PASI 2008 Pan American Advanced Studies Institute Program on Emerging Trends in Process Systems Engineering

Wednesday, August 13: Seminar on Biosystems Engineering
Mar del Plata, Argentina

Biotechnology research for biomass-based products other than bioethanol

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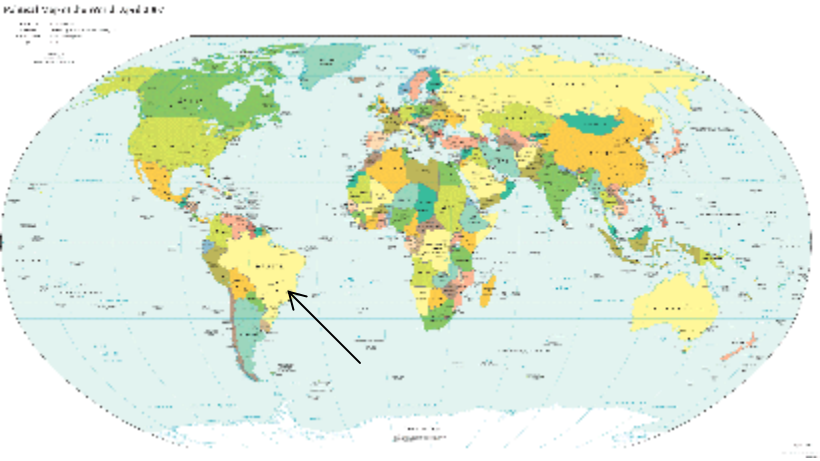

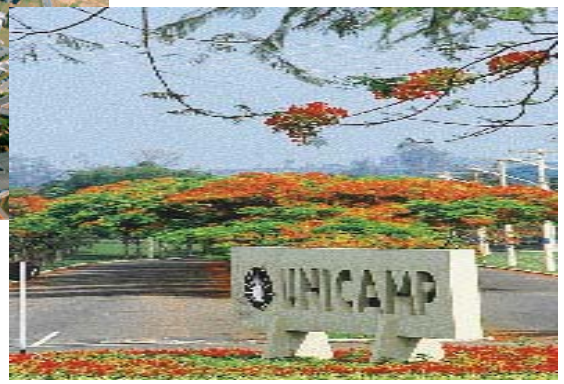

STATE UNIVERSITY OF CAMPINAS, UNICAMP created October 1966

Unicamp concentrates almost 20% of the post-graduation (Msc +PhD) of the coutry.

- **14,000 undergraduate students,**
- **14,000 post-graduate students (MsC+PhD),**
- **2,100 lecturers and professors.**
- **10,000 students on continuous education (evening /week-end courses)**
- **Chemical Engineering School → 570 bachelor and 450 PhD +MsC students**

Outline

- Sugarcane & Conventional use of sugarcane
- Sugarcane bagasse – bioethanol
- Potential for biorefinery of sugar cane
- Non-bioethanol research from sugarcane
    - Feasibility of acrylic acid production from sugars
    - Sugar acrylates by biocatalysis
    - Photobioreactors and microalgae

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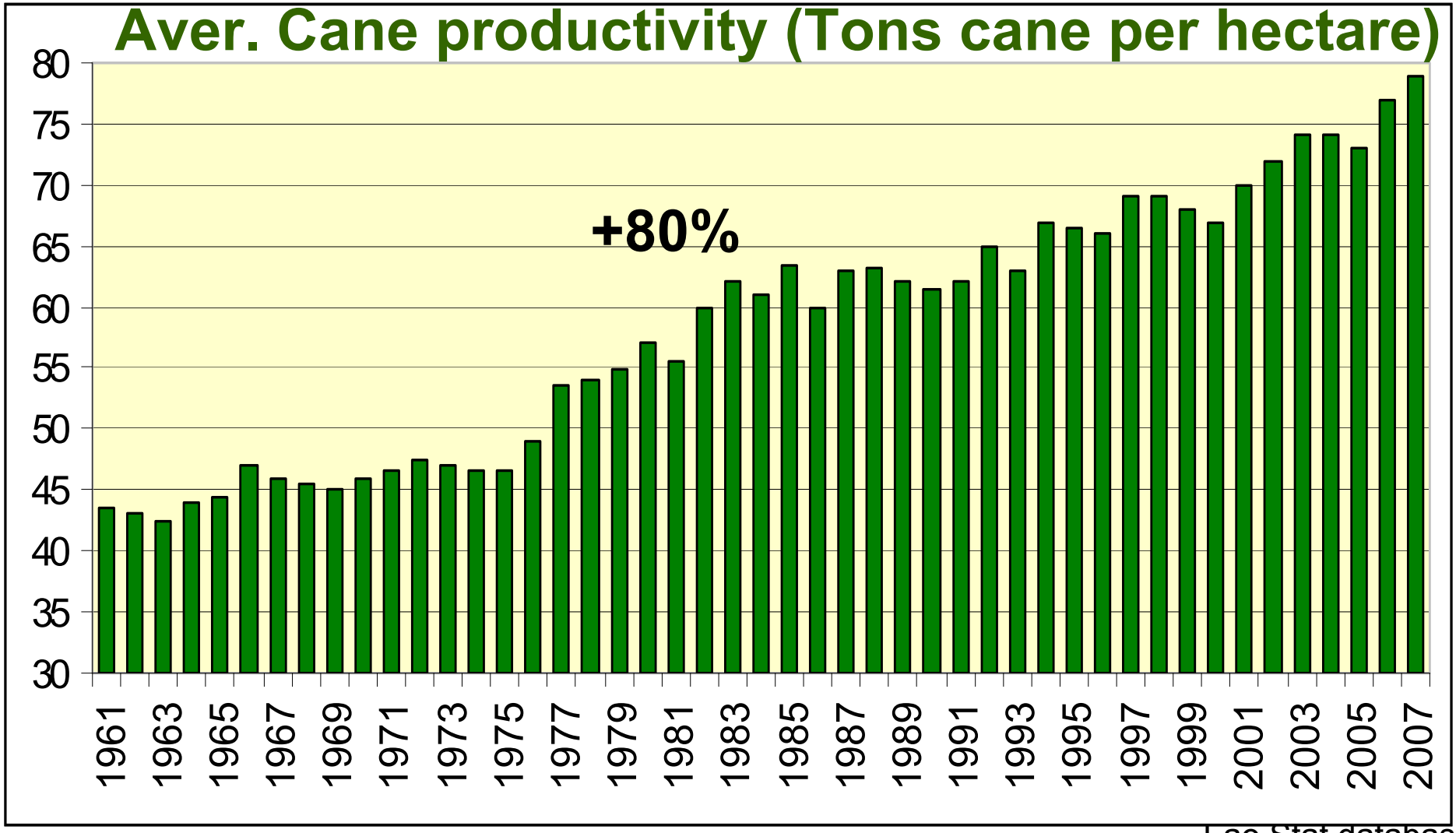
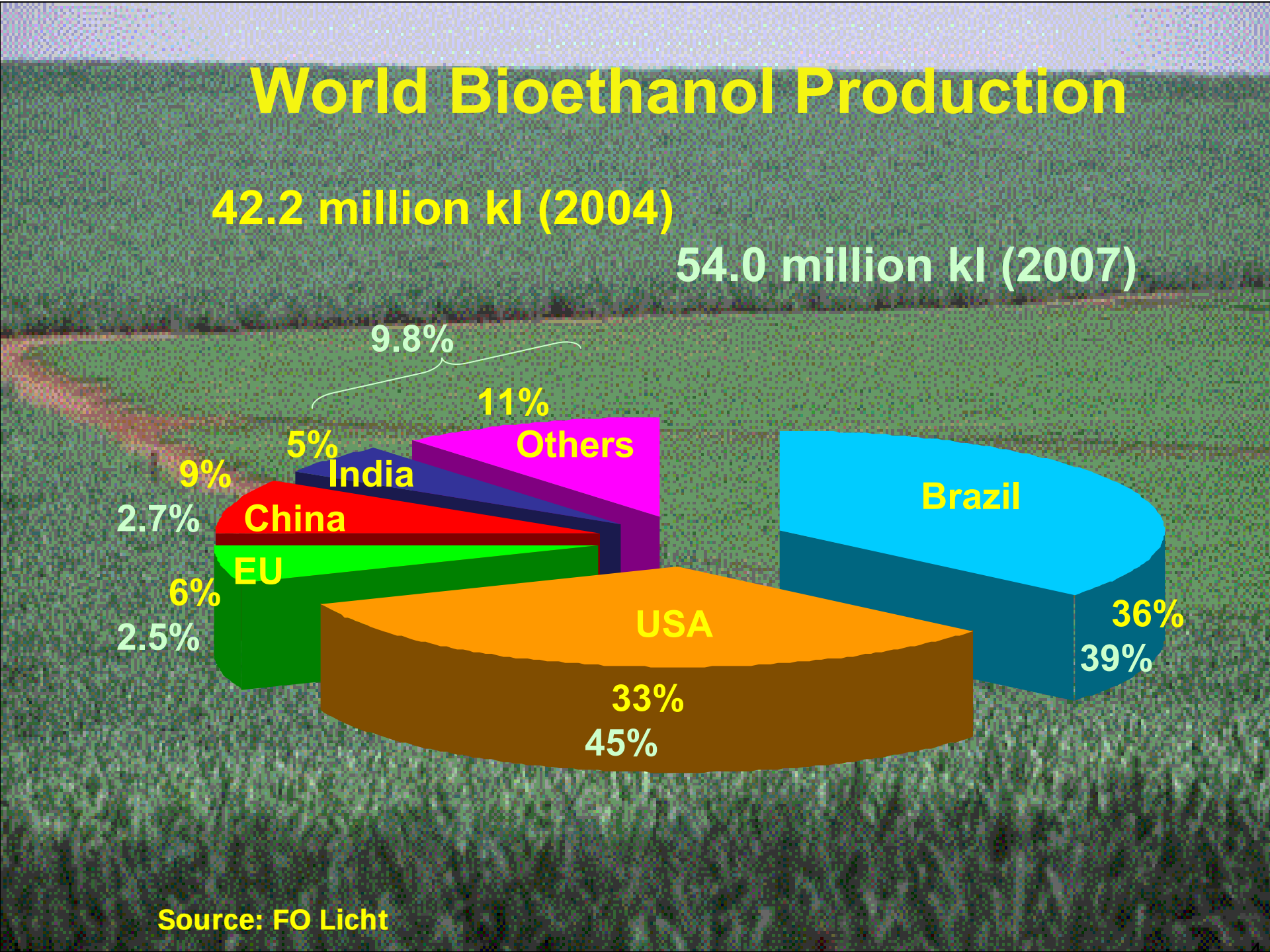
Evolution of sugarcane in Brazil



Fao Stat database

World Bioethanol Production

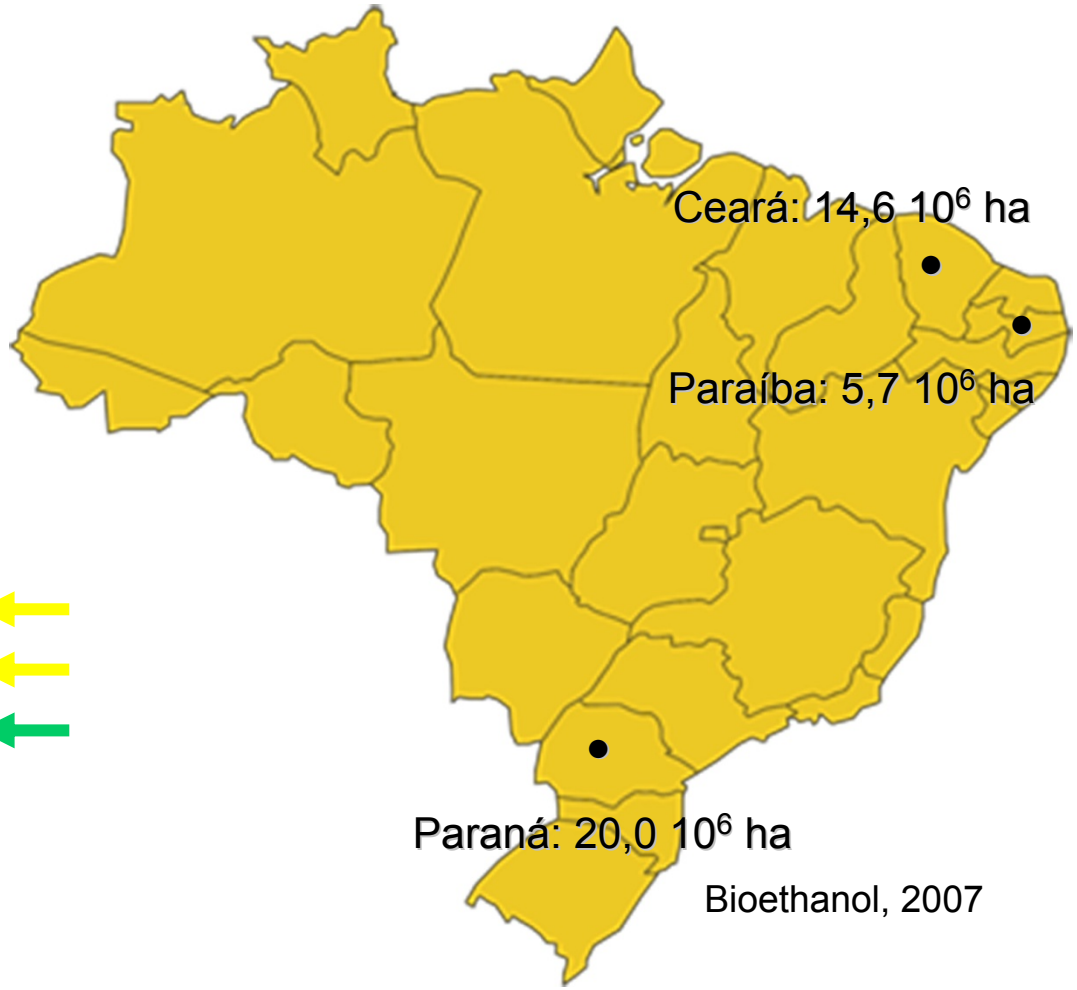


Source: FO Licht

Brazil: main crops 2004

Brazil: 851 10^6 ha

	Surface [10^6 ha]
Pasture	**150-200**
Soya	**21.5**
Corn	**12.3**
Sugarcane	**5.6**
Agric. land	**58.0**

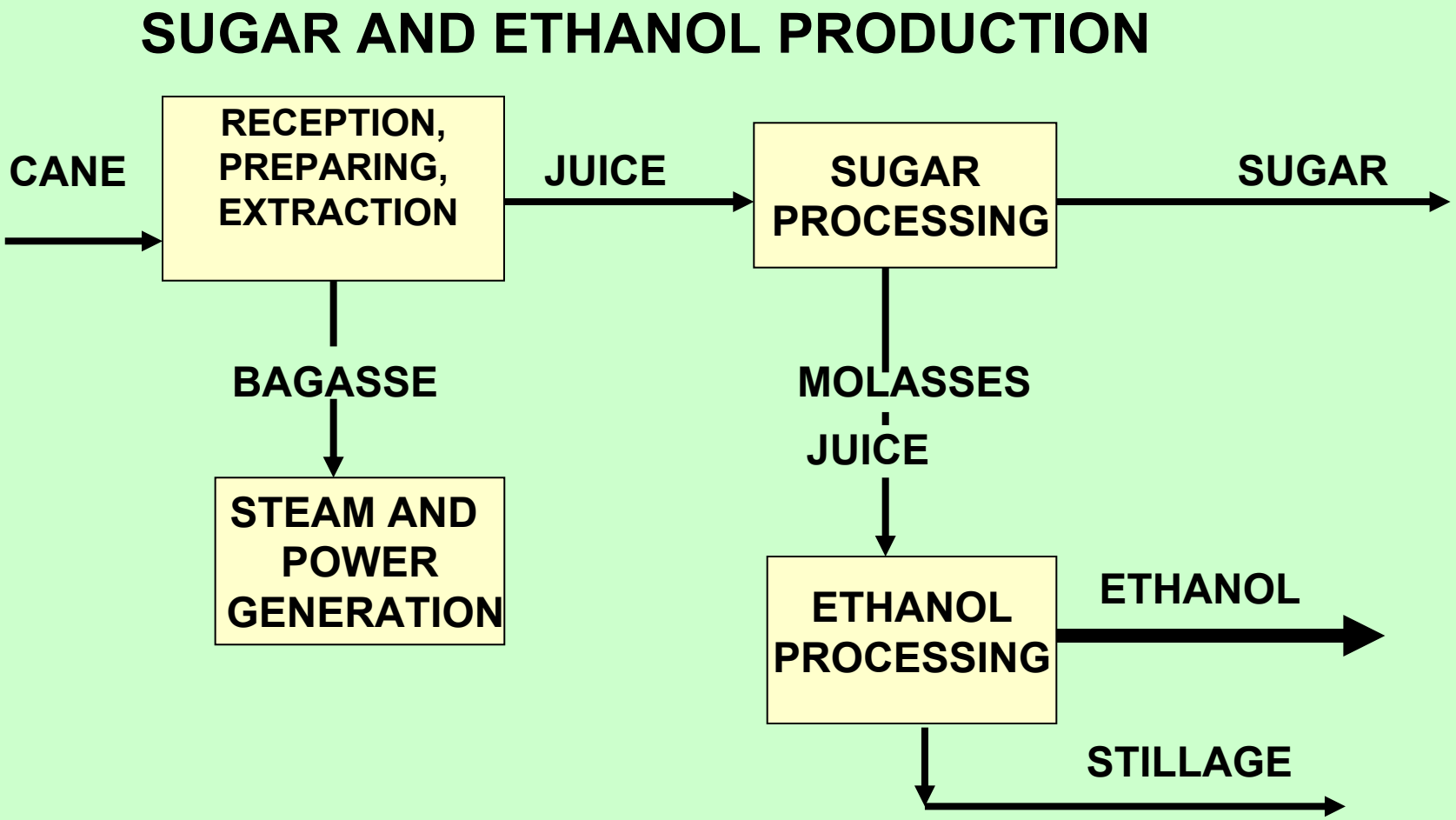


Bioethanol, 2007

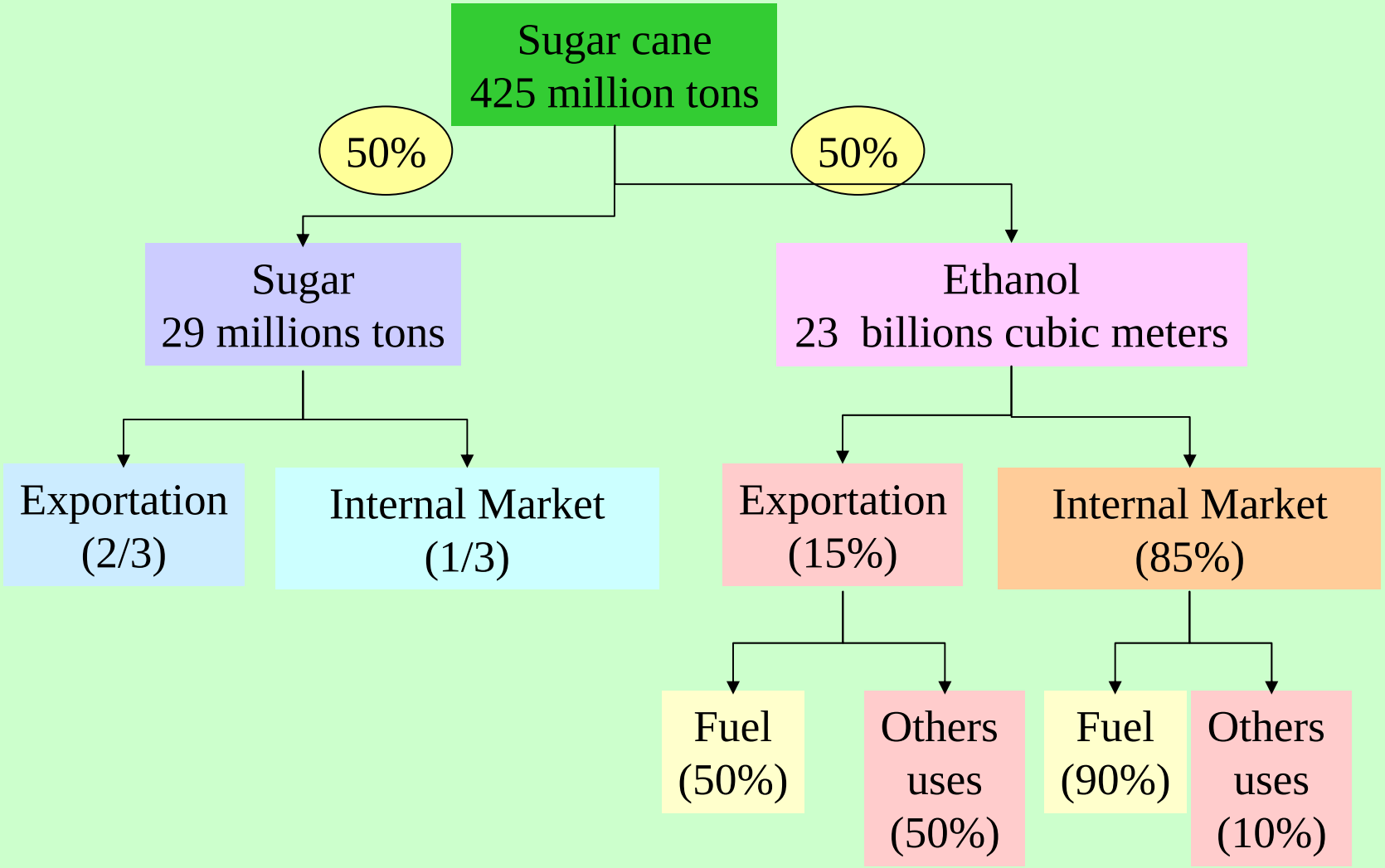
Present Location of Sugar-Etanol Mills in Brazil

Finguerutti, 2007

Existing Sugar and Ethanol Production Technology

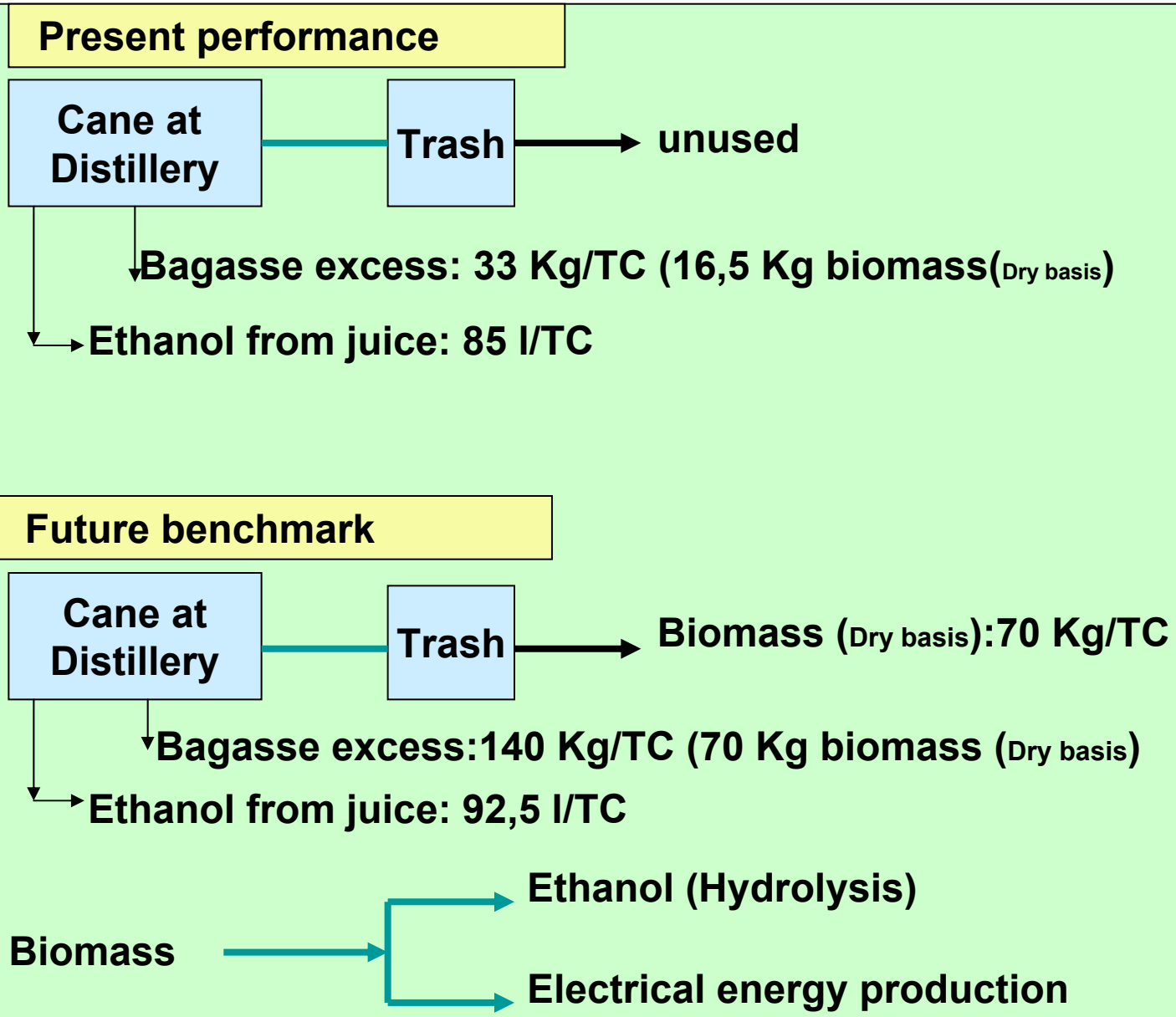


Conventional sugar and ethanol chain - Brazil

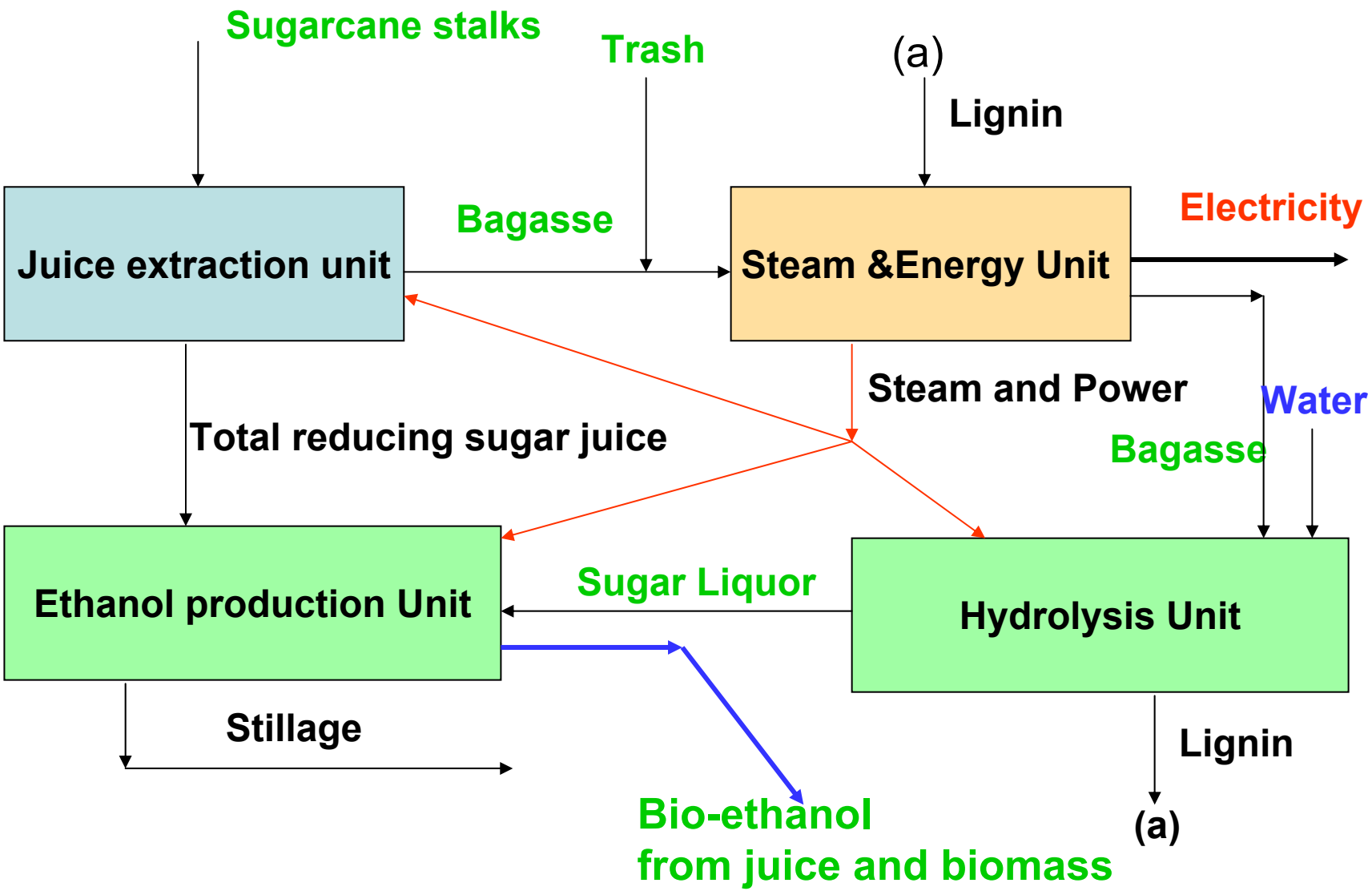


Etanol, Alcoolquímica e Biorrefinarias
BNDES Setorial, *Rio de Janeiro, n. 25, p. 5-38, mar. 2007*

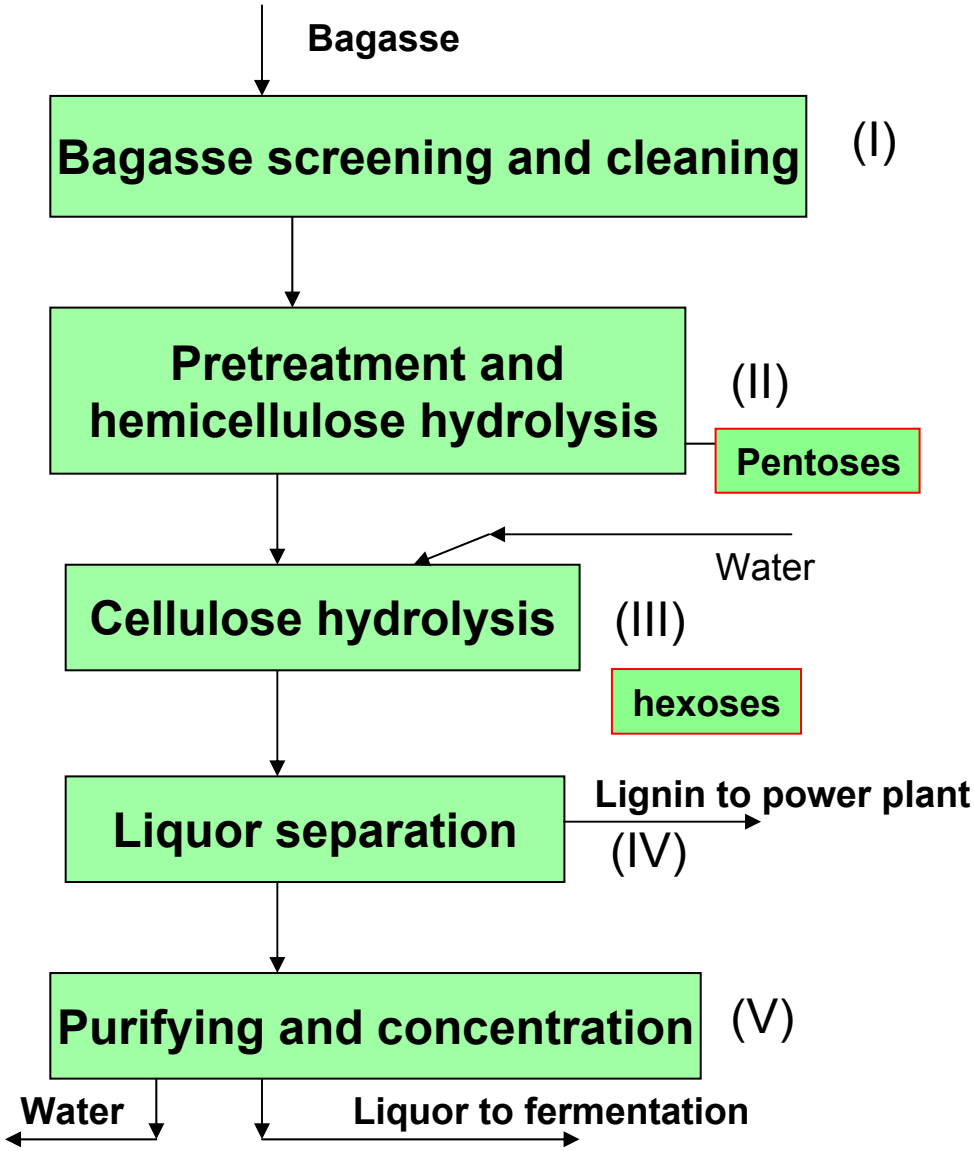
Technology for Ethanol Production



Sugarcane process to bioethanol and power introducing Hydrolysis



Hydrolysis Steps



(I) Rind, pith and sand removed from fiber

(II) Delignifying and hemicellulose hydrolysis step

(III) Cellulose conversion by enzyme catalysis

(IV) Liquor separation from lignin and washing

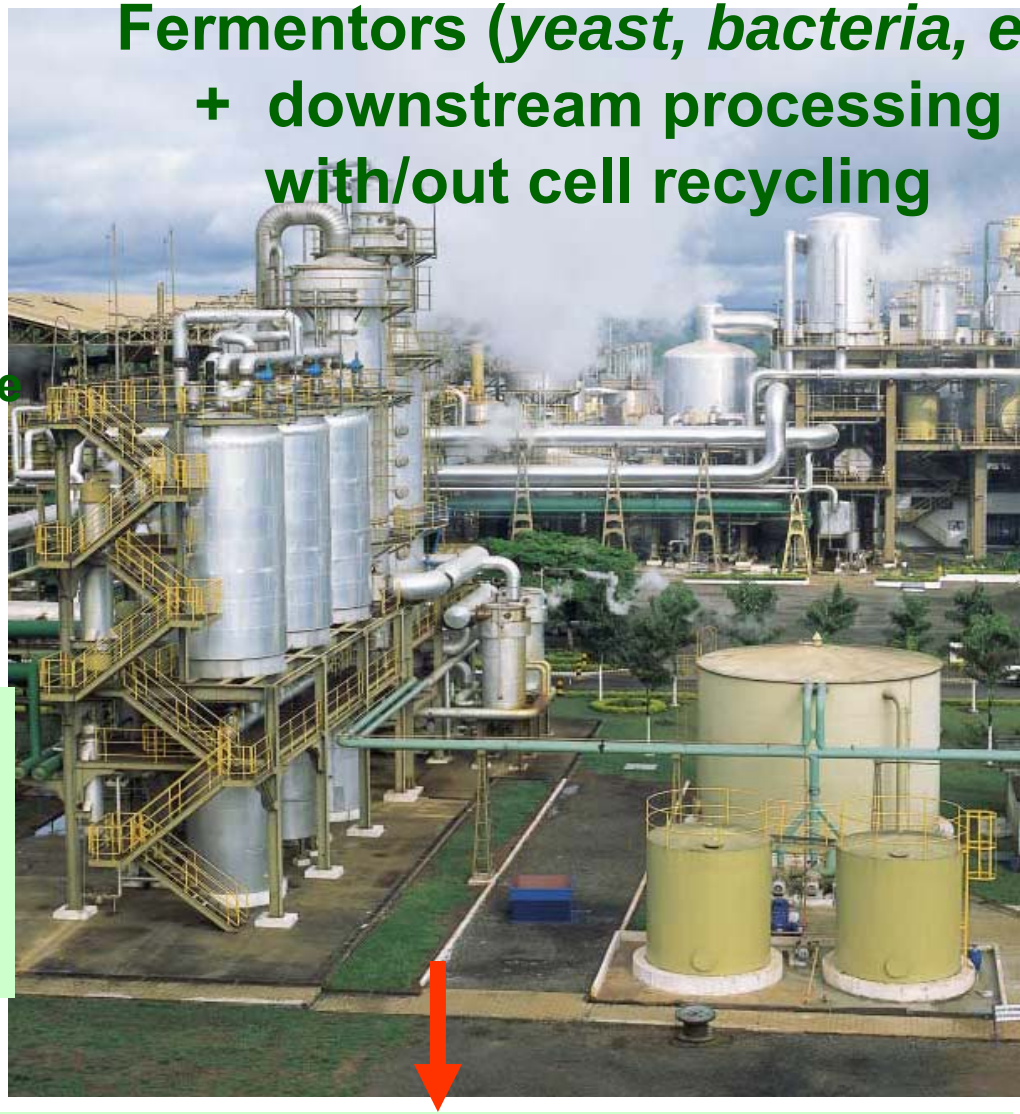
(V) Removal of inhibitors and concentration of liquor, recover of condensed water for reuse in process

Biorefinery for chemicals/biochemicals

Sugar-cane crushed

Sugar-cane (juice+ trash and bagasse)
Sucrose
Glucose
Pentoses
Lignin

Fermentors (*yeast, bacteria, e*
+ downstream processing
with/out cell recycling



Acrylic acid, ethanol, organic acids, polymers, …

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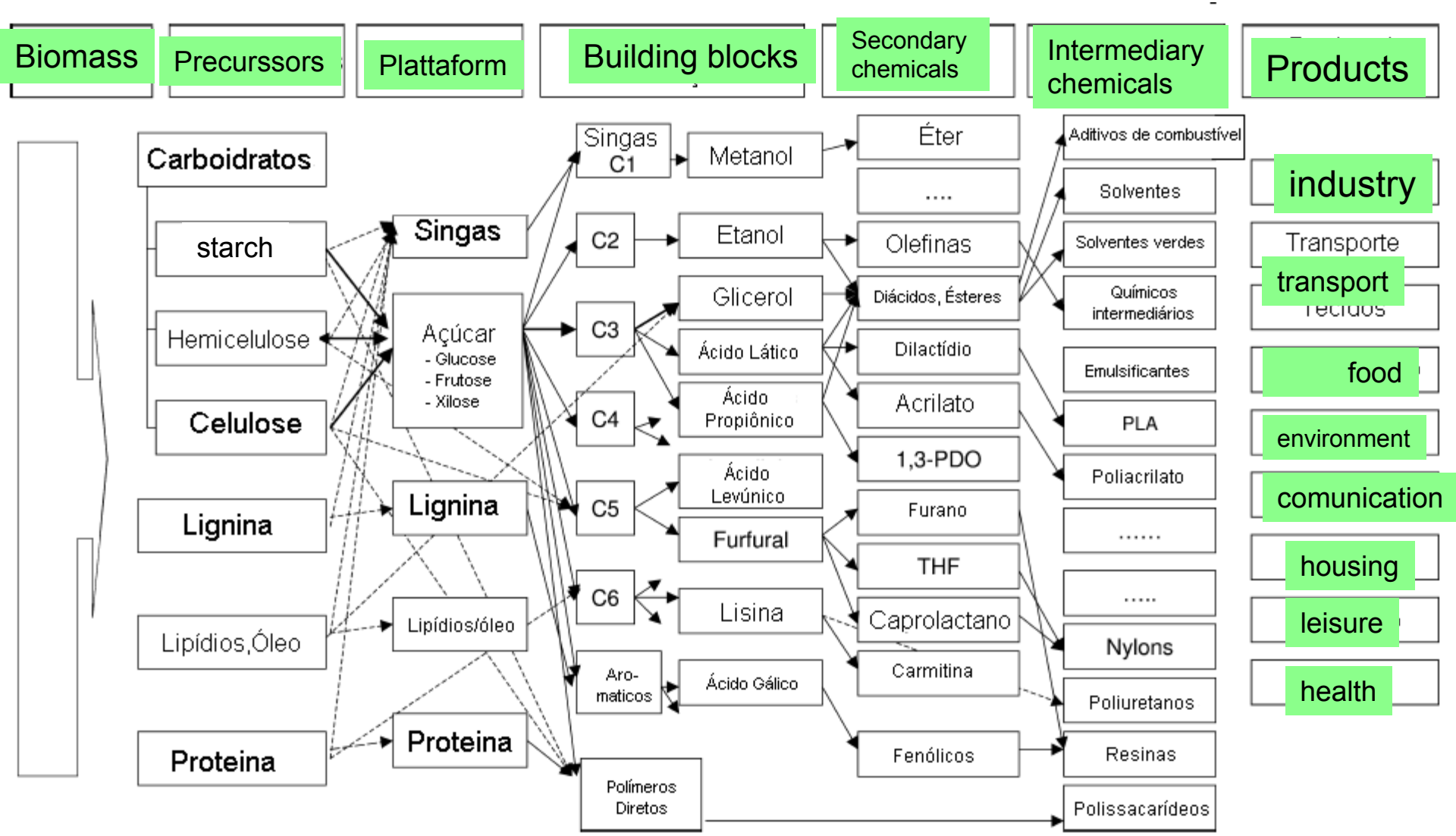
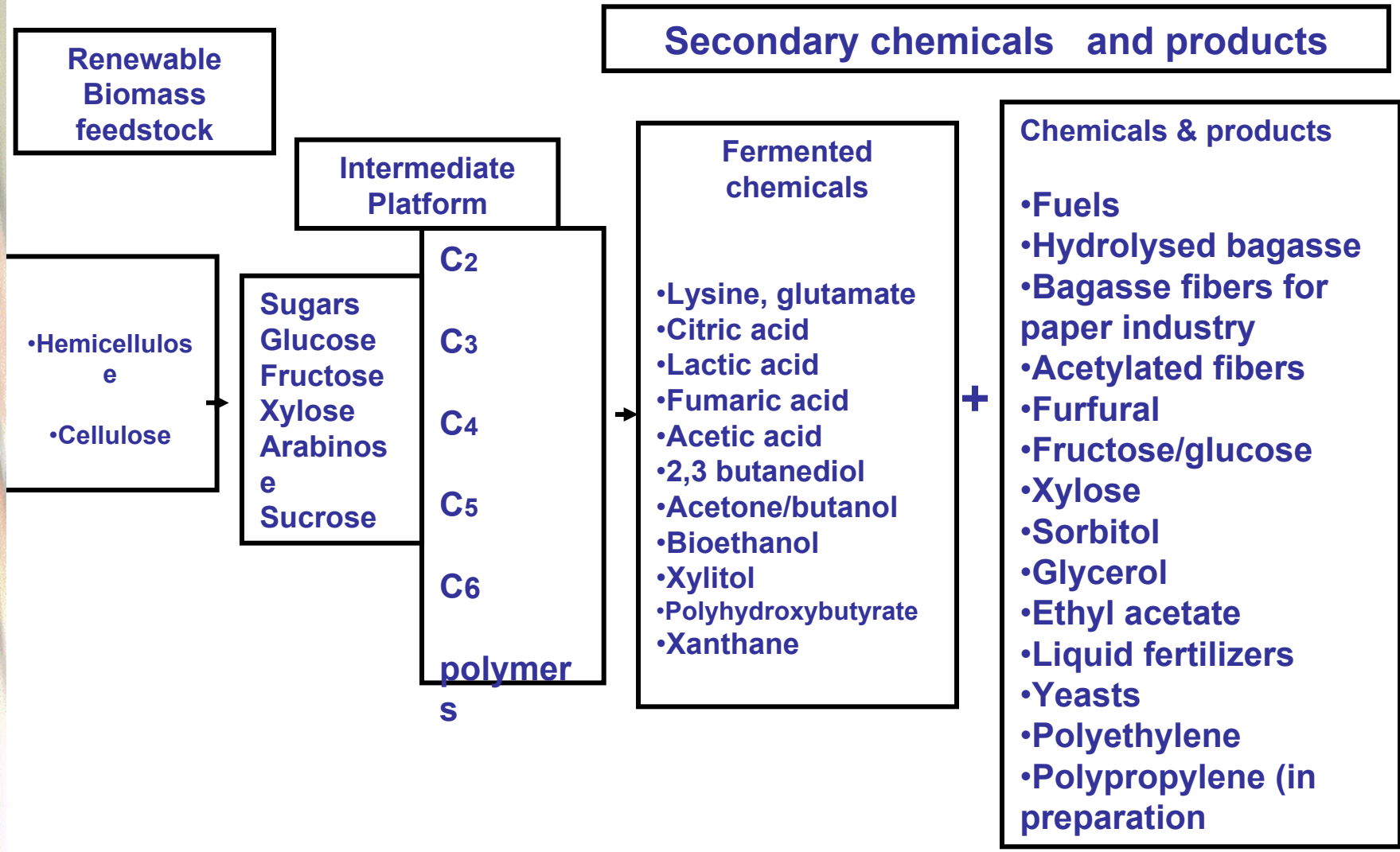
Biobased product flow-chain from biomass feedstock



Kamm & Kamm, 2006

Products from sugar-cane - Brazil



From: INDUSTRIAL PERSPECTIVES FOR BIOETHANOL. ed. Telma Teixeira Franco, Editora Uniemp, Sao Paulo, ISBN 85-98951-06-4, 2006.

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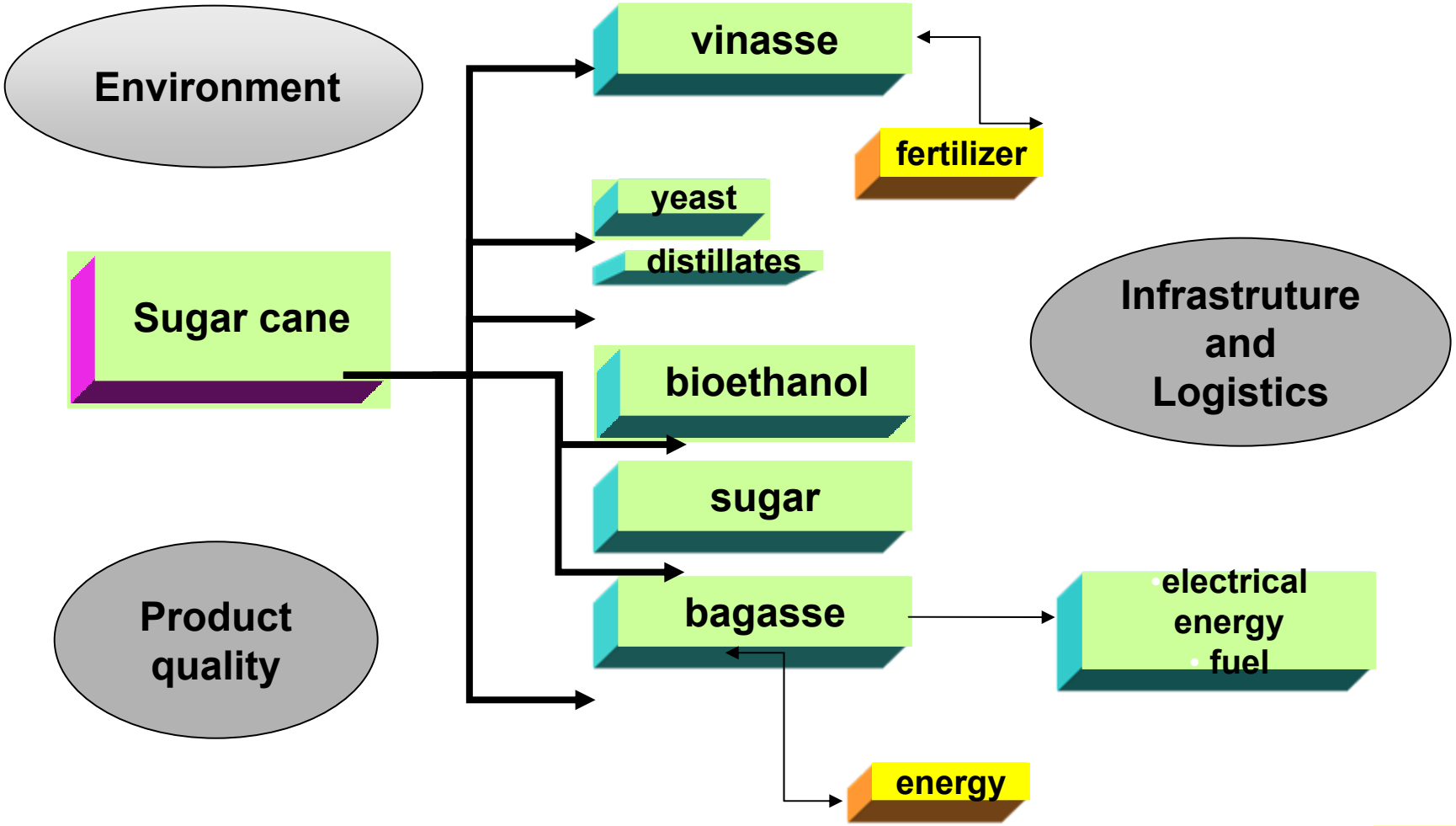
Present situation - first generation products



Bonomi, 2006

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LEBBPOR - non bioethanol activities

Material application

Cellulose & hemicellulose hydrolisate → Succinic acid; L- and D-lactic acid

Microbial acrylic acid from sugar (2005)
Sugar acrylates (sucrose, frutose, etc, from 2003.)

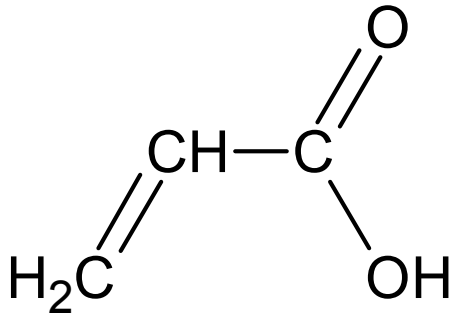
Energy application

[1] Photobioreactor Light + CO2 → Biomass

Biomass → Carbohydrates rich /Oil rich (algae)

[2] Conventional fermentation →microbial oils from hydrolizates (cells)

Acrylic acid case, started in 2002 FEQ



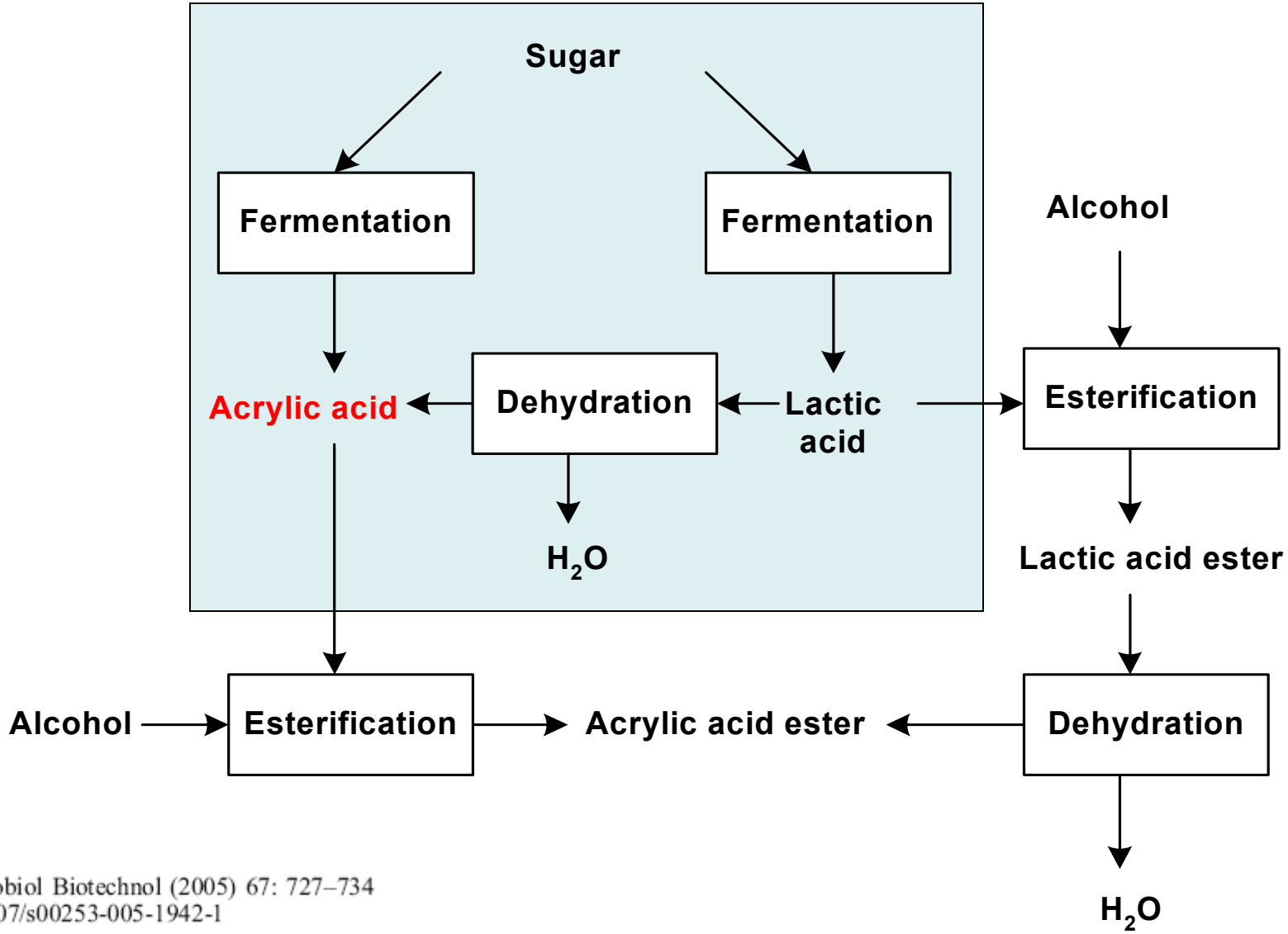
- Polymerized as acid or as methyl, ethyl or butyl ester

- Polymer for flocculants, coatings, paints, adhesives, and binders for leather and textile.

Why acrylic acid?

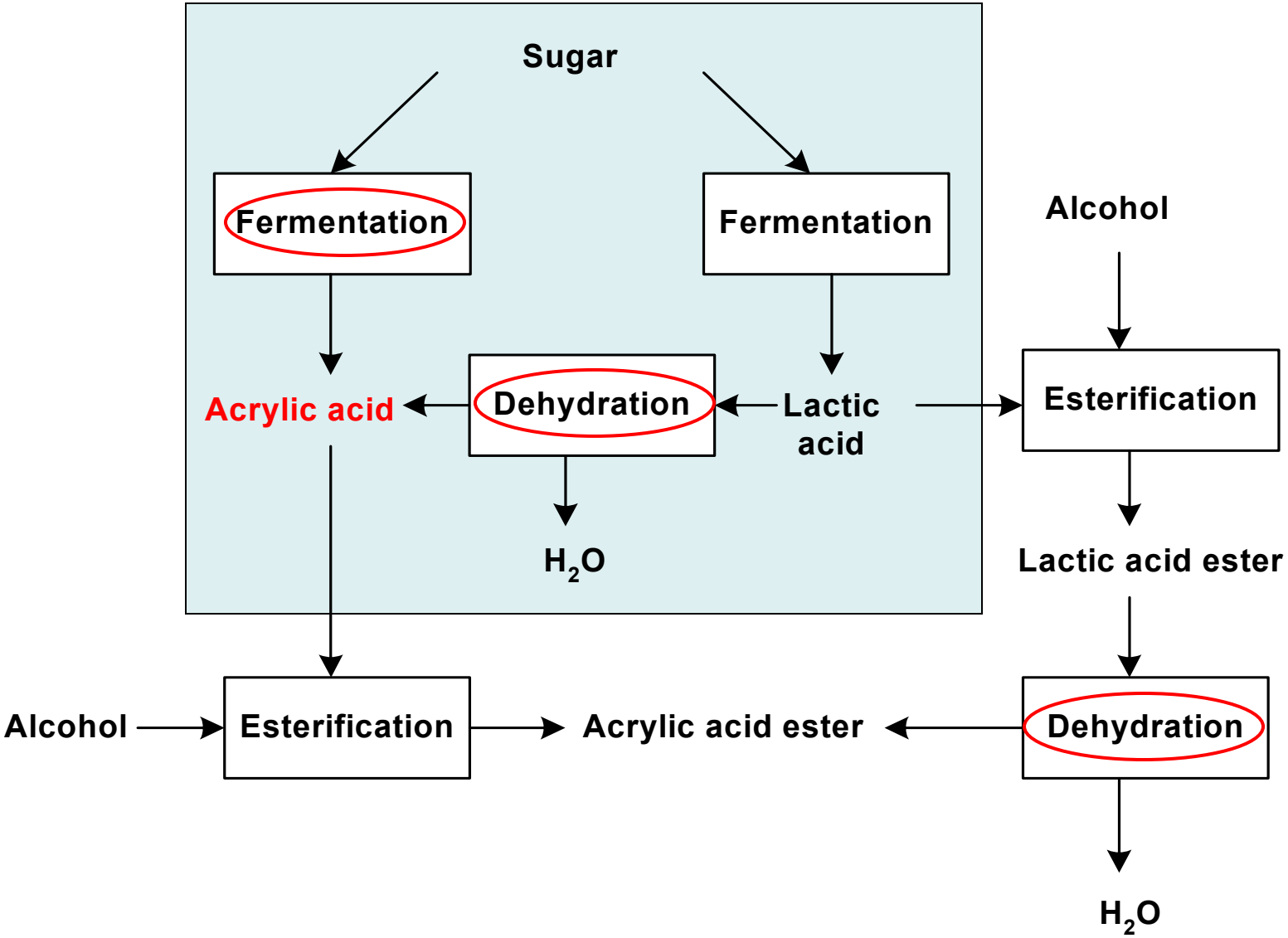
- Production capacity = 4.2 million tons (2003)
- Price = 0.85-0.90 $/lb = 1.95 $/kg (Chemical Market Reporter, 11 April 2005)
- Market size = $ 8 billion

Alternative routes



Appl Microbiol Biotechnol (2005) 67: 727–734
DOI 10.1007/s00253-005-1942-1

Directly to acrylic acid is attractive



Direct fermentation of sugars to acrylate

- Desired stoichiometry

  $C_6H_{12}O_6$ ==> 2 CH_2=CH-COOH + 2 H_2O

  (0.8 kg/kg glucose)

- ATP formation by this reaction to support growth and maintenance
- Cell retention/recycling to minimize growth requirements
- No aeration

Fermentation titers obtained for products related to acrylic acid

Acid	Final conc. (g/L)	Ferment-ation pH	Strain	Reference
Acetic	180-200	?	*Acetobacter*	(Maselli and Horwarth, 1984)
Propanoic	65	6.5	*P. acidipropionici*	(Huang et al., 2002)
Butanoic	42	6.0	*C. tyrobutyricum*	(Huang et al., 2002)
Lactic	210	6.2	*Lactobacillus lactis*	(Bai et al., 2003)
Pyruvic	135	5.0	*S. cerevisiae*	(van Maris et al., 2004a)
Fumaric	64	5.5	*Rhizopus arrhizus*	(Riscaldati et al., 2002)
Itaconic	75	2.0	*Aspergillus terreus*	(Yahiro et al., 1997)

Microbial tolerance to acrylate

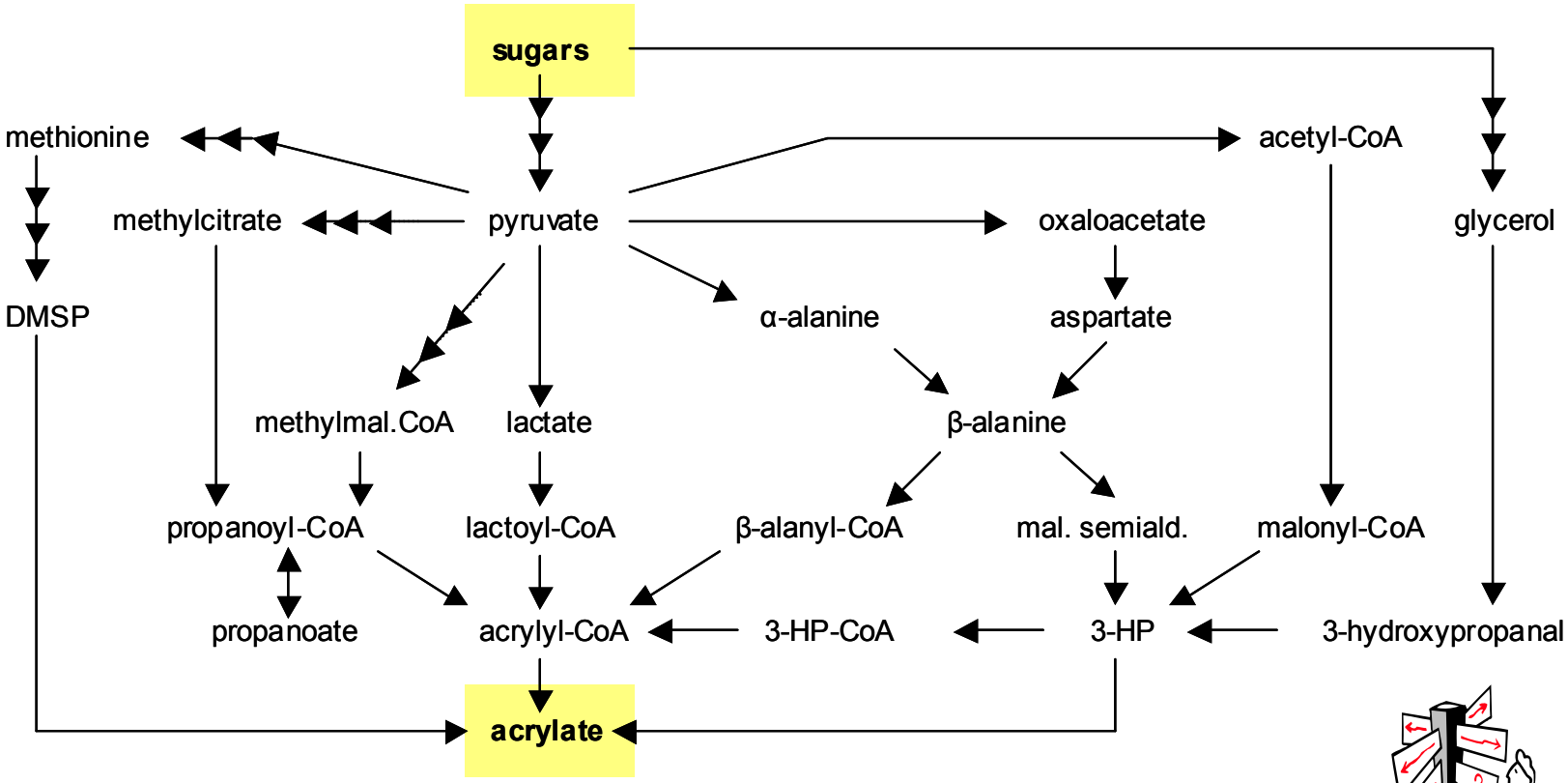
In general, a high toxicity is to be expected

BUT:

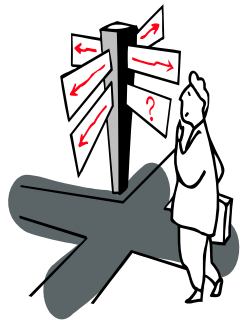
- The C=C-COOH sub-structure is present in fumarate and itaconate
- **Some cell types survive 35 g/L acrylate**

Using selective pressure, genome shuffling, etc. it is expected that **50 g/L acrylate is a realistic maximum concentration**

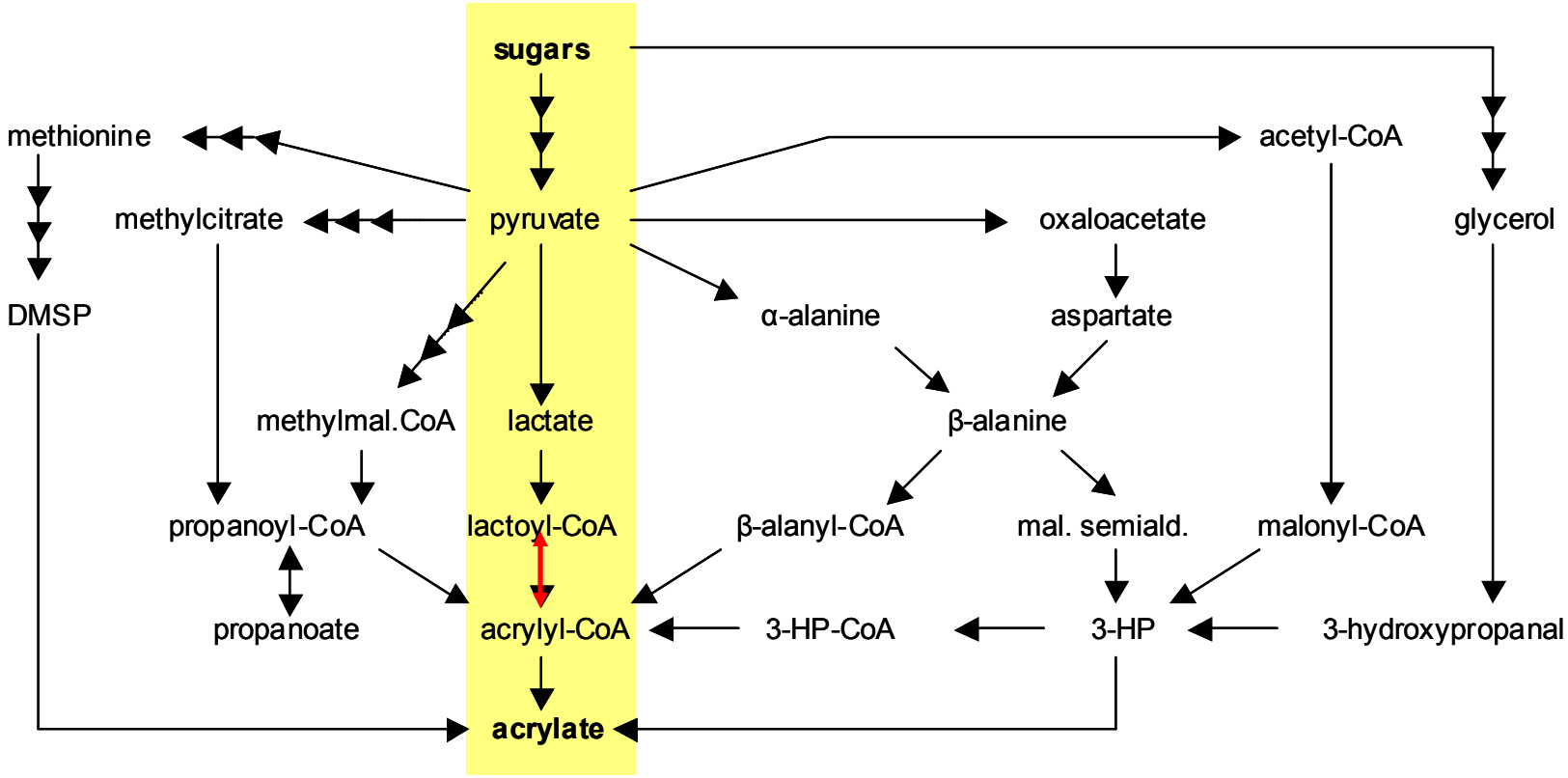
Hypothetical metabolic pathways to acrylate



Which might give a high yield?

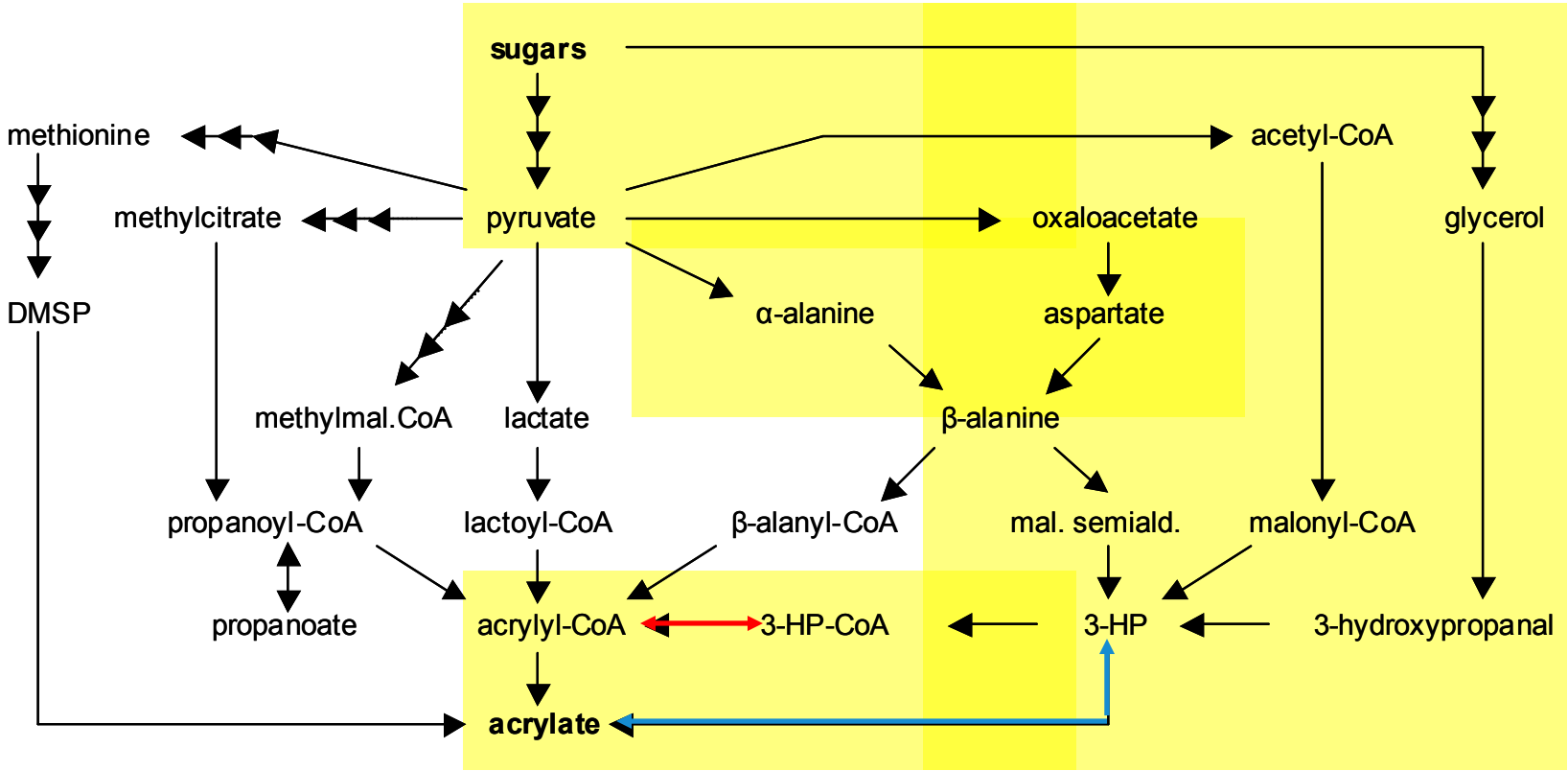


Lactate pathway



Keq [acrylylCoA]/[lactoylCoA] = 0.5 % → low yield

3-Hydroxypropanoate (3-HP) pathways



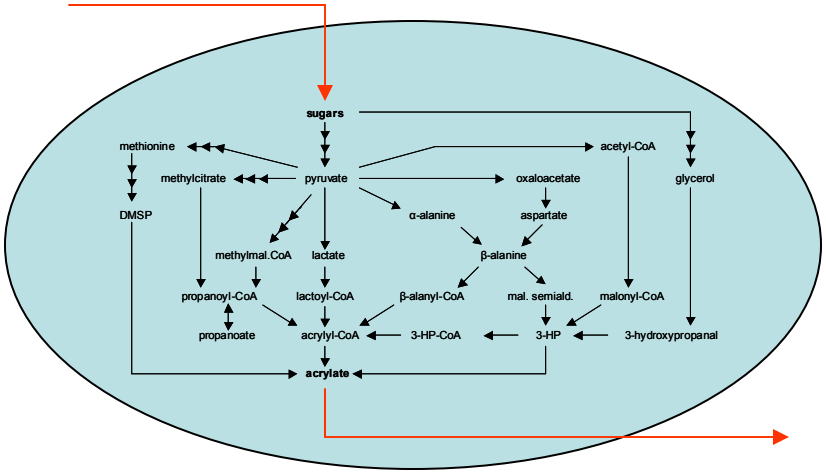
Keq [acrylylCoA]/[3-HPCoA] < 10 % ?

Keq [acrylate]/[3-HP] < 10 % ?

Export

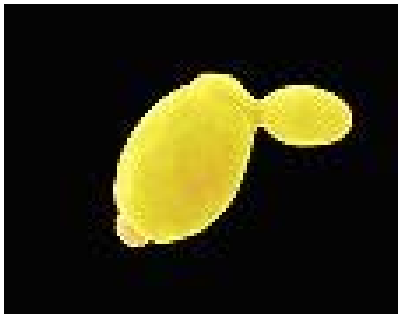
- Active excretion of acrylic acid is required
- Export should not consume all ATP

sugars



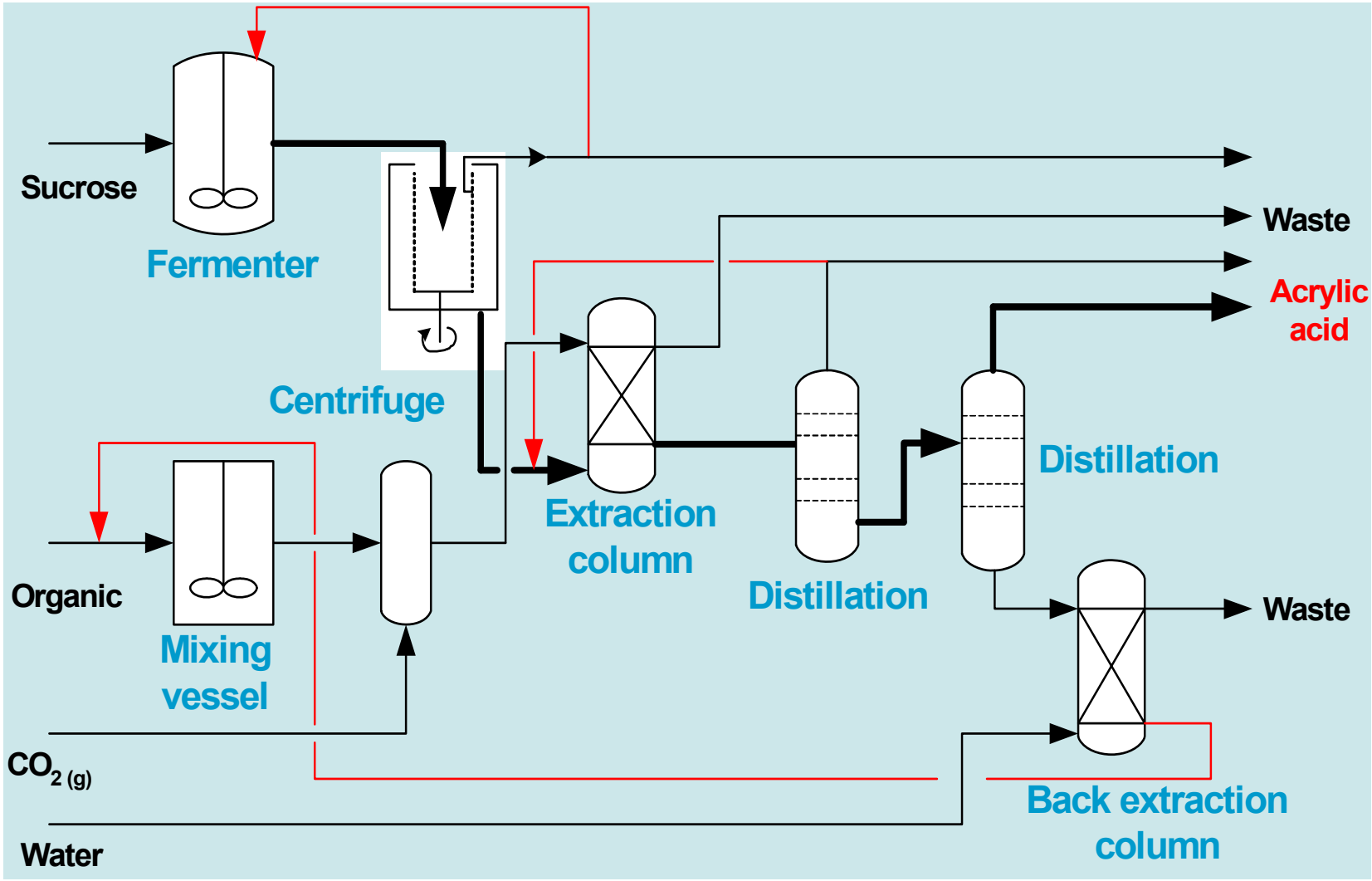
acrylic acid

Fermentation process



- Microorganism: *S. cerevisiae*
- Mode of operation: continuous
- pH = 7 (controlled by Na_2CO_3)
- Some assumptions:
  - Acrylate yield on glucose: 0.72 $g.g^{-1}$
  - Acrylate concentration: 50 $g.l^{-1}$
  - Lactate produced: 1 $g.l^{-1}$

Description of the chosen design



Conclusions

- The designed process ⇒ economically feasible
- Most interesting route:

  sugar ⇒ acrylic acid
- Preferably at low pH

- Recombinant biocatalyst might
  - survive at 50 g/L
  - produce & excrete acrylic acid
  - grow anaerobically
  - show a very high yield on sugars

- Incentive for checking these speculations

Improvements to consider

1. **If fermentation were at lower pH:**

- **less sodium carbonate**
- **less investment in extraction**
- **less waste**

2. **Sucrose costs much less, since no refined sugar is required, but probably just sugar-cane juice, as used in ethanol bioproduction.**

Main gaps in information

- Thermodynamic data of pathway intermediates
- Existence or accessibility of suitable exporter and pathway enzymes
- Metabolic consequences of blocking competing pathways
- Potential tolerance to acrylate
- Equilibrium data for extraction

JCTB1983/08-0043.R1

Journal of Chemical Technology and Biotechnology

J Chem Technol Biotechnol **83**:000–000 (2008)

Enzymatic direct synthesis of acrylic acid esters of mono- and disaccharides

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[2]*Interdisciplinary Research Center for Mass Spectrometry of Biopolymers, University of Potsdam, P.O. box 601553, D-14415 Potsdam, Germany*

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Abstract

BACKGROUND: There is an increased need to replace materials derived from fossil sources by renewables. Sugar-cane derived carbohydrates are very abundant in Brazil and are the cheapest sugars available in the market, with more than 400 million tons of sugarcane processed in the year 2007. The objective of this work was to study the preparation of sugar acrylates from free sugars and free acrylic acid, thus avoiding the previous preparation of protected sugar derivatives, such as glycosides, or activated acrylates, such as vinyl acrylate.

Building blocks from renewable resources by biocatalysis

Why sugar acrylates?

- biomedical, chemical and pharmaceutical applicability ;

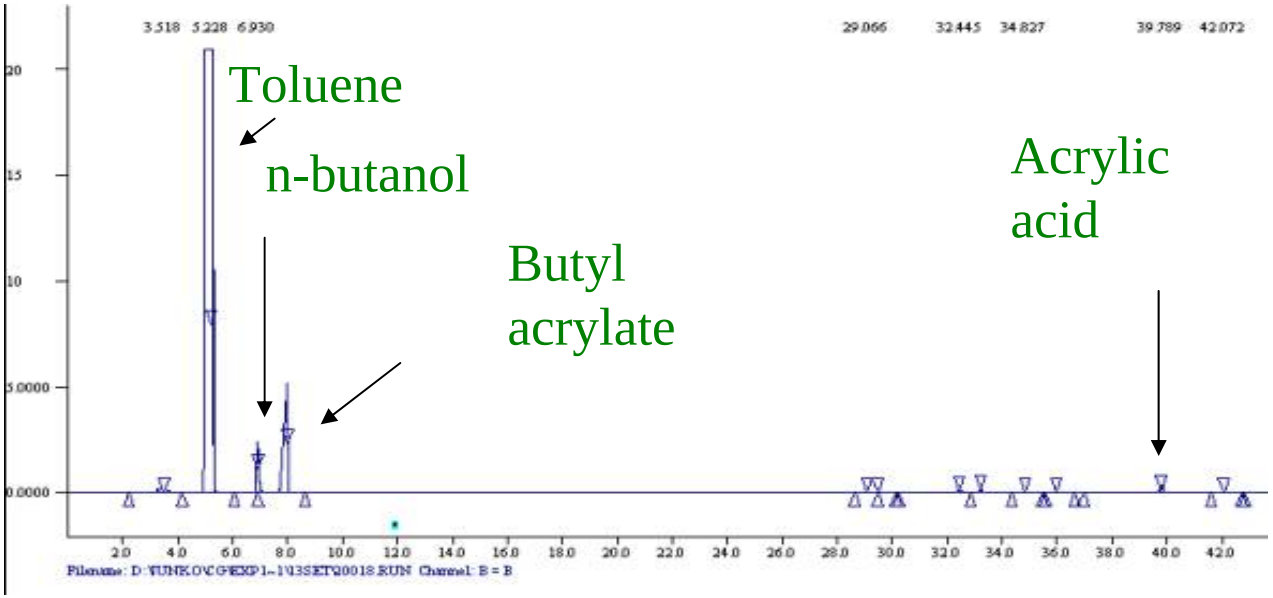
•If hydrogels – water-absorbent materials for applications such as general water absorbents, water-treatment additives;

• Enzymatic synthesis
•Sugar + fatty acid with lipase as biocatalysts – 1980's.
•Sugar + acrylic / metacrylic acid with lipase (esterification or transesterification) – 1991's
•BASF patent, indirect esterification of methyl glicosides

Enzymatic direct synthesis of acrylic acid esters of mono and disaccharides, J.Tsukamoto, PhD Thesis. Unicamp, Brazil. 2006

Initially Calb was tested to catalyse n-butanol + acrylic acid esterification.....

- Maximize the reacional conditions to increase the conversion to esters of acrylic acid using CalB ;
- Evaluate the products by HPLC, MALDI-TOF-MS and KF analysis.



Enzymatic conversion of sugars and alchools to acrylate esters

Substrates + media	**Catalyst (mass)**	**Temp. ºC/time**	**Conv.(%)**	**Byprod.**	**Ref.:**
AA (43.7 mmol) + 1-butanol (43.7 mmol) +toluene(3.5 cm^3)	CalB 60 mg	55 / 8 h	**61.6**	0	**Tsukamoto et al, 2006**
	CalB 200 mg		**94.6**	0	
AA (43.7 mmol) + 1-butanol (43.7 mmol) +toluene(5 cm^3)	$Cs_{2.5}H_{0.5}PW_{12}O_{40}$ (56 mg)	79.85 / 4 h	15.9	3*	Chen et al, 1999.
	$Cs_{2.5}H_{0.5}PW_{12}O_{40com.}$ (56 mg)		19.0	2**	
	Amberlist 15 (14 mg)		33.6	3*	
	$H_3PW_{12}O_{40}$ (25.2 mg)		83.5	3*	
	H_2SO_4 (2.8 mg)		60.2	3*	
AA/ButOH (molar ratio: 0.75)	$H_3PW_{12}O_{40}$	80 / 4 h25 m.	98.0	?	Dupont et al, 1995.
	H_2SO_4	80 / 11h17m.	98.0	?	

* 3-butoxypropionic acid; butyl 3-butoxypropanate and butyl 3-acryloxy propanoate

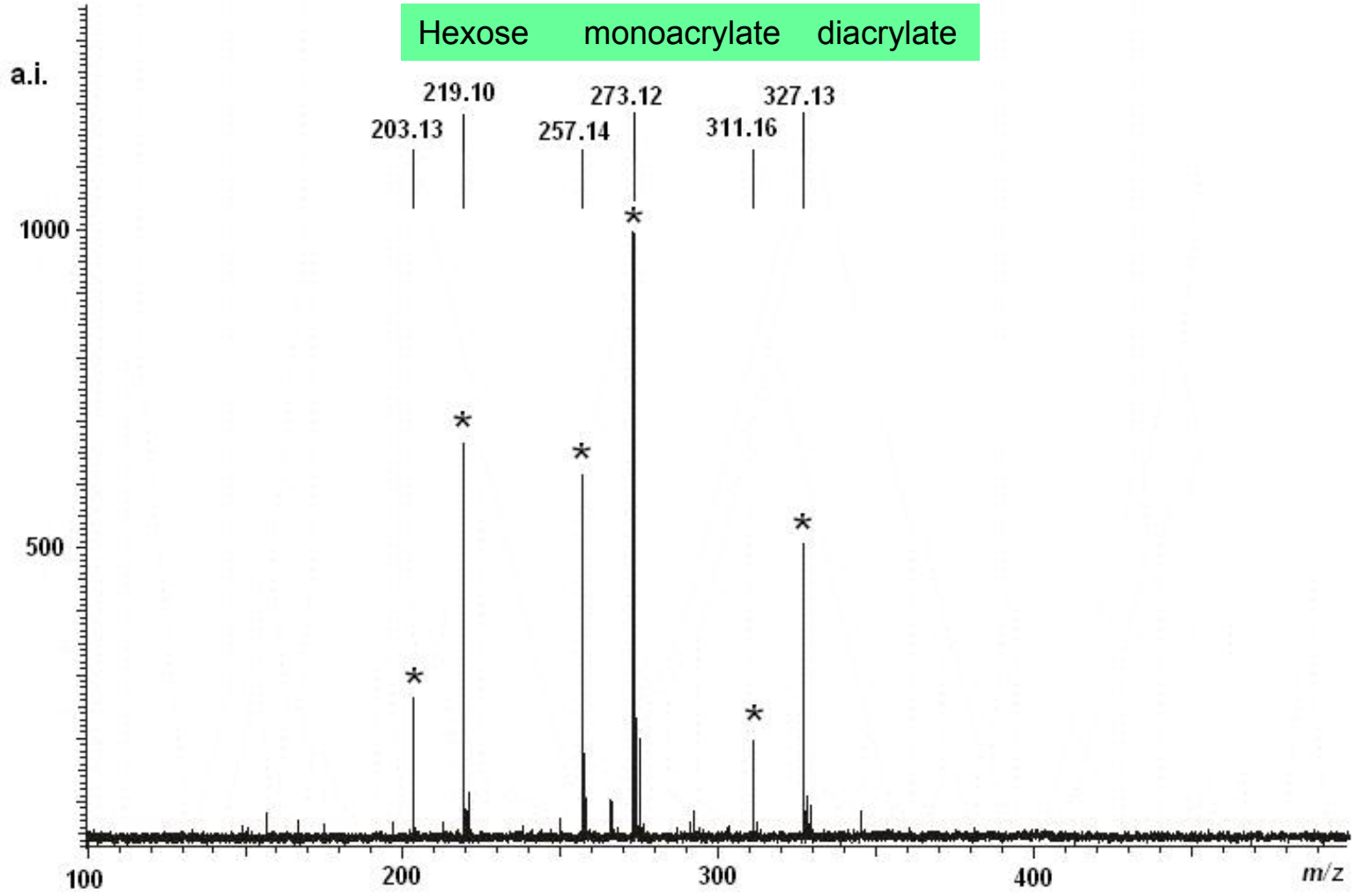
** 3-butoxypropionic acid and butyl 3-acryloxy propanoate

MALDI-TOF MS Analysis: monosaccharides

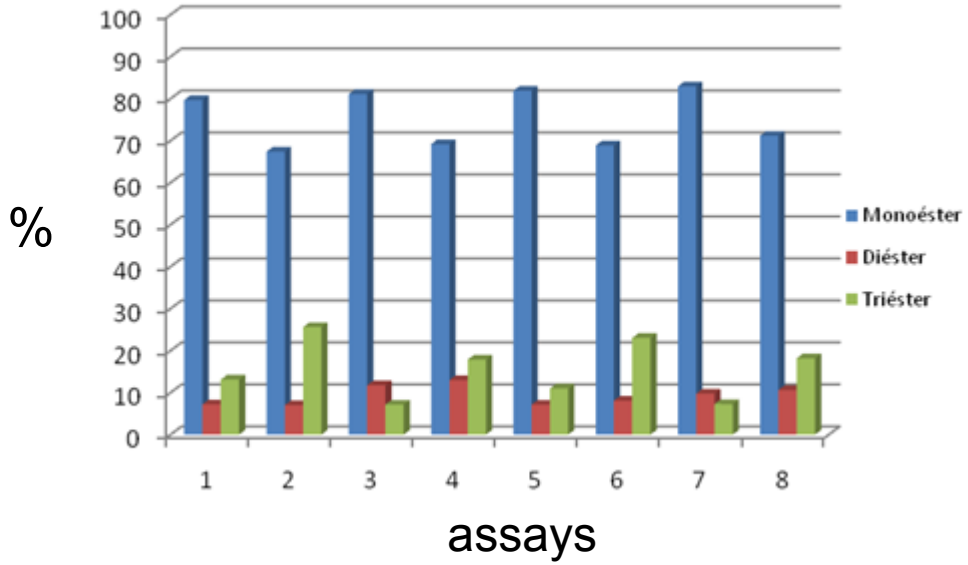
Table 2. Calculated and observed masses (*m/z*) of sodiated resp. potassiated molecular ions generated in MALDI-TOF MS of hexoses, pentoses, and corresponding acrylates (A: reaction in the presence of molecular sieves; B: in the absence of molecular sieves).

		calcd. (*m/z*)	found (*m/z*)				calcd. (*m/z*)	found (*m/z*)	
		Hexoses	D-Fructose		D-Glucose		Pentose	D-Xylose	
			A	B	A	B		A	B
Free sugars	$[M+Na]^+$	203.05	203.20	203.13	203.21	203.24	173.04	173.22	173.24
	$[M+K]^+$	219.02	219.18	219.10			189.01		
Monoacrylates	$[M+Na]^+$	257.06	257.24	257.14	257.25	257.28	227.05	227.27	227.29
	$[M+K]^+$	273.03	273.21	273.12	273.23	273.24	243.02		
Diacrylates	$[M+Na]^+$	311.07	311.27	311.16	311.28	311.31	281.06	281.32	281.34
	$[M+K]^+$	327.04	327.23	327.13			297.03		
Triacrylates	$[M+Na]^+$	365.09	365.51		365.35	365.51	335.08	335.54	
	$[M+K]^+$	381.06					351.04		
Tetraacrylates	$[M+Na]^+$	419.10	419.60		419.33	419.36	389.09		
	$[M+K]^+$	435.07	435.48				405.05		
Pentaacrylates	$[M+Na]^+$	473.11			473.65	473.68			
	$[M+K]^+$	489.08							

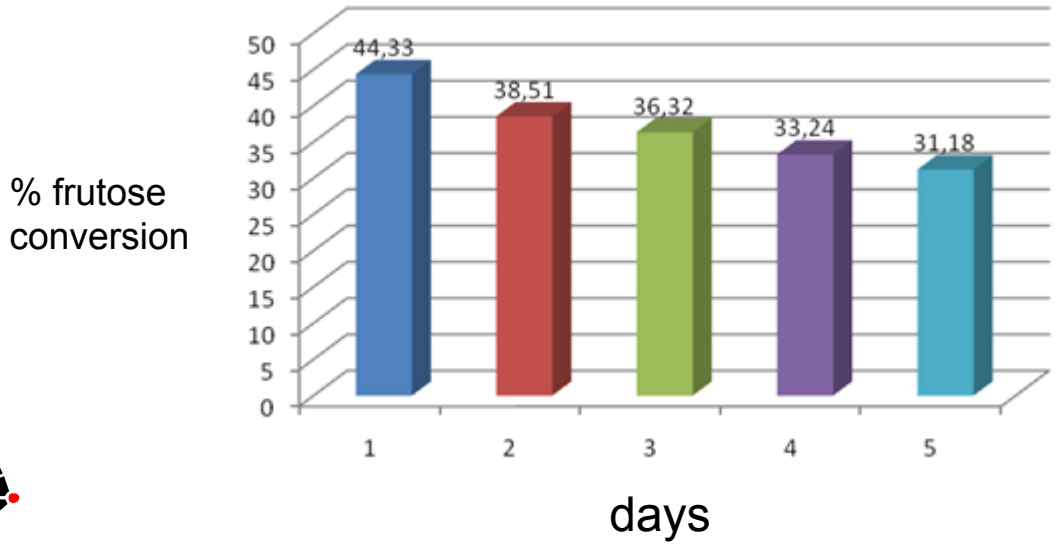
frutose



MALDI-TOF MS of the reaction mixtures of the lipase catalyzed esterifications of **D-fructose,** recorded after a reaction time of 48h. Asterisks indicate peaks from fructose and acrylates.



Product distribution



Enzyme reutilization

UNICAMP

E.Vagetti, 2008

Photobioreactor for CO_2 sequestration and microalgal biomass production

Products

biomass

Fats →biodiesel

Polysaccharides& gels

O_2

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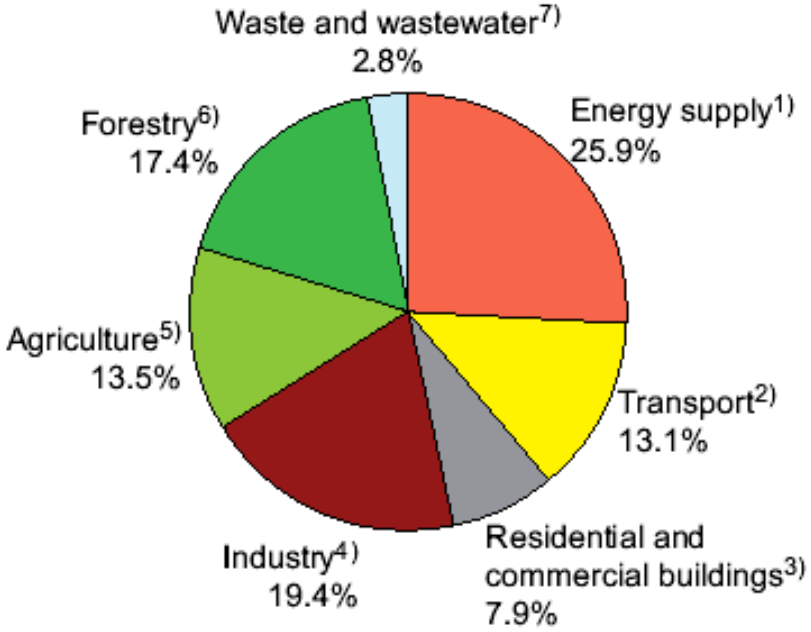
THE PROBLEM

The industrial processes most contributing to increasing atmospheric CO_2 concentrations:

- electrical and petrochemical energy generating plants,
- hydrogen and ammonia producing plants,
- cement factories, and fermentative and chemical oxidation processes.



GHG emissions by sector in 2004 (IPCC, 2007)

Global warming – possible reasons

Pollution
Gas emission

Carbon dioxide (CO_2)

Methane (CH_4)

Nitrous oxide (N2O)

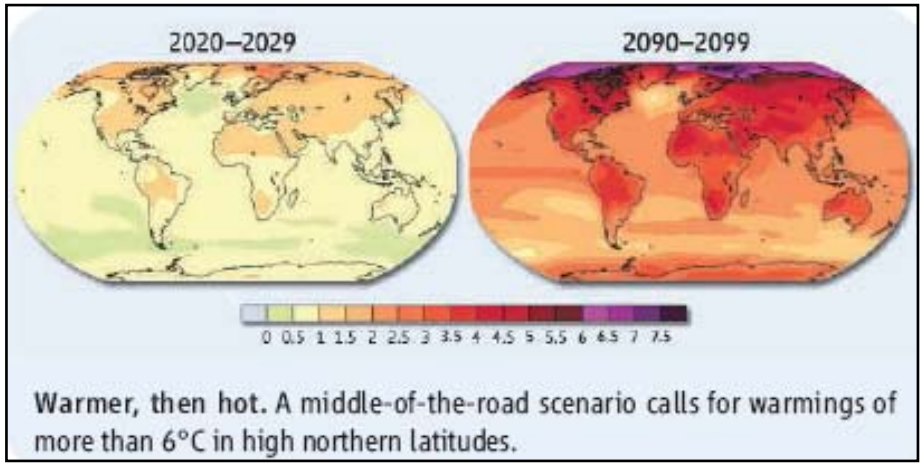
Hydrofluorcarbons (HFCs)

Perfluorcarbons (PFCs)

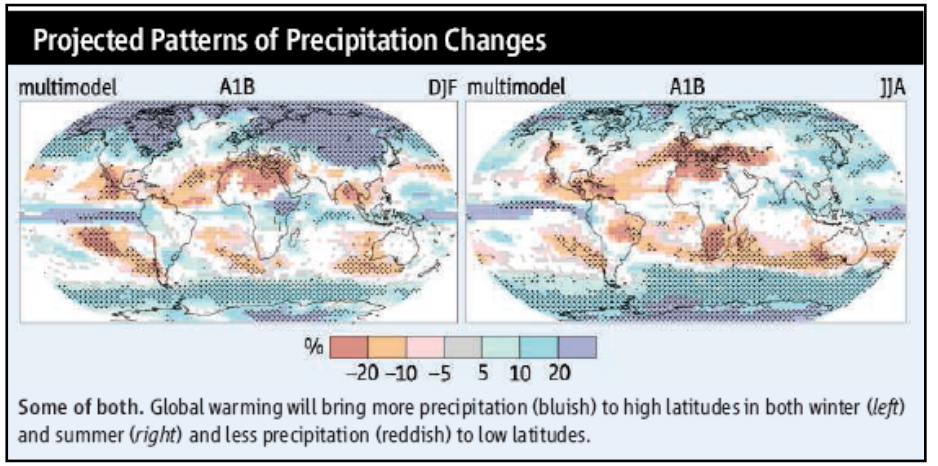
Sulphur hexafluoride (SF_6)

Global warming → consequences

"Green-house" effect



Warmer, then hot. A middle-of-the-road scenario calls for warmings of more than 6°C in high northern latitudes.



Some of both. Global warming will bring more precipitation (bluish) to high latitudes in both winter (*left*) and summer (*right*) and less precipitation (reddish) to low latitudes.

Arctic permafrost will thaw.

Savanna will replace tropical forests.

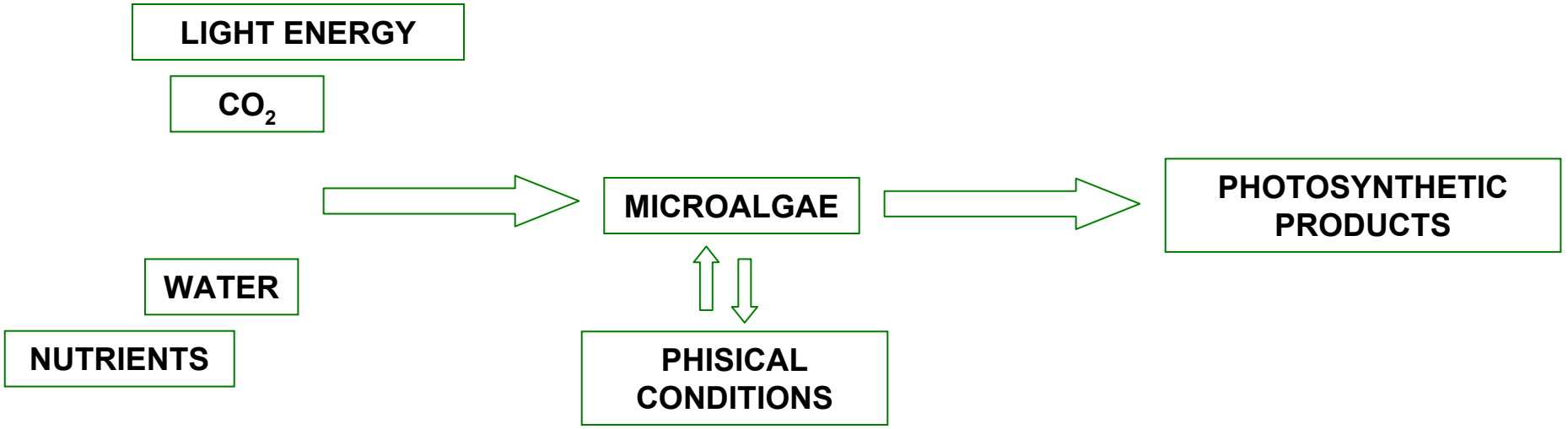
Rising sea level will increase coastal flooding.

Most corals will suffer major declines.

Science, 316, 188-190, 2007.

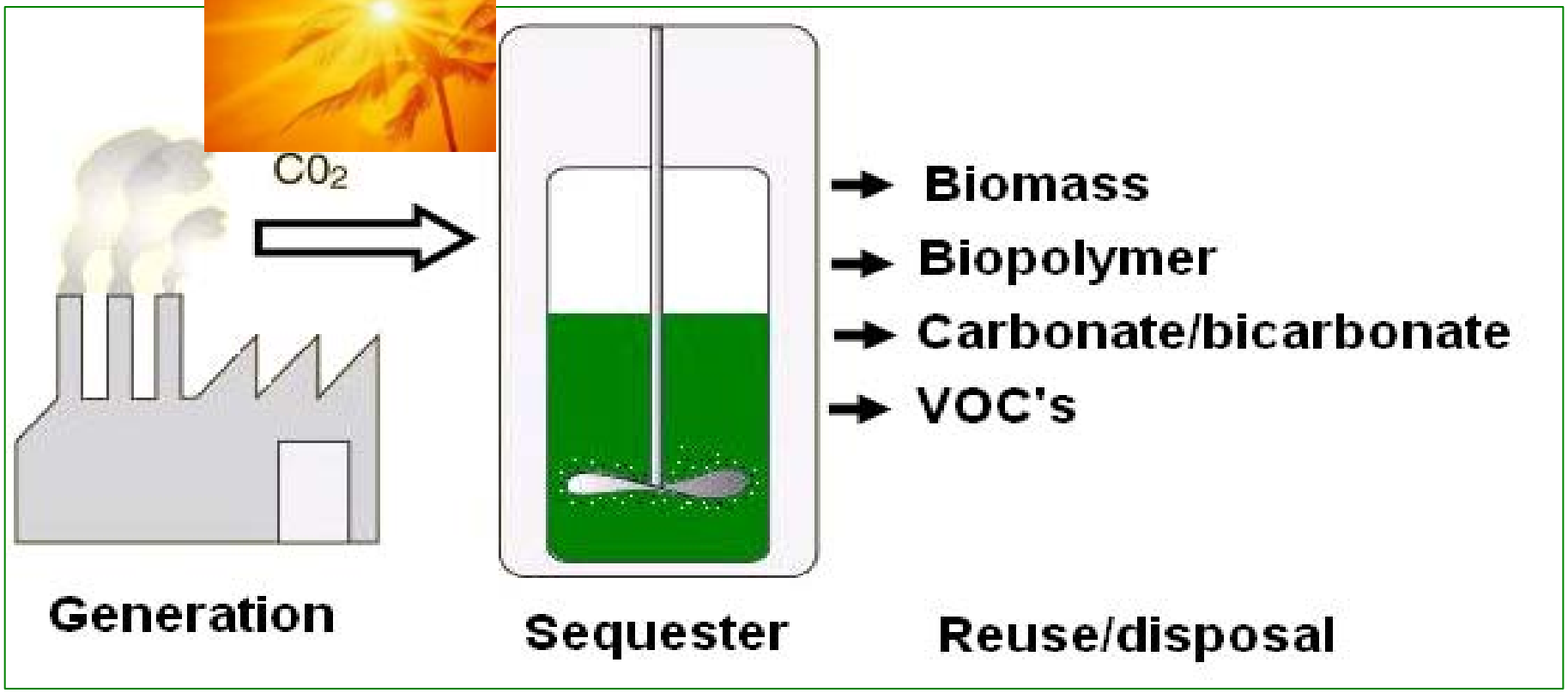
PHOTOBIOREACTOR TECHNOLOGY

- Initial studies – Japan, decade of 1990's
- Carbon dioxide fixation into microalgal biomass
- Current studies show that other products have significance in the process



THE STRATEGY

- **potentiality for application in stationary sources of carbon dioxide**



Biotechnological process for carbon dioxide sequestration

Synechococcus sp. PCC 8806, PCC 8807	Study of CO_2 mitigation by calcium carbonate formation.	Lee et al., (2006)
-	Development a feasibility model for microalgal CO_2 biofixation using photobioreactors equipped with solar collectors.	Ono & Cuello (2006)
Rhodomonas sp.	Study of biomass production and carbon fixation in batch culture of the marine microalgae.	Lafarga-De La Cruz et al., (2006)
Chlorella sp.	Study of the performance of open photobioreactors on the utilization of CO_2 by microalgae. The results indicate that about 70% of supplied CO_2 was utilized by the microalgae.	Doucha & Lívanský (2006)
Nannochlopsis oculta	Evaluation of the carbon balance in the bio-fixation of CO_2 in photobioreactors.	Hsueh et al., (2007)
Scenedesmus obliquus *Spirulina* sp.	CO_2 bio-fixation in reactors in series with three stages. The results showed mean fixation rates of 37.9% in cultures carried out with pulses of 15 min/hour at 6% CO_2 with a flow rate of 0.3VVM.	Morais & Costa (2007a)
Anabaena variabilis	Study of light transfer in photobioreactors for the production of H_2 with the simultaneous removal of CO_c.	Berberoglu et al., (2007)
Scenedesmus obliquus *Chlorella kessleri*	Selection and isolation of species for the biological removal of CO_2 from thermoelectric energy generating stations.	Morais & Costa (2007b)
*Aphanothece microscopica Nägeli (*RSMan92)	Kinetic modelling of carbon dioxide removal in tubular photobioreactors and process optimisation. The kinetic data indicated maximum removal rates of 108.56mg_{CO2}/L.min.	Jacob-Lopes et al., (2007a)
Chlorella sp.	Study of efficiency of CO_2 reduction, biomass and lipid productivity in a semicontinuous photobioreactor system. The results obtained estimated maximum elimination capacity of 17.2g_{CO2}/L.day	Chiu et al., (2007)
Chlorella vulgaris	Evaluation of the performance of four photobioreactors for CO_2 removal. Maximum carbon dioxide conversion rates of 0.275g/L.h were obtained.	Fan et al., (2007)
Chlamydomonas reinhardtii *Chlorella* sp.	Evaluation of CO_2 uptake and O_2 production in a gas-tight photobioreactor.	Eriksen et al., (2007)
Dunaliella parva	Study of fluid flow and mass transfer in a counter-current gas–liquid inclined tubes photobioreactor	Merchuk et al., (2007)
*Aphanothece microscopica Nägeli (*RSMan92)	Evaluation of the growth kinetics of cyanobacteria under different conditions of temperature, light intensity and CO_2 concentration. Maximum rates of incorporation of carbon in the biomass of 109.2mg_{carbon}/L.h were obtained.	Jacob-Lopes et al., (2007b)

Last 2 years literature

Patents related to carbon sequester processes by microalgae in photobioreactors

WO 2003094598	Photobioreactor and process for biomass production and mitigation of pollutants in flue gas.	Berzin (2003)
US 2005239182	Synthetic and biologically derived products produced using biomass produced by photobioreactors.	Berzin (2005a)
US 2005064577	Hydrogen production with photosynthetic organisms and from biomass derived there from.	Berzin (2005b)
KR 2005081766	Continuous photobioreactor for carbon dioxide removal to inhibit global warming and mass-production of microalgae.	Shin & Chae (2005)
AU 2006100045	Photobioreactor for mitigation of greenhouse gases.	Davey (2006)
WO 2006100667	A method for the enhanced production of algal biomass by sequestration of gaseous carbon dioxide.	Eyal & Raz (2006)
WO 20070111343	Photobioreactor for biomass production and mitigation of pollutants in flue gases.	Berzin & Wu (2007)
EP 1801197	Process and photobioreactor for the photosynthetic production of biogas from carbon dioxide.	Klaus et al., (2007)
WO 2007047805	Carbon neutralization system (CNS) for CO_2 sequestering.	Sheppard, (2007)

BARRIERS AND LIMITATIONS

- composition of gases
  - mixtures, NOx, SOx, CH_4, H_2, CO
  - microalgae can assimilate other forms of carbon?
- temperature of gases
  - 100 - 300ºC
  - biological reactions: ~25-35ºC
- scale-up

GREENFUEL® TECHNOLOGIES CORPORATION

GREENFUEL RAISES $13.9M FOR DEVELOPMENT AND SCALING PROJECTS

Financing Led by Access, DFJ, Polaris

Cambridge, MA – May 14, 2008 – GreenFuel Technologies Corporation, a privately held company developing algae farm technologies for recycling CO_2 emissions, has closed a $13.9M venture capital round led by Access Private Equity, Draper Fisher Jurvetson, and Polaris Venture Partners. GreenFuel intends to use these funds to prepare for algae farm technology development and scaling projects during 2008.

COMERCIAL PROJECTS

- **Solix Biofuels**
- **Greenfuel**
- **Petrosun**
- **HR Biopetroleum/Royal Dutch Shell**

HR Biopetroleum, Hawaii, USA (pilot plant, 2 ha)

CASE STUDIES of our laboratory

Fundamental work

Maximization of microalgae growth conditions

Light, CO_2, Temperature, pH variation

Maximization of CO_2 conversion and biofixation

Reactor configurations

Integration of refinery wastewater +flue gases

ELSEVIER

Biochemical Engineering Journal 40 (2008) 27–34

Biochemical Engineering Journal

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Biomass production and carbon dioxide fixation by *Aphanothece microscopica Nägeli* in a bubble column photobioreactor

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Received 2 March 2007; received in revised form 29 October 2007; accepted 18 November 2007

Objective

- evaluate the carbon dioxide biofixation and growth kinetics of *Aphanothece microscopica Nägeli* microalgae under different conditions of temperature, light intensity and CO_2 concentration

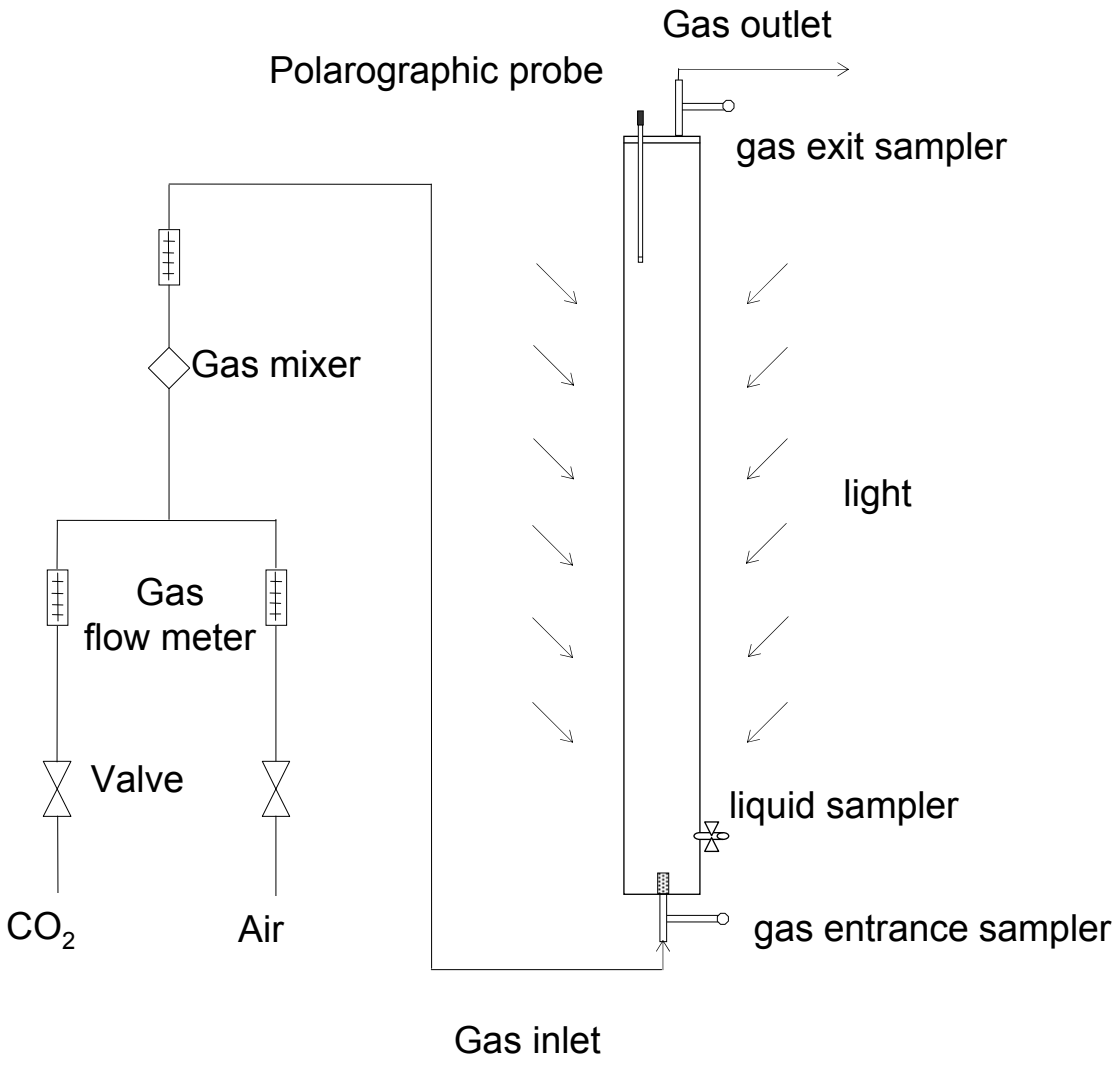
Conditions tested:

temperature: 21,5, 25, 30, 35 and 38,5ºC

light intensities: 0,96, 3, 6, 9 and 11klux

CO_2 concentration: 3, 15, 25, 50 and 62% (v/v)

Experimental apparatus



Schematic diagram of the photobioreactor

Results

IMPROVING OF CARBON DIOXIDE BIOFIXATION BY MICROALGAE

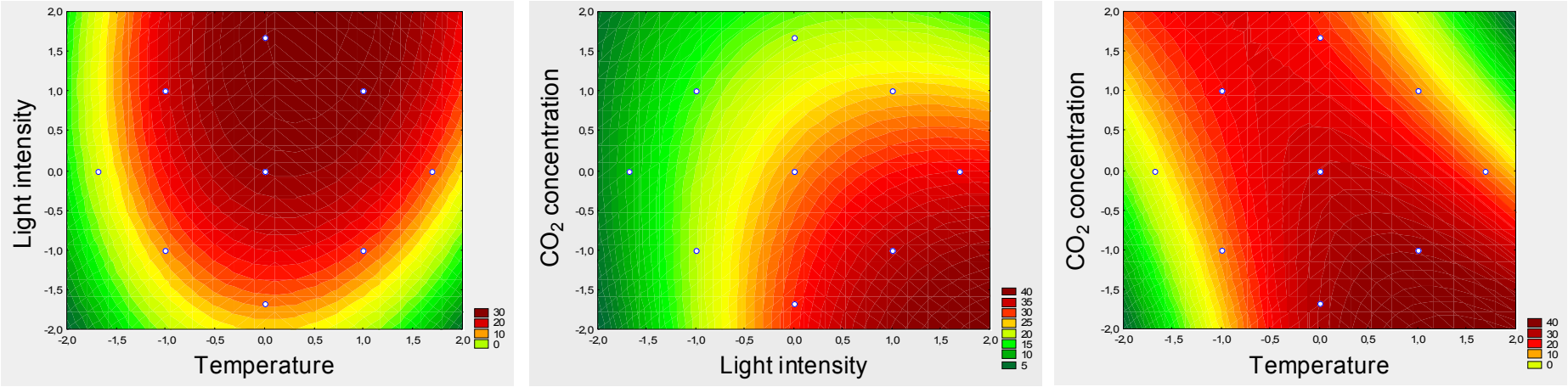
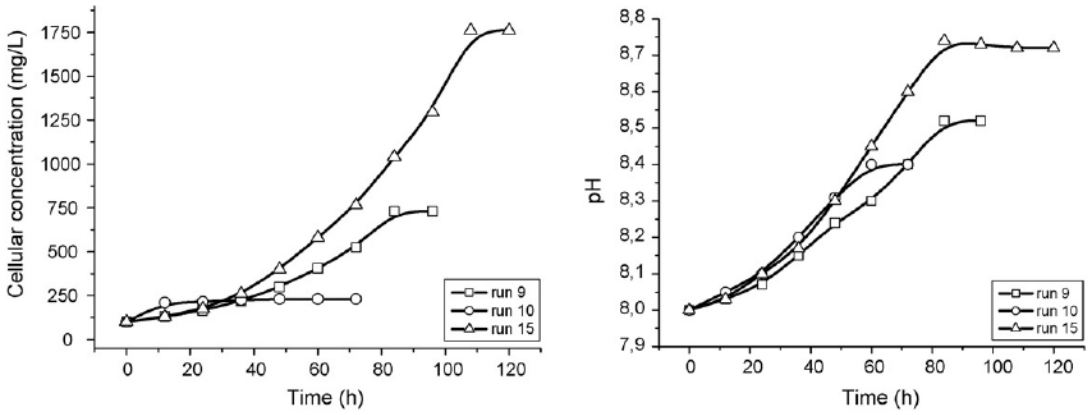


Figure 3: Contour curves for carbon fixation rate into biomass by the *Aphanothece microscopica Nägeli* (cultivations in bubble column reactor). Tested conditions: temperature (21, 25, 30, 35, 38ºC); light intensity (0.96, 3, 6, 9, 11klux) and CO_2 concentration (3, 15, 25, 50, 62%).



Kinetic parameters for process optimization

Kinetic variable	Value[a]
μ_{max} (h^{-1}) specific growth rate	0.04
t_g (h) (generation time)	17.3
t_{log} (h) duration of logarithmic growth phase	120
X_m $(mg\,L^{-1})$	5100
R_C $(mg\,L^{-1}\,h^{-1})$	109.2

[a] Mean of three replicates are shown.

best values: μ_{max}: 0.034h-1; Minimal generation time: 17 h

**** increase of 58.1% in the carbon fixation rate, no photo inhibition probably due to intracellular carbon concentration mechanism ($CO2 \rightarrow HCO_3^-$, CO_3^{-2}**

Available online at www.sciencedirect.com
ScienceDirect
Chemical Engineering and Processing 47 (2008) 1365–1373
Chemical Engineering and Processing
Process Intensification
www.elsevier.com/locate/cep

Rates of CO_2 removal by *Aphanothece microscopica Nägeli* in tubular photobioreactors

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Received 16 February 2007; received in revised form 5 June 2007; accepted 6 June 2007
Available online 22 June 2007

Objective

- evaluate the carbon dioxide removal rates in the aqueous phase of tubular photobioreactor.

Conditions tested:

temperature: 21,5, 25, 30, 35 and 38,5ºC

light intensities: 0,96, 3, 6, 9 and 11klux

CO_2 concentration: 3, 15, 25, 50 and 62% (v/v)

For the simplified reaction ($CO_2 \rightarrow$ Products), carried out in a batch reactor with constant volume, the molar balance is described by Eq. (1):

$$\frac{-\mathrm{d}[CO_2]}{\mathrm{d}t} = r_{co_2} \tag{1}$$

Assuming that the reaction rate is a function only of the carbon dioxide concentration, the rate law can be written in the following form:

$$-r_{co_2} = k[CO_2]^n \tag{2}$$

Considering a first order reaction ($n = 1$) and combining the rate law with the molar balance, Eq. (3) is obtained:

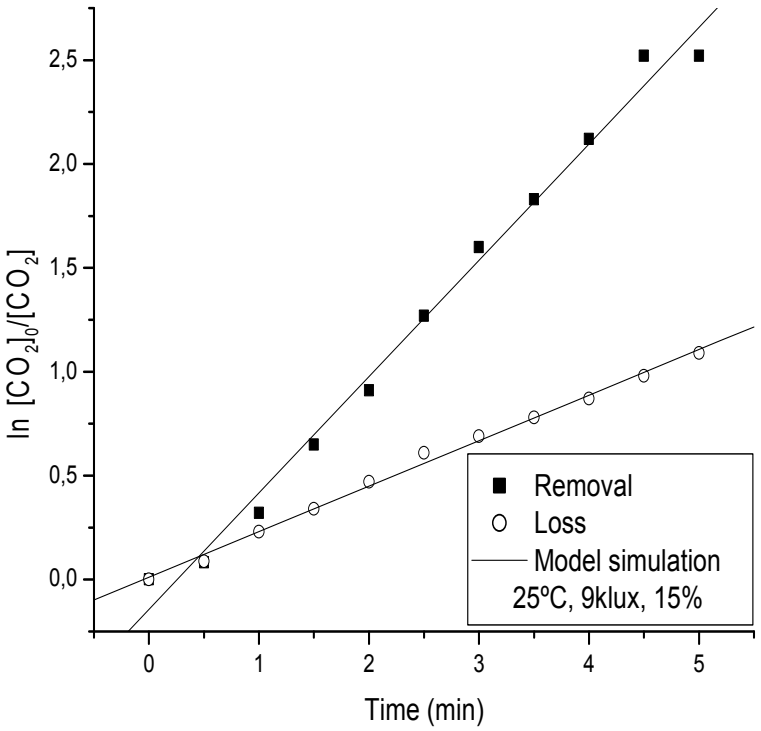
$$\frac{-\mathrm{d}[CO_2]}{\mathrm{d}t} = k[CO_2] \tag{3}$$

Integrating the differential equation, with $[CO_2] = [CO_2]_0$ at $t = 0$, Eq. (3) becomes:

$$\ln\frac{[CO_2]_0}{[CO_2]} = kt \tag{4}$$

Thus, the graph of $\ln([CO_2]_0/[CO_2])$ as a function of time should be linear, with a slope corresponding to the rate constant of the reaction (k).

However, one should consider that the variation in carbon dioxide as a function of time is not only due to biological and physicochemical removal, since part of the CO_2 is lost with the exhaustion gases (desorption). The true rate of carbon dioxide removal from the system is obtained by determining the resulting rate constant of the reaction (k_R), which corresponds to the difference between the rate constant of the reaction for the processes of absorption (k_1) and desorption (k_2). In this way, with

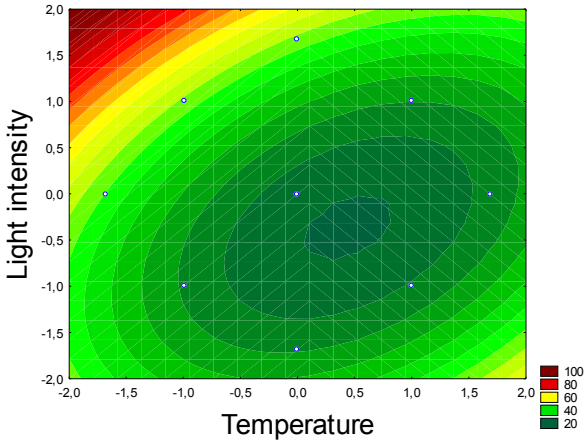


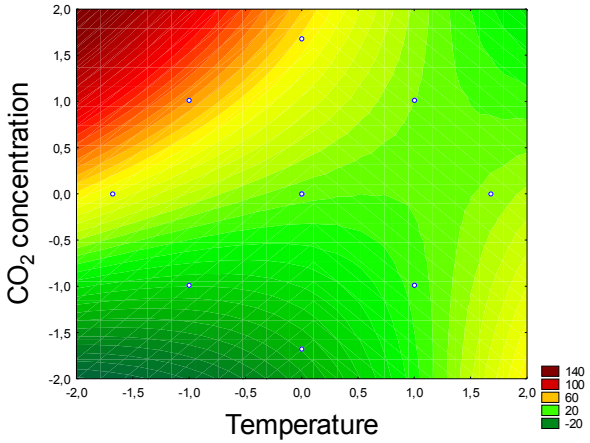
Fit of the experimental data by the integral method for the analysis of first order kinetic data

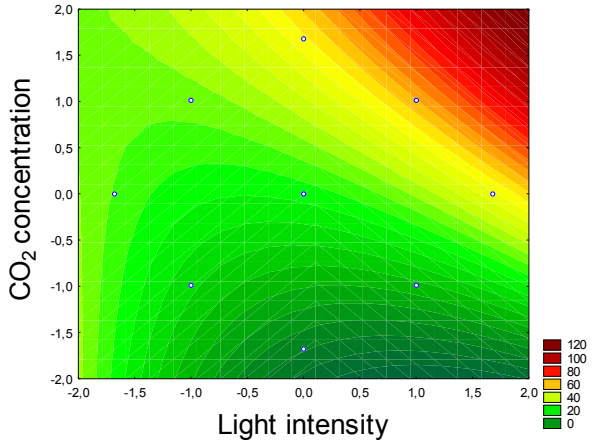
Initial cell conc. 0.1g/l

IMPROVING OF GLOBAL CARBON DIOXIDE SEQUESTRATION BY MICROALGAE

Carbon fixation rate RC_{max}= 109mg_{CO2}/L.min







Contour curves for the variable carbon dioxide removal rate.

*Global sequestration rates indicate the presence of the another routes of carbon dioxide bioconversion (apart incorporation into biomass):

- Precipitation of carbonate and bicarbonate
- Exopolymers
- Volatile organic compounds (VOC's)

Contents lists available at ScienceDirect

Chemical Engineering and Processing: Process Intensification

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Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors

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School of Chemical Engineering, State University of Campinas, UNICAMP, P.O. Box 6066, 13083-970 Campinas, SP, Brazil

Objective

➢ evaluate the effect of the photoperiod on the biomass production and carbon dioxide fixation rates

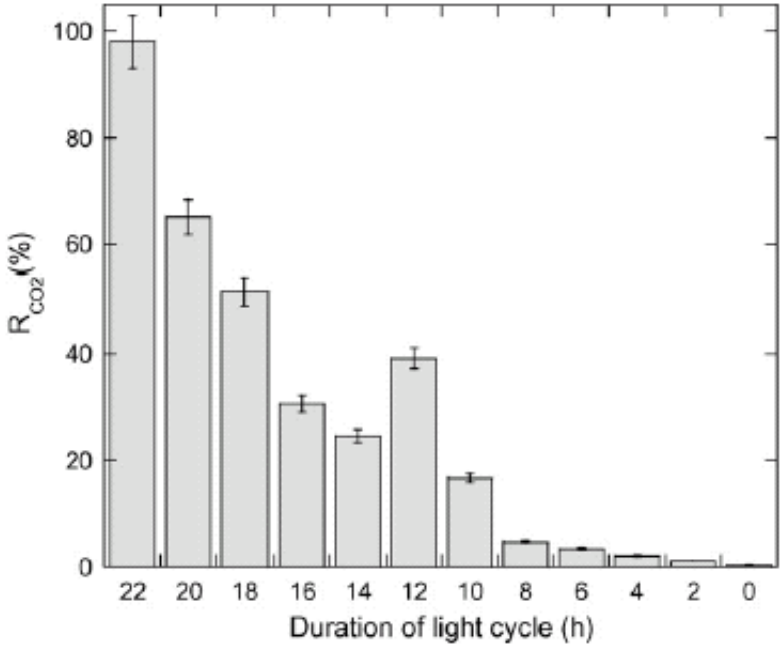
Conditions tested:

Light cycles: 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0 (night:day)

Table 1: Kinetic parameters for *Aphanothece microscopica Nägeli* in different light cycles

Photoperiod (night/day) (h)	P_X (g/L day)	X_{max} (g/L)	R_{CO_2} (g/L day)
0:24	$0.770^{a} \pm 0.038$	$5.100^{a} \pm 0.255$	$1.440^{a} \pm 0.072$
2:22	$0.764^{a} \pm 0.042$	$5.080^{a} \pm 0.305$	$1.428^{a} \pm 0.085$
4:20	$0.501^{b} \pm 0.025$	$3.400^{b} \pm 0.187$	$0.936^{b} \pm 0.065$
6:18	$0.235^{c} \pm 0.014$	$2.685^{c} \pm 0.174$	$0.439^{c} \pm 0.032$
8:16	$0.240^{d} \pm 0.016$	$1.640^{d} \pm 0.116$	$0.448^{c} \pm 0.040$
10:14	$0.189^{e} \pm 0.009$	$1.300^{e} \pm 0.052$	$0.353^{d} \pm 0.021$
12:12	$0.301^{f} \pm 0.016$	$2.060^{f} \pm 0.072$	$0.562^{e} \pm 0.025$
14:10	$0.127^{g} \pm 0,006$	$0.944^{g} \pm 0.018$	$0.237^{f} \pm 0.014$
16:8	$0.035^{h} \pm 0,002$	$0.343^{h} \pm 0.013$	$0.065^{g} \pm 0.003$
18:6	$0.026^{i} \pm 0.001$	$0.260^{i} \pm 0.013$	$0.048^{g} \pm 0.003$
20:4	$0.015^{j} \pm 0.000$	$0.200^{i} \pm 0.017$	$0.028^{g} \pm 0.001$
22:2	$0.008^{k} \pm 0.000$	$0.150^{i} \pm 0.009$	$0.015^{g} \pm 0.001$
24:0	$0.002^{l} \pm 0.000$	$0.110^{i} \pm 0.004$	$0.004^{g} \pm 0.000$

Values are mean ± S.D. of quadruplicate analysis; Within the same column, means having different superscripts (a–l) are significantly different ($p < 0.05$) by Tukey's test.



Percent carbon dioxide fixation rates (into biomass) as related to the duration of the light periods (bubble column reactor for optimized conditions).

Final considerations :

Highest CO_2 removal very often does not correspond to the highest specific growth rates,

Possibility that photosynthetic reactions also leads to the formation of extracellular products;

CO_2 is incorporated to phosphoglycerate (PGA) catalyzed by carbonic anhydrase

High levels of intracellular CO_2 (1000x)

Development of operational strategies to remove carbon dioxide in photobioreactors

Eduardo Jacob-Lopes[1], Sergio Revah[2], Sergio Hernández[3], Keiko Shirai[4] and Telma Teixeira Franco[1*]

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[2]Department of Process and Technology, Universidad Autónoma Metropolitana-Cuajimalpa, UAM-C, México DF, México.
[3]Department of Hydraulic and Process Engineering, Universidad Autónoma Metropolitana-Iztapalapa, UAM-I, Mexico DF, Mexico.
[4]Department of Biotechnology, Universidad Autónoma Metropolitana-Iztapalapa, UAM-I, Mexico DF, Mexico.

Chemical Engineerind Science, *Accepted*, 2008.

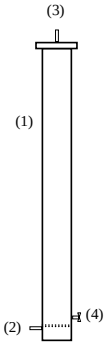
Objective

- **evaluate different operational strategies for photobioreactors in order to remove carbon dioxide using microalgae**

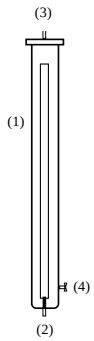
Conditions tested:

- reactors: bubble column and airlift
- operational mode: simple operation, air recirculation and two stages in series

[A-B]: (1): reactor; (2): gas entrance sampler; (3): gas exit sampler; (4): liquid sampler.

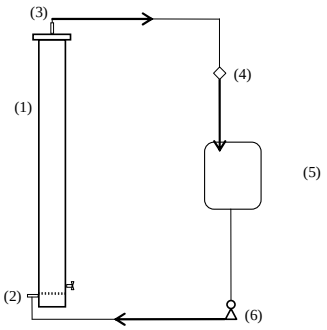


(A) BCR reactor with simple operation

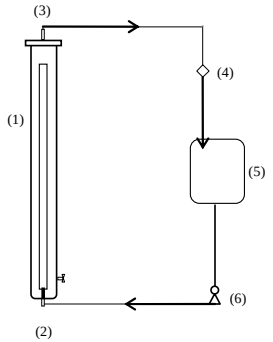


(B) ALR reactor with simple operation

[C-D] (1): reactor; (2): gas entrance sampler; (3): gas exit sampler; (4): air dehumidifier; (5): storage tank; (6): pump.

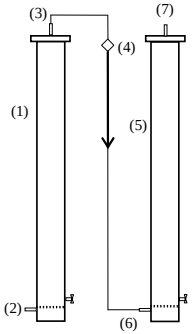


(C) BCR reactor with air recirculation

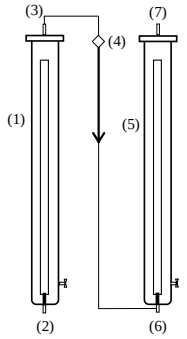


(D) ALR reactor with air recirculation

[E-F]: (1): reactor 1; (2): gas entrance sampler; (3): gas exit sampler; (4): air dehumidifier, (5): reactor 2; (6): gas entrance sampler; (7): gas exit sampler.



(E) BCR reactors in series

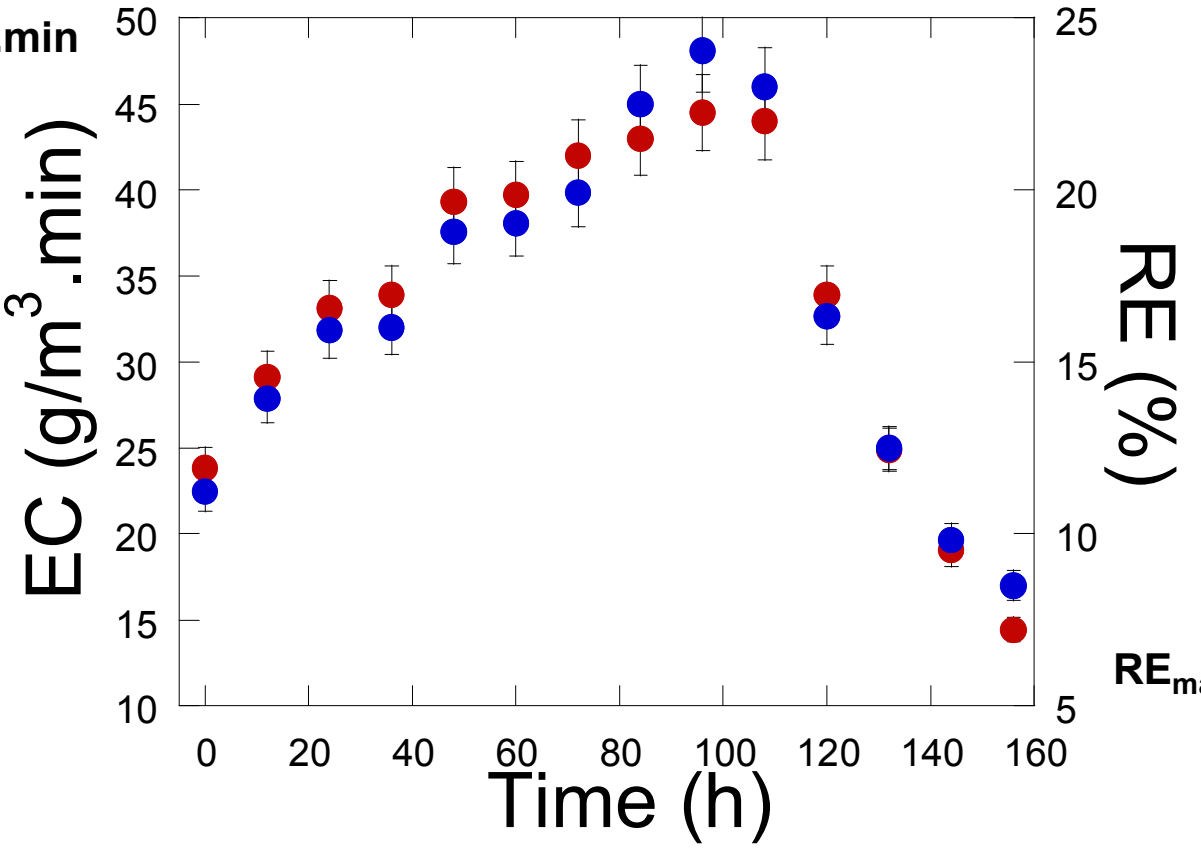


(F) ALR reactors in series

- **Airlift reactors:**

EC_{max}: 46.4g/m³.min

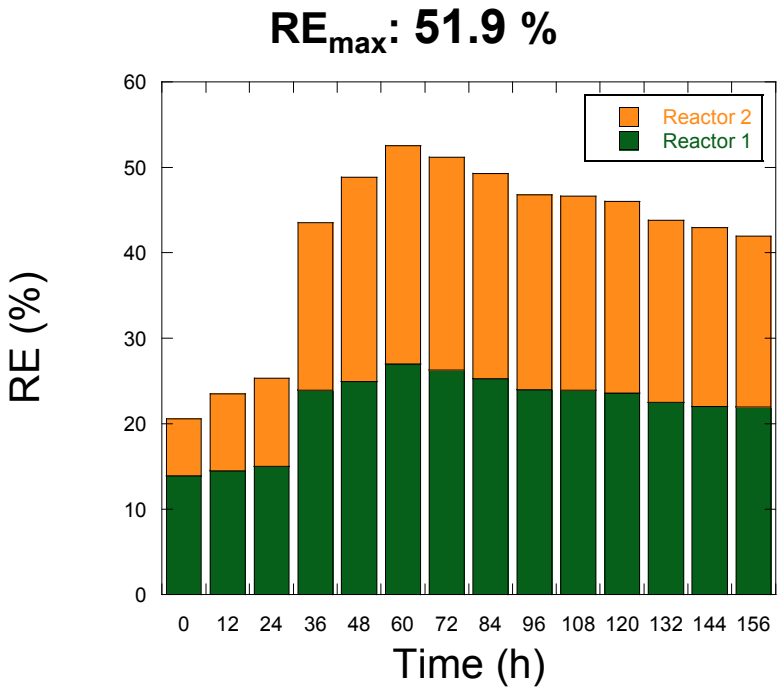
$$EC = \frac{(C_i - C_o) \times Q}{V_R}$$

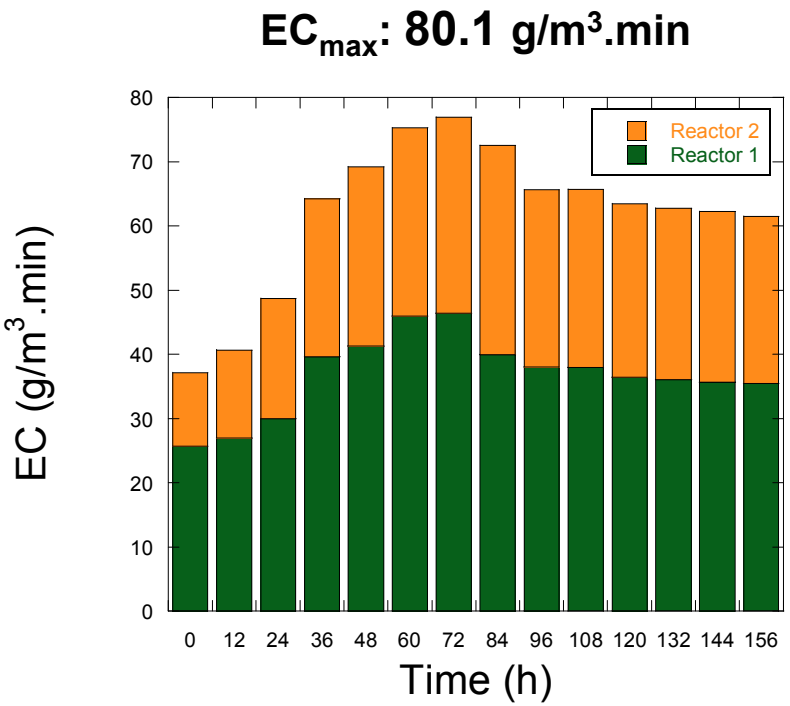


$$RE = \frac{(C_i - C_o)}{C_T} \times 100$$

RE_{max}: 26.9g/m³.min

Kinetic data for the airlift reactor with simple operation. EC: elimination capacity. RE: removal efficiency.





Kinetic data for two airlift reactors in series in the optimized conditions. Tested conditions: configuration (airlift); operational mode (simple operation, air recirculation and two reactors in series). EC: elimination capacity. RE: removal efficiency.

Daily carbon sequestering capacity of the reactors.

System	Carbon sequestered ($g_{carbon}/L_{reactor}$.day)
BCR (simple operation)	12.90 ± 0.15
BCR (operation with air recirculation)	5.55 ± 0.16
BCR (operation in series)	18.30 ± 0.18
ALR (simple operation)	14.32 ± 0.12
ALR (operation with air recirculation)	8.67 ± 0.10
ALR (operation in series)	24,13 ±0.09

BCR: bubble column reactor; ALR: airlift reactor

Industrial approach

- **refinery flue gases**
- **refinery wastewater**

Refinery wastewater improving for microalgal production and CO_2 biofixation: predictive modelling and simulation

Eduardo Jacob-Lopes[1], Carlos Henrique Gimenes Scoparo[1], Maria Isabel Queiroz[2], Kelerson Modenesi[3], Telma Teixeira Franco[1*]

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2Biotechnology Laboratory, Chemical Departament, Fundação Universidade Federal do Rio Grande, FURG, 96201-900, Rio Grande-RS, Brazil.
3Petróleo Brasileiro S/A – Replan/Petrobras, 13140-000, Paulínia-SP, Brazil.

Journal of Biotechnology, *Submited*, 2008.

Petrochemical industry

- **Generation and consumption of Energy**

- **Refinery Paulínia – Replan/Petrobras (1,04%)**

  - **2.954.022 equivalent ton CO_2/year (99% CO_2)**
  - **1.181 ton CH_4/year**
  - **33 ton N_2O**

source: Chan, 2007

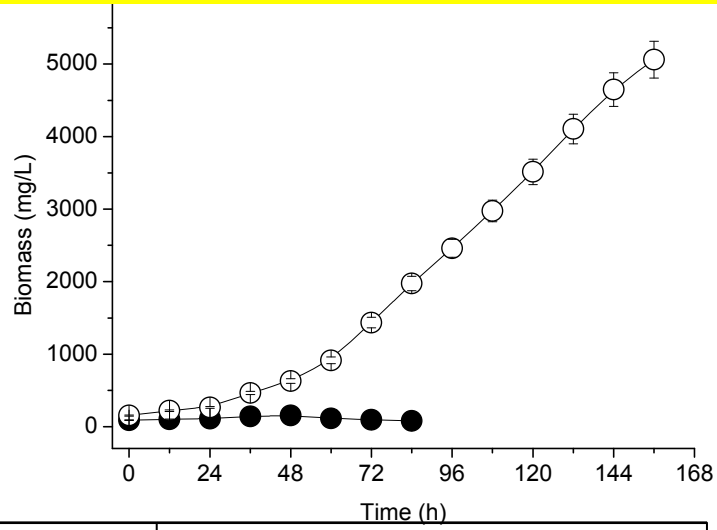
Composition of wastewater from refinery industry

***Values are means ± SD of all months considered.**

Parameter	Treated effluent*
pH	8.3 ± 0.24
Temperature (ºC)	28.1 ± 2.41
BOD (mg/L)	14.0 ± 1.36
Nitrite (mg/L)	0.1 ± 0.00
Nitrate (mg/L)	15.4 ± 0.32
Ammonia (mg/L)	1.2 ± 0.10
Phosphate (mg/L)	0.5 ± 0.00
Phenol (mg/L)	0.02 ± 0.00
Cyanide (mg/L)	0.04 ± 0.00
Oil and grease (mg/L)	4.6 ± 0.38
TSS (mg/L)	0.13 ± 0.00

Water collected from the discharge point of the activated sludge treatment for 8 months from May to December of 2007,

To evaluate the use of refinery wastewater in microalgae cultivation for CO2 biofixations



Growth curves in the refinery wastewater (closed symbols) and in the synthetic BGN medium (open symbols).

Culture Medium	Composition
M1	**refinery wastewater**
M2	**synthetic BGN medium**
M3	**75% wastewater and 25% BGN**
M4	**50% wastewater and 50% BGN**
M5	**25% wastewater and 75% BGN**
M6	**wastewater with 100% BGN salts supplementation**
M7	**wastewater with 75% BGN salts supplementation**
M8	**wastewater with 50% BGN salts supplementation**
M9	**wastewater with 25% BGN salts supplementation**

Growth data of *Aphanothece microscopica Nägeli* in different tests

Media	X_{max} (g/L)	μ_{max} (h^{-1})	pH (end)
M1	0,16	0,033	8,96
M2	5,06	0,028	9,12
M3	0,71	0,026	8,92
M4	2,28	0,040	8,95
M5	4,92	0,044	9,10
M6	4,34	0,034	8,75
M7	3,80	0,052	9,0
M8	3,43	0,047	9,31
M9	2,05	0,046	8,9

CO_2 removal rates and O_2 release rates (for M9 media)

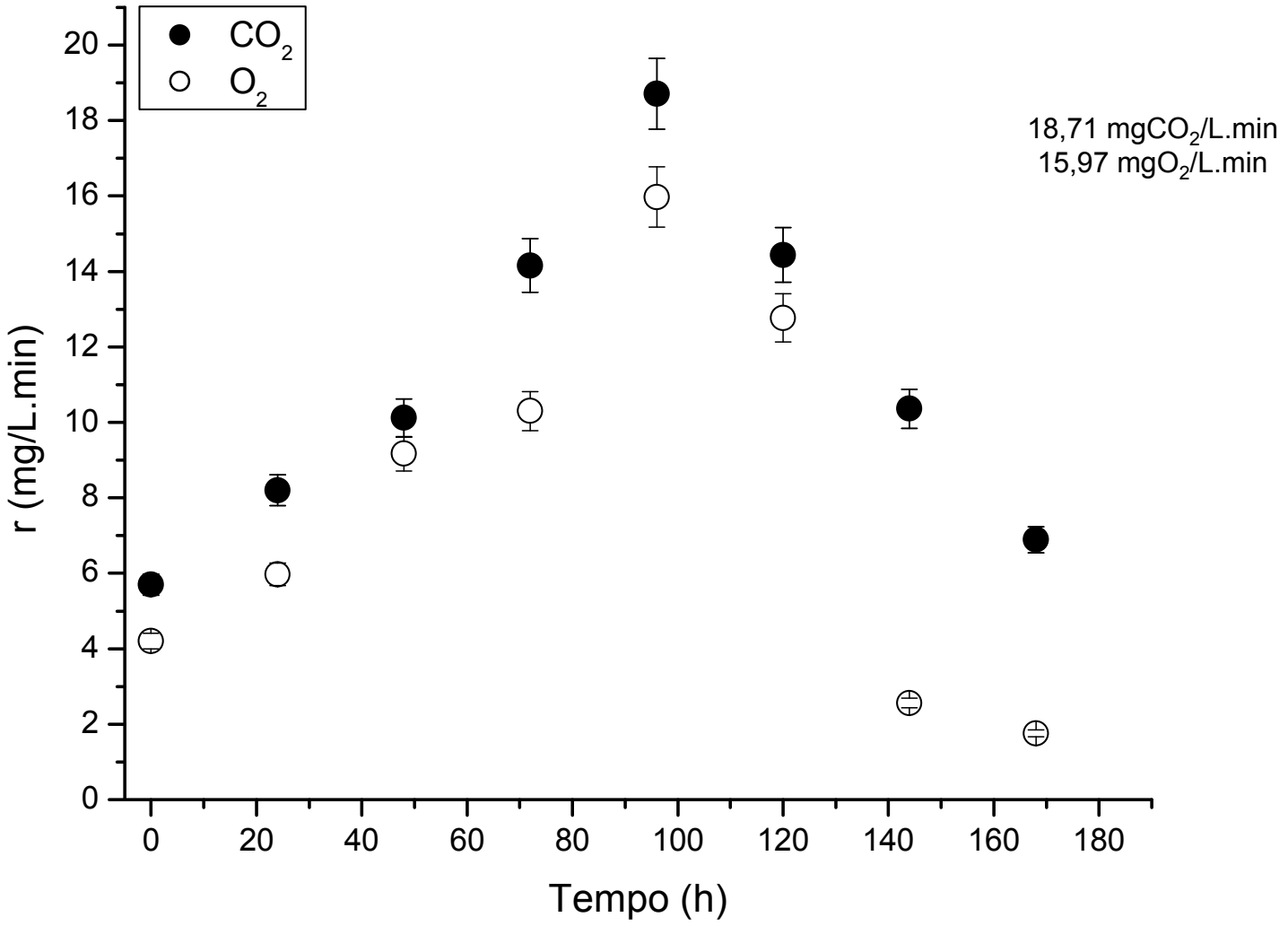
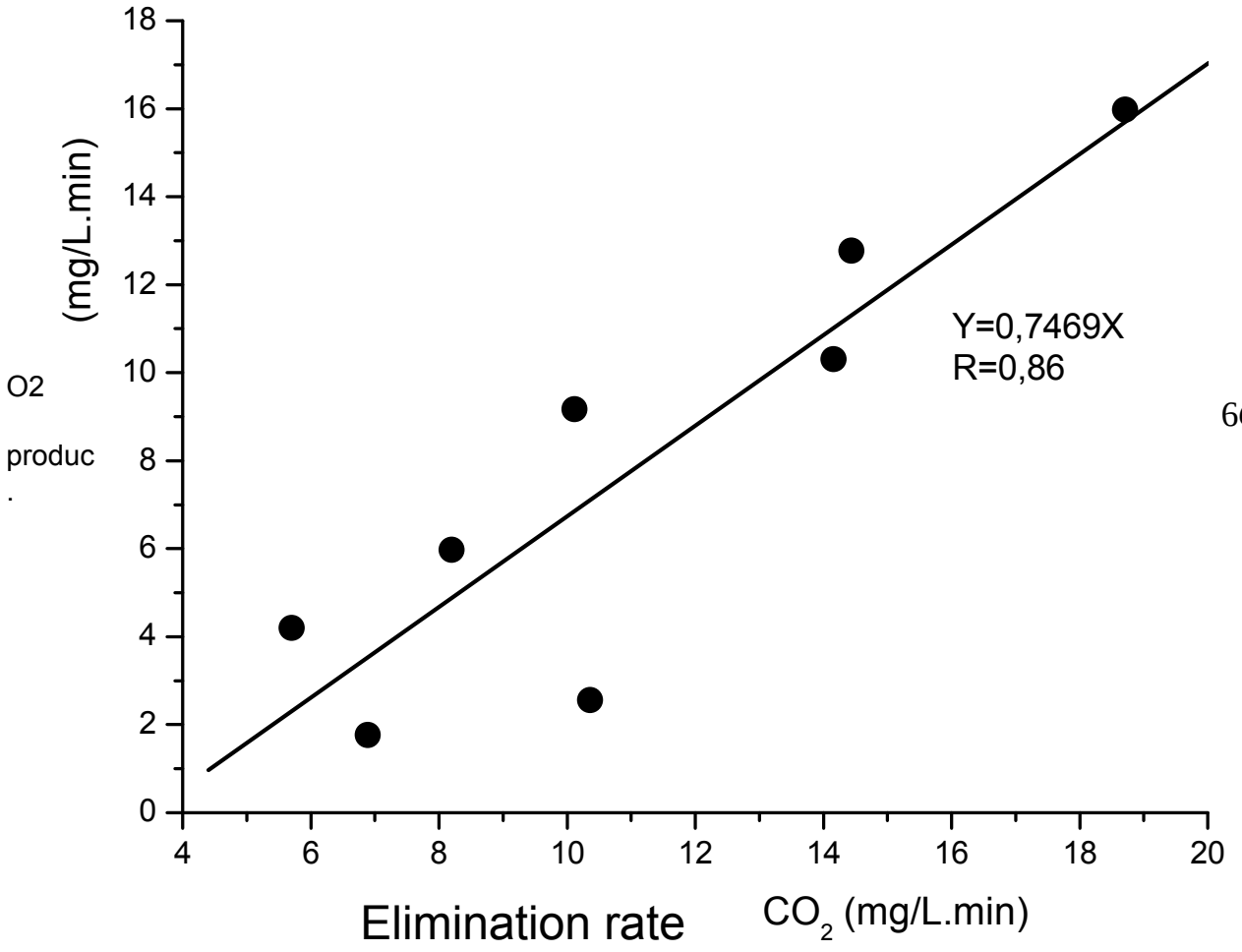


Figure 9: Carbon dioxide sequestration and oxygen release rates; ● CO2 ○ O2 (measurements in the gaseous phase)

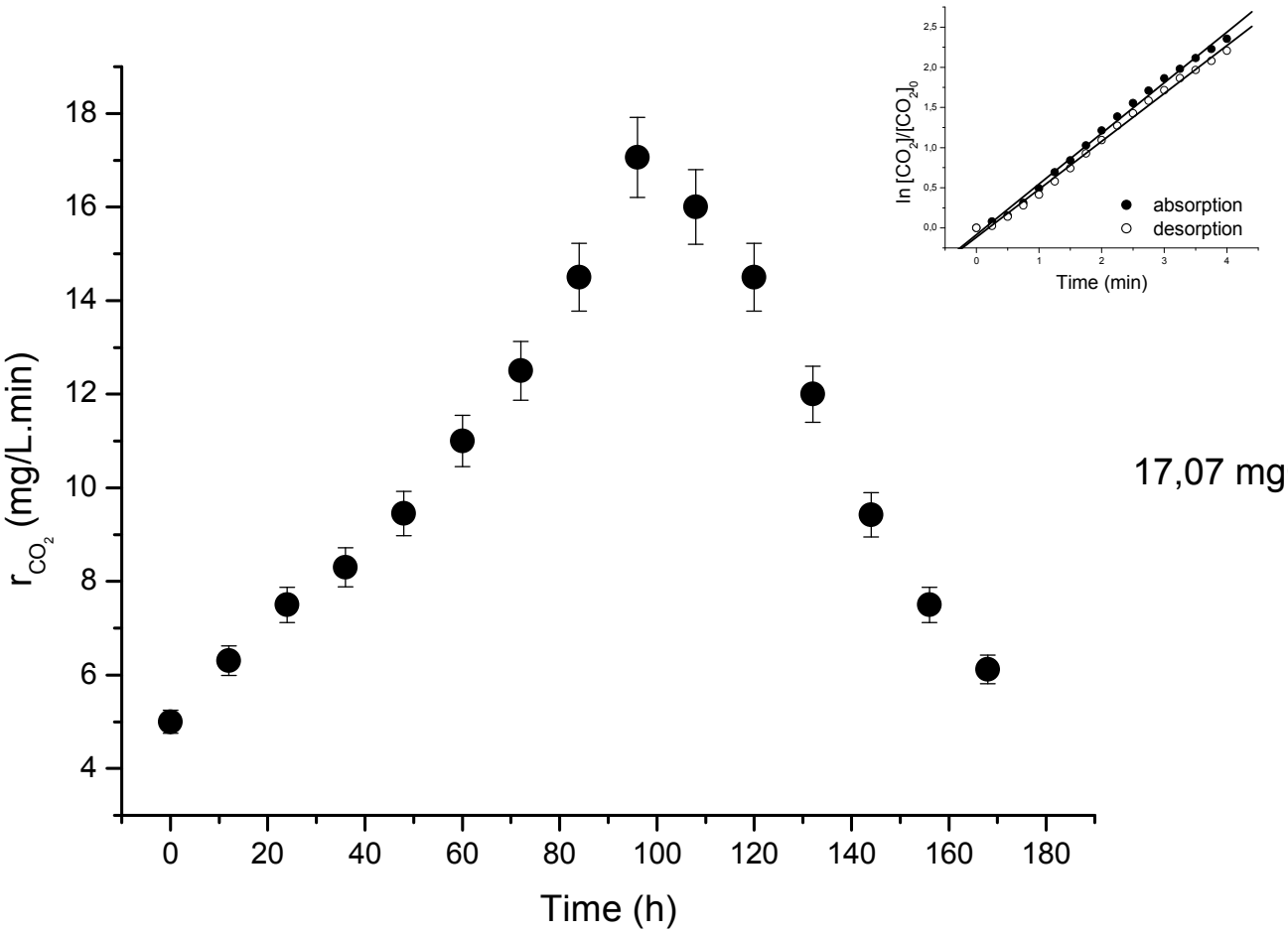
Photosynthetic quotient (PQ)



$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

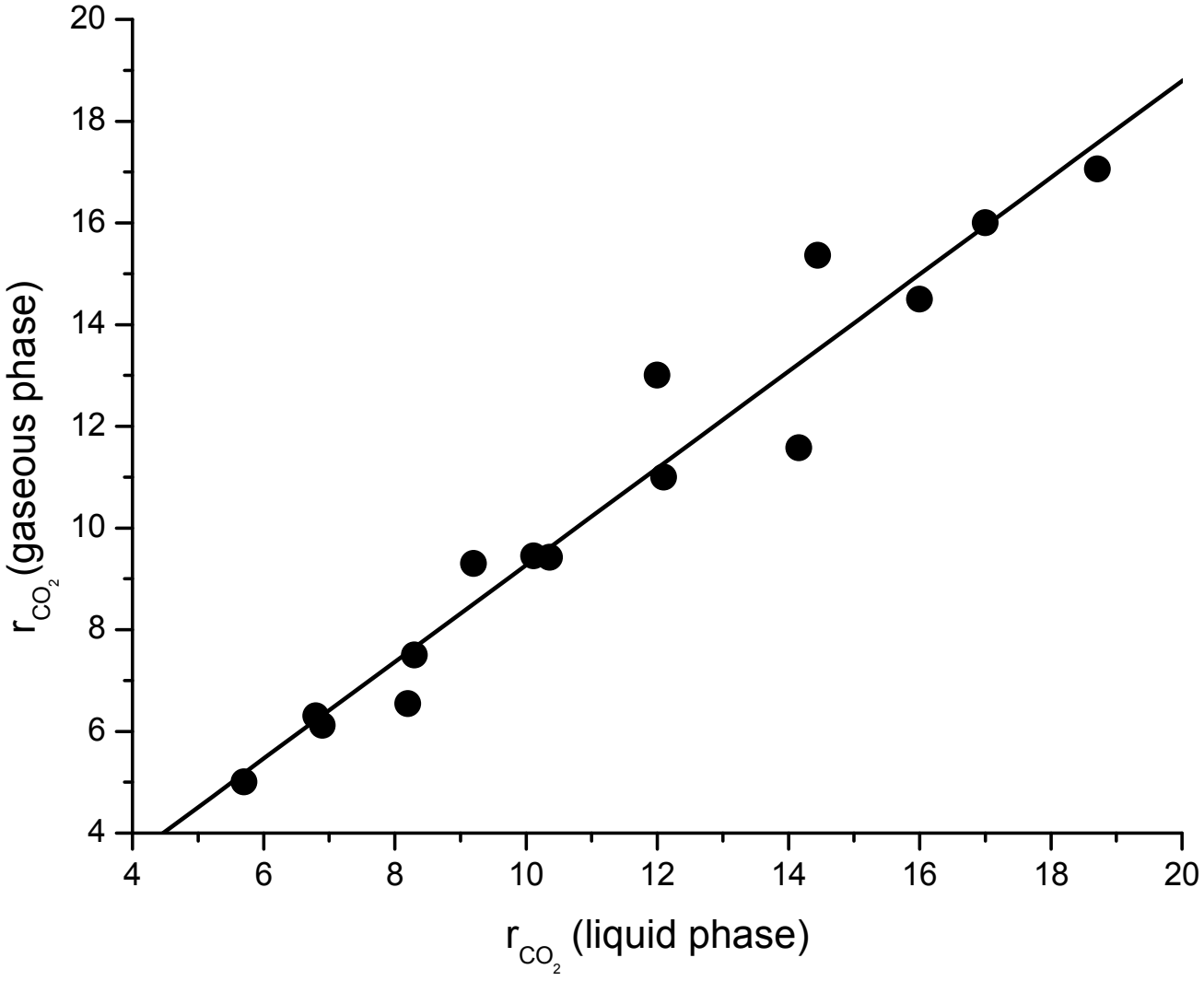
Ratio between O_2 release rate and CO_2 sequestration rate

➢ Liquid phase studies



17,07 mgCO_2/L.min

Carbon dioxide sequestration rates and fit of the experimental data by the integral method (measurements in the liquid phase)



Comparison between carbon dioxide sequestration rates evaluated in the liquid and gaseous phases

➢ Rates of carbon fixation into biomass

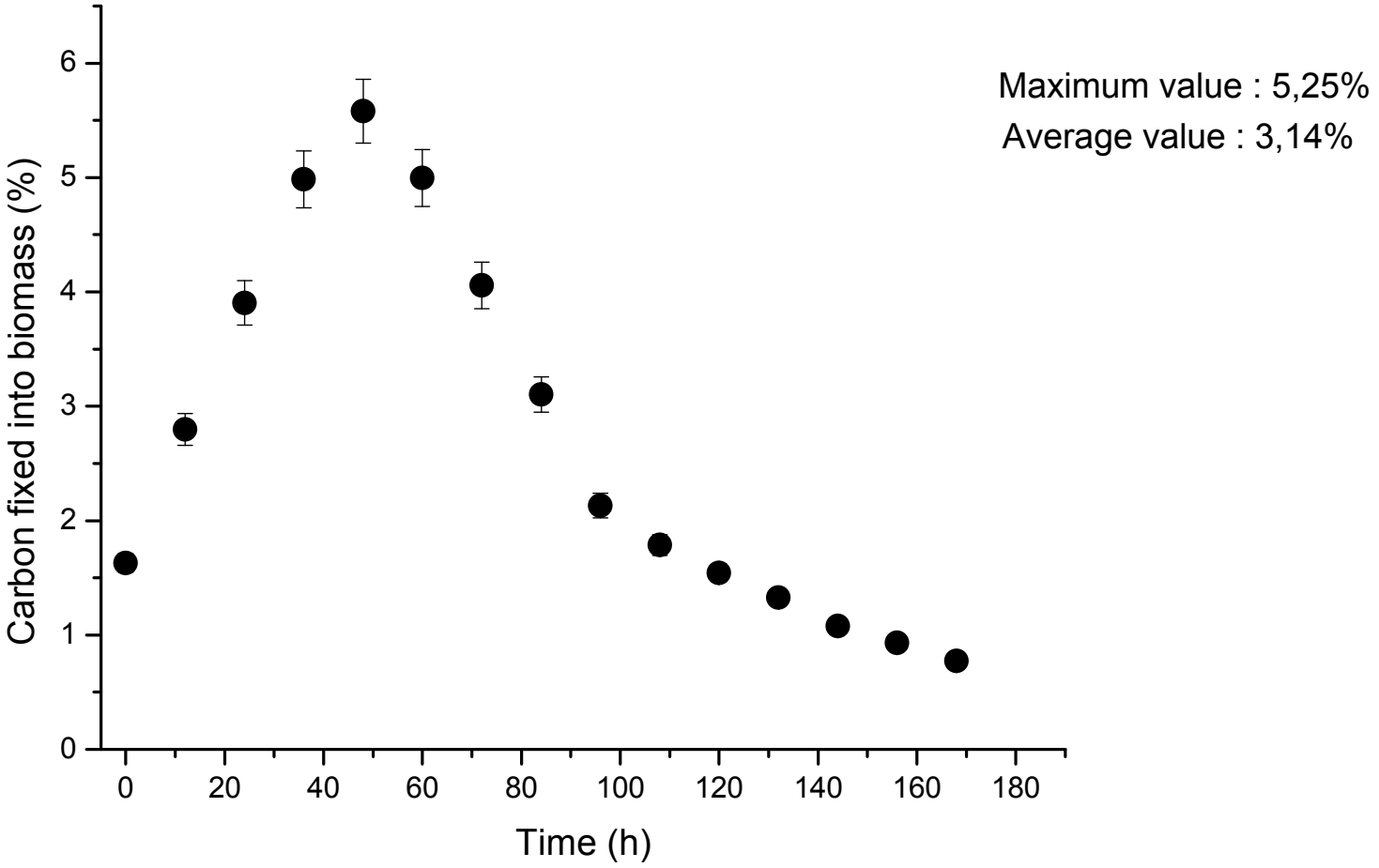
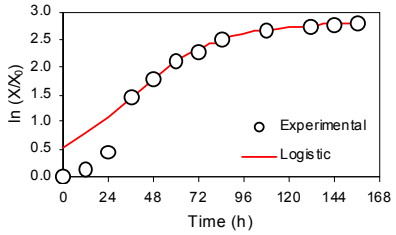
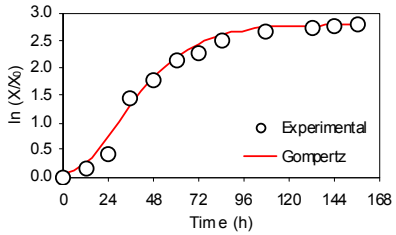
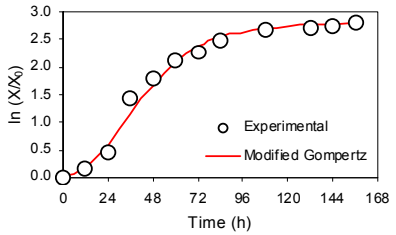
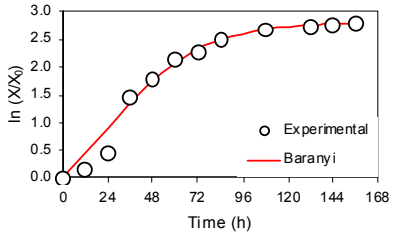


Figure 13: Percentage of carbon sequestered effectively fixed into biomass.









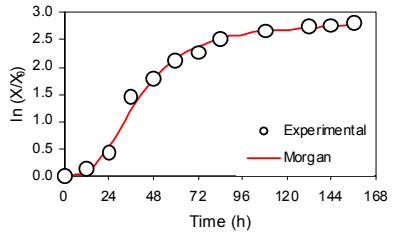


Figure 2: Fit of the models to experimental data.

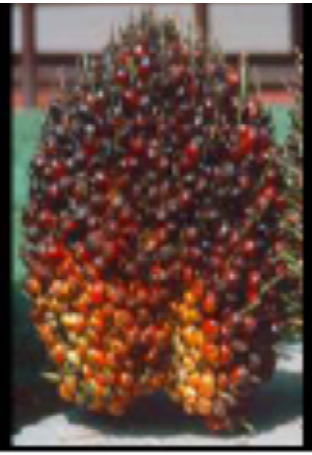
Moving to continuous operation prediction ...

According to Modifief Gompertz model → for the M9 culture medium: μ_{max}=1.22d^{-1}, λ=15h and X_{max}=2.05g/L.

Cell concentrations and biofixation were predicted (mass balance to CSTR operation)

→58.8$kg_{biomass.}m^3.day^{-1}$ with a biofixation of 110.0$kg_{CO2.}m^3.day^{-1}$;

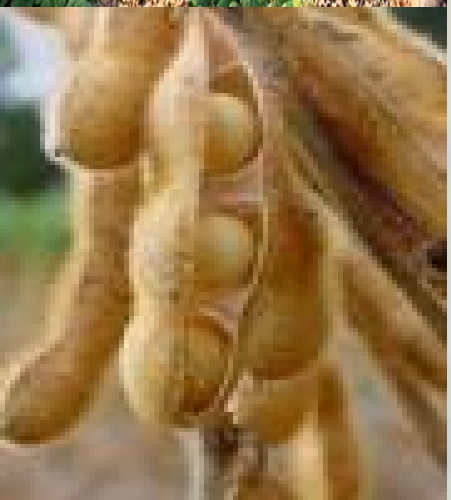
→The amount of produced oil would depend on the strain of the algae;

Ricinus oil	sunflower	soybean	Palm oil	cotton
				
Yields of the crops (year)				
1.500 kg/ha	1.500 kg/ha	3.000 kg/ha	20.000 kg/ha	3.000 kg/ha
% vegetal oil				
47%	42%	18%	20%	15%
vegetal oil (kg/ha)				
705	630	540	4.000	450

Fonte: diversas (Embrapa, MDA, IBGE, CONAB)

1st or 3th generation of biofuels?

Crop



Soybean[1]

2700 kg/ha

20% fatty

Cycle 120 days/year

$0,46g_{fatty}/m^2.day$

Microbial

***Aphanothece*[2]**

1,04 g/L.day

7,5% fatty

Cycle 120 days/year

CSTR ≈ few L/m^2

[1] EMBRAPA, www.embrapa.br, (2008)

[2] Jacob-Lopes et al. Biochem. Eng. J. (2008)

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Thank you

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