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CMC-ase Production by the Newly Isolated *Myceliophtora sp.* I-1D3b in Packed Bed Solid State Bioreactor

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Prof. Eleni Gomes – Microbiologist
Prof. Roberto da Silva – Biochemist
Prof. Mauricio Boscolo – Analytical Chemist
Prof. Gustavo Bonilla – Biochemist
Prof. João Thoméo – Chemical Engineer

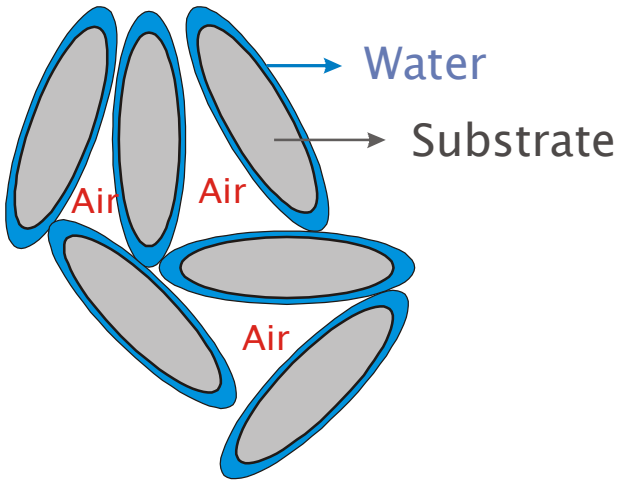
Researches

- Enzyme production: cellulases, pectinases, lipases, amylases, proteases, phytase…
- Enzyme technology
- Bioethanol
- Biodiesel
- Soil bioremediation
- Bioreactor engineering for SSF
- Animal feed
- Fungal isolation and screening

SSF: definition

- SSF: Enough water to keep microbial metabolical activity

Without exceeding the water retention capacity of the porous media

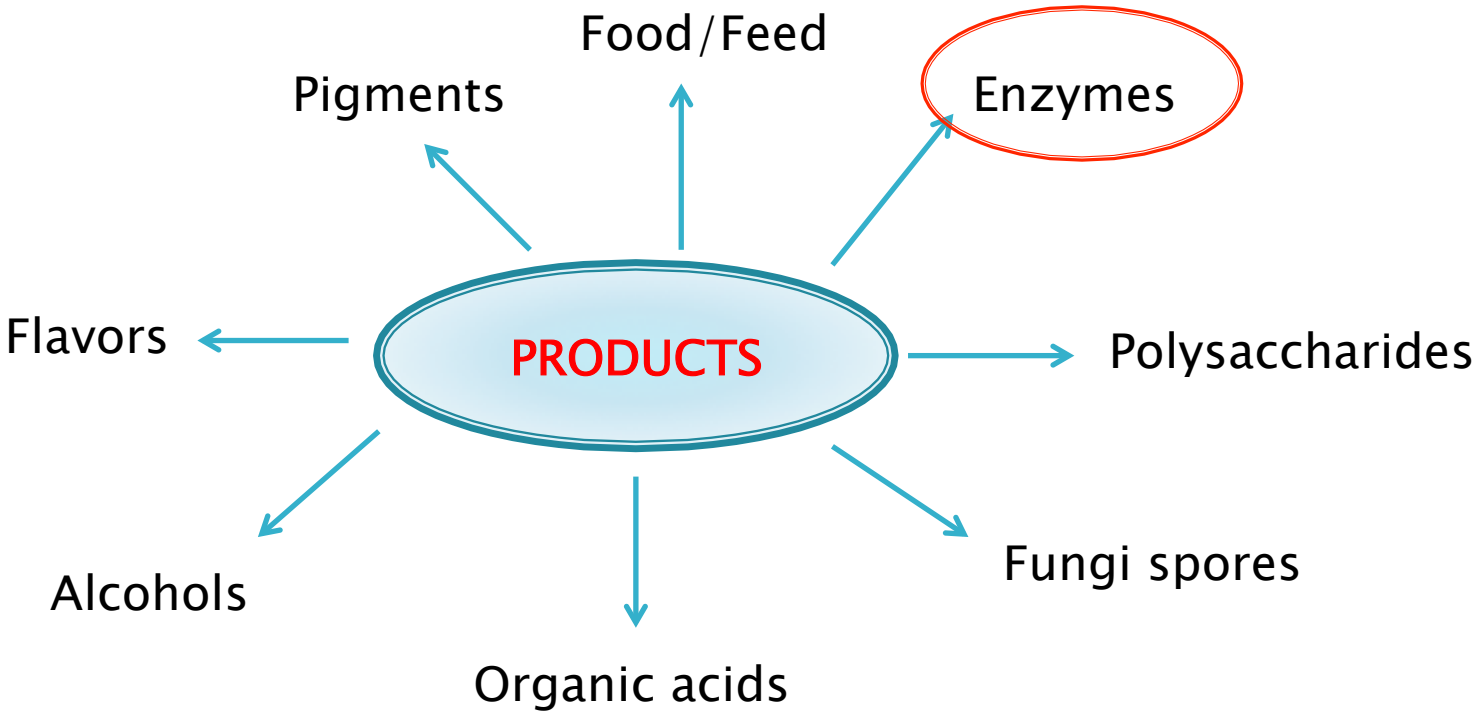


WHY SSF?

- **Disadvantages when compared to SmF**
  - Heterogeneity
  - Poor automatic control
  - Lack of equipments

- **Advantages when compared to SmF**
  - Use of solid waste
  - When the product will be use as a solid (no extraction)
  - Small units
  - Filamentous fungi

WHY SSF?


Food/Feed
Pigments
Enzymes
Flavors
PRODUCTS
Polysaccharides
Alcohols
Fungi spores
Organic acids

WHY SSF?

Algae field

Biethanol /Biodiesel production

Aquaculture center

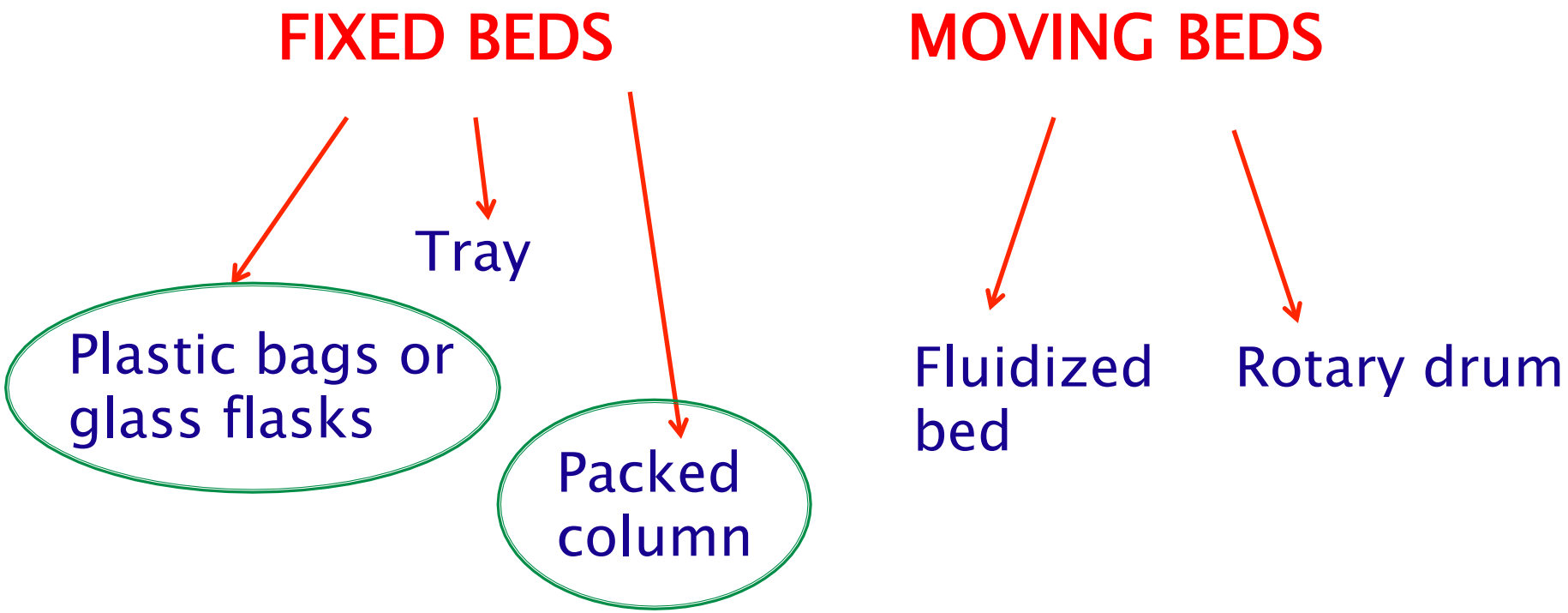
SSF

Co-generation plant

Crops and livestock

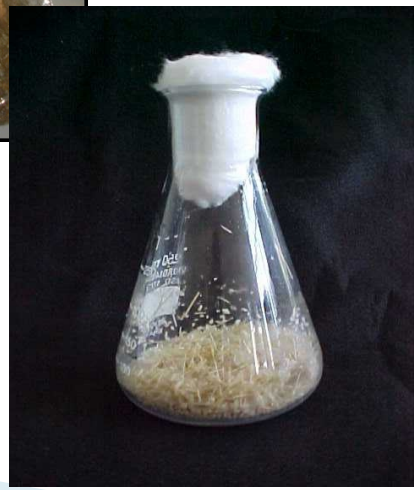
www.alltech.com

SSF Bioreactors



Function: provide the proper environment to the microorganism metabolism and avoid the entrance of contaminants

Plastic bag/glass flask

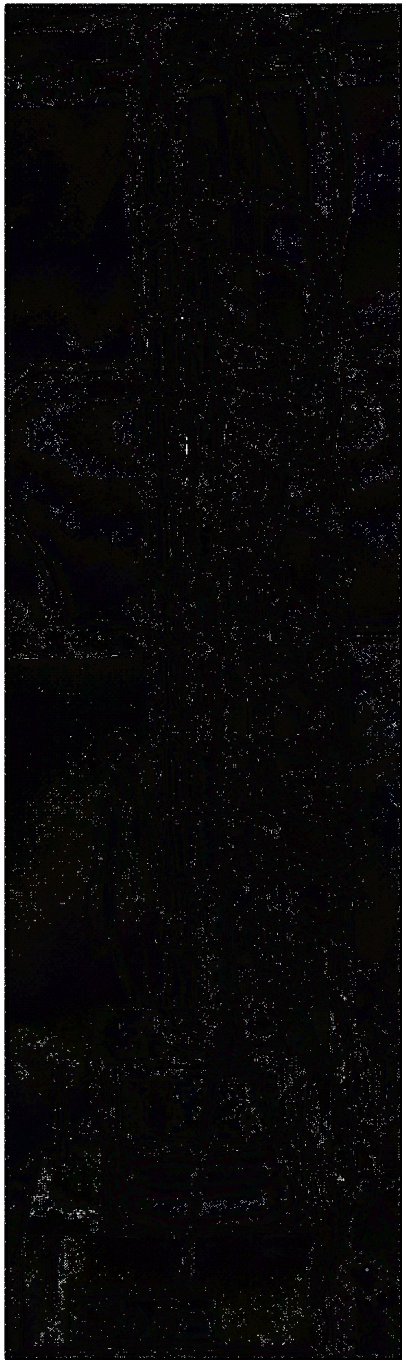


Target:

Exploratory studies

- ✓ Process viability
- ✓ Microbial adaption to the solid media
- ✓ Cultivation conditions
- ✓ Microbial growth kinetics
- ✓ Metabolites production kinetics
- ✓ Nutrient consumption kinetics

Variables: solid media composition; solid media size; moisture content; temperature, nutrient solution composition



Bench Scale Packed bed

Material: stainless steel/aluminum
Capacity: low
D: 2 ~ 5cm
L: 10 ~ 200cm
Structure: modular
Flexibility: little
Cost: low

Target: Scale-up exploratory studies

- ✓ Forced aeration effect on microbial growth and metabolite production;
- ✓ Microbial adaptation to the operational conditions
- ✓ Effect of the operational conditions on the porous medium
- ✓ Heterogeneity occurence

Variables: air flow rate; air inlet humidity and temperature; wall temperature, solid media composition; solid media size; nutrient solution composition; **reactor length**

Objectives

- Apply the newly isolated *Myceliophtora sp.* I-1D3b to the fibrolytic enzyme production in small scale (plastic bag), using sugar cane bagasse (SCB) and wheat bran (WB) as substrates;
- Verify the influence of time, temperature, SCB/WB proportion, and moisture content on the CMC-ase production;
- Apply the optimal results obtained in the small scale to a bench scale packed bed, having the temperature and the air flow rate as variables.

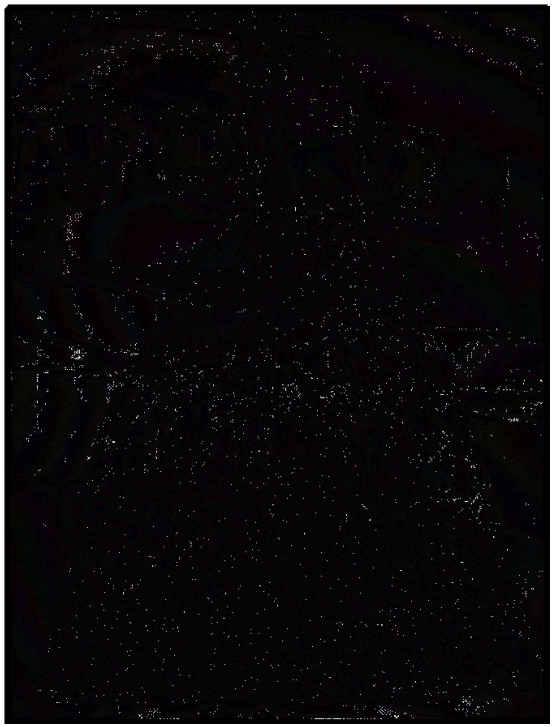
Materials and Method

- **The microbe**
  - *Myceliophtora sp.* I-1D3b: isolated from sugar cane bagasse piles in an industrial ethanol producer;
  - Cotton like filamentous mycelium;
  - Spore cocentration: 10^7/mL

- **Substrates:**
  - Sugar cane bagasse: provided by Usina Vale, Onda Verde – SP. Washed, sieved, oven dried to 4% MC (w.b.)
  - Wheat bran: bought from local retailers. MC 11%.

Materials and Method

- **Fermentation in plastic bags**



Variables

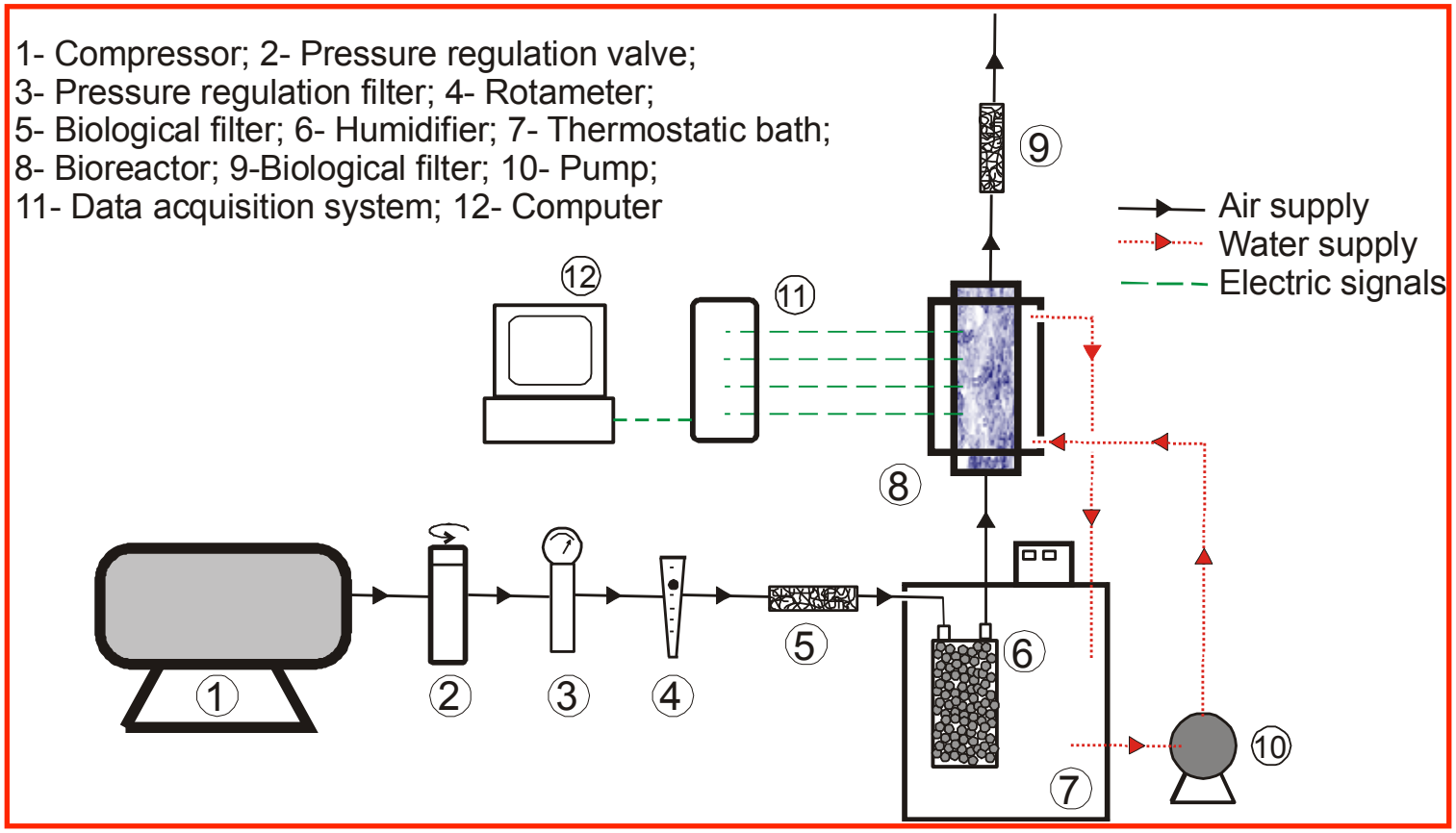
Temperature: 40, 45, 50°C
SCB/WB proportion (weight): 1:1, 7:3, 9:1
Moisture content: 75, 80, 85%
Time: 48 to 360h

- **Microbial growth**

Temperature: 45°C
Moisture content: 75%
Time: 96h
SCB/WB proportion: 7:3
Method : Kjeldahl

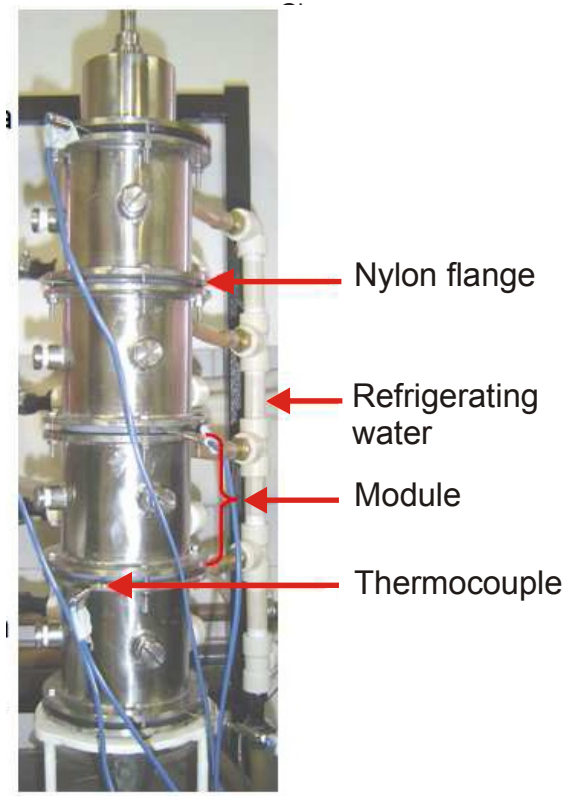
Materials and Methods

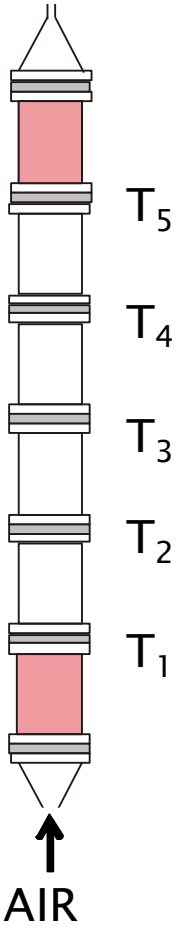
- **Bench scale packed bed**
  - Experimental set up



Materials and Methods

- **Bench scale packed bed**
  - Modular structure (10cm each module)





Physical characteristics
Material: stainless steel
D= 7.62cm (3")
Jacketed
Refrigeration: water

Fixed conditions
Moisture content: 75%
SCB/WB proportion: 7:3
Length: 40cm (+20cm)

Variables
Temperature: 45, 50°C
Flow rate: 80, 100, 120L/h
Time: 48 to 144h

Materials and Methods

- **Bench scale packed bed**
  - Modeling

$$\rho_s Cps\left(\frac{\partial T}{\partial t}\right) + (Cpg + \Phi_g \lambda)\rho_a G\left(\frac{\partial T}{\partial z}\right) = Kb\left(\frac{\partial^2 T}{\partial z^2}\right) + \rho_s (1-\varepsilon) Y \frac{\partial X}{\partial t}$$

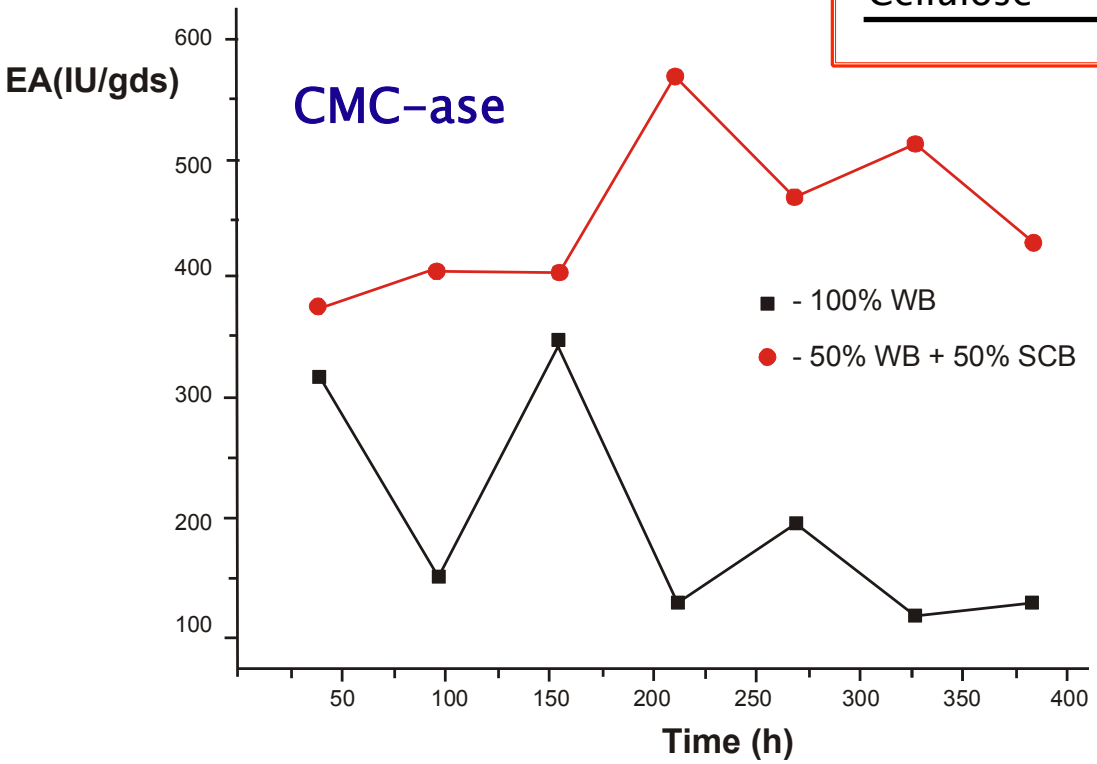
ρ_s bed density
ρ_a moist air density
K_b bed effective conductivity
ϵ bed porosity
z axial coordinate
Y energy metabolically generated
X biomass generated
t time

Fanaei and Vaziri,
Chem. Engn. & Proc., 48, 2009.

Results: plastic bags

- **Preliminary results**
  - Substrate composition (%)

	SCB	WB
Total dry solid	87,46	88,03
Total protein	0,00	17,77
Lignin	8,99	7,04
Hemycellulose	33,39	43,03
Cellulose	45,97	15,22

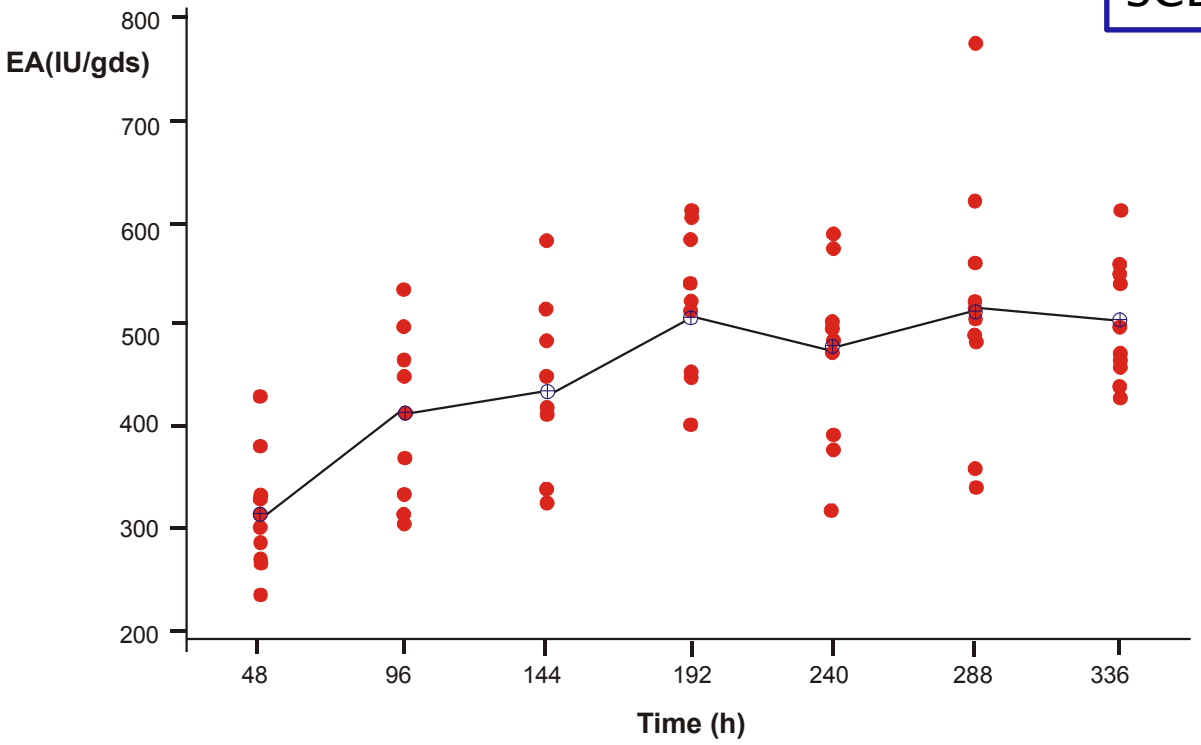


Experimental condition
Temperature: 45ºC
MC: 75%

Results: plastic bags

- **Reproducibility experiments**

Experimental condition
Temperature: 45°C
MC: 75%
SCB/WB: 7:3



Ten independent experiments

Results: plastic bags

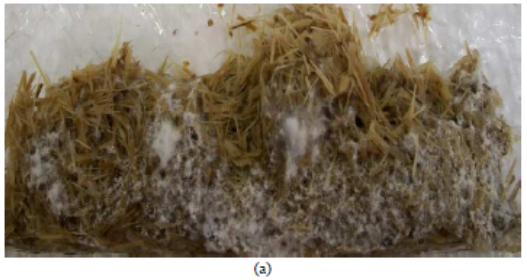
- **Temperature and SCB/WB proportion influence**

Factor	DF	TSS	ASS	F	P
Fermentation time 48h					
Temperature	1	3781	3780,8	0,43	0,52
SCB/WB	1	16280	16280,3	1,87	0,193
Interaction	1	392	392,0	0,05	0,835
Residual	14	121767	8697,6		
Fermentation time 96h					
Temperature	1	17787	17787,0	1,32	0,27
SCB/WB	1	9861	9861,0	0,73	0,407
Interaction	1	5151	5151,0	0,38	0,546
Residual	14	188627	13473		

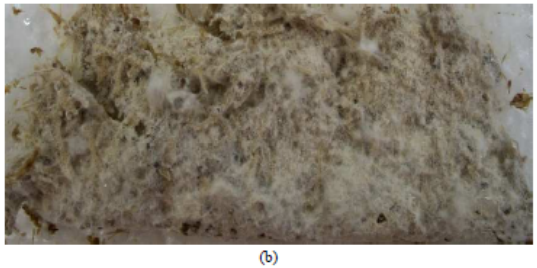
No significant factor!

Results: plastic bags

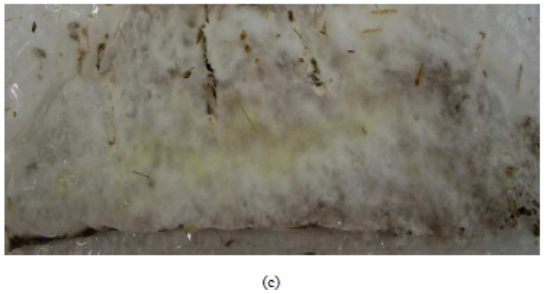
- Temperature and SCB/WB proportion influence



SCB/WB 9:1

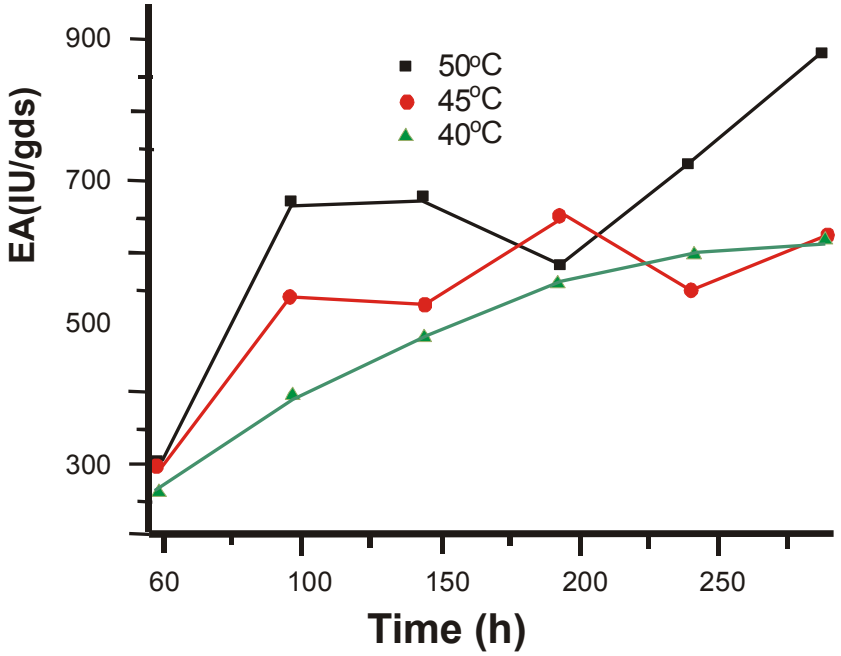


SCB/WB 7:3



SCB/WB 1:1

For the Packed Bed experiments, SCB/WB proportion was fixed in 7:3



Results: plastic bags

- **Moisture content influence**

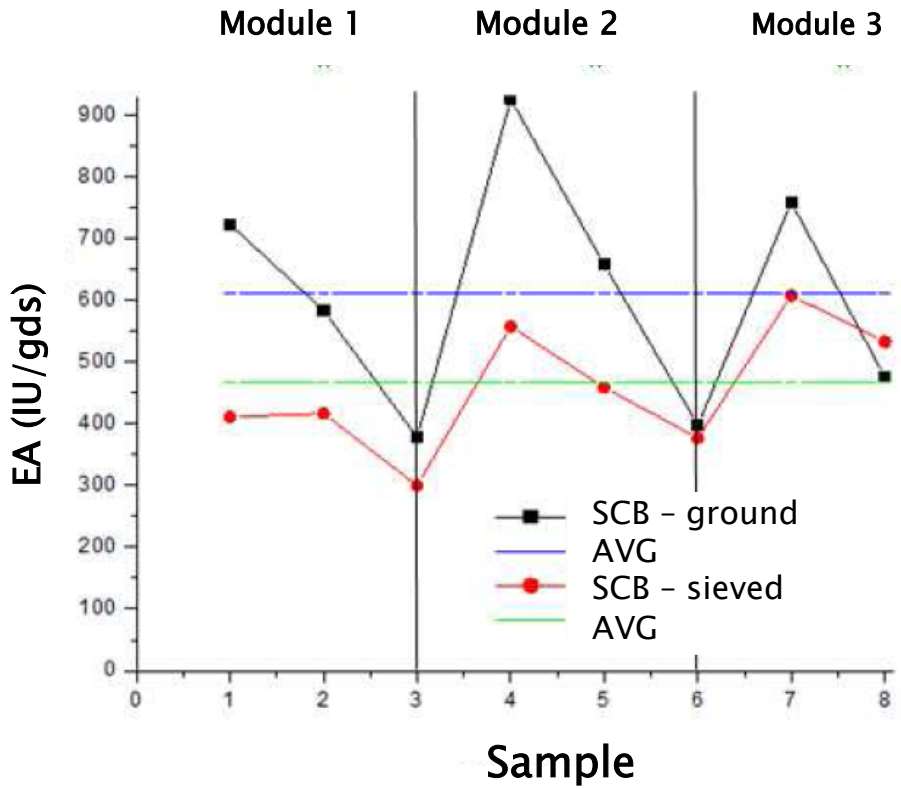
T = 45°C
SCB/WB proportion: 7:3

Time (h)	Initial MC (%)	CMC-ase activity (IU/gds)	
	85	239	218
48	80	302	289
	75	277	289
	85	227	260
96	80	336	314
	75	199	267

Tukey test: MC was not significant!

Results: packed bed

- **Preliminary results**



Packing technique: "shaking"

Cyclical Ez Acticity

Bagasse preparation: sieved or ground

- Ground best results
- Higher superficial area.
- About 30% more bagasse (weight) when ground

	Sieved	Ground
AVG	467	613
SD	95	172
Runs	8	8

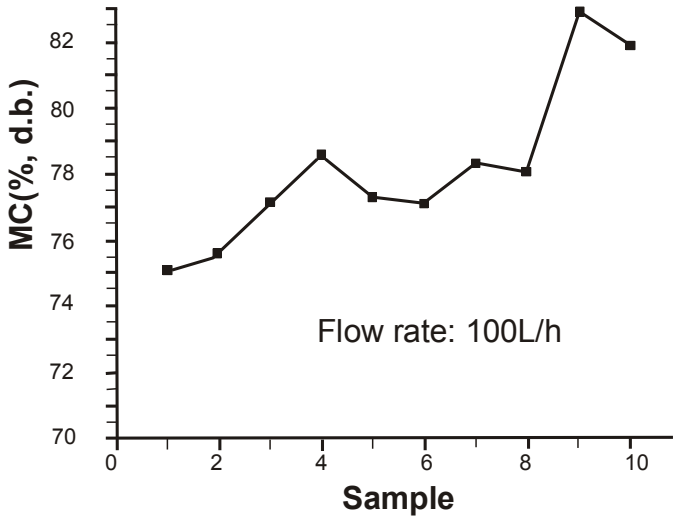
No statistical difference

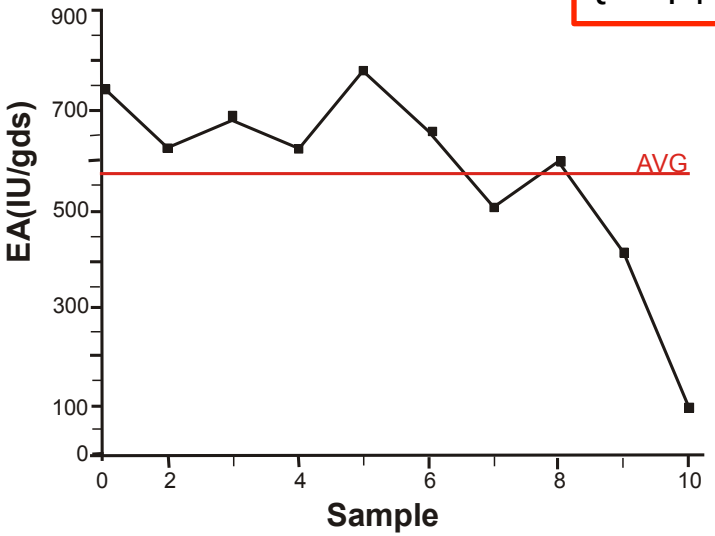
T = 45ºC, MC = 75%, t = 144h, Flow rate = 80L/h

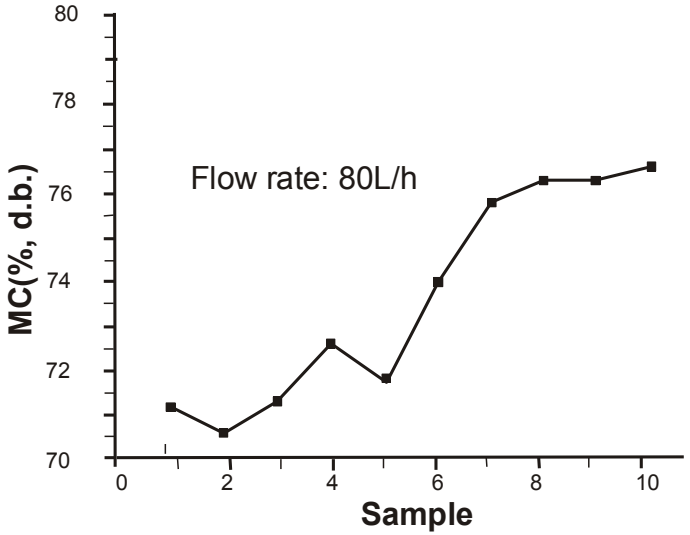
Bench Scale Packed bed

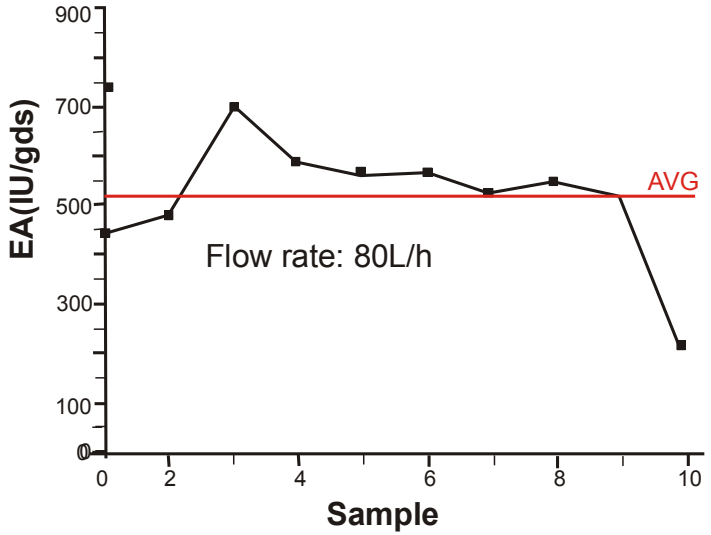
- Temperature and flow rate influence

Myceliophtora sp I-1D3b
SCB:WB (7:3)
T = 50ºC
MC = 75%
t = 144h





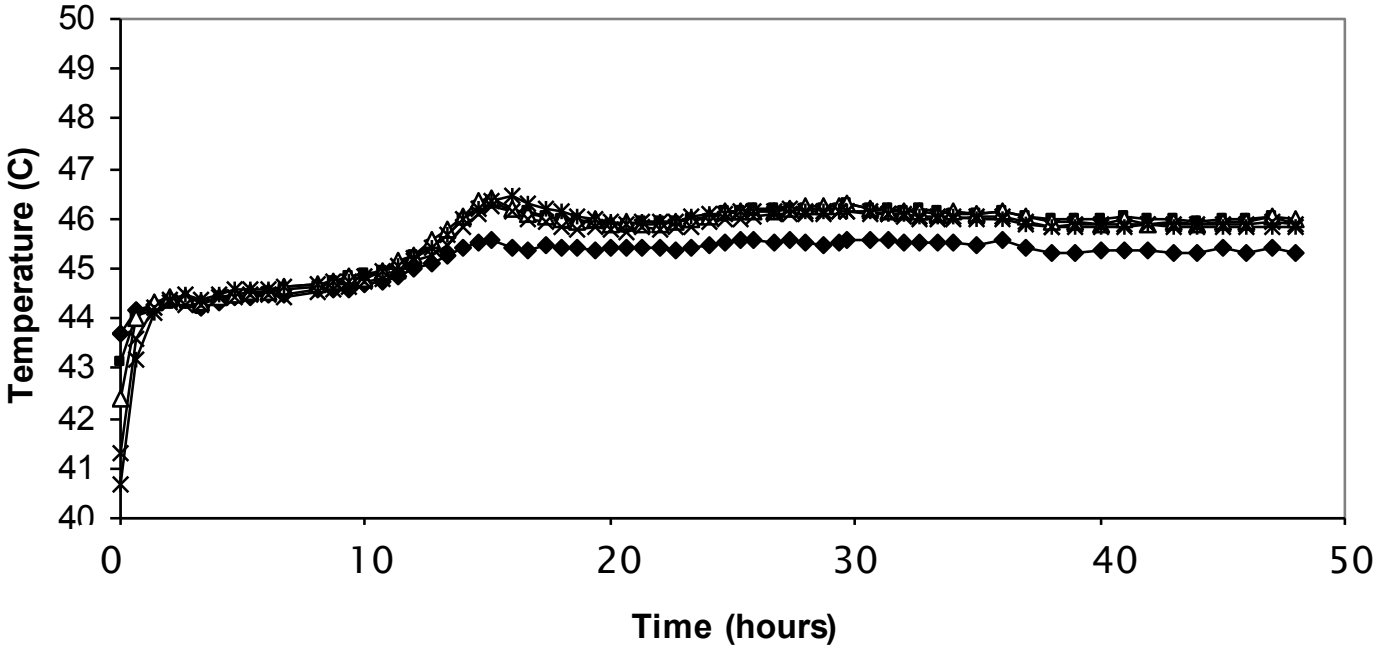




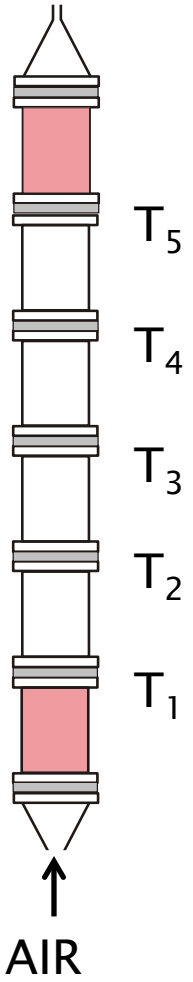
No statistical influence of temperature and flow rate

Bench Scale Packed bed

- Temperature profile

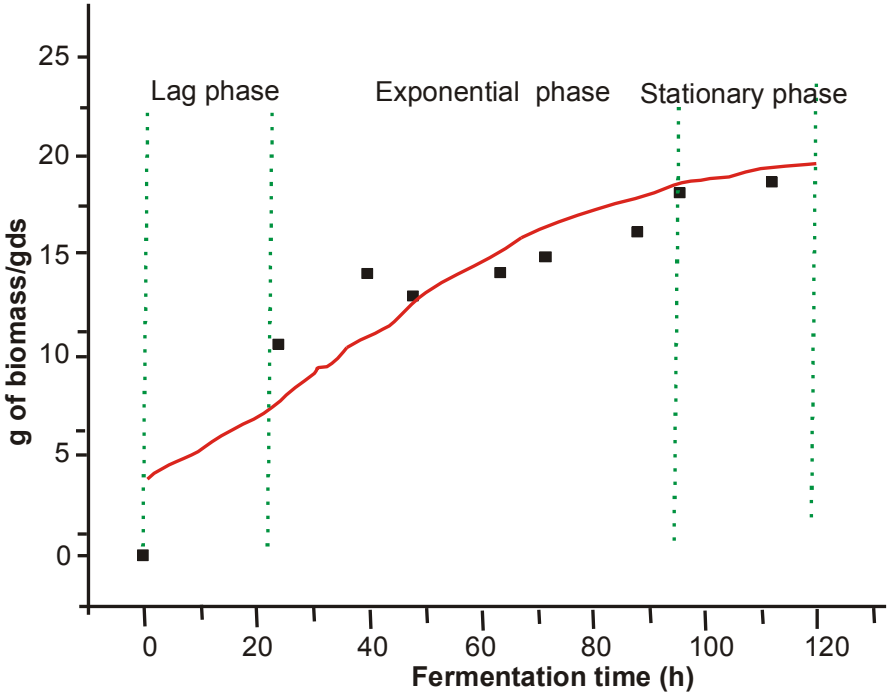


Myceliophtora sp I-1D3b
SCB:WB (7:3)
T = 45°C
MC = 75%
t = 144h



Bench Scale Packed bed

- **Modeling: fungal growth**

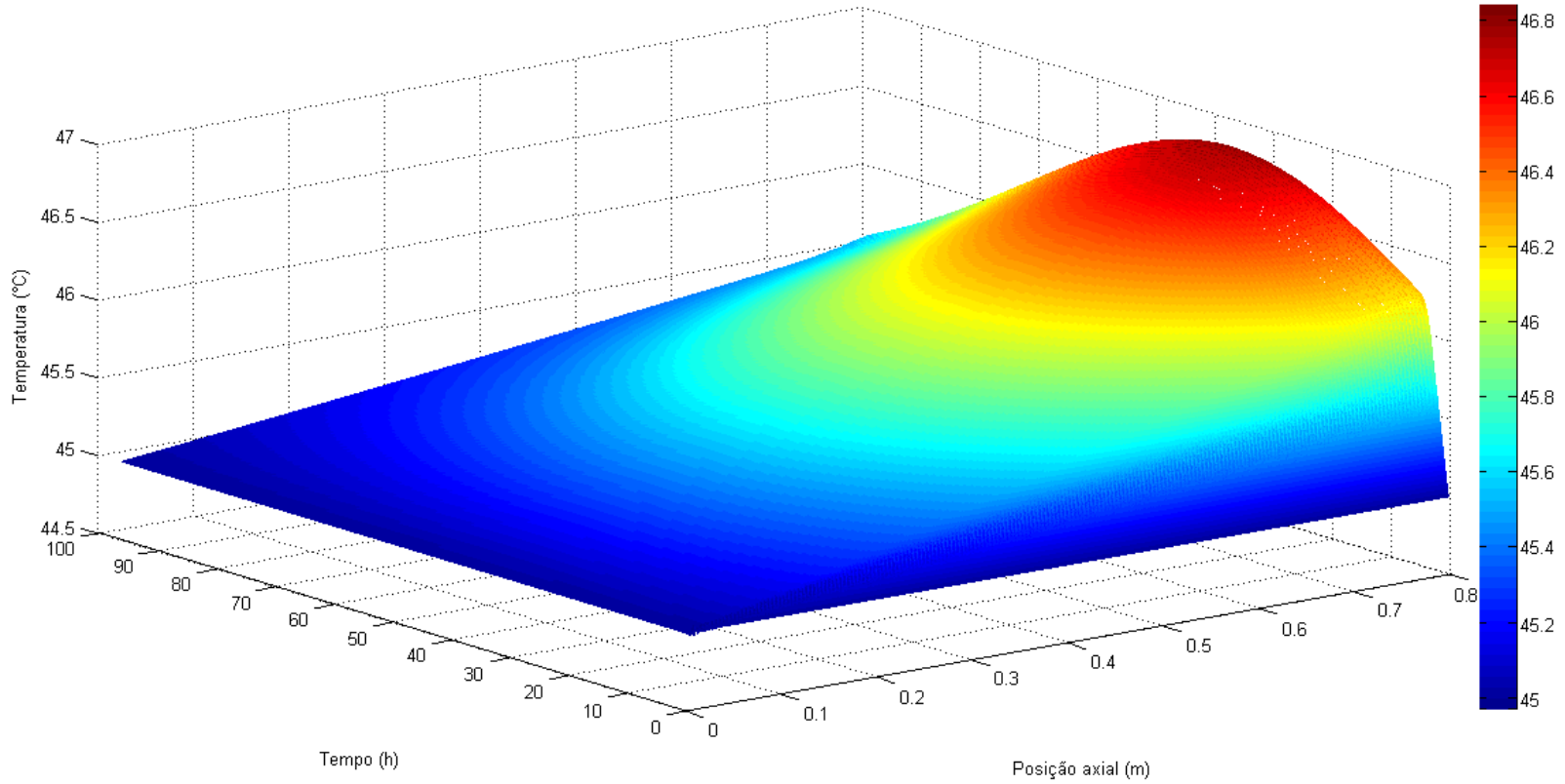


$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_m}\right)$$

Logistic model

Bench Scale Packed bed

- **Modeling: temperature profile**



Flow rate: 120L/h
T = 144h

Conclusions

- **Plastic bag experiments**
  - High CMC-ase activity
  - High variance for a same experimental condition
  - No statistical difference on EA for temperature, moisture content and SCB/WB proportion variations
- **Packed bed experiments**
  - Packing technique influences enzyme activity distribution
  - No statistical difference on EA for temperature, and flow rate variations
  - Low temperature increase during the fermentation
  - Logistic model fits well the fungal growth
  - Simulated temperature profiles satisfactorily represented the experimental results

Acknowledgments

- My students
- My colleagues
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INCT BIOETANOL

Instituto Nacional de Ciência e Tecnologia do Bioetano (INCT – Bioetanoll

Gracias!
Thank you!
Obrigado!

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