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.

$$r_P = P.\ D = Y\ .\ X\ .\ D$$

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 h^{-1}.

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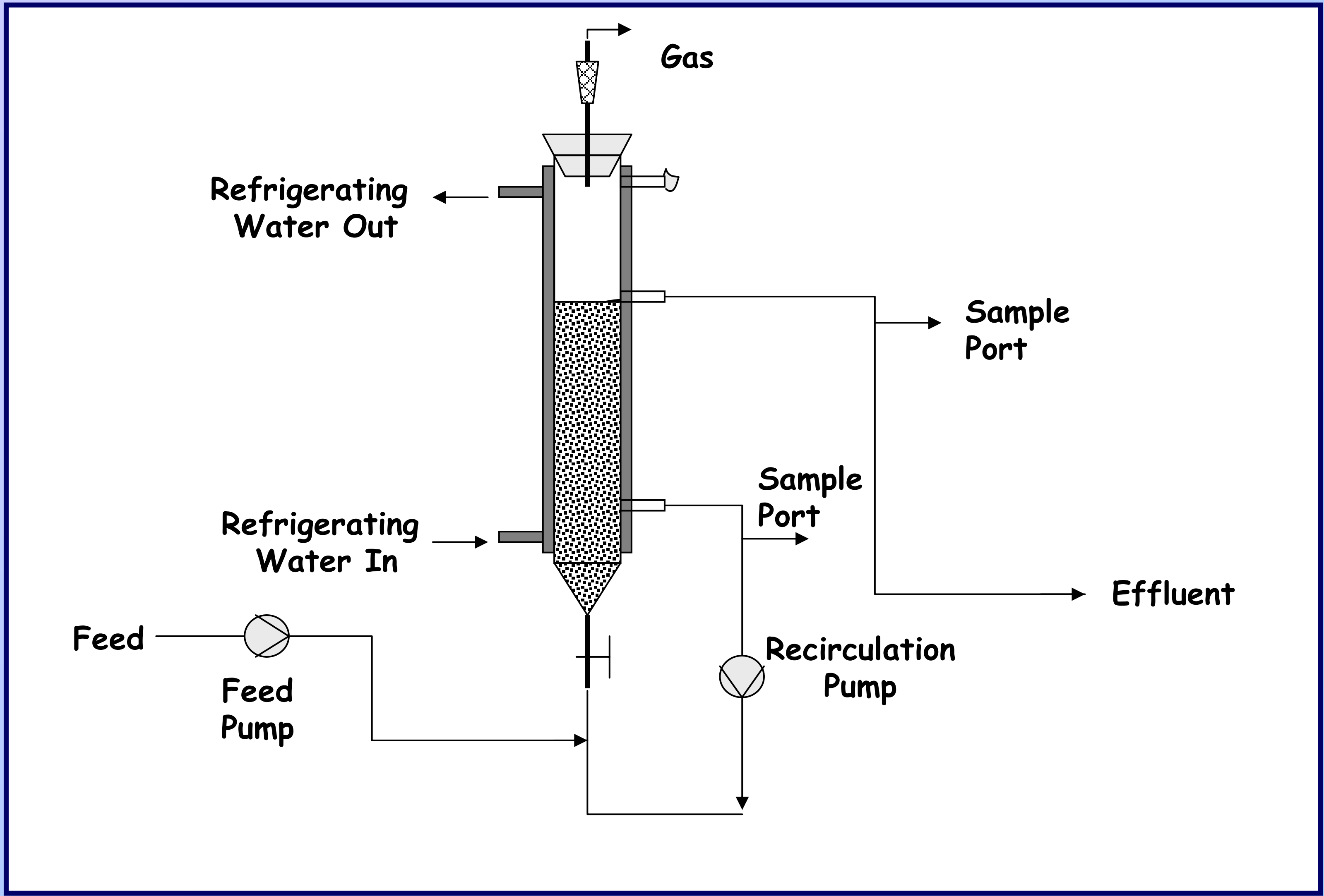
➢The Fluidized Bed Reactor (FBR)
➢with Flocculated Cells Recycling



➢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

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➢Strain Selection and Batch Tests

Strain	μ_m (h^{-1})	X_f (g/L)	P_f (g/L)	$Y_{P/S}$ (g/g)	$\langle r_P \rangle$ (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: 28ºC

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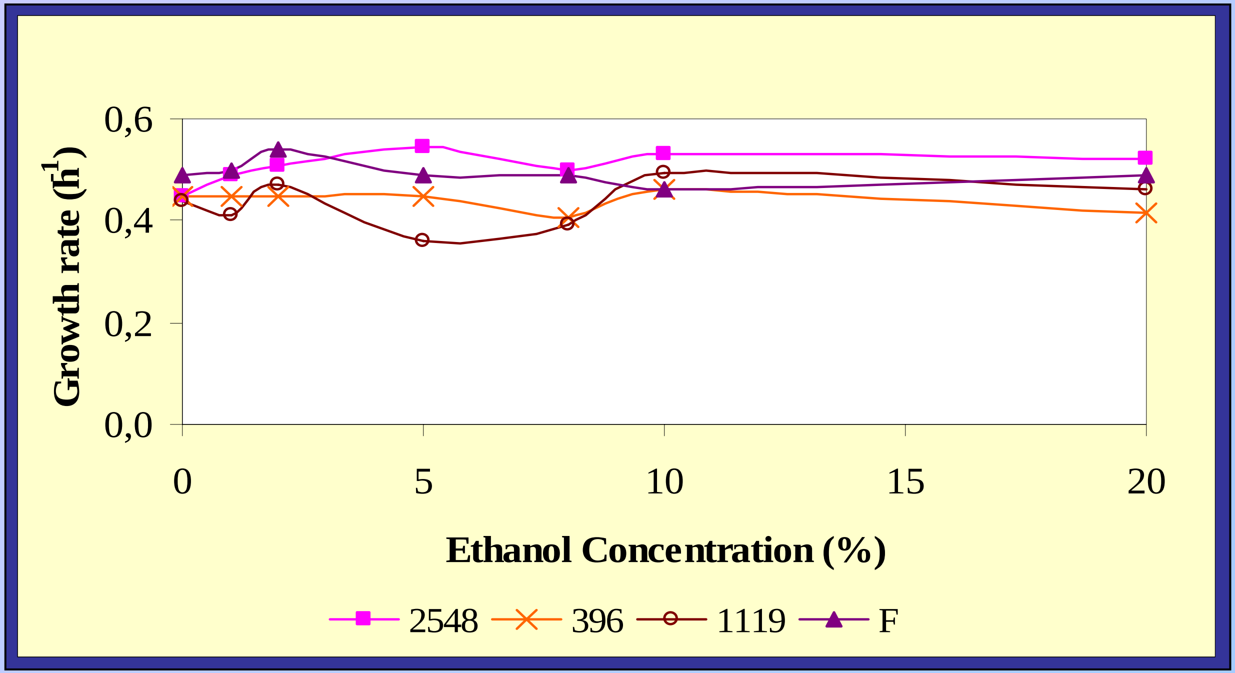
➢Strain Selection and Batch Tests



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

all strains showed good tolerance to ethanol.

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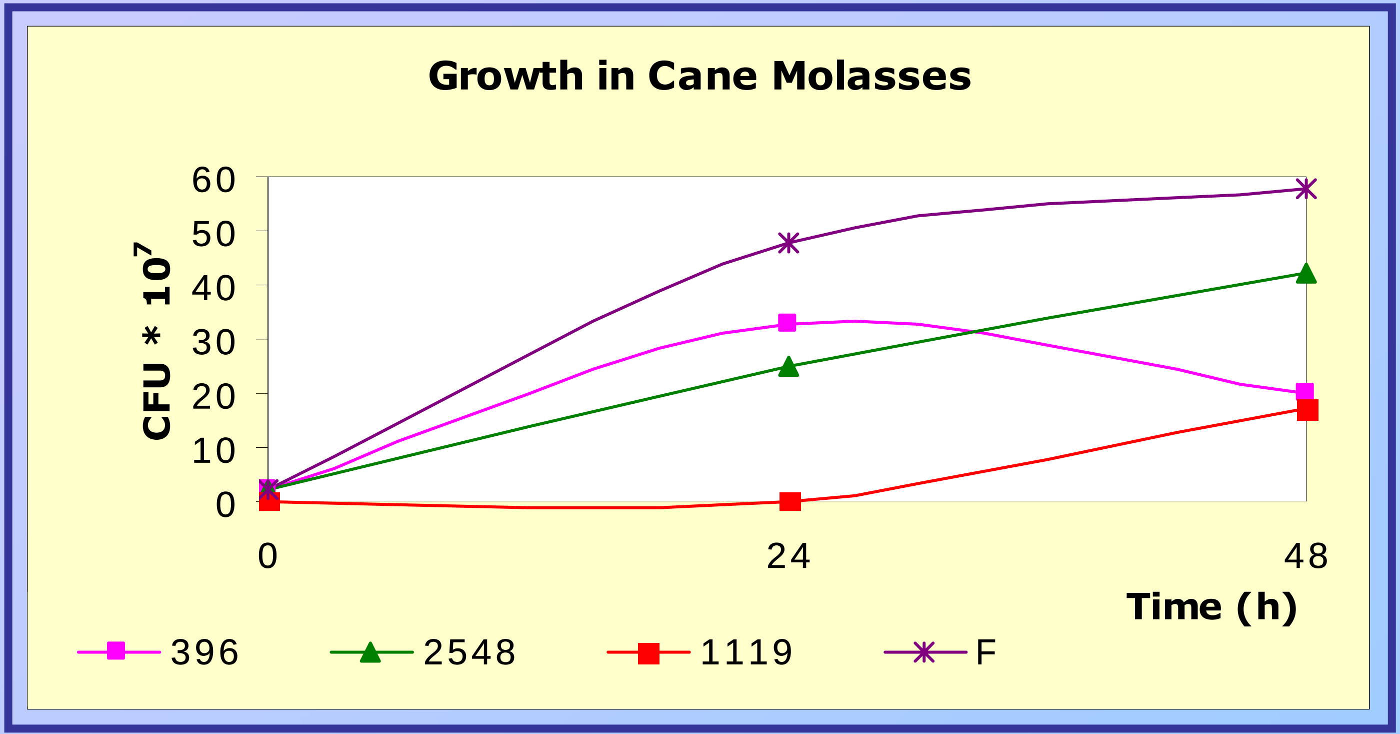
➢Strain Selection and Batch Tests



Batch Conditions:
-Initial sugar: 120 g/L
-Temperature: 28ºC
-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.

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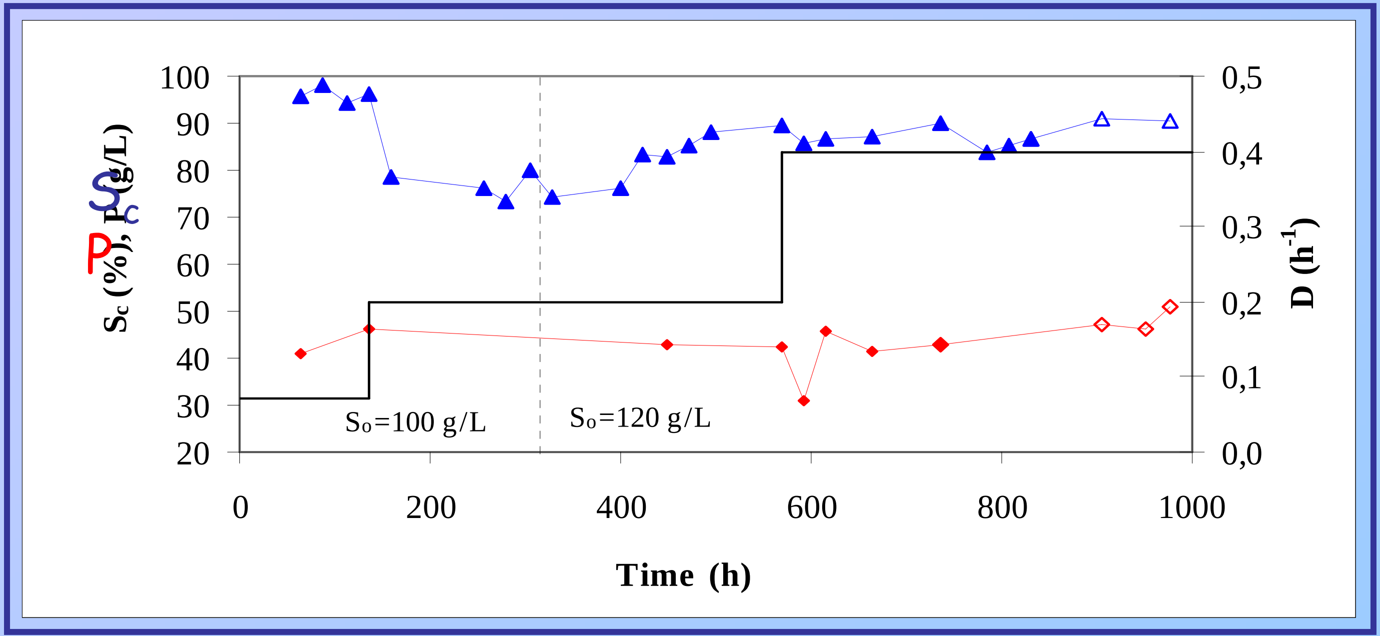
➢Strain Selection and Batch Tests:

Only strains F and 1119 showed good flocculation ability, which is very important in FBR fermentations with no solid suport to retain biomass;
1119 has more dense flocs than F.

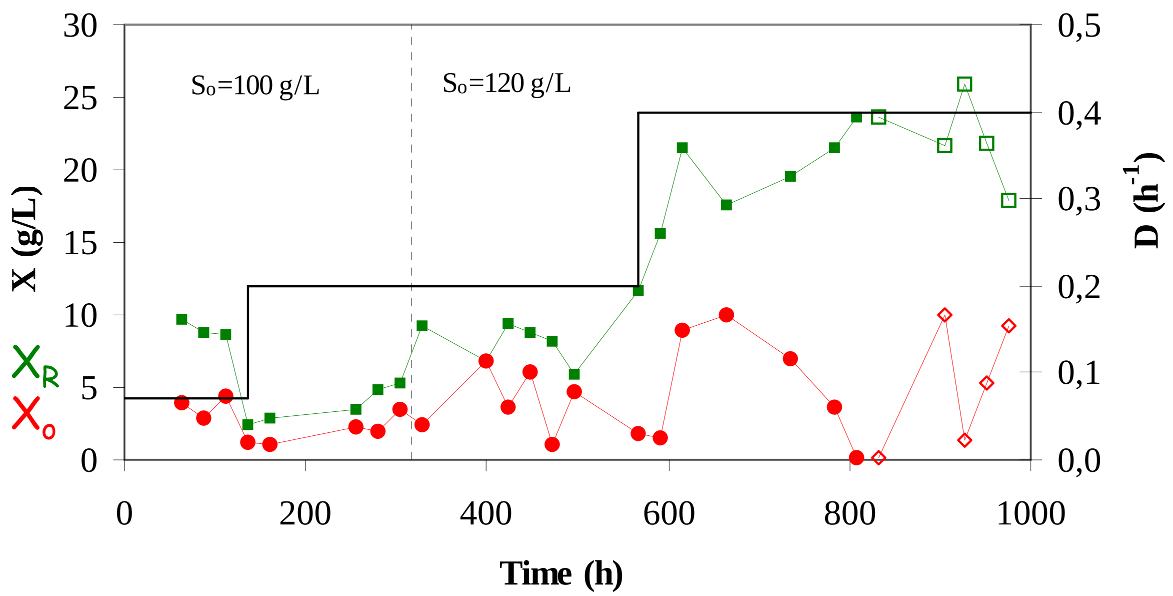
Due to higher maximum specific growth rates, ethanol yields and flocculation ability, strains F and 1119 are suitable for ethanol production in FBR

➢Continuous FBR Tests: Strain F

-Defined sucrose media
-Temperature: 28ºC



➢Continuous FBR Tests: Strain F



There is a good capacity for retention of biomass

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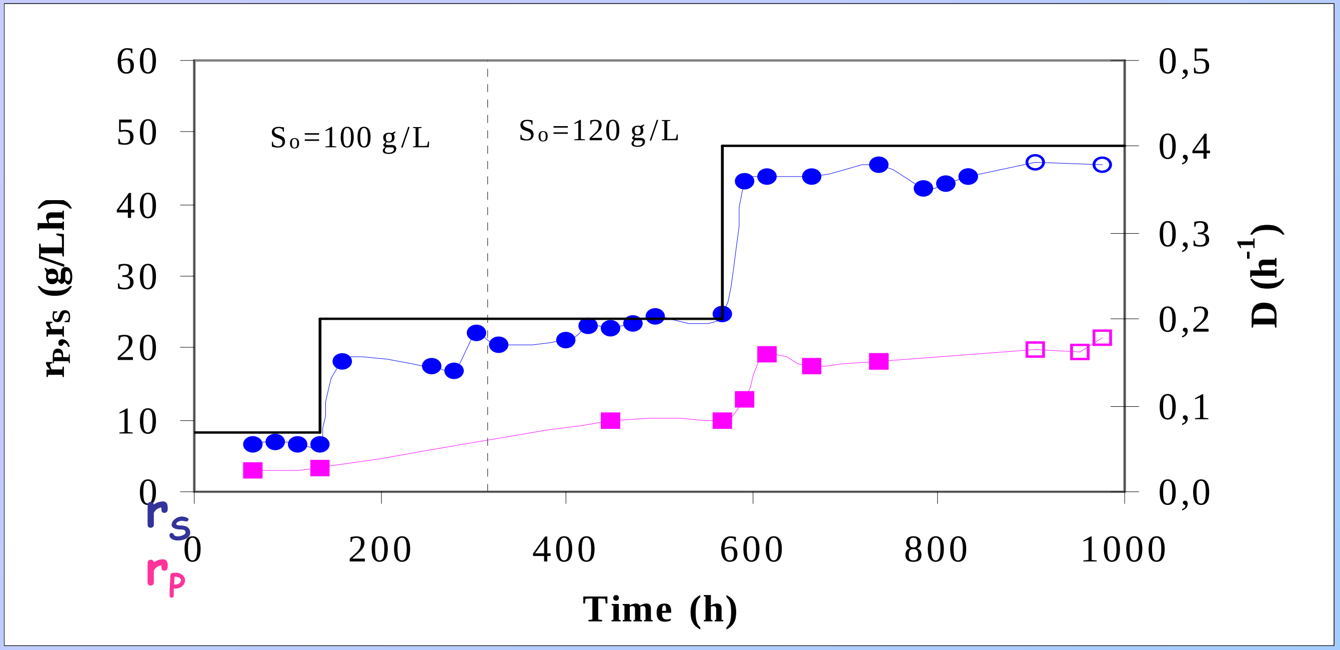
➢Continuous FBR Tests: Strain F



$Y_{P/S}$=0.47
X_R≈25 g/L
X_o <5 g/L

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

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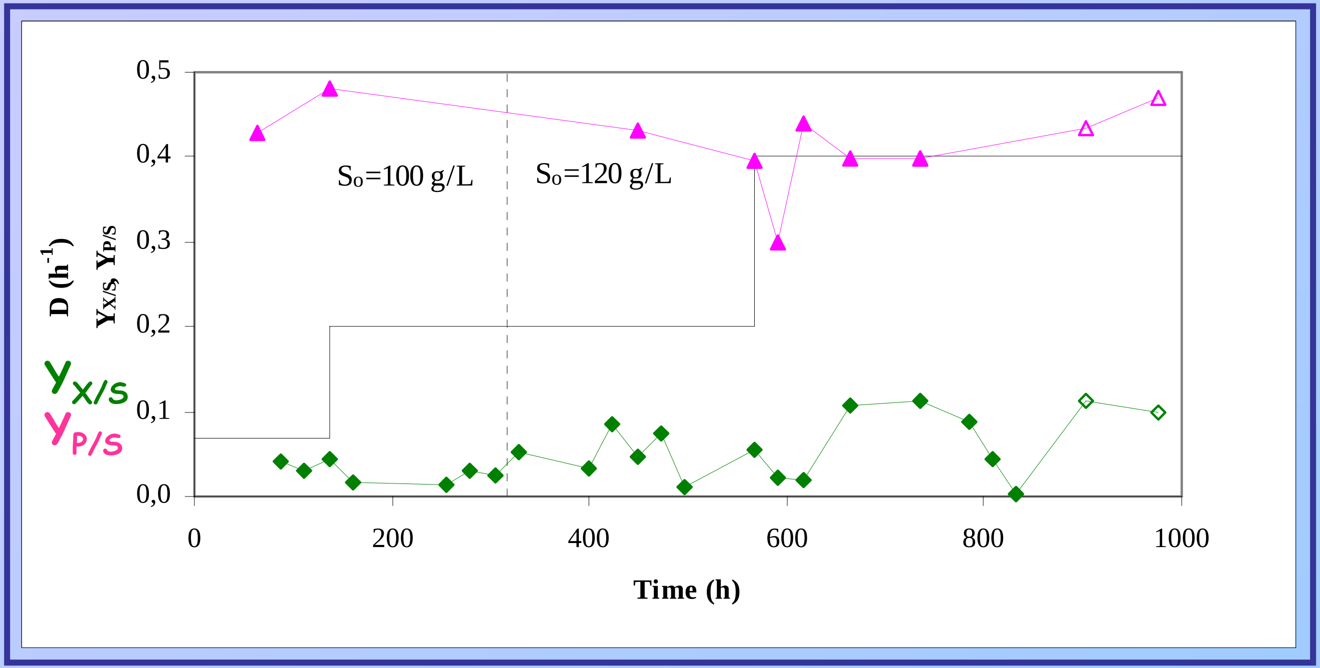
➢Continuous FBR Tests: Strain F



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

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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).

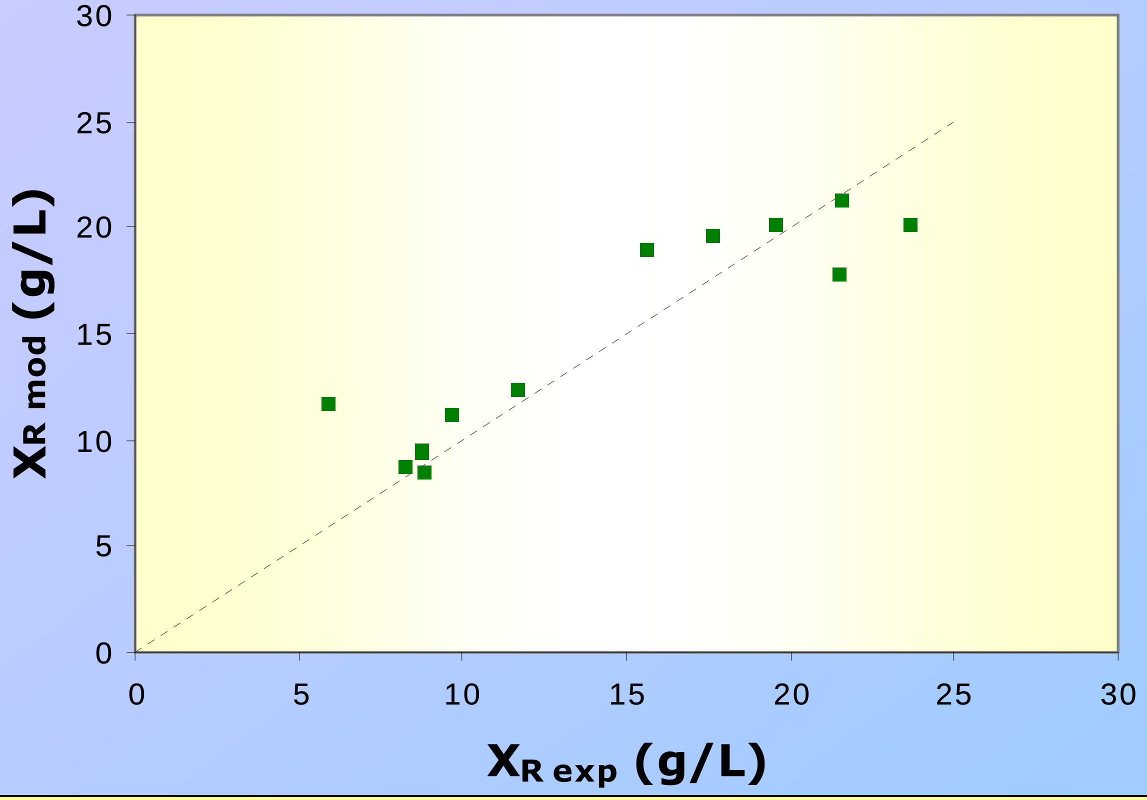
➢Construction of a biomass inferential sensor:

✓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).

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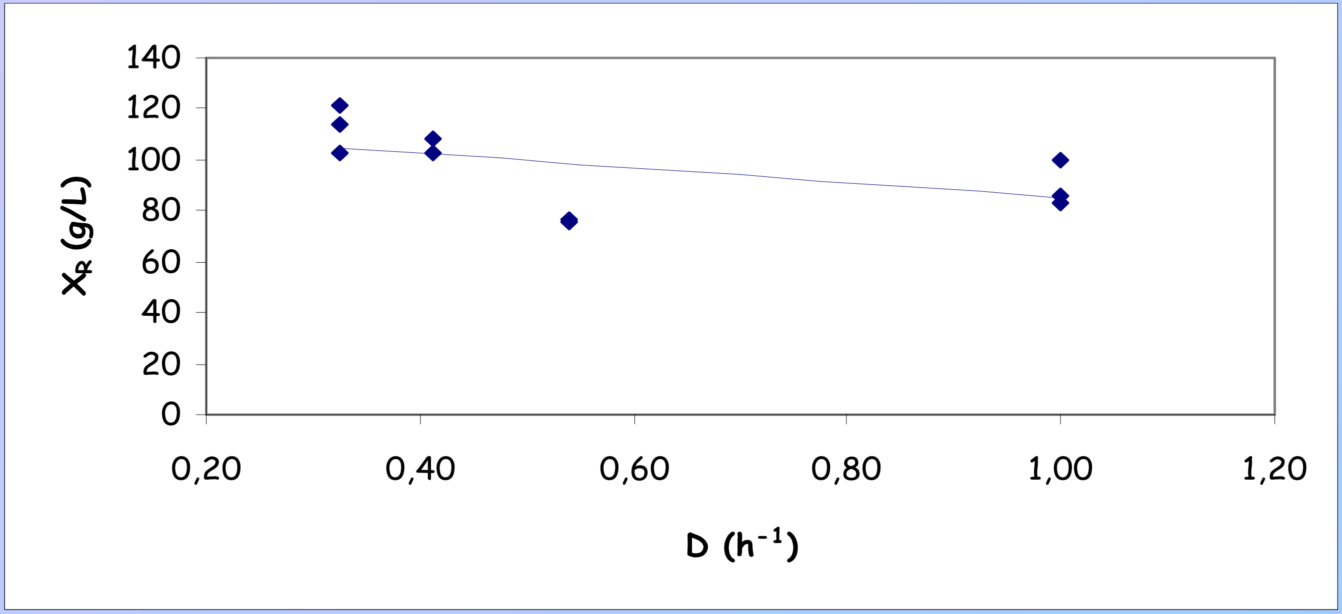
➢Continuous FBR Tests: Strain 1119



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

At all tested dilution rates cells always showed viability ab-ove 90%.

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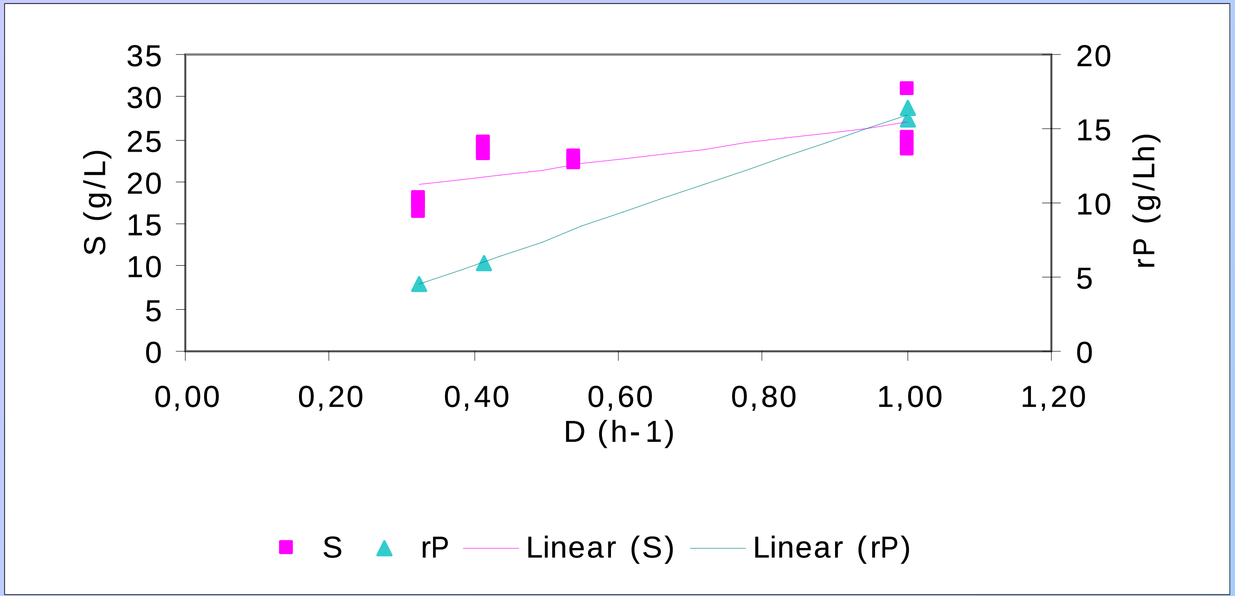
➢Continuous FBR Tests: Strain 1119



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**.

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➢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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Tomar, 5-10 March 2006