Laboratorio de Biotecnología Agroalimentaria

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GALICIA

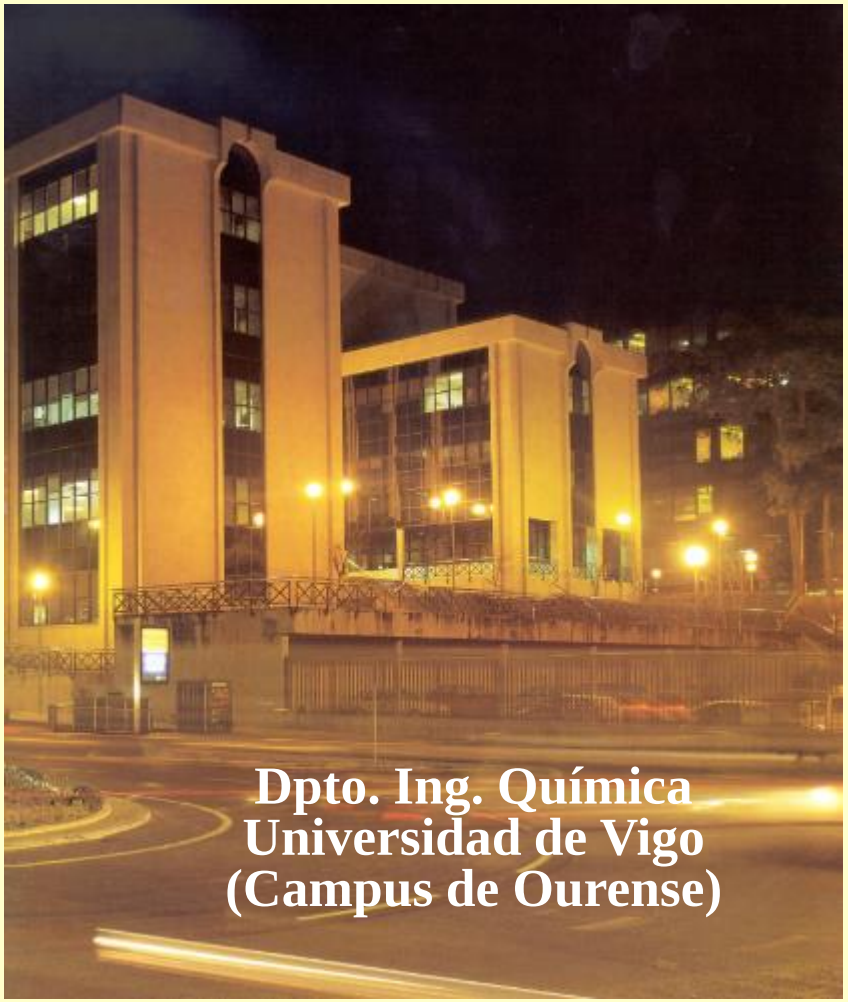
MAR CANTÁBRICO

ORTIGUEIRA
CEDEIRA
VIVEIRO
O FERROL
A CORUÑA
MALPICA
PONTED
CORME
LAXE
CARBALLO
CAMARIÑAS
VIMIANZO
ORDES
A-9
CURTIS
RÁBADE
FONSAGRADA
LUGO
FISTERRA
CEE
SANTIAGO DE COMPOSTELA
MELIDE
BECERREÁ
MUROS
NOIA
PADRÓN
LALÍN
CHANTAD
VILAGARCÍA
CALDAS DE REIS
O GROVE
PONTEVEDRA
EQ7
MONFORTE
O CARBALLIÑO
O BARCO
OURENSE
VIGO
REDONDELA
RIBADAVIA
PONTEAREAS
CELANOVA
XINZO DE LIMIA
GUDIÑA
BAIONA
TUI
A GUARDA
VERÍN
PORTUGAL
OCÉANO ATLÁNTICO

UNIVERSIDADE DA CORUÑA

UNIVERSIDADE DE VIGO

Laboratorio de Biotecnología Agroalimentaria



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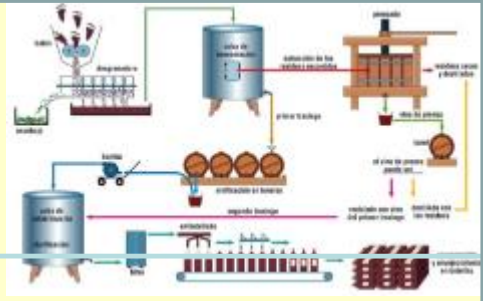


Línea de Biotecnología

Revalorización de residuos de la industria agroalimentaria

Desarrollo de procesos biotecnológicos para la obtención de aditivos alimentarios

ADITIVOS ALIMENTARIOS

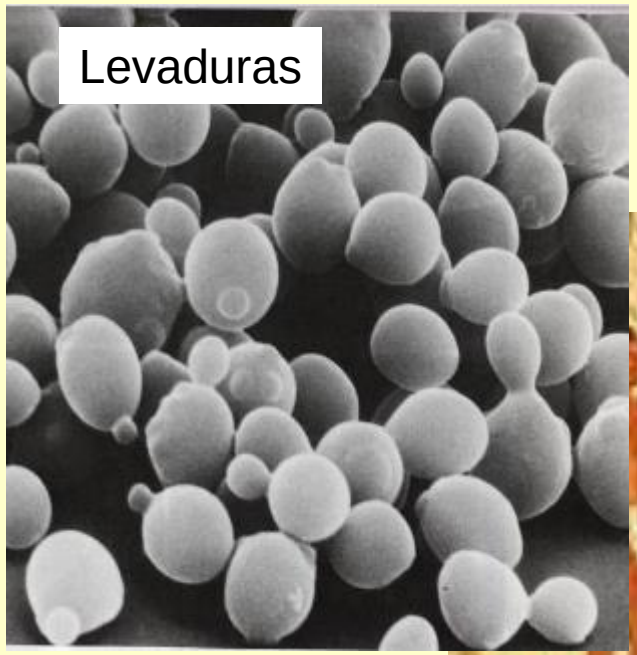
Aromas y análisis sensorial de alimentos

Elaboración de bebidas alcohólicas fermentadas y destiladas

Línea de Aromas y Sensorial

1.- Introducción

Los procesos fermentativos han sido utilizados históricamente para producir bebidas alcohólicas y alimentos fermentados en gran escala.



Biotecnología

Una fermentación es un proceso biológico o bioproceso que consiste en la descomposición de la materia orgánica por microorganismos fermentadores (bacterias y hongos).

ESQUEMA GENERAL PARA LA OBTENCIÓN DE ADITIVOS

APROVECHAMIENTO DE MATERIALES RESIDUALES

PROCESOS FERMENTATIVOS

ADITIVOS ALIMENTARIOS

Xilitol

Antimicrobianos

ÁCIDO LÁCTICO

BACTERIOCINAS

ÁCIDO FENILÁCTICO

BIOSURFACTANTES

Materiales residuales de carácter lignocelulósicos

Sustancias de origen vegetal, similares en composición

- **Celulosa**
- **Hemicelulosas y**
- **Lignina**

Fraccionamiento

Naturaleza polimérica

- **Agrícola (residuos de cosechas, paja, restos de podas)**
- **Industrial (bagazo, cebada, cortezas, serrín)**
- **Forestal (bosques, 70 % del total generado)**
- **Urbano (> 50% basuras, papel, césped)**

RESIDUOS AGROINDUSTRIALES

Definición

"Son aquellos residuos que, siendo o no peligrosos, se generan en los diversos procesos de fabricación industrial o actividad agrícola, y de los cuales quienes los producen, tienen voluntad de desprenderse"

Atractivos

- ✓ Azúcares
- ✓ Pigmentos
- ✓ Fibra alimentaria
- ✓ Proteína
- ✓ Polifenoles...

Tecnología

Microorganismos

Productos de elevado valor añadido

- ✓ Campo alimentario
- ✓ Médico
- ✓ Sector químico

Reducción del impacto ambiental

Biotecnología

Materias primas

- ✓ Renovables
- ✓ Bajo coste
- ✓ Fácil adquisición
- ✓ Utilizables como sustratos fermentables

RESIDUOS AGROINDUSTRIALES

Sector vitivinícola

Podas de sarmiento (VIDEIRA)

Industria láctea

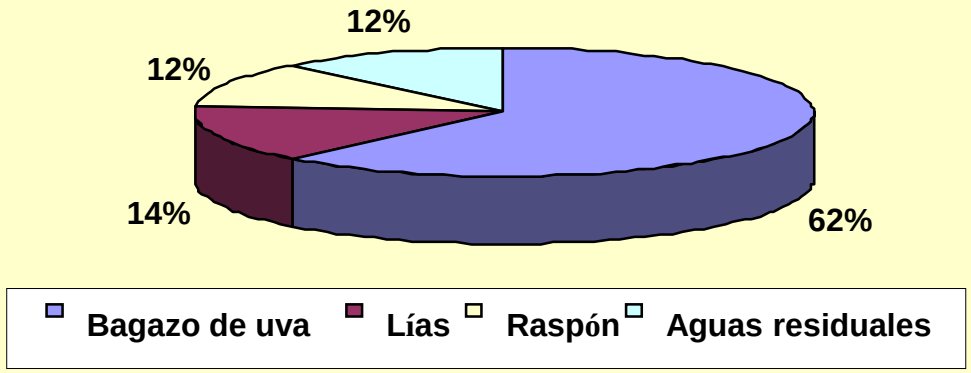
RESIDUOS AGROINDUSTRIALES

- **RESIDUOS VITIVINÍCOLAS**
  - **VITICULTURA**
    - **HOJAS**
    - **SARMIENTO**
    - **RACIMOS AGRACES**
  - **VINICULTURA**
    - **RASPONES**
    - **BAGAZO**
    - **LÍAS**

→ **Destilerías**

Sarmiento

- **2.000 a 4.000 kg/ha**
- **Galicia: 65.000 T/año**



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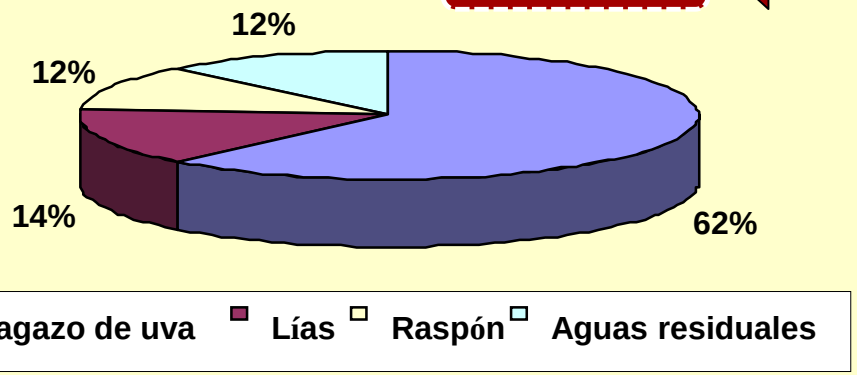
  - **VINICULTURA**
    - RASPONES
    - BAGAZO
    - **LÍAS**

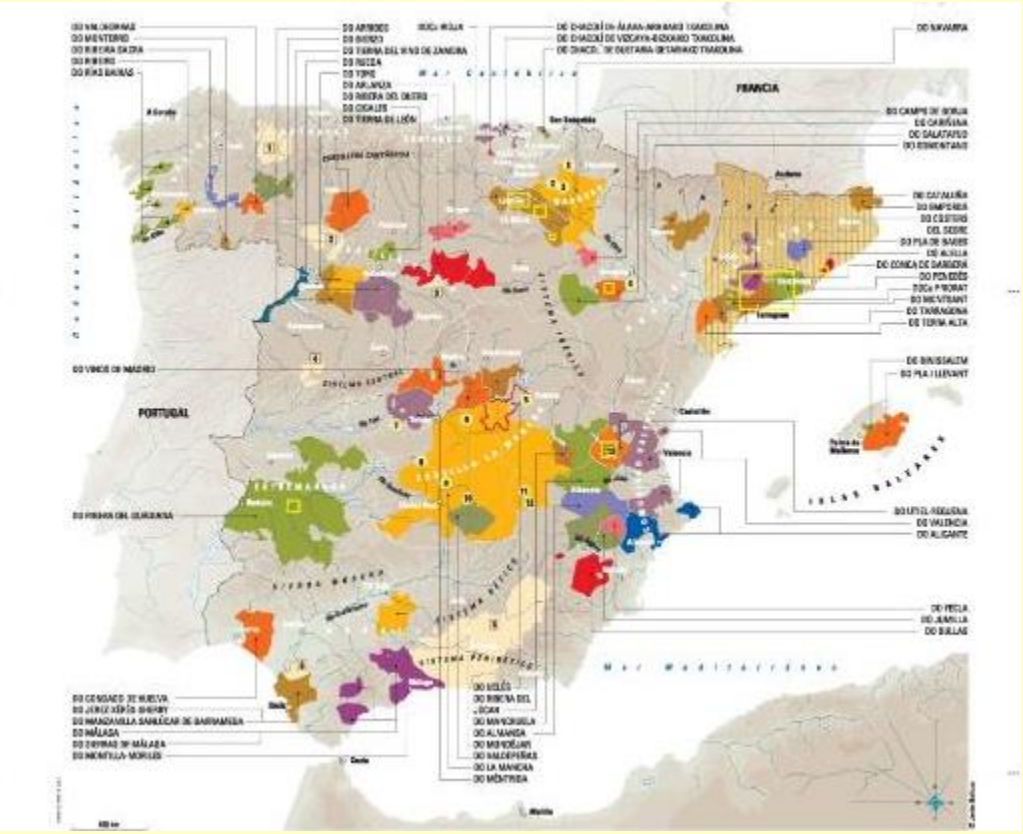
Destilerías → **VINAZAS**

Sarmiento

- **2.000 a 4.000 kg/ha**
- **Galicia: 65.000 T/año**



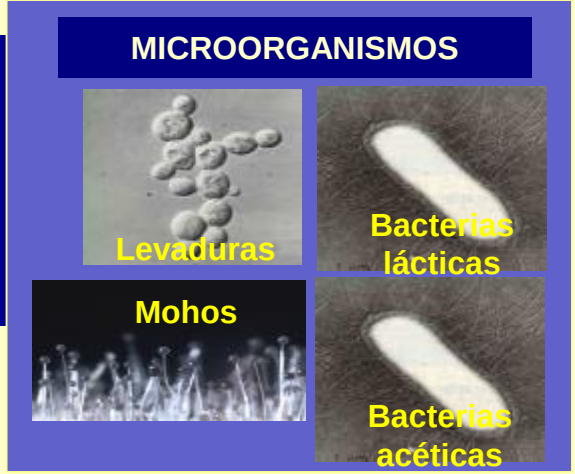
Distribución de los viñedos en España



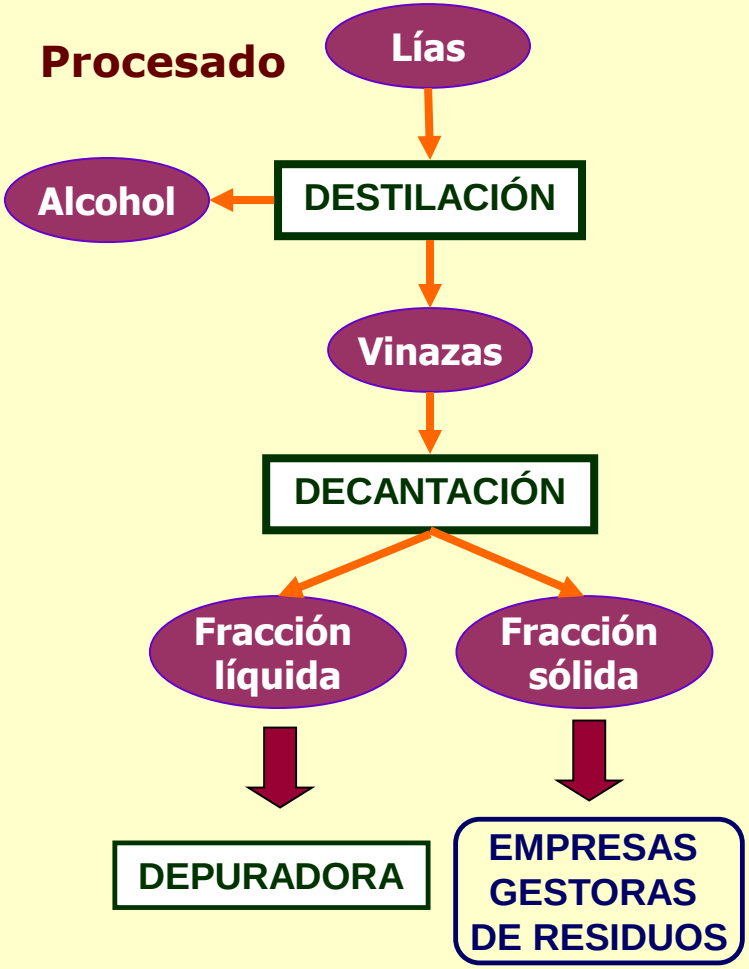
Lías vino, sidra y Cerveza

VINAZAS

pH: 3 – 6
DQO > 30,000 mg/L
Materia orgánica: 900 - 35,000 mg/L
K > 2,500 mg/L;
Componentes fenólicos: 1000 mg/L
T > 90 ºC



Procesado



Composición

BIOMASA MICROBIANA

- Levaduras/mohos
- Bacterias lácticas
- Bacterias acéticas

SUSTANCIAS EN EL VINO

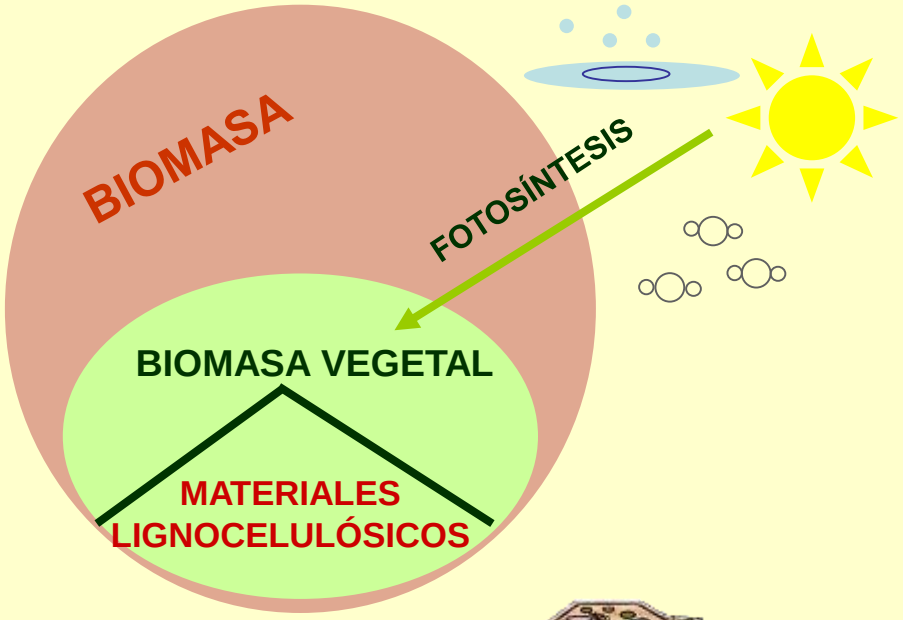
- Compuestos fenólicos
- Sales tartáricas
- Minerales

Aplicaciones

Aprovechamiento integral

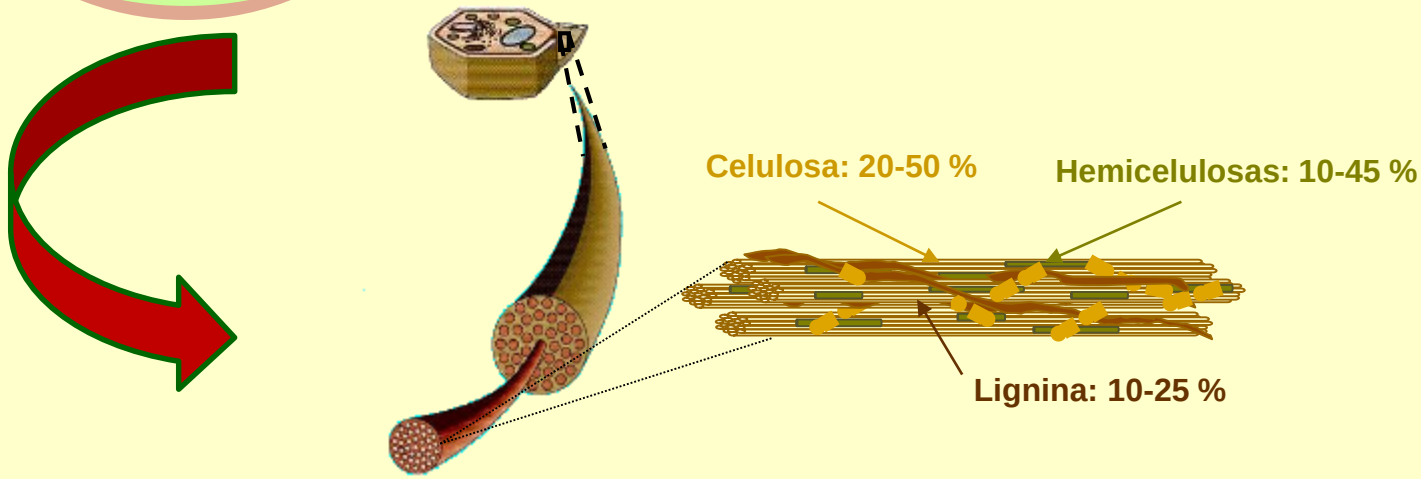
NUTRIENTE EN PROCESOS BIOTECNOLÓGICOS

PODAS DE SARMIENTO



Interés

- ✓ Abundancia
- ✓ Fácil accesibilidad
- ✓ Elevada capacidad de regeneración
- ✓ Carácter renovable
- ✓ Bajo coste por unidad de masa



PODAS DE SARMIENTO

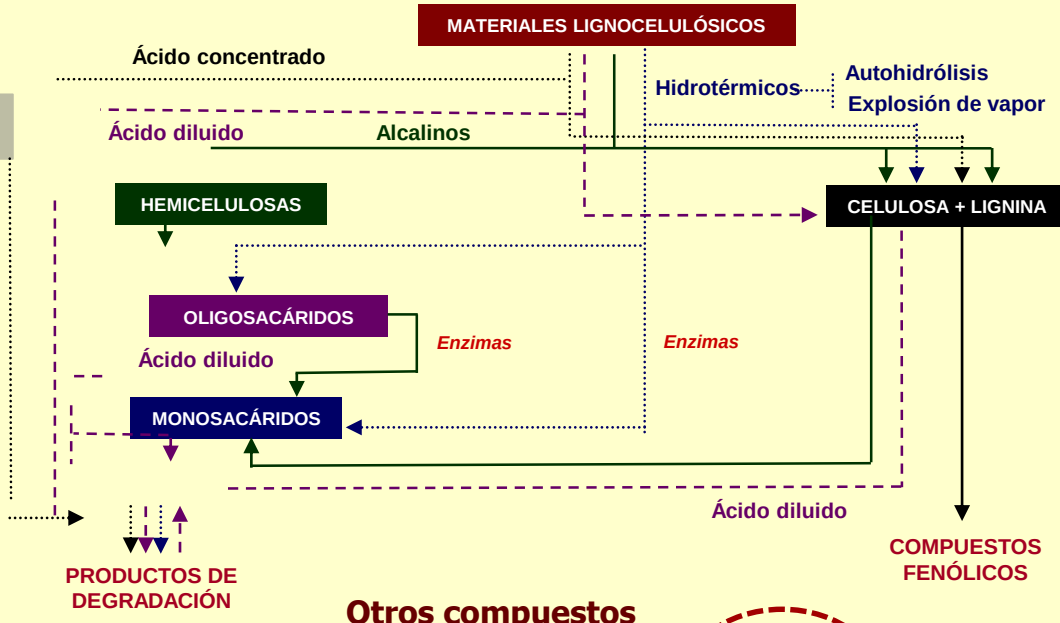
Aplicaciones

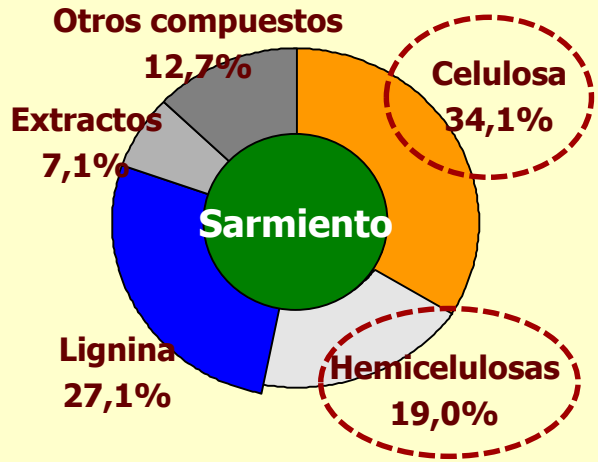
APROVECHAMIENTO BIOTECNOLÓGICO

Generación de disoluciones de azúcares

Formulación de medios fermentables

Metabolitos de interés industrial





SUERO DE QUESERÍAS

55 %

SUERO

- ✓ Lactosa (45-50 g/L)
- ✓ Proteínas (6-10 g/L)
- ✓ Lípidos (4-5 g/L)
- ✓ Sales minerales (8-10 % extracto seco)

SUSTRATO BIOTECNOLÓGICO → Procesos microbianos → **Productos de valor añadido**

El suero de queserías es un subproducto de la industria láctea que representa entre un 80-90% del volumen lácteo transformado y constituye uno de los residuos más severos que existen.

Este suero retiene cerca del 55 % de los nutrientes de la leche de partida.

Debido a esto puede ser utilizado como sustrato en una gran variedad de procesos microbianos para la obtención de productos de valor añadido.

LAS BACTERIAS LÁCTICAS

- **Son un grupo heterogéneo de compleja taxonomía**
- **Alta tolerancia acidez**

Ácido láctico

PRINCIPALES GÉNEROS

- ***Lactobacillus***
- ***Leuconostoc***
- ***Pediococcus***
- ***Streptococcus***

- **Son un grupo heterogéneo de compleja taxonomía**
- **Alta tolerancia acidez**
- **Requieren complejos medios de cultivo**

Ácido láctico

REQUERIMIENTOS NUTRICIONALES

- Carbono
- Nitrógeno
- P y S
- Otros minerales
- Iones metálicos
- Factores de crecimiento
- Vitaminas

MEDIO COMERCIAL "MERCIER"

Fuente de Nitrógeno:

Peptona y Extracto de levadura

Alto coste

OTROS:

CSL/Vinazas

Menor coste

- **Obtienen la energía a nivel de sustrato, a través de la fermentación de azúcares**
- **En función de cómo lleven a cabo esta fermentación:**

Fermentación homofermentativas

Fermentación heterofermentativas

Ácido láctico $Y_{Teórico(AL/S)}$ = 1 g producto/g sustrato

Ácido láctico + Otros productos $Y_{Teórico(AL/S)}$ = 0,5 g producto/g sustrato

CO_2, EtOH y/o AcH

Fermentación Homoláctica

(Embden Meyerhof-Parnas)

GLUCOSA
ATP
ADP
glucosa-6-P
fructosa-6-P
ATP
ADP
fructosa-1,6-DP
gliceraldehido-3-P ⇌ dihidroxiacetona-P
Pi
2NAD
2NADH+2H+
2 1,3-difosfoglicerato
2ADP
2ATP
2 3-fosfoglicerato
2 2-fosfoglicerato
2 H_2O
2 fosfoenolpiruvato
2ADP
2ATP
2 piruvato
2NADH+2H+
2NAD+
2 LACTATO

Fermentación Heteroláctica

GLUCOSA
ATP
ADP
glucosa-6-P
NAD+
NADH+H+
6-fosfogluconato
NAD+ NADH+H+
ribulosa-5-P
PENTOSAS
(Pentosas Fosfato)
ATP
ADP
xilulosa-5-P
Pi
acetilfosfato
CoA
Pi
acetil-CoA
NADH+ H+
NAD+
CoA
acetaldehído
NADH+H+
NAD+
ETANOL
ADP
ATP
CoA
ACETATO
gliceraldehído-3-P
2ADP
2ATP
NAD+
NADH+H+
piruvato
NADH+H+
NAD+
LACTATO

- **Son un grupo heterogéneo de compleja taxonomía**
- **Alta tolerancia acidez**
- **Requieren complejos medios de cultivo**

Ácido láctico

REQUERIMIENTOS NUTRICIONALES

- Carbono
- Nitrógeno
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MEDIO COMERCIAL "MERCIER"

Fuente de Nitrógeno:

Peptona y Extracto de levadura

Alto coste

OTROS:

CSL/Vinazas

Menor coste

- **Además de estos productos pueden producir, por fermentación, otros metabolitos como es el caso de las bacteriocinas y los biosurfactantes**

ADITIVOS ALIMENTARIOS

- ***Aditivo*: Compuesto que se añade a un producto intencionadamente con el objetivo de mejorar sus características así como de facilitar su proceso de elaboración y/o conservación**

- **Desempeñan un papel fundamental en el mercado de abastecimiento actual:**
  - **Obtener cualidades**
  - **Mantener calidades**
  - **Proporcionar características**

Consumidores
Legislación

- **Estos aditivos a menudo son producto del metabolismo microbiano**

Bacterias lácticas
Levaduras
Mohos

Ácido láctico

Ácido feniláctico

Biosurfactantes

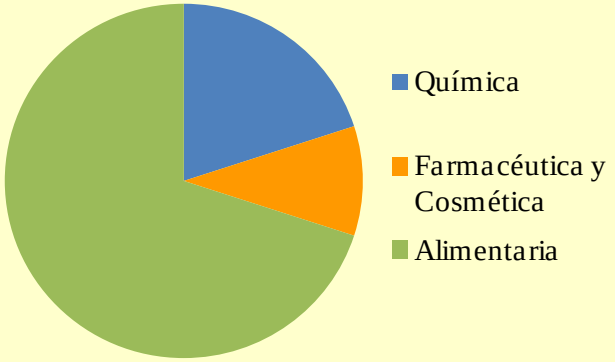
Bacteriocinas

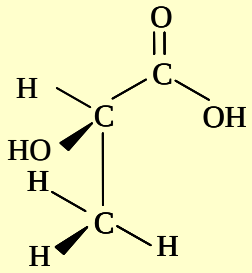
ÁCIDO LÁCTICO

El ácido láctico es un producto intermedio del metabolismo, principalmente del ciclo de los carbohidratos, con numerosas aplicaciones industriales:

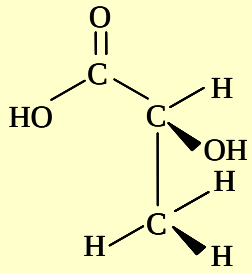
Aplicaciones

- **Industria alimentaria** — **Agente acidulante, estabilizante y conservante**
- **Industria Farmacéutica y Cosmética** — **Fabricación de numerosos productos relacionados**
- **Industria Química** — **Precursor para la síntesis de diferentes moléculas**
  Obtención de polímeros biodegradables y biocompatibles





Isómero D(-)

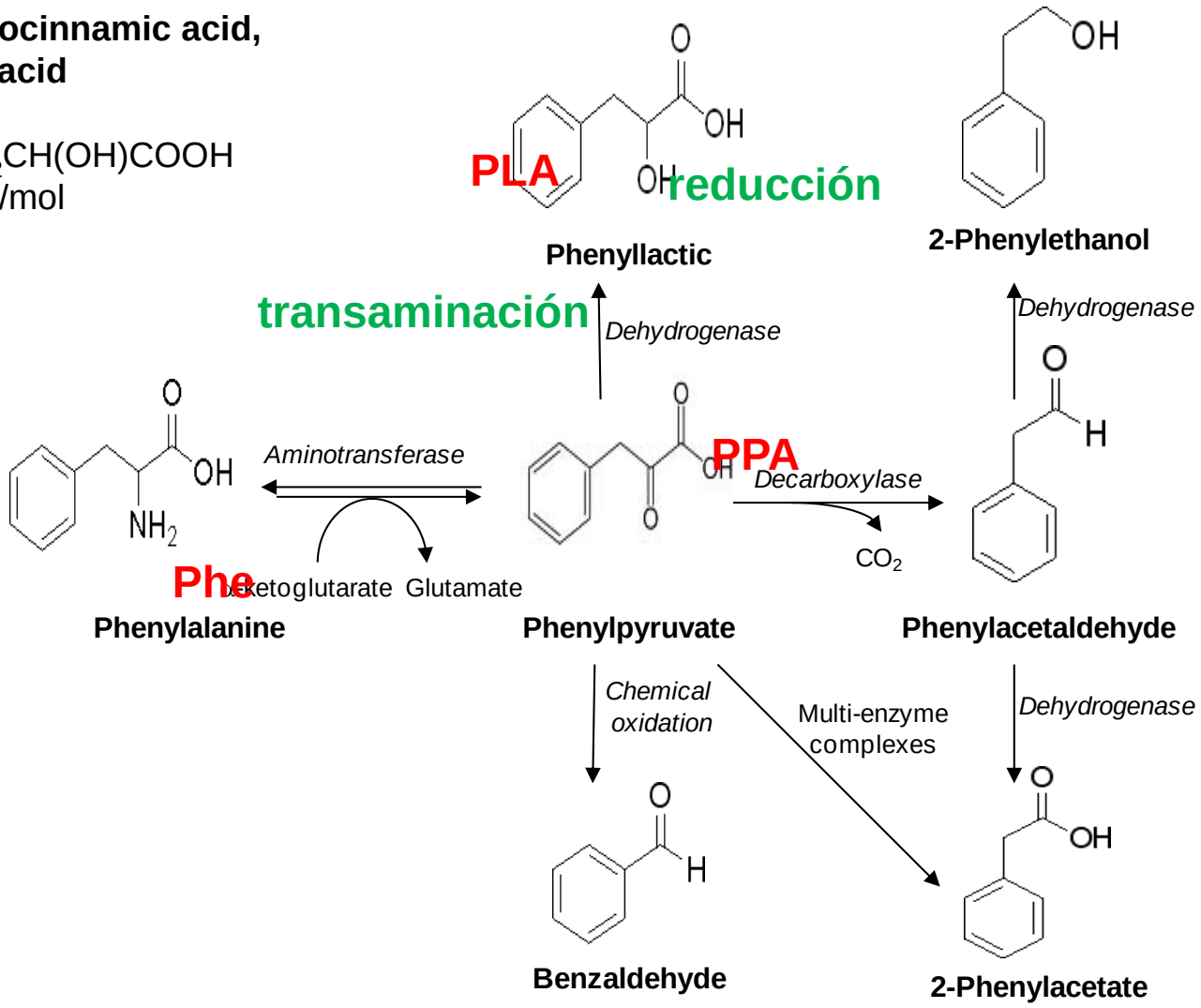


Isómero L(+)

ÁCIDO FENILÁCTICO

- **Sinónimos:**
  - **(±)-2-Hydroxy-3-phenylpropionic acid,**
  - **DL-α-Hydroxyhydrocinnamic acid,**
  - **DL-β-Phenyllactic acid**
- Número CAS: 828-01-3
- **Fórmula** Lineal: $C_6H_5CH_2CH(OH)COOH$
- Peso molecular: 166.17 g/mol

Ruta metabólica



❑ **El ácido feniláctico es un ácido orgánico producto del metabolismo de la fenilalanina.**

ÁCIDO FENILÁCTICO

Aplicaciones

Agente antiséptico

↑ vida útil

APLICACIÓN DIRECTA EN ALIMENTACIÓN

- ✓Leche UHT
- ✓Masa fermentada de pan
- ✓Dieta diaria de cerdos, gallinas ponedoras y pollos de engorde

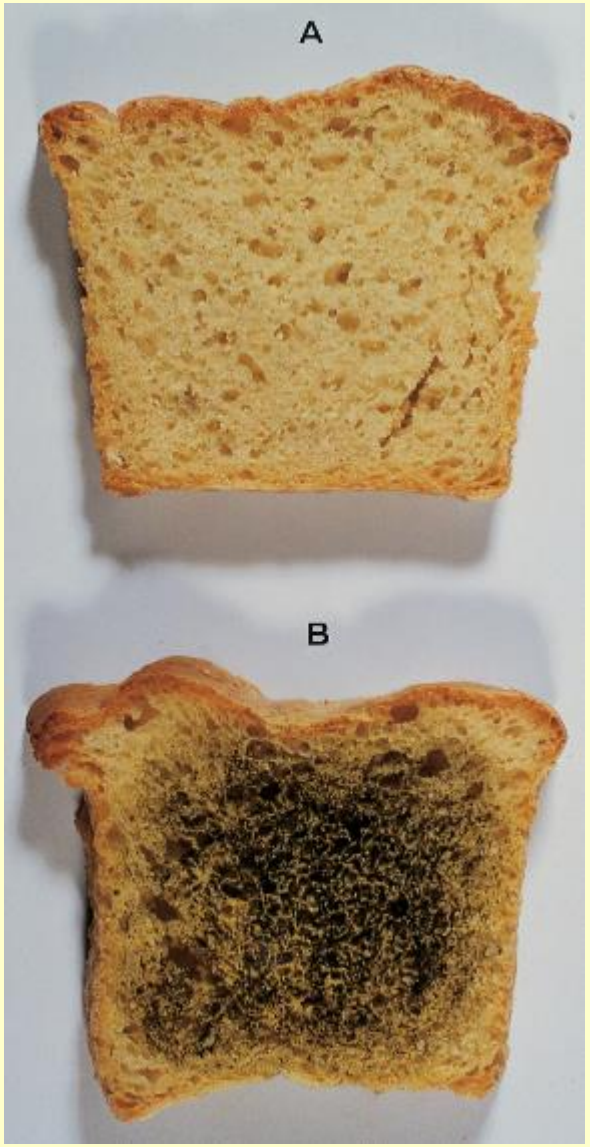
Precursor de la síntesis de fármacos

❑Su principal aplicación se basa en su potencial como agente antiséptico, incrementando la vida útil de los productos sobre los que se aplica.

❑ Existen pocos trabajos sobre su aplicación directa en alimentación, en este sentido se probó su efecto en leches UHT y en masa fermentada de pan, incrementado significativamente su vida útil, además se probó su aplicación directa en la dieta diaria de cerdos, gallinas ponedoras y pollos de engorde, remplazando de este modo los tradicionales antibióticos empleados para el control de enfermedades.

❑ Además es útil como precursor para la síntesis de importantes fármacos.

Efecto antimicrobiano

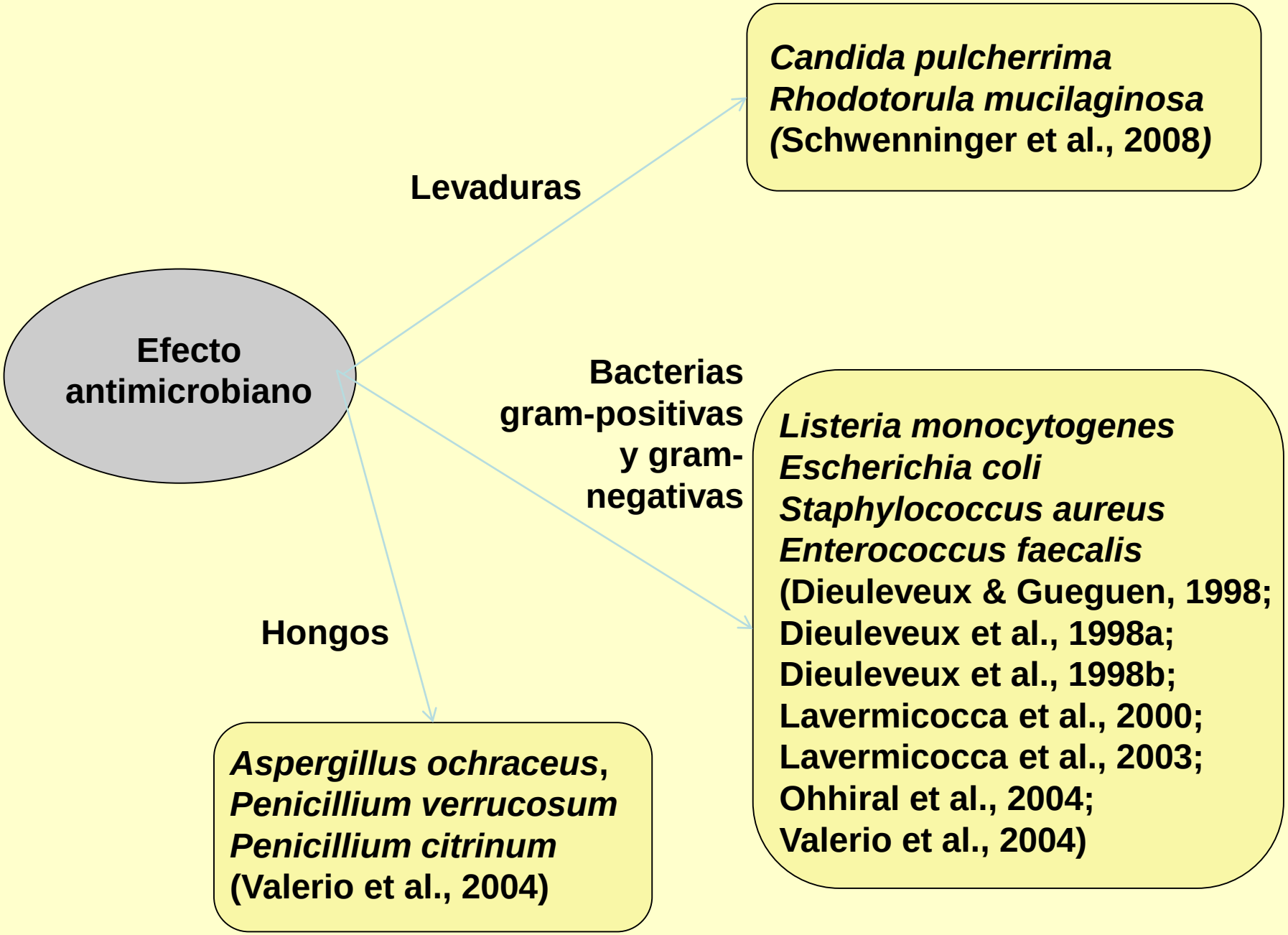


Growth of *Aspergillus niger FTDC3227 in bread*

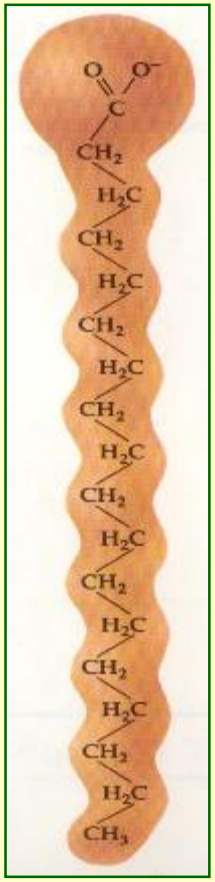
(A) started with S. cerevisiae* 141 and *Lactobacillus plantarum 21B after 7 days of storage

(B)* started with *Saccharomyces cerevisiae 141 alone after 2 days of storage.

LAVERMICOCCA et al., (2000)

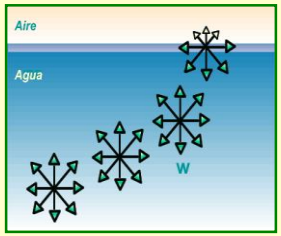

Efecto antimicrobiano
Levaduras
Candida pulcherrima
Rhodotorula mucilaginosa
(Schwenninger et al., 2008)
Bacterias gram-positivas y gram-negativas
Listeria monocytogenes
Escherichia coli
Staphylococcus aureus
Enterococcus faecalis
(Dieuleveux & Gueguen, 1998;
Dieuleveux et al., 1998a;
Dieuleveux et al., 1998b;
Lavermicocca et al., 2000;
Lavermicocca et al., 2003;
Ohhiral et al., 2004;
Valerio et al., 2004)
Hongos
Aspergillus ochraceus,
Penicillium verrucosum
Penicillium citrinum
(Valerio et al., 2004)

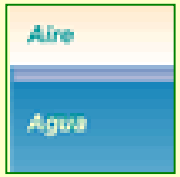
SURFACTANTES



Polar

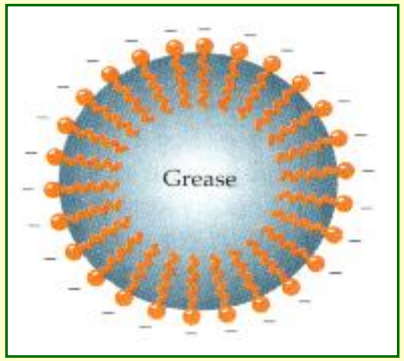
Apolar





- **Interfases de fluidos con diferentes grados de polaridad**
- **Interfases de líquidos con sólidos**

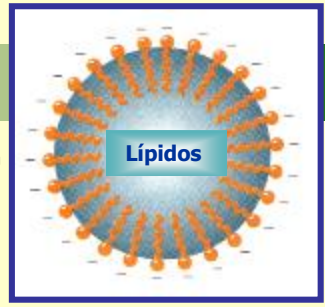
↓TS del medio



❑ **Los surfactantes son moléculas anfipáticas constituidas por una parte polar (pudiendo ser iónica o neutra) y otra apolar, normalmente una cadena hidrocarbonada.**

❑ **Debido a esto se suelen colocar en las interfases de fluidos con diferentes grados de polaridad e incluso en las interfases de líquidos con sólidos, reduciendo sustancialmente la TS del medio en el que se encuentran.**

BIOSURFACTANTES



Microorganismos

- Bacterias: *Pseudomonas, Basillus subtilis, Lactobacillus*
- Hongos
- Levaduras: *Candida lipolytica Candida bambicola*

Naturales → Biosurfactantes

Sintéticos → Detergentes

Importancia e Interés

- Propiedades comunes
- Menor concentración para reducir la tensión superficial
- Toleran cambios de pH, temperatura y fuerza iónica
- Son biodegradables
- Baja toxicidad

METABOLISMO

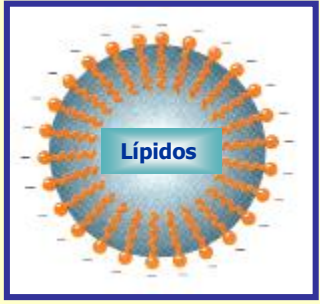
Biosurfactantes:

- Extracelulares
- Intracelulares

PBS

❑ Cuando estos surfactantes son sintetizados por microorganismos y no químicamente, se les denomina biosurfactantes.

Los Biosurfactantes



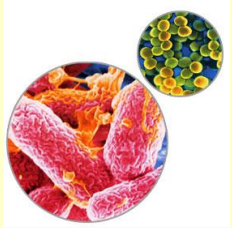
En panadería, pastelería e industria cárnica

- Agentes antimicrobianos
- Agentes dispersantes

Food industry

Cosmetic industry

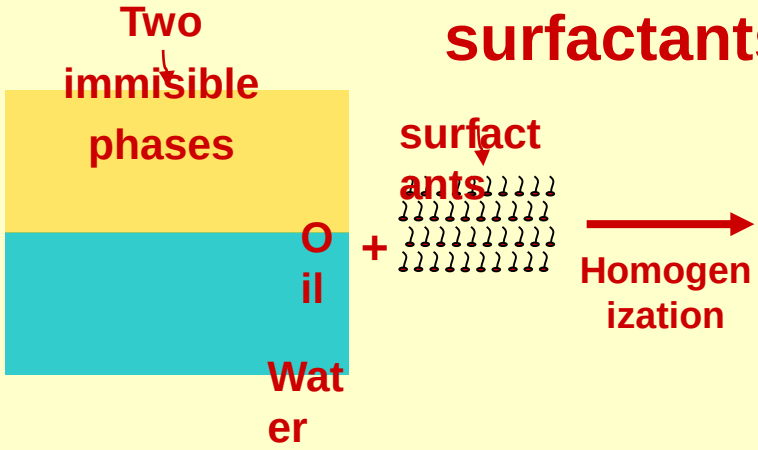
Therapeutic applications



Agriculture

Applications of biosurfactants

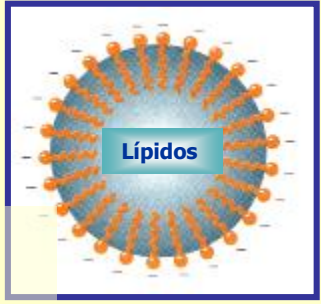
Emulsion formation by surfactants



Oil industry

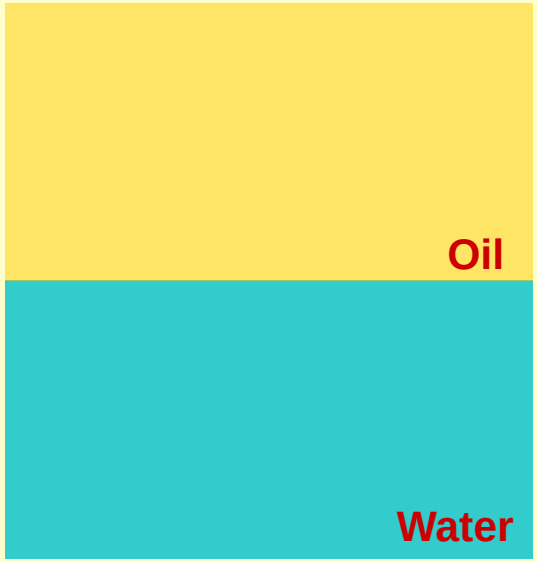
Biorremediation

Los Biosurfactantes

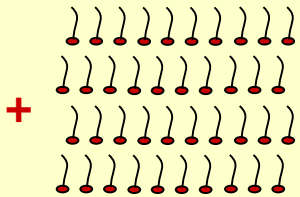


Emulsion formation by surfactants

Two immisible phases

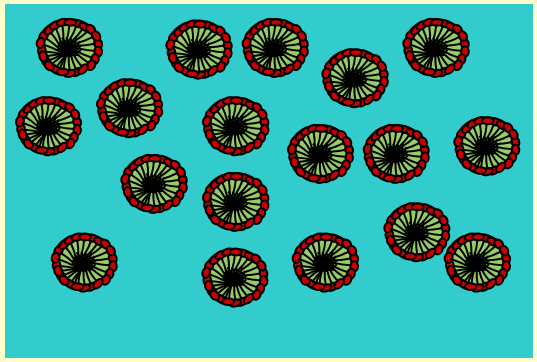


surfactants



Homogenization

Oil droplets trapped in water by surfactants



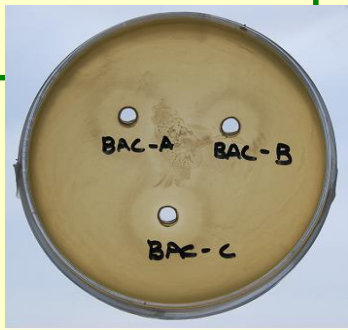
BACTERIOCINAS

❑ Las bacteriocinas son agentes antimicrobianos de naturaleza peptídica, segregados por un gran número de bacterias para inhibir competidores.

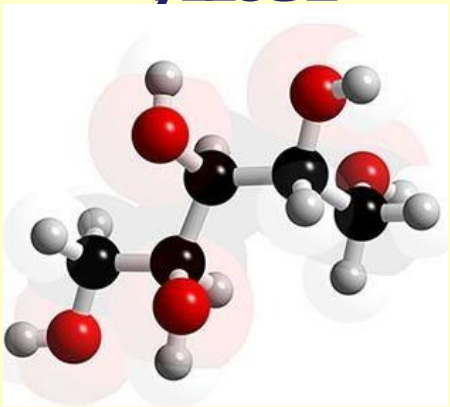
❑ Su empleo en la industria alimentaria se basa en la inhibición de especies bacterianas asociadas a la producción así como para suavizar tratamientos aplicados. Además, su empleo permite reducir la cantidad de nitritos adicionados en carnes.

❑ Sus Aplicaciones clínicas se basan principalmente como alternativa al uso de antibióticos en Control de cepas multirresistentes, en Tratamientos de úlceras, ginecológicos, infecciones de la cavidad oral, infecciones cutáneas, tuberculosis…, entre otras y como antiviral y antifúngico.

- **Industria alimentaria**
  - Inhibir desarrollo especies de *Clostridium*, así como *Listeria monocytogenes*, asociados a la producción
  - Suavizar los tratamientos aplicados
  - Permite reducir la cantidad de nitrito efectiva en carnes
- **Aplicaciones clínicas**
  - Control de cepas multirresistentes
  - Tratamientos de úlceras, ginecológicos, infecciones de la cavidad oral, infecciones cutáneas, tuberculosis…
  - Antiviral y antifúngico



ylitol



Polialcohol ($C_5H_{12}O_5$)

High sweetening power

Food energy ~1.6-2 kcal/g

High negative heat of solution

Anticaries properties

. ood harmaceutical osmetic .ndustry

“Free sugar “ Products

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- ✓ andy
- ✓ akery

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- ✓ . oisturizer

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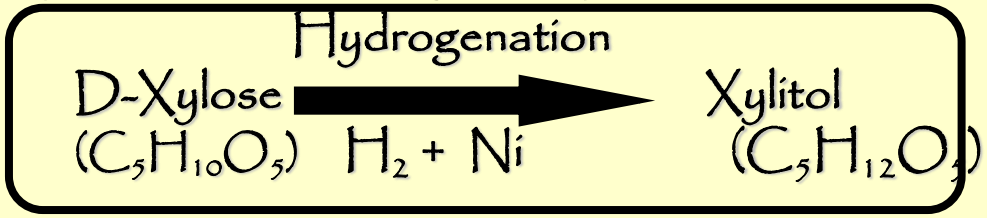
harmaceutical roducts

- ✓ itamins
- ✓ . ineral
- ✓ ntiacids
- ✓ ough
- ✓ ntibiotic

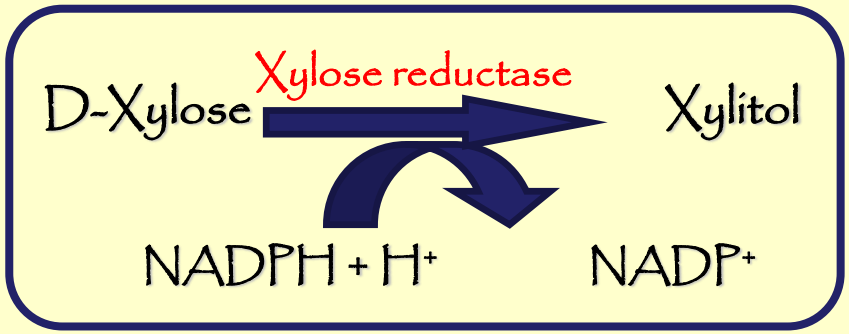
ow

y solid
(. =.°, . >. atm, catalyst: . i)

hemical synthesis from .
(. =.°, . >. atm, catalyst: . i)



√ **ioconversion (=°, = . atm, catalyst: easts)**



2.- Bioprocesos

procesos biológicos *VS.* **procesos basados en síntesis químicas**

Ventajas:

- ❑ preferencia de los consumidores por productos "*naturales*",
- ❑ condiciones operativas más suaves,
- ❑ uso de materias primas renovables,
- ❑ menor impacto ambiental,
- ❑ mayor especificidad y eficiencia del proceso catalítico,
- ❑ etc,

Mayores costes

Desventajas:

- ❑ la generación de mezclas complejas que necesitan complejos sistemas de **purificación**,
- ❑ la obtención de un **producto en baja concentración**,
- ❑ los bajos valores de las **constantes de velocidad** (D↓),
- ❑ problemas de **contaminación** o infección con otros microorganismos, y
- ❑ los costes energéticos asociados a la **esterilización** de los equipos.

3.- Biorreactores

Un **biorreactor** puede ser definido como un ambiente biológicamente controlado en el que se pretende un **crecimiento celular** o un **producto**, con una eficacia óptima.

El principal objetivo de un biorreactor es, por tanto, lograr una **alta productividad a bajos costes operativos**.

Para ello se necesita:

a) Una elevada productividad global **(↑ Q_P)**.

b) Una elevada conversión de las materias primas **(↑ $Y_{P/S}$)**.

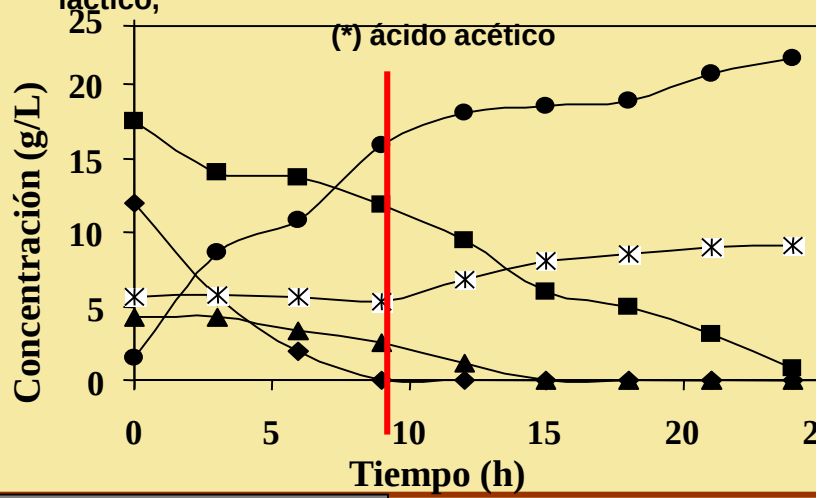
c) Una reducción de los costes operativos **(↓ Costes)**.

d) Una simplificación de los procesos de separación **(↑ sencillez)**.

Fermentación en discontinuo con hidrolizados hemicelulósicos (de podas de sarmiento) y *L. pentosus*

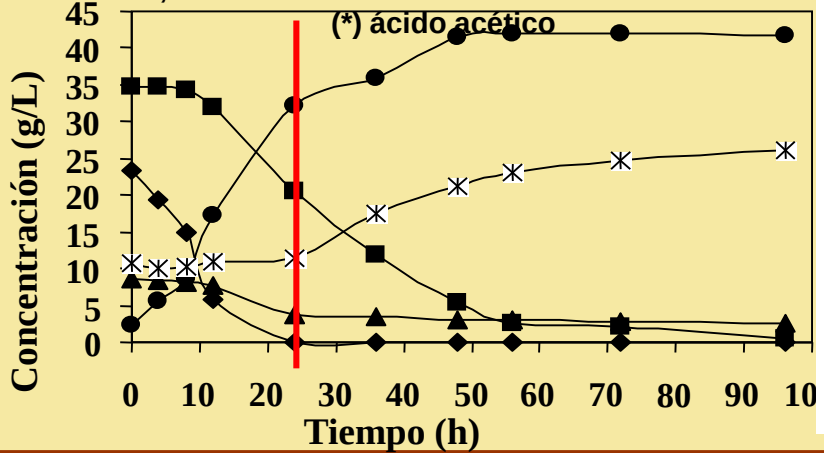
Medio sin concentrar 1/1

(♦) glucosa, (■) xiosa, (▲) arabinosa, (●) ácido láctico,



Concentrado 1/2

(♦) glucosa, (■) xiosa, (▲) arabinosa, (●) ácido láctico,



Biotechnol. Prog. 2005, 21, 793–798 793

Influence of the Metabolism Pathway on Lactic Acid Production from Hemicellulosic Trimming Vine Shoots Hydrolyzates Using *Lactobacillus pentosus*

Gundalupe Bustos, Ana Belén Moldes, José Manuel Cruz, and José Manuel Domínguez*

Departmento de Ingeniería Química, Universidad de Vigo (Campus Ourense), As Lagoas s/n, 32004 Ourense, Spain

Hemicellulosic hydrolyzates from trimming wastes of vine shoots were proposed as a carbon source for lactic acid production by *Lactobacillus pentosus* CECT-4023T (ATCC-8041). These hydrolyzates are composed mainly of glucose (12.0 g/L), xylose (17.5 g/L) and arabinose (4.3 g/L). Acetic acid, the main subproduct, started to be produced after all of the glucose was completely depleted, showing that the acetic acid coproduction came only from the xylose and arabinose consumption. In the absence of glucose, the *L. pentosus* pathway shifts from homo to heterofermentative. Thus, *L. pentosus* can be considered a facultative heterofermentative organism, degrading hexoses (glucose) via the Embden–Meyerhoff–Parnas pathway and pentoses (xylose and arabinose) via the phosphoketolase pathway. Hydrolyzates were vacuum evaporated to increase the initial sugars concentration up to 35.4 g/L of glucose, 52.3 g/L of xylose, and 13.0 g/L of arabinose. Under these conditions the lactic acid concentration reached 46.0 g/L (Q_P = 0.933 g/L·h, $Y_{P/S}$ = 0.78 g/g; $Y_{P/S}$ theoretical = 91.7%) and a clear product inhibition was observed. Additional experiments with synthetic sugars, in the absence of inhibitory compounds, indicate that this inhibition must be attributed to the metabolic pathway but not to the inhibitory compounds present in the fermentation broth.

Introduction

Lactic acid bacteria (LAB) are industrially important microbes that are used worldwide to produce lactic acid (LA), a commercially viable product (the world consumption is estimated to be more than 60,000 metric tons per year) that is used for meat and poultry preservation, cosmetics, oral and health care products, baked goods and during cheese and yogurt elaboration. Besides their LA-forming capacity, LAB also have the ability to contribute to other product characteristics such as flavor, texture and nutrition. The most important application of LAB is undoubtedly in the dairy industry, although LAB are also applied at an industrial scale in the fermentation of other food raw materials such as meat and vegetables. The biosynthetic capacity and metabolic versatility of LAB is generally quite limited and their physiology is relatively simple, which are important factors making these organisms suitable objects for metabolic engineering (*1*). In addition, the processes of energy metabolism and biosynthesis are almost completely separate in LAB, allowing interference in, for example, sugar catabolism without affecting biosynthesis and vice versa. Besides, an extensive knowledge on their physiology and genetics has contributed to the effectiveness of metabolic engineering in these bacteria. For example, Zhang et al. (*2*), developed a *Lactobacillus* strain called Mont4++pxyABmod, which was able to convert xylose into LA by including a plasmid in its genomic structure, and Isizaki and Ueda (*3*, *4*) also modified genetically the strain *Lactococcus lactis* named *Lactococcus lactis* IO-1 in order to obtain improved results during xylose fermentation.

LAB are capable of generating LA by homo- or heterofermentative degradation of sugars. During anaerobic growth of obligatory homofermentative LAB in the presence of excess substrate, energy sources such as glucose are converted into pyruvate via the Embden–Meyerhoff–Parnas pathway (EMP-P), and the pyruvate is further metabolized to lactate. Under optimal conditions *Lactobacillus* can convert different carbon sources, mainly hexoses, into LA with 100% yield (*5*). Thus, Bustos et al. (*6*) using *L. pentosus* CECT-4023T (ATCC-8041) reported 84.7 g/L of LA from 91.1 g/L of commercial glucose, following exclusively the Embden–Meyerhoff–Parnas pathway, as no acetic acid or ethanol were detected. During the heterofermentative pathway, the degradation of sugars is metabolized via the phosphoketolase pathway (PK-P), which results in equimolar amounts of CO_2, lactate, and acetate or ethanol. Heterolactic LAB can be divided into obligatory heterofermentative species, in which both hexoses and pentoses are fermented via the PK-P, and facultative heterofermentative organisms, which degrade hexoses via the EMP-P and pentoses via the PK-P (*7*) following the overall stoichiometry for both pathways:

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3$$

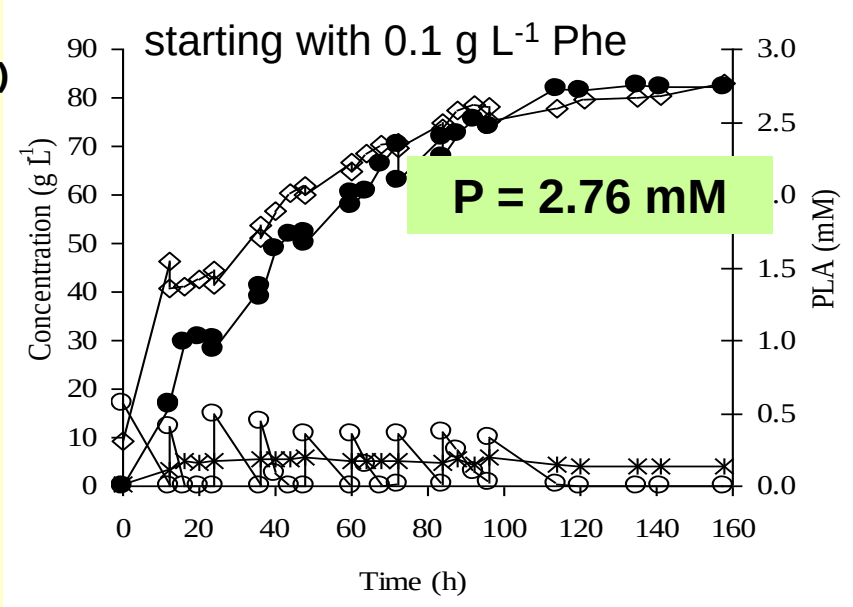
$$C_5H_{10}O_5 \rightarrow C_3H_6O_3 + CH_3COOH$$

End products and proportions can vary depending upon

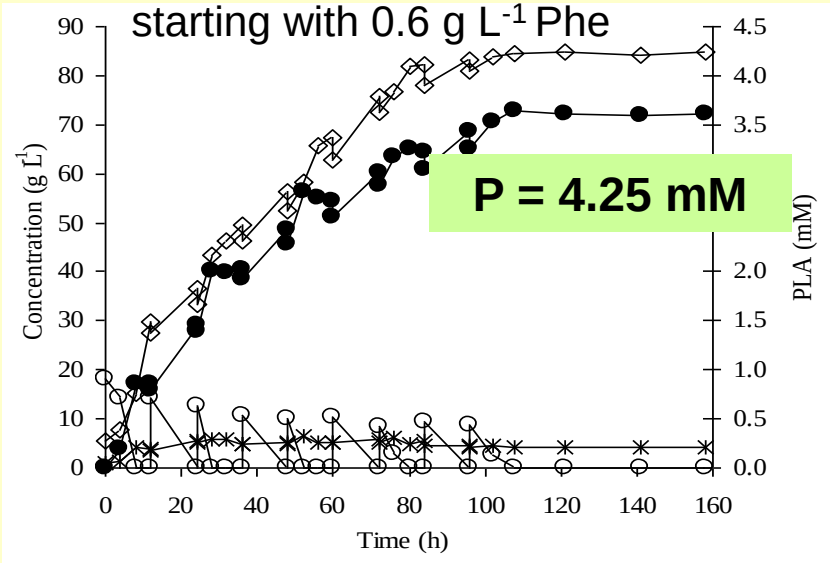
10.1021/bp040063v CCC: $30.25
Published on Web 03/19/2005

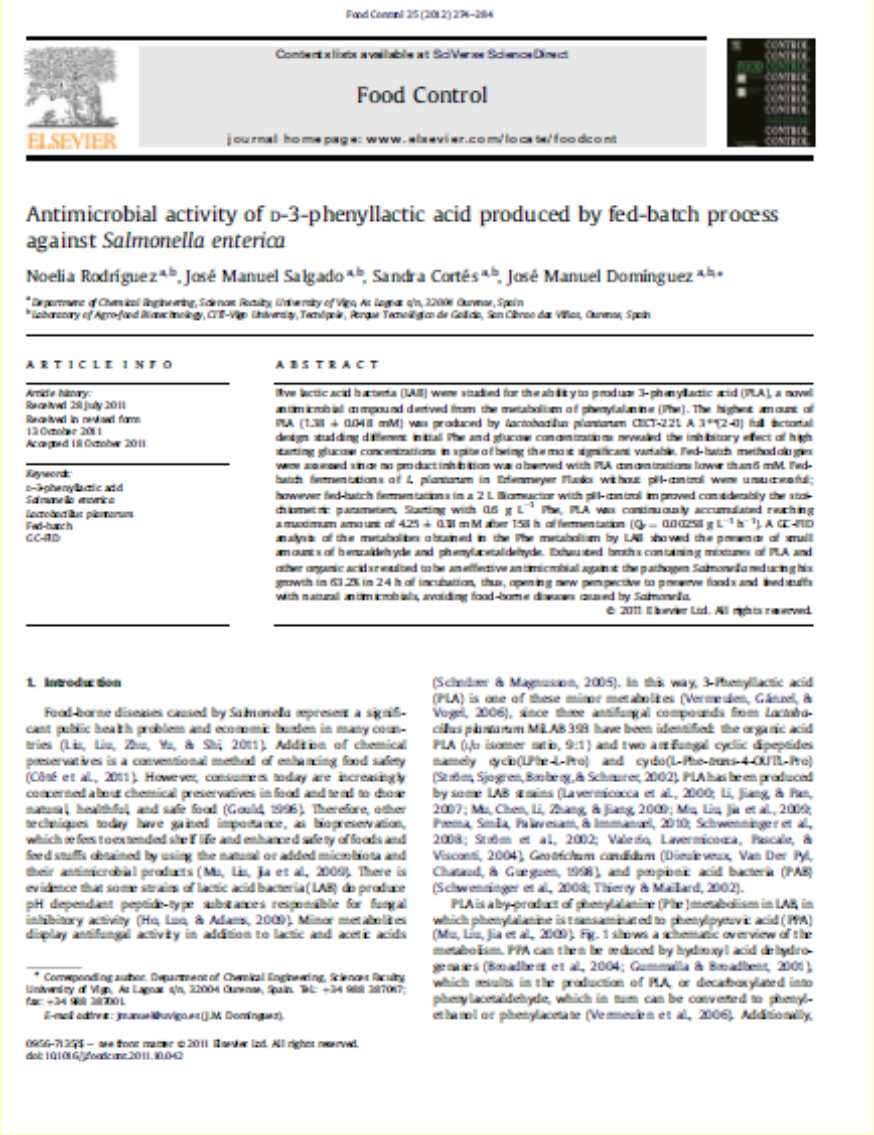
Fed-batch fermentations of Lactobacillus plantarum CECT-221 using intermittent feeding schemes in a 2L Biorreactor

a)



b)





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Antimicrobial activity of D-3-phenyllactic acid produced by fed-batch process against *Salmonella enterica*

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Lactobacillus plantarum
Fed-batch
GC-FID

ABSTRACT

Five lactic acid bacteria (LAB) were studied for the ability to produce 3-phenyllactic acid (PLA), a novel antimicrobial compound derived from the metabolism of phenylalanine (Phe). The highest amount of PLA (1.38 ± 0.04 mM) was produced by *Lactobacillus plantarum* CECT-221. A $3^{*}(2-0)$ full factorial design studding different initial Phe and glucose concentrations revealed the inhibitory effect of high starting glucose concentrations in spite of being the most significant variable. Fed-batch methodologies were assessed since no product inhibition was observed with PLA concentrations lower than 6 mM. Fed-batch fermentations of *L. plantarum* in Erlenmeyer Flasks without pH-control were unsuccessful; however fed-batch fermentations in a 2 L Bioreactor with pH-control improved considerably the stoichiometric parameters. Starting with 0.6 g L^{-1} Phe, PLA was continuously accumulated reaching a maximum amount of 4.25 ± 0.11 mM after 158 h of fermentation (Q_p = 0.00258 g L^{-1} h^{-1}). A GC-FID analysis of the metabolites obtained in the Phe metabolism by LAB showed the presence of small amounts of benzaldehyde and phenylacetaldehyde. Exhausted broths containing mixtures of PLA and other organic acids resulted to be an effective antimicrobial against the pathogen *Salmonella* reducing his growth in 63.2% in 24 h of incubation, thus, opening new perspective to preserve foods and feedstuffs with natural antimicrobials, avoiding food-borne diseases caused by *Salmonella*.

1. Introduction

Food-borne diseases caused by *Salmonella* represent a significant public health problem and economic burden in many countries (Liu, Liu, Zhu, Yu, & Shi, 2011). Addition of chemical preservatives is a conventional method of enhancing food safety (Côté et al., 2011). However, consumers today are increasingly concerned about chemical preservatives in food and tend to those natural, healthful, and safe food (Gould, 1996). Therefore, other techniques today have gained importance, as biopreservation, which refers to extended shelf life and enhanced safety of foods and feed stuffs obtained by using the natural or added microbiota and their antimicrobial products (Mu, Liu, Jia et al., 2009). There is evidence that some strains of lactic acid bacteria (LAB) do produce pH dependant peptide-type substances responsible for fungal inhibitory activity (Ho, Luo, & Adams, 2009). Minor metabolites display antifungal activity in addition to lactic and acetic acids (Schnürer & Magnusson, 2005). In this way, 3-Phenyllactic acid (PLA) is one of these minor metabolites (Vermeulen, Gänzle, & Vogel, 2006), since three antifungal compounds from *Lactobacillus plantarum* MiLAB 393 have been identified: the organic acid PLA (LD isomer ratio, 9:1) and two antifungal cyclic dipeptides namely cyclo(LPhe-L-Pro) and cyclo(L-Phe-trans-4-OHPL-Pro) (Ström, Sjogren, Broberg, & Schnürer, 2002). PLA has been produced by some LAB strains (Lavermicocca et al., 2000; Li, Jiang, & Pan, 2007; Mu, Chen, Li, Zhang, & Jiang, 2006; Mu, Liu, Jia et al., 2009; Pernia, Sirilla, Palaveesam, & Immanuel, 2010; Schweninger et al., 2008; Ström et al., 2002; Valerio, Lavermicocca, Pascale, & Visconti, 2004), *Geotrichum candidum* (Dieuleveux, Van Der Pyl, Chataud, & Gueguen, 1998), and propionic acid bacteria (PAB) (Schweninger et al., 2008; Thierry & Maillard, 2002).

PLA is a by-product of phenylalanine (Phe) metabolism in LAB, in which phenylalanine is transaminated to phenylpyruvic acid (PPA) (Mu, Liu, Jia et al., 2009). Fig. 1 shows a schematic overview of the metabolism. PPA can then be reduced by hydroxyl acid dehydrogenases (Broadbent et al., 2004; Gummalla & Broadbent, 2001), which results in the production of PLA, or decarboxylated into phenylacetaldehyde, which in turn can be converted to phenylethanol or phenylacetate (Vermeulen et al., 2006). Additionally,

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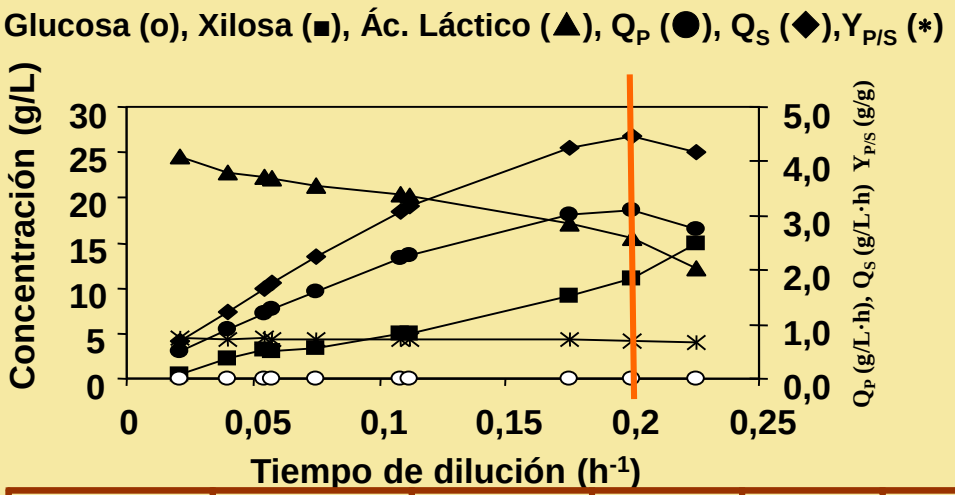
Glucose (o); LA (•); PLA (◊); Biomass (*).

Fermentación en continuo con hidrolizados hemicelulósicos (de podas de sarmiento) y *L. pentosus*



Caudal (mL/min)	Velocidad dil. (h^{-1})	Glucosa (g/L)	Xilosa (g/L)	P (g/L)	Q_P (g/L·h)	Q_S (g/L·h)	$Y_{P/S}$ (g/g)
0.20	0.021	0.0	0.5	24.5	0.514	0.691	0.74
0.40	0.040	0.0	2.3	22.8	0.904	1.234	0.73
0.50	0.054	0.0	3.2	22.3	1.212	1.645	0.74
0.58	0.058	0.0	3.1	22.1	1.276	1.752	0.73
0.70	0.075	0.0	3.3	21.4	1.604	2.249	0.71
1.00	0.108	0.0	4.9	20.4	2.209	3.086	0.72
1.12	0.112	0.0	5.0	20.2	2.261	3.176	0.71
1.75	0.175	0.0	9.2	17.2	3.010	4.240	0.71
2.00	0.200	0.0	11.1	15.5	3.100	4.460	0.70
2.25	0.225	0.0	14.9	12.2	2.745	4.163	0.66

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Revalorization of hemicellulosic trimming vine shoots hydrolyzates trough continuous production of lactic acid and biosurfactants by *L. pentosus*

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Available online 6 December 2005

Abstract

This work proposes the utilization of trimming vine shoots hydrolyzates by *Lactobacillus pentosus* for lactic acid employing continuous culture and the evaluation of biosurfactants production. The treatment of hydrolyzates with sulphuric acid (prehydrolysis) allowed the solubilization of hemicelluloses to give liquors containing 18 g/L of xylose, 11.1 g/L of glucose, 4.3 g/L of arabinose and 4 g/L of acetic acid. Continuous fermentation of hemicellulosic hydrolyzates gave a $Q_P = 3.10$ g of lactic acid/L·h and $Y_{P/S} = 0.70$ g of lactic acid/g of sugars for a dilution rate of 0.02 h^{-1} using 10 g/L of corn steep liquor and 10 g/L of yeast extract as nutrients. When nutrients were replaced by 20 g/L of distilled lees from white wine production the same results were obtained without interfering, lees, in the lactic acid recovery from the fermentation medium. On the other hand, *L. pentosus* cells were submitted to extraction process with PBS to ...unification lees or corn steep liquid plus yeast extract, the extracted biosurfac... ...8 in 23.5 and 25.5 m N/m, respectively. No extracellular biosurfactants were

...; Biosurfactants; Lees

...ping of ...to the ...green- ...ommu- ...es are ...f fossil ...wastes,

...es (J.M. ...erved.

which are usually burned in the field, releasing carbon dioxide to the atmosphere, as well as some other toxic lignin compounds. This aspect is really important if we consider the big amount of pruning wastes of vine-stocks generated worldwide. Just in Galicia, northwestern Spain, the amount of vineshoots produced per year is estimated in approximately 65,000 tonnes.

Pruning wastes of vine shoots are lignocellulosic residues (Bustos, Moldes, Cruz, & Domínguez, 2004, 2005). Upon chemical and/or enzymatic stages, the polymers can be hydrolyzed to the constitutive monomers to obtain sugar solutions which are potential renewable sources for the biotechnological lactic acid production without giving

4. Tipos de Biorreactores

1.- Biorreactores completamente mezclados agitados mecánicamente.

1.1.- FCTA (Fermentador Continuo de Tanque Agitado).

1.2.- FCTAs en Serie.

1.3.- Fermentadores de Membrana.

2.- Biorreactores basados en el concepto de flujo en pistón (FCFP).

2.1.- Reactores de Lecho Fijo.

2.2.- Biorreactores Pulsantes.

3.- Biorreactores agitados por fluidos.

3.1.- Columnas de Burbujeo.

3.2.- Fermentadores Air-lift.

4.1.1.- FCTA (Fermentador Continuo de Tanque Agitado)

procesos no inhibidos por producto

Lactobacillus plantarum

Lactobacillus pentosus

Appl Biochem Biotechnol (2013) 169:2175–2188
DOI 10.1007/s12010-013-0126-1

Biotechnological Production of Phenyllactic Acid and Biosurfactants from Trimming Vine Shoot Hydrolyzates by Microbial Coculture Fermentation

Noelia Rodríguez-Pazo · José Manuel Salgado · Sandra Cortés-Diéguez · José Manuel Domínguez

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Abstract Coculture fermentations show advantages for producing food additives from agroindustrial wastes, considering that different specified microbial strains are combined to improve the consumption of mixed sugars obtained by hydrolysis. This technology dovetails with both the growing interest of consumers towards the use of natural food additives and with stricter legislations and concern in developed countries towards the management of wastes. The use of this technology allows valorization of both cellulosic and hemicellulosic fractions of trimming vine shoots for the production of lactic acid (LA), phenyllactic acid (PLA), and biosurfactants (BS). This work compares the study of the potential of hemicellulosic and cellulosic fractions of trimming vine shoots as cheaper and renewable carbon sources for PLA and BS production by independent or coculture fermentations. The highest LA and PLA concentrations, 43.0 g/L and 1.58 mM, respectively, were obtained after 144 h during the fermentation of hemicellulosic sugars and simultaneous saccharification and fermentation (SSF) carried out by cocultures of *Lactobacillus plantarum* and *Lactobacillus pentosus*. Additionally, cell-bond BS decreased the surface tension (ST) in 17.2 U; meanwhile, cell-free supernatants (CFS) showed antimicrobial activity against *Salmonella enterica* and *Listeria monocytogenes* with inhibition halos of 12.1± 0.6 mm and 11.5±0.9 mm, respectively.

Keywords Coculture · Trimming vine wastes · Phenyllactic acid · Biosurfactants · Lactic acid · Lactic acid bacteria

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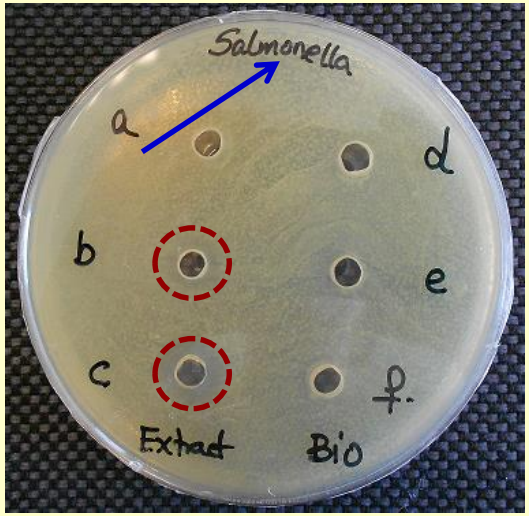
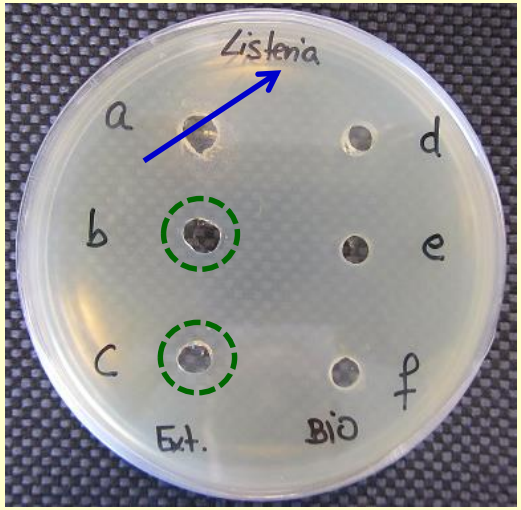
Actividad antimicrobiana

A



B



a,d) Fracción hemicelulósica
b,e) Fracción celulósica
c,f) Ambas fracciones

- ✓ ***Salmonella enterica subsp. enterica***
- ✓ ***Pseudomonas aeruginosa***
- ✓ ***Staphylococcus aureus subsp. aureus***
- ✓ ***Listeria monocytogenes***

	Diámetro halo inhibición (mm)	
	S. enterica	***L. monocytogenes***
Fracción celulósica	9,5 ± 0,3	9,0 ± 0,7
Cultivo mixto	12,1 ± 0,6	11,5 ± 0,9

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ELSEVIER

Coupling two sizes of CSTR-type bioreactors for sequential lactic acid and xylitol production from hemicellulosic hydrolysates of vineshoot trimmings

Research Paper

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This study develops a system for the efficient valorisation of hemicellulosic hydrolysates of vineshoot trimmings. By connecting two reactors of 2 L and 10 L, operational conditions were set up for the sequential production of lactic acid and xylitol in continuous fermentation, considering the dependence of the main metabolites and fermentation parameters on the dilution rate. In the first bioreactor, *Lactobacillus rhamnosus* consumed all the glucose to produce lactic acid at 31.5°C, with 150 rpm and 1 L of working volume as the optimal conditions. The residual sugars were employed for the xylose to xylitol bioconversion by *Debaryomyces hansenii* in the second bioreactor at 30°C, 250 rpm and an air-flow rate of 2 L min^{-1}. Several steady states were reached at flow rates (F) in the range of 0.54–5.33 mL min^{-1}, leading to dilution rates (D) ranging from 0.032 to 0.320 h^{-1} in Bioreactor 1 and from 0.006 to 0.064 h^{-1} in Bioreactor 2. The maximum volumetric lactic acid productivity ($Q_{P\,LA}$ = 2.908 g L^{-1} h^{-1}) was achieved under D = 0.266 h^{-1} (F = 4.44 mL min^{-1}); meanwhile, the maximum production of xylitol (5.1 g L^{-1}), volumetric xylitol productivity ($Q_{P\,xylitol}$ = 0.218 g L^{-1} h^{-1}), volumetric rate of xylose consumption ($Q_{S\,xylose}$ = 0.398 g L^{-1} h^{-1}) and product yield (0.55 g g^{-1}) were achieved at an intermediate dilution rate of 0.043 h^{-1} (F = 3.55 mL min^{-1}). Under these conditions, ethanol, which was the main by-product of the fermentation, was produced in higher amounts (1.9 g L^{-1}). Finally, lactic acid and xylitol were effectively recovered by conventional procedures.

Introduction

Lactic acid and xylitol are two additives widely used worldwide in the food sector. The main applications of lactic acid include not only its use as a food acidulant and preservative, but also its use in preparing emulsifying agents. Additionally, lactic acid has applications in other relevant sectors, such as the pharmaceutical and cosmetic industries, and more recently in the production of biodegradable polylactic acids [1,2]. Despite its widespread use, with worldwide consumption estimated to fluctuate between 130,000 and 150,000 metric tonnes/year, the costs of production must be reduced considerably, taking into account that the price of the raw materials usually employed can represent up to 40–70% of the final cost [3,4]. The commercial price of food grade lactic acid is 1.38 US$ kg^{-1} for lactic acid with 50% purity and 1.54 US$ kg^{-1} for lactic acid with 88% purity [5]. In addition, the price of biological lactic acid is considerably higher than that of chemical lactic acid, largely due to the high price of the carbohydrate sources [6].

The sweetness of the natural carbohydrate xylitol is similar to that of sucrose; new applications for xylitol are occurring daily in the food sector for the development of chewing gums and confectioneries. Furthermore, xylitol shows anticarcinogenic properties and is insulin-independent when metabolised by humans [7]. Moreover, its use is increasingly widespread in other important non-food industries, such as pharmaceuticals, due to

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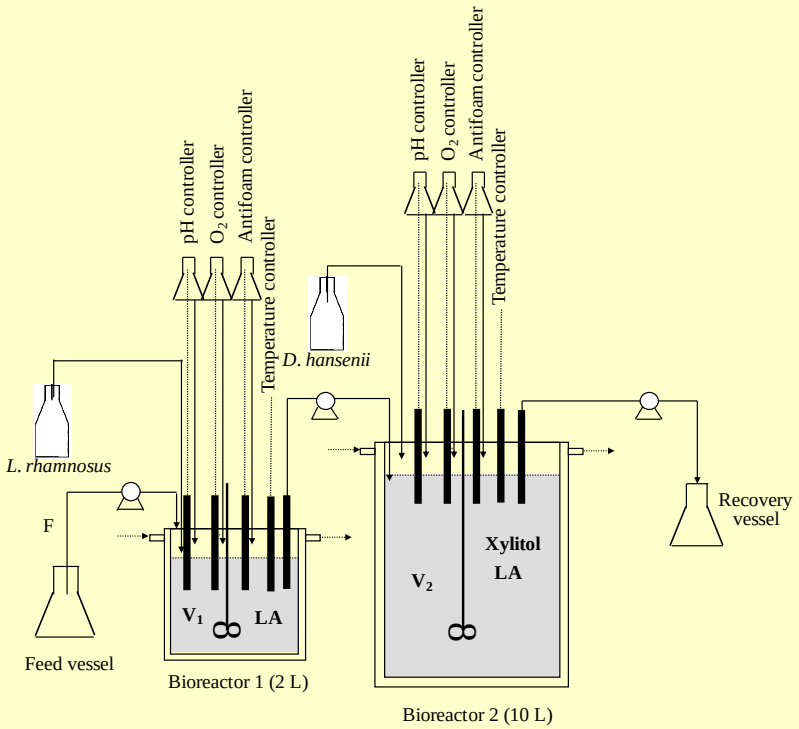
1871-6784/$ - see front matter doi:10.1016/j.nbt.2011.07.003

4.1.2.- FCTAs en Serie



4.1.3.- Fermentadores de Membrana

Biotechnology Letters **22**: 1895–1898, 2000.

Xylitol production from barley bran hydrolysates by continuous fermentation with *Debaryomyces hansenii*

J.M. Cruz, J.M. Domínguez*, H. Domínguez & J.C. Parajó
Department of Chemical Engineering, University of Vigo (Campus Ourense), Faculty of Science, Polytechnical Building, As Lagoas, 32004 Ourense, Spain
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Received 21 July 2000; Revisions requested 4 August 2000; Revisions received 26 September 2000; Accepted 26 September 2000

Key words: cell recycle, continuous fermentation, *Debaryomyces hansenii*, xylitol

Abstract

The continuous bioconversion of xylose-containing solutions (obtained by acid hydrolysis of barley bran) into xylitol was carried out using the yeast *Debaryomyces hansenii* under microaerophilic conditions with or without cell recycle. In fermentations without cell recycle, the volumetric productivities ranged from 0.11–0.6 g l^{-1} h^{-1} were obtained for dilution rates of 0.008–0.088 h^{-1}. In experiments performed with cell recycle after membrane separation, the optimum xylitol productivity (2.53 g l^{-1} h^{-1}) was reached at a dilution rate

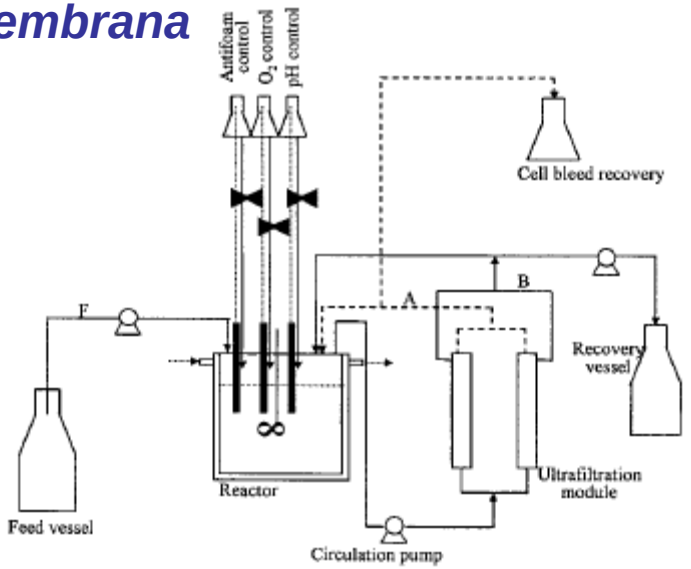


Fig. 1. Process scheme. F = feed; A = retentate; B = permeate.

Introduction

Xylitol is used in the food industry for its high sweetening power and because of its anticaries properties, its tolerance by diabetics and its high negative heat of solution. For these reasons, xylitol has been employed in the manufacture of sugar-free confections and food (Pepper & Olinger 1988).

Xylitol is formed as a metabolic intermediate in the fermentation of D-xylose by yeasts, which can be converted into xylitol by NAD(P)H-dependent xylose reductase (Höfer *et al.* 1971). *Debaryomyces hansenii* has been widely studied for this purpose, particularly in batch fermentation (Gírio *et al.* 1990, 1994, Heikkilä *et al.* 1991, Parajó *et al.* 1995, 1996, Domínguez *et al.* 1997a,b, 1999, Cruz *et al.* 2000a,b).

Fermentation productivities can be increased by using high cell concentrations by retaining cells in the fermentation system (for example, using cell immobilization or cell recycle). Cell immobilization in packed-bed reactors has been investigated for producing xylitol with various yeasts (Silva & Afschar 1994, Roca *et al.* 1996, Domínguez *et al.* 1999, Silva *et al.* 1999). To our knowledge, no systematic studies on the continuous xylitol fermentation from hydrolysates in fermenters with cell recycle have been reported.

This work deals with the xyl
tinuous fermentation of xylos
obtained from barley bran hydr
out biomass recycle. The depen
mentation parameters (includin
concentrations, productivity a
the dilution rate has been asse
technologies.

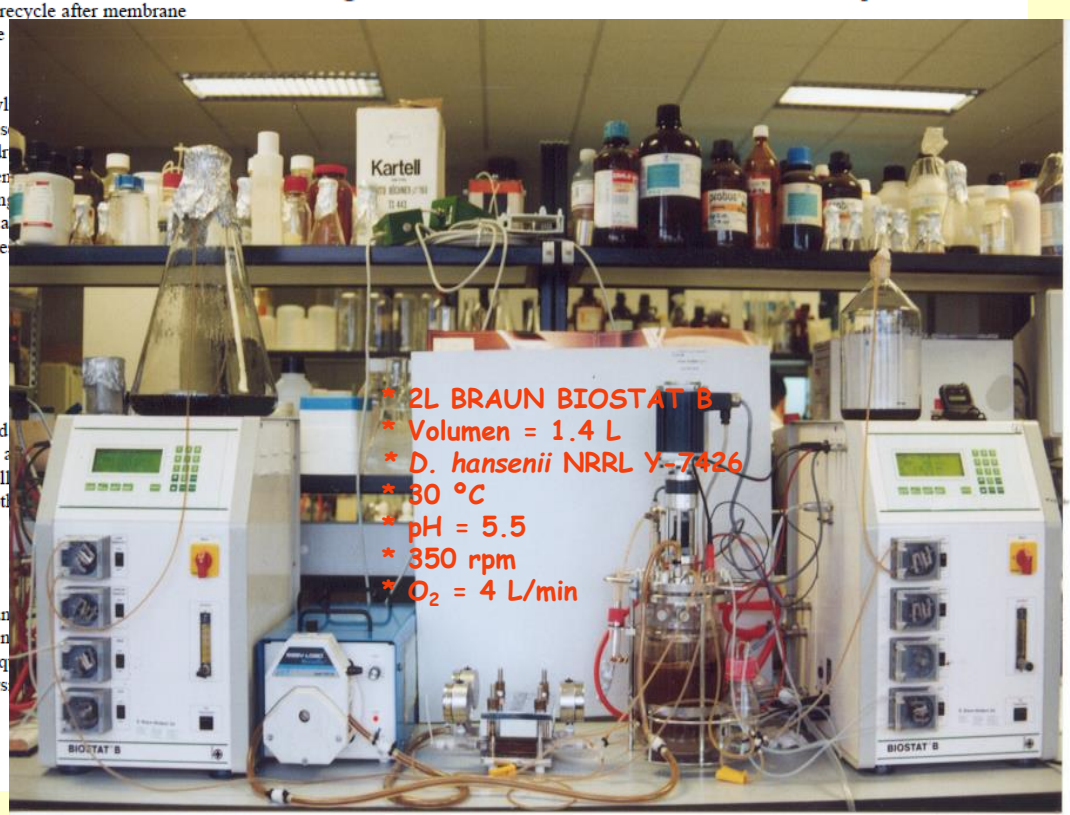
Materials and methods

Raw material

Barley bran samples, obtained
industry, were dried, milled to a
1 mm. They contained 23% cell
lose, 21% lignin and 29% of ot
basis).

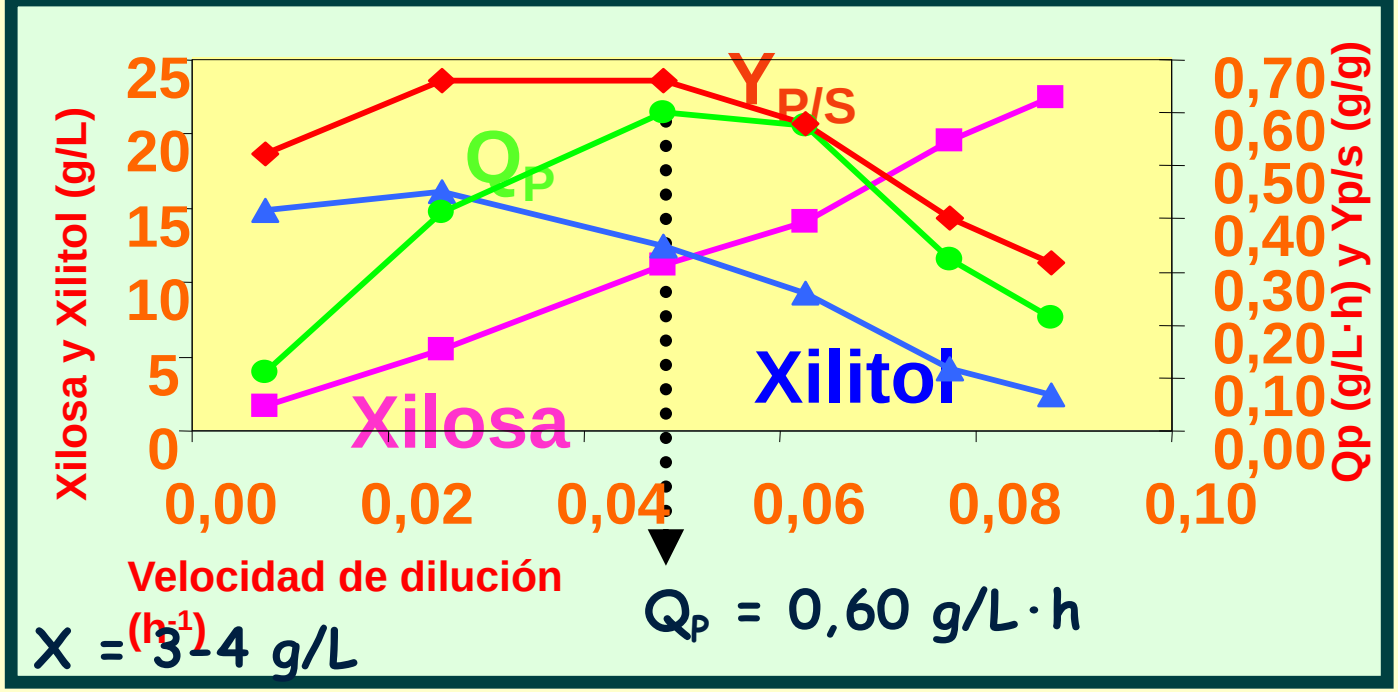
Acid hydrolysis

Acid hydrolyses of barley bran
out under optimized condition
130 °C for 15 min at a solid/liq
The liquid phase from hydrolys

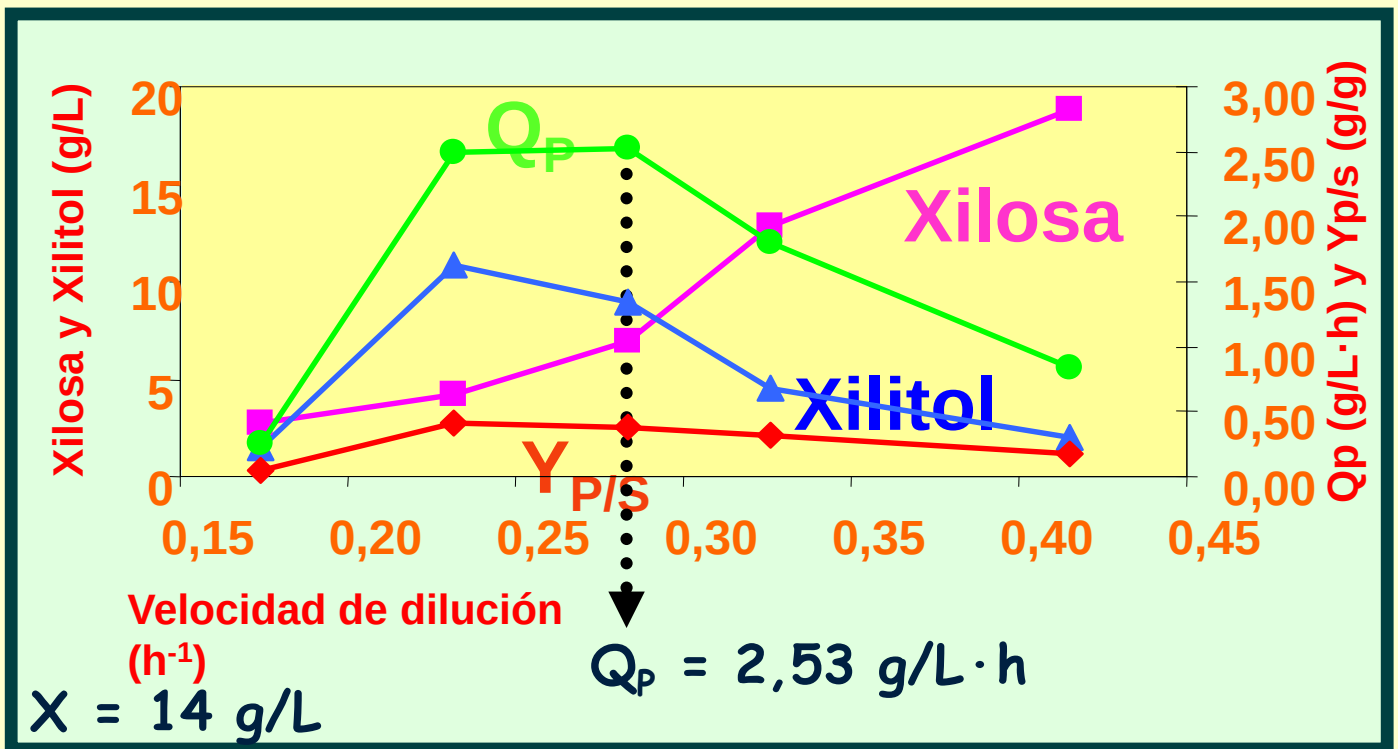


* 2L BRAUN BIOSTAT B
* Volumen = 1.4 L
* *D. hansenii* NRRL Y-7426
* 30 °C
* pH = 5.5
* 350 rpm
* O_2 = 4 L/min

I) Producción de xilitol en continuo SIN recirculación de bimasa



II) Producción de xilitol en continuo CON recirculación de bimasa



Sistema de retención.

dvantages

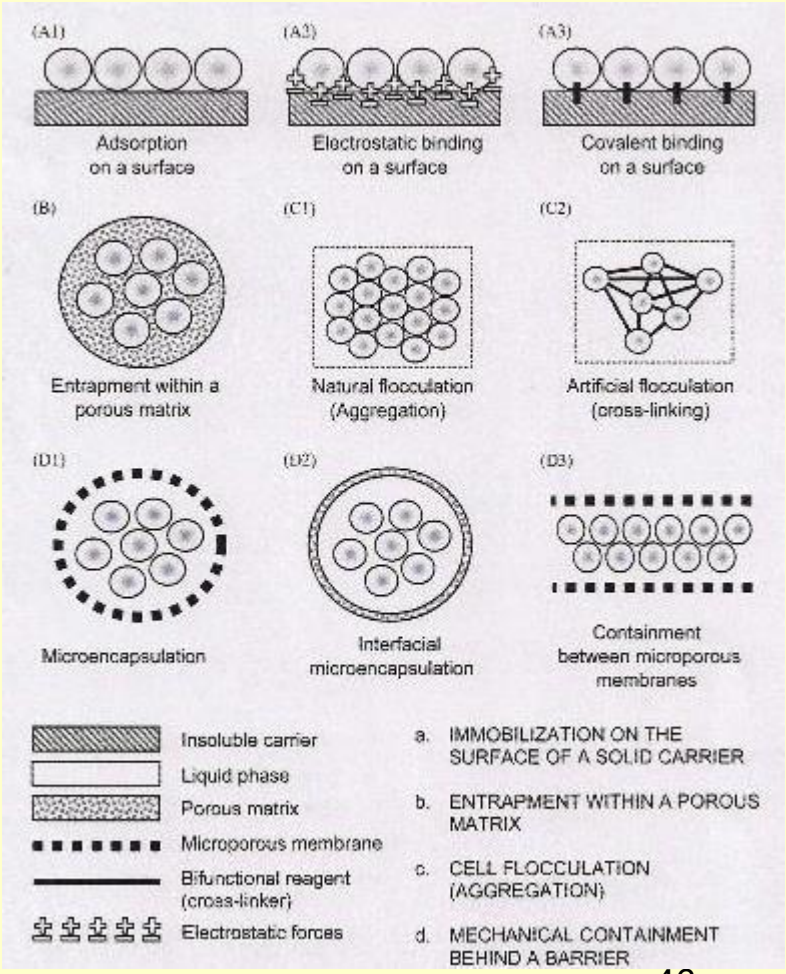
- ✓ igh
- ✓ igh . ermentation roductivities
- ✓ ell
- ✓ \esistance

Cada sistema de retención empleado supone una característica diferenciada del biorreactor. De los cuatros métodos principalmente se emplean dos:

1) Atrapamiento: Se basa en la retención *"inmovilización"* de los microorganismos o enzimas en el interior de una matriz, frecuentemente de polisacáridos.

2) Adhesión: Los sistemas basados en el fenómeno de adhesión *"inmovilización"* se conocen como biofilms o biopelículas.

Estos sistemas permiten operar con una **elevada concentración microbiana** desde el principio de la operación así como controlar más eficazmente la liberación y crecimiento de **células libres** en el caldo de fermentación.



0273-2289/99/81/0119/$13.00

Atrapamiento

Estudio de la condiciones de gelificación sobre la producción de xilitol:

- **Optimización de la concentración de alginato sódico (1% - 5%)**
- **Efecto del ión trivalente (Al^{+3}) como agente endurecedor**

Xylitol Production from Wood Hydrolyzates by Entrapped *Debaryomyces hansenii* and *Candida guilliermondii* Cells

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ENRIQUE ROCA,[2] HERMINIA DOMÍNGUEZ,[1]
AND JUAN CARLOS PARAJÓ[1]

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Abstract

Debaryomyces hansenii cells were entrapped in Ca-alginate beads and used for producing xylitol from wood hydrolyzates. Batch experiments showed that bioconversion was severely hindered when Ca-alginate beads were hardened with Al^{3+} solutions. As an alternative to Al^{3+} hardening, the improvements in both mechanical stability of bioparticles and fermenting ability of the immobilized system derived from using increased concentrations of sodium alginate were assessed. The best results were obtained using a 4% (w/v) Na-alginate solution in the gelification step. This concentration was selected to perform continuous fermentations in a packed-bed reactor using raw or charcoal-treated hydrolyzates (15.5 g of xylose/L) with two different yeasts: *Candida guilliermondii* and *Debaryomyces hansenii*. With a final cell concentration of about 50 g of cells/L (0.075 g of cells/g of beads), the volumetric productivities reached with these yeasts in media made from charcoal-treated hydrolyzates were 0.58 and 0.91 g/L·h, respectively.

Index Entries: Ca-alginate; *Candida guilliermondii*; *Debaryomyces hansenii*; hemicellulose hydrolyzate; xylitol.

Introduction

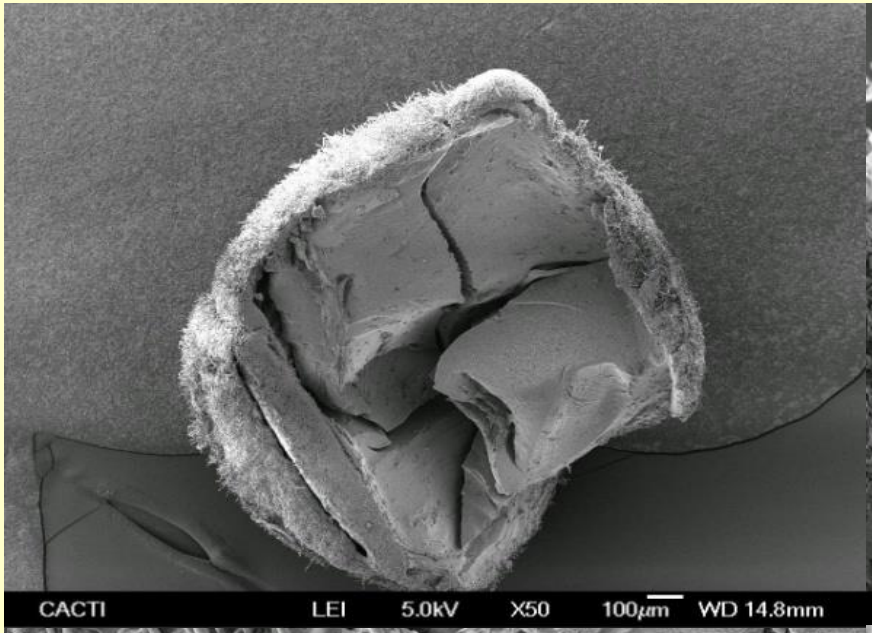
Xylitol, a five-carbon polyol with high sweetening power, is tolerated by diabetics, has anticariogenic properties, and has been recommended for

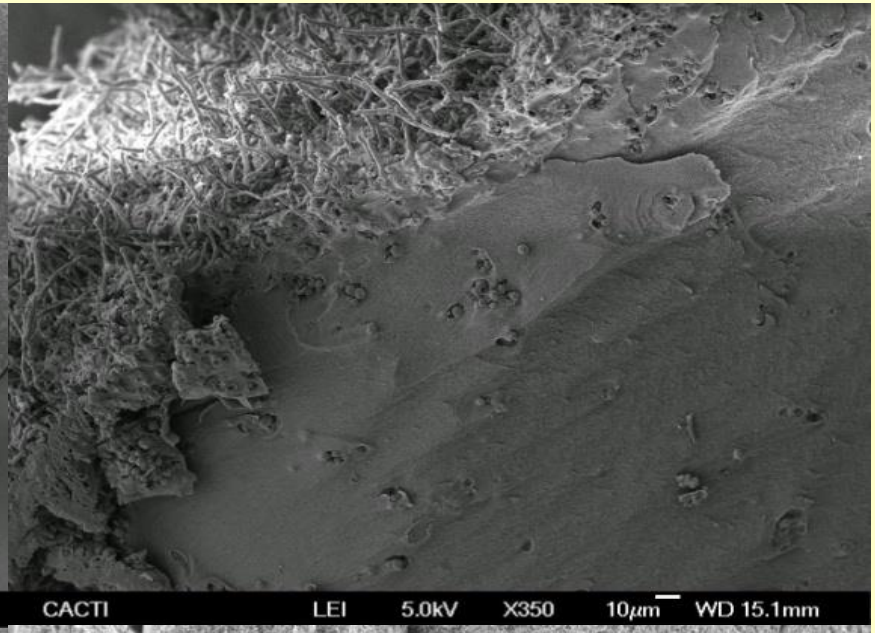
*Author to whom all correspondence and reprint requests should be addressed.

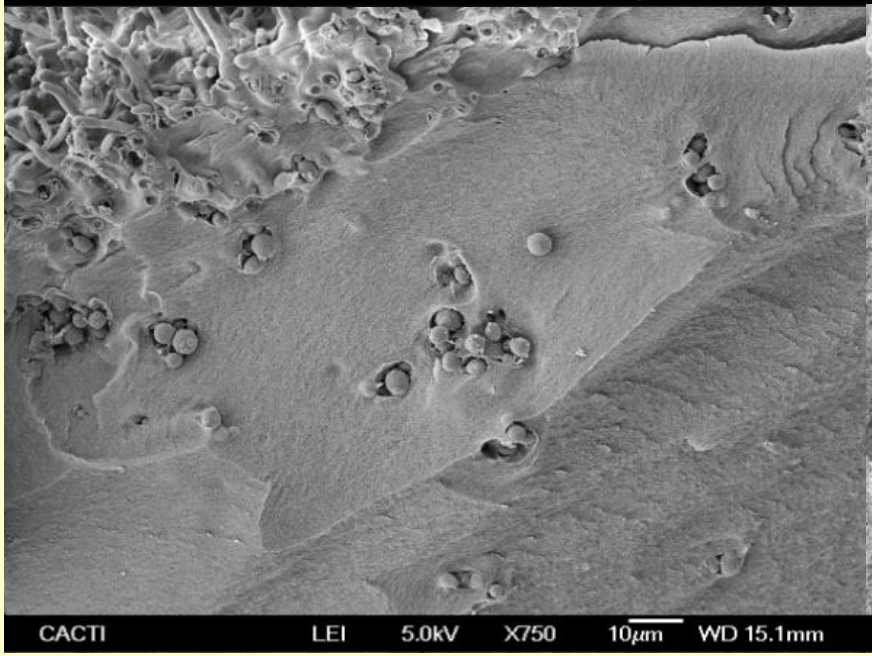
Madera de eucalipto: Producción en continuo con células inmovilizadas.

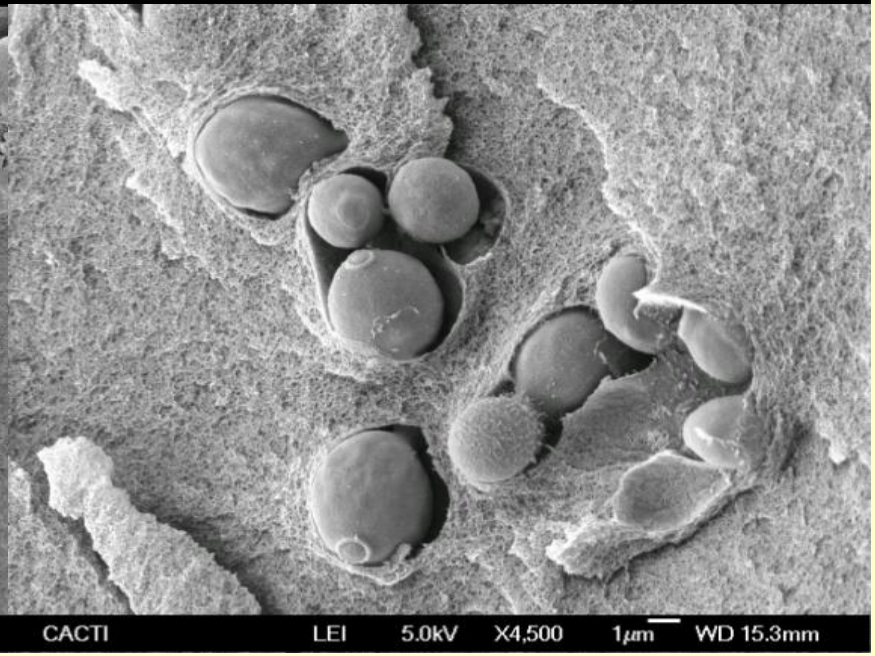
Hidrolizados destoxificados con:

Carbón activo


CACTI LEI 5.0kV X50 100μm WD 14.8mm


CACTI LEI 5.0kV X350 10μm WD 15.1mm


CACTI LEI 5.0kV X750 10μm WD 15.1mm


CACTI LEI 5.0kV X4,500 1μm WD 15.3mm

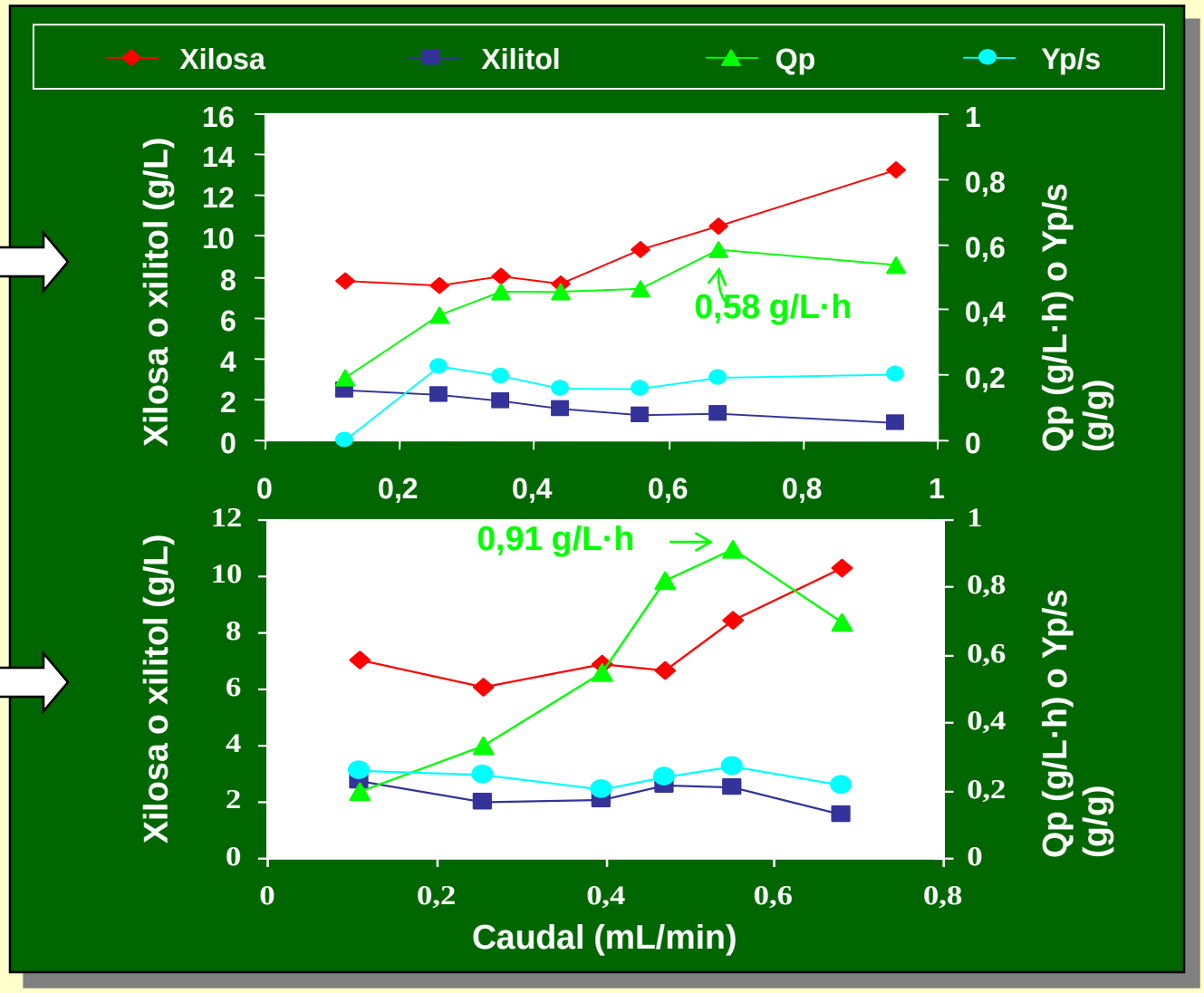
Madera de eucalipto: Producción en continuo con células inmovilizadas.

Destoxificación:

Carbón activo

Candida guilliermondii

Debaryomyces hansenii



Appl Microbiol Biotechnol (2014) 98:151–161
DOI 10.1007/s00253-013-5280-4

BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Study of the potential of the air lift bioreactor for xylitol production in fed-batch cultures by *Debaryomyces hansenii* immobilized in alginate beads

Belinda Pérez-Bibbins · Ricardo Pinheiro de Souza Oliveira · Ana Torrado · María Guadalupe Aguilar-Uscanga · José Manuel Domínguez

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Abstract Cell immobilization has shown to be especially adequate for xylitol production. This work studies the suitability of the air lift bioreactor for xylitol production by *Debaryomyces hansenii* immobilized in Ca-alginate operating in fed-batch cultures to avoid substrate inhibition. The results showed that the air lift bioreactor is an adequate system since the minimum air flow required for fluidization was even lower than that leading to the microaerobic conditions that trigger xylitol accumulation by this yeast, also maintaining the integrity of the alginate beads and the viability of the immobilized cells until 3 months of reuses. Maximum productivities and yields of 0.43 g/l/h and 0.71 g/g were achieved with a xylose concentration of 60 g/l after each feeding. The xylose feeding rate, the air flow, and the l
ning of the fed-batch op
parameters for achievin
Although a maximum xyl
tained, product inhibitio
ments, which allowed esti
for xylitol productivity ar
able production of glyce
noticeable, which could r
regulatory function of this
ic pressure caused by hig
the role of the glycerol sy
of NAD^+ in conditions of
insufficient aeration or hig
mass concentrations were

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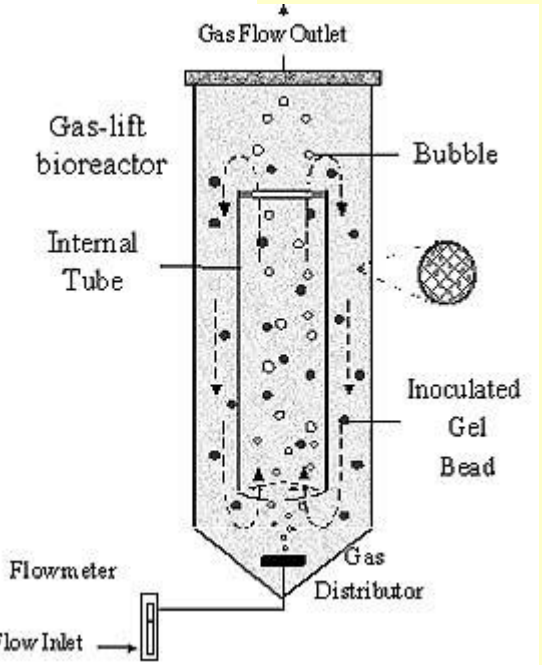
Keywords Xylitol · Glyc
Debaryomyces hansenii ·



Introduction

Xylitol is a pentahydroxy sugar alcohol derived from xylose. Because of its high sweetening power (Castillo et al. 2005), its suitability as diabetic sweetener (Bär 1991), and its interesting properties for the biomedical sector (Mussatto and Roberto 2002), xylitol has received a great attention by the food, pharmaceutical and cosmetic industries (Cunha et al. 2007). In this sense, the development of highly productive and scalable systems is a critical target for improving the economic competitiveness of the microbiological production of xylitol (Parajó et al. 1998) in comparison with the chemical synthesis.

The generation of microaerobic conditions is the most critical condition for directing to xylitol accumulation the metabolism of a group of yeasts, including *Debaryomyces hansenii*

hy ir

dvantages of ir

- ✓ . ess
- ✓ etter
- ✓ etter

Biomass (x)
Xylose (△)
Xylitol (●)
Glycerol (○)
Total **X**ylose consumed (▲)

Xylitol, xylose, xylose consumed (g/L)

Biomass, glycerol (g/L)

Time (h)

0
50
100
150
200
250
300
350

0
5
10
15
20
25
30

0
250
500
750
1000

Brigado
Gracias
Thank you