Unidad de Innovación Docente
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MEMORIA FINAL

Actuaciones Avaladas para la Mejora Docente, Formación del Profesorado y Difusión de Resultados
Modalidad A

Identificación de la actuación	
Código:	AAA_13_008
Título:	INTRODUCCIÓN DEL INGLÉS EN LAS ASIGNATURAS DEL GRADO EN BIOTECNOLOGÍA

Responsable	
Apellidos y nombre:	Gómez Montes de Oca, José Manuel
Correo electrónico:	josemanuel.montesdeoca@uca.es
Departamento:	Ingeniería Química y Tecnología de Alimentos

1. Describa la contribución a la actuación de cada uno de los participantes. Copie y pegue las líneas que necesite para contemplarlos a todos y disponga del espacio que necesite.

Apellidos y nombre:	Gómez Montes de Oca, José Manuel
Coordinación del proyecto, participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Cantero Moreno, Domingo
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Cabrera Revuelta, Gema
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Ramírez Muñoz, Martin
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Cantoral Fernández, Jesús Manuel
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Rebordinos González, Laureana
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Rodríguez Jiménez, Mª Esther
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Cross Pacheco, Ismael
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Ayuso Vilacides, Jesús
Participación en el curso de inglés, elaboración de material, realización de exposición oral en inglés, participación en las reuniones del proyecto.	

Apellidos y nombre:	Cabrera Castro, Remedios
Participación ocasional en el curso de inglés y realización de exposición oral en inglés	

Apellidos y nombre:	Bolívar Pérez, Jorge
Participación ocasional en el curso de inglés.	

Apellidos y nombre:	Merlo Torres, Manuel Alejandro
Participación ocasional en el curso de inglés.	

Apellidos y nombre:	Álvarez Saura, Jose Ángel
Participación ocasional en el curso de inglés.	

2. Describa de manera precisa los resultados obtenidos a la luz de los objetivos y compromisos reflejados en la solicitud. Copie y pegue tantas tablas como necesite y tenga en cuenta que la extensión de este apartado no podrá sobrepasar el de un folio (2 páginas).

Objetivo 1: Desarrollar la competencia idiomática en el profesorado del Grado en Biotecnología
Actividades realizadas y resultados obtenidos: Se ha realizado un curso de actualización lingüística de 45 horas de duración que ha permitido el desarrollo de esta competencia con un programa de inglés específico para esta titulación.

Objetivo 2: Preparar materiales docentes en un segundo idioma (inglés)
Actividades realizadas y resultados obtenidos: El profesorado participante ha preparado material docente en inglés relativo a las asignaturas que imparte en el Grado. Parte de ese material se ha ido incorporando a la docencia de sus asignaturas.

Objetivo 3: Favorecer la formación del profesorado mediante habilidades comunicativas en un segundo idioma
Actividades realizadas y resultados obtenidos: Durante el desarrollo del proyecto se han trabajado situaciones cotidianas que pueden darse en la vida universitaria, que han servido de punto de partida para realizar debates y coloquios entre los participantes del proyecto. Estas situaciones han servido para incrementar el vocabulario de los participantes, así como favorecer la comunicación en inglés. Igualmente, las presentaciones realizadas por los participantes han supuesto un coloquio sobre temas específicos de Biotecnología en algunas de las sesiones.

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ANEXO: Presentaciones realizadas por los participantes en el proyecto

Counterfeit in olive oils

The AIM: To develop a screening method for counterfeit in olive oils

Virgin Olive Oil, VOO, the object of the presentation, is an essential component of the Mediterranean diet.

It is part of the Andalusia culture since ancient times and

is considered as product of high reputation,

highly appreciated in gastronomy.

Jesús Ayuso,

Dpt. of chem. Phys.

Subject: Thermodynamic and kinetic.

Counterfeit in olive oils

Before starting, a little introduction about the types of olive oil is necessary.

Counterfeit in olive oils

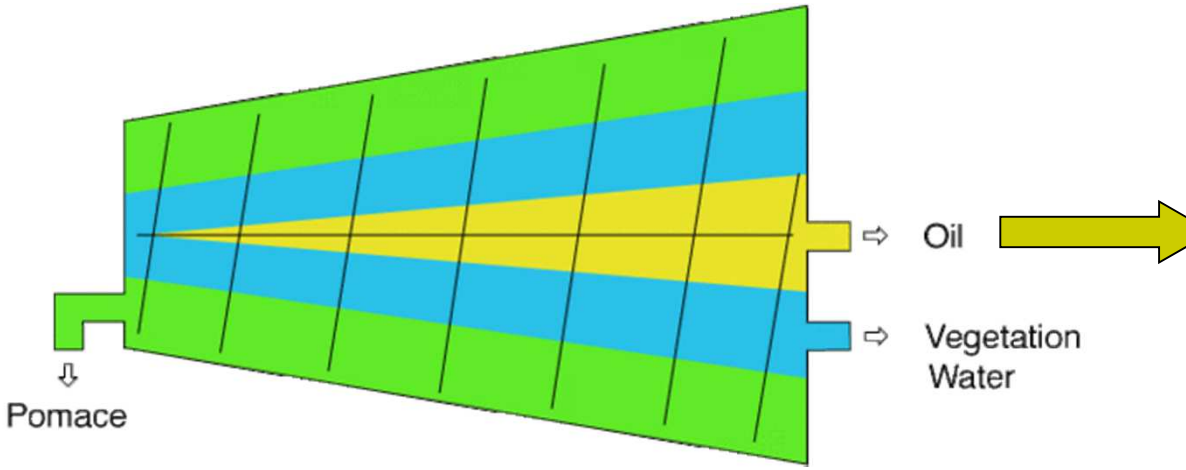
Olive harvest: from late november to early february.

Olive milling

Paste of crushed olives

Counterfeit in olive oils

Olive oil extraction by Decanter centrifugation



Counterfeit in olive oils

The oil can be stored and filtered, as well.

Finally the oil is bottled and sold

First cold pressed oil

Counterfeit in olive oils

Based on the productions process, olive oils are classified as:

"Virgin olive oil" which is defined by the International Olive Oil Council (IOOC) as the oil obtained from the fruit of the olive with **only minimal treatments such as washing, pressing, decantation, centrifugation or filtration**. If the VOO contains a free acidity lower than 2% of free fatty acid, it can be suitable for consumption without any treatment.

Additionally, "extra virgin olive oil", EVOO, must have a free acidity percentage of less than 0.8% and keeps the flavour characteristics of the original olive fruit as well .

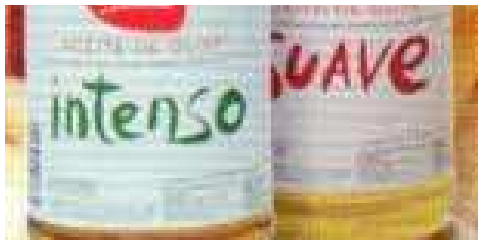
When the VOO contains a higher free acidity, this oil is known as "lampante olive oil" and needs to be refined in order to become edible.

Counterfeit in olive oils

Other olive oils are:

“Refined olive oil” is referred to those oils that had some defect (loss of aroma), and need to be processed in order to be edibles. The refining process can not alter the initial triglyceride composition.

As edible olive oils, there are:



“Pure olive oils”, also labeled “Olive oils”, are blends of refined oils and VOO and must possess the free acidity of no more than 0.3% for the “suave” or smooth taste oil and less than 1% for “intenso” or strong taste oil.

“Pomace olive oil“ is obtained by solvent extraction, heat treatment, esterification, or refining. The free acidity must be lower than 1% when is blended with VOO.

Counterfeit in olive oils

OIL SEEDS

Sunflower

Corn

Soybean

Counterfeit in olive oils

Average annual production of olive oil

Países	Prod. Mundial (x 1.000 Tm)	Prod. Mundial %
Unión Europea	1.536,8	75,57
Túnez	161,5	7,94
Turquía	98,2	4,83
Siria	83,5	4,10
Marruecos	54,3	2,67
Argelia	28,7	1,41
Otros	70,7	3,48
Total	2.033,7	100,00

Counterfeit in olive oils

The **health benefits** together with the **fine aroma** and a pleasant taste, are the reasons that make the VOO highly appreciated and so, it cost more than other commonly used vegetable oils.

Price of oils:

Oil	€/L
EVOO-VOO	From 3 € to 18 € (and more)
Olive Oil	From 2,5 € to 3 €
Pomace Olive Oil	From 1,5 € to 2,5 €
Sunflower Oil	From 1,0 € to 3 €
Corn Oil	From 1,0 € to 2 €
Soybean Oil	From 1,0 € to 2 €
Oil from other seeds	From less than 1,0 €

Therefore, the production of olive oil is **a big business** that is prone to **fraudulent marketing attempts.**

LETTER FROM ITALY

SLIPPERY BUSINESS

The trade in adulterated olive oil.

BY TOM MUELLER

AUGUST 13, 2007

On August 10, 1991, a rusty tanker called the Mazal II docked at the industrial port of Ordu, in Turkey, and pumped twenty-two hundred tons of hazelnut oil into its hold. The ship then embarked on a meandering voyage through the Mediterranean and the North Sea. By September 21st, when the Mazal II reached Barletta, a port in Puglia, in southern Italy, its cargo had become, on the ship's official documents, Greek olive oil. It slipped through

Counterfeit in olive oils

http://en.wikipedia.org/wiki/Olive_oil_regulation_and_adulteration

In March 2008, 400 Italian police officers conducted "Operation Golden Oil," arresting 23 people and confiscating 85 farms after an investigation revealed a large-scale scheme to relabel oils from other Mediterranean nations as Italian.[14] In April 2008, another operation impounded seven olive oil plants and arrested 40 people in nine provinces of northern and southern Italy for adding chlorophyll to sunflower and soybean oil and selling it as extra virgin olive oil, both in Italy and abroad. 25,000 liters of the fake oil were seized and prevented from being exported.[15]

On December 22, 2008, the Guardia Civil in La Rioja (Spain) warned about the possible sale of adulterated olive oil in the area. This warning came after 550 litres of oil was found in a large container labelled 'Astispumante 1510' in Rincón de Soto and after the theft of 1,750 litres of oil was reported in the area on December 18, 2008.[16]

On March 15th, 2011, the Florence, Italy prosecutor's office, working in conjunction with the forestry department, indicted two managers and an officer of Carapelli, one of the brands of the Spanish company Grupo SOS (which recently changed its name to Deoleo). The charges involved falsified documents and food fraud. Carapelli lawyer Neri Pinucci said the company was not worried about the charges and that "the case is based on an irregularity in the documents." [17]

And to be continued…

Counterfeit in olive oils

http://en.wikipedia.org/wiki/Olive_oil_regulation_and_adulteration

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And to be continued…

Counterfeit in olive oils

One kind of fraud, the counterfeit is made by colouring cheaper edibles oils with some colorants like the natural pigment chlorophyll (E-140) or the complex Cu-chlorophyll (E-141i).

Since the oil used in the forgeries may have a yellowish colour, it needs add some green pigments in order to be coloured as a VOO.

But sometimes ß-carotene (colourant E-160) is added to get a more similar colour.

Counterfeit in olive oils

Natural colourants

(1) Normal seed oil. (2) The same oil coloured with E151, (3) or carotene, E160. (4) A mix of (2) and (3) in order to mimic an actual VOO (5).

Counterfeit in olive oils

Of Course, an extensive literature on the identification of mixtures of oils can be found, which describe methods of very high precision, to detect small olive oil adulteration.

But these high accuracy analytical methods have some disadvantages:

- They are big expensive, from the ecological and economical point of view (instruments and solvents).
- A very experienced annalist is needed.
- These analyses are not fast.
- Neither, it can be applied in situ.

Counterfeit in olive oils

The **cost of analyses is triggered if these have to be routine**, because the countless number of possible samples to analyze.

This is why a **screening method** is necessary and, hence, the interest of this study.

Counterfeit in olive oils

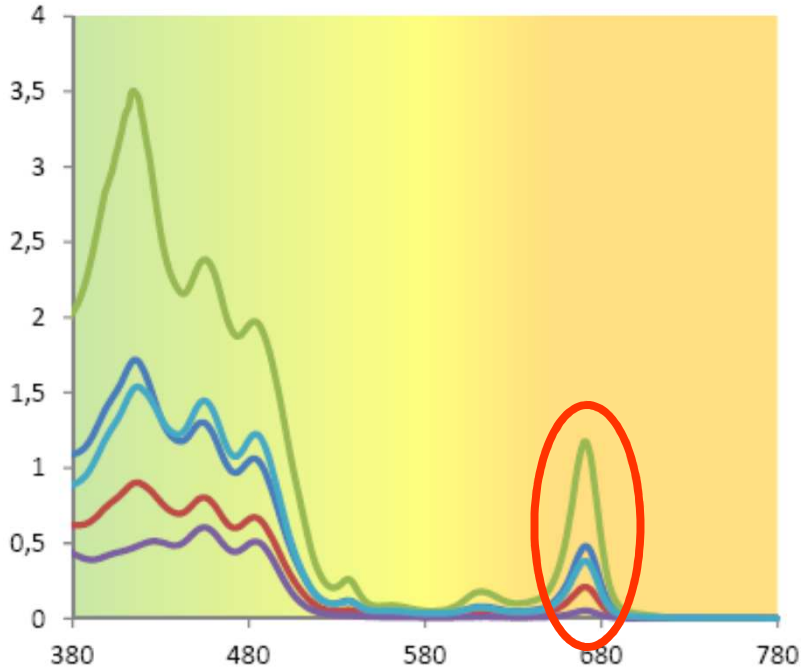
The good screening tests must be:

- fast,
- cheap,
- used to alert for suspect oils,
- not destructive, and
- transportable (they can apply in situ).

After detecting a possible adulteration, the sample must be analysed more detailed.

Counterfeit in olive oils

To cope with this study, visible spectroscopy was employed.

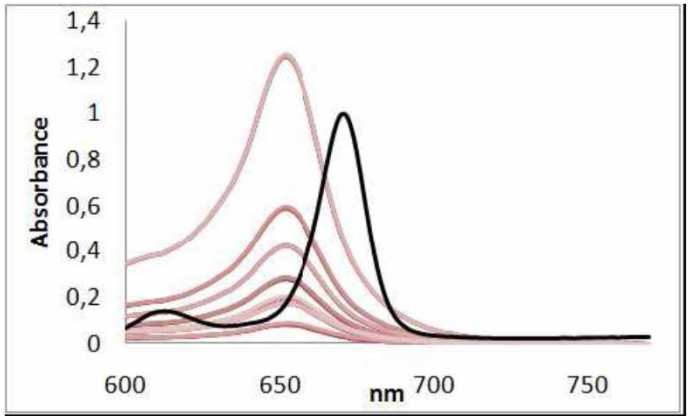


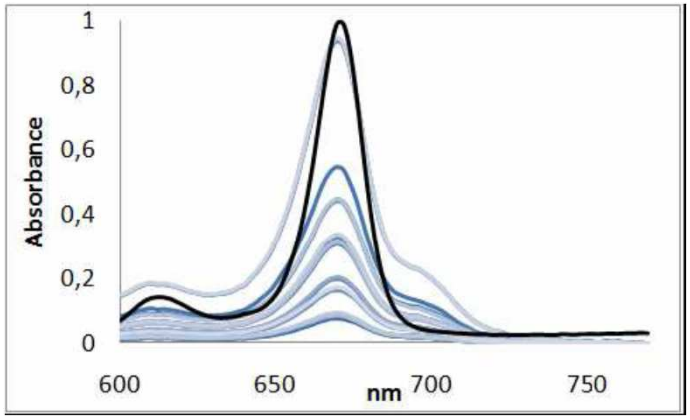
Counterfeit in olive oils

These are spectra of several oils with natural pigment chlorophyll, E-140 or Mg-Chl, added (right figure) and other oils coloured with the complex Cu-chlorophyll, E-141i, (left figure).

For both figures the spectrum of a VOO is showed in order to compare.

Within the selected spectral range, we can see the clear differences among the three types of oils: the actual VOO and the two kinds of counterfeits.



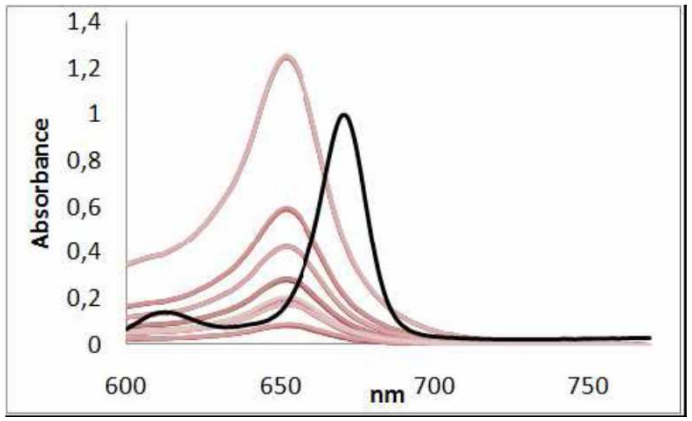


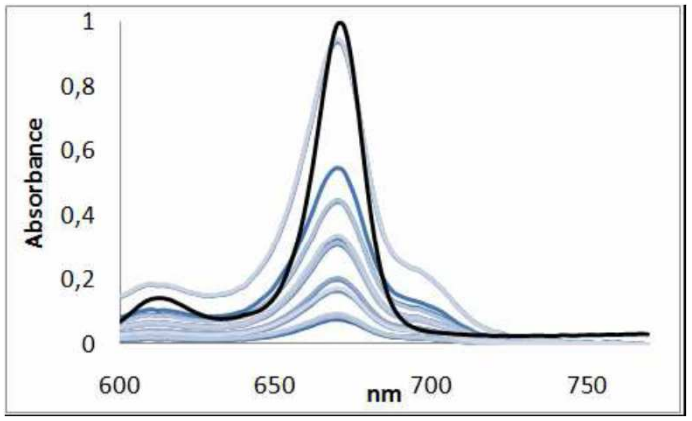
Counterfeit in olive oils

The spectra for oils coloured with Cu-Chl show the maximum of its most intensive band at 650 nm.

But if oils are coloured with the natural colourant of chlorophyll (Mg-Chl) the maximum band is coincident with the same of the VOO. However, in this case the spectra show a shoulder at 700 nm.

Therefore, the colourants used can be distinguished by measuring the absorbance ratios: p1 = A650/A670 and p2 = A700/A670.





Counterfeit in olive oils

Method:

These estimators, p1 and p2, were used with the purpose to distinguish among coloured oils with Chlorophylls and VOO.

The usefulness of these estimators is tested by a Hierarchical Cluster Analysis (HCA).

The cluster analysis method is based on the grouping of the samples into subsets or clusters with similar values of certain parameters, being in this case, p1 and p2.

Experimental:

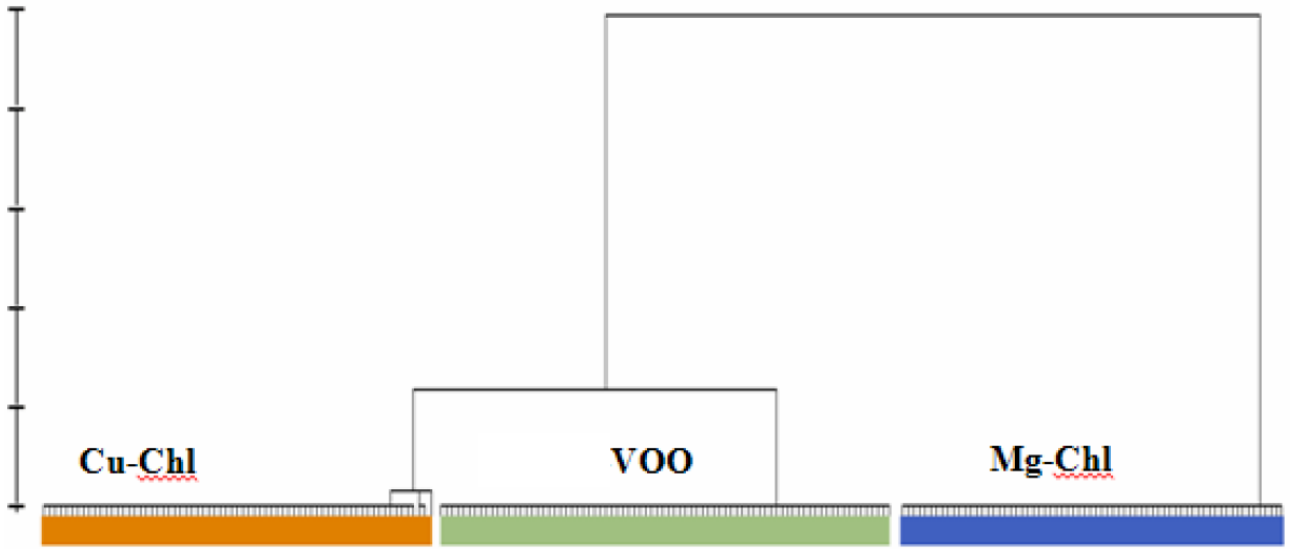
The samples used were 146 coloured oils and 81 VOO oils.

Counterfeit in olive oils

Results:

The next figure shows a dendrogram with the distances at which obtained clusters are combined.

When the cluster is stopped in three groups, we obtain a correct classification of samples into three clusters: one just containing all the samples of **VOO**, and the other two containing **Cu-Chl** and **Mg-Chl** coloured oils, respectively



Counterfeit in olive oils

Conclusions:

Visible spectroscopy, combined with the definition of useful parameters like **p1** and **p2** were able to distinguish between counterfeit oils Cu-Chl or Mg-Chl.

Advance:

The good results obtained indicate that the methodology could be applied to other many cases of fake oil or even other type of food.

THANK YOU!

International Water Association

10th Conference on Biofiltration for Air Pollution Control
March 4-7, 2013. San Francisco, USA

UCA
Universidad de Cádiz

H_2S REMOVAL FROM BIOGAS BY A PILOT ANOXIC BIOTRICKLING FILTER. EFFECT OF FLOW RATE OF BIOGAS AND RECIRCULATION MEDIUM.

F. Almenglo, M. Ramírez, J.M Gómez and D. Cantero
Department of Chemical Engineering and Food Technologies. Faculty of Sciences. University of Cádiz. 11510 Puerto Real (Cádiz), Spain. Campus de Excelencia Agroalimentario ceiA3.

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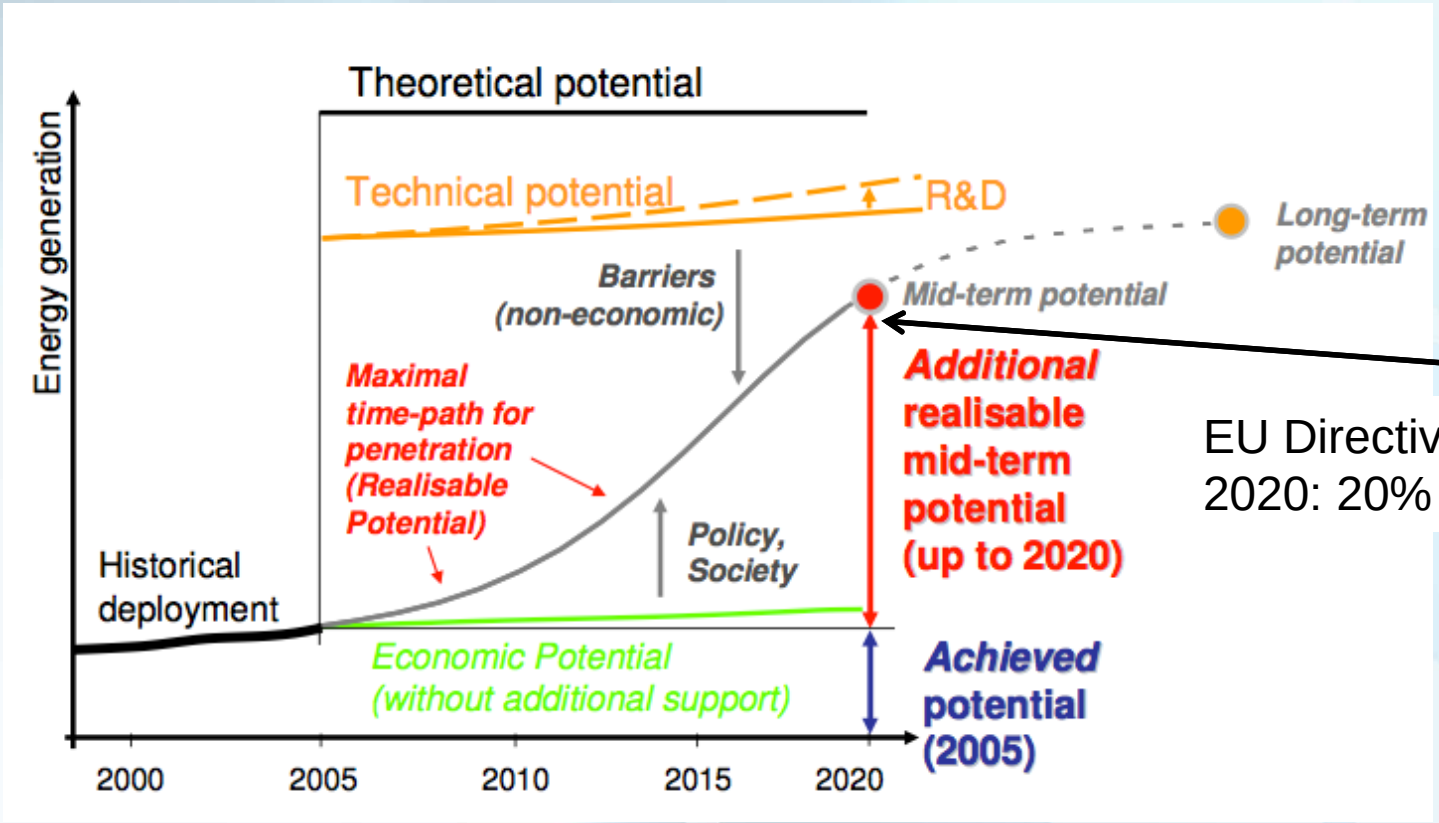
http://www.uca.es/grupos-inv/TEP105/ResearchID

Rbe
Reactores biológicos y enzimáticos

Background



EU Directive 2009/28/CE
2020: 20% renewable energy

Financing Renewable Energy in the European Energy Market
(http://ec.europa.eu/energy/renewables/studies/doc/renewables/2011_financing_ren

Background

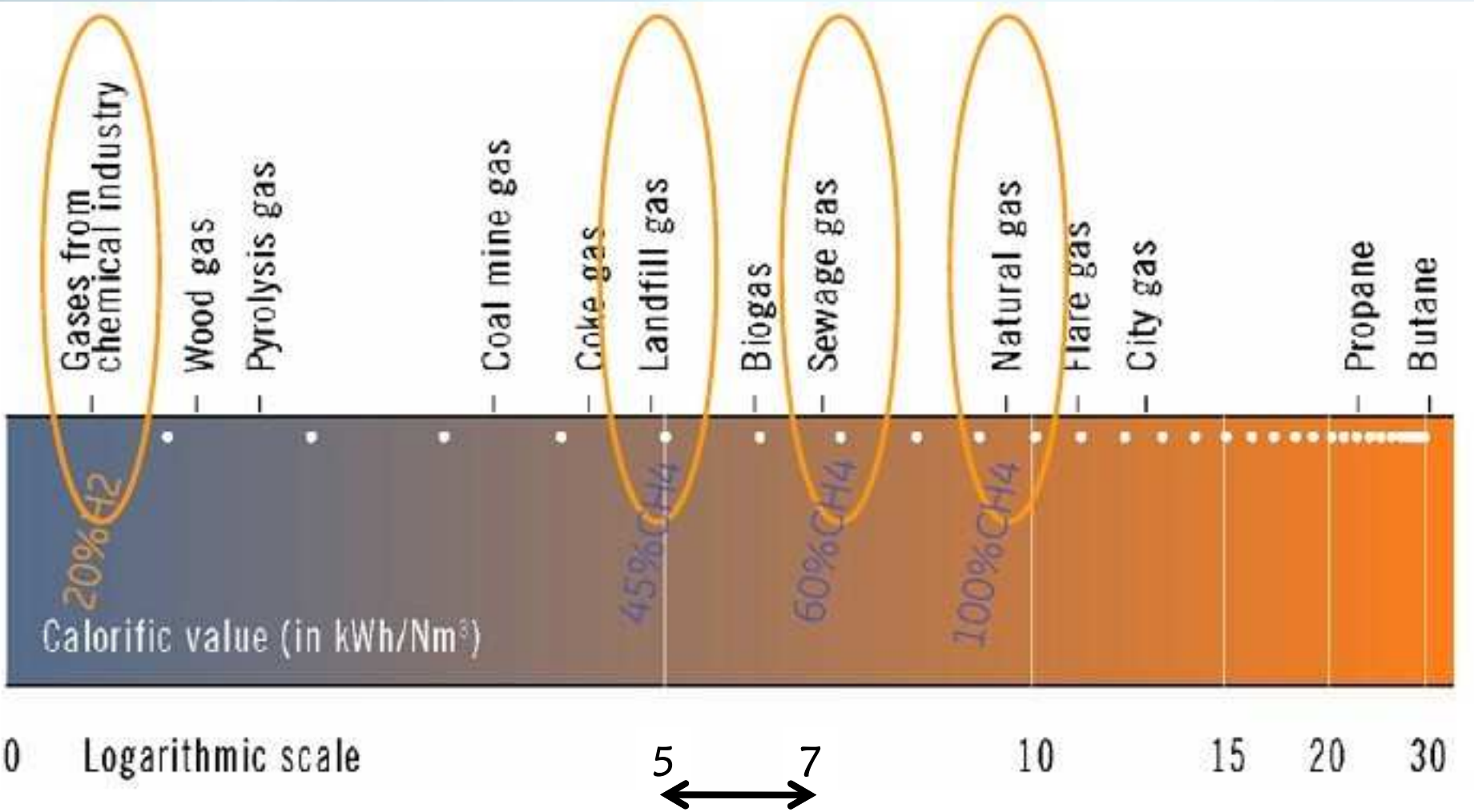
- Biogas is a natural fuel, not fossil with high calorific power.
- It is produced by organic matter biodegradation under anaerobic conditions

Type		Concentration
Sewage sludge digester	CH_4 CO_2 N_2	55-65% 35-45% <1%
Organic digester	CH_4 CO_2 N_2	60-70% 30-40% <1%
Landfill	CH_4 CO_2 N_2	45-55% 30-40% 5-15 %

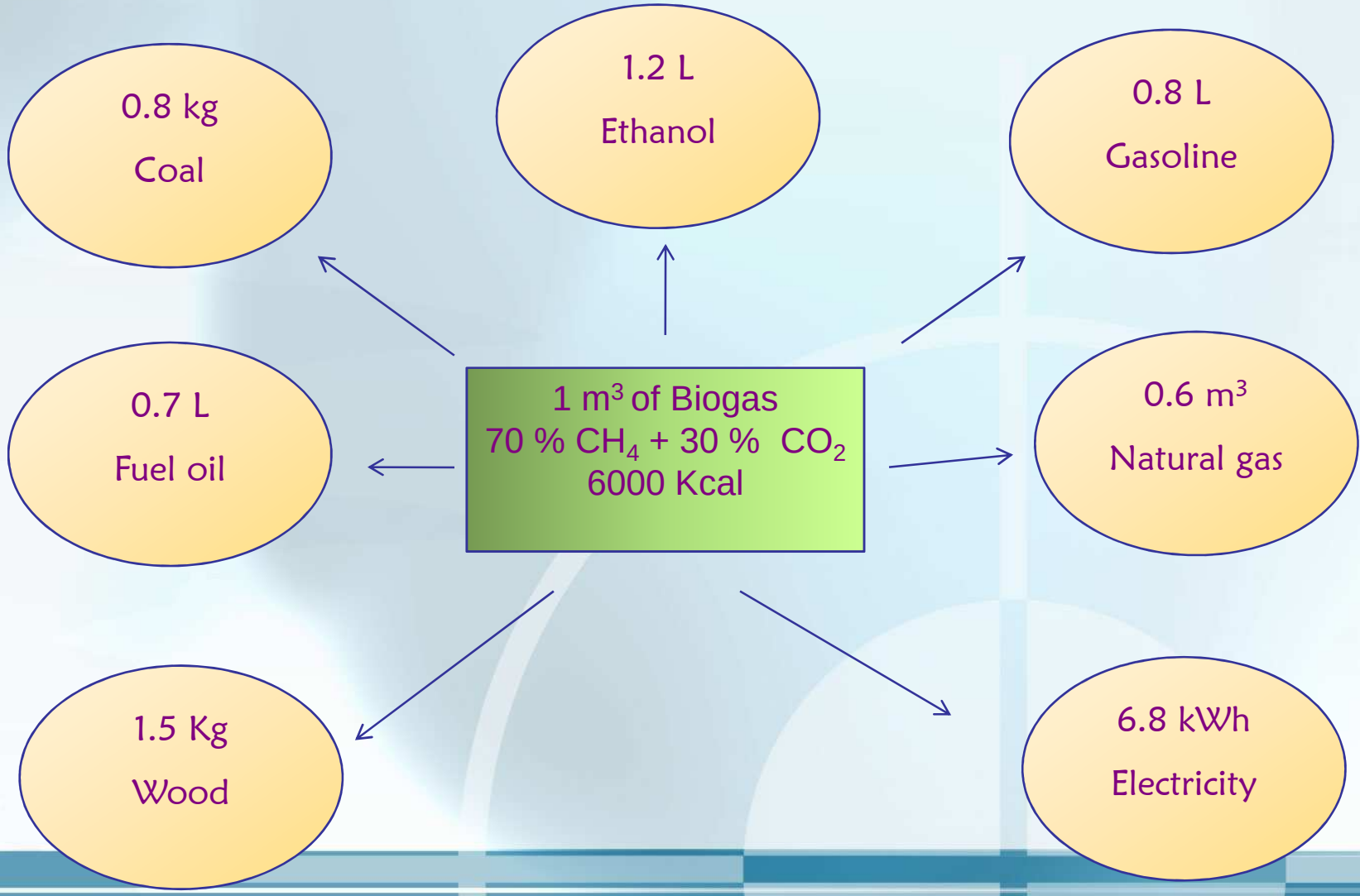
Main Pollutants	Concentration
Hydrogen Sulphide	0-20.000 ppmv
Mercaptans	0-100 ppm
Ammonia	0.1-1%
Siloxanes (Landfill)	0-100 mg/m^3
COV's	1-2%

Background

Inferior Calorific Power (ICP)



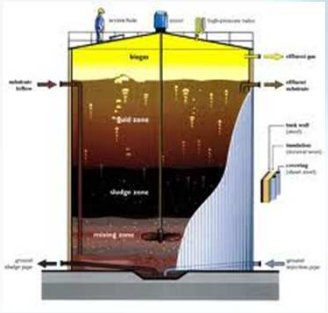
Background


0.8 kg
Coal
1.2 L
Ethanol
0.8 L
Gasoline
0.7 L
Fuel oil
1 m3 of Biogas
70 % CH4 + 30 % CO2
6000 Kcal
0.6 m3
Natural gas
1.5 Kg
Wood
6.8 kWh
Electricity

Background

Landfill

Anaerobic digesters

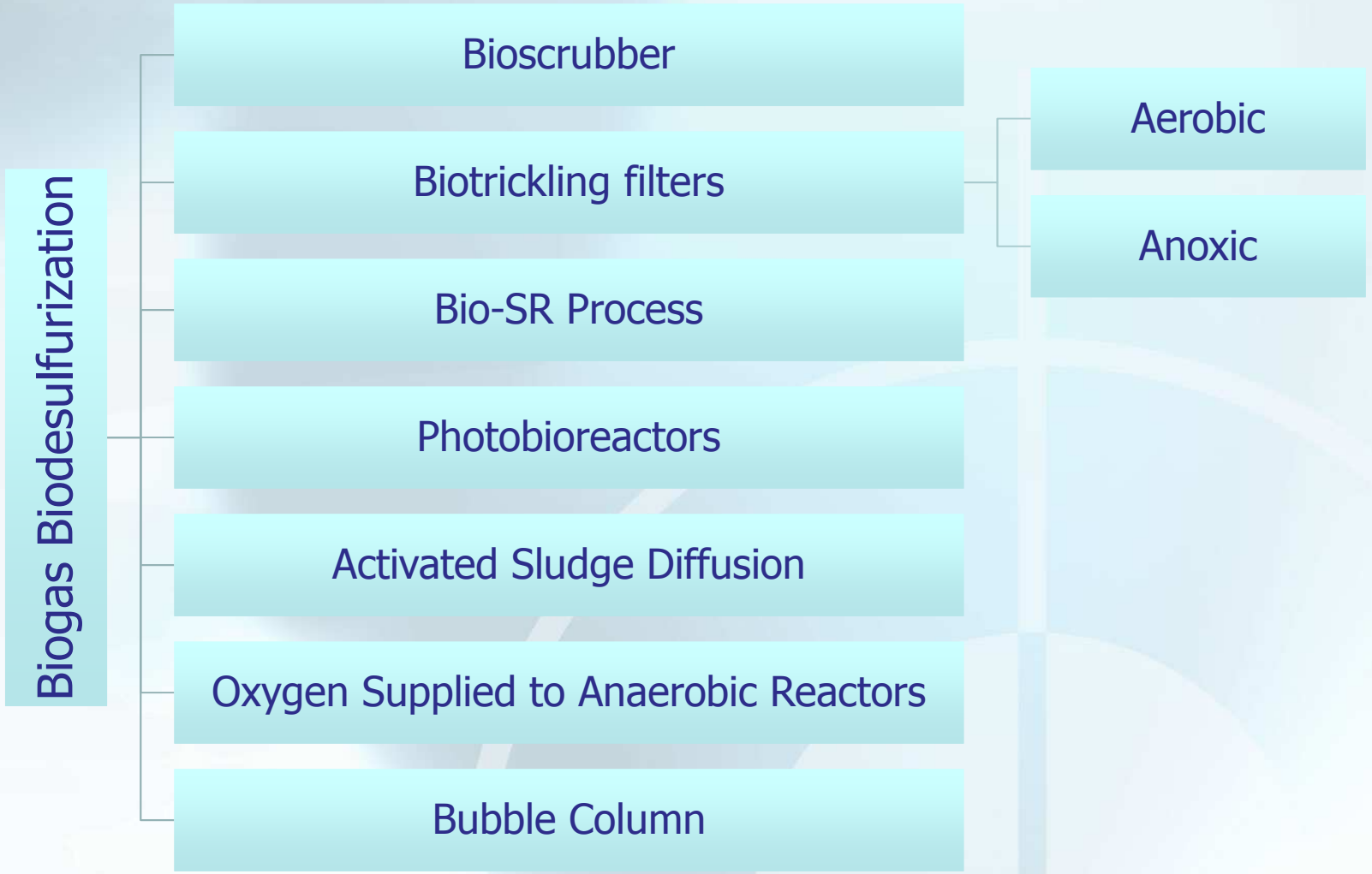


H_2S REMOVAL

Biogas

- Heat
- Heat and electricity
- Pipe grade quality
- Gas fuel for cars
- Fuel cells

Background



M. Ramírez, F. Almenglo, M. Fernández, J.M. Gómez, D. Cantero, Bio-desulphurisation of H_2S - bearing industrial gas streams, in: L. Gonzaga, D. Monteiro, C.E. Gomes (Eds.) Biohydrometallurgical Processes: A practical approach, CETEM/MCTI, Rio de Janerio, Brazil, 2011, pp. 271-290.

Background

	Advantages	Disadvantages
Aerobic Biotrickling filter	- Lower operational cost (oxygen provide from air)	- Reduction in methane concentration - High water consumption - Discharge of acid water
Anoxic biotrickling filter	- No reduction in methane concentration - Best control of ratio oxidation	- Higher operational cost (Nitrate feeding)

Background

Ref	V (L)	Carrier	EBRT (min)	EC_{CRIT} ($gS\ m^{-3}\ h^{-1}$)	RE (%)	EC_{MAX} ($gS\ m^{-3}\ h^{-1}$)	RE (%)	TLV ($m\ h^{-1}$)
[1]	12	Plastic fibre	18	10.5-11.8	99	12.5 -14.5	75 - 43	1.7
[2]	12	Plastic fibre	18	4.9	>99			1.7
[3]	6.7	Plastic fibre	5.4 - 16	7.9	95			1.7
	6.7	Lava rocks	5.4 - 16	8.6	95			1.7
[4]	6.7	Plastic fibre	17	9.2	98.9			1.7
[5]			6	50				1.7
[6]	2.25	OPUF	2	84	98	142	47	7
[7]	167	OPUF	7	50	98			7.63

[1] Soreanu, G. et al., 2008. Journal of Environmental Engineering and Science, 7(5): 543-552
[2] Soreanu, G. et al., 2008. Water Science & Technology, 57(2): 201-207
[3] Soreanu, G. et al., 2009. Environ. Technol. 30(12): 1249-1259
[4] Soreanu, G. et al., 2010. Bioresource Technology, 101(23): 9387-9390
[5] Soreanu, G. et al., 2010. Proceeding of IWA World Water Congress and Exhibition, Canada. Paper#IWA-2653
[6] Montebello, A.M. et al., 2012. Chem. Eng. J., 200-202: 237-246.
[7] Almenglo, F. et al. 2011. Proceeding of I Worksohp on Bioprocess for the Mining Industry and Environmental. Sao Paulo, pp. 41-54

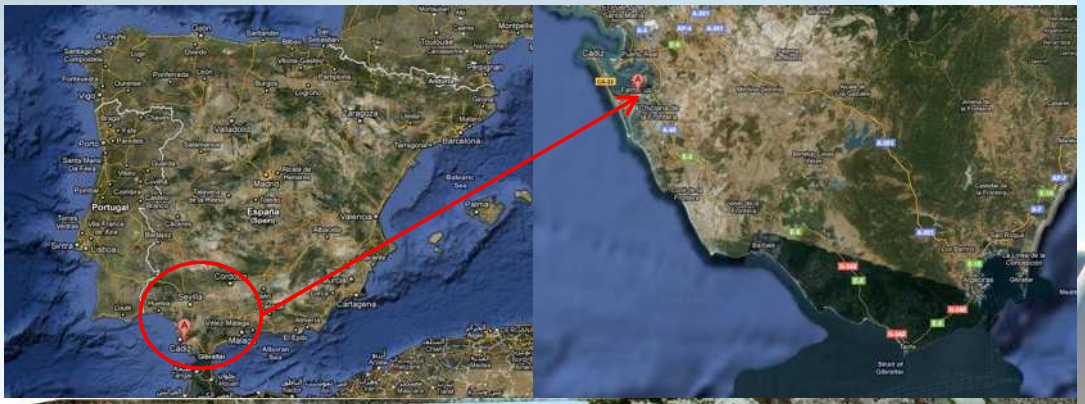
Aims

Study the effect on H_2S removal efficiency (RE) of:

- Biogas Flow Rate (Q) / H_2S inlet load (L)
- Liquid recirculation flow rate (QR)
- Nitrate concentration ([$N\text{-}NO_3^-$])

Experimental Methods

Wastewater Treatment plant in Cádiz (Spain): “UTE EDAR Bahía de Cádiz”



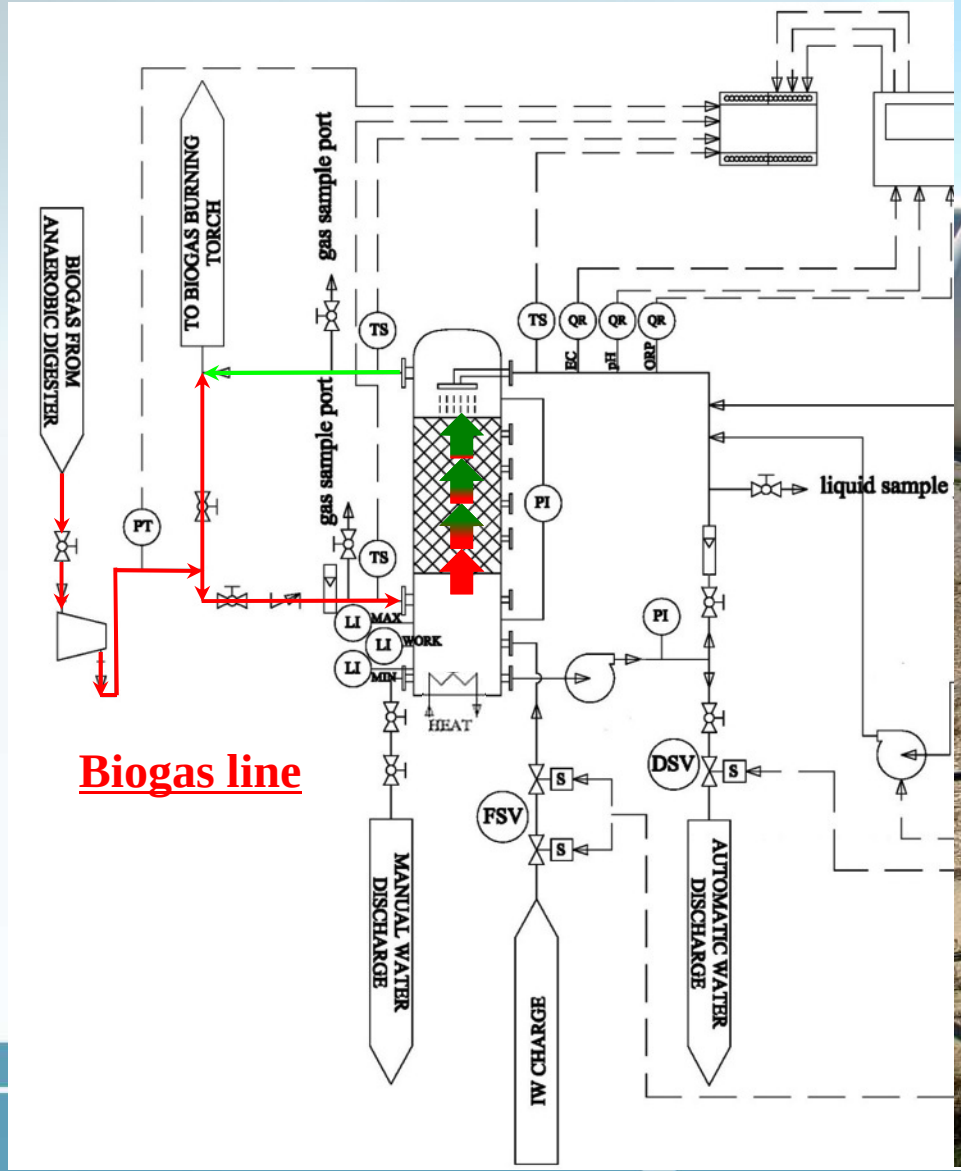
Experimental Methods

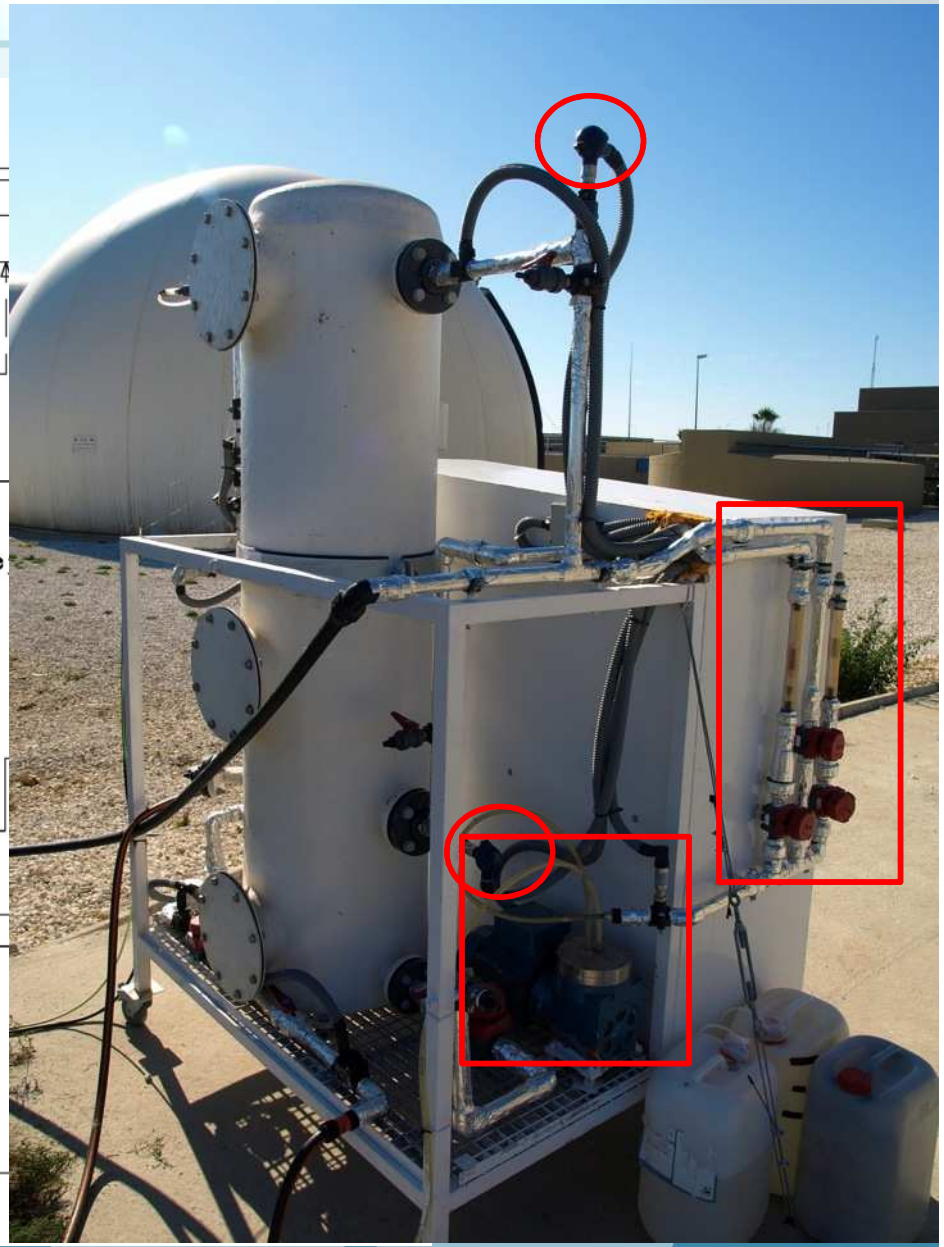
Material: Fiberglass reinforced polyester (FRP)
Diameter: 0.5 m
Height: 2.1 m
Bed height: 0.85 m
Packing volume: 0.167 m^3
Packing material: Cubes of Open pore polyurethane foam (OPUF)

Filtren TM25450
(Recticel Iberica, Spain)
- 50 mm side length
- 600 $m^2\ m^{-3}$
- 35 kg m^{-3}

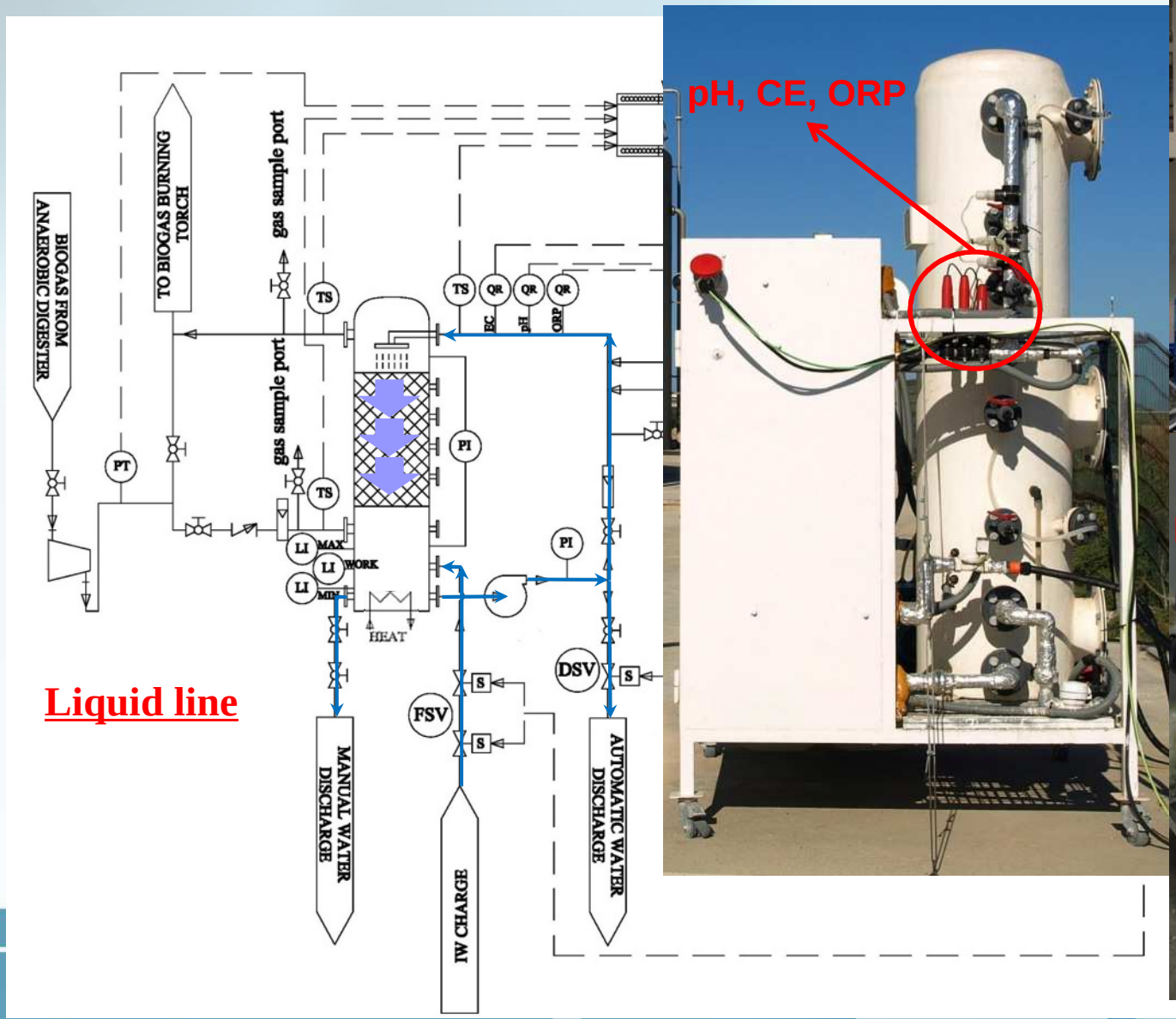
Biogas composition:
- $[H_2S] = 5472 \pm 737$
- $[CH_4] = 64 \pm 1.6$
- $[CO_2] = 33 \pm 1.7$

Experimental Methods





Experimental Methods

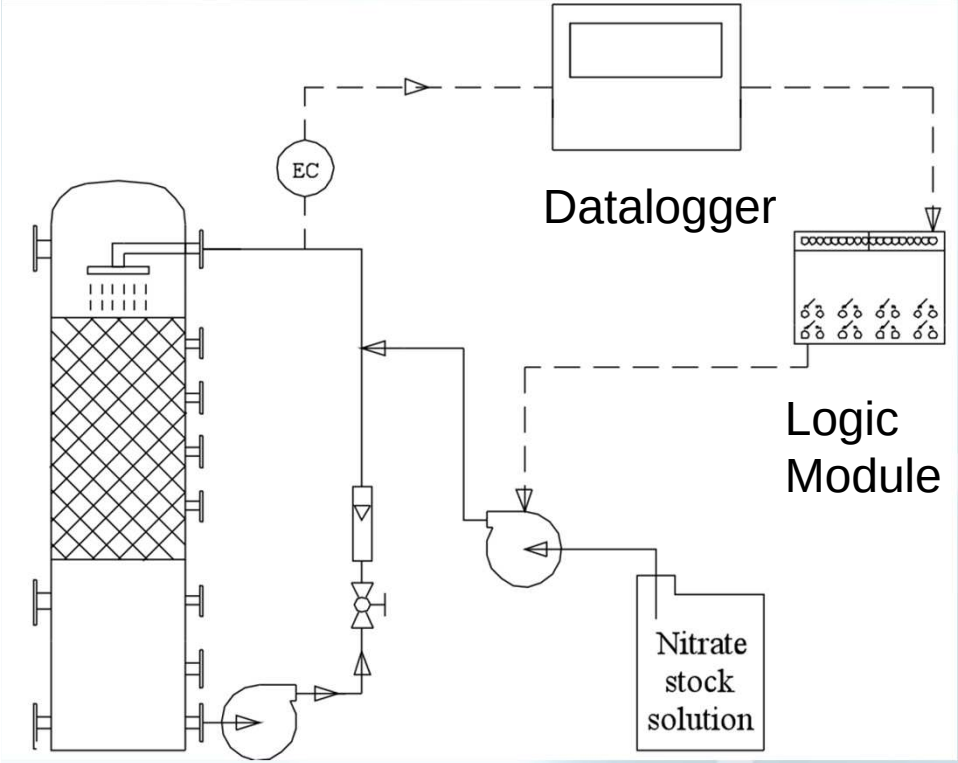


Experimental Methods

Control

Experimental Methods

Dosage of nitrate stock solution:



ORP Set-point: -365 mV

Volume stock solution added:
From 0.14 to 0.7 L
(L from 33 to 177 gS $m^{-3}h^{-1}$)

=> Nitrate depletion time: 3-4 h

Composition nitrate stock solution:

- $NaNO_3$: 500 g L^{-1} (82.4 g $N\text{-}NO_3^-$ L^{-1})
- KH_2PO_4: 10 g L^{-1}
- NH_4Cl: 5 g L^{-1}
- $MgSO_4 \cdot 7H_2O$: 4 g L^{-1}
- 10 mL of solution $FeSO_4 \cdot 7H_2O$ (2 g in 1 L of H_2SO_4 0.1 N)
- Trace elements: 5 mL (SL-4 and S-6; according to medium ATTC 1255)

- Sulfate concentration: constant 8.9 ± 1.6 $gS\text{-}SO_4$ L^{-1}
- Nitrite concentration: between 0.1 – 10 mg $N\text{-}NO_2^-$ L^{-1}

Experimental Methods

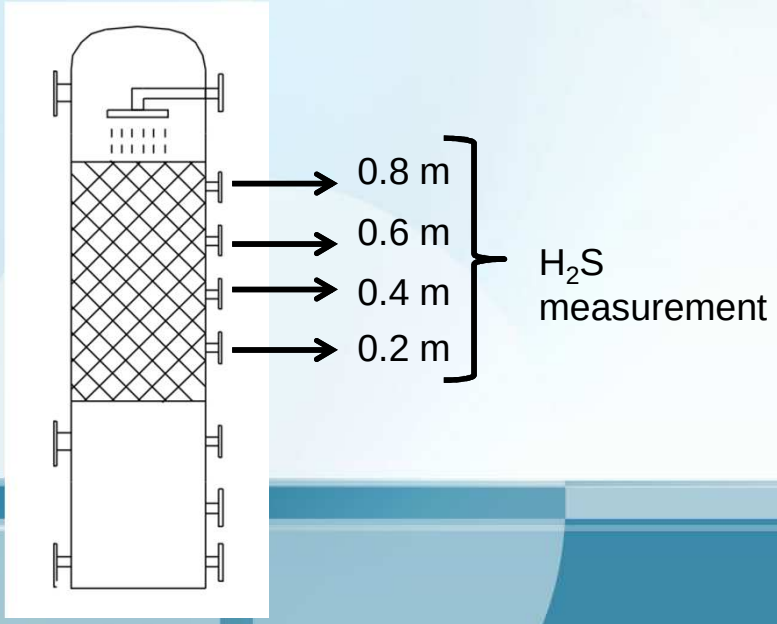
Analytical techniques:

- Nitrate: ultraviolet spectrophotometric method (4500-NO_3^- B, APHA, 1999)
- Nitrite: colorimetric method (4500-NO_2^- B, APHA, 1999)
- Sulfate: Turbidimetric method (4500-SO_4^{2-} E, APHA, 1999)
- H_2S gas concentration:
  - 0-500 ppmv: GasBadge® Pro
  - 500-5000 ppmv: GA2000PLUS® equipped with external H_2S sensor
  - >5000 ppmv: gas chromatograph 450-GC (Bruker) with TCD

GasBadge®Pro

GA2000Plus

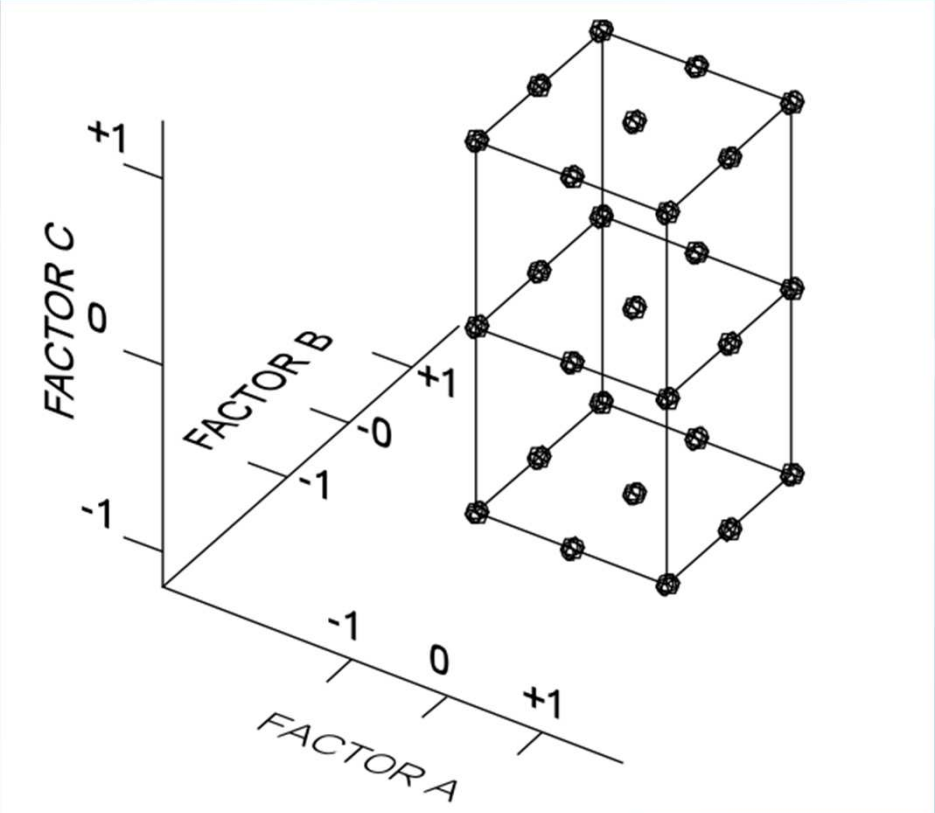
450-GC



Experimental methods

Nitrate concentration effect:

Full factorial design 3^3 with two replicates at the central point



Factor A: L (gS m^{-3} h^{-1})
Factor B: QR (m^3 h^{-1})
Factor C: [N-NO_3^-] (mg N-NO_3^- L^{-1})

FAC.	Level (-1)	Level (0)	Level (+1)
A*	35.3 ± 1.4	109.1 ± 7.8	171.7 ± 3.1
B	1	2	3
C	37 ± 12	122 ± 20	258 ± 17

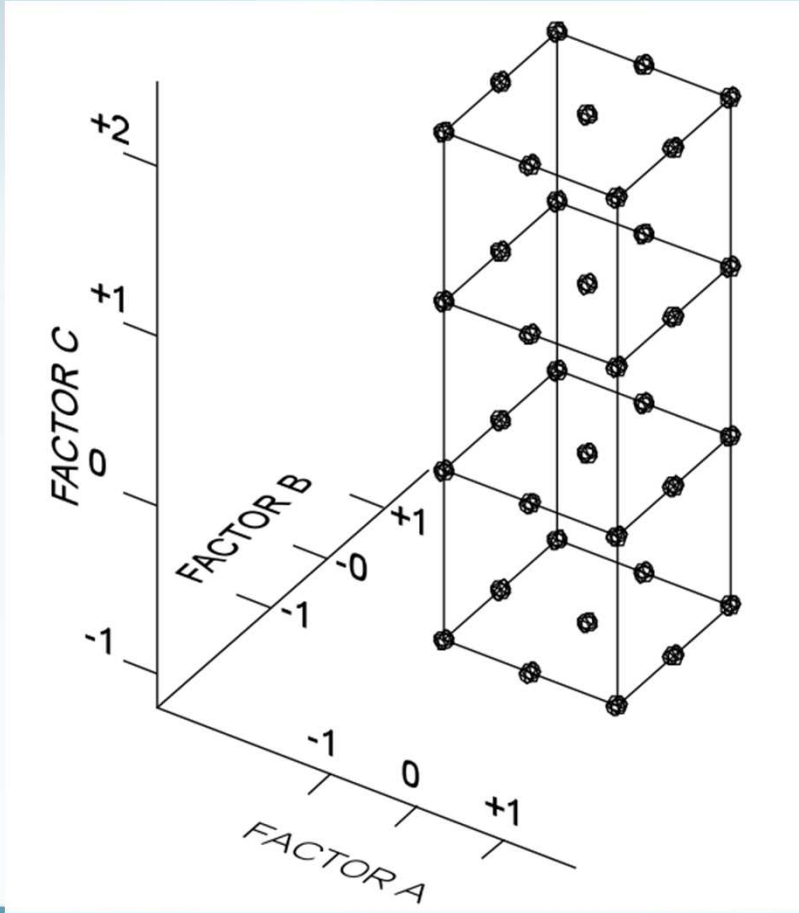
*EBRT of 600, 200 and 120 s

29 EXPERIMENTS

Experimental methods

Effect of Height:

Multilevel factorial design:



Factor A: Q ($m^3 h^{-1}$)
Factor B: QR ($m^3 h^{-1}$)
Factor C: H (m)

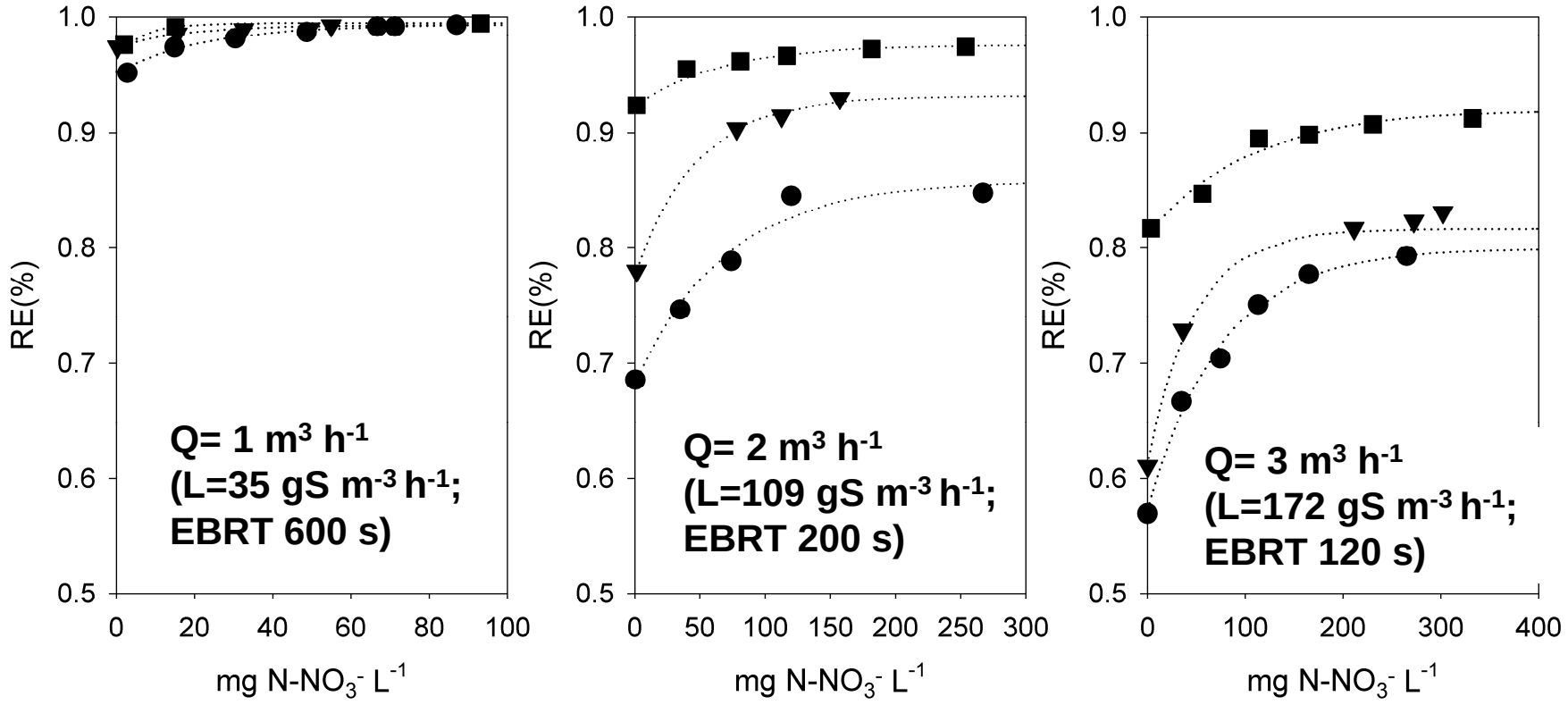
FAC.	Level (-1)	Level (0)	Level (+1)	Level (+2)
A*	1	3	5	
B	1	2	3	
C	0.2	0.4	0.6	0.8

*EBRT of 600, 200 and 120 s

36 EXPERIMENTS

Results

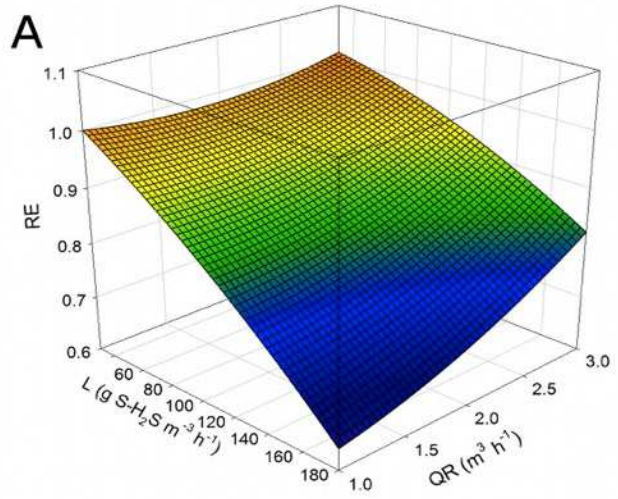
QR: ● 1 $m^3 h^{-1}$ [5 $m h^{-1}$] ▼ 2 $m^3 h^{-1}$ [10 $m h^{-1}$] , ■ 3 $m^3 h^{-1}$ [15 $m h^{-1}$]

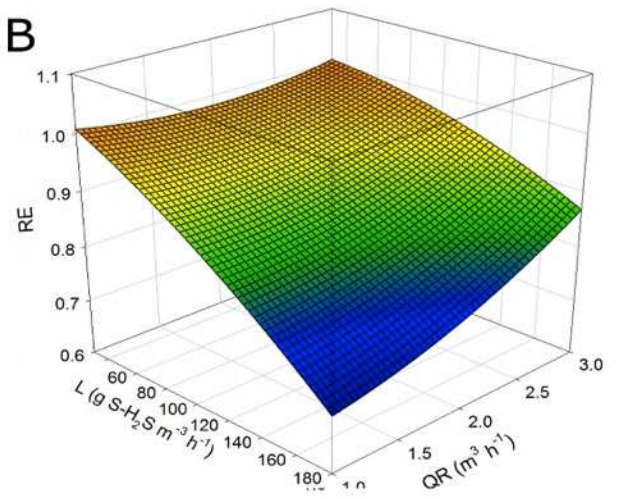


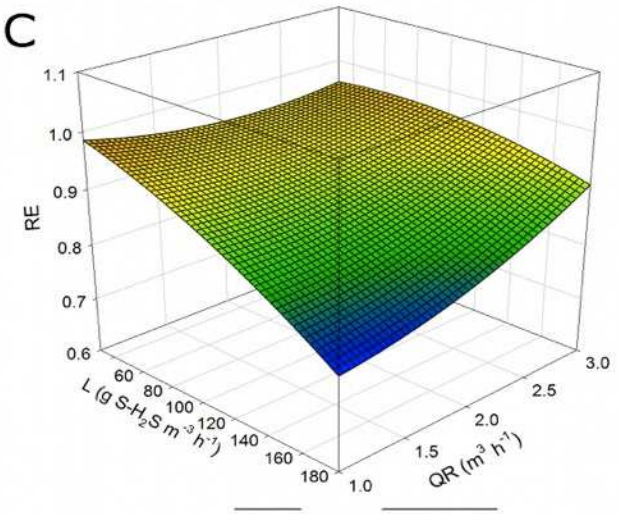
Results

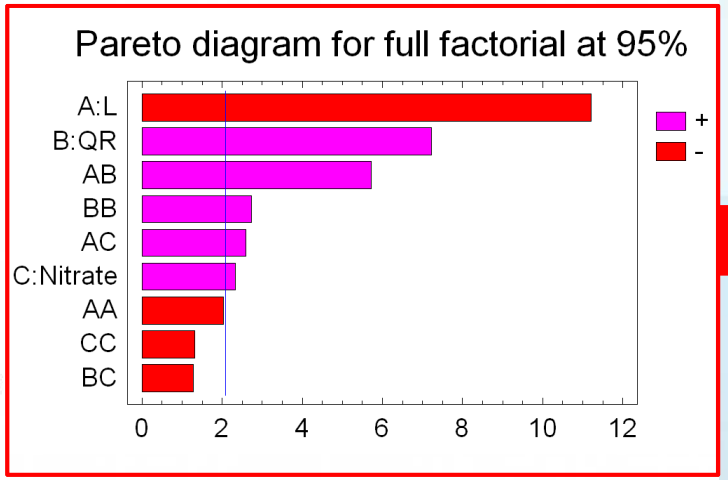
Model coefficients	
constant	= 1.12
A:L	= -2.24 × 10^{-3}
B:QR	= -1.03 × 10^{-1}
C:Nitrate	= 2.33 × 10^{-4}
AA	= -4.26 × 10^{-6}
AB	= 5.649 × 10^{-4}
AC	= 4.25 × 10^{-6}
BB	= 2.40 × 10^{-2}
BC	= -8.89 × 10^{-5}
CC	= -1.14 × 10^{-6}

Regression coefficient: 96.65%.







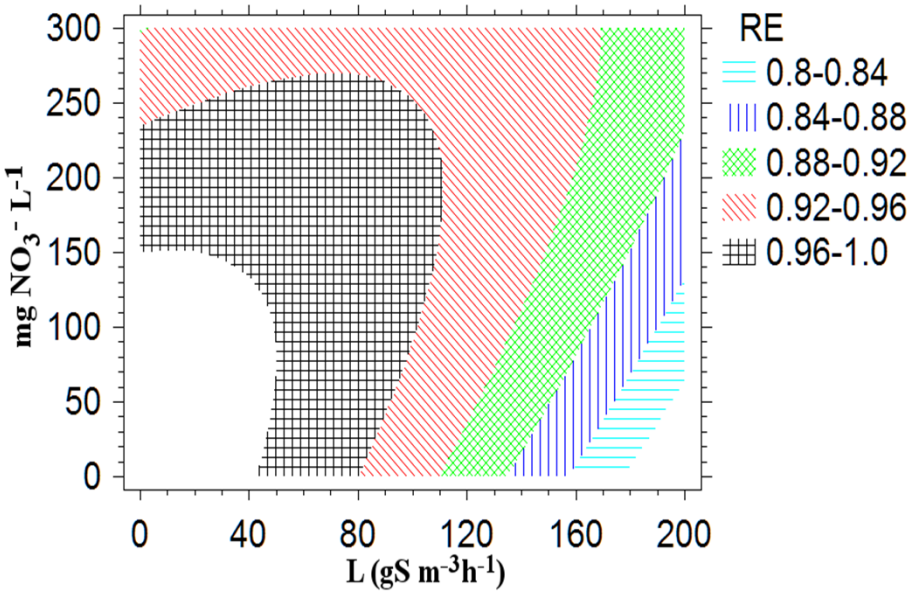


- L
- QR
- L x QR
- QR^2
- L x $[NO_3^-]$
- $[NO_3^-]$

A. 37 mg N-NO_3^-; B. 122 mg N-NO_3^-; C 258 mg N-NO_3^-

Results

Contour surface for QR=3 m^3h^{-1} (15 m h^{-1})



<u>For L < 80 gS m^{-3} h^{-1}</u>

Nitrate concentration has low effect on RE

<u>For 80 < L < 90 m^{-3} h^{-1}</u>

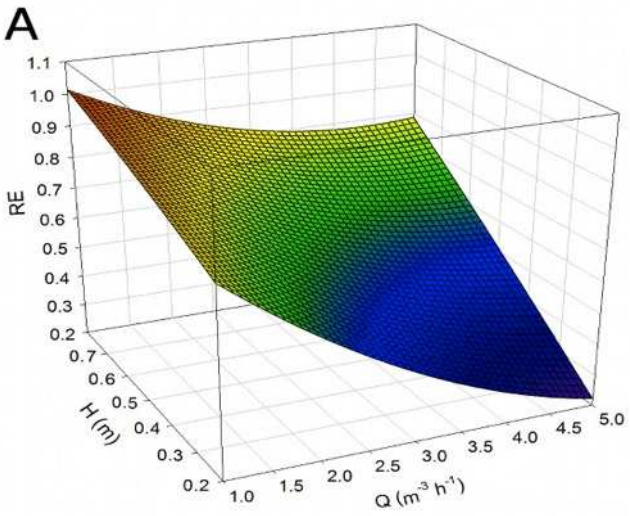
Nitrate has high effect on RE

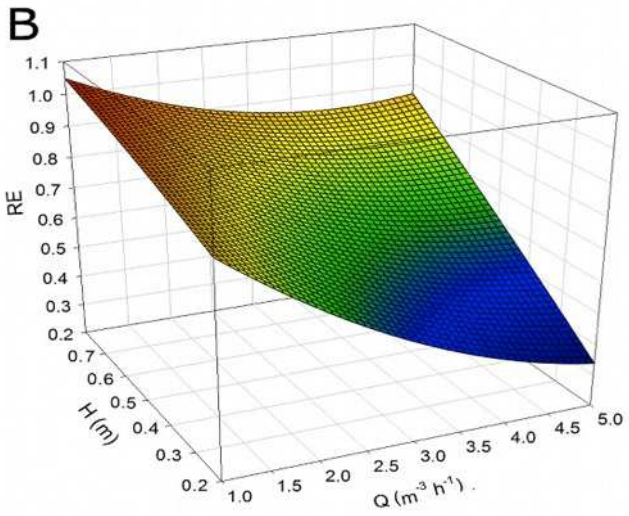
RE > 95 % → [$N\text{-}NO_3^-$] > 35 mg $N\text{-}NO_3^-$ L^{-1}

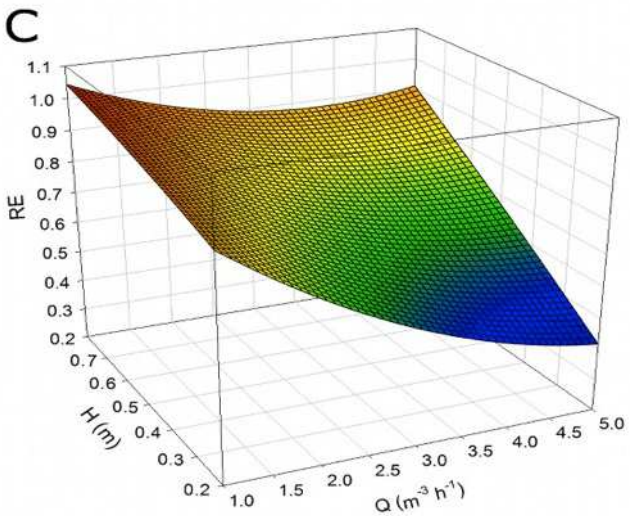
<u>For 90 < L < 100 m^{-3} h^{-1}</u>

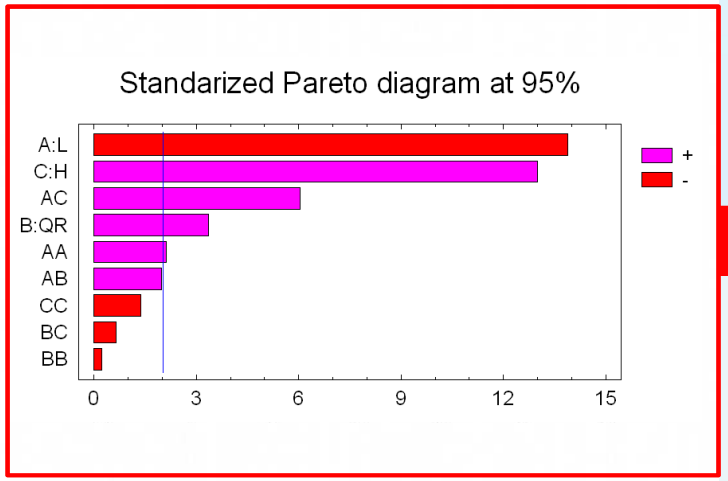
RE > 95 % → [$N\text{-}NO_3^-$] > 95 mg $N\text{-}NO_3^-$ L^{-1}

Results









Model coefficients	
constant	= 8.69 × 10^{-1}
A:Q	= -3.10 × 10^{-1}
B:QR	= 1.31 × 10^{-1}
C:H	= 3.80 × 10^{-1}
AA	= 2.14 × 10^{-2}
AB	= 1.15 × 10^{-2}
AC	= 1.43 × 10^{-1}
BB	= -1.98 × 10^{-2}
BC	= -6.07 × 10^{-2}
CC	= -9.29 × 10^{-2}

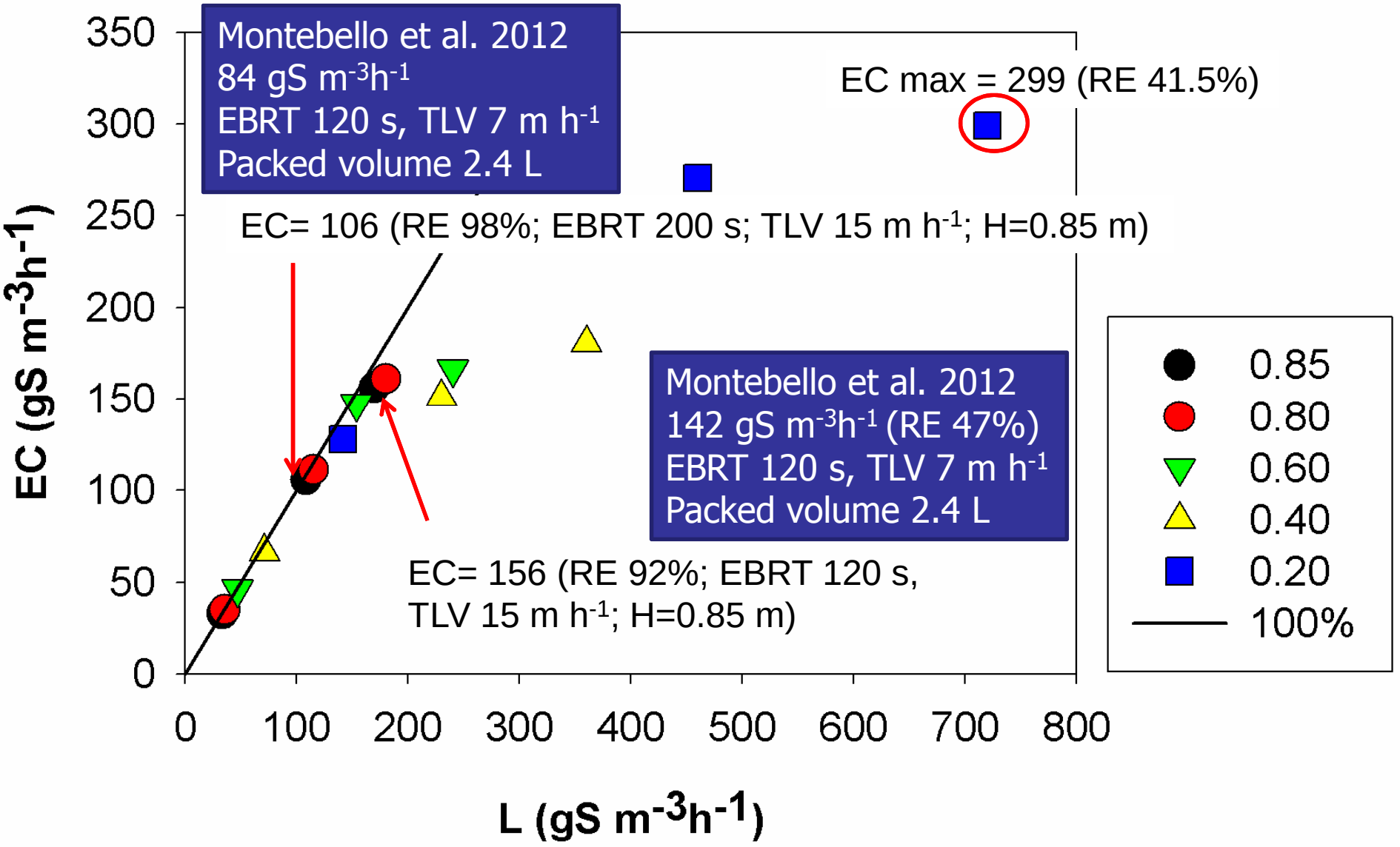
Regression coefficient: 90.90%.

- Q
- H
- Q x H
- QR
- Q^2

RE = 0.2 RE = 0.3 RE = 0.4 RE = 0.5 RE = 0.6 RE = 0.7 RE = 0.8 RE = 0.9 RE = 1.0

QR. A. 1 m^3h^{-1}; B. 2 m^3h^{-1}; C 3 m^3h^{-1}

Results



EV vs. L at constant QR of 3 m h^{-1}

Conclusions

- ORP measurement allows an efficient control of nitrate dosage
- For $L < 80$ gS m^{-3} h^{-1} the most important parameters were H and TLV. High RE were reached at low nitrate concentration.
- Nitrate concentration has high effect on RE at $L > 80$ gS m^{-3} h^{-1}
- The optimal operational conditions were:
  - QR = 3 m^3 h^{-1} (TLV = 15 m h^{-1})
  - $L < 80$ gS m^{-3} h^{-1}
  - EBRT between 120-200 s

Thank you for your attention

Picture: Conil de la Frontera (Cádiz). Spain

Acknowledgments:

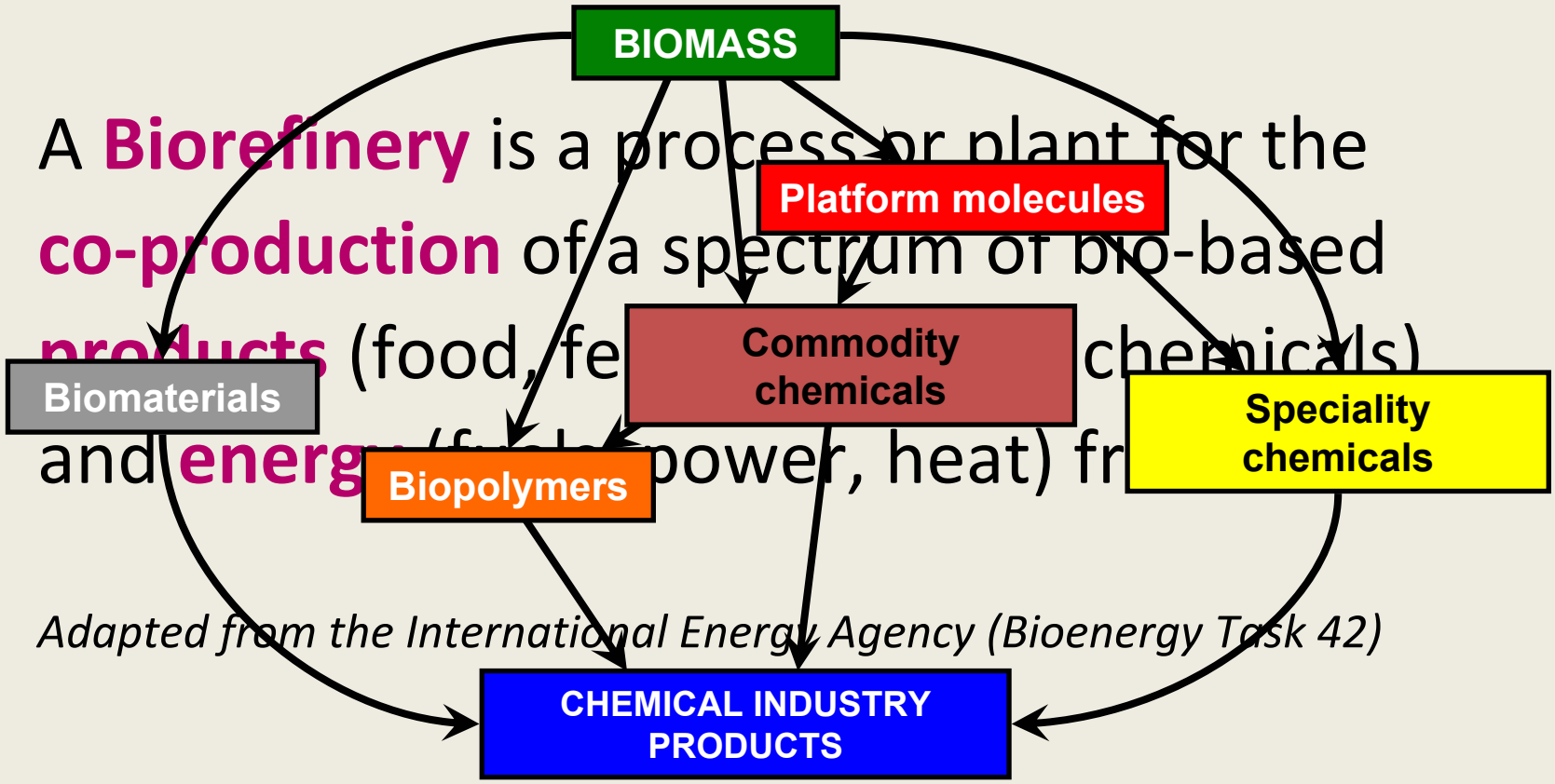
• Spanish Ministry of Science and Innovation. Projec "Desulfuration of energy rich gases in biotrickling filters: process development and optimization unde anoxic and aerobic conditions" (CTM2009-14338-C03 02)

• RRTO of University of Cádiz Project "Procedure fo inoculation of industrial biotrickling filters" (PROTO-05 2010)

• WWTP "UTE EDAR Bahía de Cádiz".

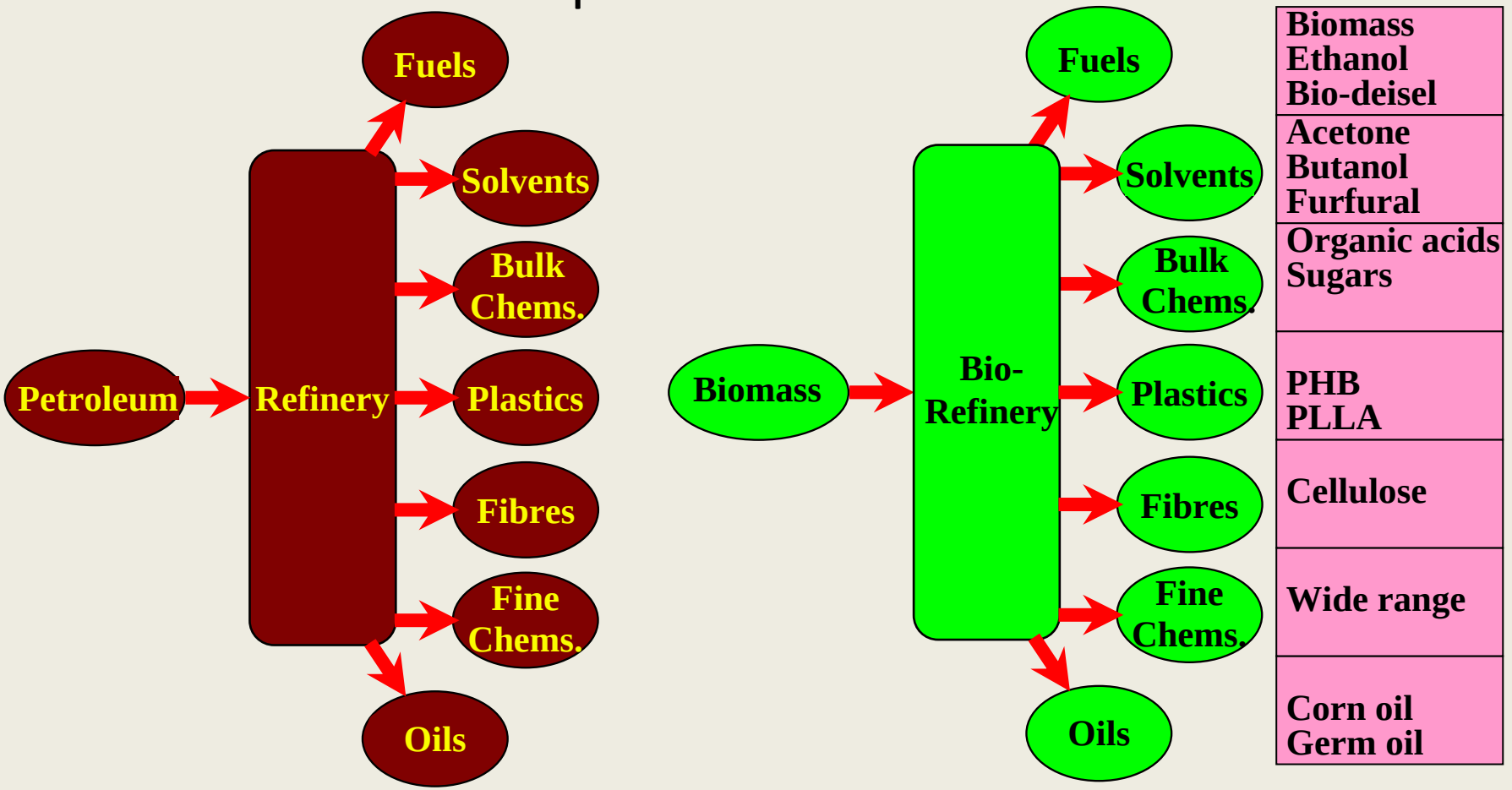
What is a biorefinery?

A **Biorefinery** is a process or plant for the **co-production** of a spectrum of bio-based **products** (food, fe chemicals) and **energy** (fuels, power, heat) fr



Adapted from the International Energy Agency (Bioenergy Task 42)

Most types of chemical produced from petroleum can be produced from biomass



Many of these can be produced through bioprocessing (fermentation).

Fermentation Processes

four groups:

- producing microbial cells
- producing microbial enzymes
- producing microbial metabolites
- carrying out a transformation

Fermentation Process Characteristics

- the “REACTION” is not defined by the “REACTANTS”
- “REACTANTS” are actually FOOD
- “PRODUCT” is incidental to the micro-organism
- “CATALYST” is produced during the fermentation
- the fermentation must be “SEEDED”

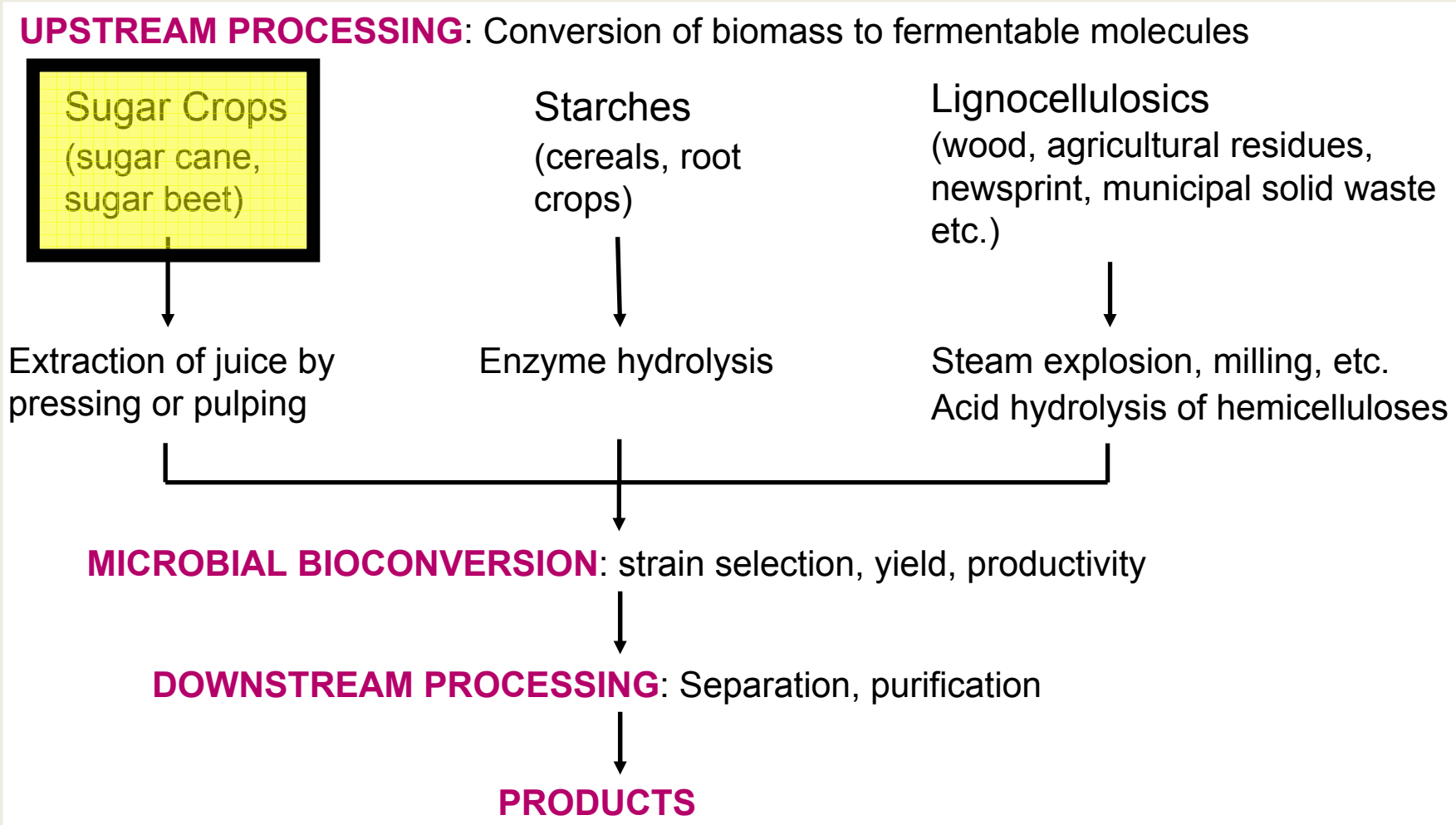
Fermentation Process Characteristics

- raw material shelf life is limited
- process must be carried out under MILD conditions
- “REACTIONS” are very SLOW
- Concentrations are very LOW (<10%)
- Fermenter operation is almost always BATCH

Nutrient requirement for a fermentation medium

- Carbon source
- Nitrogen source
- Minerals:
  - Macronutrients: P, S, Mg, Fe, K, Ca;
  - Micronutrients: Mo, Zn, Mn, Co, Ni, Cu, I, Br, V
- Growth factors (vitamins, amino acids, purines, pyrimidines)

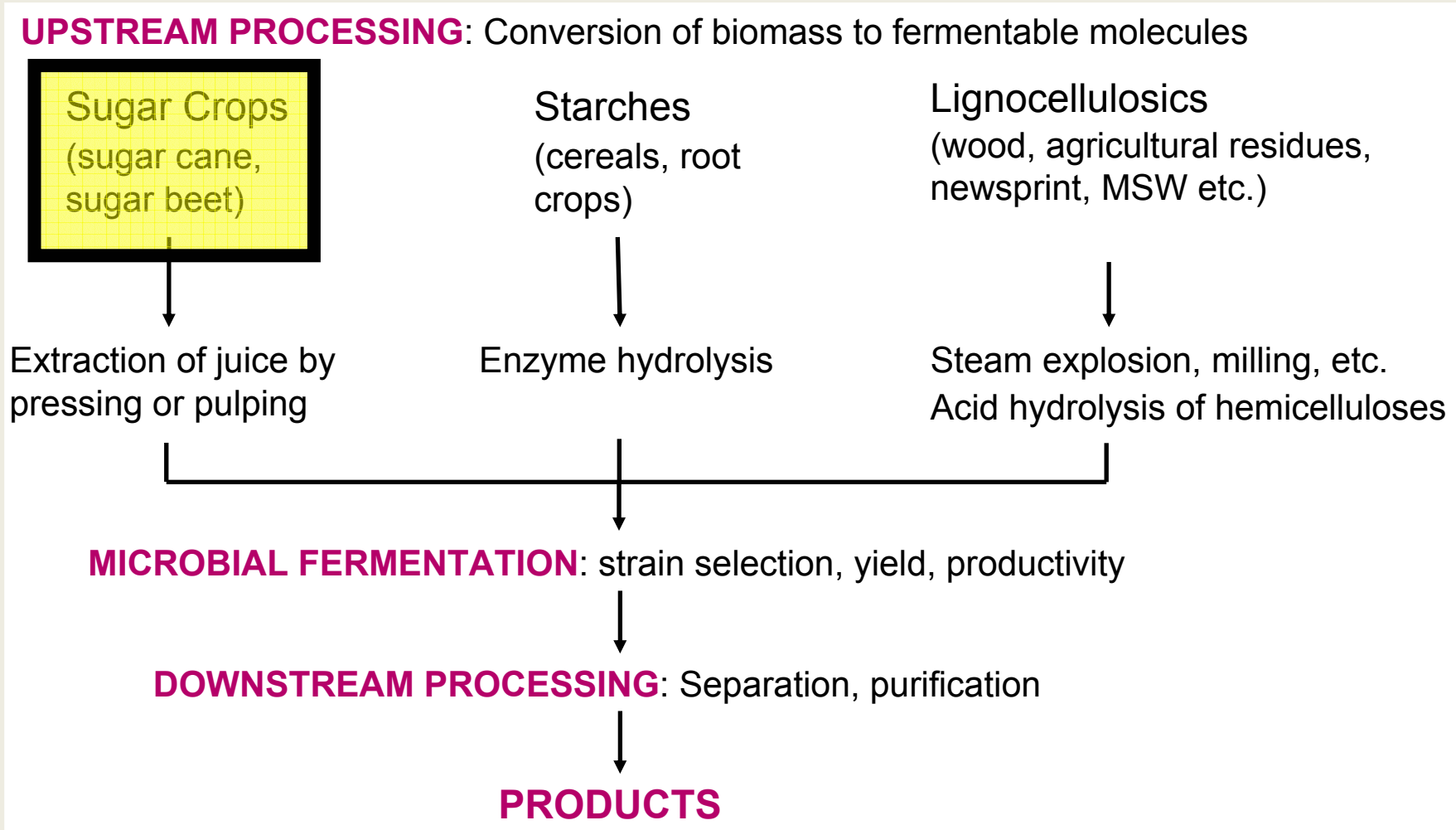
General Bioprocess Scheme



Sugar crops and molasses

- Sugar cane and sugar beet (20% sucrose, 75% water, 5% cellulose and 1% inorganic salts)
- Micro-organisms can only grow on sucrose if they produce the enzyme invertase
- Sugar cane is utilised for ethanol production in Brazil and it is a particularly favourable substrate for yeasts

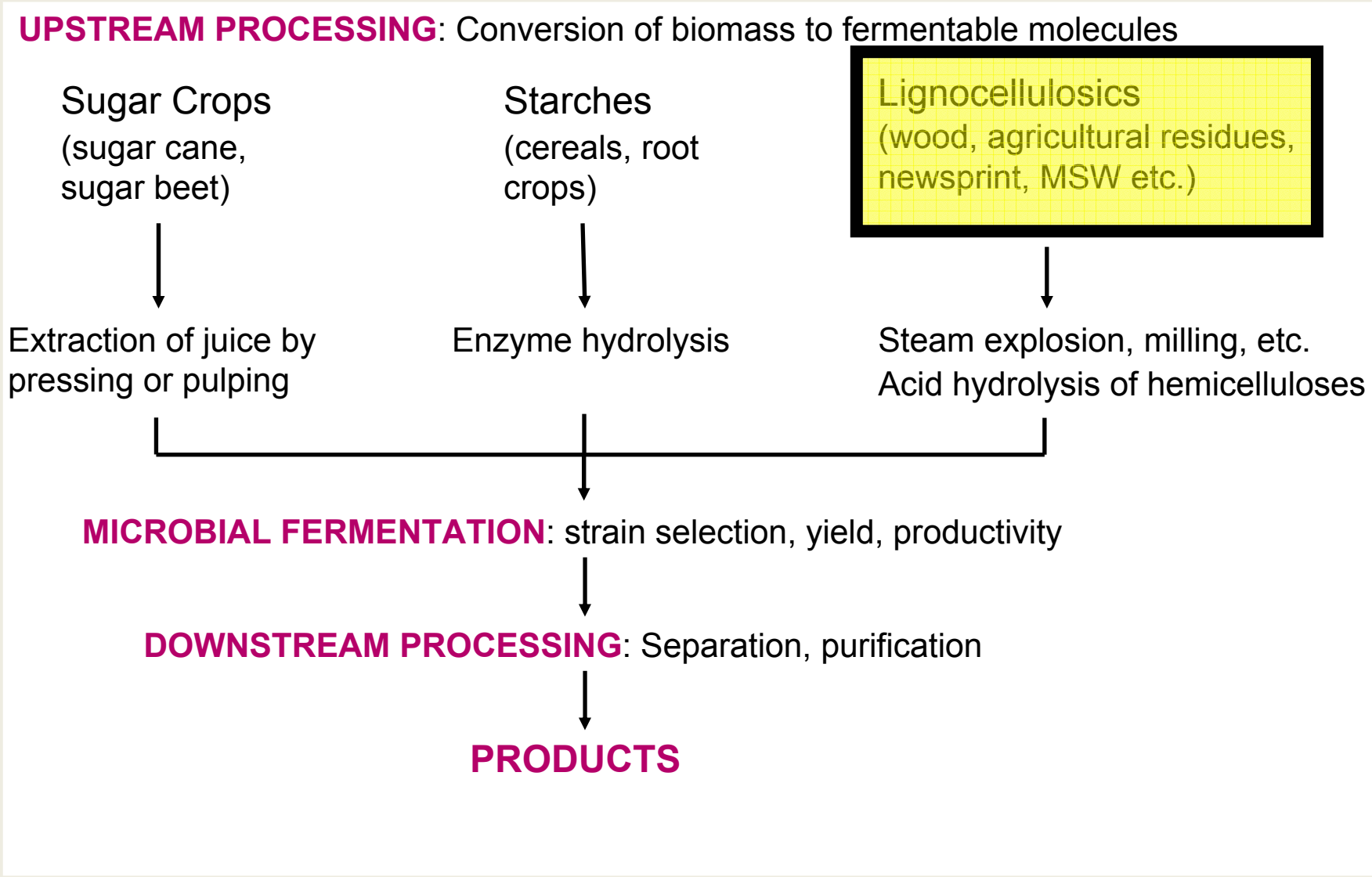
General Bioprocess Scheme



Lignocellulosic raw materials

- Cellulose (35-50%), Hemicellulose (20-35%) & Lignin (10-25%), intimately linked by physical and chemical bonds, which require pre-treatment prior to (expensive) enzymatic hydrolysis.
- Cellulose hydrolysis is carried out by three main enzyme types:
  - endo-1,4-β-D-glucanases,
  - 1,4-β-D-glucan cellobiohydrolases
  - cellobiases (or β-D-glucosidases).
- Xylans are common hemicelluloses hydrolysed by
  - endo-β-xylanases
  - β-xylosidases.

General Bioprocess Scheme



Starch based raw materials

- Mainly cereals (e.g. wheat, maize)
- Ideal, with low water content, high energy density, easy cultivation and good infrastructure
- Cereal macromolecules (starch, protein) must be hydrolysed to glucose, amino acids, peptides
- Starch is hydrolysed by
  - α-amylases
  - β-amylases
  - Amylogucosidase
- Proteins are hydrolysed by proteases

How much land is there?

- Global surface area: 510 million km^2
- Global land area: 149 million km^2 (29%)
- Agricultural land: ~40% of global land
- Arable land: ~25% of agricultural land (about 1.4 billion hectares)
- With a global population of ~7 billion there is only 0.2 hectares per person

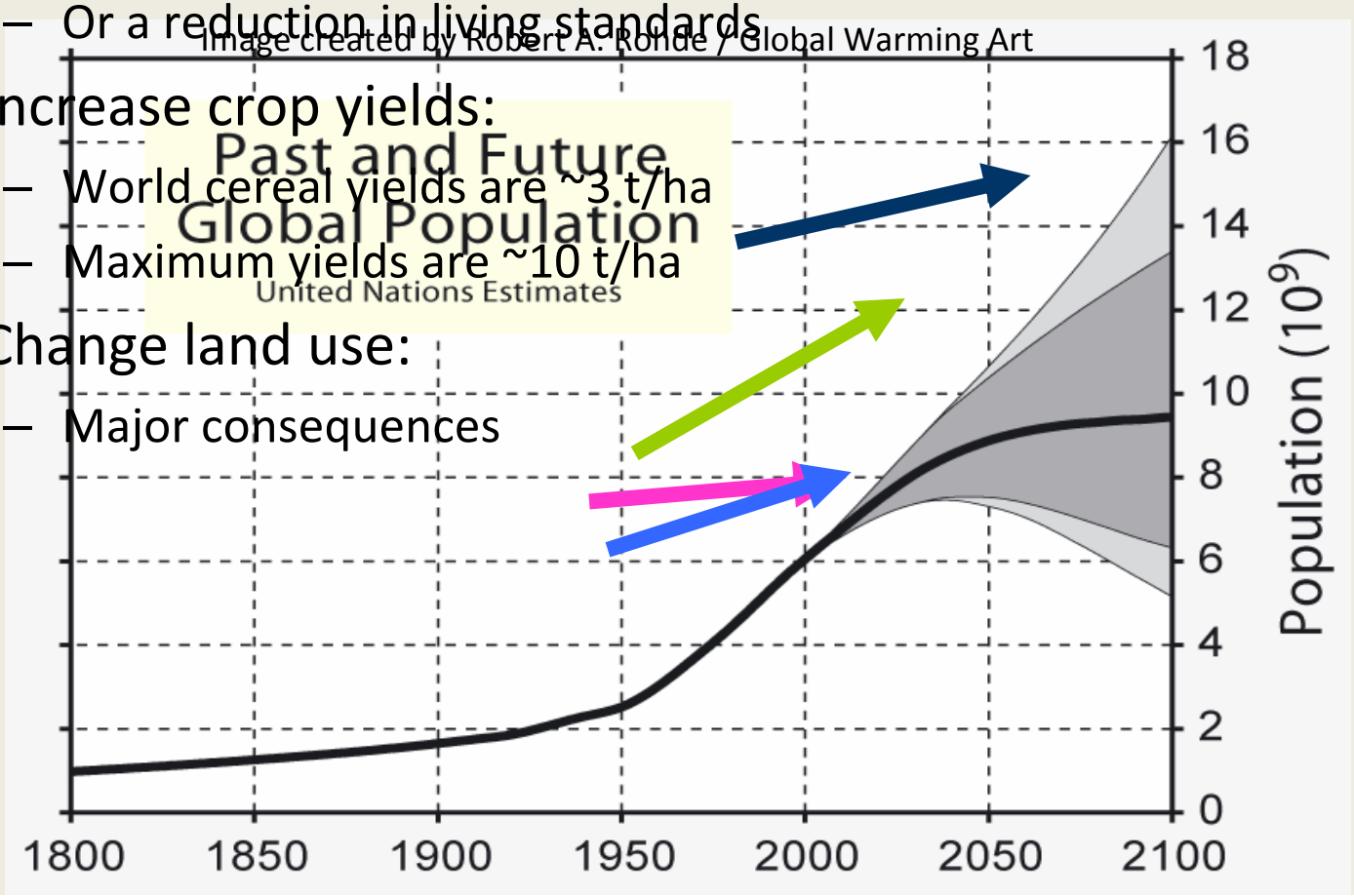
Is it enough?

- Global grain consumption is ~2 billion tonnes (wheat, rice, maize, barley, sorghum and soy)
- This requires ~0.7 billion hectares (50% of available arable land)
- If all remaining arable land was also used to produce grains this would generate enough starch to produce about 70 kg ethanol per person.
- This would be enough to drive about 1000 km per year (less than 10% of the UK average)

…better just to drink it!

How can we meet demands?

- Decrease demands:
  - Requires a reduction in global population
  - Or a reduction in living standards
- Increase crop yields:
  - World cereal yields are ~3 t/ha
  - Maximum yields are ~10 t/ha
- Change land use:
  - Major consequences



- “We cannot afford to abandon biofuels as part of a low carbon transport future.
- Equally, we cannot continue producing biofuels which are ultimately more environmentally and socially damaging than the fossil fuels they seek to replace.”

Biorefinery Exercise

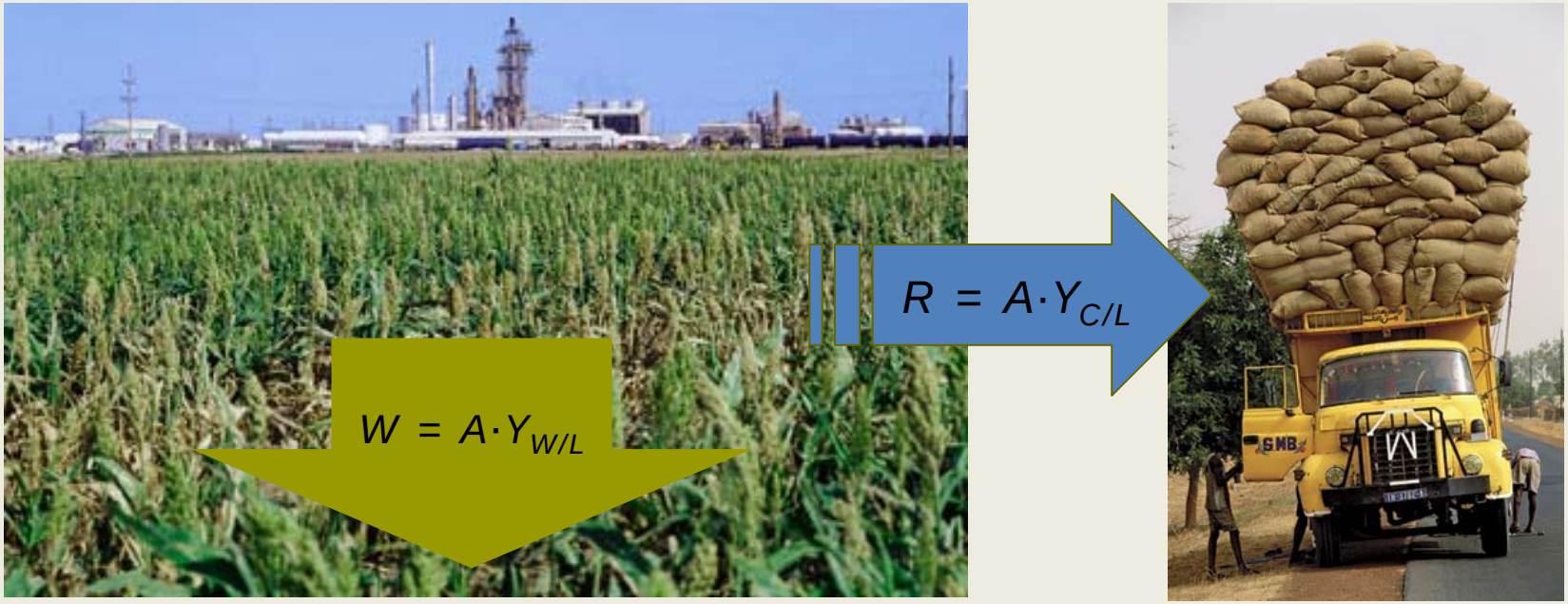
ethanol/biodiesel from **wheat/rapeseed**

Biorefinery Exercise

- A biorefinery is to be sited in the middle of an agricultural region producing **wheat/rapeseed** over a 20km radius. Estimate the annual production of **ethanol/biodiesel** and carry out a preliminary process design, including material balances.
- How much of the biofuel produced would be required for essential transportation associated with the biorefinery?
- What would be the key differences in the process if the biorefinery was producing a range of chemical products rather than just the biofuel?

Raw materials supply

- The amount of raw material available (*R*) depends on:
  - Area of land cultivated – *A*
  - Yield of crops in the field – $Y_{C/L}$

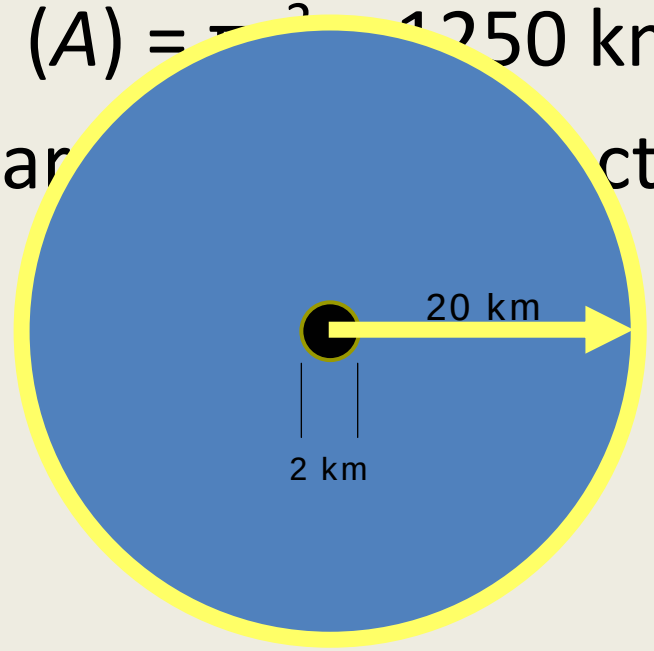


Typical crop yields

Crop	$Y_{C/L}$ (tonne/hectare)	*Residue* $Y_{W/L}$ (tonne/hectare)
Sugar (cane)	68.5	12
Sugar (beet)	50	10
Oilseed Palm	14	9
Corn	11	10
Jatropha	8	4
Rapeseed	3.5	1.8
Wheat	3.5	3.4
Soya	3.2	4.5

Crop production:

- Radius (km) = 20 (minus ? for factory etc.)
- Area of land (A) = πr^2 = 1250 km^2 (1256.6)
- Area in hectares = ctares
- $Y_{C/L}$ = ?



Yields around the world

	Wheat $Y_{C/L}$ (tonne/hectare)	*Rape* $Y_{C/L}$ (tonne/hectare)
UK (2006)(2002)	8.0	3.4
France (2002)(2003)	6.8	3.3
EU (2009)	**5.3**	**3.0**
Mexico (2002)	5.0	-
World (2008)(2003)	**3.1**	**1.5**
China (2009)	3.8	1.5
India (2009)	2.9	1.0
Russia (2009)	2.0	1.25

Crop production (bioethanol):

- Radius (km) = 20 (minus ? for factory etc.)
- Area of land (A) = $\pi r^2 \approx 1250$ km^2 (1256.6)
- Area in hectares ≈ 125000 hectares
- $Y_{C/L}$ = 5.3 tonnes of wheat/hectare
- So, Raw material, (R) ≈ 662.5 ktonne/yr
- For 350 days operation ≈ 1893 tonne/day

Crop production (biodiesel):

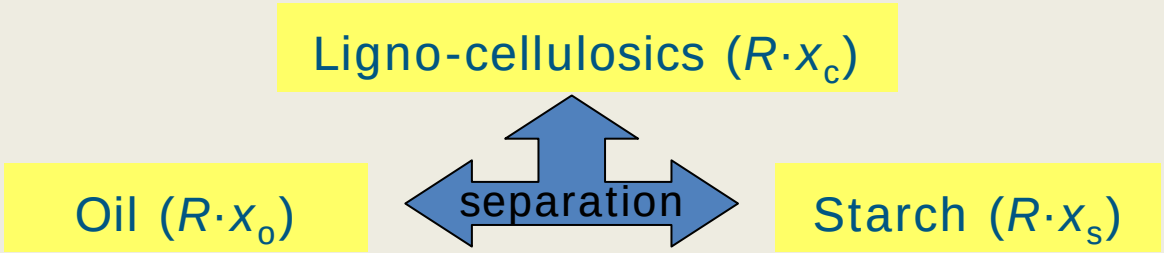
- Radius (km) = 20 (minus ? for factory etc.)
- Area of land (A) = $\pi r^2 \approx 1250$ km^2 (1256.6)
- Area in hectares ≈ 125000 hectares
- $Y_{C/L}$ = 3.0 tonnes of rapeseed/hectare
- So, Raw material, (R) ≈ 375 ktonne/yr
- For 350 days operation ≈ 1071 tonne/day

Raw materials

- The amount of processable material, $R \cdot x_i$ (e.g. starch, oil, sugar…) depends on the extractable composition (milling, pressing etc.):

$$\Sigma R \cdot x_i = R$$

  - Where x_i is the fraction of component *i* in the raw material (x_i wheat starch ≈ 0.6, x_i rape oil ≈ 0.36)
- Different components might be processed in different ways:



Conversion to intermediate

- Oil can be processed directly to biodiesel but starch must be converted to glucose before ethanol production.
- The amount of 'primary reactant' that can be converted from the processable material (e.g. starch to glucose) will depend on stoichiometry and conversion efficiency (i.e. $\alpha_i \cdot yc_i$)
  - Production of glucose from grain starch: Theoretical yield (yc_i) = 1.11 (by mass) achievable ≈ 95% of this (i.e. $\alpha_i \approx 0.95$)

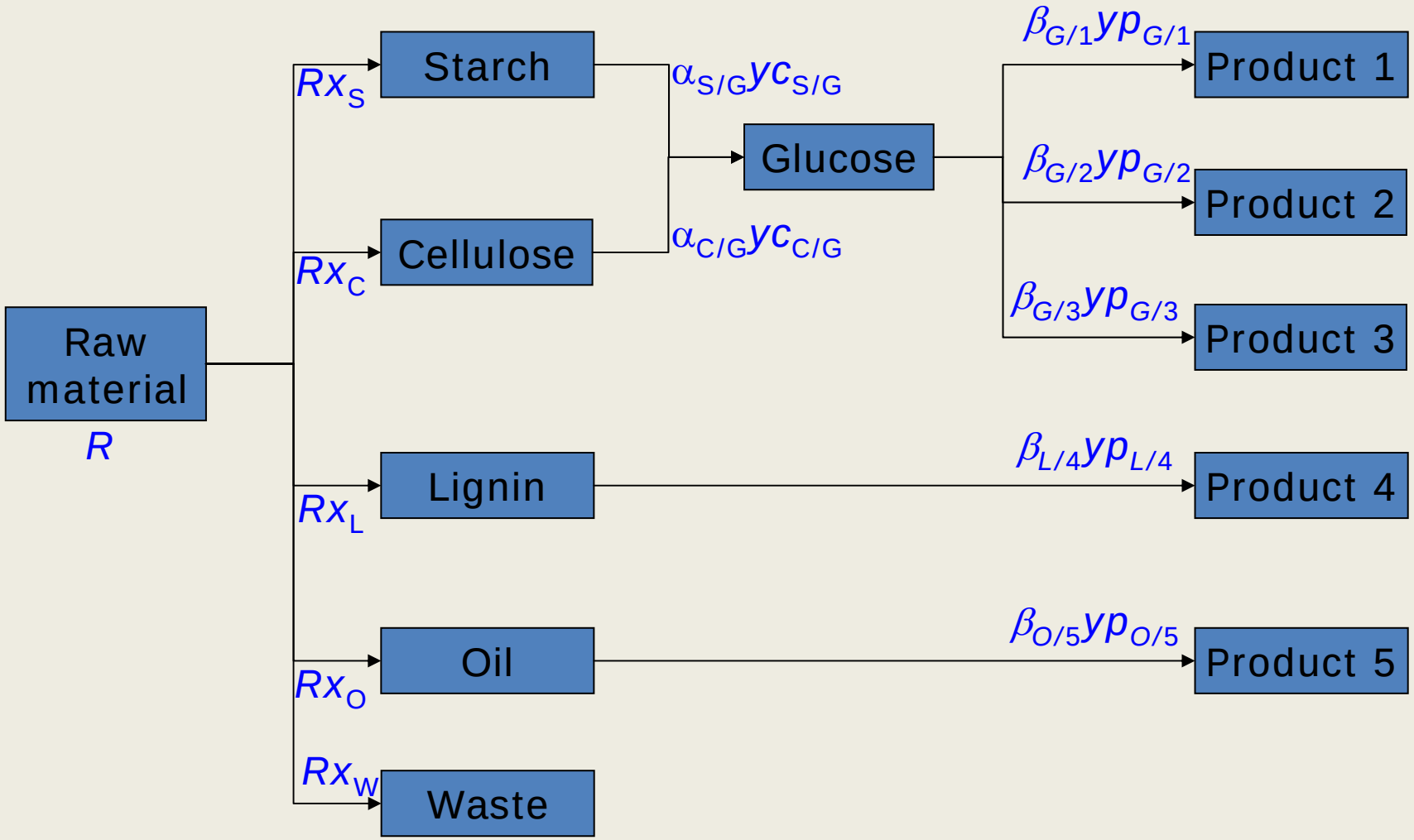
Product formation

- The amount of product produced will depend on many factors such as the complexity of the transformation process
- Overall yields might be based on simple stoichiometry and a conversion factor (e.g. $\beta_i \cdot yp_i$)
- For each product (P_j) conversions from each 'primary reactant' should be summed.

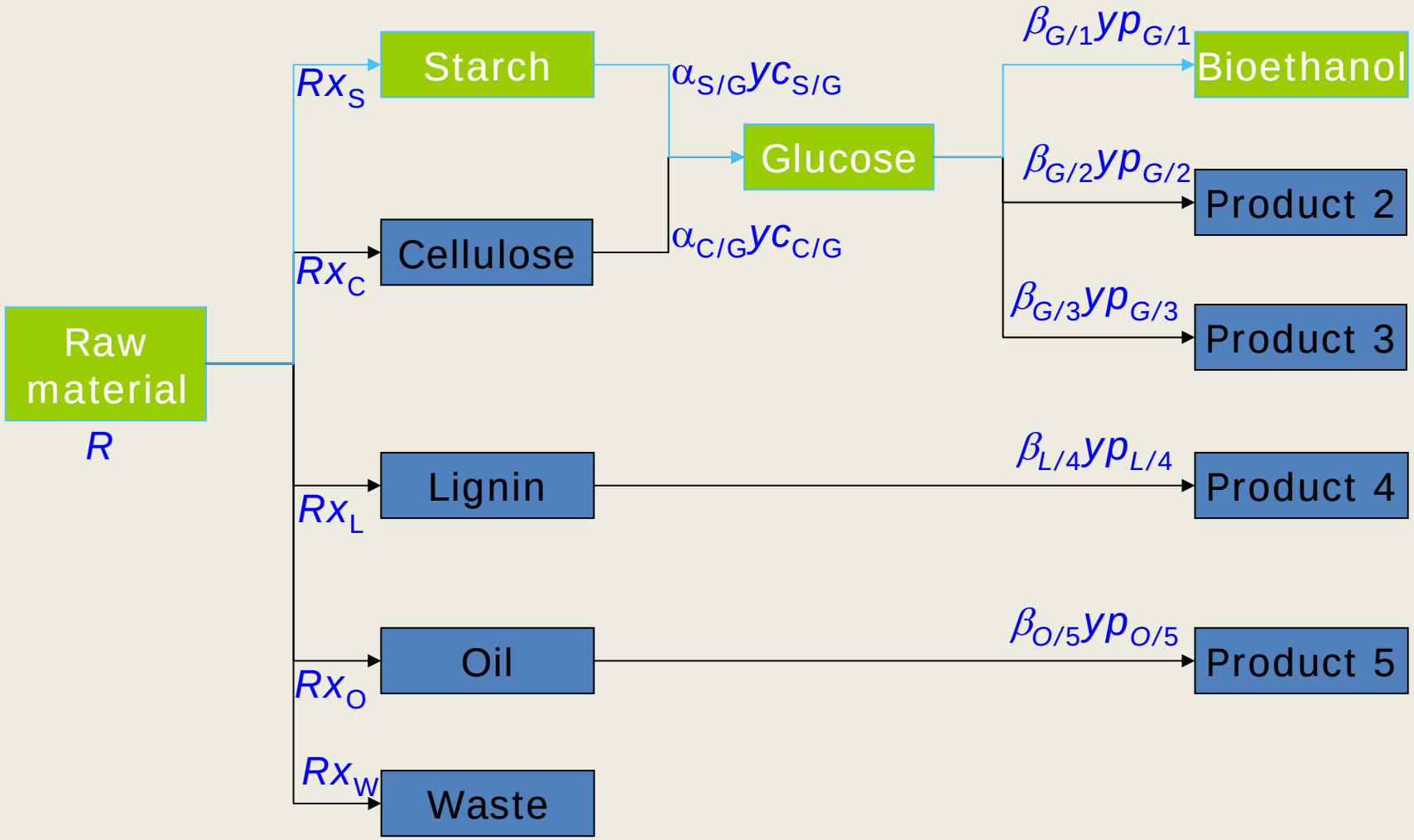
  So: $P_j = \Sigma(R \cdot x_i \cdot \alpha_i \cdot yc_i \cdot \beta_{ij} \cdot yp_{ij})$

  And total production from *R,* is: $P = \Sigma P_j$

Biorefinery material balance:



Wheat to bioethanol:



Wheat to bioethanol:

- A = 125000 hectares
- $Y_{C/L}$ = 5.3 t/ha
- X_s = 0.6 t/t
- $yc_{S/G}$ = 1.11 t/t
- $\alpha_{S/G}$ = 0.95
- $yp_{G/E}$ = 0.51 kg/kg
- $\beta_{G/E}$ = 0.98

So production ≈ 209,500 tpa (+ 200,000 tpa CO_2)
(almost 600 tonne per day)

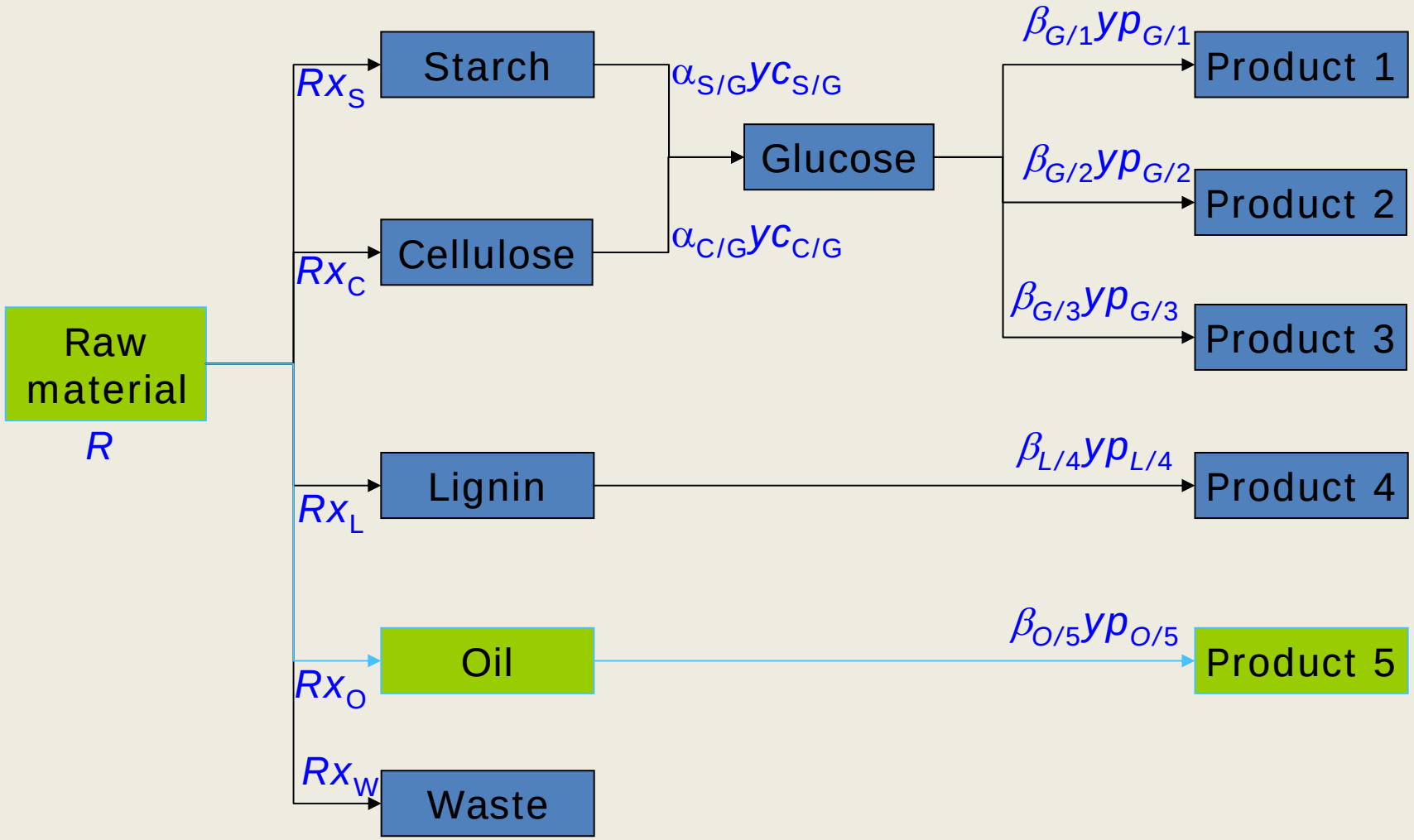
We should also estimate…

- Enzymes required for hydrolysis
- Yeast required for fermentation
- Water required for fermentation
- Distillation yields etc.
- Number of fermenters required
- Waste solids production (yeast & bran)

And…

- Amount of additive required for azeotropic distillation (e.g. cyclohexane)
- Energy requirements
  - for milling,
  - heating,
  - distillation
  - other operations.

Rapeseed to biodiesel:



Rapeseed to biodiesel:

- A = 125000 hectares
- $Y_{C/L}$ = 3.0 t/ha
- X_s = 0.36 t/t
- $yp_{G/E}$ = 0.88 kg/kg
- $\beta_{G/E}$ = 0.97

So production ≈ 115,236 tpa (+ 11,340 tpa glycerol)

(almost 330 tonne per day)

We can also estimate…

- Methanol required and recovered
- KOH catalyst required
- Production of mono- and di-glyceride
- Number of reactors required
- Energy requirements

Comparing biofuels…

	Bioethanol	Biodiesel
Raw materials	1893 t/day (wheat)	1071 t/day (rapeseed)
Product	598 t/day (ethanol)	330 t/day (biodiesel)
Energy	16,000 GJ/day (ethanol)	12,000 GJ/day (biodiesel)

Fuel for transportation/processing?

..

USE OF GLYCEROL AS RAW MATERIAL FOR ENERGY PRODUCTION

José Manuel Gómez

Introduction

- 80% of energy comes from fossil fuels.
- Fossil fuels are no-renewable energy sources.
- Combustion of fossil fuels causes high environmental impacts (greenhouse gases)
- Goverments and international organizations are driving strategies to develop process for energy production from renewable energy sources.

Alternative: Bioenergy

Chemical energy in organic materials that can be converted into usable energy sources by biological, mechanical or thermochemical processes.

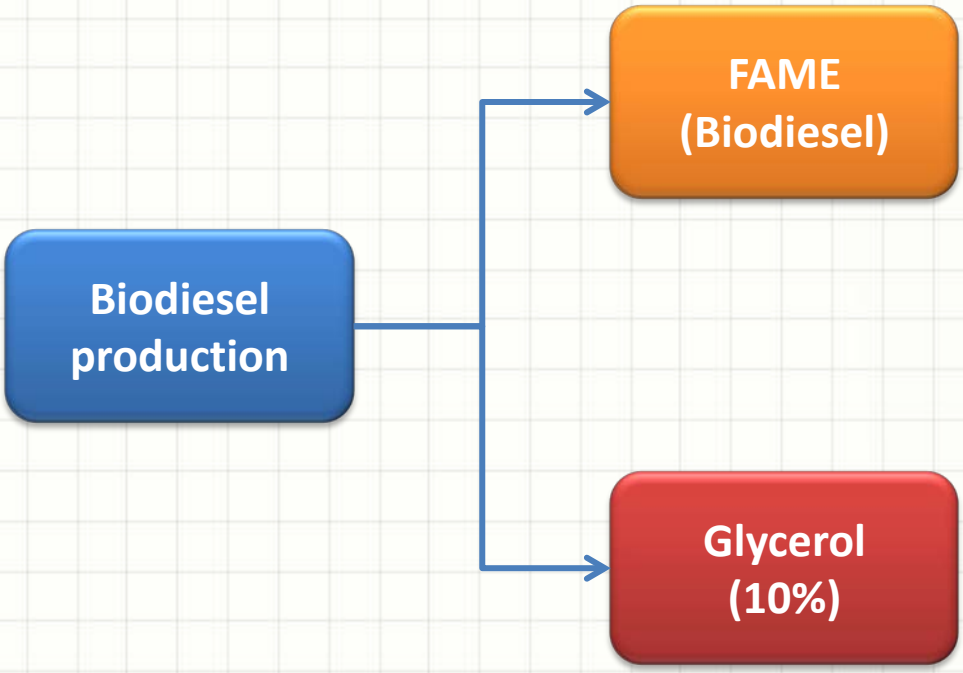
Nowadays, bioenergy is the most important renewable and powerful energy to go replacing the use of fossil fuels

Liquid and gaseous biofuels derived from organic matter can play an important role in reducing CO_2 emissions abatement.

Biofuels obtained from biomass may contributes to the environmental and economic optimization of the complete value chain ---> BIOREFINERY

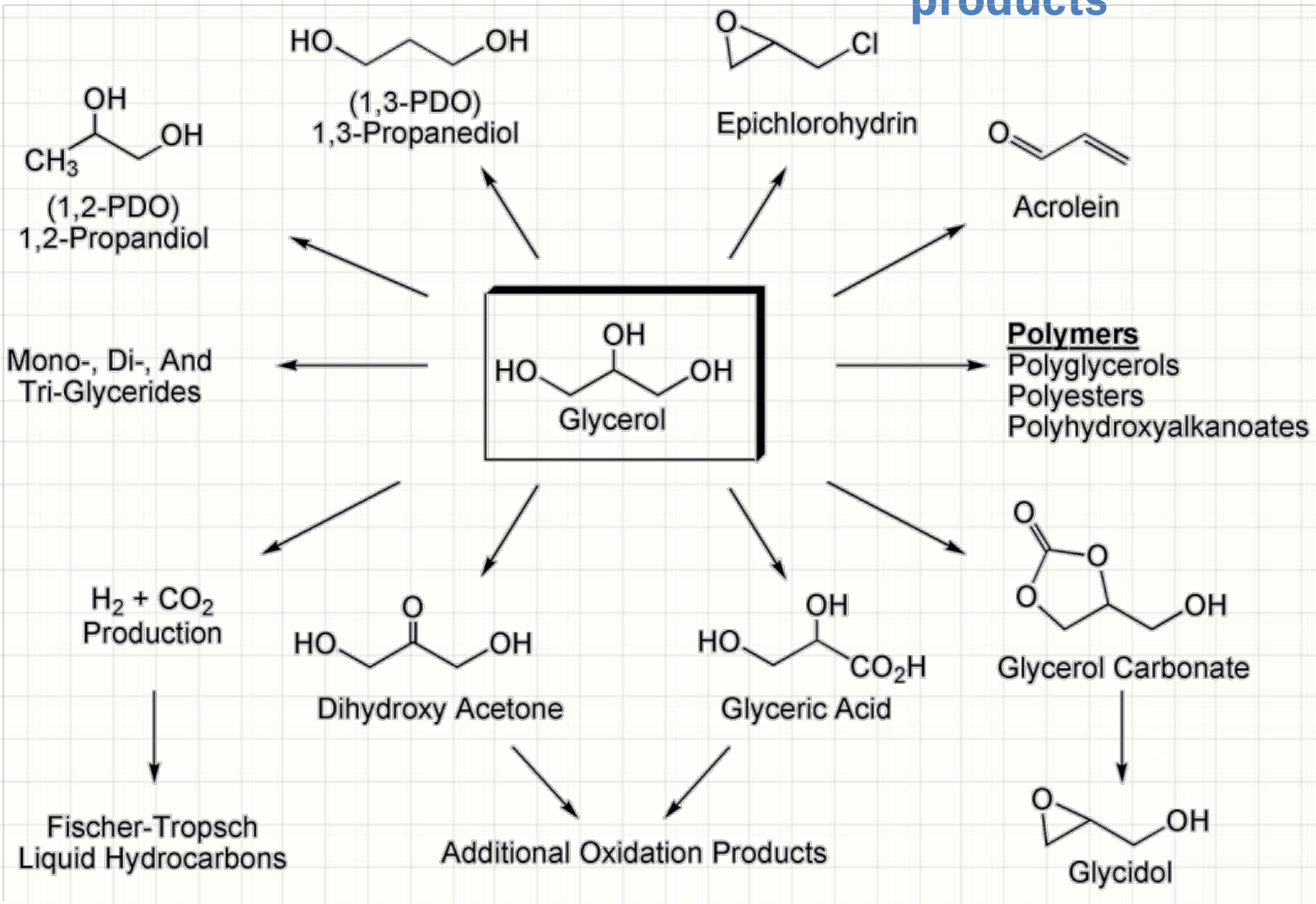
Use of biomass has lower investment costs, compared with other renewable sources.

The diversity of raw materials and processes that can takes place offers a wide range of possibilities.


Biodiesel production
FAME (Biodiesel)
Glycerol (10%)

Glycerol

Primary building block for other value added products



Platform chemicals derived from glycerol.

Current glycerol production can not be absorbed by traditional industries, so its price has fallen. Some chemical companies (Dow Chemical or Procter & Gamble) have closed their plants.

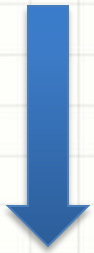
Glycerol has a high level of reduction (4.67 vs 4 for glucose or xylose).

It can be possible the use of this compound as carbon source for different fermentation processes to obtain high value-added compounds.

Glycerol can be converted chemically or biologically in to some products as dyhydroxyacetone, pyruvic acid, tartaric acid, oxalic acid,…

Biological vs Chemical transformation

- Biological doesn't need large energy requirements (moderate temperature and pressure conditions).
- Glycerol is very competitive towards other sugars commonly used in microbial fermentation.



Conversion of phosphoenolpyruvate from glycerol is generated twice reducing equivalents compared to what is produced via glucose or xylose

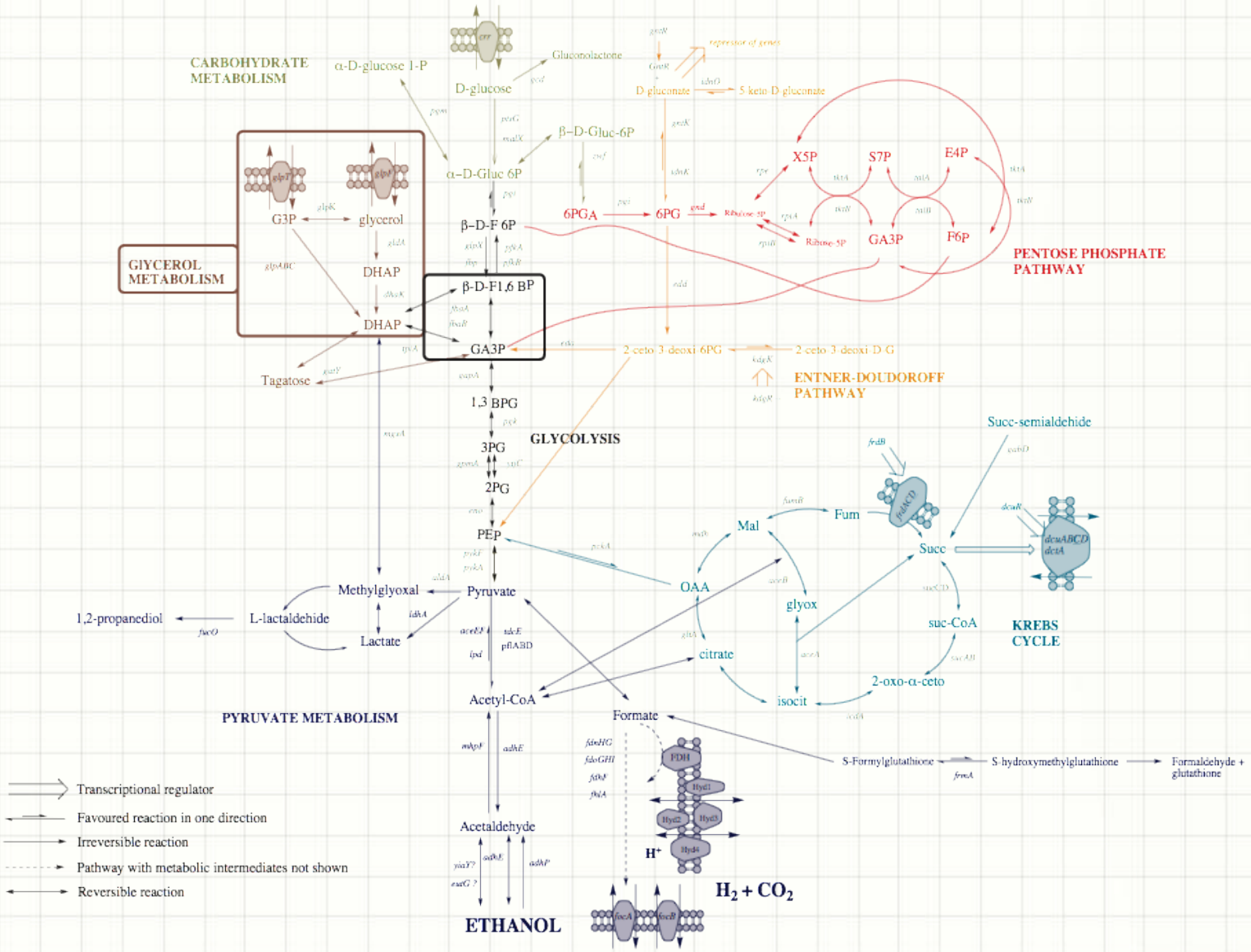
Hypothesis

1. Use of glycerol from biodiesel industry.
2. Bioconversion to reduced compounds such as ethanol, xylitol or hydrogen
3. Important opportunity for biofuels and energy from a cheap and easily available substrate

Biotechnological use

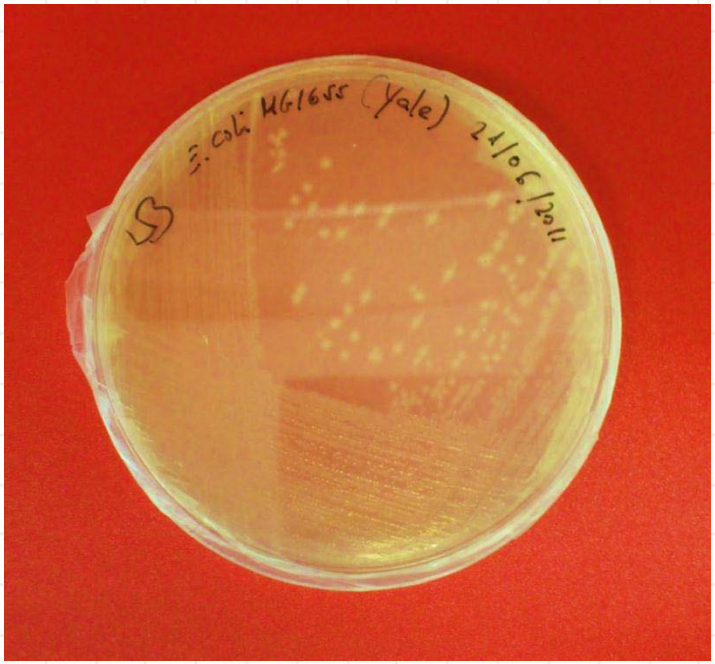
- Some authors have been previously reported
- Rise in the use of this compound as raw material has occurred in last five years.
- Microorganisms used: *Klebsiella, Citrobacter, Enterobacter, Clostridium, Lactobacillus, Propionibacterium or* mixed cultures.
- From industrial applications, some of them are pathogens, (subject) needs high nutritional requirements or (plural)have difficulties in genetic manipulation
- *Escherichia coli* presents interesting advantages that enhanced industrial use: versatility in the use of different carbon sources, complete knowledge of its genome, “workhorse” of the modern biotechnology

METABOLIC PATHWAYS of *Escherichia coli* BW25113



E. coli

Anaerobic conditions



Ethanol, hydrogen

Bioproduction of ethanol and hydrogen

- Some of the works have been developed with pure (grade) glycerol to avoid inhibition by the presence of impurities.

- Pure glycerol is much more expensive than crude glycerol (purification process).

- Use of pure glycerol doesn't solve the problem of excess of crude glycerol obtained in biodiesel industry.

- Most of the studies have been worked out in small reactor (minor than 1 L) in batch mode and little feasibility configurations (bioelectrochemical cells).

So, it’s necessary to deepen the knowledge of the process for bioconversion of crude glycerol to ethanol and hydrogen.

Our experience

- Development and optimization a suitable culture medium that increases bacterial production by reducing the number of component media.

Element	Compound
C	Glycerol
N	Ammonium chloride
P	Dipotasium hydrogen phospahte
S	Sodium sulphate
K	Dipotasium hydrogen phosphate
Na	Sodium chloryde
Mg	Magnesium sulphate
Cl	Sodium chloryde
Fe	Ferric sulphate
Mn	Manganese sulphate
Cu	Copper sulphate
Zn	Zinc sulphate
Mo	Amonium molybdate
Co	Cobalt chloryde
	Peptone

Optimized culture medium

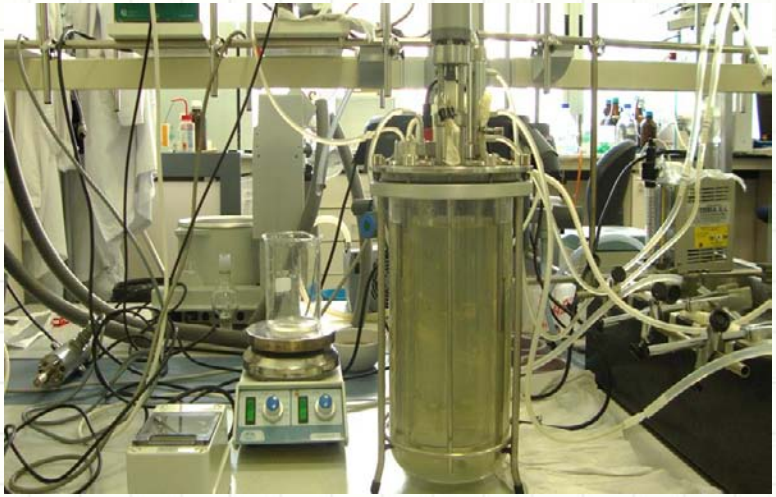
Component	Unidad	Valor
Glycerol	g L^{-1}	10
Na_2SO_4	g L^{-1}	0,0806
NaCl	g L^{-1}	0,0152
$MgSO_4 \cdot 7H_2O$	g L^{-1}	0,0310
Peptone	g L^{-1}	4,25

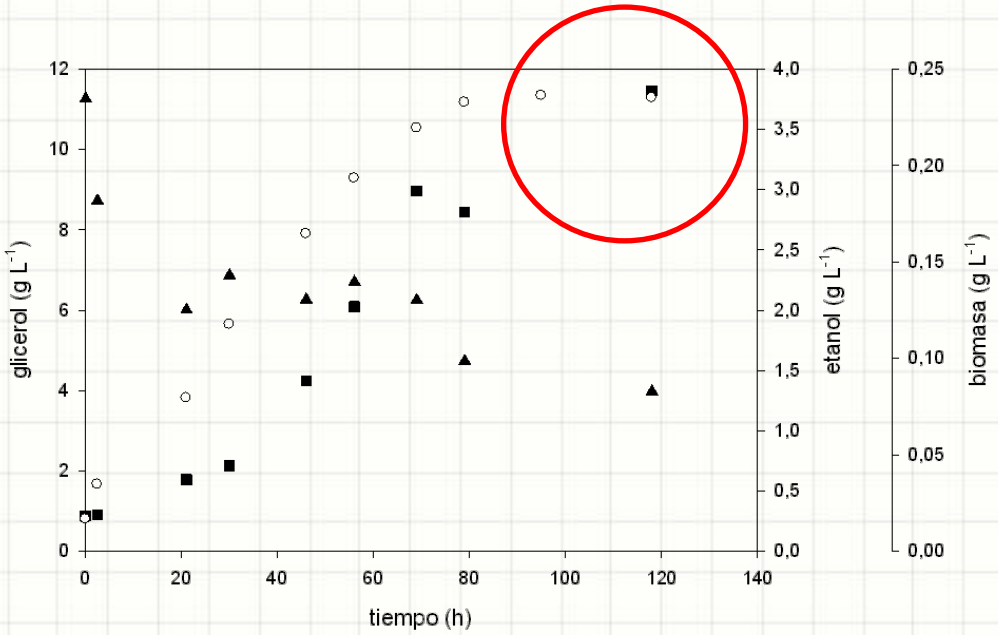
Original culture medium

Experiments in 5 L reactors

Batch mode

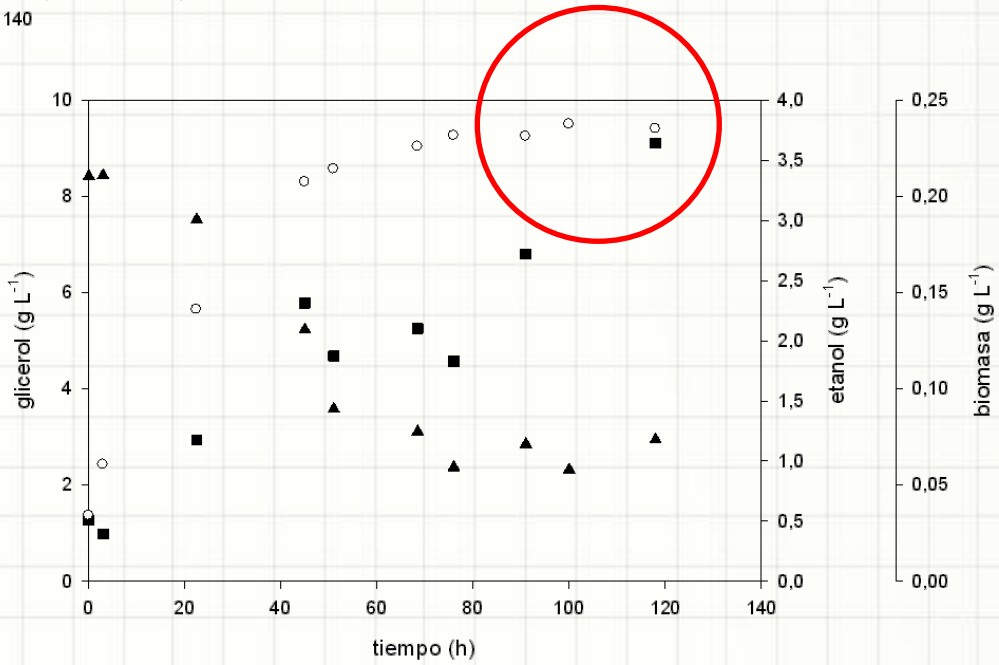
Parameter	Value	Unit
pH	6.3	
Temperature	37	°C
Inert gas adding	≈0.0025	L min^{-1} L^{-1}
Fermentation time	≈ 90	H
Working volume	5.5	L
Agitation speed	150	Rpm





Profiles of fermentation obtained for R5BGFGAr (initial concentration of glycerol 10 g L^{-1}, 37°C, 150 rpm, pH=6,3). (■) ethanol, (▲) glycerol, (○) biomass.

Profiles of fermentation obtained for R5BGCAr (initial concentration of glycerol 10 g L^{-1}, 37°C, 150 rpm, pH=6,3). (■) ethanol, (▲) glycerol, (○) biomass.



Experiments in 5 L reactors Fed-Batch mode

	Substrate	So (g L^{-1})	Feeding modality	Ar bubbling	Code
FB	CG	10	Constant	+	R5FCG10C
FB	CG	30	Constant	+	R5FCG30C
FB	CG	10	Exponential	+	R5FCG10E
FB	CG	30	Exponential	+	R5FCG30E
FB	CG	50	Exponential	+	R5FCG50E

Fermentation	Initial (g)	Fed (g)	Final (g)	Consum(g)	% Consumed
R5FGC10C	17,45	36,50	26,43	27,52	51,01
R5FGC30C	12,03	109,50	92,89	28,62	23,56

Fermentation	Initial (g)	Fed (g)	Final (g)	Consum (g)	% Consumed
R5FGC10E	0	36,31	0,55	35,76	98,48
R5FGC30E	0	106,62	24,24	82,38	77,27
R5FGC30E*	0	106,62	0	106,62	100
R5FGC50E	0	177,7	69,76	107,94	60,70
R5FGC50E*	0	177,7	28,17	149,53	84,14
*: considered step of feed 88 h + post batch of 72 h					

Fermentation	$Y_{P/S}$ g g^{-1}	$Y_{X/S}$ g g^{-1}	Ethanol g L^{-1}
R5FGC10E	0,478	0,044	4,12
R5FGC30E	0,331	0,038	6,28
R5FGC30E*	0,341	-	7,58
R5FGC50E	0,385	0,022	8,59
R5FGC50E*	0,281	-	8,68
*: considered step of feed 88 h + post batch of 72 h			

Experiments in 200 L reactor Pilot plant scale

No inhibition of *E. coli* by crude glycerol was detected.

Fermentation	Initial (g)	Fed (g)	Final (g)	Consum (g)	% Consumed
RPFGC30E	0	4425	2092	2333	52,72
RPFGC30E*	0	4425	0	4425	100
*: considera etapa de alimentación + post batch					

Hydrogen production:

- **449,18 mmol (0,19 mmol H_2 $g_{glicerol}^{-1}$, a 160 h)**

Overexpression of enzymes

- Pyruvate formate lyase (PFL), pyruvate formate lyase activase ($PFL_{activase}$) and phosphoenolpyruvate carboxykinase (PECPK) has been overexpressed in *E. coli* TOP10.
- Increase in H_2 production over strain not expressed has been obtained.
- Starts the study of mutants in order to increase the production of ethanol and hydrogen by crude glycerol utilization.

Future challenges

- Consideration of *E. coli* genetically modified to maximize ethanol and hydrogen production.
- Optimize the production of ethanol and hydrogen at laboratory and pilot scale for industrial applications.

Thanks

Questions?

Genetics and Biotechnology: an introduction

Dr. Ismael Cross Pacheco
Department of Biomedicine, Biotechnology and Public Health
Area of Genetics
University of Cadiz

PRENSA

PRENSA

PRENSA

PRENSA

PRENSA

Clonan al
La

a hacer un

Maiz transgénico con tres vitaminas 'made in Spain'

NEWS!!

Los genes del colesterol

El 'milagro' de Andy y Ángel

Un ensayo confirma la eficacia de la terapia genética en una enfermedad rara. Los pequeños, que están haciendo una vida normal, han sido los menores españoles

Avances en Genética

Avances en Genética

Avances en Genética

Andy and Colesterol genes are discovered in Spain'

Introduction

These and many others related news appear frequently in press, but usually nobody knows well what they consist of, or what they are based in.

- In this presentation, some application of the biotechnology will be explained, focusing mainly on genetics and health.

- Biotechnology and environment.

- DNA and the flow of genetic information towards the proteins.

- And finally, we will see some curious or interesting applications of the biotechnology: transgenic animals and plants, synthetic genomes…


Health and Genes

Medicines that improve our health

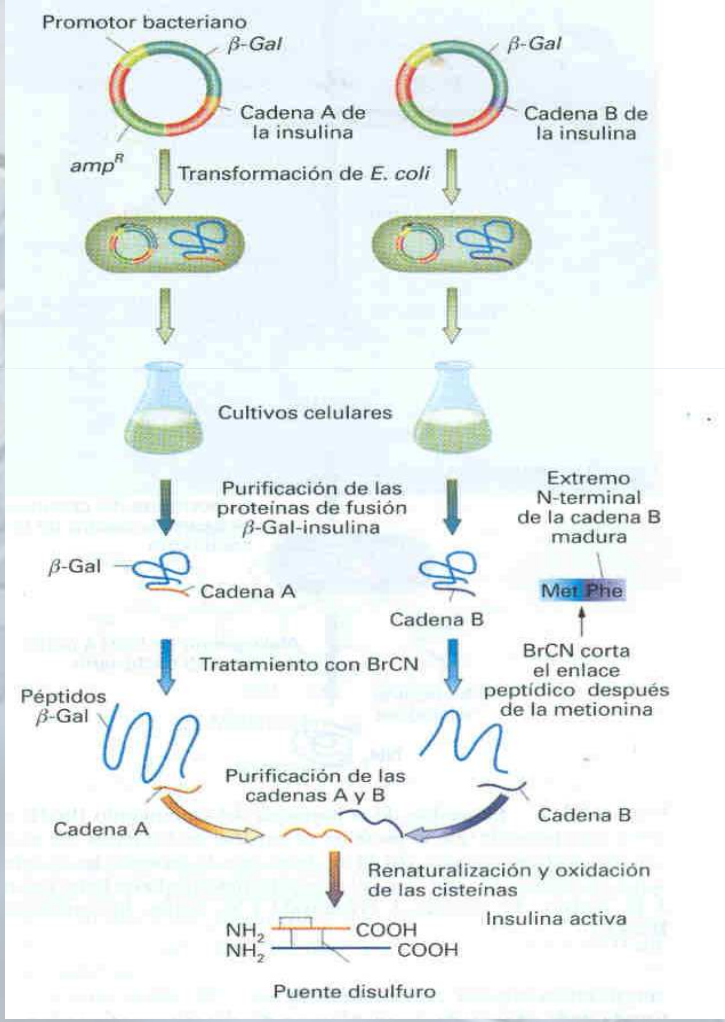
Insulin: protein

- From a human insulin **GENE**, the insulin **protein** can be produced, but … How can it be done??

-The gene is introduced in bacteria or yeast, and they produce human insulin.

-These microorganisms are then cultured in bioreactors.

- Finally, insulin is marketed and used by diabetics .



Pharmaceuticals applied to human health arising from GMOs (I)

Producto	Sistema de producción	Indicación terapeutica
Factores de coagulación		
Factor VIII	Cultivo de células de mamífero	Hemofilia A
Factor IX	Cultivo de células de mamífero	Hemofilia B
Factor VIIa	Cultivo de células de mamífero	Ciertas formas de hemofilia
Anticoagulantes		
Activador del plasminógeno tisular	Cultivo de células de mamífero	Infarto de miocardio
Activador del plasminógeno tisular	E. coli	Infarto de miocardio
Hirudina	Levaduras	Trombocitopenia y prevención de trombosis
Hormonas		
Insulina	Levaduras	Diabetes mellitus
	E. coli	
Hormona de crecimiento	E. coli	Deficiencia de la hormona en niños, acromegalia, síndrome de Turner
Folículo-estimulante	Cultivo de células de mamífero	Infertilidad, anovulación y superovulación
Paratiróidea	E. coli	Osteoporosis
Gonadotrofina coriónica	Cultivo de células de mamífero	Reproducción asistida
Tirotrofina	Cultivo de células de mamífero	Detección /tratamiento de cáncer de tiroides
Lutelnizante	Cultivo de células de mamífero	Ciertas formas de infertilidad
Calcitonina	E. coli	Enfermedad de Paget
Glucagon	Levaduras	Hipoglucemia

Nature Biotechnology, 2003, vol. 21 Nº8

Pharmaceuticals applied to human health arising from GMOs (II)

Factores hematopoyéticos		
Eritropoyetina (EPO)	Cultivo de células de mamífero	Anemia
Factor estimulante de colonias de granulocitos/macrófagos (GM-CSF)	E. coli	Netropenia, transplante autólogo de médula
Interferón e Interleuquinas		
Interferón alfa (IFN alfa)	E. coli	Hepatitis B y C, distintos tipos de cáncer
Interferón beta (IFN beta)	Cultivo de células de mamífero	Esclerosis múltiple
Interferón gamma (IFN gamma 1b)	E. coli	Enfermedad granulomatosa crónica
Interleuquina 2 (IL-2)	E. coli	Cáncer de riñón
Vacunas		
Anti-hepatitis B	Levaduras	Inmunización contra la hepatitis B
Anti-hepatitis A	Levaduras	Inmunización contra la hepatitis A
Anti-enfermedad de Lyme	E. coli	Inmunización contra la enfermedad de Lyme
Anticuerpos monoclonales recombinantes		
Anti-IgE (recombinante)	Cultivo de células de mamífero	Asma
Anti-TNF (recombinante)	Cultivo de células de mamífero	Arthritis reumatoidea
Anti-IL2	Cultivo de células de mamífero	Prevención del rechazo agudo de transplante de riñón
Otros productos recombinantes		
Proteína morfogénica del hueso-2	Cultivo de células de mamífero	Fractura de tibia
Galactosidasa	Cultivo de células de mamífero	Enfermedad de Fabry (deficiencia en alfa-galactosidasa)
Iaronidasa	Cultivo de células de mamífero	Mucopolisacaridosis
Proteína C	Cultivo de células de mamífero	Gepsis severa
Beta-glucocerebrosidasa	E. coli	Enfermedad de Gaucher
DNAsa	Cultivo de células de mamífero	Fibrosis quística

Nature Biotechnology, 2003, vol. 21 Nº8

Predictive medicine: Towards a molecular individualized profile

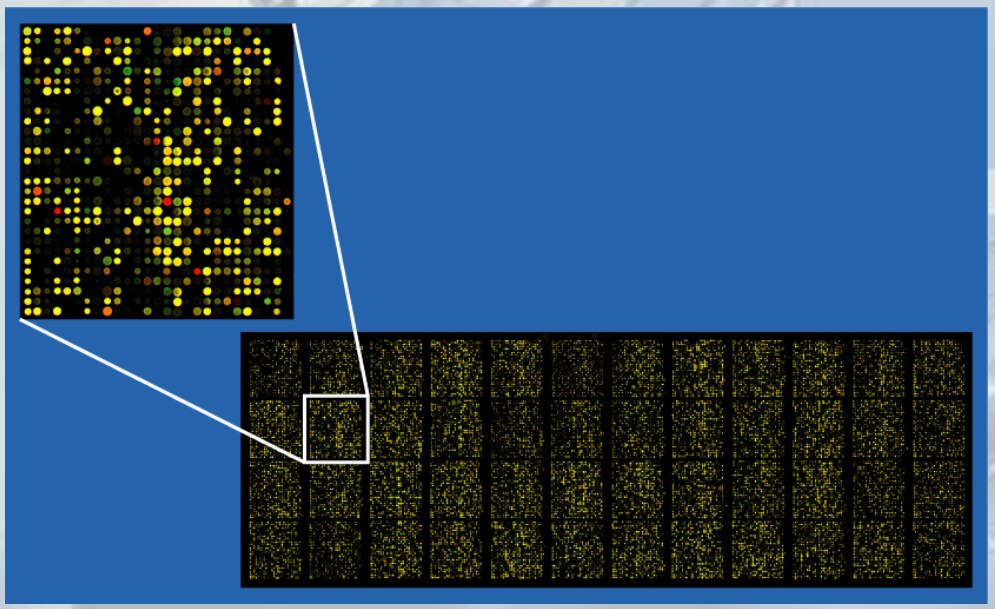
The genetic profile allows a differential prediction and the individualization of the treatments.

Applications of DNA chips

It allows to differentiate subtypes of a disease (cholesterol, cancer …)→ More successful and safe prognosis

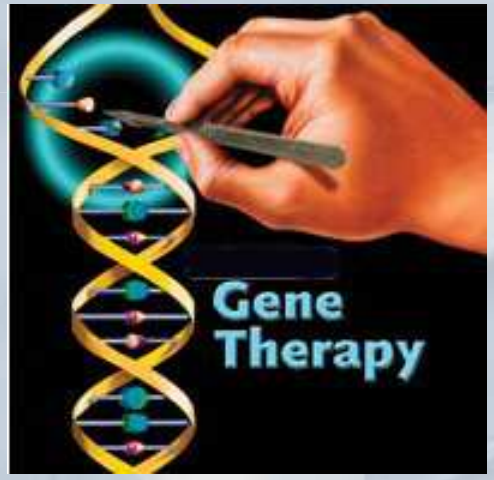
Genes that are actives in an organisms or tissue, can be analyzed at a given time. At past, it was for a few genes, but nowadays for thousands at a time.

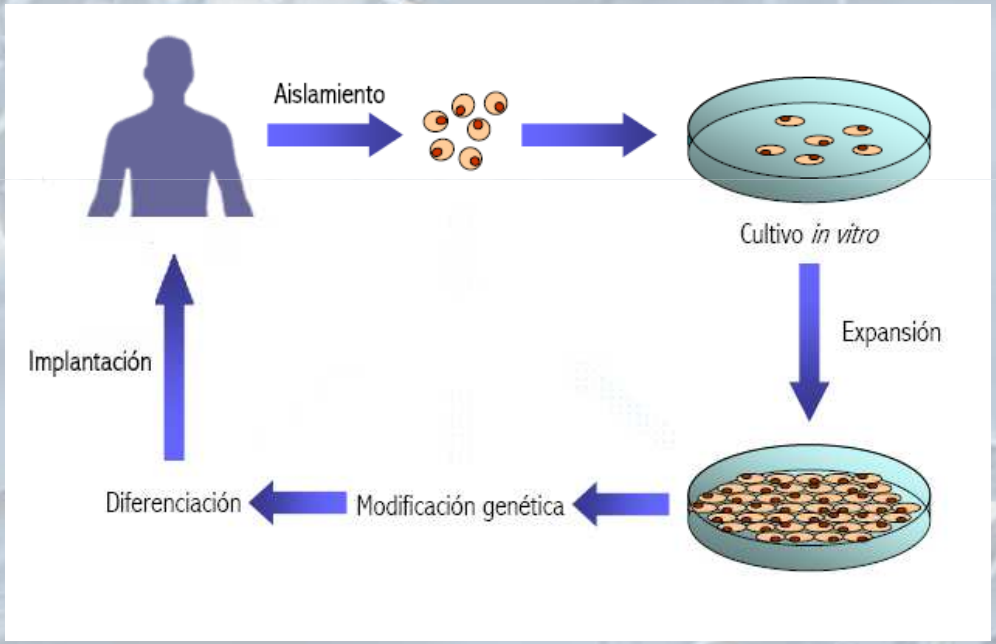
Useful for identifying individuals at risk of developing a disease, and to choose the most suitable pharmacological treatment personalized.



Gene Therapy

Gene therapy is an technique that uses genes to treat or prevent diseases. The most common form of gene therapy involves inserting a normal gene to replace an abnormal gene.





Regeneration of infarcted hearts (and other tissues) with adult stem cells

ASÍ ES EL PROCESO

Un estudio de la Clínica Universitaria con pacientes que sufren de insuficiencia cardiaca tras un infarto antiguo pone de manifiesto las posibilidades curativas de las células madre adultas

1 Estudios previos
Para determinar si el paciente puede correr algún riesgo con el tratamiento:
- Análisis de sangre
- Resonancia Magnética
- Ecografía
- Electrocardiograma de 24 horas

2 Plasmaféresis — Paciente → Sangre → Plasma
Se extrae el plasma sanguíneo, que se utilizará para cultivar las células

Células sanguíneas

Suero

Las células sanguíneas se retornan al paciente en suero fisiológico

3 Biopsia de músculo — Músculo
Se obtienen 10 gramos de músculo del muslo, en una sencilla operación con anestesia local. El paciente recibe el alta el mismo día.

Digestión

4 Digestión mecánica y enzimática
Para disgregar el tejido

Suspensión celular

5 Obtención de células madre — Mioblastos (células madre musculares)
Por suspensión en medios de densidad adecuada, se separan las células que nos interesan

Células musculares

Fibroblastos (tejido fibroso)

6 Cultivo celular
Durante seis meses, las células crecen en el plasma del propio paciente

La técnica reduce el riesgo de alergias, infecciones y otras reacciones no deseadas con sueros ajenos al paciente

7 Implante en el corazón
Cuando hay suficientes células, se introduce un catéter que inyecta las células madre en el área lesionada

8 Mejora de la función cardiaca
Las células implantadas liberan factores de crecimiento que estimulan la creación de vasos en la zona y disminuyen la fibrosis, lo que mejora la función del área infartada.
El estudio pasará a Fase I de seguridad con éxito y comienza ahora la Fase II de eficacia

Aorta
Aurícula izquierda
Ventrículo izquierdo
Aurícula derecha
CORAZÓN
Ventrículo derecho
Aguja retirada
Aguja
Células madre
De 10 a 15 inyecciones en la zona infartada
El catéter se introduce por la arteria femoral
Miocardio

deberán cultivarse durante un mes hasta conseguir el número suficiente de células para realizar el trasplante.

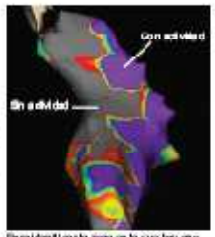
El cultivo de las células se practica en el laboratorio GMP (Good Manufacturing Practice) de la Clínica Universitaria, y está basado en la utilización de suero del propio paciente, a diferencia de los cultivos realizados en otros estudios que usan suero de origen animal, con lo que se reduce el riesgo de reacciones adversas. "De este modo se evitan hipotéticas infecciones, alergias o reacciones inmunológicas, ya que no se introducen proteínas extrañas", describe el doctor Juan José Gavira, cardiólogo de la Clínica. El suero se obtiene mediante la realización de una plasmaféresis, técnica con la que se consigue el plasma en el que se cultivarán los mioblastos.

Transcurrido el mes necesario para el crecimiento de la población celular, las células obtenidas se inyectan en el corazón del paciente mediante cateterismo con un catéter especial de inyección. Las células se implantan en las regiones de músculo cardiaco infartadas y alrededor de éstas.

Uno de los requisitos que deben cumplir los pacientes incluidos en el ensayo clínico es que no precisen de ninguna otra terapia invasiva adyuvante de forma simultánea. De este modo, apunta el especialista, "los resultados que se obtienen son mucho más puros, ya que en ellos no interfiere ningún otro tratamiento". Obtenidas las células madre adultas, se analiza una muestra en el Laboratorio de Microbiología para descartar la existencia de infecciones y certificar su perfecto estado.

EL IMPLANTE DE CELULAS MADRE. Para implantar los mioblastos es necesario hacer una reproducción anatómica del ventrículo izquierdo, que es la zona que se va a tratar. La reproducción virtual se realiza mediante un sistema de navegación denominado

PASA A LA PÁG. 20 >>



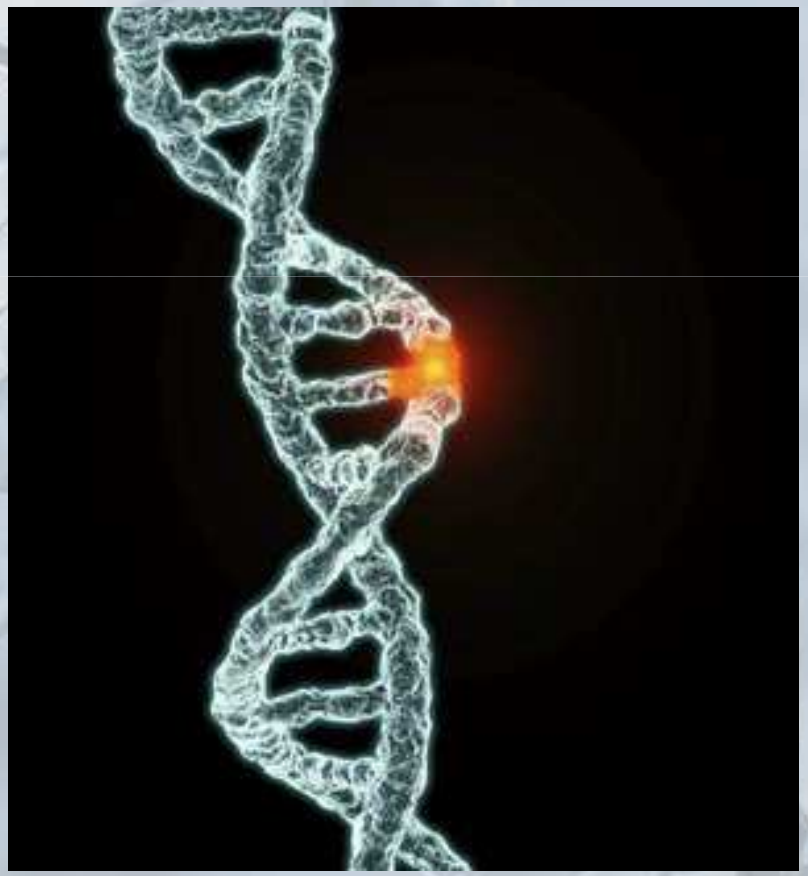
Para identificar la zona en la que hay que implantar las células madre se emplean imágenes tridimensionales de la actividad eléctrica del corazón.

Fighting cancer with Biotechnology

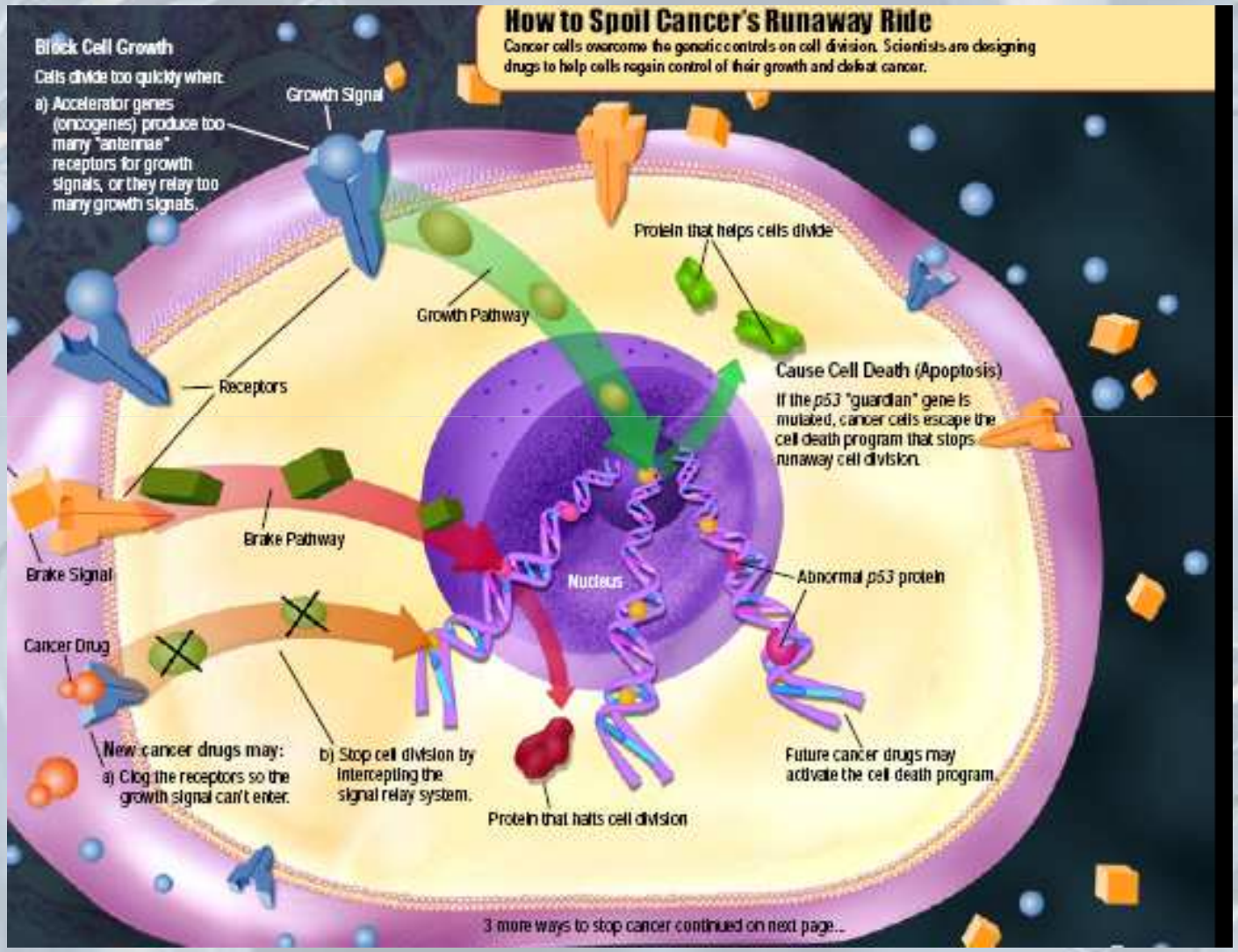
Cancer is a disease caused by uncontrolled cell proliferation due to the accumulation of mutations in cells.

-Currently, dozens of different genes associated with specific tumor types are known.

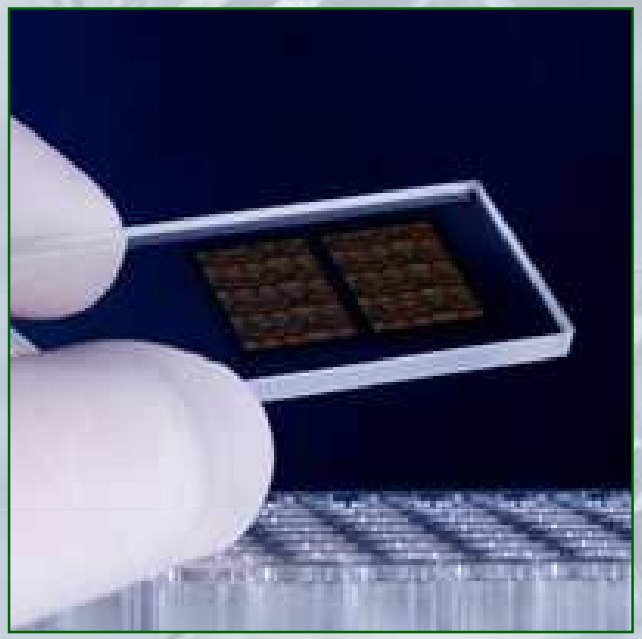
- To understand how a normal cell becomes cancerous, it is necessary to understand the mechanisms that control cell number.

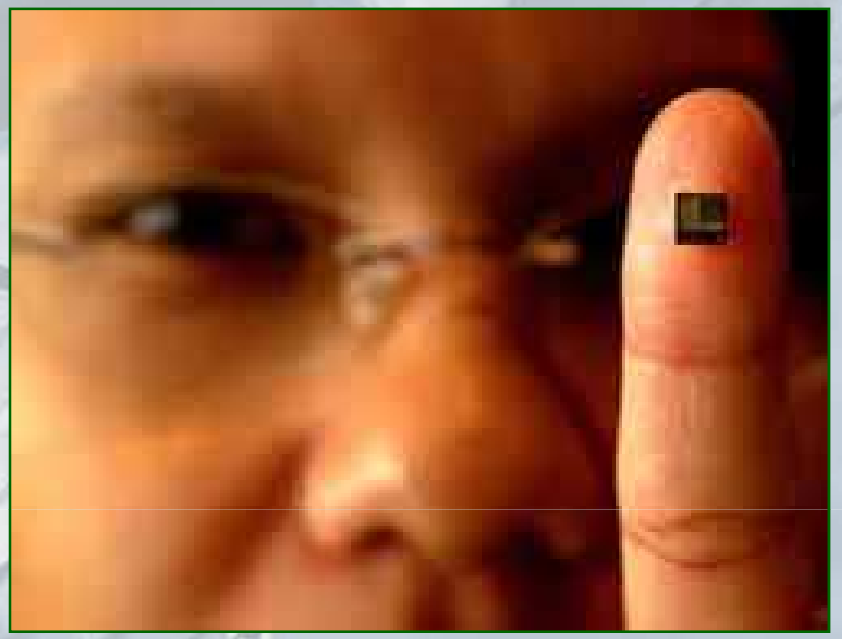


Strategies to fight cancer



DNA Chips for detecting and analyzing tumor

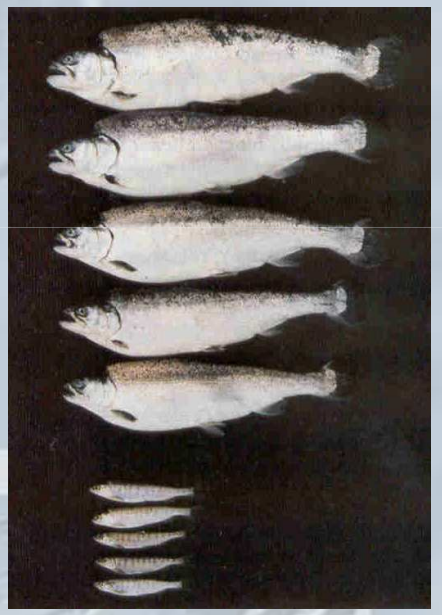




The accuracy for DNA chips is such that it may have tens of thousands of DNA fragments that detect tens of thousands of possible mutated sequences.


Transgenic organisms(GMOs)

Transgenic organism: It is an organism whose genome has been modified by the introducing new exogenous DNA.



Just some applications

Genetically Modified Organisms (GMO)

- ✓ Bacteria: insulin, interferon, Growth Hormone..
- ✓ Yeasts: vaccine for hepatitis A and B, insulin. …
- ✓ Plants: herbicide-resistant, enriched in vitamins…etc
- ✓ Animals: model organism to study diseases (cancer among others), bioreactors of medical interest proteins, growth, cold-resistance, applications in environmental biomonitoring …

All these applications, research and discoveries are based in the knowledge of genetic material: Genes.

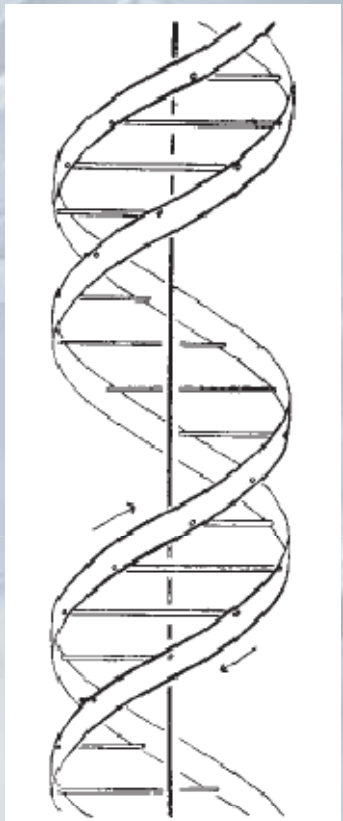
-But:

- **What are genes??**
- **What are they make of?**
- **How does the genetic information flow among cells, organisms, generations or individuals?**
- **What is the protein-gene relationship??. Can genes be cut, copied, pasted or combinated?**
- **Can DNA be *in vitro* synthesized?**

It is not possible to answer these and other essential questions in this presentation…


..but: Let´s talk about DNA

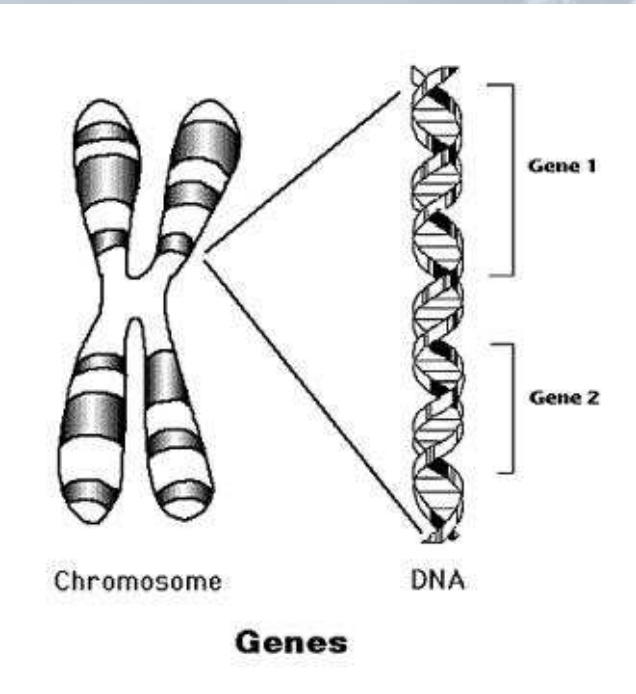
DNA structure (Watson and Crick, Nature, 1953)

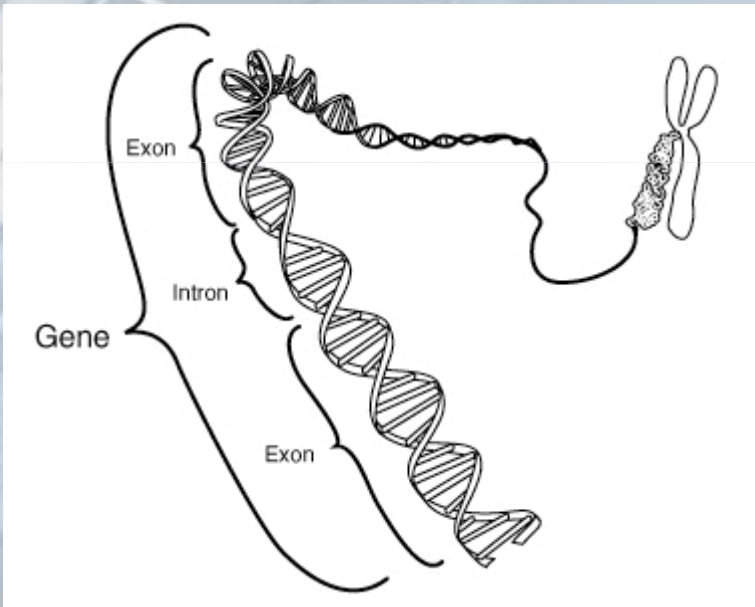


This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Genes and DNA

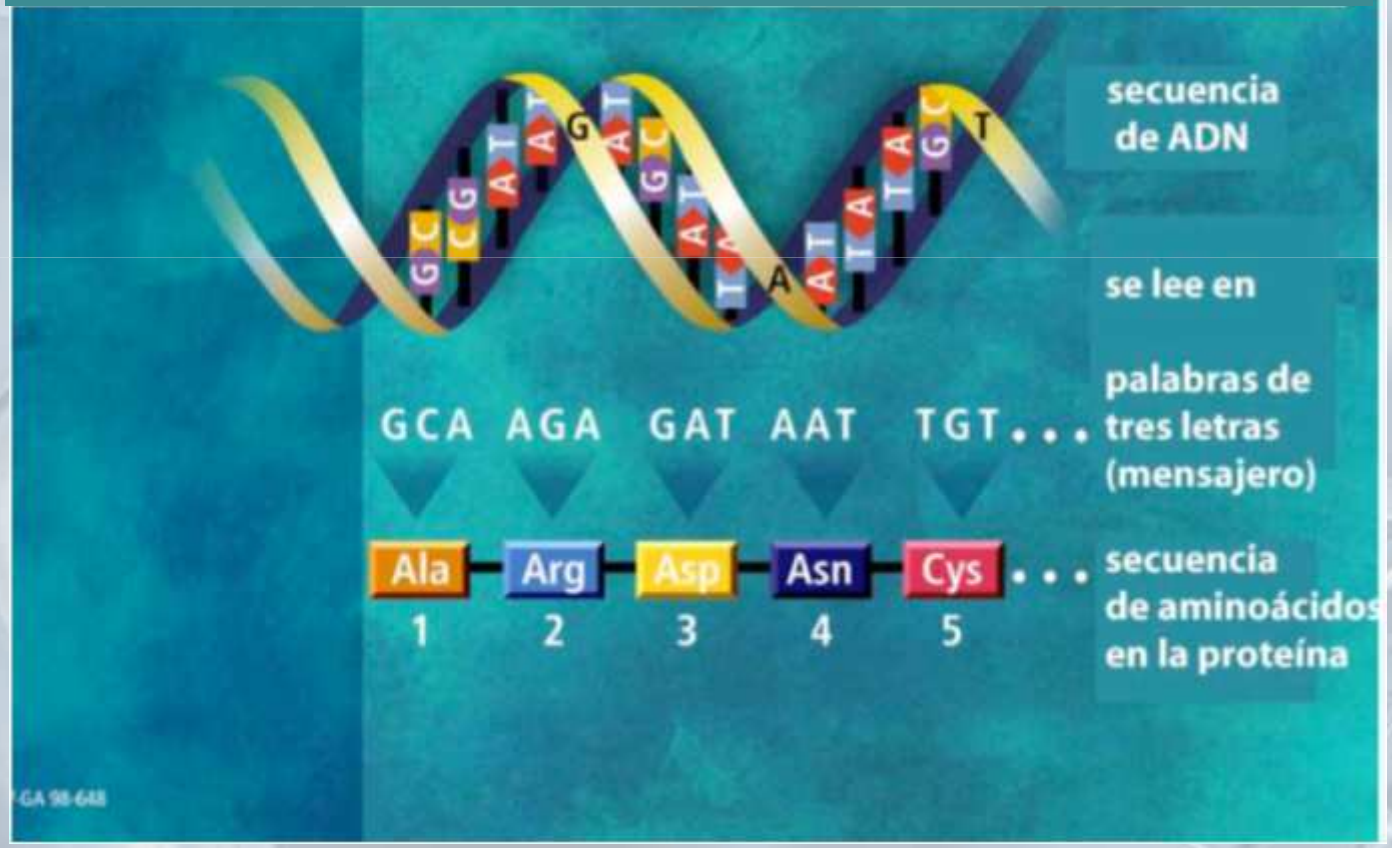
Gene definition: it is a region of genomic sequence (DNA), corresponding to an unit of inheritance, which is associated with regulatory regions, transcribed regions, and other functional sequence regions.





How does the life work?

THE DNA SEQUENCE DETERMINES THE AMINO-ACIDS SEQUENCE IN THE PROTEIN.



How does the life work?


Just a minute to speak about..

1.- Transgenic mammal producing human insulin protein

INS insulin [*Homo sapiens*]

The polypeptide hormone insulin is required for normal glucose homeostasis. Insulin deprivation results in diabetes, a disease affecting up to 5% of the human population.

>gi|224589802:c2182654-2180794 Homo sapiens chromosome 11, GRCh37.p10 Primary Assembly

```
CTAATGACCCGCTGGTCCTGAGGAAGAGGTGCTGACGACCAAGGAGATCTTCCCACAGACCCAGCACCAG
GGAAATGGTCCGGAAATTGCAGCCTCAGCCCCCAGCCATCTGCCGACCCCCCCACCCCAGGCCCTAATGG
GCCAGGCGGCAGGGGTTGAGAGGTAGGGGAGATGGGCTCTGAGACTATAAAGCCAGCGGGGGCCCAGCAG
CCCTCAGCCCTCCAGGACAGGCTGCATCAGAAGAGGCCATCAAGCAGGTCTGTTCCAAGGGCCTTTGCGT
CAGGTGGGCTCAGGATTCCAGGGTGGCTGGACCCCAGGCCCCAGCTCTGCAGCAGGGAGGACGTGGCTGG
GCTCGTGAAGCATGTGGGGGTGAGCCCAGGGGCCCCAAGGCAGGGCACCTGGCCTTCAGCCTGCCTCAGC
CCTGCCTGTCTCCCAGATCACTGTCCTTCTGCCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGC
TGCTGGCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGT
GGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGACCCGCCGGGAGGCAGAG
GACCTGCAGGGTGAGCCAACTGCCCATTGCTGCCCCTGGCCGCCCCCAGCCACCCCCTGCTCCTGGCGCT
CCCACCCAGCATGGGCAGAAGGGGGCAGGAGGCTGCCACCCAGCAGGGGGTCAGGTGCACTTTTTTAAAA
AGAAGTTCTCTTGGTCACGTCCTAAAAGTGACCAGCTCCCTGTGGCCCAGTCAGAATCTCAGCCTGAGGA
CGGTGTTGGCTTCGGCAGCCCCGAGATACATCAGAGGGTGGGCACGCTCCTCCCTCCACTCGCCCCTCAA
ACAAATGCCCCGCAGCCCATTTCTCCACCCTCATTTGATGACCGCAGATTCAAGTGTTTTGTTAAGTAAA
GTCCTGGGTGACCTGGGGTCACAGGGTGCCCCACGCTGCCTGCCTCTGGGCGAACACCCCATCACGCCCG
GAGGAGGGCGTGGCTGCCTGCCTGAGTGGGCCAGACCCCTGTCGCCAGGCCTCACGGCAGCTCCATAGTC
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TGTGGGTGACCCTCCCTCTAACCTGGGTCCAGCCCGGCTGGAGATGGGTGGGAGTGCGACCTAGGGCTGG
CGGGCAGGCGGGCACTGTGTCTCCCTGACTGTGTCCTCCTGTGTCCCTCTGCCTCGCCGCTGTTCCGGAA
CCTGCTCTGCGCGGCACGTCCTGGCAGTGGGGCAGGTGGAGCTGGGCGGGGGCCCTGGTGCAGGCAGCCT
GCAGCCCTTGGCCCTGGAGGGGTCCCTGCAGAAGCGTGGCATTGTGGAACAATGCTGTACCAGCATCTGC
TCCCTCTACCAGCTGGAGAACTACTGCAACTAGACGCAGCCCGCAGGCAGCCCCACACCCGCCGCCTCCT
GCACCGAGAGAGATGGAATAAAGCCCTTGAACCAGCCCTGCTGTGCCGTCTGTGTGTCTTGGGGGCCCTG
GGCCAAGCCCCACTTCCCGGCACTGTTGTGAGCCCCTCCCAGCTCTCTCCACGCTCTCTGGGTGCCCACA
GGTGCCAACGCCGGCCAGGCCCAGCATGCAGTGGCTCTCCCCAAAGCGGCCATGCCTGTCGGCTGCCTGC
TGCCCCCACCCTGTGGCTCAGGGTCCAGTATGGGAGCTGCG
```

Link: Insulin DNA Sequence

1.- Transgenic mammal producing human insulin protein

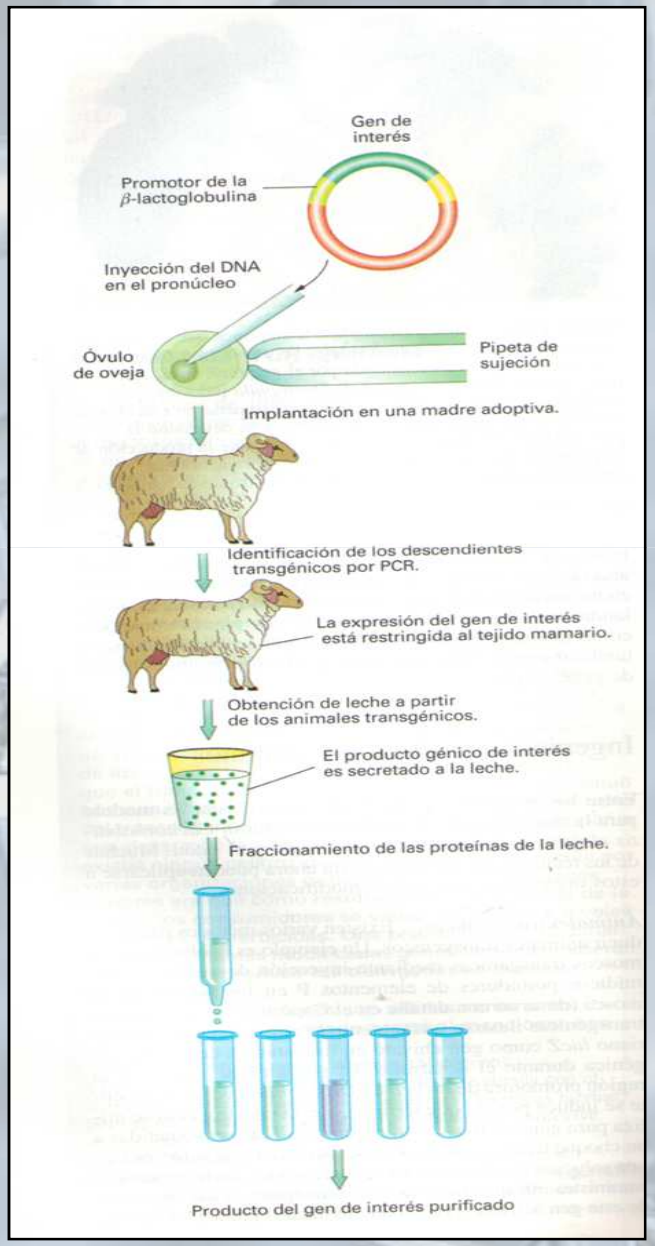
Steps:

1.- Make recombinant DNA including human insulin gene and lactoglobulin promotor

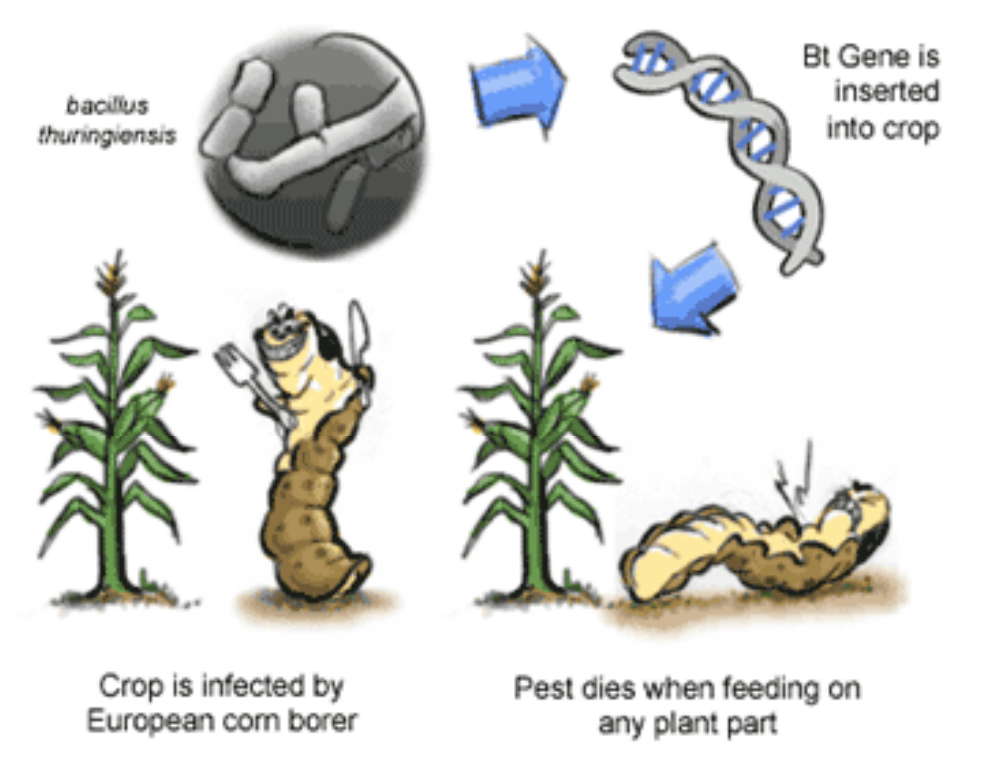
2.- Introduction in an sheep's ovum

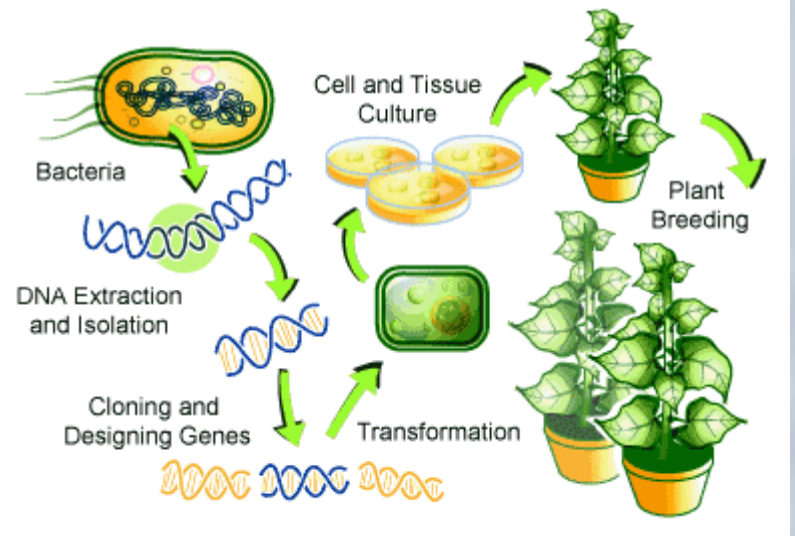
3.- Identification of transgenic offsprings

4.- The human insulin is produced in the milk and then purified.



2.- Making transgenic plants





- From a bacteria (*Bacilus thuringiensis*), the Bt gene is extracted, purified and inserted into crop.
- This gene produces a lethal protein that kills the insect when the plant is infected.

3.- Biotechnology and Environment

1.- Genetic engineering can combine different characteristics of microorganisms that bio-degrades toxic chemicals, to increase efficiency or recombinant microbes that produce new features

2.- Removal of heavy metals

3.- Oil spills control.

…

4.- Synthetic genomes to "create" (*new?*) organisms

RESEARCH ARTICLE

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

In 1977, Sanger and colleagues determined the complete genetic sequence of phage φX174 (*1*), the first DNA genome to be completely sequenced. Eighteen years later, in 1995, our team was able to read the first complete genetic sequence of a self-replicating bacterium, *Haemophilus influenzae* (*2*). Reading the genetic sequence of a wide range of species has increased exponentially from these early studies. The ability to rapidly digitize genomic information has increased by more than eight orders of magnitude over the past 25 years (*3*). Efforts to understand all this new genomic information have spawned numerous new computational and experimental paradigms, yet our genomic knowledge remains very limited. No single cellular system has all of its genes understood in terms of their biological roles. Even in simple bacterial cells, do the chromosomes contain the entire genetic repertoire? If so, can a complete genetic system be reproduced by chemical synthesis starting with only the digitized DNA sequence contained in a computer?

Our interest in synthesis of large DNA molecules and chromosomes grew out of our efforts over the past 15 years to build a minimal cell that contains only essential genes. This work was inaugurated in 1995 when we sequenced the genome of *Mycoplasma genitalium*, a bacterium with the smallest complement of genes of any known organism capable of independent growth in the laboratory. More than 100 of the 485 protein-coding genes of *M. genitalium* are dispensable when disrupted one at a time (*4*–*6*).

We developed a strategy for assembling viral-sized pieces to produce large DNA molecules that enabled us to assemble a synthetic *M. genitalium* genome in four stages from chemically synthesized DNA cassettes averaging about 6 kb in size. This was accomplished through a combination of in vitro enzymatic methods and in vivo recombination in *Saccharomyces cerevisiae*. The whole synthetic genome [582,970 base pairs (bp)] was stably grown as a yeast centromeric plasmid (YCp) (*7*).

Several hurdles were overcome in transplanting and expressing a chemically synthesized chromosome in a recipient cell. We needed to improve methods for extracting intact chromosomes from yeast. We also needed to learn how to transplant these genomes into a recipient bacterial cell to establish a cell controlled only by a synthetic genome. Because *M. genitalium* has an extremely slow growth rate, we turned to two faster-growing mycoplasma species, *M. mycoides* subspecies *capri* (GM12) as donor, and *M. capricolum* subspecies *capricolum* (CK) as recipient.

To establish conditions and procedures for transplanting the synthetic genome out of yeast, we developed methods for cloning entire bacterial chromosomes as centromeric plasmids in yeast, including a native *M. mycoides* genome (*8*, *9*). However, initial attempts to extract the *M. mycoides* genome from yeast and transplant it into *M. capricolum* failed. We discovered that the donor and recipient mycoplasmas share a common restriction system. The donor genome was methylated in the native *M. mycoides* cells and was therefore protected against restriction during the transplantation from a native donor cell (*10*). However, the bacterial genomes grown in yeast are unmethylated and so are not protected from the single restriction system of the recipient cell. We overcame this restriction barrier by methylating the donor DNA with purified methylases or crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (*8*, *9*, *11*). One was the genome donor used by Lartigue *et al.* [GenBank accession CP001621] (*10*). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1-*AttpelTres* [GenBank accession CP001668] (*8*). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reliable, there are 95 sites at which they differ. We began to design the synthetic genome before both sequences were finished. Consequently, most of the cassettes were designed and synthesized based on the CP001621 sequence (*11*). When it was finished, we chose the sequence of the genome successfully transplanted from yeast (CP001668) as our design reference (except that we kept the intact *tpeIIres* gene). All differences that appeared biologically significant between CP001668 and previously synthesized cassettes were corrected to match it exactly (*11*). Sequence differences between our synthetic cassettes and CP001668 that occurred at 19 sites appeared harmless and so were not corrected. These provide 19 polymorphic differences between our synthetic genome (JCVI-syn1.0) and the natural (nonsynthetic) genome (YCpMmyc1.1) that we have cloned in yeast and use as a standard for genome transplantation from yeast (*8*). To further differentiate between the synthetic genome and the natural one, we designed four watermark sequences (fig. S1) to replace one or more cassettes in regions experimentally demonstrated [watermarks 1 (1246 bp) and 2 (1081 bp)] or predicted [watermarks 3 (1109 bp) and 4 (1222 bp)] to not interfere with cell viability. These watermark sequences encode unique identifiers while limiting their translation into peptides. Table S1 lists the differences between the synthetic genome and this natural standard. Figure S2 shows a map of the *M. mycoides* JCVI-syn1.0 genome. Cassette and assembly intermediate boundaries, watermarks, deletions, insertions, and genes of the *M. mycoides* JCVI syn1.0 are shown in fig. S2, and the sequence of the transplanted mycoplasma clone sMmYCp235-1 has been submitted to GenBank (accession CP002027).

Synthetic genome assembly strategy. The designed cassettes were generally 1080 bp with 80-bp overlaps to adjacent cassettes (*11*). They were all produced by assembly of chemically

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GENOMICS

Synthetic Genome Brings New Life to Bacterium

For 15 years, J. Craig Venter has chased a dream: to build a genome from scratch and use it to make synthetic life. Now, he and his team at the J. Craig Venter Institute (JCVI) in Rockville, Maryland, and San Diego, California, say they have realized that dream. In this week's *Science* Express (www.sciencemag.org/cgi/content/abstract/science.1190719), they describe the stepwise creation of a bacterial chromosome and the successful transfer of it into a bacterium, where it replaced the native DNA. Powered by the synthetic genome, that microbial cell began replicating and making a new set of proteins.

This is "a defining moment in the history of biology and biotechnology," says Mark Bedau, a philosopher at Reed College in Portland, Oregon, and editor of the scientific journal *Artificial Life*. "It represents an important technical milestone in the new field of synthetic genomics," says yeast biologist Jef Boeke of Johns Hopkins University School of Medicine in Baltimore, Maryland.

The synthetic genome created by Venter's team is almost identical to that of a natural bacterium. It was achieved at great expense, an estimated $40 million, and effort, 20 people working for more than a decade. Despite this success, creating heavily customized genomes, such as ones that make fuels or pharmaceuticals, and getting them to "boot" up the same way in a cell is not yet a reality. "There are great challenges ahead before genetic engineers can mix, match, and fully design an organism's genome from scratch," notes Paul Keim, a molecular geneticist at Northern Arizona University in Flagstaff.

The "synthetic" bacteria unveiled this week have their origins in a project headed by Venter and JCVI colleagues Clyde Hutchison III and Hamilton Smith to determine the minimal instructions needed for microbial life and from there add genes that could turn a bacterium into a factory producing compounds useful for humankind. In 1995, a team led by the trio sequenced the 600,000-base chromosome of a bacterium called *Mycoplasma genitalium*, the smallest genome of a free-living organism. The microbe has about 500 genes, and researchers found they could delete 100 individual genes without ill effect (*Science*, 14 February 2003, p. 1006).

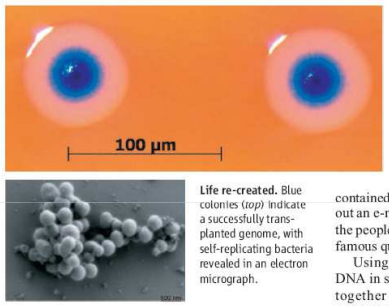
But confirming the minimal genome suggested by those experiments required synthesizing a full bacterial chromosome and getting it to work in a recipient cell, two steps that have taken years because the technology to make and manipulate whole chromosomes did not exist. In 2007, Venter, Smith, Hutchison, and colleagues finally demonstrated that they could transplant natural chromosomes from one microbial species to another (*Science*, 3 August 2007, p. 632). By 2008, they showed that they could make an artificial chromosome that matched *M. genitalium*'s but also contained "watermark" DNA sequences that would enable them to tell the synthetic genome from the natural one (*Science*, 29 February 2008, p. 1215).

But combining those steps became bogged down, in part because *M. genitalium* grows so slowly that one experiment can take weeks to complete. The team decided to change microbes in midstream, sequencing the 1-million-base genome of the faster-growing *M. mycoides* and beginning to build a synthetic copy of its chromosome. Last year, they showed they could extract the *M. mycoides* natural chromosome, place it into yeast, modify the bacterial genome, and then transfer it to *M. capricolum*, a close microbial relative (*Science*, 21 August 2009, p. 928; 25 September 2009, p. 1693). The next step was to show that the synthetic copy of the bacterial DNA could be handled the same way.

The researchers started building their synthetic chromosome by going DNA shopping. They bought from a company more than 1000 1080-base sequences that covered the whole *M. mycoides* genome; to facilitate their assembly in the correct order, the ends of each sequence had 80 bases that overlapped with its neighbors. So that the assembled genome would be recognizable as synthetic, four of the ordered DNA sequences contained strings of bases that, in code, spell out an e-mail address, the names of many of the people involved in the project, and a few famous quotations.

Using yeast to assemble the synthetic DNA in stages, the researchers first stitched together 10,000-base sequences, then 100,000-base sequences, and finally the complete genome. However, when they initially put the synthetic genome into *M. capricolum*, nothing happened. Like computer programmers debugging faulty software, they systematically transplanted combinations of synthetic and natural DNA, finally homing in on a single-base mistake in the synthetic genome. The error delayed the project 3 months.

After months of unsuccessfully transplanting these various genome combinations, the team's fortune changed about a month ago when the biologists found a blue colony of bacteria had rapidly grown on a lab plate over the weekend. (Blue showed the cells were using the new genome). Project leader Daniel Gibson sent Venter a text message declaring success. "I took my video camera in and filmed [the plate]," says Venter.



Life re-created. Blue colonies (*top*) indicate a successfully transplanted genome, with self-replicating bacteria revealed in an electron micrograph.

CREDITS (TOP TO BOTTOM): J. CRAIG VENTER INSTITUTE; T. DEERINCK AND M. ELLISMAN/NCMIR, UNIVERSITY OF CALIFORNIA, SAN DIEGO


Thank you!

USE OF GLYCEROL AS RAW MATERIAL FOR ENERGY PRODUCTION

José Manuel Gómez

Introduction

- 80% of energy comes from fossil fuels.
- Fossil fuels are no-renewable energy sources.
- Combustion of fossil fuels causes high environmental impacts (greenhouse gases)
- Goverments and international organizations are driving strategies to develop process for energy production from renewable energy sources.

Alternative: Bioenergy

Chemical energy in organic materials that can be converted into usable energy sources by biological, mechanical or thermochemical processes.

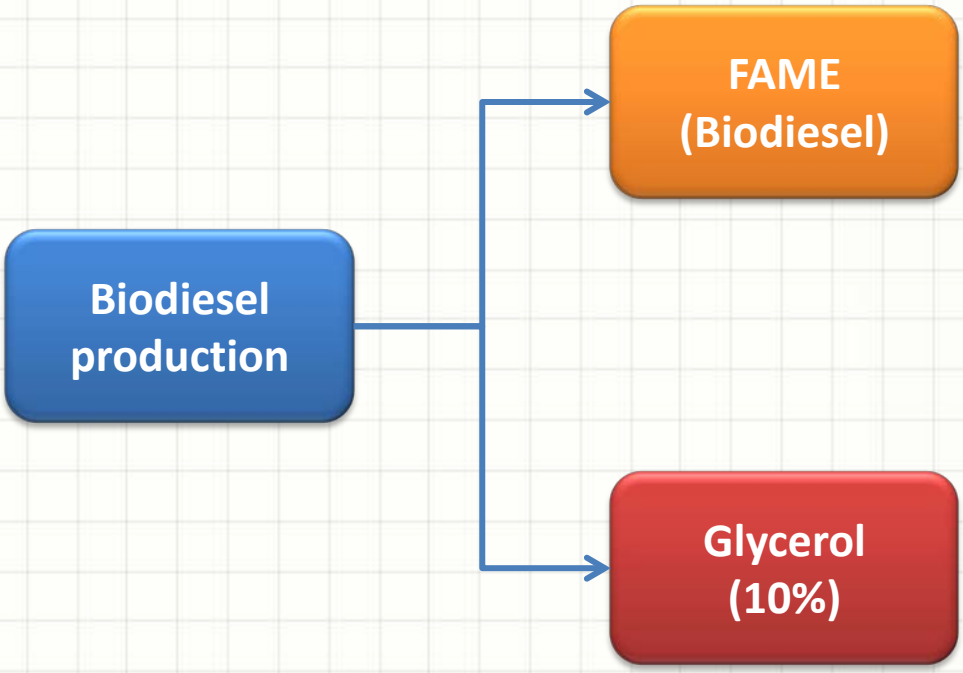
Nowadays, bioenergy is the most important renewable and powerful energy to go replacing the use of fossil fuels

Liquid and gaseous biofuels derived from organic matter can play an important role in reducing CO_2 emissions abatement.

Biofuels obtained from biomass may contributes to the environmental and economic optimization of the complete value chain ---> BIOREFINERY

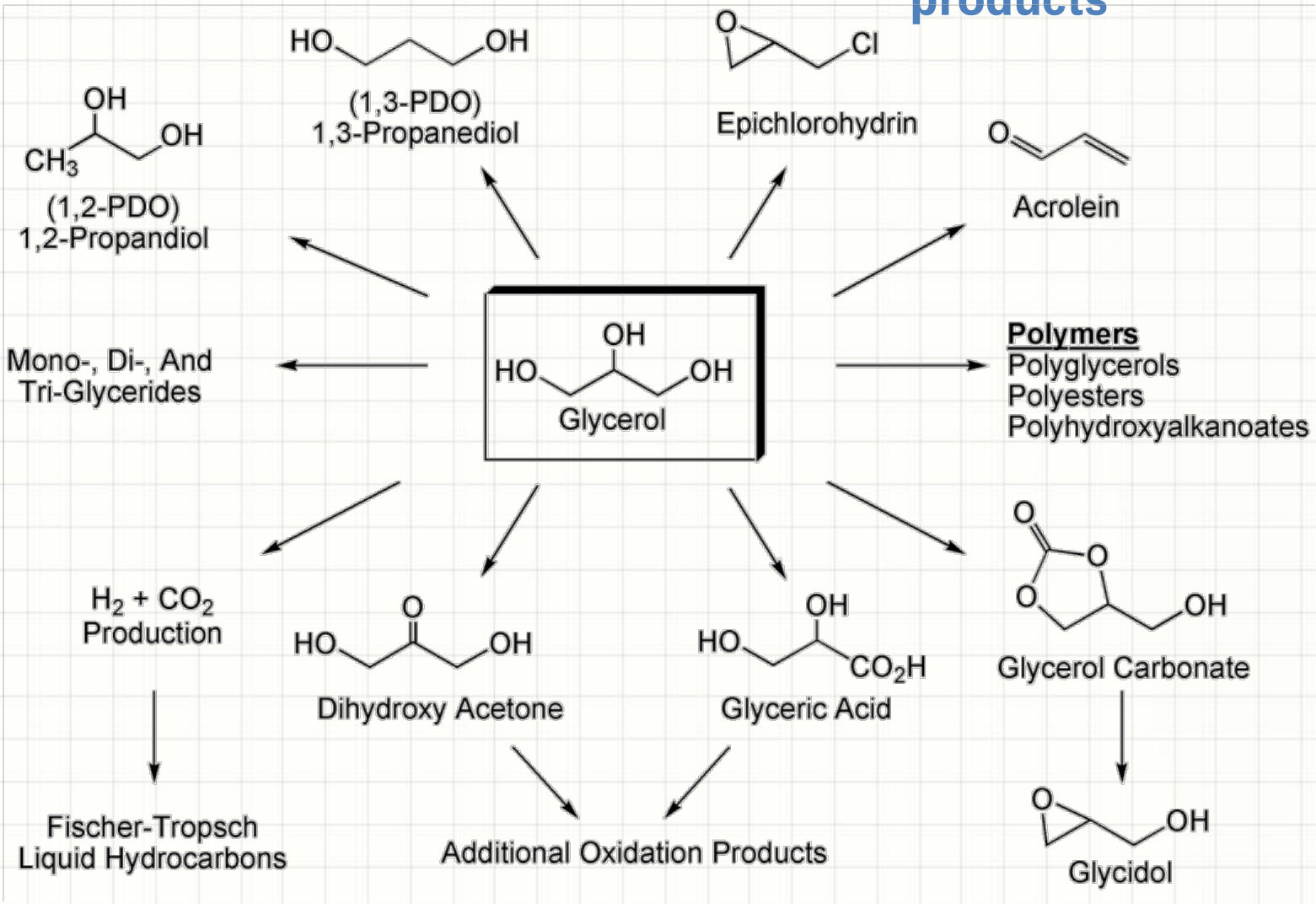
Use of biomass has lower investment costs, compared with other renewable sources.

The diversity of raw materials and processes that can takes place offers a wide range of possibilities.


Biodiesel production
FAME (Biodiesel)
Glycerol (10%)

Glycerol

Primary building block for other value added products



Platform chemicals derived from glycerol.

Current glycerol production can not be absorbed by traditional industries, so its price has fallen. Some chemical companies (Dow Chemical or Procter & Gamble) have closed their plants.

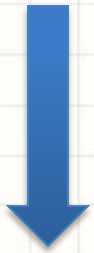
Glycerol has a high level of reduction (4.67 vs 4 for glucose or xylose).

It can be possible the use of this compound as carbon source for different fermentation processes to obtain high value-added compounds.

Glycerol can be converted chemically or biologically in to some products as dyhydroxyacetone, pyruvic acid, tartaric acid, oxalic acid,…

Biological vs Chemical transformation

- Biological doesn't need large energy requirements (moderate temperature and pressure conditions).
- Glycerol is very competitive towards other sugars commonly used in microbial fermentation.



Conversion of phosphoenolpyruvate from glycerol is generated twice reducing equivalents compared to what is produced via glucose or xylose

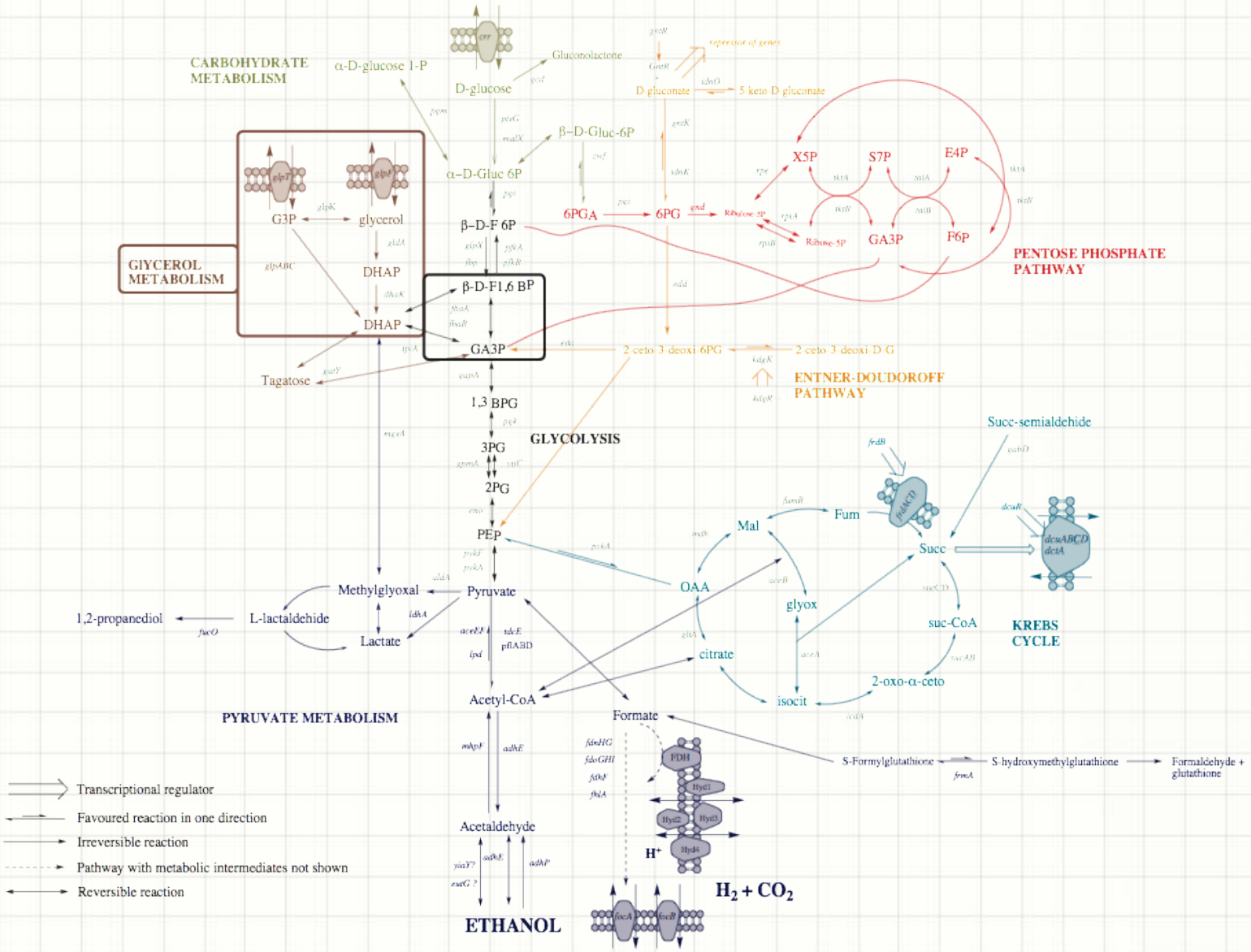
Hypothesis

1. Use of glycerol from biodiesel industry.
2. Bioconversion to reduced compounds such as ethanol, xylitol or hydrogen
3. Important opportunity for biofuels and energy from a cheap and easily available substrate

Biotechnological use

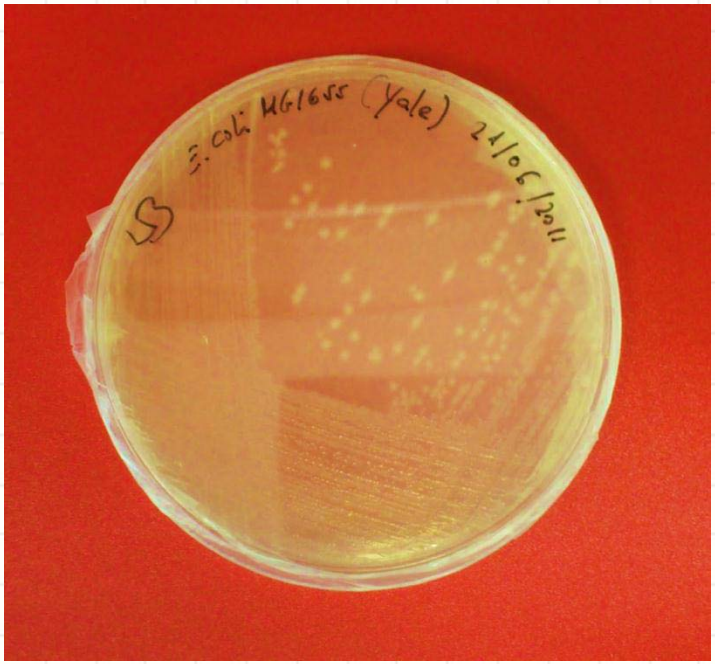
- Some authors have been previously reported
- Rise in the use of this compound as raw material has occurred in last five years.
- Microorganisms used: *Klebsiella, Citrobacter, Enterobacter, Clostridium, Lactobacillus, Propionibacterium or* mixed cultures.
- From industrial applications, some of them are pathogens, (subject) needs high nutritional requirements or (plural)have difficulties in genetic manipulation
- *Escherichia coli* presents interesting advantages that enhanced industrial use: versatility in the use of different carbon sources, complete knowledge of its genome, “workhorse” of the modern biotechnology

METABOLIC PATHWAYS of *Escherichia coli* BW25113



E. coli

Anaerobic conditions



Ethanol, hydrogen

Bioproduction of ethanol and hydrogen

- Some of the works have been developed with pure (grade) glycerol to avoid inhibition by the presence of impurities.

- Pure glycerol is much more expensive than crude glycerol (purification process).

- Use of pure glycerol doesn't solve the problem of excess of crude glycerol obtained in biodiesel industry.

- Most of the studies have been worked out in small reactor (minor than 1 L) in batch mode and little feasibility configurations (bioelectrochemical cells).

So, it’s necessary to deepen the knowledge of the process for bioconversion of crude glycerol to ethanol and hydrogen.

Our experience

- Development and optimization a suitable culture medium that increases bacterial production by reducing the number of component media.

Element	Compound
C	Glycerol
N	Ammonium chloride
P	Dipotasium hydrogen phospahte
S	Sodium sulphate
K	Dipotasium hydrogen phosphate
Na	Sodium chloryde
Mg	Magnesium sulphate
Cl	Sodium chloryde
Fe	Ferric sulphate
Mn	Manganese sulphate
Cu	Copper sulphate
Zn	Zinc sulphate
Mo	Amonium molybdate
Co	Cobalt chloryde
	Peptone

Optimized culture medium

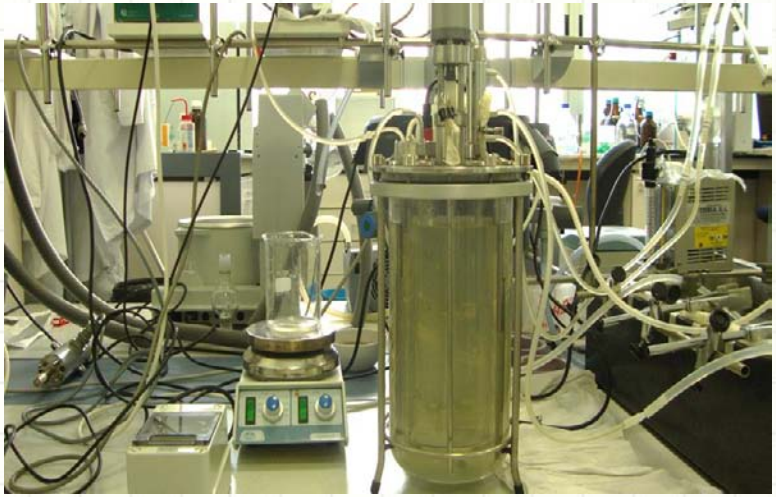
Component	Unidad	Valor
Glycerol	g L^{-1}	10
Na_2SO_4	g L^{-1}	0,0806
NaCl	g L^{-1}	0,0152
$MgSO_4 \cdot 7H_2O$	g L^{-1}	0,0310
Peptone	g L^{-1}	4,25

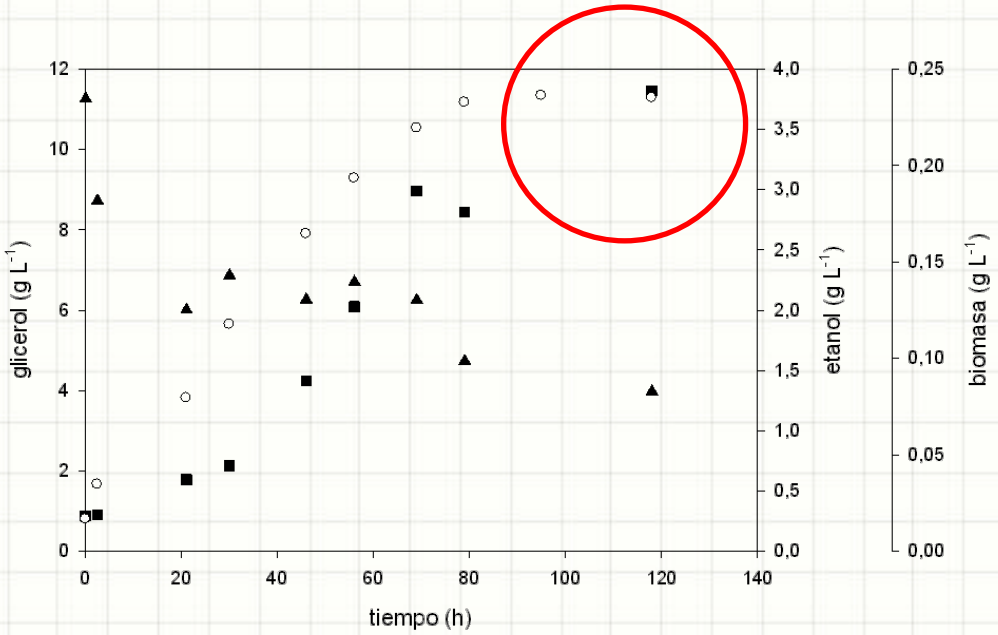
Original culture medium

Experiments in 5 L reactors

Batch mode

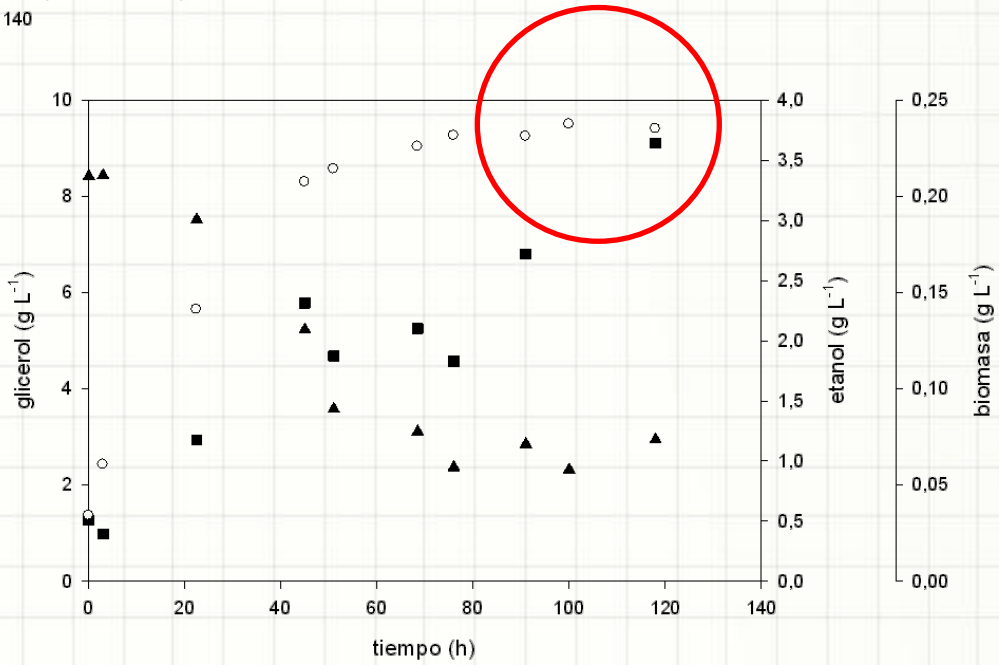
Parameter	Value	Unit
pH	6.3	
Temperature	37	°C
Inert gas adding	≈0.0025	L min^{-1} L^{-1}
Fermentation time	≈ 90	H
Working volume	5.5	L
Agitation speed	150	Rpm





Profiles of fermentation obtained for R5BGFGAr (initial concentration of glycerol 10 g L^{-1}, 37°C, 150 rpm, pH=6,3). (■) ethanol, (▲) glycerol, (○) biomass.

Profiles of fermentation obtained for R5BGCAr (initial concentration of glycerol 10 g L^{-1}, 37°C, 150 rpm, pH=6,3). (■) ethanol, (▲) glycerol, (○) biomass.



Experiments in 5 L reactors Fed-Batch mode

	Substrate	So (g L^{-1})	Feeding modality	Ar bubbling	Code
FB	CG	10	Constant	+	R5FCG10C
FB	CG	30	Constant	+	R5FCG30C
FB	CG	10	Exponential	+	R5FCG10E
FB	CG	30	Exponential	+	R5FCG30E
FB	CG	50	Exponential	+	R5FCG50E

Fermentation	Initial (g)	Fed (g)	Final (g)	Consum(g)	% Consumed
R5FGC10C	17,45	36,50	26,43	27,52	51,01
R5FGC30C	12,03	109,50	92,89	28,62	23,56

Fermentation	Initial (g)	Fed (g)	Final (g)	Consum (g)	% Consumed
R5FGC10E	0	36,31	0,55	35,76	98,48
R5FGC30E	0	106,62	24,24	82,38	77,27
R5FGC30E*	0	106,62	0	106,62	100
R5FGC50E	0	177,7	69,76	107,94	60,70
R5FGC50E*	0	177,7	28,17	149,53	84,14
*: considered step of feed 88 h + post batch of 72 h					

Fermentation	$Y_{P/S}$ g g^{-1}	$Y_{X/S}$ g g^{-1}	Ethanol g L^{-1}
R5FGC10E	0,478	0,044	4,12
R5FGC30E	0,331	0,038	6,28
R5FGC30E*	0,341	-	7,58
R5FGC50E	0,385	0,022	8,59
R5FGC50E*	0,281	-	8,68
*: considered step of feed 88 h + post batch of 72 h			

Experiments in 200 L reactor Pilot plant scale

No inhibition of *E. coli* by crude glycerol was detected.

Fermentation	Initial (g)	Fed (g)	Final (g)	Consum (g)	% Consumed
RPFGC30E	0	4425	2092	2333	52,72
RPFGC30E*	0	4425	0	4425	100
*: considera etapa de alimentación + post batch					

Hydrogen production:

- **449,18 mmol (0,19 mmol H_2 $g_{glicerol}^{-1}$, a 160 h)**

Overexpression of enzymes

- Pyruvate formate lyase (PFL), pyruvate formate lyase activase ($PFL_{activase}$) and phosphoenolpyruvate carboxykinase (PECPK) has been overexpressed in *E. coli* TOP10.
- Increase in H_2 production over strain not expressed has been obtained.
- Starts the study of mutants in order to increase the production of ethanol and hydrogen by crude glycerol utilization.

Future challenges

- Consideration of *E. coli* genetically modified to maximize ethanol and hydrogen production.
- Optimize the production of ethanol and hydrogen at laboratory and pilot scale for industrial applications.

Thanks

Questions?

GENETIC MAPPING OF BAC CLONES FROM SENEGALESE SOLE CONTAINING GENES OF INTEREST IN AQUACULTURE

Senegalese Sole

Data Base	
Database name	Direct links
Nucleotide	483
Nucleotide EST	10,631
Protein	271
Genome Sequences	1
Genome Projects	1
Popset	14
GEO Datasets	11
UniSTS	78
PubMed Central	32
Gene	13
Taxonomy	1

Culture Optimization

Improved production

Reproduction

Metamorphosis

Comparison of genetic, cytogenetic and physical maps

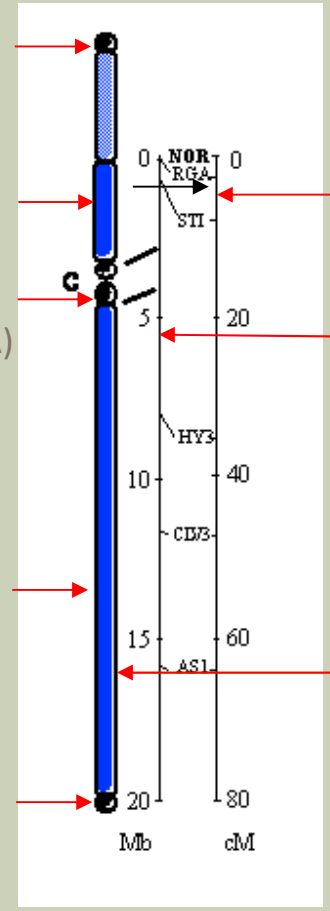
Telomeres
(repetitive DNA)

Euchromatin
(high gene density)

Centromere
(repetitive DNA)

Euchromatin
(high gene density)

Telomeres
(repetitive DNA)

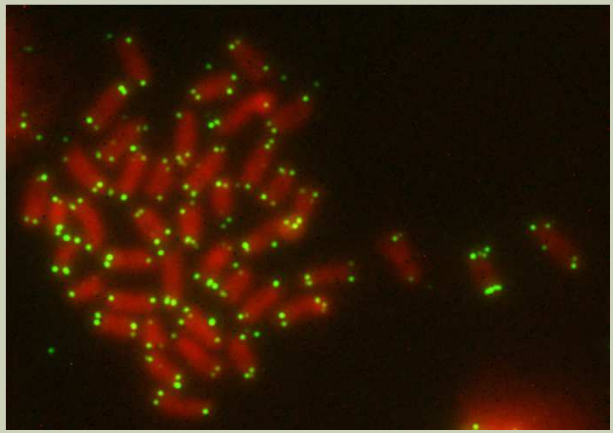


Linkage map (in cM)

Physical map (in Mb)

Cytogenetic map (Chromosome)

Telomeres
Hybridized with TTAGGG



Alignment of overlapping BAC clones and anchoring on the genetic map

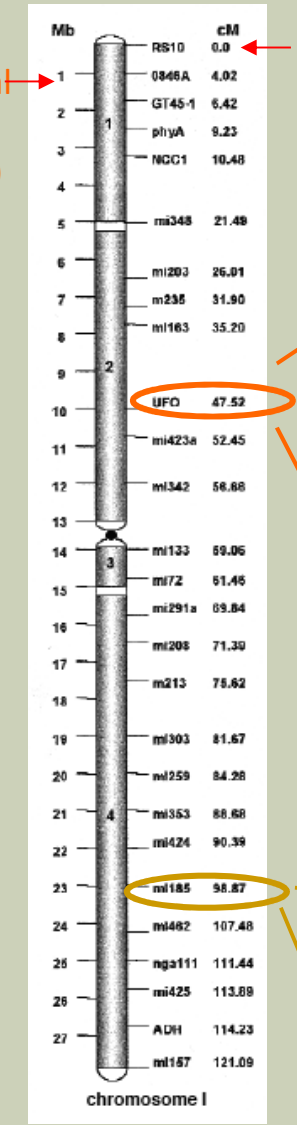
Physical map (in Mb)

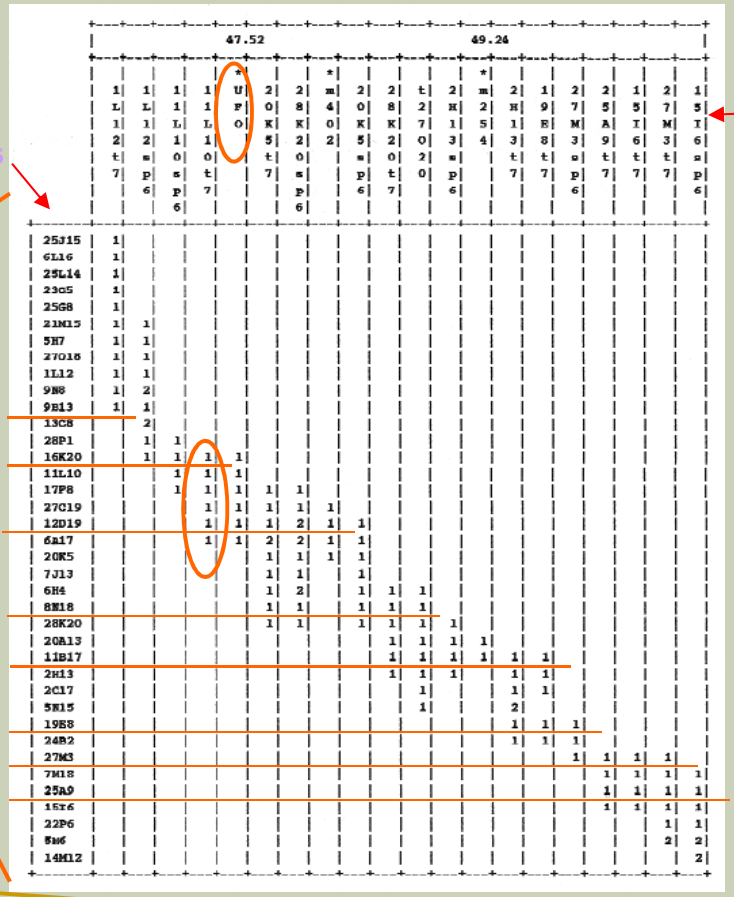
Linkage map (in cM)

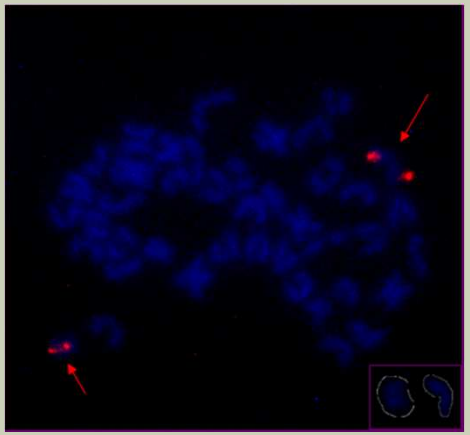
BAC clones

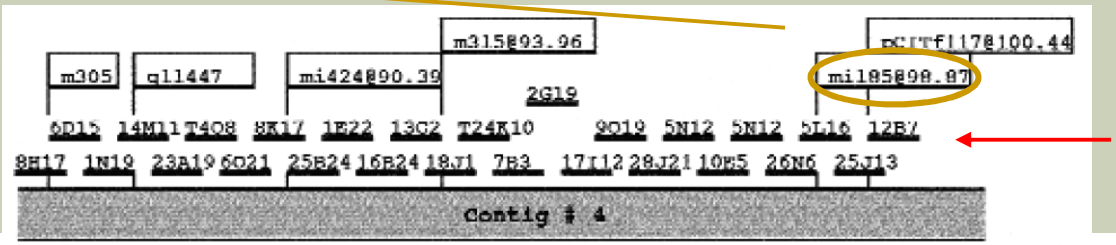
BAC ends

BAC –FISH





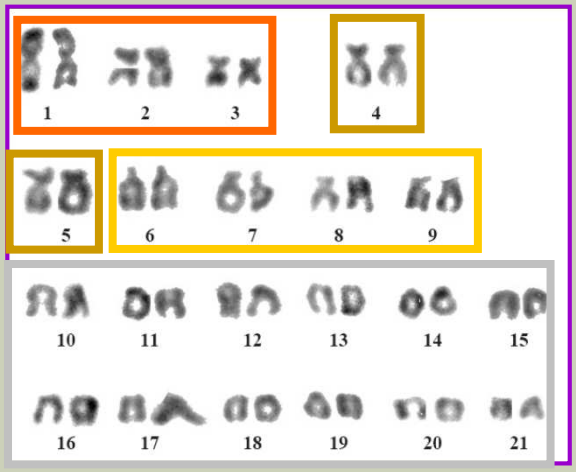




BAC clones

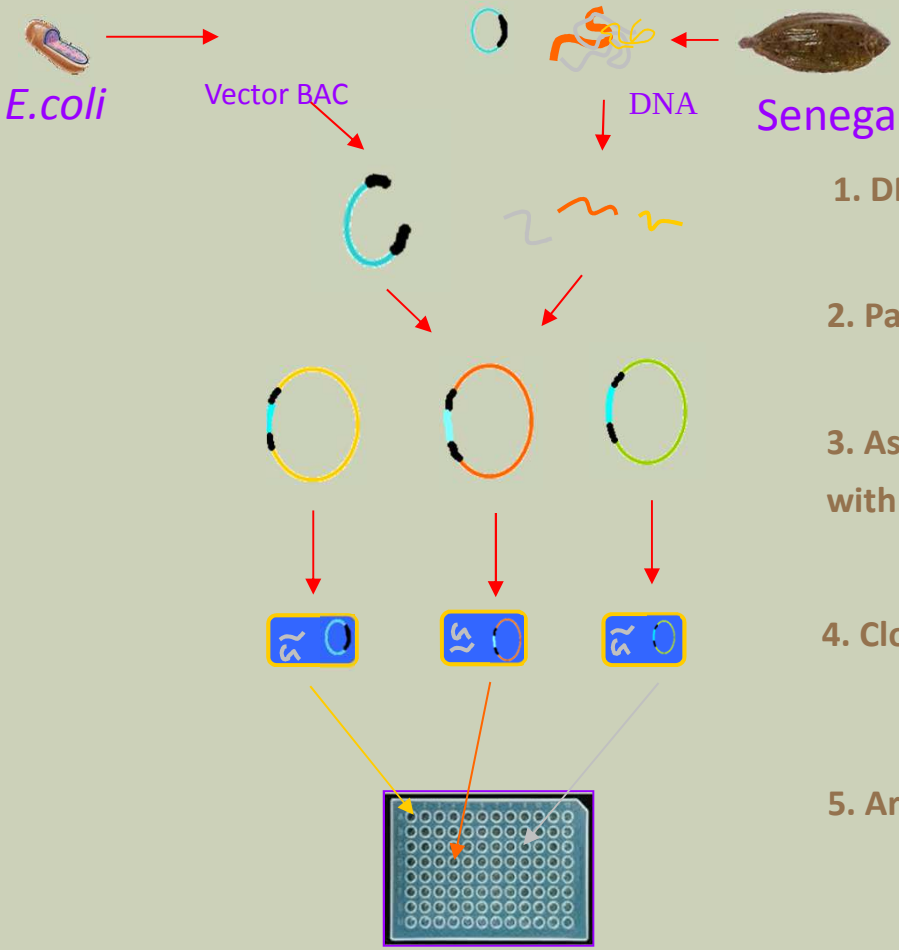
KARYOTYPE OF *S. SENEGALENSIS*

2n = 42



Vega et al. 2002

BAC libraries



1. DNA extraction

2. Partial digestion

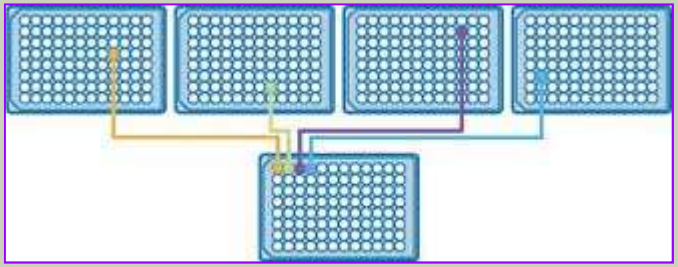
3. Assembly of Bacterial Artificial Clones with digested DNA

4. Clone inserts into BAC vector

5. Array into libray in microliter plates

Isolation of genes from the BAC library

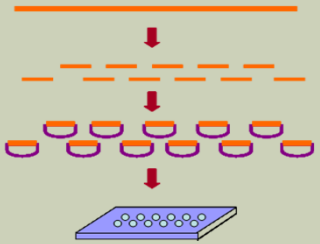
BAC library screening



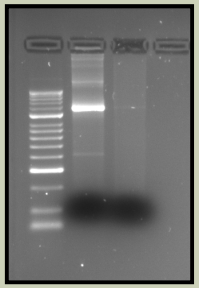
Location of genes of interest in aquaculture

- Innate Immunologycal system
  - Lysozyme
  - Hepcidin
  - Semaphorin 7 A
- Metamorphosis
  - Thyroid Hormone Receptors:
    - TRαA
    - TRαB
    - TRß

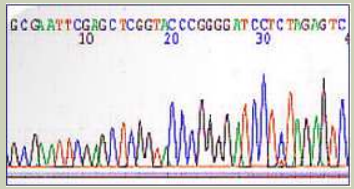
Characterization of BACs and FISH



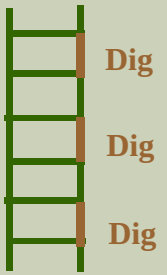
BAC DNA extraction



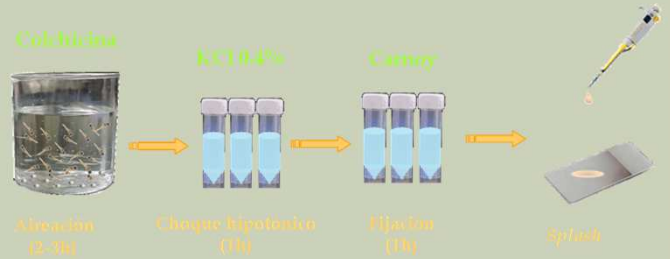
Digestion and electrophoresis

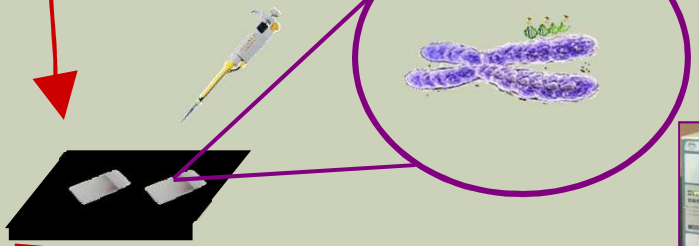


BAC sequencing



Labelling Nick-Translation or PCR

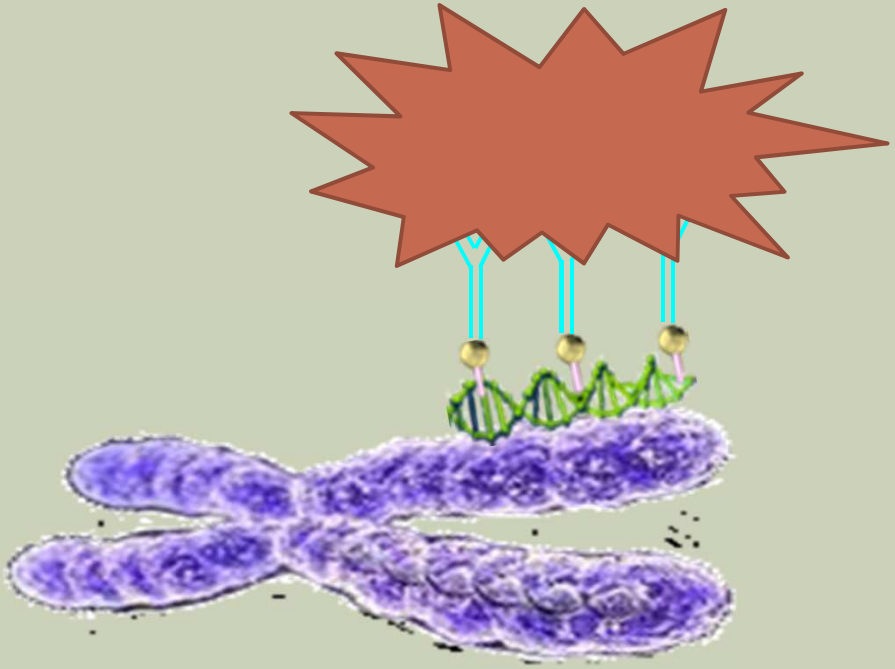




Denaturation

Hybridization

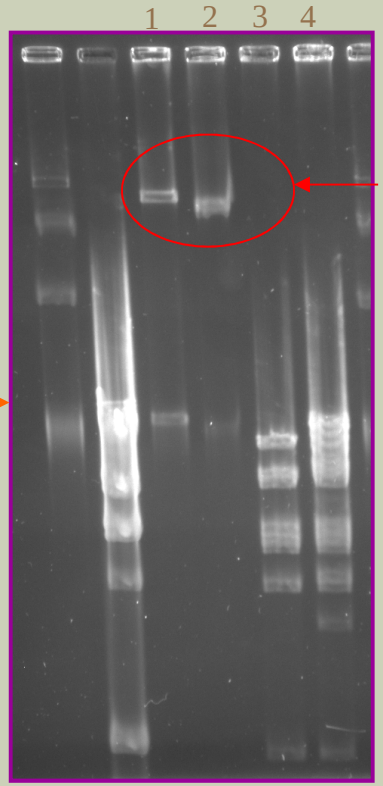
BAC-FISH



Genetic characterization of BAC clones

Pulsed field electrophoresis(CHEF)

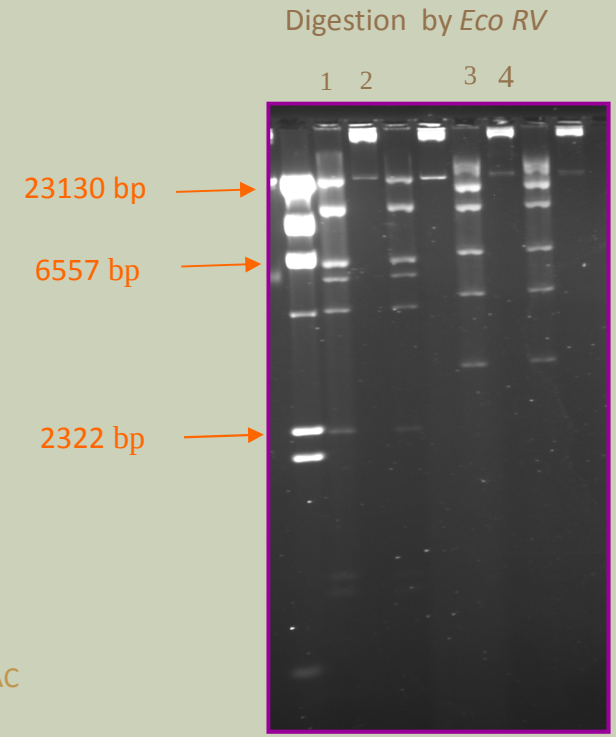
Digestion with ***Eco RV***



1. Undigested *Lysozyme* BAC
2. Undigested *Semaphorin 7* A BAC
2. Digested *Lysozyme* BAC
3. Digested *Semaphorin BAC*

Genetic characterization of BAC clones

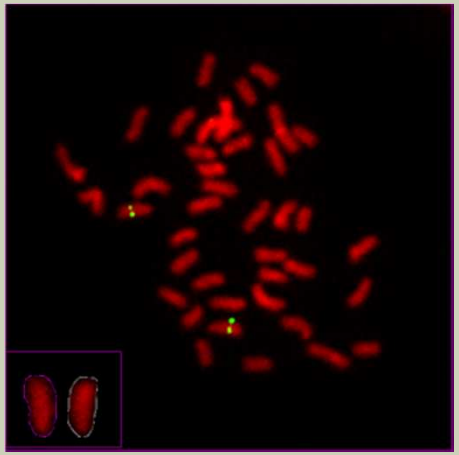
Standard electrophoresis



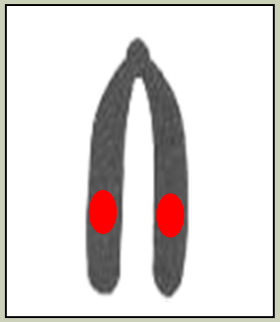
1. Digested Lysozyme BAC
2. Undigested Lysozyme BAC
3. Digested Semaphorin 7 A BAC
4. Undigested Semaphorin 7 A BAC

Location of BAC clones by FISH

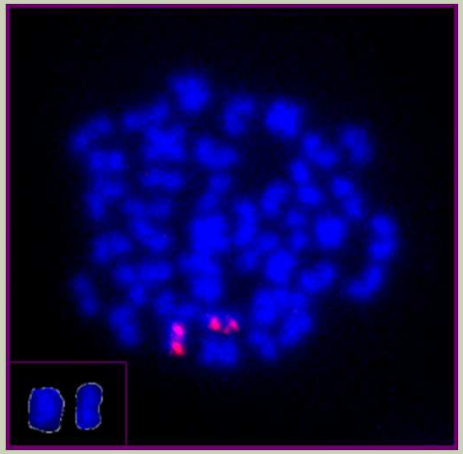
Lysozyme



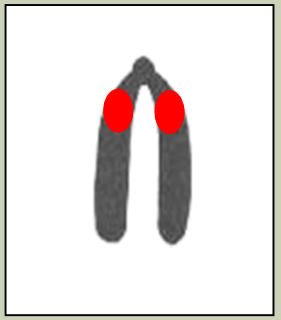
Intestitial position in a medium size chromosome pair



Hepcidin

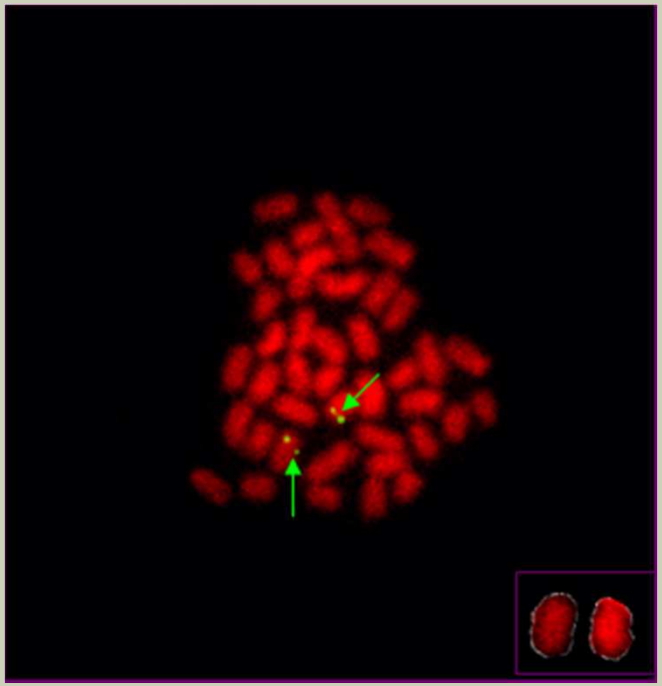


Sutelomeric position of a small chromosome pair

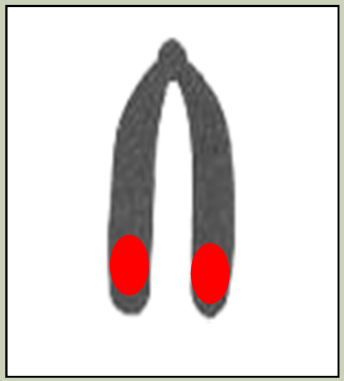


Location of BAC clones by FISH

Semaphorin 7 A

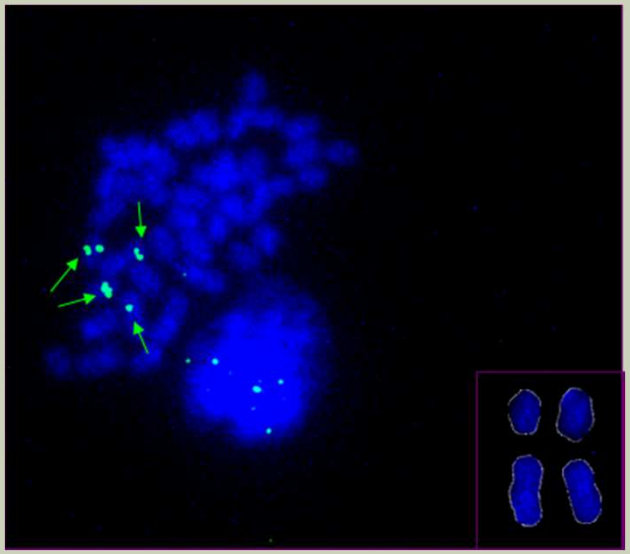


Semaphorin 7a shows signal in one achrocentric chromosomal pair in a telomeric position.

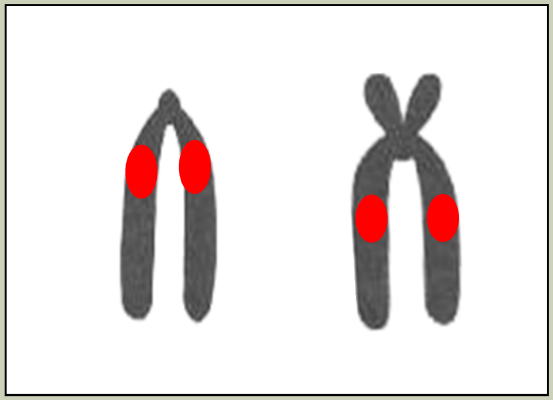


Location of BAC clones by FISH

TRß

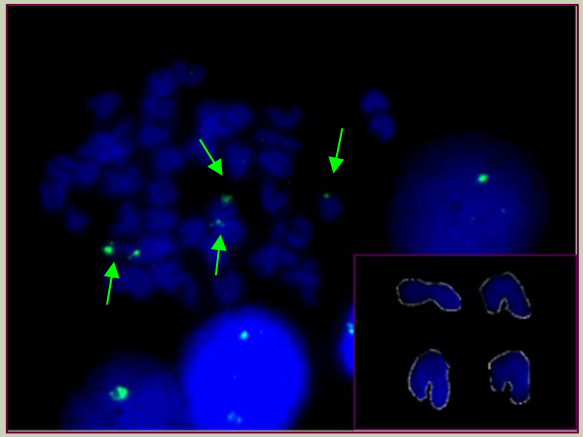


In one pair it shows a bigger signal that locates in a interstitial position of an achrocentric chromosome pair. The other signal is smaller and locates in a centromeric pisition in an achrocentric chromosome pair

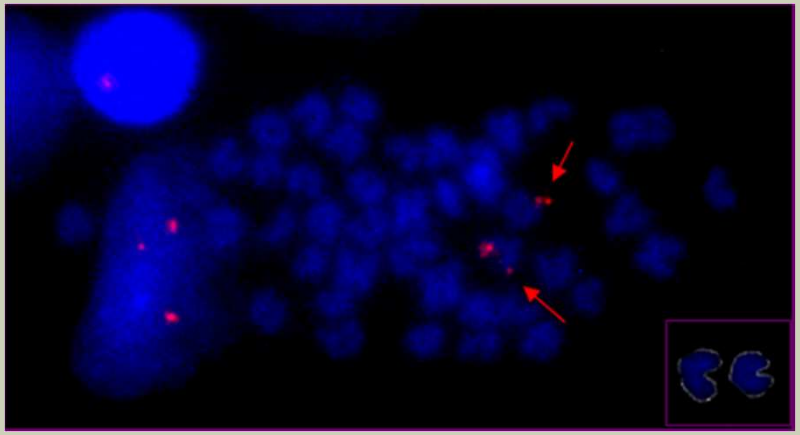


Location of BAC clones by FISH

TRαB

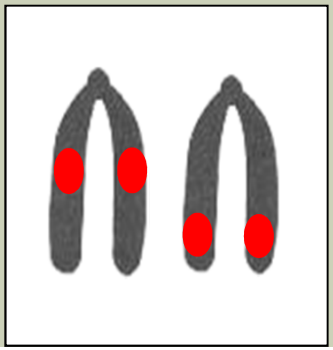


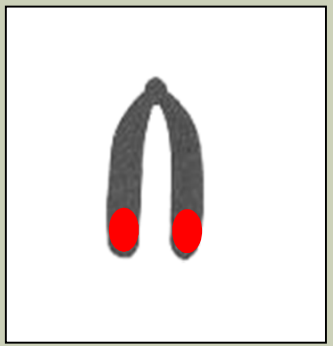
TRαA



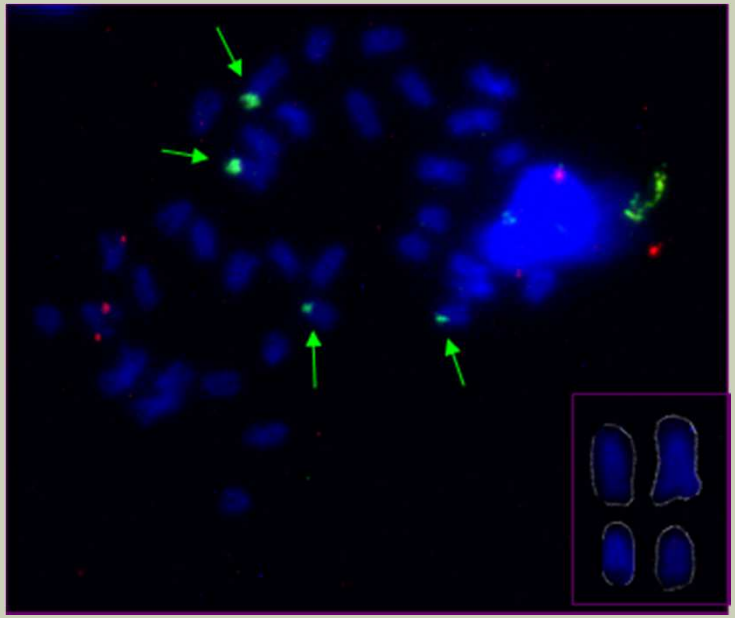
TRαB appears in one or two chromosome pairs. In one pair the signals locate in an intesticial position of an achrocentric chromosome pair, and the other signal locate in a telomeric position of an achrocentric pair

TRαA shows signal in one achrocentric chromosome pair in a telomeric position.

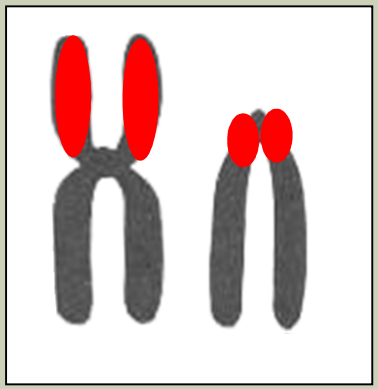




Location of ribosomal DNA 5S of Senegalese sole



The bigger signal locates in a subcentromeric position in a submetacentric chromosome pair and the smaller signal locates in a centromeric position of an achrocentric chromosome pair

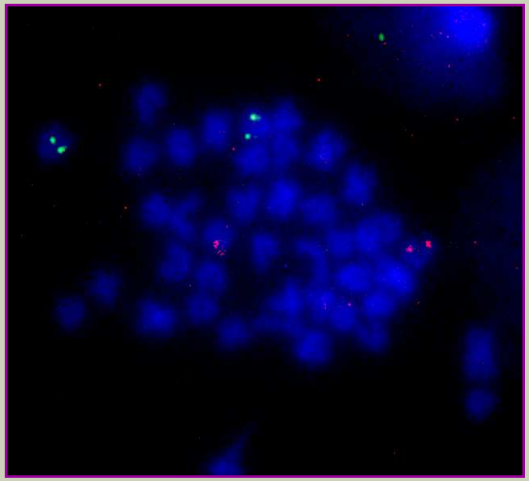


Double colour FISH-BAC in *S. senegalensis* chromosomes

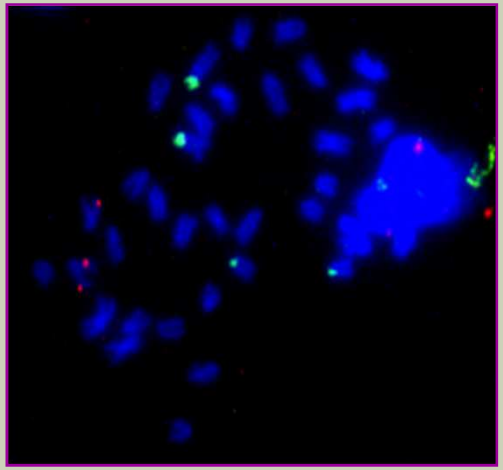
	5S	Lys	Sem	Hep	TRß	TRαA	TRαB
5S		X	X	X	X	X	X
Lys			X	X	X	X	X
Sem				X	X	X	X
Hep					X	X	X
TRß						X	X
TRαA							X
TRαB							

Double colour FISH-BAC in *S. senegalensis* chromosomes

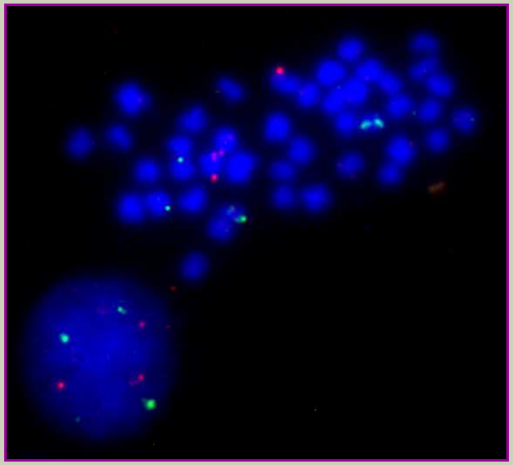
Lys and Sem



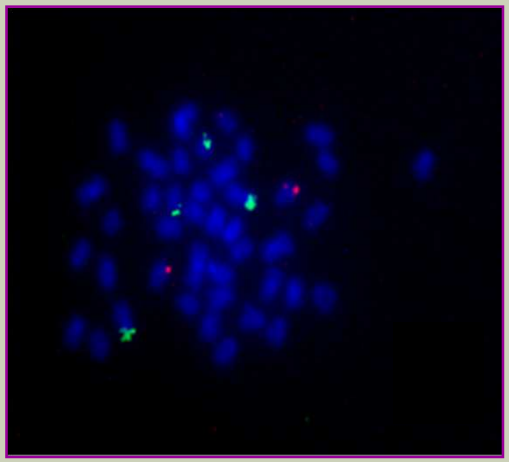
Sem and 5S rDNA



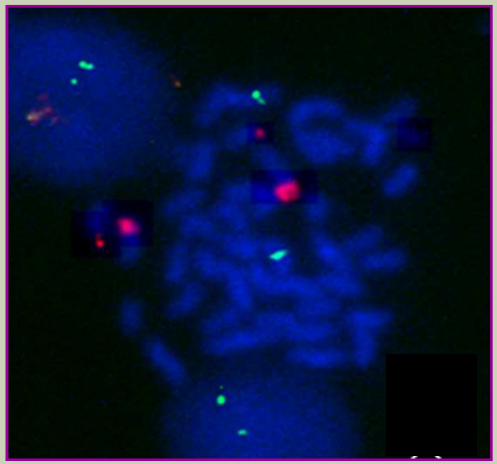
Lys and TRß



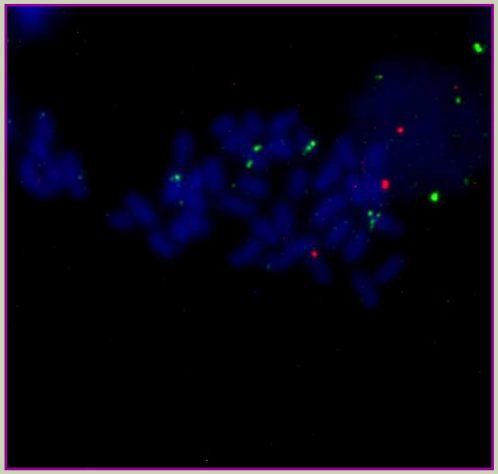
Lys and 5S rDNA



5S and Lys

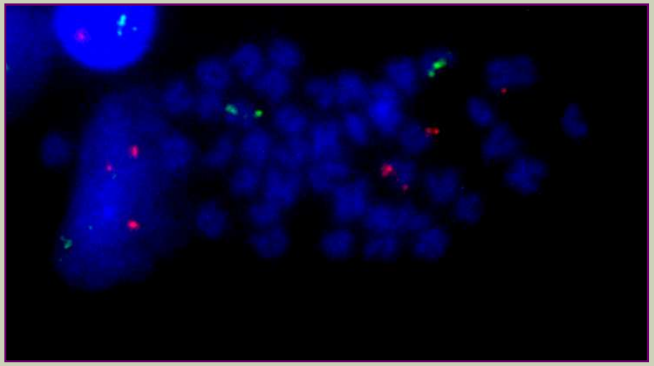


Sem and TRß

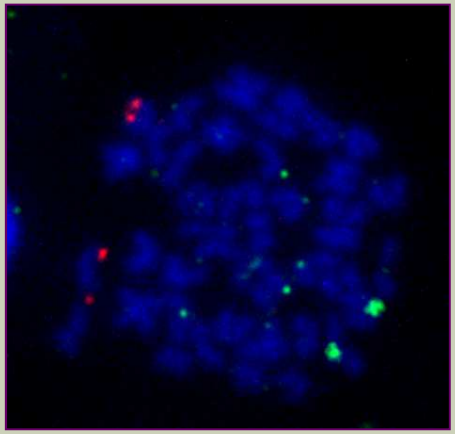


Double colour FISH-BAC in *S. senegalensis* chromosomes

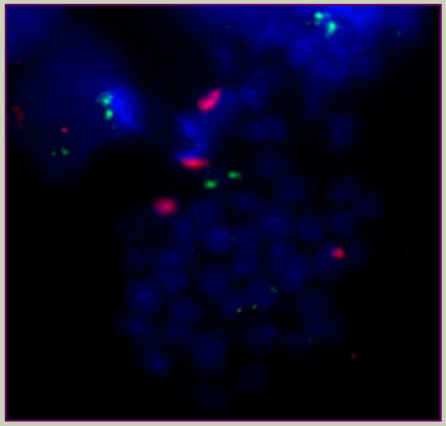
TRαA and Hep



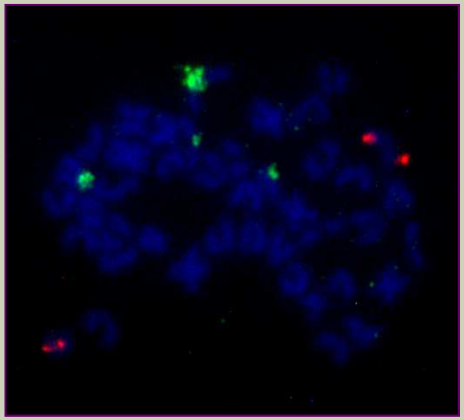
5S and TRαA



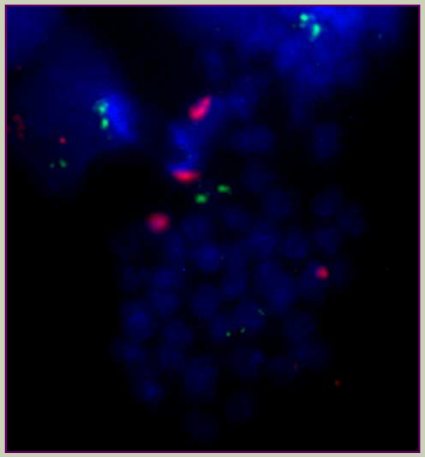
Hep and **5S**



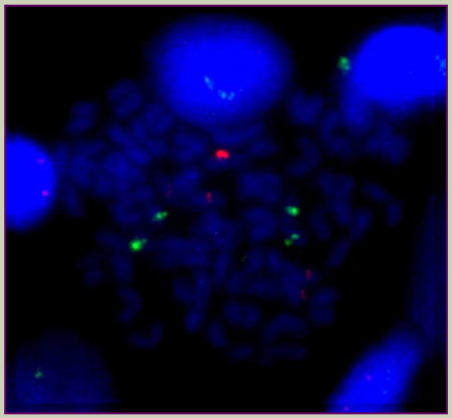
5S and TRαB



Hep and **5S**

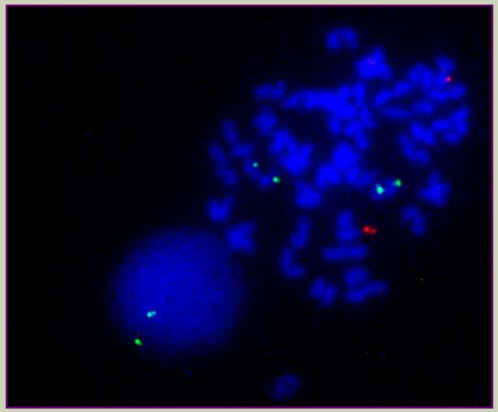


Lys and **Hep**

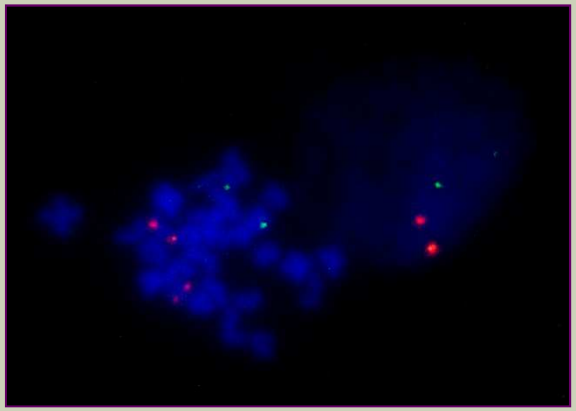


Double colour FISH-BAC in *S. senegalensis* chromosomes

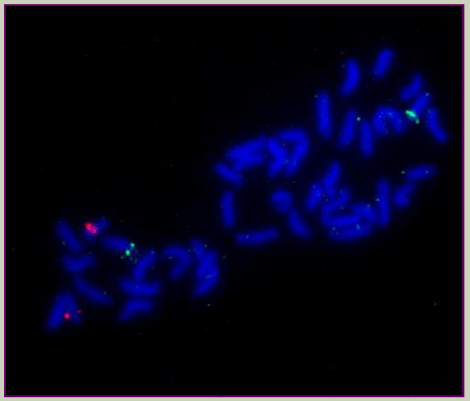
TRαA and Lys



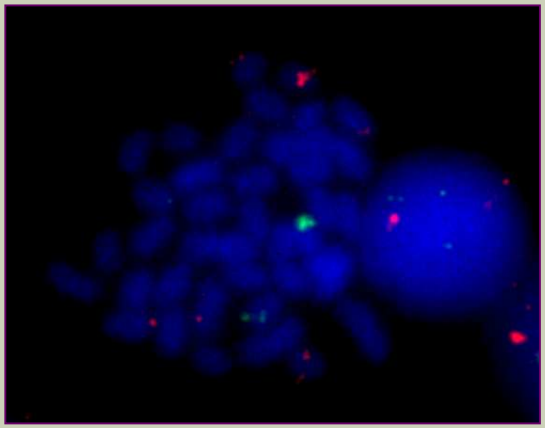
TRαB and Lys



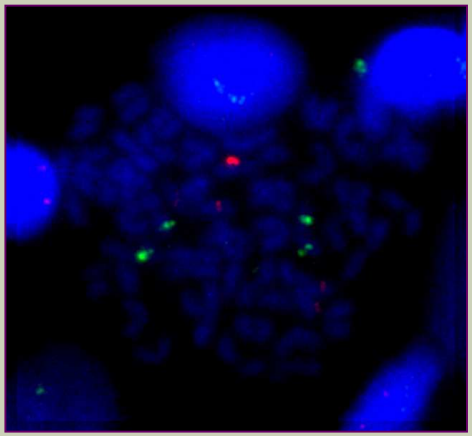
Sem and TRαB



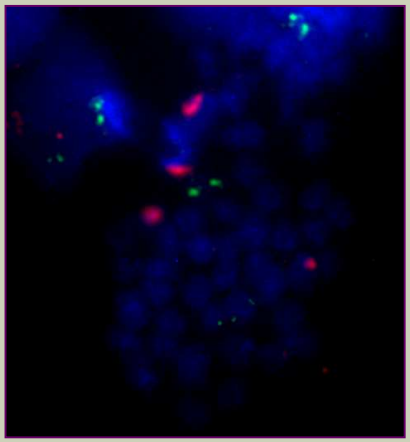
TRαA and TRαB



Lys and Hep

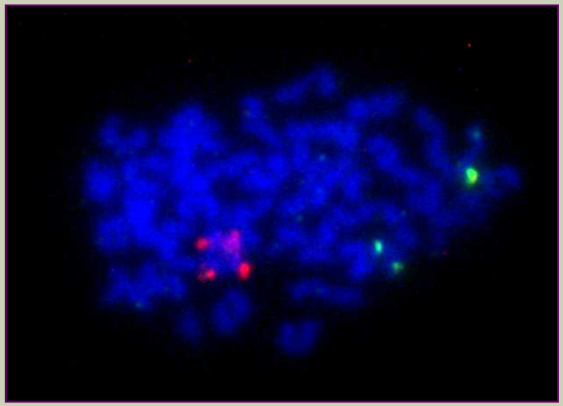


Hep and 5S

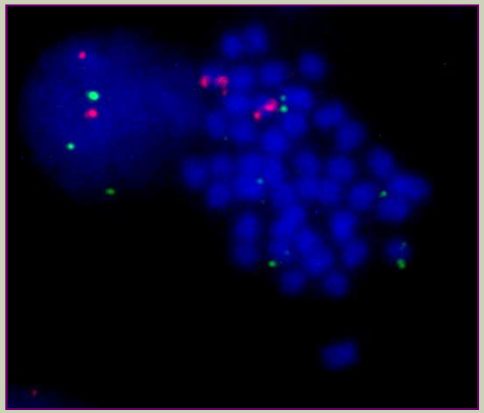


Double colour FISH-BAC in *S. senegalensis* chromosomes

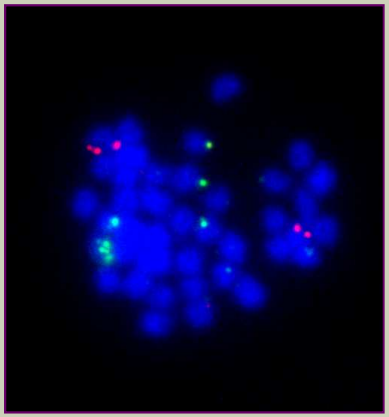
Hep and TRαB



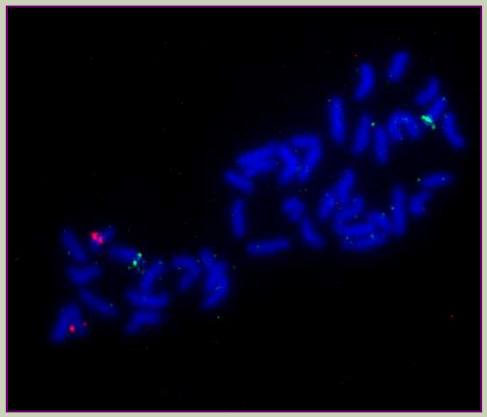
Hep and TRαB



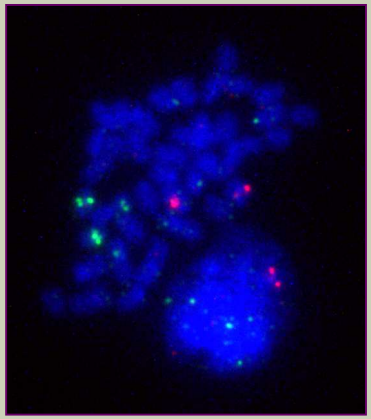
TRαA and TRαß



Sem and TRαB



Lys and TRαB



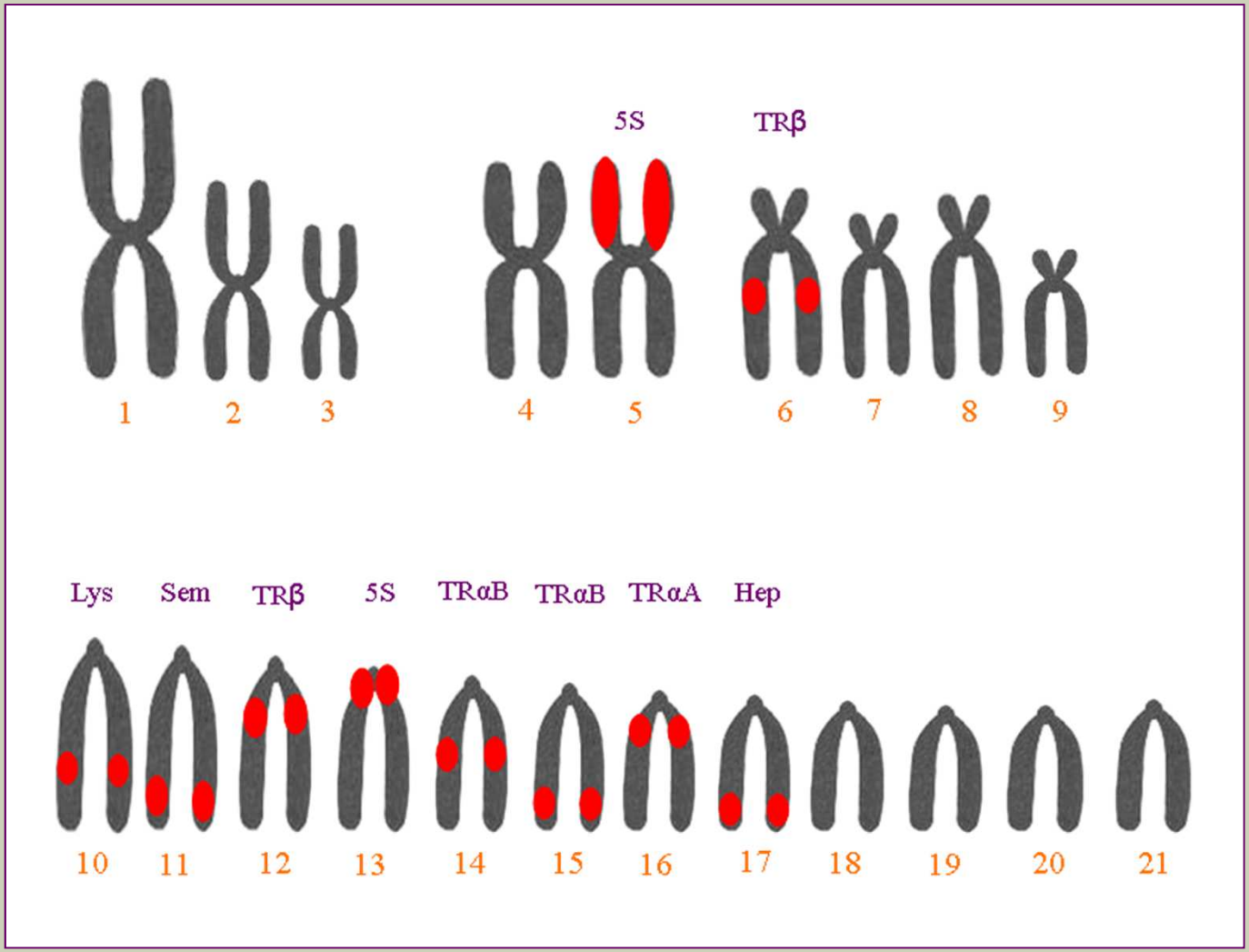
Double colour FISH-BAC combinations of Senegalese sole

	Sem	*Lysozyme*	*TRβ*	*TRαA*	*TRαB*	*Hepcidin*
Sem		1 chromosome pair each	Sem=1 chromosome pair TRβ=2 chromosome pair	1 chromosome pair each	TRαB=2 chromosome pair Sem=1 chromosome pair	1 chromosome pair each
Lysozyme			TRβ=2 chromosome pair Lys=1 chromosome pair	1 chromosome pair each	TRαB=2 chromosome pair Lys=1 chromosome pair	1 chromosome pair each
TRβ				1 chromosome pair each	2 chromosome pair each	Hepc=1 chromosome pair TRβ=2 chromosome pair
TRαA					TRαB=2 chromosome pair TRαA=1 chromosome pair	1 chromosome pair each
TRαB						1 chromosome pair each
Hepcidin						

Gene Number of chromosome pairs Colocate

Double colour FISH-BAC combinations of Senegalese sole

CONCLUSIONS: IDEOGRAM

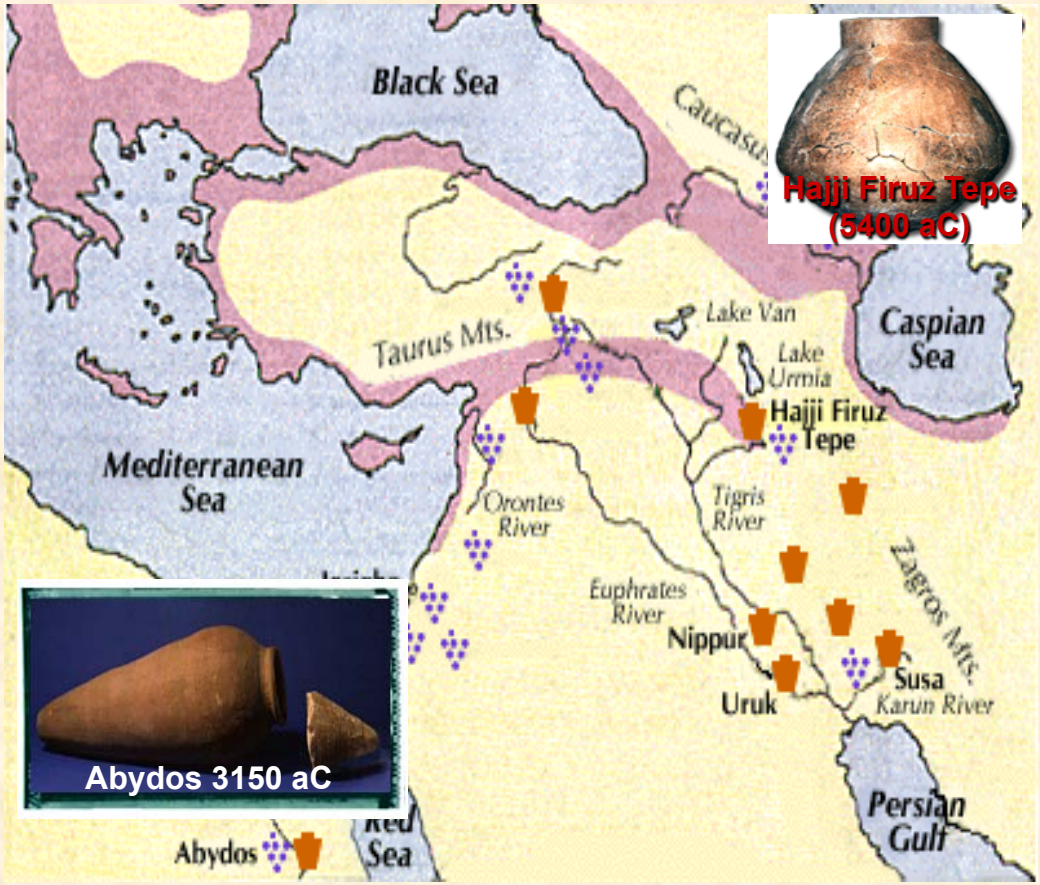


THANK YOU

WINE AND YEASTS

María Esther Rodríguez Jiménez

Vinification in ancient civilizations



-Neolithic: 7000 years ago

-Egiptian and Fenicia: 5000 bC

-Abydos: 3150 bC

-Greece and Crete: 2000 bC

In Spain

Evidences of vinification in IV century bC

Rests of a winery in the Ruinas del Castillo de Doña Blanca (El Puerto de Santa María)

Presence of phoenicians 3000 years ago aprox., in Gades (Cádiz)

VINIFICATION PROCESS

Sept.

Oct.

Nov.

Dec.

Yeasts:

√ *Cryptococcus*
√ *Rhodotorula*
√ *Hanseniaspora*
√ *Metschnikowia*
√ *Candida*

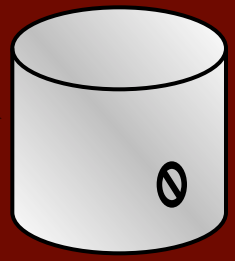
Bacteria
Filamentous fungi

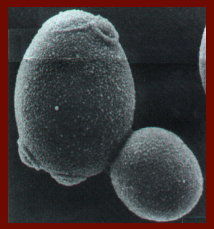
PRESSING

SO_2

TARTARIC ACID

GRAPE JUICE





Yeasts:
√ *Saccharomyces*

ACOHOLIC FERMENTATION

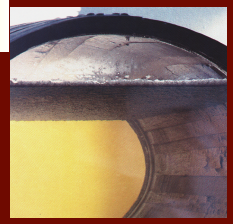
YOUNG WINE (11%)

Adding alcohol

14-15%

18%

Yeasts:
√ *Saccharomyces of flor velum*



BIOLOGICAL AGING

FINOS Y MANZANILLAS

Physico-chemical aging

Olorosos

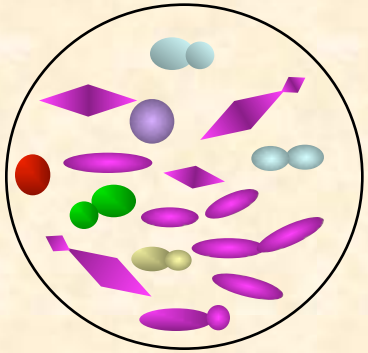
HOW IT IS POSSIBLE IMPROVE WINE QUALITY IN AN INDUSTRIAL PROCESS?

ACTING IN THE FERMENTATION PROCESS

FERMENTATION

Spontaneous fermentation is a complex microbiological process, in which are implicated a high diversity of yeasts

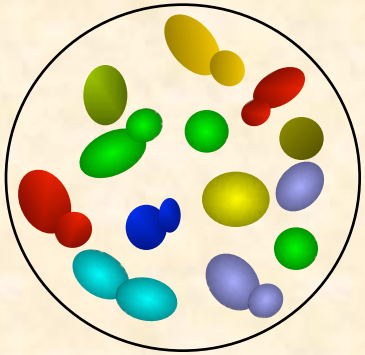
Low Alcoholic grade



1st Stage

Hanseniaspora,
Pichia,
Candida,
Torulaspora,
Metschnikowia,
Zygosaccharomyces, etc.

High Alcoholic Grade



2nd Stage

Saccharomyces cerevisiae,

3th Stage

Saccharomyces uvarum

This diversity of yeast strains provides complexity and typicity in the sensorial cahracteristics of the wine

To improve the fermentation process

1. The first step is to study the genetic diversity of the yeast strains

2. The second step is to choose the most representative yeasts within the process

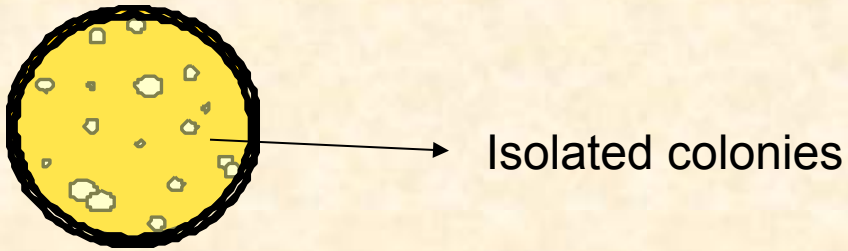
3. The third step is to analyse the enological properties of these yeast (fermentative capacity, flavour and taste in microvinifications, consumption of the sugar, etc.) to choose the most adequate yeasts for the process

1 Analysis of genetic diversity in spontaneous fermentation

➔ Sampling

➔ Dilution and plating in YPD laboratory medium

➔ Selection of repesentative number of colonies (20 aproximately)

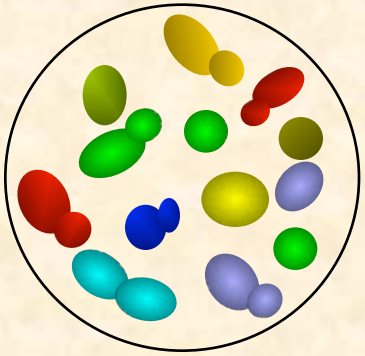


➔ Characterization by electrophoretic karyotype

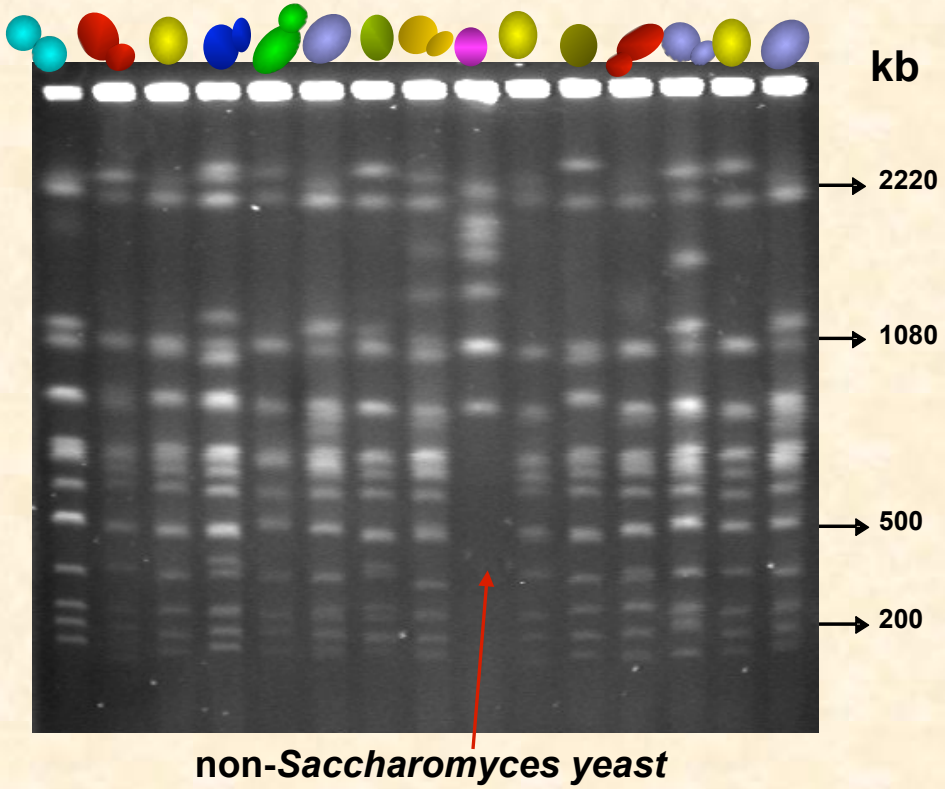
Molecular Techniques

Identification and characterization of wine yeast

Pulsed Field Gel Electrophoresis (PFGE)

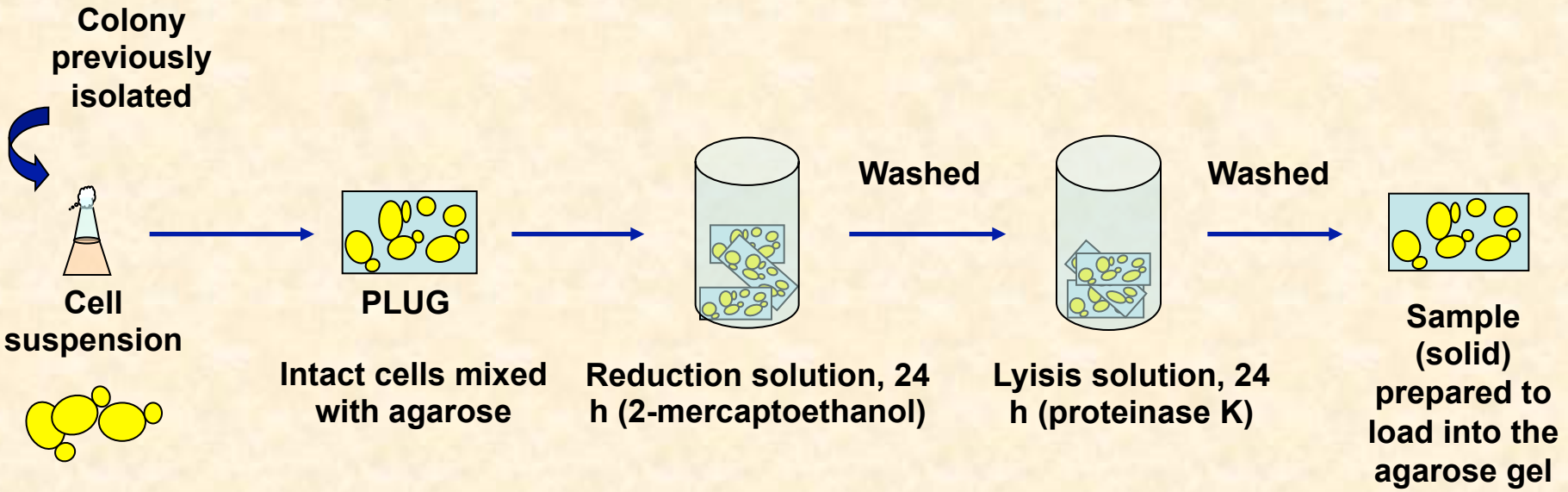


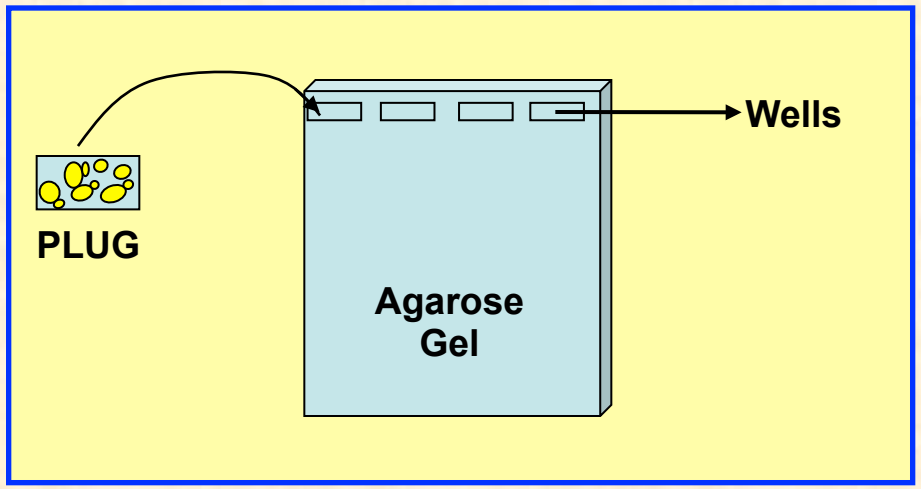
Strains are not possible to differenciate by classical microbiological techniques



Pulsed Field Gel Electrophoresis (PFGE)

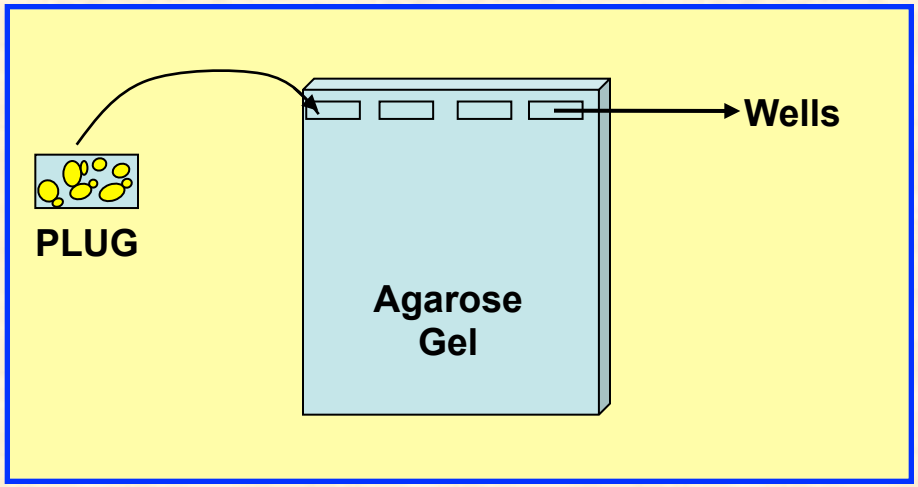
Preparation and treatment of the samples

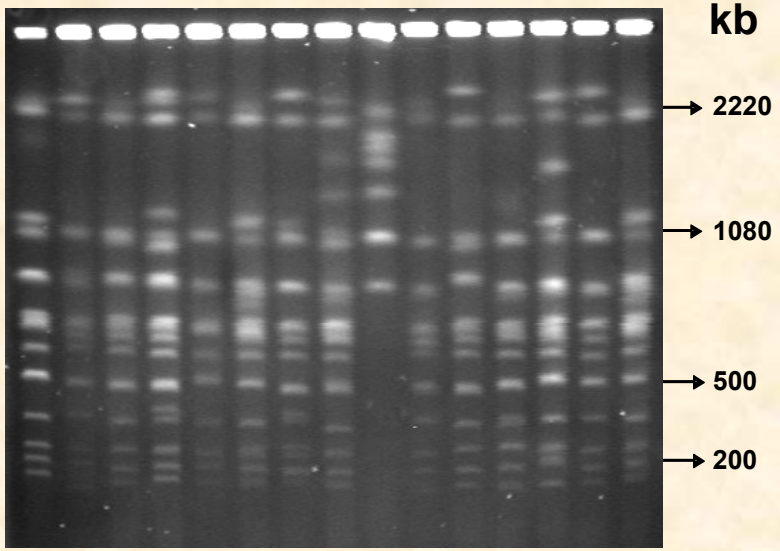




Pulsed Field Gel Electrophoresis (PFGE)

Foto aparato





Application of this technique allows:

-Characterization and identification of the yeast strains during all fermentation process
-Show the dynamics of the yeast population
-Show genetic diversity of different strains belonged to the same yeast specie

2 **The second step in improving wine is to choose the most representative yeasts within the process**

3 **Application of different selection criteria**

1) % Ethanol inhibition

2) Fermentative capacity

3) Rate of sugar consumption

4) Killer phenotype

5) Capacity of each yeast strain to compete within the mixed population in a fermentation vessel under semi-industrial condition

6) Sensorial analysis of the wines produces in pures micro-vinifications

SELECTION OF THE YEASTS

INOCLATION OF INDUSTRIAL FERMENTATIONS

White wine

Bodegas Barbadillo

Castillo de San Diego

Red wine

Bodega Dominio de Pingus

INOCULATION OF THE WINE PRODUCTION SYSYTEM WITH SELECTED AUTOCHTHONOUS YEAST STRAIN

Scheme for white wine

In laboratory conditions

Selected yeast strain

500 mL

a b

2 L

20 L

In winery

Addition of cultures (40 L)

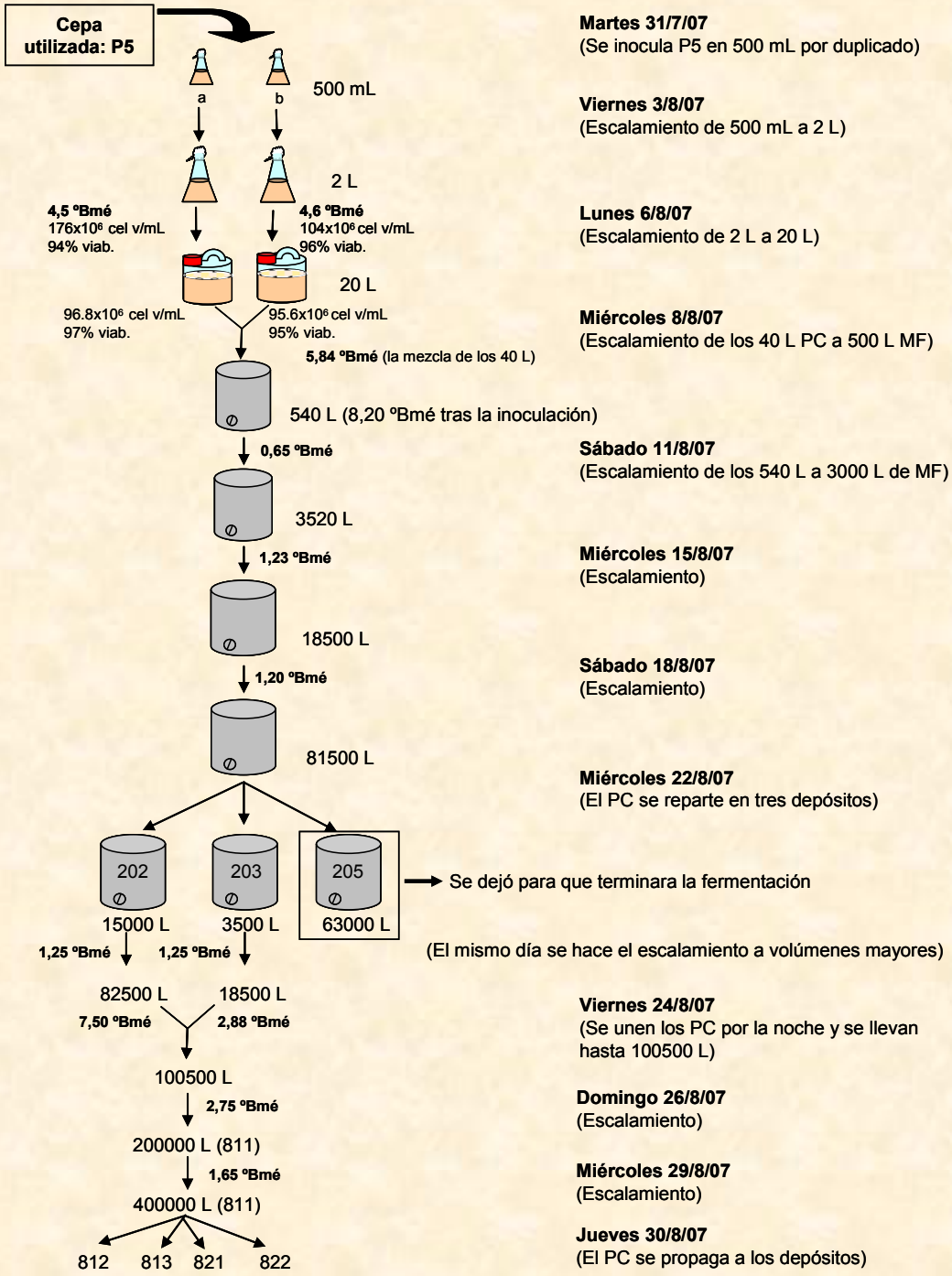
251 L

1096 L

Refill of must

400.000 L

VINO BLANCO



Martes 31/7/07
(Se inocula P5 en 500 mL por duplicado)

Viernes 3/8/07
(Escalamiento de 500 mL a 2 L)

Lunes 6/8/07
(Escalamiento de 2 L a 20 L)

Miércoles 8/8/07
(Escalamiento de los 40 L PC a 500 L MF)

Sábado 11/8/07
(Escalamiento de los 540 L a 3000 L de MF)

Miércoles 15/8/07
(Escalamiento)

Sábado 18/8/07
(Escalamiento)

Miércoles 22/8/07
(El PC se reparte en tres depósitos)

Viernes 24/8/07
(Se unen los PC por la noche y se llevan hasta 100500 L)

Domingo 26/8/07
(Escalamiento)

Miércoles 29/8/07
(Escalamiento)

Jueves 30/8/07
(El PC se propaga a los depósitos)

Use of yeasy of *S. cerevisiae* or *(S. uvarum)* together with non-*Saccharomyces* yeast

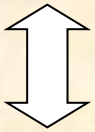
MIXED CULTURES

SEQUENTIAL CULTURES

- To obtain a major complexity in the sensorial characteristics of the wines

-New styles of wines

Control of the fermentation process by proper inoculation + autochthonous yeast strain



Improve on the quality of the wine

thanks for your attention!

“You … and microorganisms”

… The exciting world of Microbiology through the history …

Jesús Manuel Cantoral

SUMMARY:

-If we coul talk to de Microorganisms

- Life on Earth

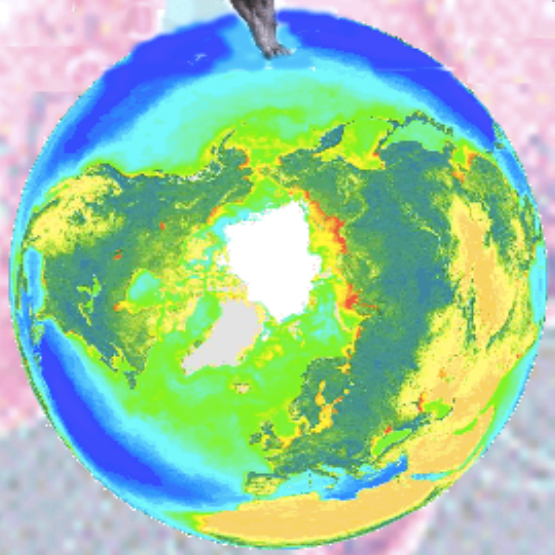
- A litle History

- Stage Pre-Scientific

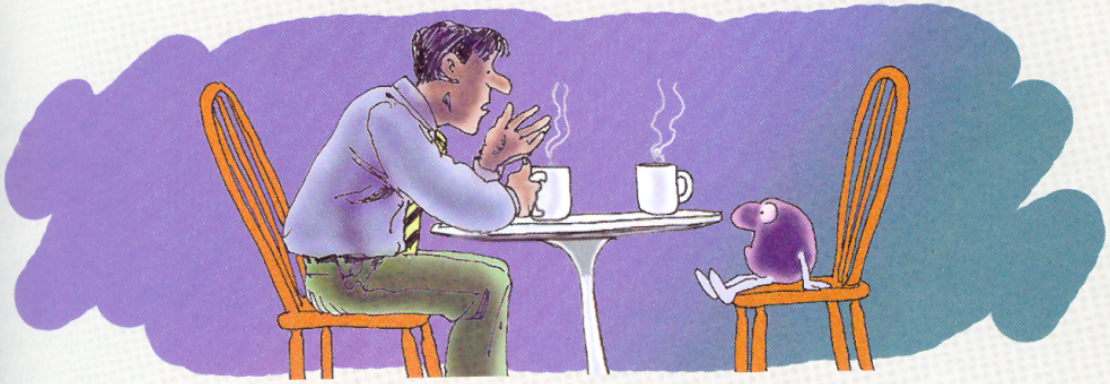
- L. Pasteur, R. Cock, A. Fleming

-Present and Future of Microbiology

-How will the Microbiology of the XXI century?



If We Could Talk to the Microbes



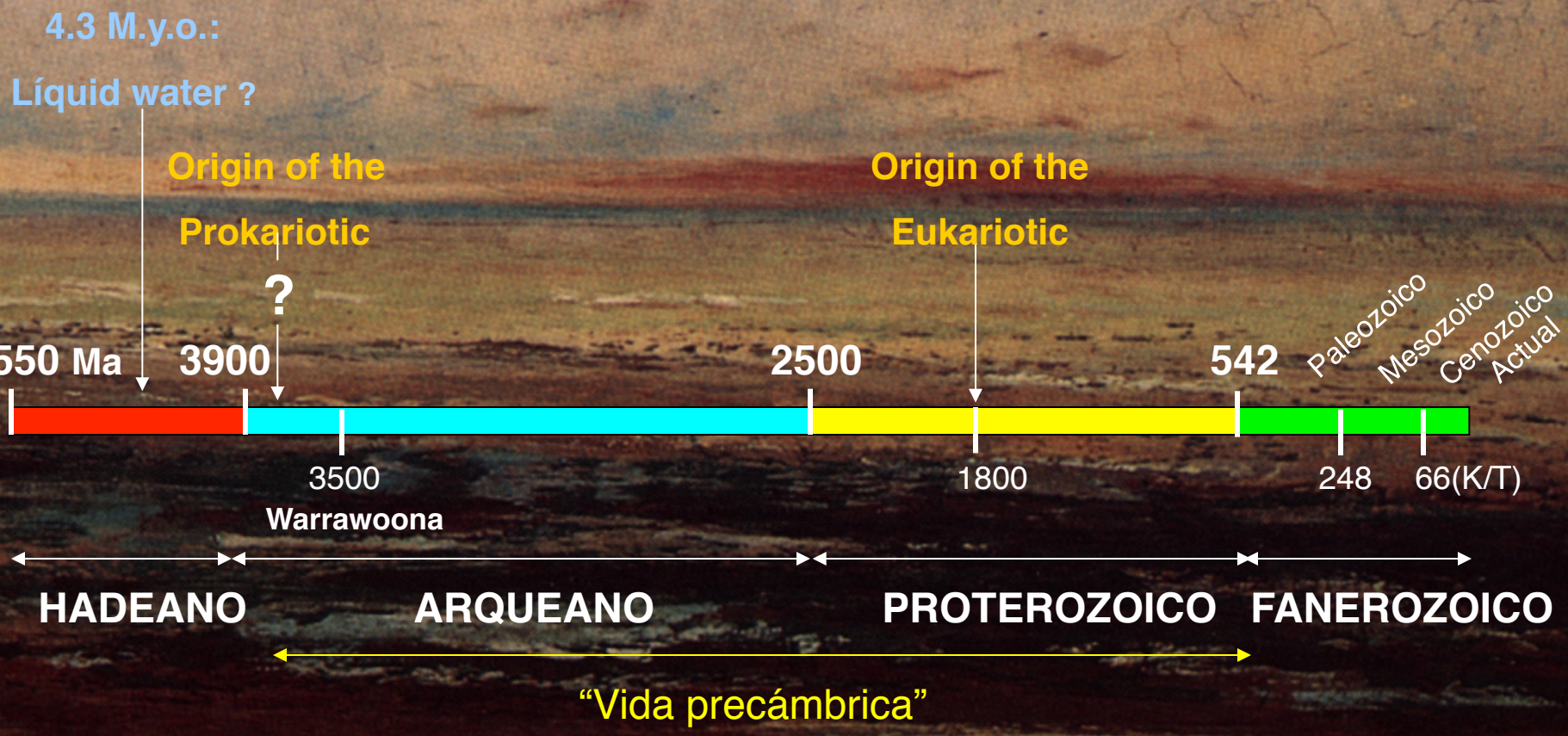
"Microorganisms do communicate. They do it chemically."

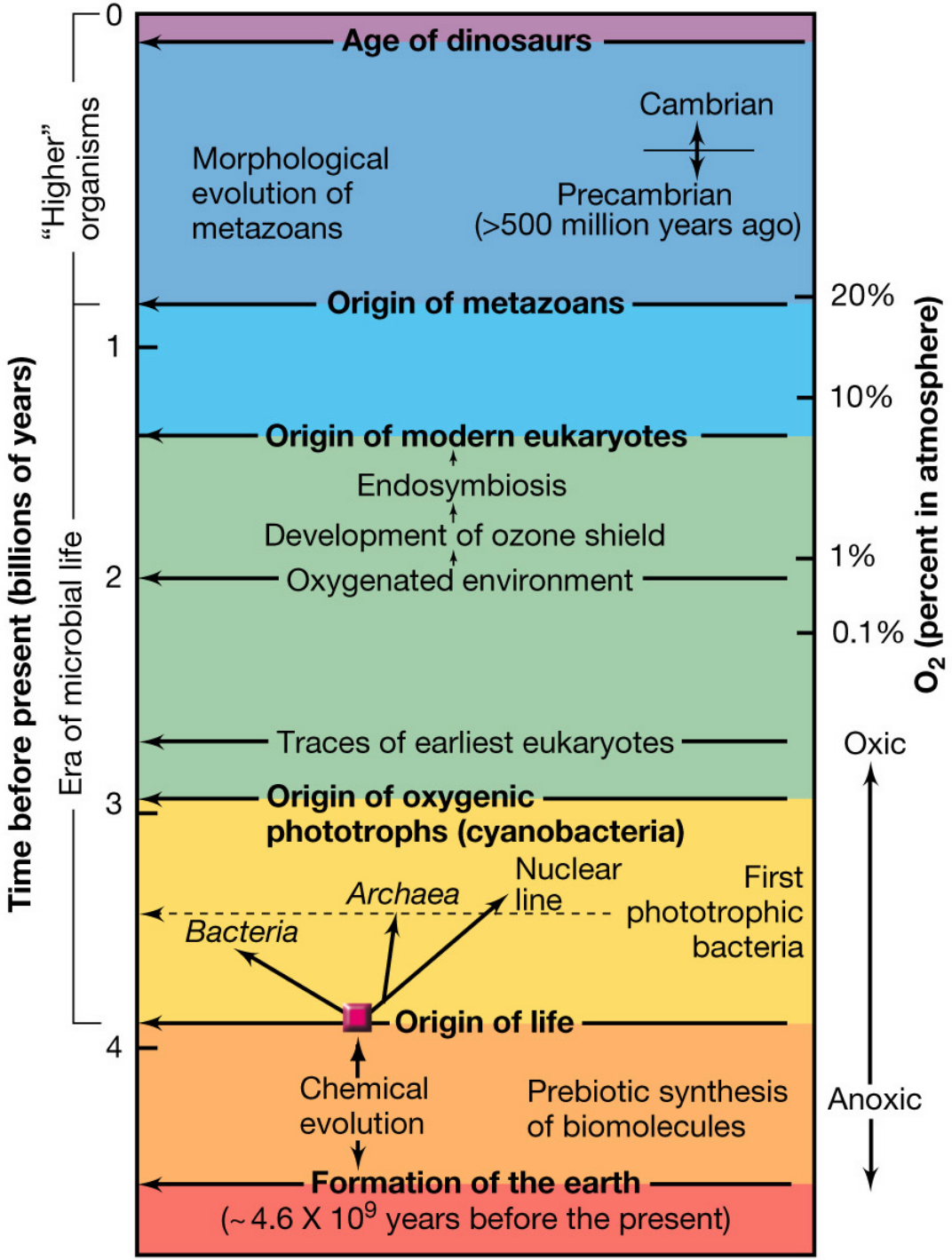
	Length		Relación
Human	1,70 m		
World	12.756 km		$7,5 \times 10^6$
Human	1,70 m		
M. genitalium	0,23 μm		$7,4 \times 10^6$

All living things on Earth depends on the microorganisms

Interactions

Life on Earth




Time before present (billions of years)
0
1
2
3
4
"Higher" organisms
Era of microbial life
Age of dinosaurs
Cambrian
Precambrian
(>500 million years ago)
Morphological evolution of metazoans
Origin of metazoans
Origin of modern eukaryotes
Endosymbiosis
Development of ozone shield
Oxygenated environment
Traces of earliest eukaryotes
Origin of oxygenic phototrophs (cyanobacteria)
Archaea
Bacteria
Nuclear line
First phototrophic bacteria
Origin of life
Chemical evolution
Prebiotic synthesis of biomolecules
Formation of the earth
(~4.6 X 10^9 years before the present)
20%
10%
1%
0.1%
O2 (percent in atmosphere)
Oxic
Anoxic

A little History:

1. Stage Pre-Scientific: "art of fermenting since ancient”:

- Fermentative processes: 10,000 years
- Domestication of microorganisms by man
- Egyptians, beer, bread: 3,000-5,000 years
- Derivatives lactic: 5,000 Years
- Recipes and customs that have come down to us (cheese making)

A brief history of wine

“ … I would imagine the time, lost in the mists of time, when an unknown cave dweller, perhaps a benefactor dark kingdom of Mesopotamia successful, happily discovered that fruit juices are much tastier if you let it sit a while. Perhaps at the same time, some unknown ancestors of *Saccharomyces cerevisiae* fermenters made his fortuitous survival was enhanced by association with the man, and decided to leave the unsafe environment and become the first domesticated organism “

(Drs. Vaughan and Martini)

Origin of Champagne

Come quickly brothers, I'm drinking stars!

Dom Perignon. France 1638-1715

1. Stage Pre-Scientific: "art of fermenting since ancient":

2. Development of Microbiology

- 1.664: Observations of Robert Hooke

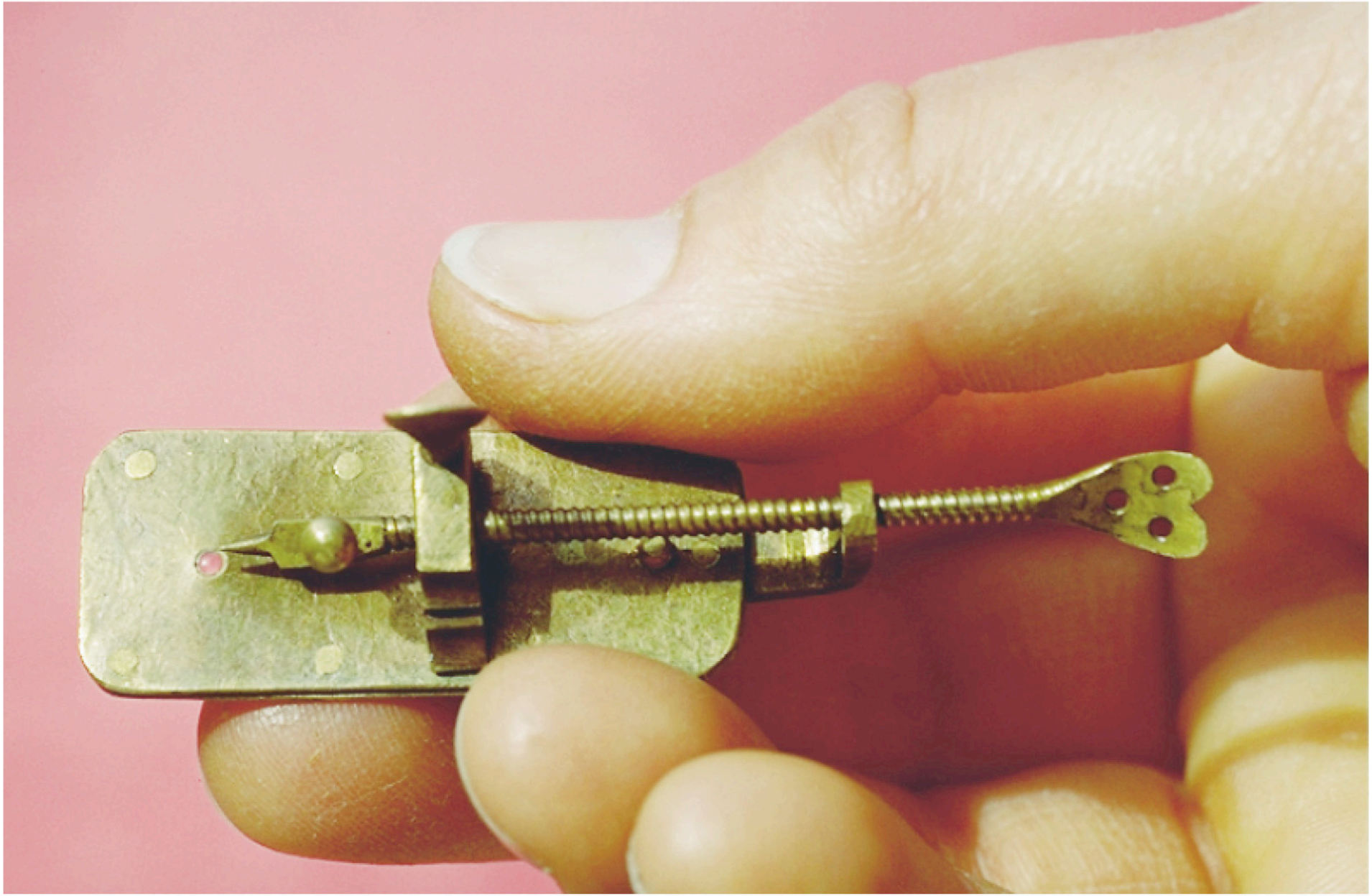
2.1. Discovery of the microscope:

Antony van Leewenhoek: 1.677

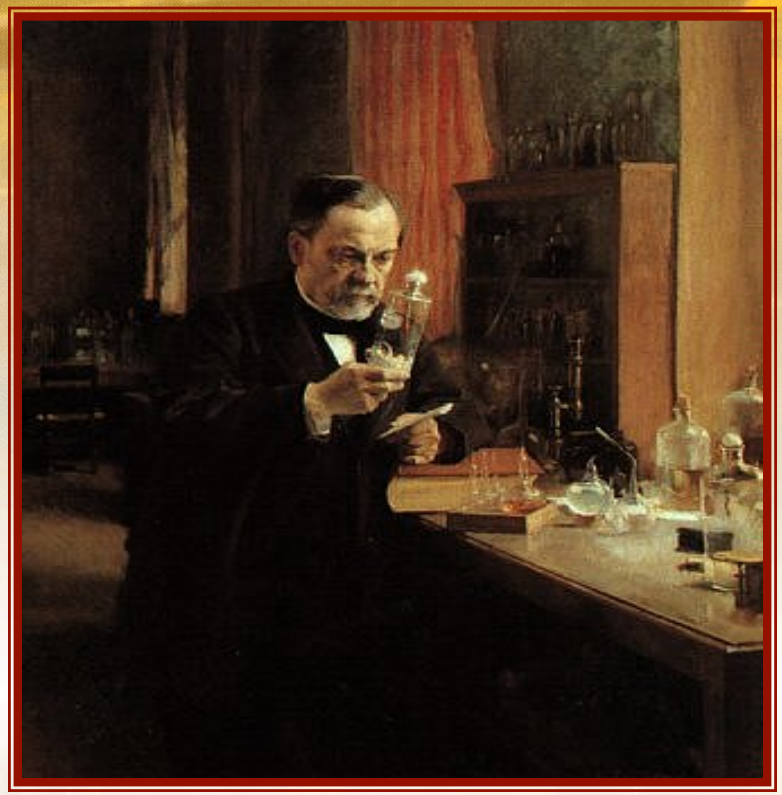
- Delft 1.632-1.723. Cloth merchant. He had an innate curiosity
-1680: member of the "Royal Society" in London
- does with his hands: 300 microscopes (50-300) increases
- discover the World of "animalcules" he surprised the great wealth

… "I've had several ladies home very interested in seeing small eelworms vinegar, but some of them were so little taste the show they promised not to use vinegar.

However, what if you were to say to these people in the future, in the lining of the teeth from the mouth of a man's more live animals people around a kingdom? " …



Fundamentals of Microbiology:



Louis Pasteur 1.822-1.895

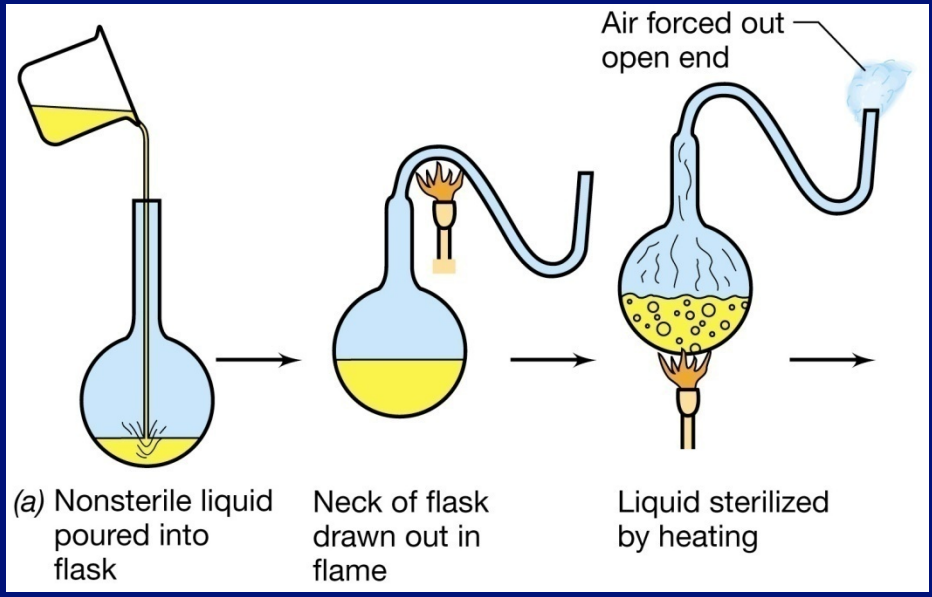
1.860: the air contains "Organized bodies“

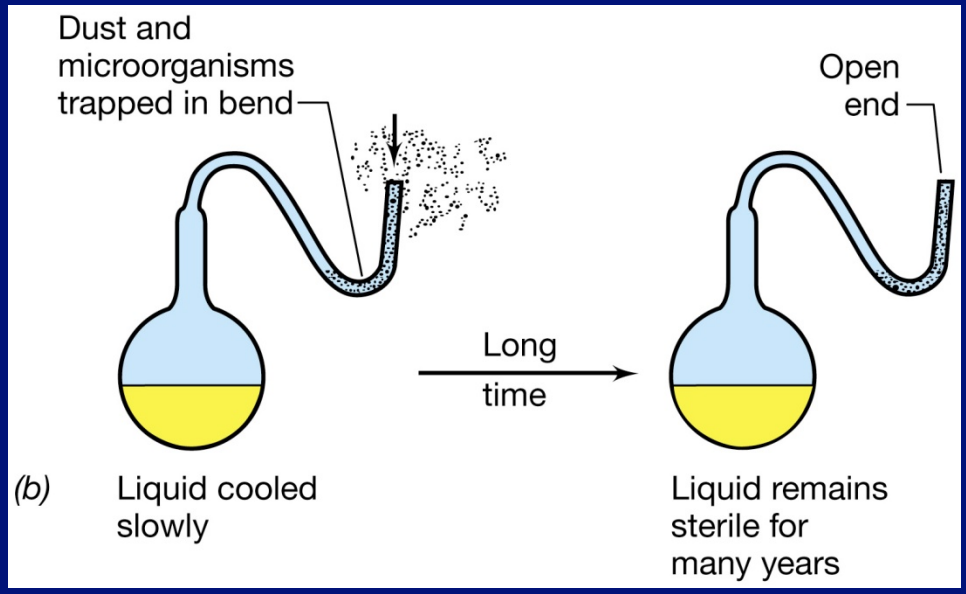
Swan neck flasks

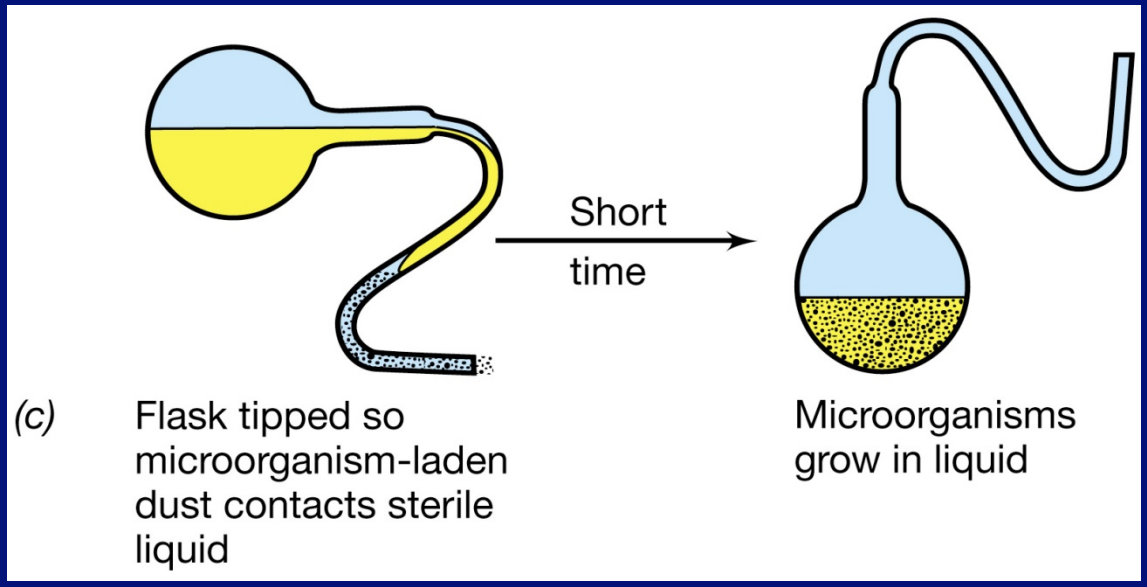
Anaerobes. Fermentation is life without air: Postulates of Pasteur

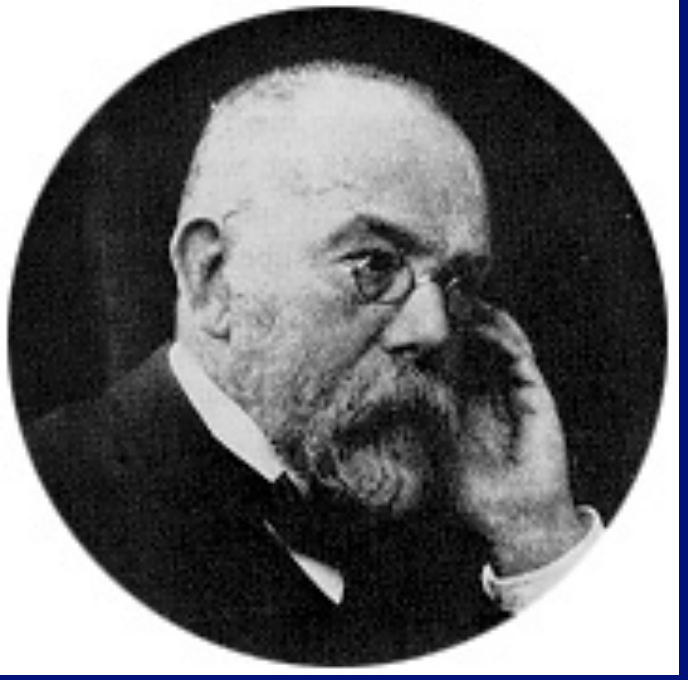
One. Fermentation is only produced by living organisms
Two. Each fermentation is produced by a particular organism
Three. The fermentation does not occur spontaneously

Techniques: Sterilization, Pasteurization or Vaccination


Air forced out
open end
(a) Nonsterile liquid poured into flask
Neck of flask drawn out in flame
Liquid sterilized by heating

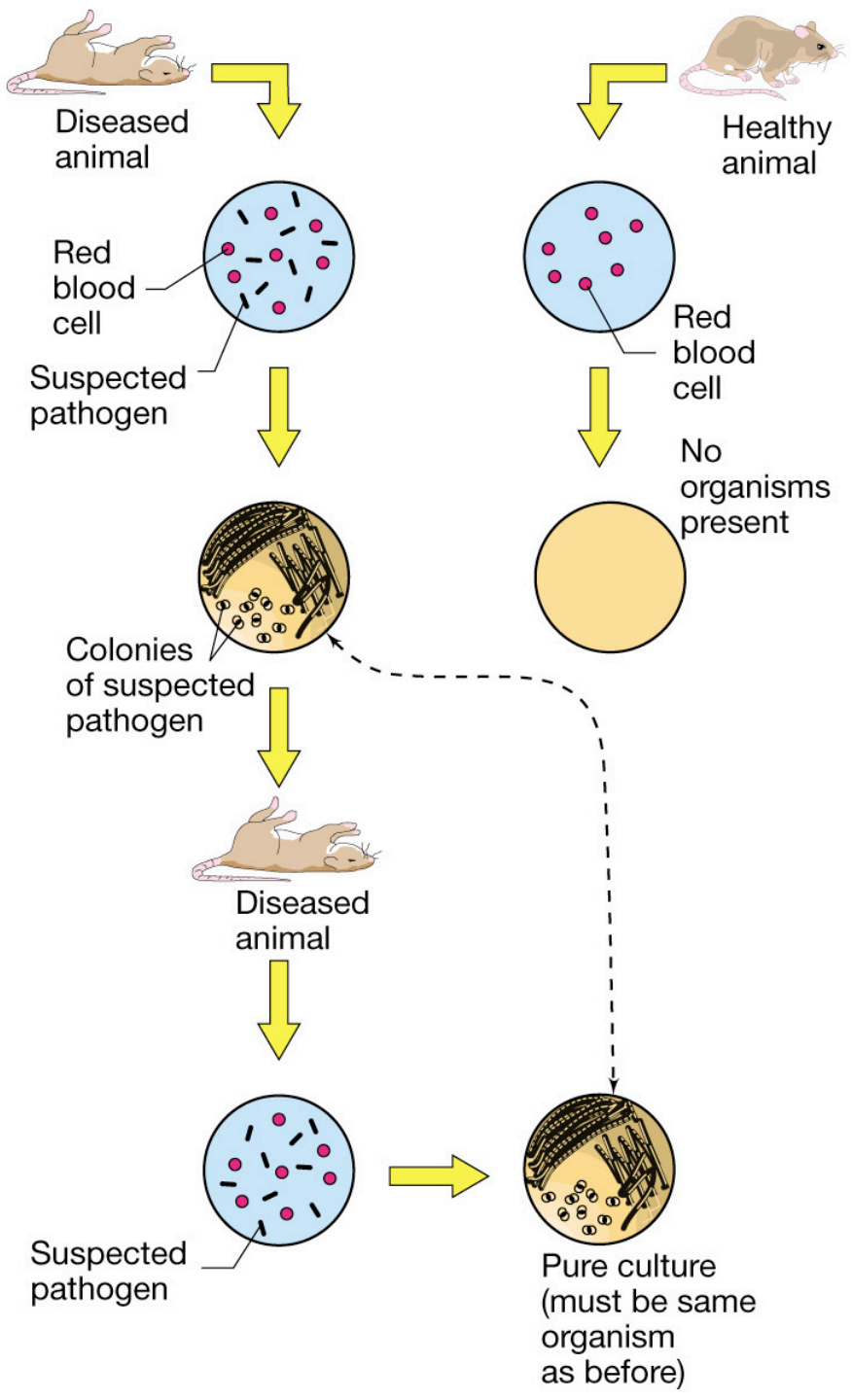

Dust and microorganisms trapped in bend
Open end
Long time
(b)
Liquid cooled slowly
Liquid remains sterile for many years

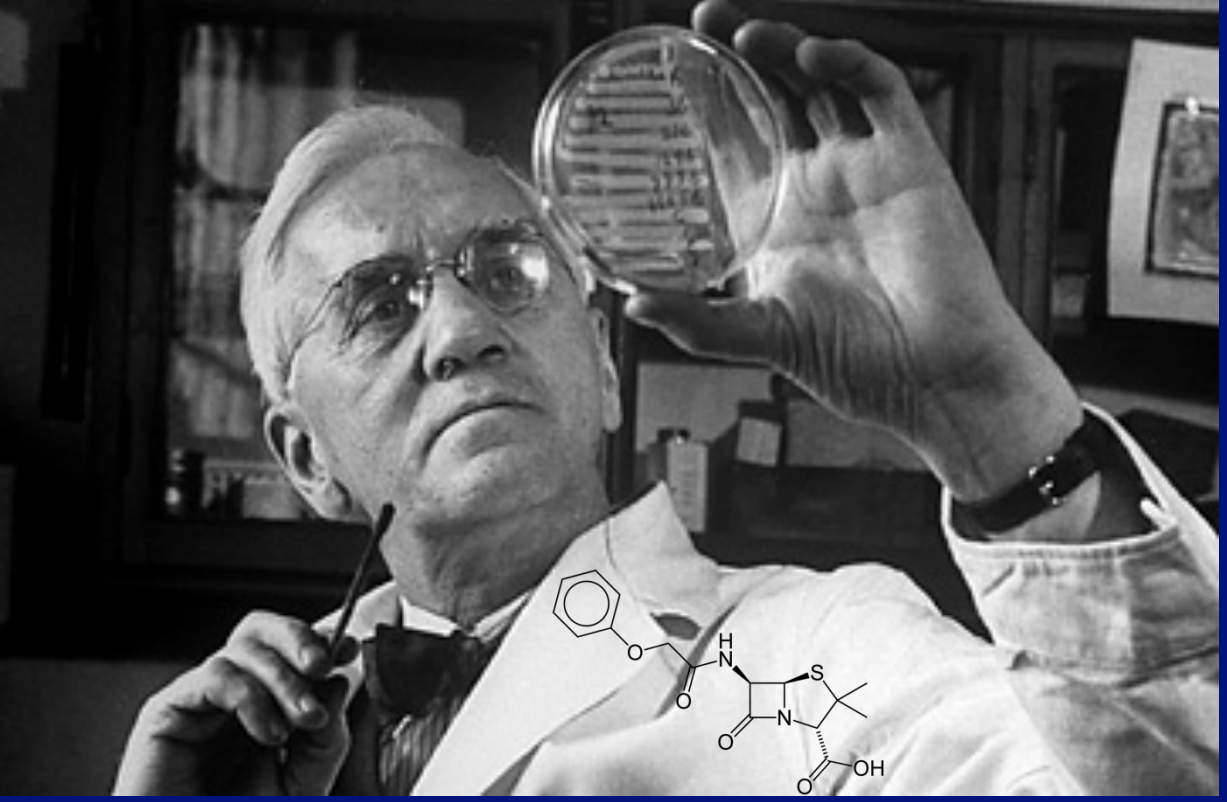

Short time
(c)
Flask tipped so microorganism-laden dust contacts sterile liquid
Microorganisms grow in liquid

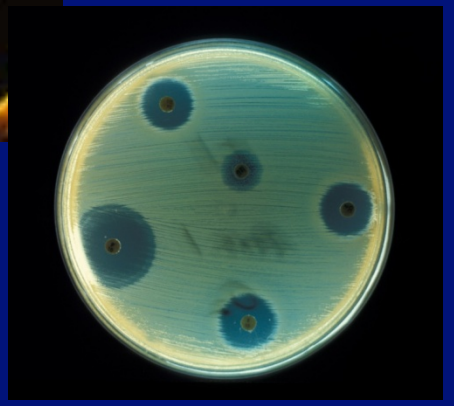


Robert Koch (1843-1910)

With its postulates demonstrated the relationship between a particular disease of microbial origin and the organism that produces it:







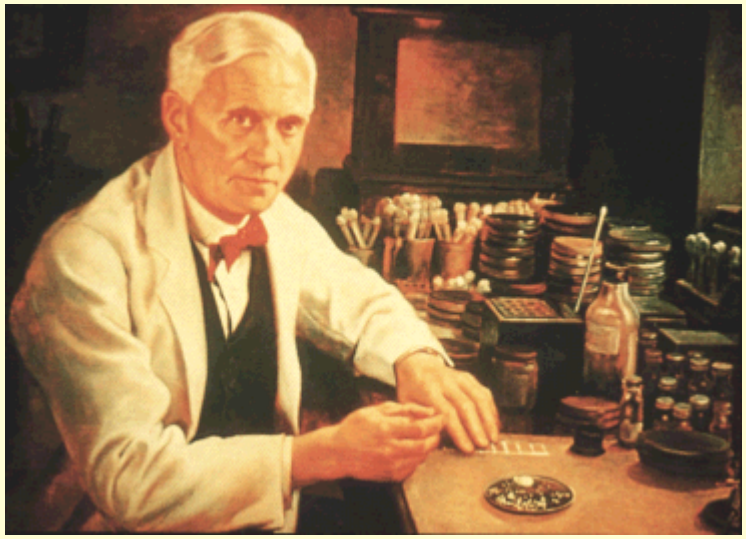
Alexander Fleming (1881-1955)

- 1928: antibiotic penicillin *Penicillium notatum*

- 1940: Nobel Prize with E. Chain and W. Florey

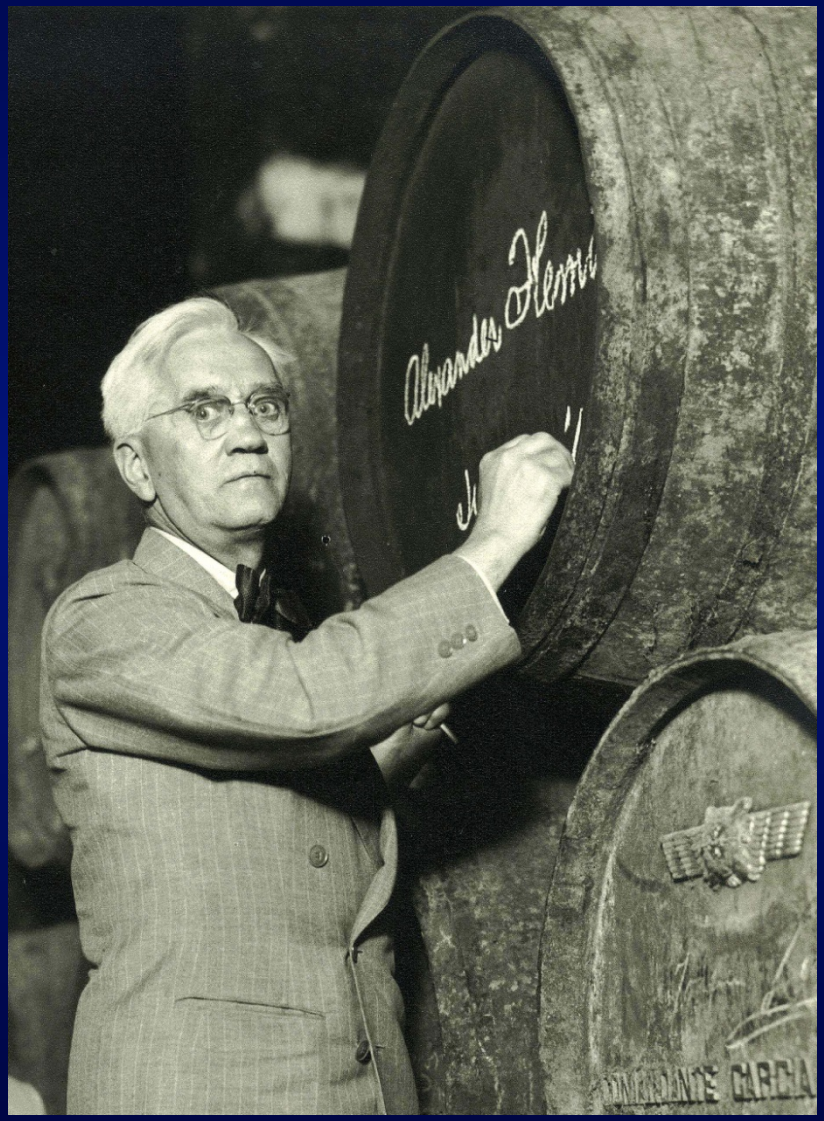
Not patent penicillin and can reach everyone

As said Sir Alexander Fleming



"Penicillin is the humans who cures, but wine that maketh glad the heart of man "

As said Sir Alexander Fleming



"Is that cures penicillin to humans, but it is wine that maketh glad the heart of man"

" If penicillin saves the sick, the wine "odorous" resurrects the dying"

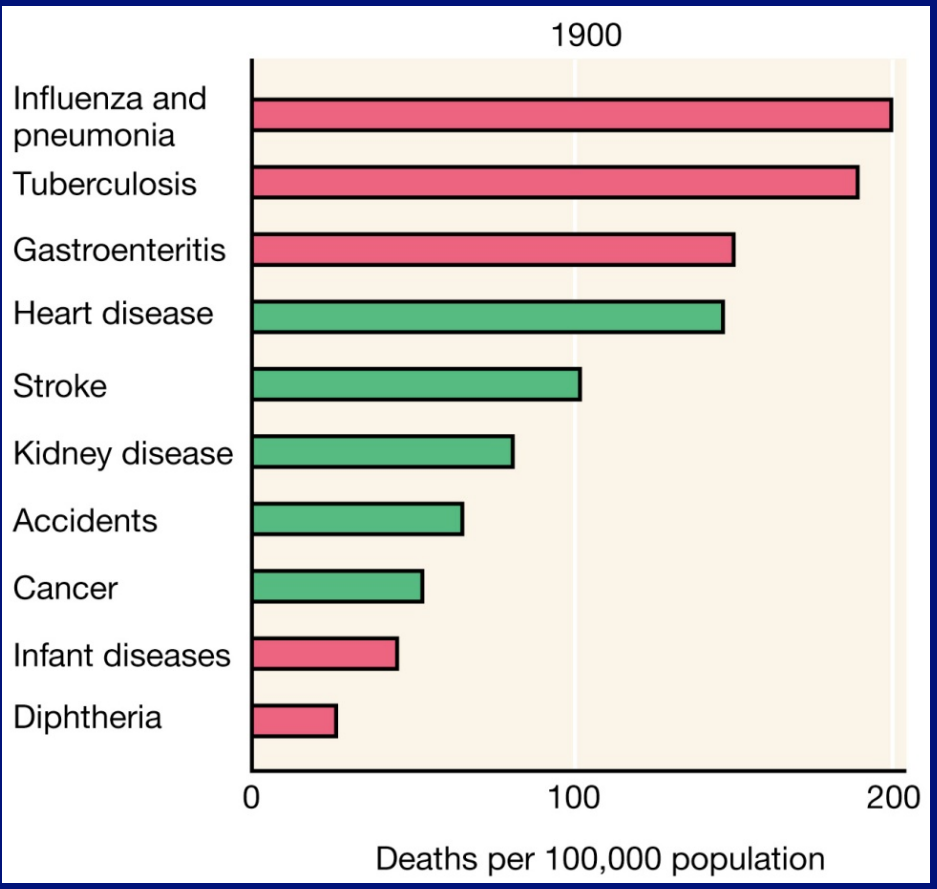
4. Present and Future of Microbiology:

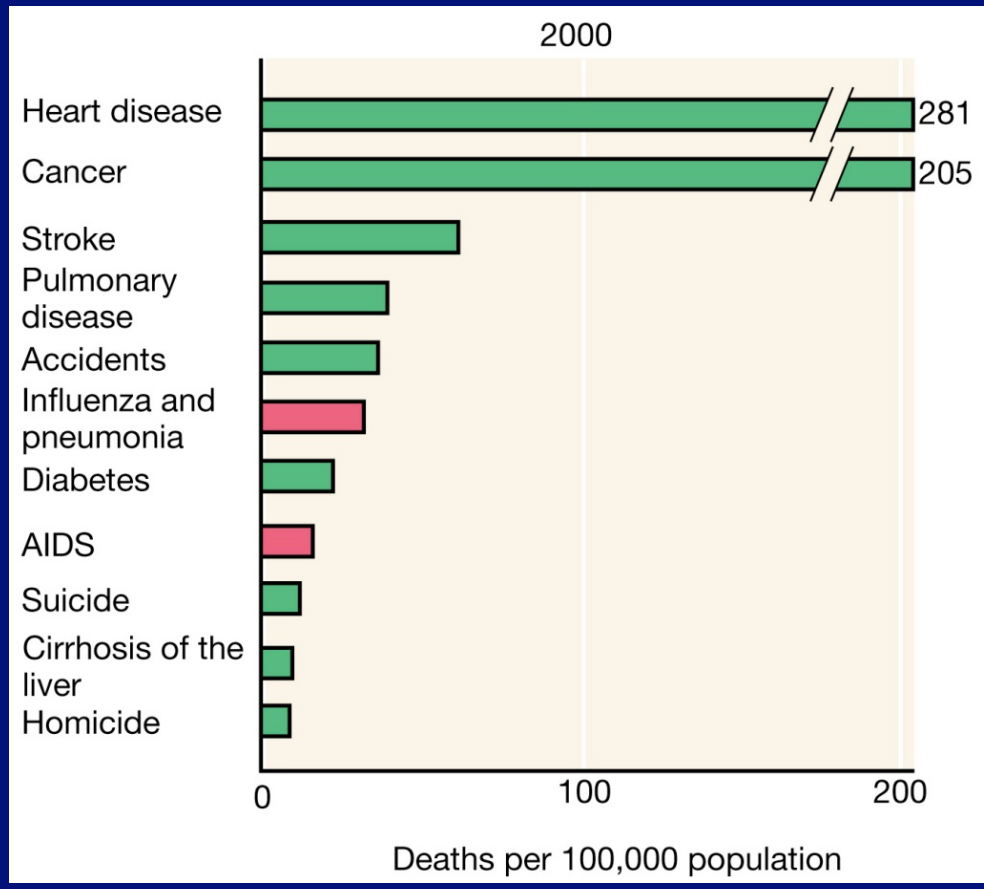
In less than two centuries Microbiology has developed as an important part of Biology. So is this in:
health and medicine, industry, agriculture, environment, etc.

Microbiology has especially contributed to the development of Molecular Biology and interconnection with other life sciences, engineering and computer science as Biotechnology

It has made possible:

- **The genetic manipulation of microorganisms**
- **Cloning genes: insulin, growth factors**
- **Development of new vaccines: safer, more specific and cheaper**
- **The creation of new antibiotics, anticancer, antitumor**
- **Use of microorganisms (*Thiobacillus*) in industrial processes: biomining**
- **Development of new fermentation processes: cheaper clean**
- **Environment applications: such as wastewater treatment**

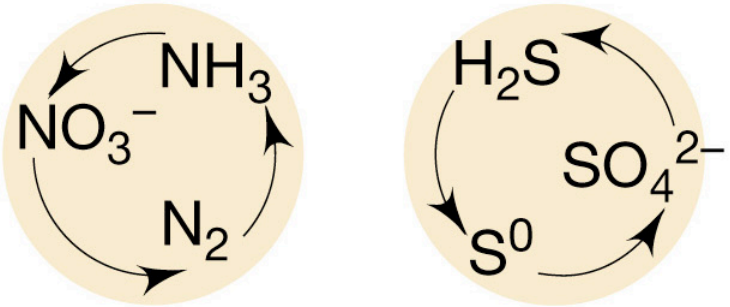

1900
Influenza and pneumonia
Tuberculosis
Gastroenteritis
Heart disease
Stroke
Kidney disease
Accidents
Cancer
Infant diseases
Diphtheria
0
100
200
Deaths per 100,000 population


2000
Heart disease
281
Cancer
205
Stroke
Pulmonary disease
Accidents
Influenza and pneumonia
Diabetes
AIDS
Suicide
Cirrhosis of the liver
Homicide
0
100
200
Deaths per 100,000 population

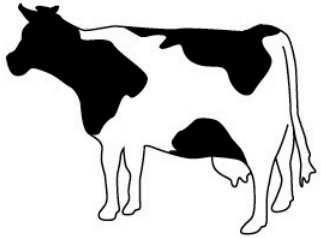
Agriculture

N_2 fixation ($N_2 \rightarrow 2NH_3$)

Nutrient cycling



Animal husbandry



Cellulose $\rightarrow$ CO_2 + CH_4 + animal protein

Rumen

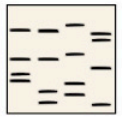
Food

Food preservation (heat, cold, radiation, chemicals)

Fermented foods

Food additives (monosodium glutamate, citric acid, yeast)

Disease

Identify new disease 

Treatment, cure, and prevention 

Energy/Environment

Biofuels (CH_4)

Fermentation
(Corn → Ethanol) 

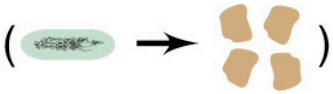
O_2
Bioremediation (spilled oil → CO_2)

(organic pollutants → CO_2)

Microbial mining ($CuS \rightarrow Cu^{2+} \rightarrow Cu^0$)

Biotechnology

Genetically modified organisms 

Production of pharmaceuticals (insulin and other human proteins) 

Gene therapy for certain diseases

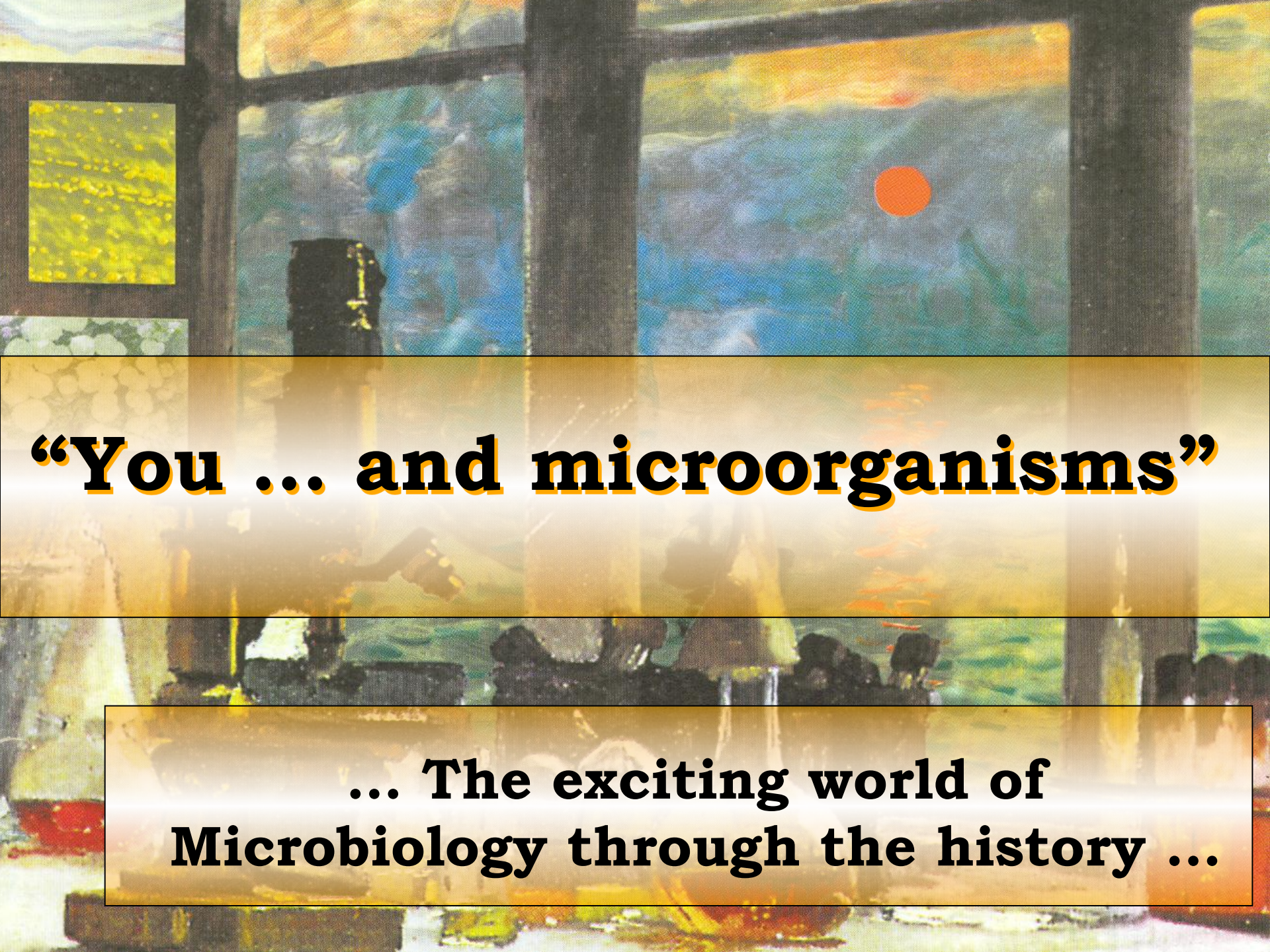
(person with disease → correct genetic lesion)

… How will the Microbiology of the XXI century?

The most prudent will repeat the phrase of Louis Pasteur:

… "Microorganisms which will have the final word" ("Les microorganismes qui auront le dernier mot» …)

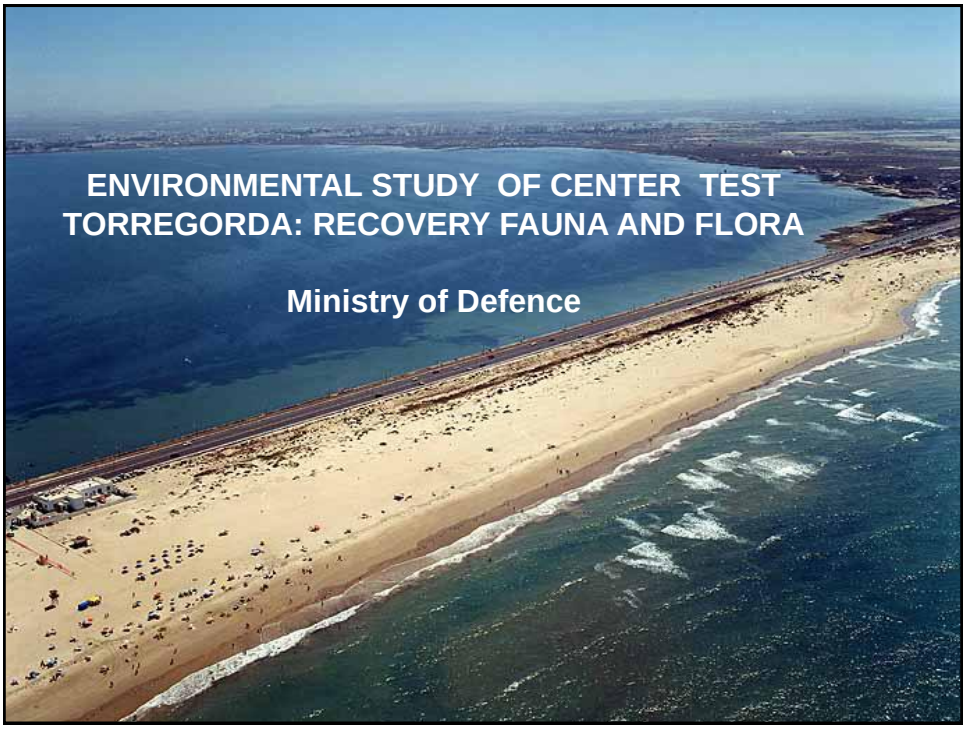

... Thank you very much for your attention!!!
...
... Thank you very much for your patience!!!



“You … and microorganisms”

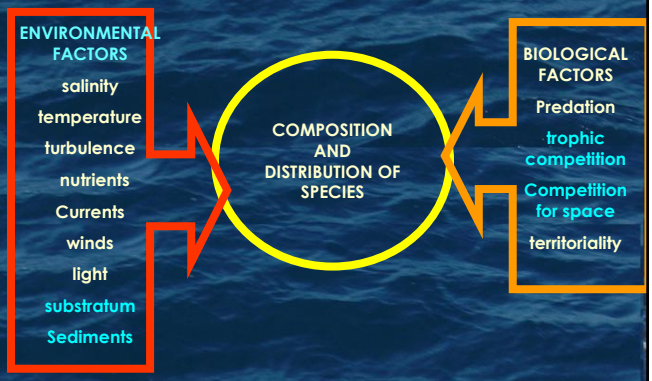
… The exciting world of Microbiology through the history …


UCA
Universidad de Cádiz
DINÁMICA DE POBLACIONES DE PECES
Departamento de Biología
UCA
Facultad de Ciencias del Mar y Ambientales
INTERTIDAL ASSEMBLAGES IN THE GULF OF CADIZ
Remedios Cabrera Castro


ENVIRONMENTAL STUDY OF CENTER TEST TORREGORDA: RECOVERY FAUNA AND FLORA
Ministry of Defence

INTRODUCTION

Many species of intertidal fish are territorial. Determining the microhabitat preferences may explain one of the components that allow coexistence.



The rocky intertidal species characteristic of the area, in general, have spawning benthic. The temporal segregation of reproductive cycles can be a key element in the reproductive success of the species coexist.

INTERTIDAL ICHTYOFAUNA OF A ROCKY PLATFORM OF GULF OF CADIZ

OBJECTIVES

FINAL OBJECTIVE:

✓ Determine the factors that allow different species of fish coexist in an area with such harsh abiotic conditions and fluctuating as is the rocky intertidal.

SPECIFIC OBJECTIVES:

➢ Determine resident fish species that inhabit the intertidal zone off the coast of Cadiz.

➢ To determine the factors affecting the spatial distribution of intertidal fish stocks.

➢ Compare the differences in the intertidal fish assemblages between two areas with similar characteristics, one of them subjected to intense human pressure and other protected.

METHODOLOGY

Field Sampling

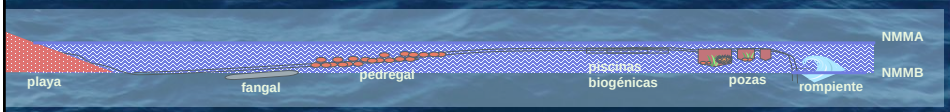
Location of the study area

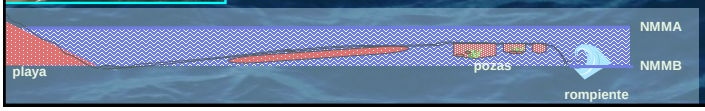
Sampling was conducted monthly, coinciding with the diurnal tide alive

Field sampling

There is little variation in the degree of exposure and other physical characteristics (temperature and salinity) and biological (algal cover, density of sea urchins, etc.) in vertical gradient, so that the samples were developed in parallel to the coastline.

Took the location of each pool sampled with a GPS.





Field Sampling

Pools tidal

SIZE

wide (a)
length (b)
maximum depth (c)

ATTRIBUTES MICROHABITATS

Rock pool

Sand pool

with pebbles

with algae

with sea urchins

For CAPTURE OF EXEMPLARY, use natural clove eugenol 87% (40 mg L-1 (Griffiths, 2000)) and hand landing nets.

Once captured fish preserved in ice water until the laboratory where they freeze.

Laboratory work

DATA COLLECTION

GROWTH

- ✓ TL: total lenght
- ✓ SL: standard length

WEIGHT

- ✓ TW: total weight
- ✓ EW: gutted weight
- ✓ DW (DW_{ll}-DW_v): digestive weight (full – empty)
- ✓ GonW: gonad weight

Laboratory work

DATA PROCESSING

GROWTH	LENGTH-WEIGHT RELATIONSHIP	$W = a * L^b$
	CONDITION INDEX	$K = (W/ L^3) * 10^5$
	VICEROSOMATIC INDEX	$VSI = (EP / L^3) * 10^5$
	GONADSOMATIC INDEX	$GSI = (W_{gon} / TW) * 100$
DIVERSITY	Jaccard similarity index	$S_{ij} = a / (a+b+c)$
	SHANNON-WIENER INDEX	$H = -\Sigma p_i \log^2 p_i$

USING STATISTICAL PROGRAMS (STATGRAPHICS 5.0, SPSS 11.5, FiSAT)

CHARACTERISTICS OF POOLS

Size of the pools

	TORREGORDA	The CHATO	DIFFERENCES
Average volume of the pools (L)	371	399	NO
Average area of the pools (m2)	1.54	1.90	YES
Average depth of the pools (m)	0.25	0.20	YES
Number of pools sampled	87	79	
Sampled TOTAL VOLUME (L)	32.307	31.564	

CHARACTERISTICS OF PONDS

Physiography of the pools

1. with stones-pebles
2. with algae
3. with algae and sea urchins
4. with pebbles and algae cover less than 50%
5. with pebbles and abundant algal cover
6. with pebbles
7. with pebbles and algae
8. bare sand

Physiography different are not associated to a specific pool size.

No statistically significant differences (ANOVA $p > 0.05$) on the depth, area and volume of the pool with the physiography, in both areas separately and in the Platform.

TORREGORDA: most abundant rock pools with abundant algae, many with sea urchins (Type 2 and 3, 60%).

EL CHATO: more than 70% of pools with sand and pebbles and vegetation, very few rock pools and absent those with sea urchins

PLATFORM : most abundant pools as those formed as a combination of 3 attributes set (types 3 and 7), and unless they are composed only of sand or rock

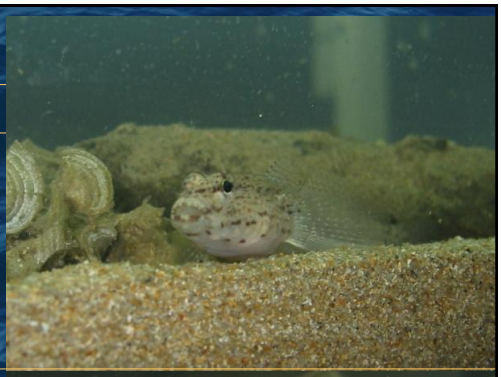
ABUNDANCE AND OCCURRENCE

ESPECIE	ABUNDANCIA COMO %DE CAPTURA			%OCCURRENCIA SOBRE EL TOTAL DE POZAS		
	Torregorda	El Chato	Plataforma	Torregorda	El Chato	Plataforma
Gobius paganellus	36,25	62,72	51,44	71.26	98.67	81.93
Gobius cobitis	4,71	2,25	3,30	19.54	8.86	14.46
Gobius bucchichi	0,90	7,09	4,45	4.60	48.10	25.30
Lipophrys pholis	9,20	6,84	7,85	32.18	27.85	30.12
Lipophrys canevae	0,90	0,83	0,86	5.75	6.33	6.02
Paralipophrys trigloides	9,88	2,34	5,55	48.28	22.78	36.14
Parablennius incognitus	15,49	5,50	9,76	48.28	30.38	39.76
Parablennius sanguindentus	1,12		0,48	5.75		3.01
Coryphoblennius galerita	5,95	1,17	3,21	29.89	10.13	20.48
Salaria pavo	6,73	4,17	5,26	25.29	29.11	27.11
Clinitrachus argentatus	0,90	2,50	1,82	8.05	22.78	15.06
Tripterygion melanurus	0,11		0,05	1.15		0.60
Tripterygion delaisi	1,35	2,00	1,72	8.05	15.19	11.45
Lepadogaster lepadogaster	0,45	0,17	0,29	3.45	2.53	3.01
Lepadogaster purpurea	3,59		1,53	16.09		8.43
Lepadogaster candolii	0,34	0,17	0,24	3.45	1.27	2.41
Symphodus roissali	2,13	2,25	2,20	5.75	21.52	13.25

Gobius paganellus

Gobius cobitis

Gobius bucchichi



Lipophrys pholis

Paralipophrys trigloides

Parablennius incognitus

Salaria pavo

Coryphoblennius galerita

OTHER SPECIES

- *Lipophrys canevae*
- *Parablennius sanguinolentus*
- *Clinitrachus argentatus*
- *Tripterygion delaisi*
- *Familia Gobiesocidae*
- *Symphodus roissali*

USE OF SPACE

Size of the pools

		ABUNDANCE For family	ABUNDANCE For species
DEPTH OF THE POOLS	WEALTH species	*Blennidae*	
SURFACE POOLS	TOTAL ABUNDANCE WEALTH species El Chato	*Gobiidae*	*G. paganellus* *G. bucchichi* *S. roissali* *L. canevae*
VOLUME OF THE POOLS	TOTAL ABUNDANCE WEALTH species	*Gobiidae, Bleniidae*	*G. paganellus, G. bucchichi* *S. roissali, G. cobitis* *P. incognitus*

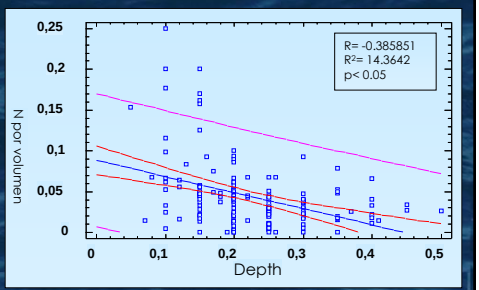
USE OF SPACE

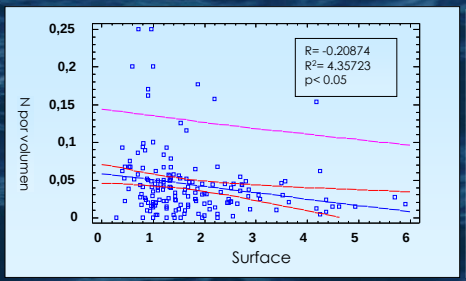
Size of the pools

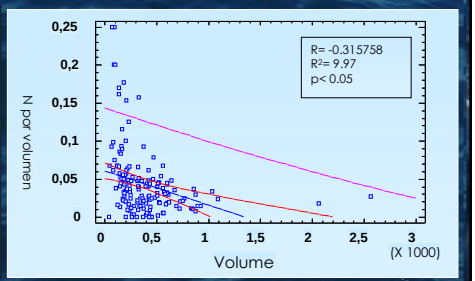
DENSITY

Although the abundance increases with the size of the pool, the density decreases.

Each pool offers a limited number of shelters for each species and size, and those left without shelter, have to travel and seek other pools.



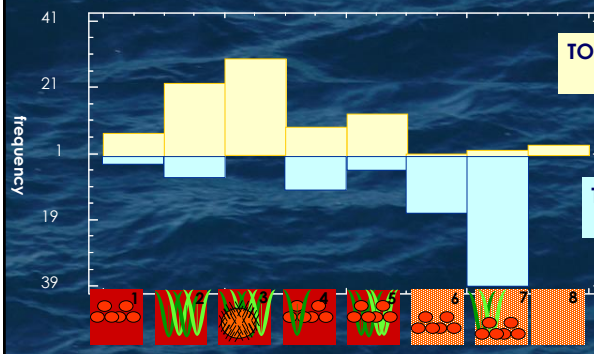




USE OF SPACE

Physiography

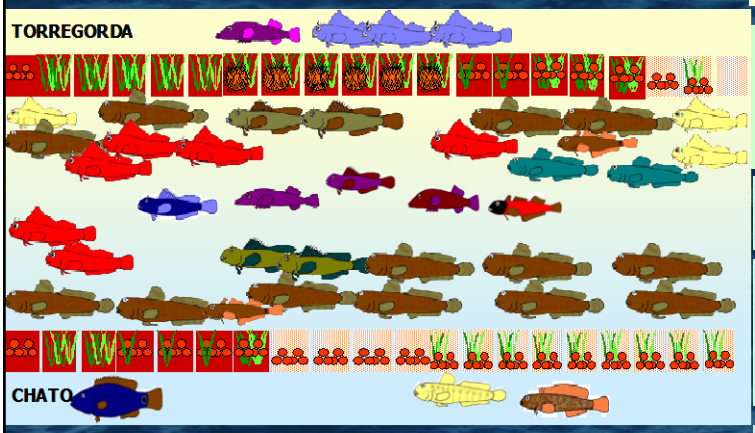
NO STATISTICALLY SIGNIFICANT DIFFERENCES (ANOVA p-value> 0.05) OR HALF OF PLENTY BY DENSITY OF POOLS PHYSIOGRAPHY)



NUMBER OF POOLS PlatForm	9	29	29	19	17	18	42	3
AVERAGE NUMBER OF SPECIES PlatForm	2.2	3.9	3.7	3.4	3.8	3.9	3.1	2

USE OF SPACE

Fisiografía



C. galerita
L. purpurea

FREQUENTLY
G. paganellus
L. pholis
P. trigloides
P. incognitus
S. pavo

LESS FREQUENTLY
G. cobitis
T. delaisi

RARE
L. canevae
P. sanguinolentus
T. melanurus
L. lepadogaster
L. candolii

S. roissali
G. bucchichi
C. argentatus

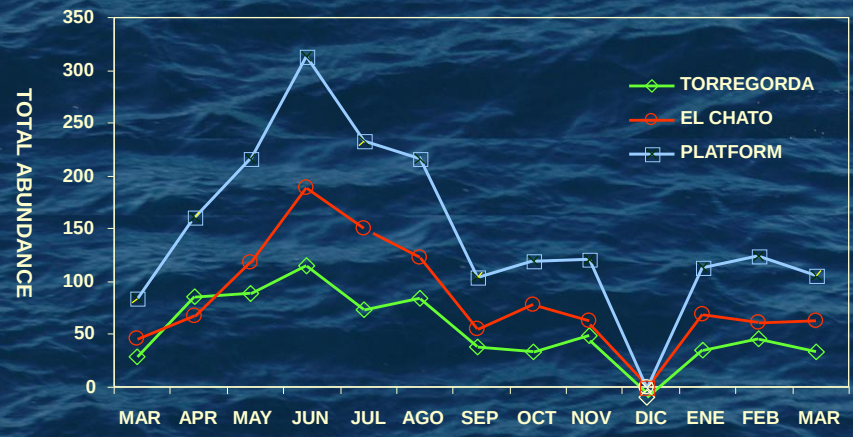
USE OF SPACE

Physiography

Statistically significant differences

USE OF SPACE

Temporal variation



USE OF SPACE

Temporal variation

ANNUAL

G. paganellus
G. cobitis
G. bucchichi
L. pholis
P. trigloides
P. incognitus
S. pavo
L. purpurea
T. delaisi
S. roissali

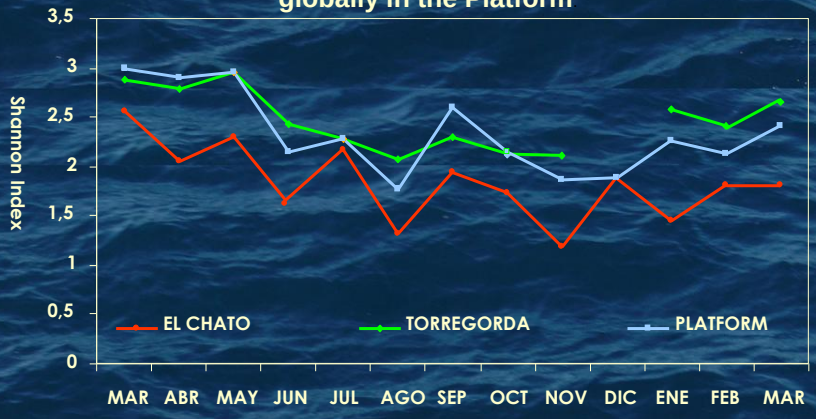
SEASONAL

C. galerita
L. canevae
C. argentatus
L. candolii

SUMMARY

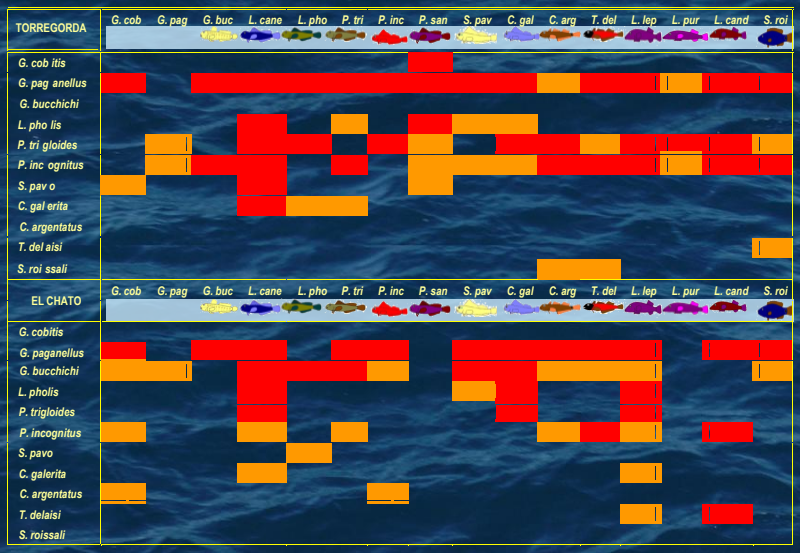
Diversity

Monthly evolution of diversity (H) in Torregorda, El Chato and globally in the Platform



SUMMARY

Co-ocurrence



SUMMARY

G. paganellus

G. cobitis

L. pholis

P. trigloides

C. galerita

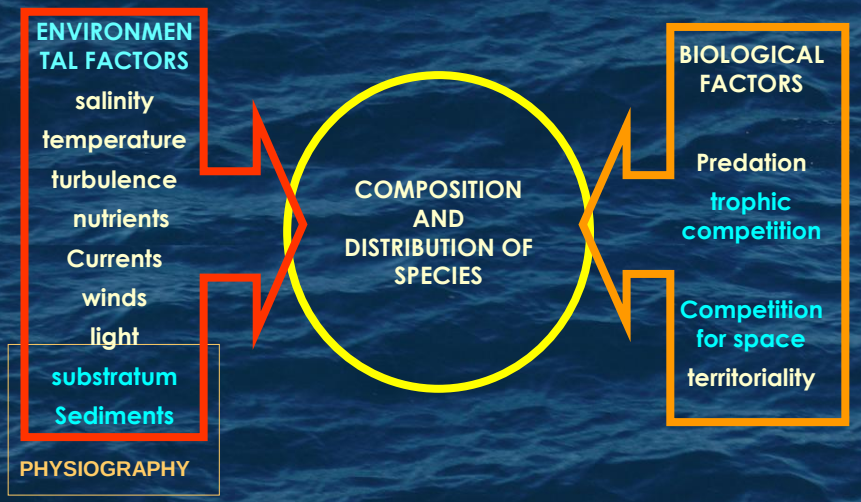
concerning latitudes populations↑

T^a

- COME ON THE TIME TO START
- DILATES IN TIME IS THE TIME TO START
- WILL EXTEND THE PERIOD OF RECRUITMENT
- FIRST SIZE OF MATURITY
- MAXIMUM SIZE SMALL

RESULTS

SUMMARY



CONCLUSIONS

- **In the temporal evolution of the catch is a greater abundance in the warmer months from late spring to August, reaching maximum abundance in June, and the lowest in December, possibly by an effect flight to deeper water by the high rainfall.**

- **Torregorda monthly exhibits greater diversity Chato and greater stability throughout the year. The highest values are reached in the spring months of March to May, possibly because in these months the proportion of *G. paganellus* is lower.**

- **The species present in the pools of Torregorda (restricted area) and Chato (free access), at low tide are virtually the same except for *P. sanguinolentus* and *L. purpurea*. The latter is associated with the pools with sea urchins, so its absence in El Chato may be due to the pressure of suffering this area shellfish.**

CONCLUSIONS

- The assembly of rocky intertidal fish Torregorda can represent the original assembly area, as the area is not affected by human impacts (shellfish, beach nourishment, ...) that are affecting Chato itself.

- **For its abundance, occurrence and temporary occupation of the intertidal zone, we consider *G. paganellus, G. bucchichi, G. cobitis, Salaria pavo, Lipophrys pholis, Paralipophrys trigloides and Parablennius incognitus,* are the dominant species in the assembly, while *G. bucchichi* is associated with sandy bottom pools of Chato.**


Thank you
very much