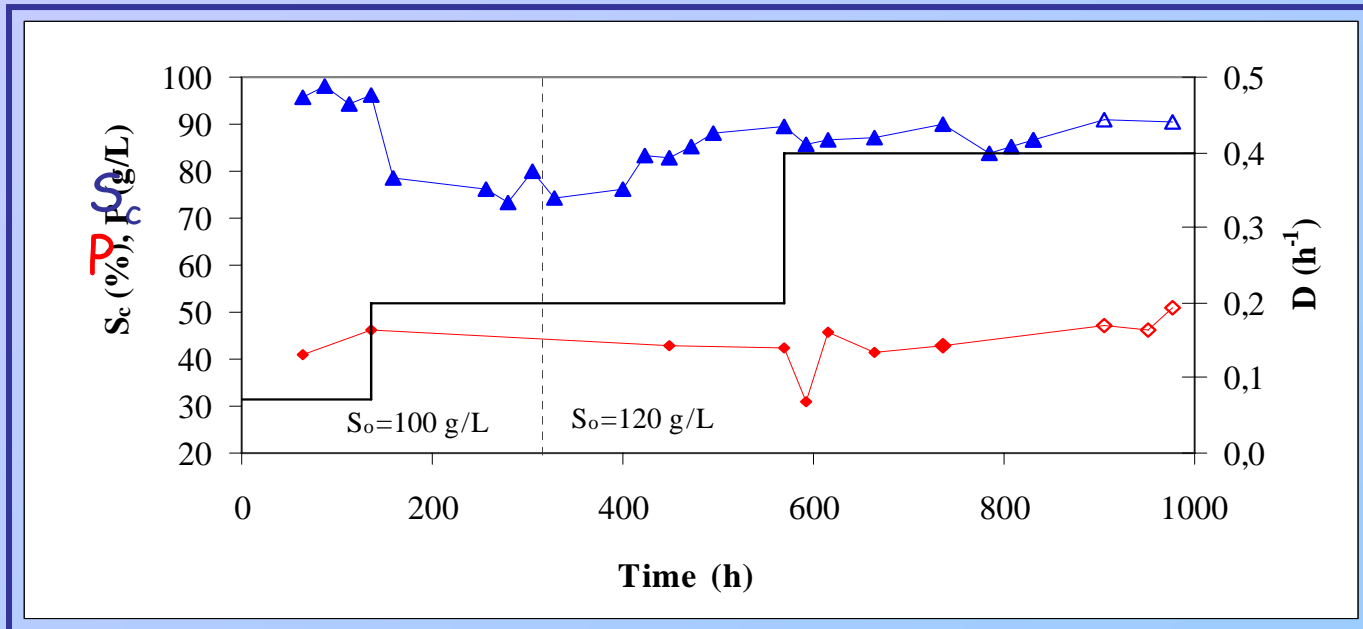
strain F showed a better performance in growing in cane molasses medium. Strain **1119 cell concentration is the lowest** but that is due to the high sedimentation velocity, which difficults a representative sampling.

➤ Strain Selection and Batch Tests:

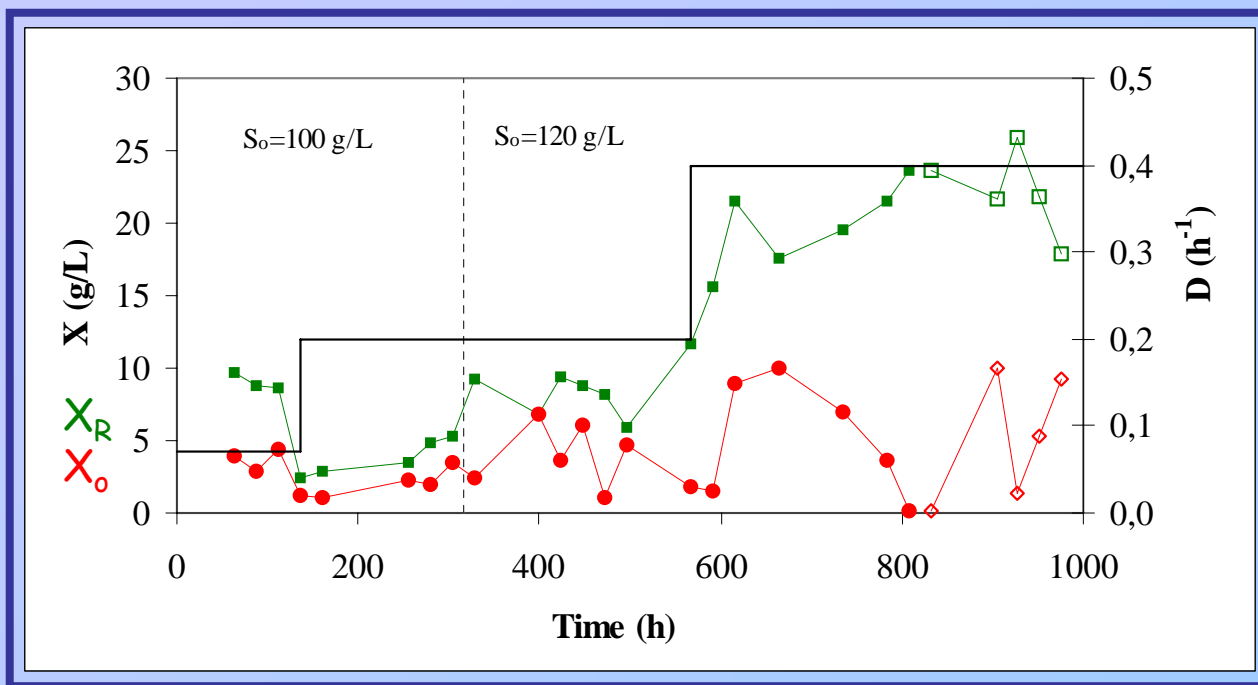
- ➔ **Only strains F and 1119 showed good flocculation ability**, which is very important in FBR fermentations with no solid support to retain biomass;
1119 has more dense flocs than F.
- ➔ **Due to higher maximum specific growth rates, ethanol yields and flocculation ability, strains F and 1119 are suitable for ethanol production in FBR**

➤ Continuous FBR Tests: Strain F

-Defined sucrose media
 -Temperature: 28°C

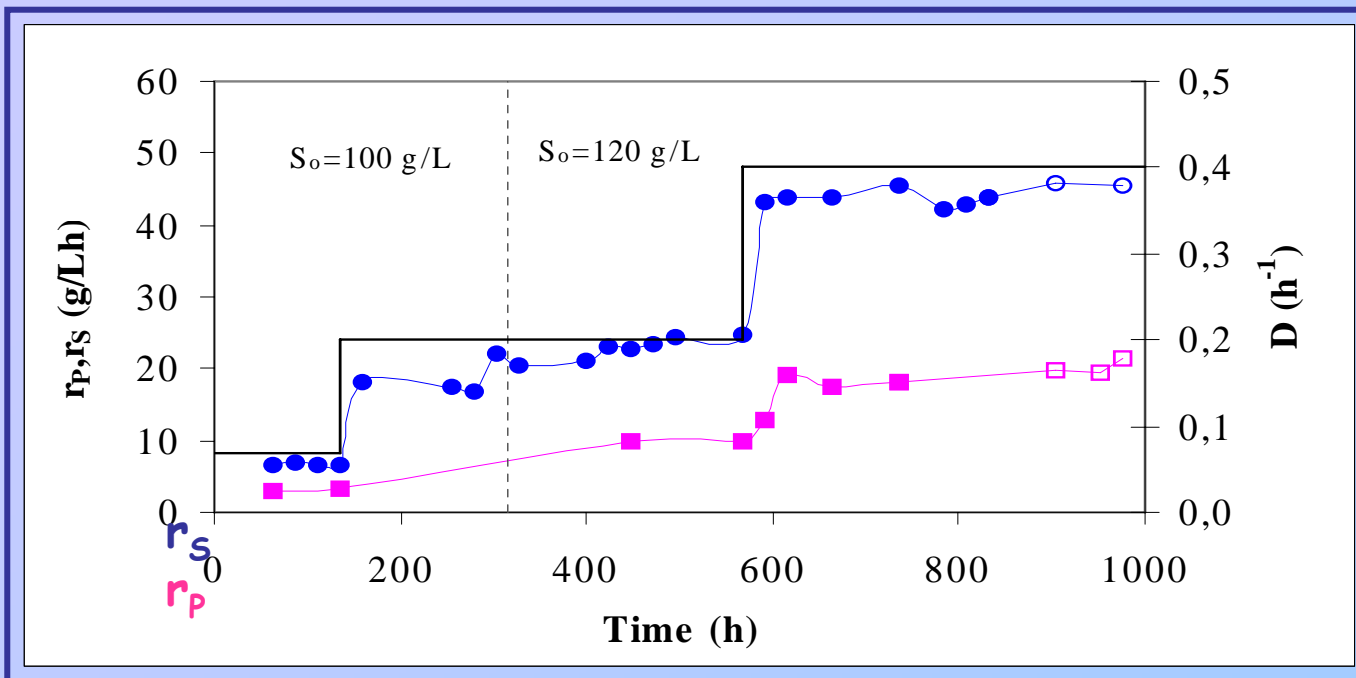


➤ Continuous FBR Tests: Strain F



There is a good capacity for retention of biomass

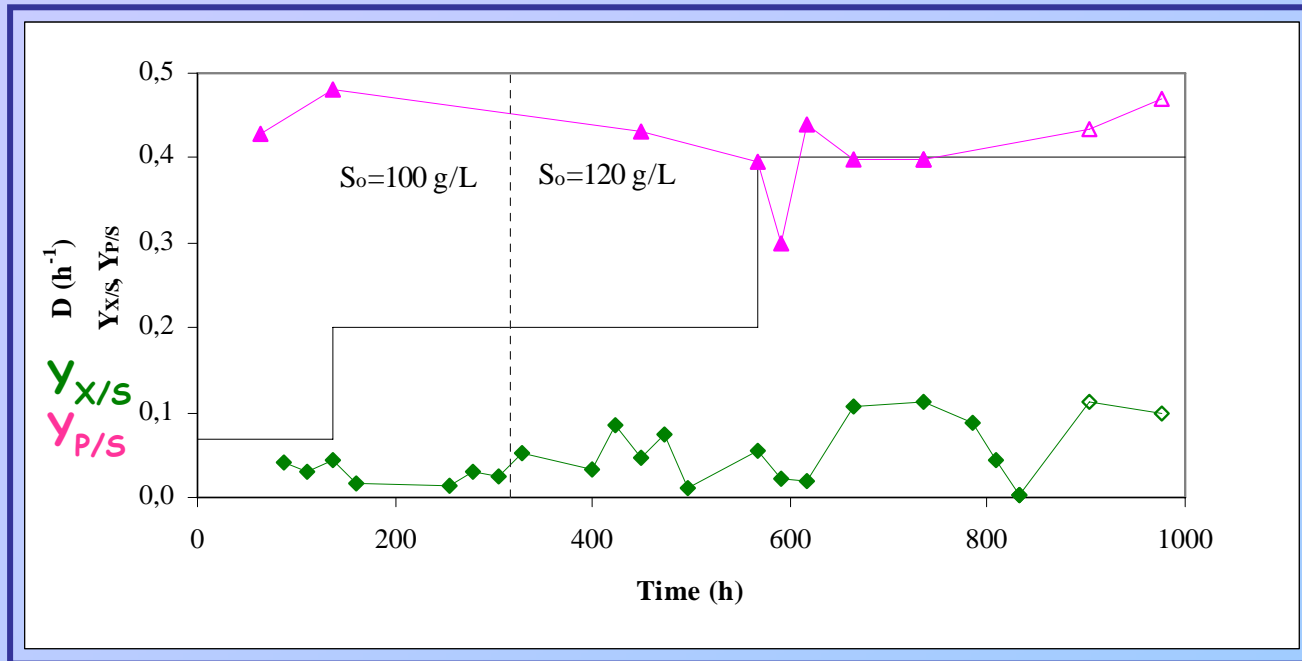
➤ Continuous FBR Tests: Strain F



$Y_{P/S} = 0.47$
 $X_R \approx 25$ g/L
 $X_0 < 5$ g/L

$D = 0.4$ h^{-1}
 $S_c = 90\%$
 $P = 50.8$ g/L
 $r_p = 20.3$ g/Lh

➤ Continuous FBR Tests: Strain F

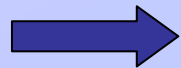


Handicap?: Strain F forms small flocs with low sedimentation velocity.



Studying reactors with high flocculated cells concentration in real time is not easy to accomplish:

- Unsteady regimes develop often
- Biomass viability and production yield may change quickly.



Practical problem:

How to measure in real time biomass concentration inside the reactor to monitor steady states?

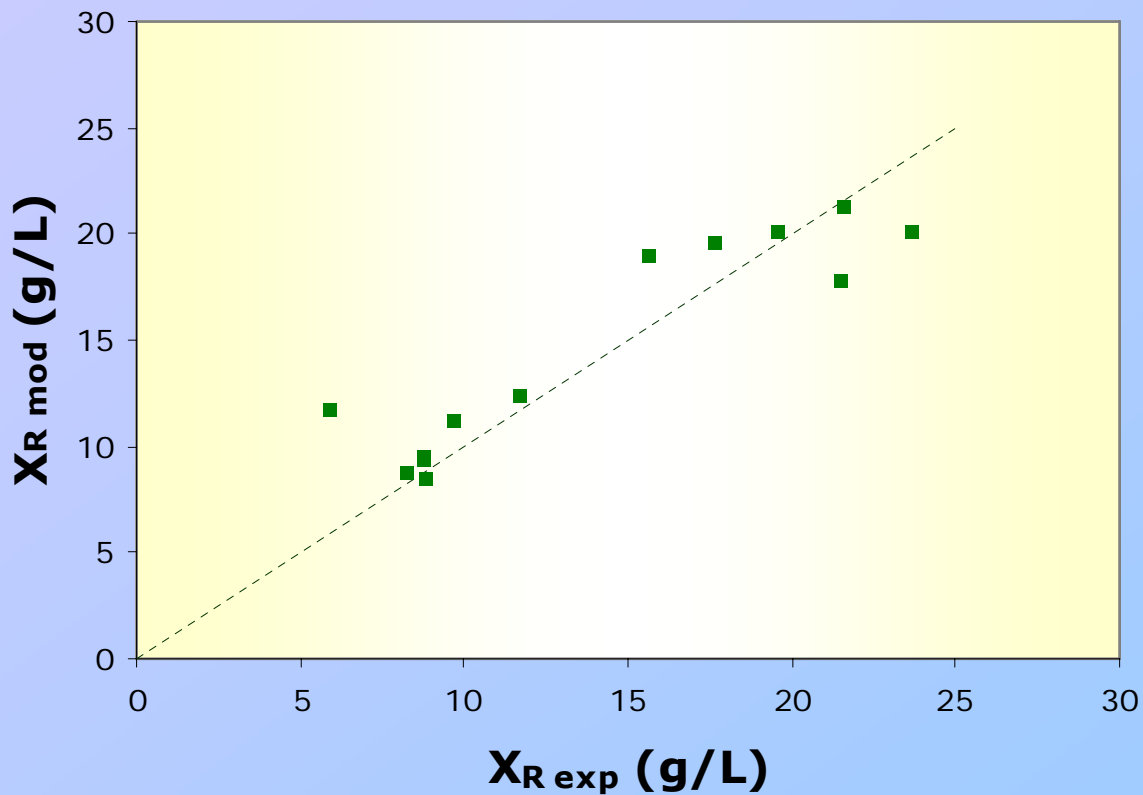


Strategy: Construction of an inferential sensor to estimate biomass, based on other properties of the systems easier to monitor in real time (such as nutrients and products concentrations, operational conditions, etc).

➤ **Construction of a biomass inferential sensor:**

- ✓ **For statistical fitting of data, only steady-state values were used concerning experiments with strain F.**
- ✓ **Biomass concentration within the broth was related to ethanol, biomass concentration in the outflow, dilution rate and percentage of consumed sugar.**
- ✓ **Only linear correlations were tested and the best one was obtained through an Ordinary Linear Square (OLS) Regression.**

➤ **Construction of a biomass inferential sensor:**



$$X_R = 37.64 D + 0.35 S_c + 0.08 X_o + 0.42 P - 46.54$$

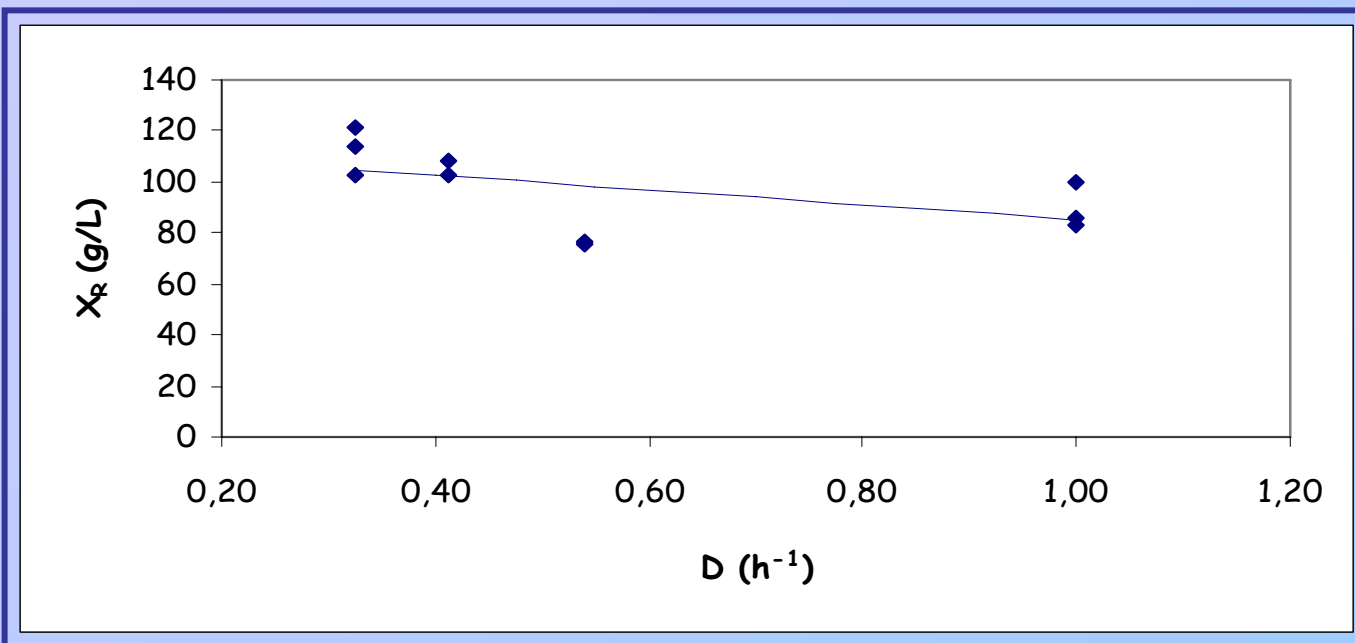
X_R , X_o and P are expressed as g/L;
 S_c is expressed as %

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➤ Construction of a biomass inferential sensor:

- ✓ The constructed biomass inferential sensor permits to predict biomass concentration within the bioreactor by the quicker measurement of other variables.
- ✓ The correlation fitted well the used data, expressed by the Root Mean Square Error (RMSQ=0.68).
- ✓ The correlation is capable of predicting biomass concentration in a quite reasonable way, as the R^2_{cv} parameter expresses ($R^2_{cv}=0.67$).
- ✓ The most important predictor is dilution rate (37.64) and the least important is biomass concentration in the outflow (0.08).

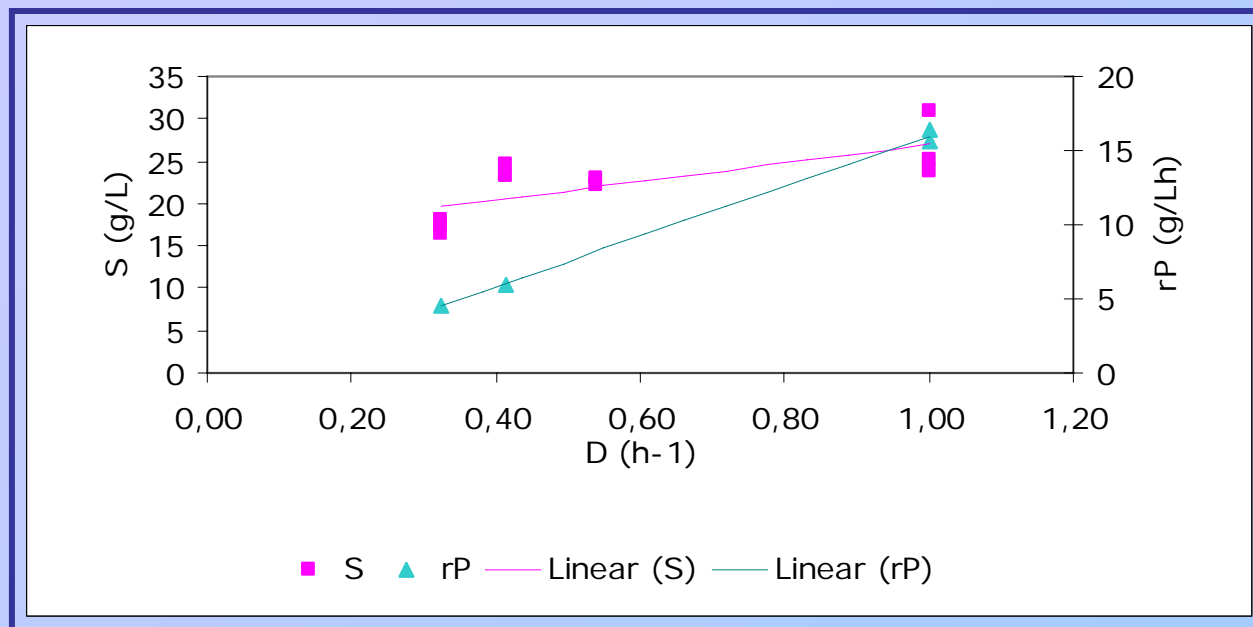
➤ Continuous FBR Tests: Strain 1119



Strain 1119 has bigger flocs and higher sedimentation velocity, which allows it to stay inside the FBR at higher dilution rates ($D > \mu_m$).

At all tested dilution rates cells always showed viability above 90%.

➤ Continuous FBR Tests: Strain 1119



Ethanol productivity of more than **15 g/Lh** was attained, but the yield $Y_{S/P}$ was of 0.2 (aprox.). If we can raise $Y_{S/P}$ till near the theoretical one (0.5) ethanol productivity will be near to **40 g/Lh**.

➤ Continuous FBR Tests: Strain 1119

➤ Some pictures of FBR in operation



Strain F



Strain 1119

➤ **Continuous FBR Tests: Microbial Resistance**

FBR showed a natural resistance concerning microbial contamination, mainly due to:

- ✓ **high dilution rates**
- ✓ **high cells density**

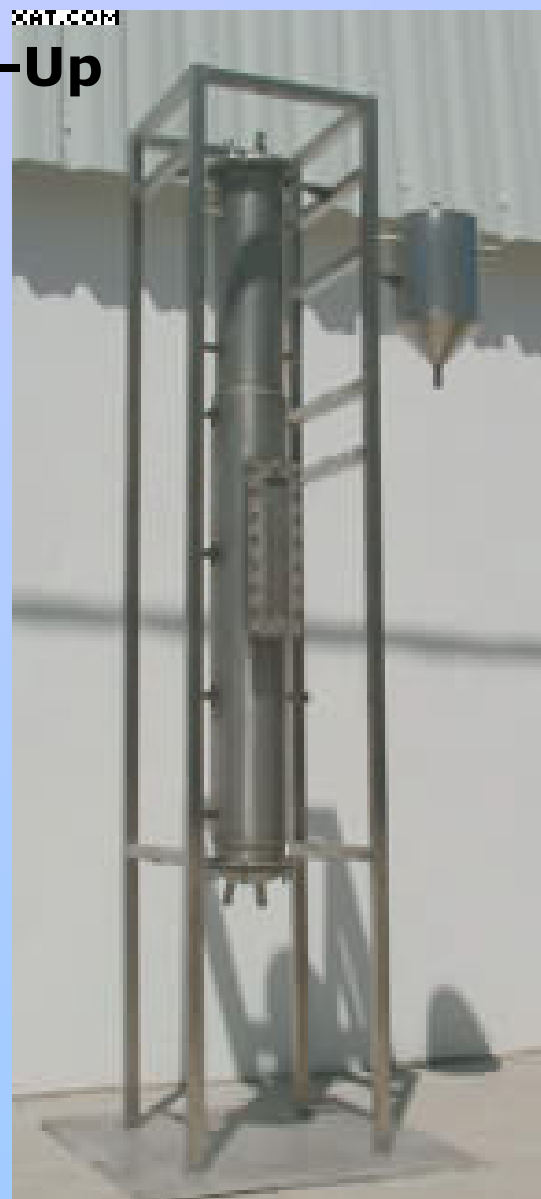
This means:

- ✓ **longer fermentations**
- ✓ **reduced need for sterility**

➤ Continuous FBR Tests: Scale-Up

Prototypes (**4;25;400;4000 L**) were built and tested for long-term operation with good results.

25L-prototype: a tubular air-lift reactor with an external settler;



Bioenergy-I: From Concept to Commercial Processes

Thank you for your attention!

For any enquiries email me:

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