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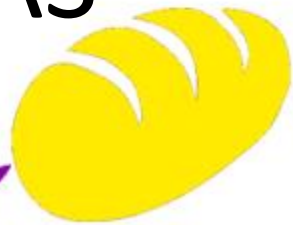
INTRODUCCIÓN A LA BIOTECNOLOGÍA DE ENZIMAS





BIOTECNOLOGÍA

- Aplicación de organismos, sistema o procesos biológicos para las industrias de manufacturas y servicios.

- Involucra el descubrimiento y la subsecuente optimización de los procesos biológicos y bioquímicos necesarios para explotar las materias primas naturales

Características y áreas de aplicación de los procesos biotecnológicos:

- Bajas demandas de energía
- Utilización de fuentes de energía renovables
- Conversión de materiales crudos naturales en productos de mayor interés económico
- Tratamiento de desechos con la consiguiente producción de biomasa útil para alimentación en animales, o de productos químicos útiles

Ciencias que aportan a la Biotecnología:

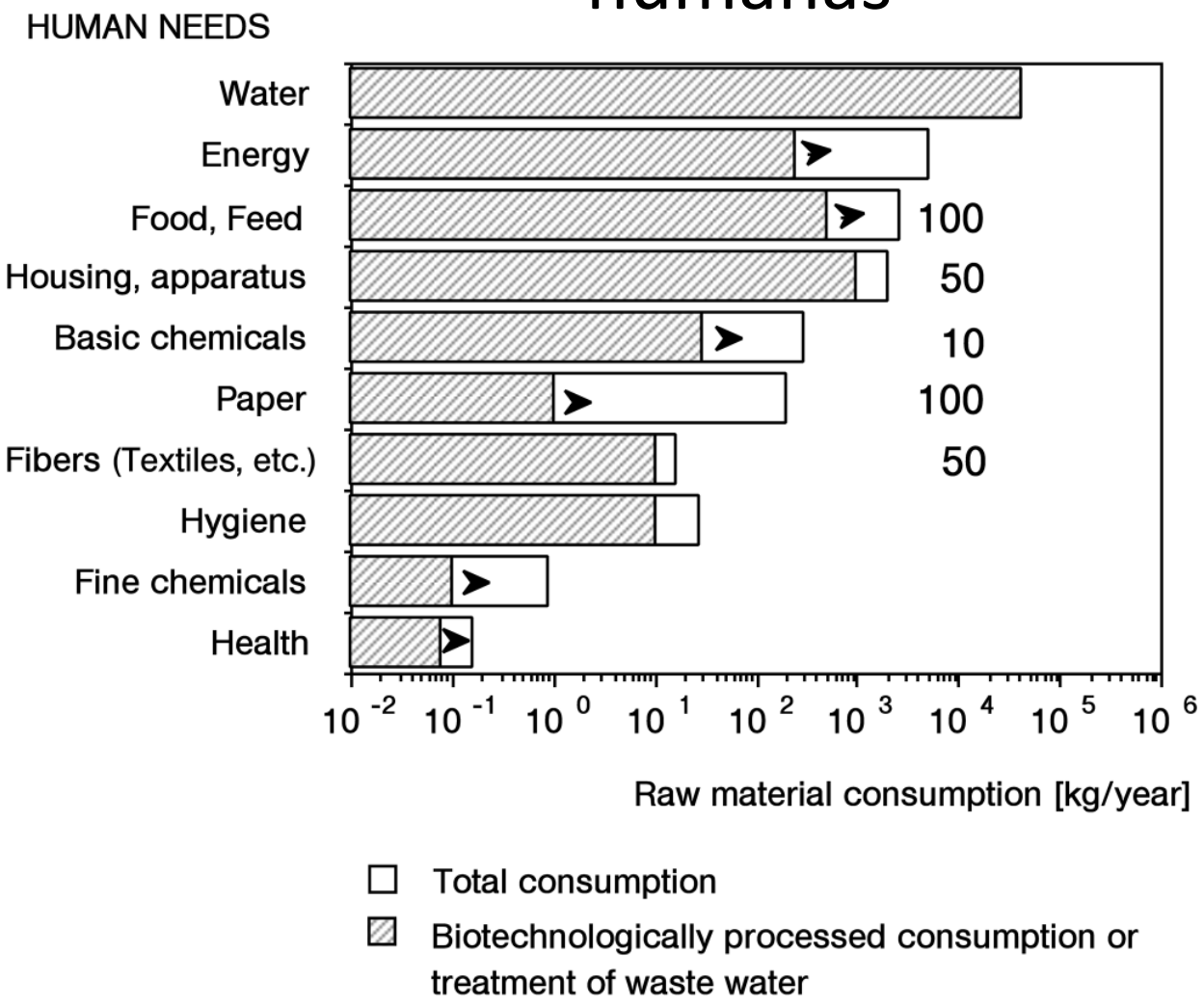
- Microbiología
- Ingeniería química
- Bioquímica
- Química

El éxito en Biotecnología significa no sólo éxito científico sino también éxito económico

OBJETIVO

- Productos competitivos y sustentables
- Sustentabilidad: …desarrollo para satisfacer las necesidades del presente sin comprometer la habilidad de las futuras generaciones de satisfacer sus propias necesidades
- Nuevos procesos diseñados para reducir el consumo de reservas, materiales de desecho y para aumentar el reciclado de basura por kg de producto

Distribución de biomasa (materias primas renovables) frente a distintas demandas humanas



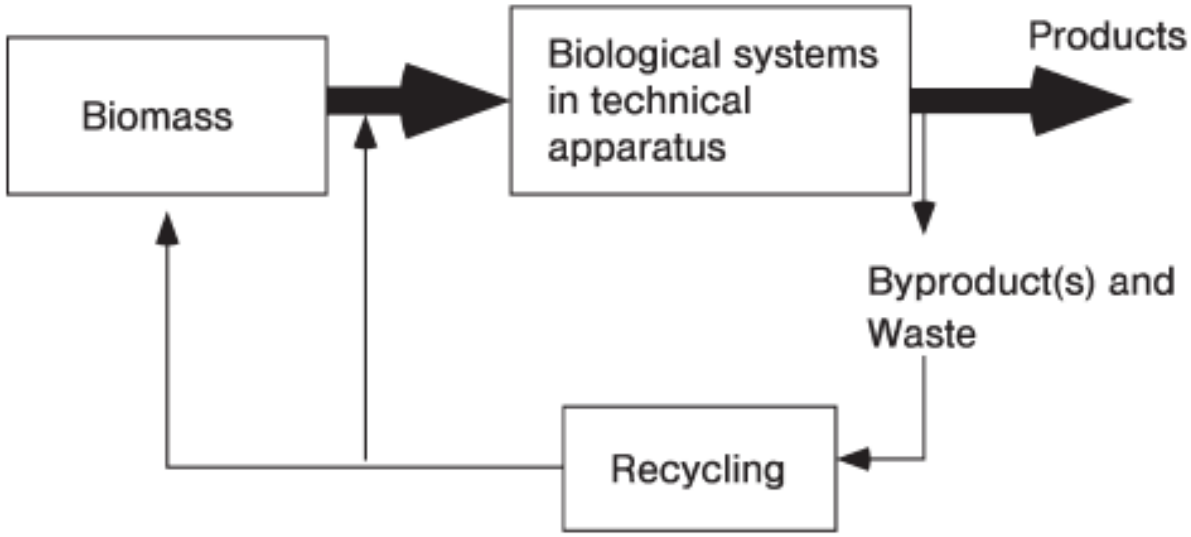


Fig. 1.2 Schematic view of an ideal sustainable biotechnological production process. Biomass as a regenerable resource is converted into desired products with minimal waste and byproduct production. The waste and byproducts must be completely recycled.

PERSPECTIVA HISTÓRICA

- Preservación de comida y bebidas alcohólicas: antiguas pinturas egipcias
- Conversión de leche a queso: 400 ac (Homero en la Ilíada)
- Según Atkinson (1974) el desarrollo de la Biotecnología se divide en tres etapas:

Pre 1800: se utilizan procesos biológicos pero se ignora el mecanismo

1800-1900: comprensión de las bases biológicas y bioquímicas de la bioconversión

Post 1900: desarrollo industrial

AGREGAMOS:

Post 1970: modificación biológica específica directa

- TECNOLOGÍA DE ENZIMAS (sub-rama de la Biotecnología): nuevos procesos son desarrollados para manufacturar productos utilizando enzimas como biocatalizadores, en orden de satisfacer las demandas en alimentación (manteca, queso, vinagre, cerveza), químicos finos (aminoácidos, vitaminas) y productos farmacológicos. También se utilizan las enzimas en la industria del lavado, procesos ambientales y con propósitos de diagnóstico y analíticos.

Enzymes and other proteins are part of everyday life

Examples of enzymes and other commercial proteins

Wastewater treatment
- Environmentally-friendly treatment of organic and toxic waste

Enzyme market size $50mm+[1]

Biopharmaceuticals
- Used to fight diseases such as rheumatoid arthritis and diabetes

Enzyme market size $800mm+[1]

Food and beverage
- Improves flavor/quality
- Facilitates digestion, improves nutritional value and reduces potential allergic reactions

Enzyme market size $1bn

Detergents
- Enhance detergents' characteristics such as cleaning ability, color fading prevention and performance at gentler washing
- Less energy needed for cleaning

Enzyme market size $750mm

Bio-based chemicals
- Reduce use of petroleum-based products
- Reduce pollution and improve product safety

End market size $10bn[2]

Textiles/Leather
- Used in the production of fabrics for clothing, furniture and other household items
- Replaces conventional chemical methods

Enzyme market size $350mm

Cosmetics and personal care
- Enhance quality/properties of toiletry products
- Increase the moisturizing factor of ingredients in cosmetics
- Reduce usage of chemical load

Active ingredient market size $550mm

Nutraceuticals
- Dietary supplements, herbal products, processed foods

End market size $20bn

Pulp & paper
- Used for bleaching, refining, de-inking and the removal of adhesives
- Enhance desired characteristics such as strength, bulk or softness
- Reduce usage of water, energy

Enzyme market size $50mm[1]

Biofuels
- Reduce use of petroleum-based products
- Large existing food-based ethanol market
- Developing cellulosic biofuel market positioned for rapid growth

Food-based market size $600mm
Cellulosic market size $5bn[3]

Animal health & nutrition
- Improve the digestibility of dietary components

Enzyme market size $600mm

Legend

Existing company markets with additional growth opportunities	Markets readily penetrable by company technologies

Source: Freedonia Group, Frost & Sullivan, Verenium press releases, equity research, company estimates
Note: Market size numbers represent latest available current global estimates, unless otherwise indicated
[1] US market size only; [2] Total market for bio-based chemicals; [3] Projected 2022 cellulosic biofuels enzyme market

ENZIMA

1878, introducción del término. Nomenclatura sistemática: sufijo «asa» seguido del nombre del sustrato

1913, descripción cinética de la actividad enzimática por Michaelis y Menten

1926, primera preparación de una enzima pura cristalizada

1953, se desarrollaron técnicas de unión covalente de enzimas a resinas de poliamino-poliestireno.

El empleo de enzimas en gran escala se desarrolló lentamente hasta que se obtuvieron avances en las técnicas de inmovilización de enzimas.

INMOVILIZACIÓN: estabilidad y reutilización

MERCADO GLOBAL

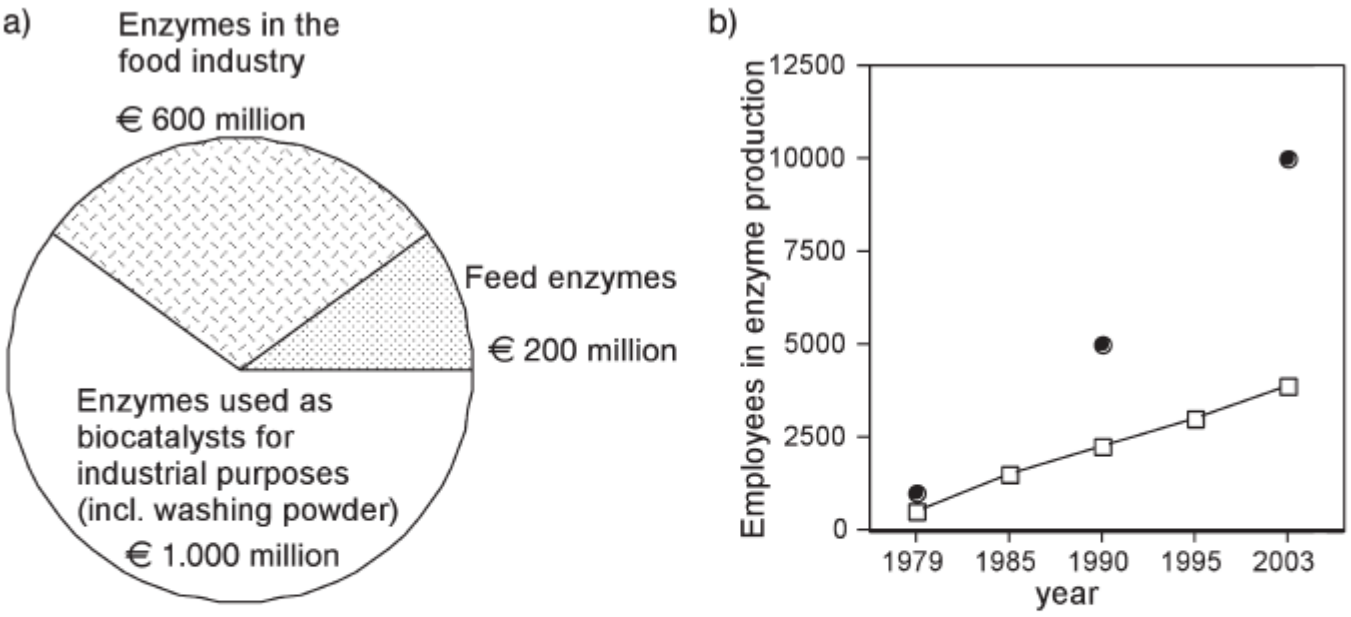


Fig. 1.5 Current market for enzymes for different purposes (a) and the increase in the application of enzymes reflected in the number of employees in the enzyme-producing industry (b) (for Novozymes (□) from yearly reports; worldwide (●) estimated).

Procesos industriales catalizados por enzimas

Table 1.2 Products produced in quantities larger than 1000 t year^{-1} by different companies with enzymes as biocatalysts. Some other products produced in the range 10 to 1000 t year^{-1} in recently developed enzyme processes are also included.

Product	*Enzyme*	*Free or immobilized enzyme*	*Companies*
> 10 000 000 t a^{-1}			
HFCS	amylase	free	several
	glucoamylase	free	
	glucose isomerase	immobilized	
Ethanol (gasoline additive)	amylase, glucoamylase	free free	several
> 10 000 t a^{-1}			
Acrylamide	nitrilase	immobilized cells	Nitto, DSM
6-Aminopenicillanic acid (6-APA)	penicillin amidase	immobilized	several
Cacao butter	lipase	immobilized	Fuji Oil, Unilever
Isomaltulose	sucrose mutase	in immobilized cells	Südzucker
Lactose-free milk or whey	β-galactosidase	free or immobilized	several
> 1000 t a^{-1}			
7-Aminocephalosporanic acid (7-ACA)	(*R*)-amino oxidase	immobilized	several
	glutaryl amidase	immobilized	
7-Aminodesacetoxycephalosporanic acid (7-ADCA)	glutaryl amidase (modified (?))	immobilized	DSM
(*S*)-Aspartic acid	aspartase	immobilized (?)	Tanabe
Aspartame	thermolysisn	immobilized	Toso, DSM
(*S*)-Methoxyisopropyl amine	lipase	immobilized	BASF
(*R*)-Pantothenic acid	aldolactonase		Fuji chem. Ind.
(*R*)-Phenylglycine	hydantoinase, carbamoylase	immobilized	several
(*S*)-Amino acids	aminoacylase	free	Degussa, Tanabe
1000 > 10 t a^{-1}			
Amoxicillin	penicillin amidase	immobilized	DSM
Cephalexin	penicillin amidase	immobilized	DSM
(*S*)-DOPA	β-tyrosinase	immobilized	Ajinomoto
Human insulin	carboxypeptidase A	free	Aventis
	lysyl endopeptidase	free	several
	trypsin	free	BASF
Sterically pure alcohols and amines	lipase	immobilized	BASF
(*R*)-Mandelic acid	nitrilase	immobilized	BASF

PROCESOS BIOTECNOLÓGICOS: enzimas o microorganismos?

Varias enzimas y regeneración de cofactores ⇨procesos de fermentación con células vivas

MICROORGANISMOS LIBRES

- Operación sencilla
- Regeneración
- A veces no requieren esterilidad
- Operación en estado estacionario, eficiencia catalítica constante
- Productos complejos
- Limitaciones: Productos no deseados (otras vías metabólicas). Producción de biomasa adicional.

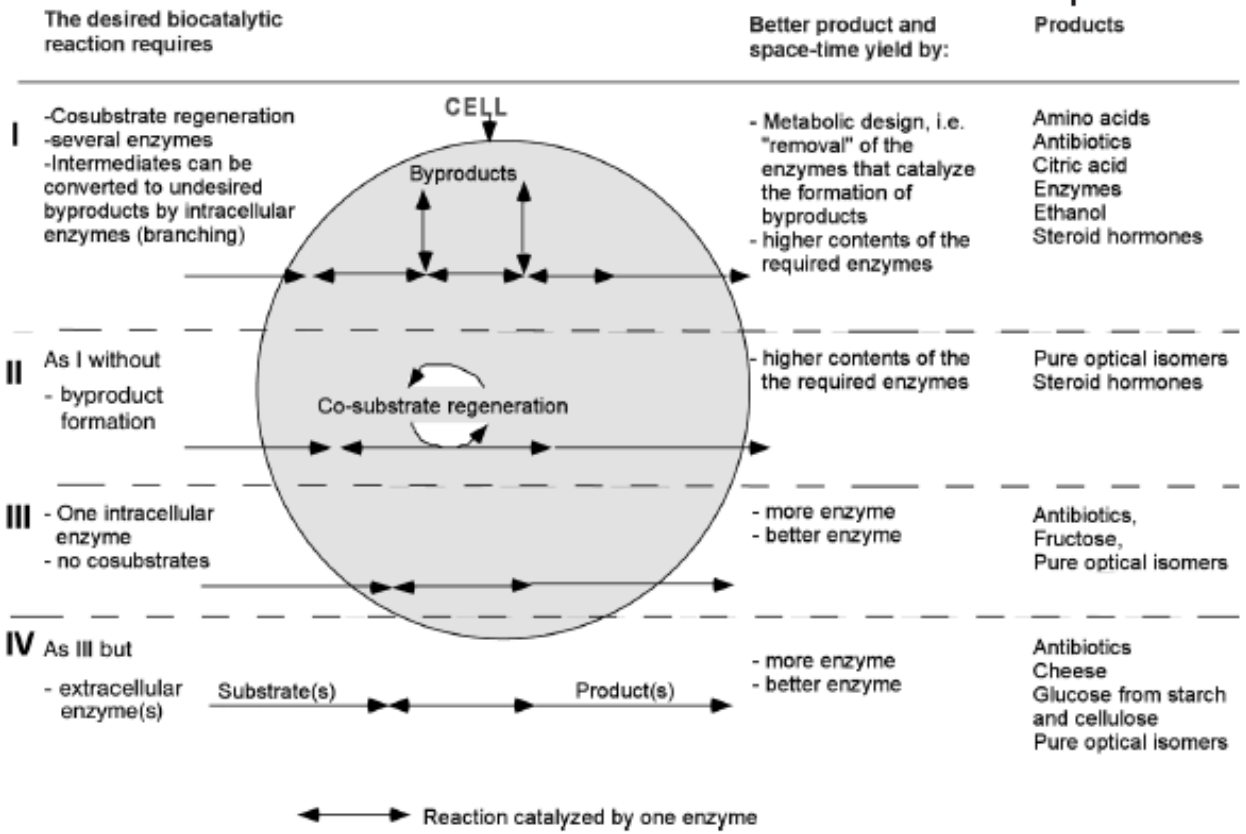


Fig. 1.6 Classification of biocatalytic processes with enzymes as biocatalysts. I and II must be performed with enzymes in living cells; III can be performed with enzymes in dead cells or as IV with isolated enzymes. Processes II–IV will be covered in this book. Process I will only be treated in connection with waste water and exhaust air treatment with immobilized cells (see Chapter 7).

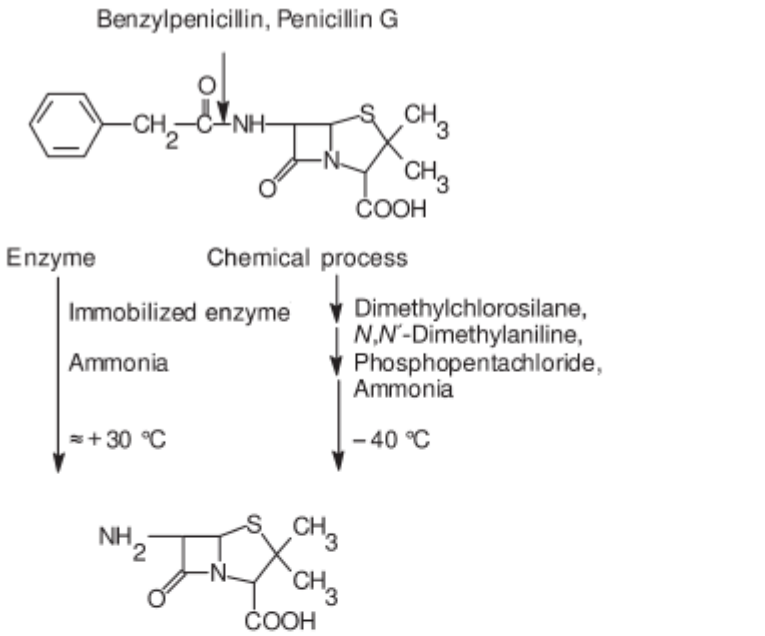
Pocas enzimas, sin cosustratos ⇨enzimas aisladas o células

CÉLULAS INMOVILIZADAS

- Situación intermedia

ENZIMAS

- Eficiencia catalítica
- Requerimientos de T, pH y presión
- Especificidad para el sustrato, reacción y producto
- Purificación por razones vinculadas al proceso(eliminación de otras enzimas) o legales (eliminación de componentes tóxicos y contaminantes)
- Estabilidad
- Reutilización



Required for the production of 500 t 6-APA

For hydrolysis:

1000 t	penicillin G	1000 t	penicillin G
45 t	ammonia	300 t	dimethylchlorosilane
≈ 1 t	immobilized enzyme	800 t	*N,N*-Dimethylaniline
10000 m^3	water	600 t	phosphopentachloride
		160 t	ammonia
		4200 m^3	dichloromethane
		4200 m^3	*n*-butanol

For downstream processing:

acetone	hydrochloric acid
ammonium bicarbonate	butylacetate
	acetone

Fig. 1.7 Comparison of the old (chemical) and the new (enzyme) process for the hydrolysis of penicillin G. The product, 6-aminopenicillinanic acid (6-APA), is used for the synthesis of semisynthetic penicillins with side chains other than phenylacetic acid. In the enzyme process, the byproduct phenylacetic acid can be recycled in the production of penicillin by fermentation (from Tischer, 1990).

Box 1.1 Advantages and disadvantages of cells and enzymes as biocatalysts in comparison with chemical catalysts.

Advantages:

- Stereo- and regioselective
- Low temperatures (0–110 °C) required
- Low energy consumption
- Active at pH 2–12
- Less byproducts
- Non-toxic when correctly used
- Can be reused (immobilized)
- Can be degraded biologically
- Can be produced in unlimited quantities

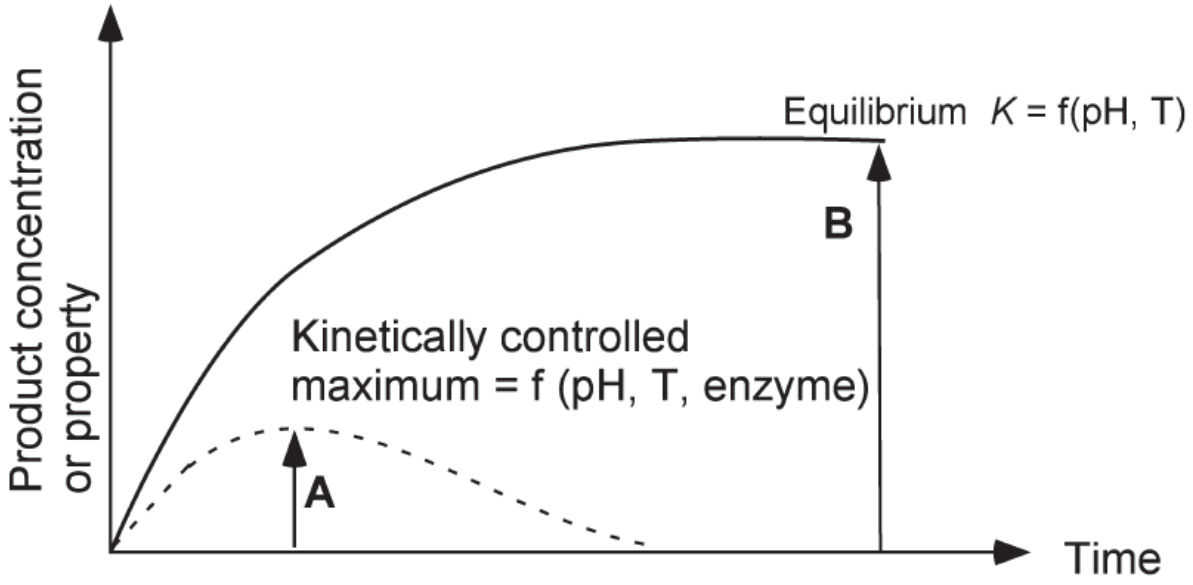
Disadvantages:

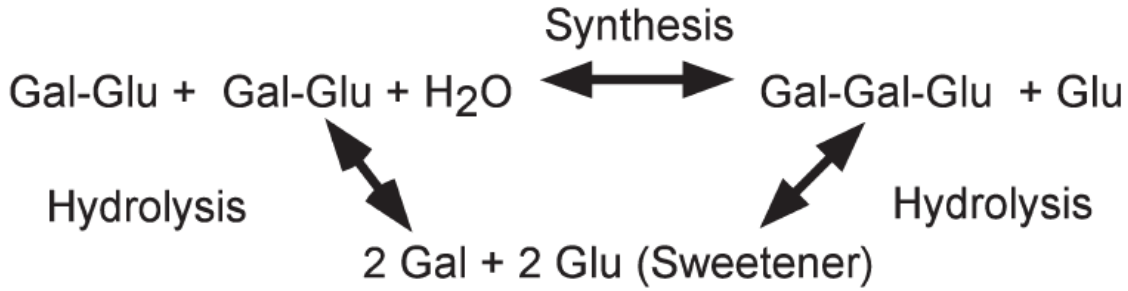
- Cells and enzymes are
  - unstable at high temperatures
  - unstable at extreme pH-values
  - unstable in aggressive solvents
  - inhibited by some metal ions
  - hydrolyzed by peptidases
- Some enzymes
  - are still very expensive
  - require expensive cosubstrates
- When inhaled or ingested enzymes are, as all foreign proteins, potential allergens

Table 1.3 Economic and environmental sustainability goals that can be realized in enzyme processes (modified from Uhlig, 1998).

Goals	*Means to achieve the goals*	*Products/Processes*
Cost reduction	Yield increase	Penicillin-Cephalosporin C hydrolysis
	Biocatalyst reuse and increased productivity by immobilization	Glucose isomerization
	Better utilization of the raw material	Isomaltulose production Juice and wine production
	Reduction of process costs for • filtration • energy • desizing of fibers • cheese ripening • malting in beer production	Sterile filtration of plant extracts; Low temperature washing powder Desizing with enzymes Increase rate of process with enzymes
	Reduction of residence time in starch processing	
Improvement of biological properties and quality	Produce only isomers with the desired biological property	Racemate resolution
	Improved preservation of foods	Juice concentrates
	Improvement of technical properties	Protein modification, flour for baking, transesterification of vegetable oils, biodiesel
	Improved taste (sweeteness)	Glucose isomerization to glucose-fructose syrup
Utilization of new regenerable sources of raw materials	Utilization of wastes from food and wood industry (whey, filter cakes with starch and protein from vegetable oil production, cellulose)	Drinks from whey Ethanol, biodiesel Animal feed
Reduction of environmental impact	Reduction of non-recyclable waste	Penicillin-, Cephalosporin C hydrolysis, leather production, paper bleaching
	Waste recycling	Utilization of whey

Hidrólisis de la Lactosa





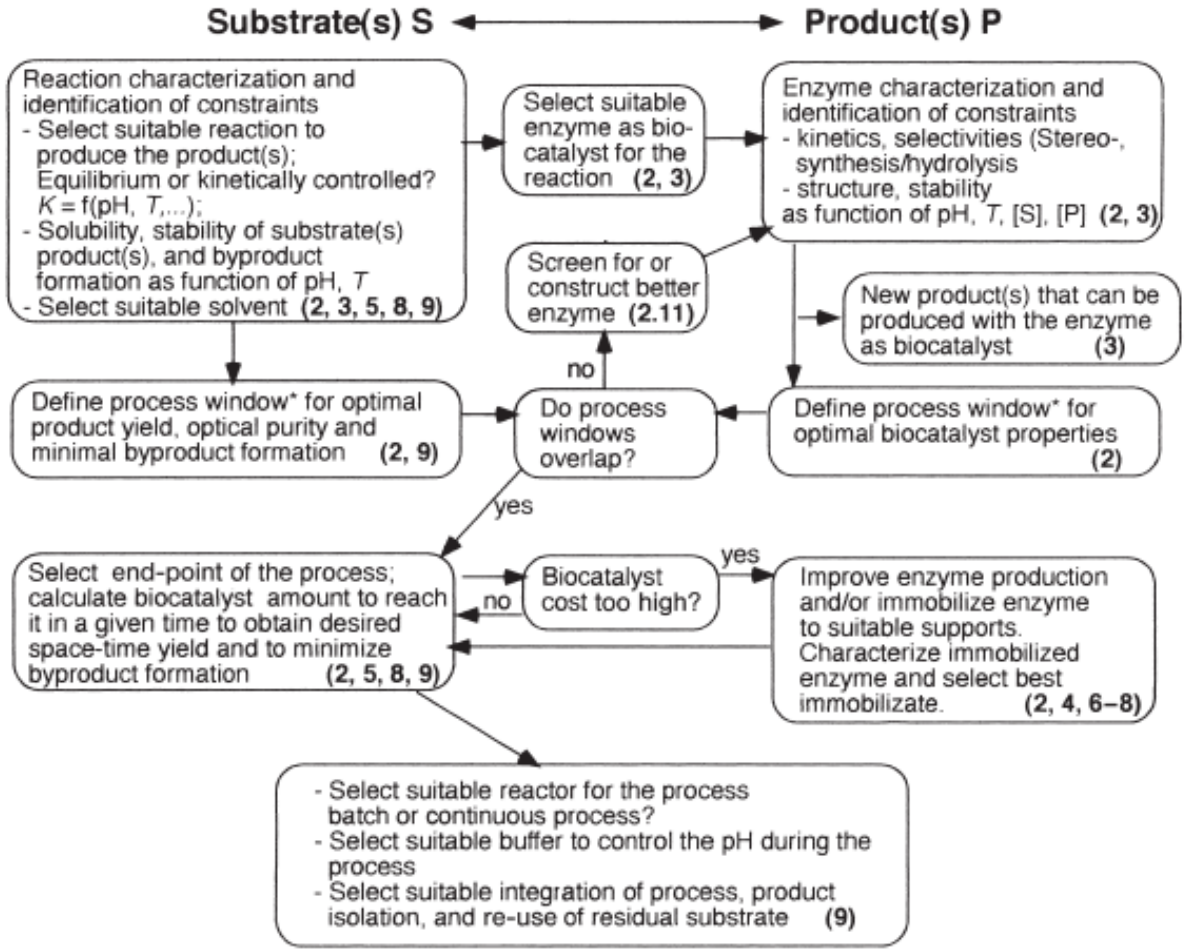


Fig. 1.10 Steps to consider in the design of an enzyme process to produce existing or new products (bold numbers refer to chapters in this book). *Process window = the range in a pH-*T*- (or pH-[S]-, pH-[P]-, *T*-[S]-, *T*-[P]-) plane where the reaction can be carried out with a given yield or optical purity, and where the properties (activity, selectivity, stability) of the biocatalyst are optimal.