Domestication of Microbes – Applied and Industrial Microbiology

• Empirical biotechnology: microbial processes used long (>4000 years) before development of microbiology as a science

remnants of a fermented drink in fragments of 9,000-year-old Chinese vessels

• Louis Pasteur
  1857 Microbiology of lactic acids fermentations
  1860 Role of yeast in ethanolic fermentation
• advances in applied microbiology led the development of microbiology

Selman Waksman (~1945)
"There is no field of human endeavor…
…where the microbe does not play an important and often dominant part."

Microbial Applications

1. **Food and beverage biotechnology**
   • fermented foods, alcoholic beverages (beer, wine)
   • flavors
2. **Enzyme technology**
   • production and application of enzymes
3. **Metabolites from microorganisms**
   • amino acids
   • antibiotics, vaccines, biopharmaceuticals
   • bacterial polysaccharides and polyesters
   • specialty chemicals for organic synthesis (chiral synthons)
4. **Biological fuel generation**
   • ethanol or methane from biomass, single cell protein, production of biomass
   • microbial recovery of petroleum
5. **Environmental biotechnology**
   • water and wastewater treatment
   • composting (and landfilling) of solid waste
   • biodegradation/bioremediation of toxic chemicals and hazardous waste
6. **Agricultural biotechnology**
   • soil fertility
   • microbial insecticides, plant cloning technologies
7. **Diagnostic tools**
   • testing & diagnosis for clinical, food, environmental, agricultural applications
   • biosensors

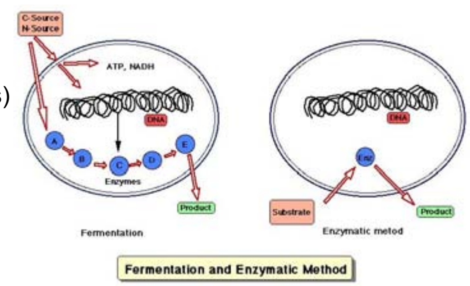
Control of Microorganisms

1. Preventing food spoilage
- sterilization methods, canning
- chemical & physical control of growth
- water activity, acidity, pickling, etc.
- fermented foods

2. Sanitation, prevention of waterborne disease
- disinfectants, antiseptics, etc.
- water treatment (filtration, chlorination…)
- wastewater treatment

3. Prevention of biodeterioration

1.Microbial cells or cell products
2. Enzymatic biotransformation products
3.Fermentation products (de novo synthesis)



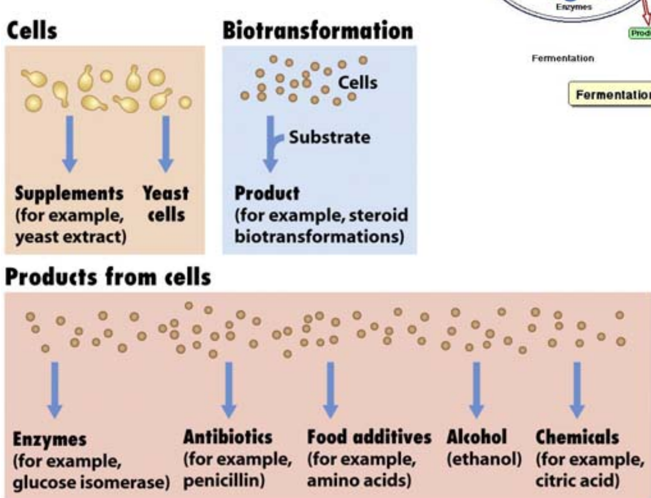


Figure 30-1 Brock Biology of Microorganisms 11/e
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Major commercial products obtained from microbes

- I. Foods, flavoring agents and food supplements, and beverages
  - Foods
    - Fermented meat
    - Cheeses and milk products
    - Edible mushrooms
    - Leavened bread-baker's yeast
    - Coffee
    - Pickles, olives, sauerkraut
    - Single-cell protein
  - Flavoring agents and food supplements
    - Vinegar
    - Nucleosides
    - Amino acids
    - Vitamins
  - Beverages
    - Wines
    - Beer, ale
    - Whiskey
  - Vitamins
    - B_{12}
    - Riboflavin
- II. Organic acids
  - Citric acid
  - Itaconic acid
- III. Enzymes and microbial transformations
  - Commercial enzymes
  - Sterol conversions
- IV. Inhibitors
  - Biocides
  - Antibiotics
- V. Products of genetically engineered microbes
  - Insulin
  - Human growth factor

Primary vs. Secondary metabolism

Primary metabolites:

- produced during active growth
- generally a consequence of energy metabolism and necessary for the continued growth of the microorganism

  Substrate A ➡ Product

  Substrate A ➡ B ➡ C ➡ Product
- ethanol, lactic acid,…

Secondary metabolites:

- synthesized after the growth phase nears completion
- a result of complex reactions that occur during the latter stages of primary growth

  Substrate A ➡ B ➡ C ➡ Primary metabolism (no product)

  ↘

  D ➡ E ➡ Product of secondary metabolism

  Substrate A ➡ B ➡ C ➡ Primary metabolism (no product)

  afterwards, the product is formed by metabolism of an intermediate

  C ➡ D ➡ Product

- growth phase = trophophase
- idiophase = phase involved in production of metabolites
- citric acid, antibiotics,…

Growth in batch culture

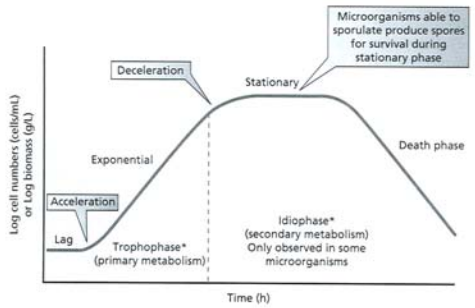
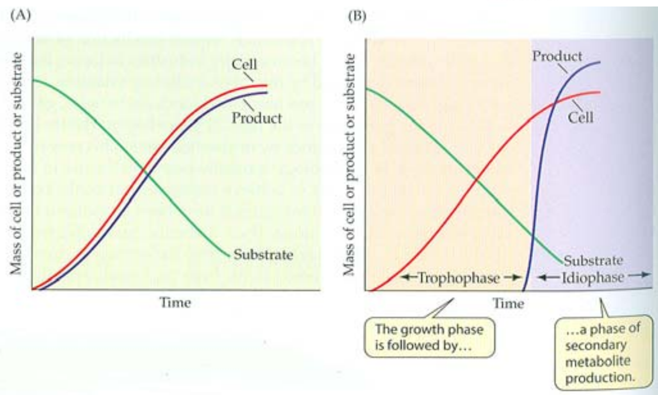


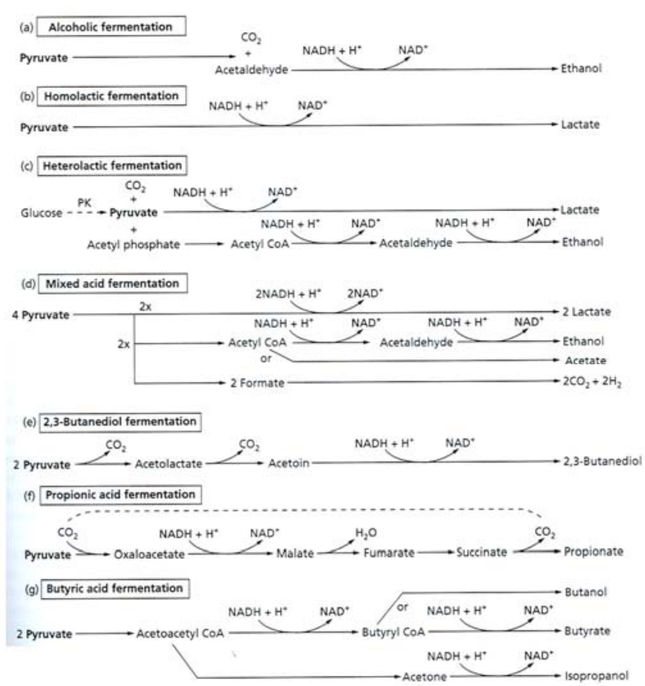
Fig. 2.1 Growth of a microorganism in a batch culture. *Trophophase and idiophase: see Chapter 3, Secondary metabolism.

Primary metabolite

Secondary metabolite



Fermentation products from pyruvate



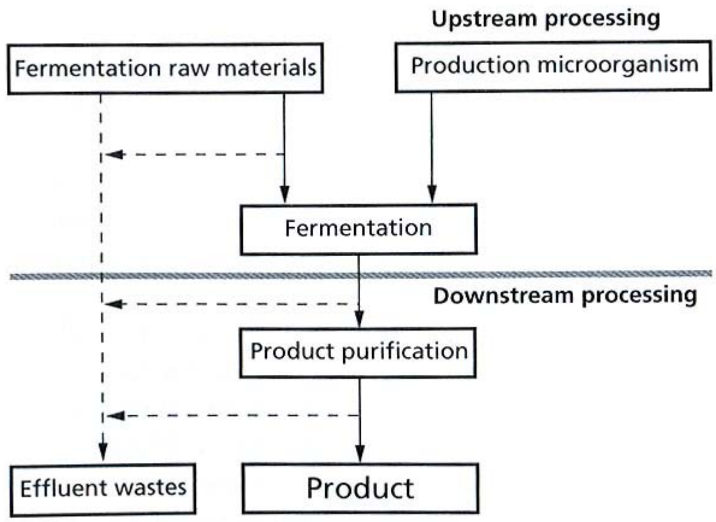


Fig. i Outline of a fermentation process.

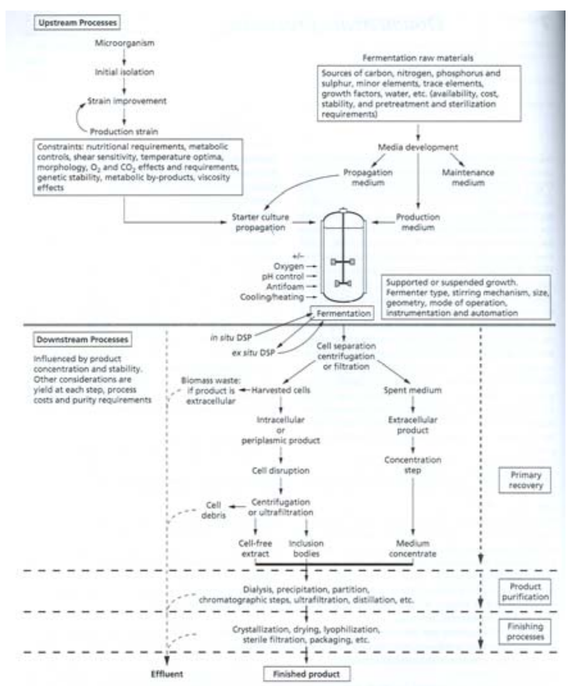
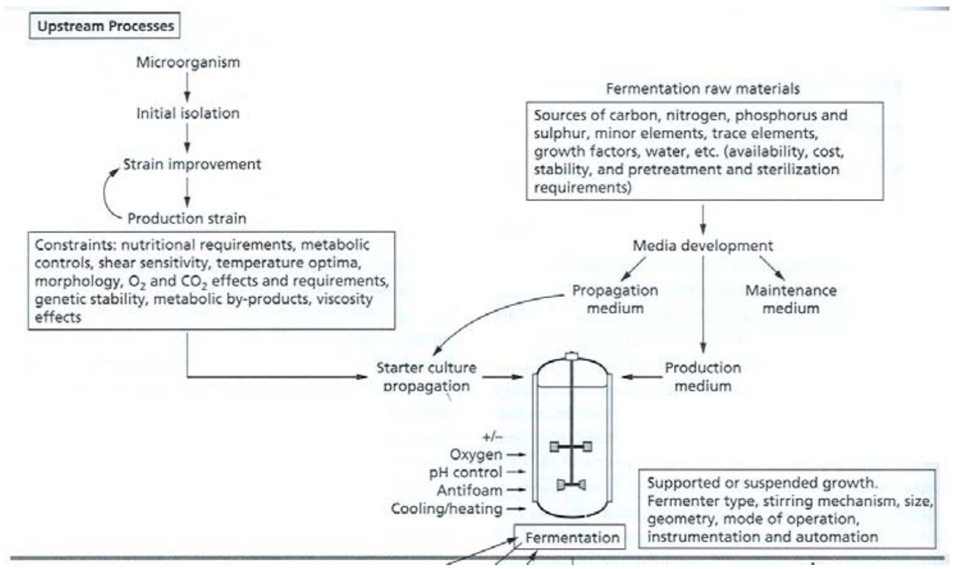


Fig. 7.1 An outline of upstream and downstream processing operations.


Upstream Processes
Microorganism
Initial isolation
Strain improvement
Production strain
Constraints: nutritional requirements, metabolic controls, shear sensitivity, temperature optima, morphology, O2 and CO2 effects and requirements, genetic stability, metabolic by-products, viscosity effects
Fermentation raw materials
Sources of carbon, nitrogen, phosphorus and sulphur, minor elements, trace elements, growth factors, water, etc. (availability, cost, stability, and pretreatment and sterilization requirements)
Media development
Propagation medium
Maintenance medium
Starter culture propagation
Production medium
+/–
Oxygen
pH control
Antifoam
Cooling/heating
Fermentation
Supported or suspended growth. Fermenter type, stirring mechanism, size, geometry, mode of operation, instrumentation and automation

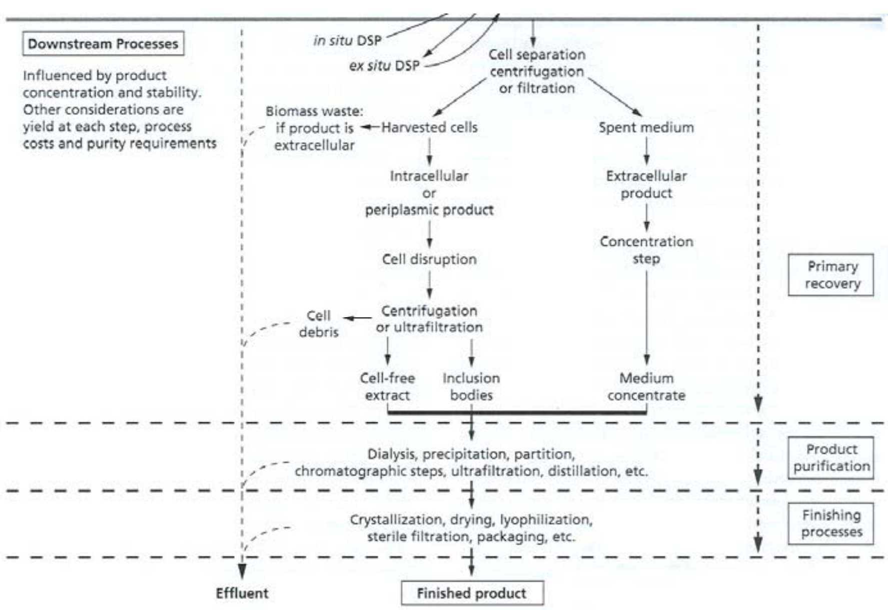

Downstream Processes
Influenced by product concentration and stability. Other considerations are yield at each step, process costs and purity requirements
in situ DSP
ex situ DSP
Cell separation centrifugation or filtration
Biomass waste: if product is extracellular
Harvested cells
Spent medium
Intracellular or periplasmic product
Extracellular product
Concentration step
Cell disruption
Primary recovery
Cell debris
Centrifugation or ultrafiltration
Cell-free extract
Inclusion bodies
Medium concentrate
Dialysis, precipitation, partition, chromatographic steps, ultrafiltration, distillation, etc.
Product purification
Crystallization, drying, lyophilization, sterile filtration, packaging, etc.
Finishing processes
Effluent
Finished product

Table 4.2 Examples of microorganisms classified as GRAS (generally regarded as safe)

Bacteria
Bacillus subtilis
Lactobacillus bulgaricus
Lactococcus lactis
Leuconostoc oenos
Yeasts
Candida utilis
Kluyveromyces marxianus
Kluyveromyces lactis
Saccharomyces cerevisiae
Filamentous fungi
Aspergillus niger
Aspergillus oryzae
Mucor javanicus (Mucor circinelloides f. *circinelloides)*
Penicillium roqueforti

Note: Normally, these microorganisms require no further testing if used under acceptable cultivation conditions.

Ethanol

• *the* major microbial biotechnology: beer, wine, distilled beverages, industrial ethanol

Saccharomyces (sugar fungus)
• alcoholic (ethanolic) fermentation, principally by yeasts in the genus *Saccharomyces*
• Embden-Meyerhof-Parnas, glycolytic pathway

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i \rightarrow 2\ \text{EtOH} + 2\ CO_2 + 2\ \text{ATP}$$

• not a facultative anaerobe, cannot grow anaerobically indefinitely (unsaturated fatty acids and sterols can be synthesized only under aerobic conditions)
• when oxygen present glucose oxidized via the Krebs cycle to CO_2 and water (much biomass and little alcohol produced)

Zymomonas mobilis
• Alphaproteobacterium
• osmotic tolerance, relatively high alcohol tolerance
• higher specific growth rate than yeast
• anaerobic carbohydrate metabolism through the Entner-Doudoroff pathway, yielding only 1 mol of ATP per mol of glucose ➡ more glucose converted to EtOH
• limited substrate use, only 3 carbohydrates: glucose, fructose and sucrose
• genetic engineering to expand substrate range

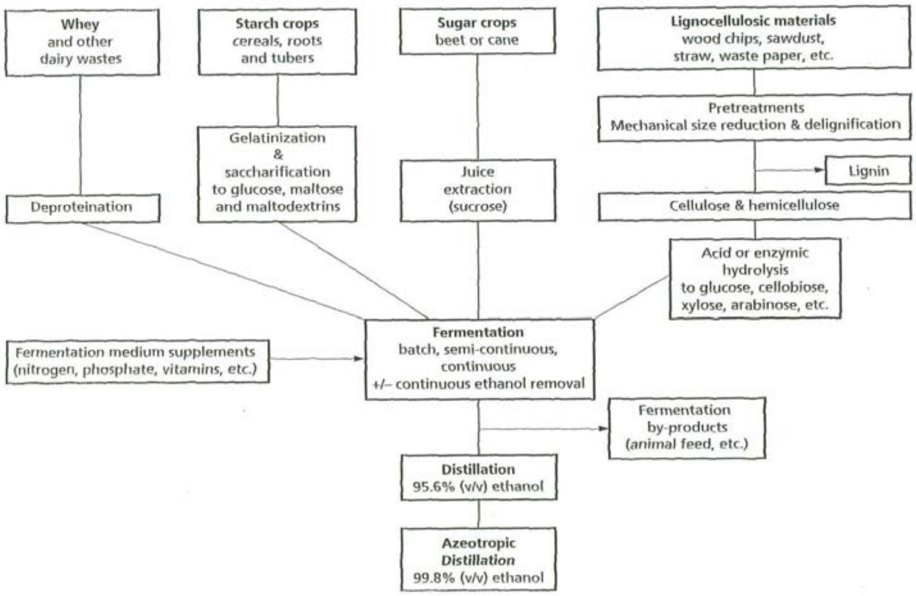
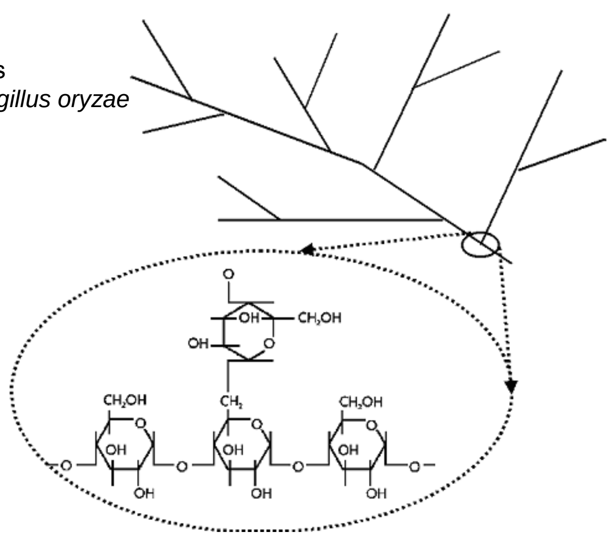


Fig. 10.3 Industrial ethanol production from various substrates.

Conversion of starch to fermentable sugars

1. Malting
   - germination of barley to induce production of amylases
   - beer

2. Amylolytic molds and yeasts
   - filamentous fungus *Aspergillus oryzae*
   - Japanese sake



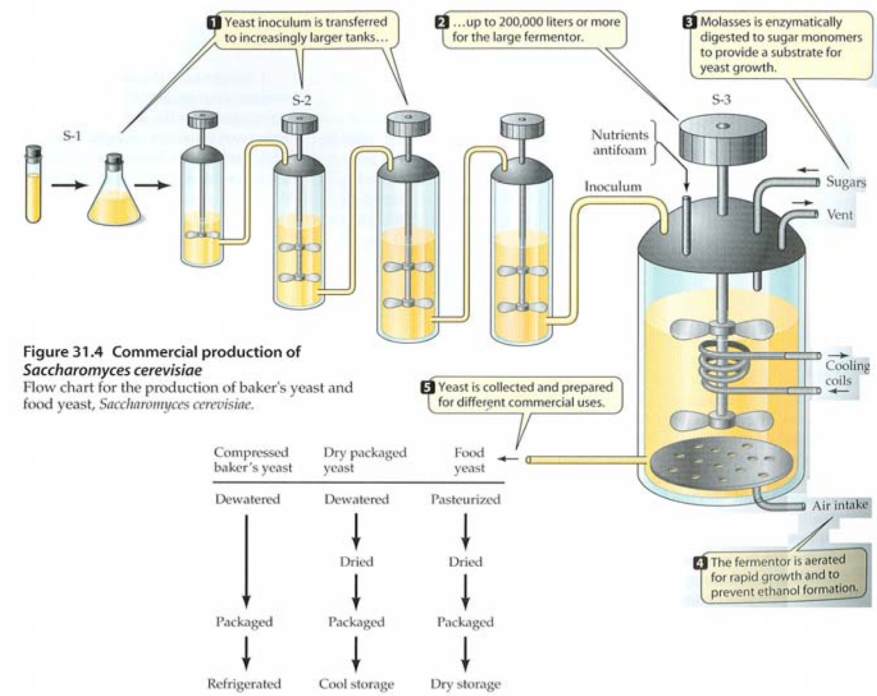
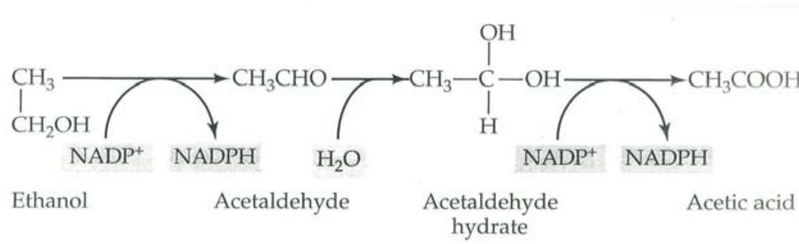


Figure 31.4 Commercial production of *Saccharomyces cerevisiae*
Flow chart for the production of baker's yeast and food yeast, *Saccharomyces cerevisiae*.

Vinegar

- sour (spoiled) wine, vinegar (from French): vin and aigre (sour)
- production in the US about 160 million gallons per year
  (2/3 used in commercial products such as sauces and dressings, production of pickles and tomato products)
- the acetic acid bacteria divided into two genera:
  Acetobacter aceti and *Gluconobacter oxydans*
- obligate aerobes that oxidize sugar, sugar alcohols and ethanol with the production of acetic acid as the major end product
- ethanol oxidation occurs via two membrane-associated dehydrogenases: alcohol dehydrogenase and acetaldehyde dehydrogenase



The organisms employed in commercial vinegar production are *Acetobacter aceti* and *Gluconobacter oxydans*.

Industrial Production of Acetic Acid

Trickling filter
- vinegar manufacturing industry near Orleans in 14th century
- trickling filter, wooden bioreactor (volume up to 60 m3) filled with beechwood shavings, acetic acid bacteria grow as biofilm
- the ethanolic solution is sprayed over the surface and trickles through the shavings into a collection basin, and recirculated
- temperature maintained at 29-35°C
- about 12% acetic acid produced in 3 days
- the life of a well-packed and maintained generator is about 20 years

Submerged, batch process (Frings acetator)
- stainless steel tank with a high-speed mixer
  microbes, air, ethanol and nutrients are mixed to provide a favorable environment for microbial growth
- 30°C maintained by circulation of cooling water
- 12% acetic acid in about 35 h
- production rate per m3 over 10 times higher than with surface “fermentation” and over 5% higher than with trickling filter

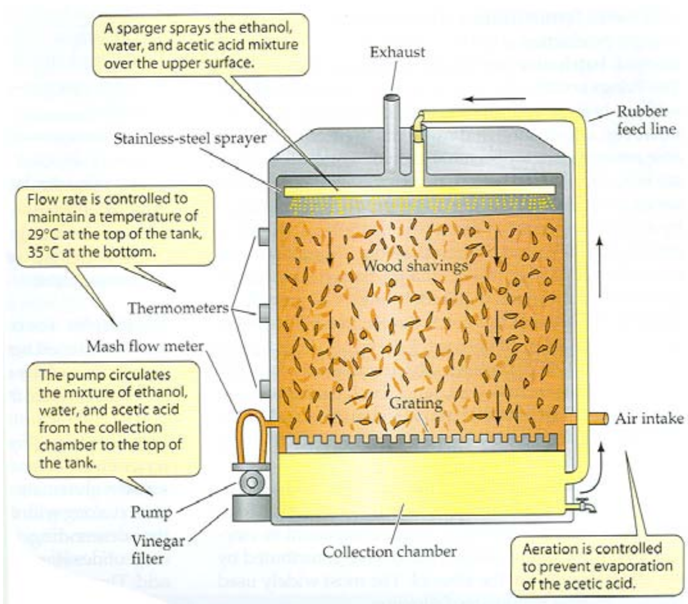


Figure 31.5 Manufacture of vinegar by continuous fermentation
Frings-type trickling generator for the commercial production of vinegar.

Table 42.10
Major Organic Acids Produced by Microbial Processes

Product	Microorganism Used	Representative Uses	Fermentation Conditions
Acetic acid	*Acetobacter* with ethanol solutions	Wide variety of food uses	Single-step oxidation, with 15% solutions produced; 95–99% yields
Citric acid	*Aspergillus niger* in molasses-based medium	Pharmaceuticals, as a food additive	High carbohydrate concentrations and controlled limitation of trace metals; 60–80% yields
Fumaric acid	*Rhizopus nigricans* in sugar-based medium	Resin manufacture, tanning, and sizing	Strongly aerobic fermentation; carbon-nitrogen ratio is critical; zinc should be limited; 60% yields
Gluconic acid	*Aspergillus niger* in glucose-mineral salts medium	A carrier for calcium and sodium	Uses agitation or stirred fermenters; 95% yields
Itaconic acid	*Aspergillus terreus* in molasses-salts medium	Esters can be polymerized to make plastics	Highly aerobic medium, below pH 2.2; 85% yields
Kojic acid	*Aspergillus flavus-oryzae* in carbohydrate-inorganic N medium	The manufacture of fungicides and insecticides when complexed with metals	Iron must be carefully controlled to avoid reaction with kojic acid after fermentation
Lactic acid	Homofermentative *Lactobacillus delbrueckii*	As a carrier for calcium and as an acidifier	Purified medium used to facilitate extraction

Lactic acid fermentation

- pyruvate is reduced to lactic acid with the coupled reoxidation of NADH to NAD^+
- lactic acid bacteria (e.g. *Lactobacillus*, *Streptococcus*) involved in many food fermentations
- fermented milk, cheese, fermented vegetables

Homolactic fermentation
- glucose degraded via EMP pathway, with lactic acid as the only end product

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i \rightarrow 2\ \text{lactic acid} + 2\ \text{ATP}$$

- carried out by *Streptococcus, Pediococcus, Lactococcus, Enterococcus* and various *Lactobacillus* species
- important in dairy industry (yogurt, cheese)

Heterolactic fermentation
- glucose degraded via pentose phosphate pathway
- in addition to lactic acid, also ethanol and CO2 produced

$$\text{glucose} + \text{ADP} + P_i \rightarrow \text{lactic acid} + \text{ethanol} + CO_2 + \text{ATP}$$

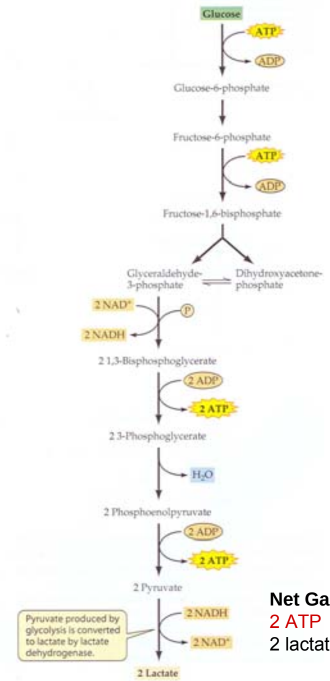
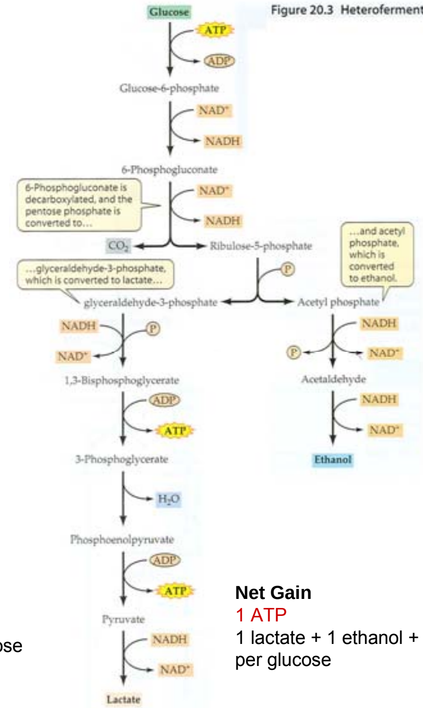


Figure 20.2 Homofermentation pathway

Net Gain
2 ATP
2 lactate per glucose

Figure 20.3 Heterofermentation pathway



Net Gain
1 ATP
1 lactate + 1 ethanol + 1 CO_2 per glucose

Table 31.1 Microorganisms involved in the manufacture of cheeses and fermented milks

Products	Principal acid producers	Intentionally introduced secondary microflora
Cheeses		
Colby, Cheddar, cottage, cream	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis*	None
Gouda, Edam, Havarti	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis*	*Leuconostoc* sp., Cit+ *Lactococcus lactis* subsp. *lactis*
Brick, Limburger	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis*	*Geotrichum candidum, Brevibacterium linens, Micrococcus* sp.
Camembert	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis*	*Penicillium camemberti,* sometimes *Brevibacterium linens*
Blue	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis*	Cit+ *Lactococcus lactis* subsp. *lactis, Penicillium roqueforti*
Mozzarella, provolone, Romano, Parmesan	*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lb. helveticus*	None; animal lipases added to Romano for picante or rancid flavor
Swiss	*Streptococcus thermophilus, Lactobacillus helveticus, Lb. delbrueckii* subsp. *bulgaricus*	*Propionibacterium freudenreichii* subsp. *shermanii*
Fermented milks		
Yogurt	*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*	None
Buttermilk	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis*	*Leuconostoc* sp., Cit+ *Lactococcus lactis* subsp. *lactis*
Sour cream	*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp.	None

Cheese making

Pre-ripening
- inoculation with starter culture of lactic acid bacteria
- acidification by fermentation of lactose to lactic acid

Coagulation of milk proteins (casein)
- addition of chymosin (rennin), an acid protease from calves' stomach - *renneting*
  since 1980s also recombinant enzyme produced in yeast
- catalyzes specific hydrolysis of κ-casein → leads to coagulation in the presence of Ca^{2+} to form a gel (curd)
- also traps bacteria that continue lactic acid fermentation
- removal of liquid whey from the curd

- addition of secondary microbiota
- treatments vary for different cheese varieties

Separation of curd and whey
- accelerated by decrease in pH
- salting
- curd pressed and placed into mold

Ripening

- storage at controlled humidity at ~9°C for up to a year
- modification of proteins and fats by proteases and lipases
- complex development of flavor

Table 30.3 Amino acids used in the food industry[a]

Amino acid[b]	Annual production worldwide (metric tons)	Uses	Purpose
L-Glutamate (monosodium glutamate, MSG)	370,000	Various foods	Flavor enhancer; meat tenderizer
L-Aspartate and alanine	5,000	Fruit juices	"Round off" taste
Glycine	6,000	Sweetened foods	Improves flavor; starting point for organic syntheses
L-Cysteine	700	Bread Fruit juices	Improves quality Antioxidant
L-Tryptophan + L-Histidine	400	Various foods, dried milk	Antioxidant, prevent rancidity; nutritive additives
Aspartame (made from L- phenylalanine + L-aspartic acid)	7,000	Soft drinks, chewing gum, many other "sugar-free" products	Low-calorie sweetener
L-Lysine	70,000	Bread (Japan), feed additives	Nutritive additive
DL-Methionine	70,000	Soy products, feed additives	Nutritive additive

[a] Data from Glazer, A. N., and H. Mikaido. 1995. *Microbial Biotechnology*, W. H. Freeman, New York.
[b] The structures of these amino acids are shown in Figure 3.12.

Table 30-3 Brock Biology of Microorganisms 11/e

Amino acids

- annual worldwide production of over 400,000 tons
- uses as food additives, medicines, starting material in chemical synthesis
  - glutamic acid (80% of total), lysine (10%)
- production of glutamic acid (MSG) for use in foods; other amino acids (L-lysine)
- *Corynebacterium glutamicum*

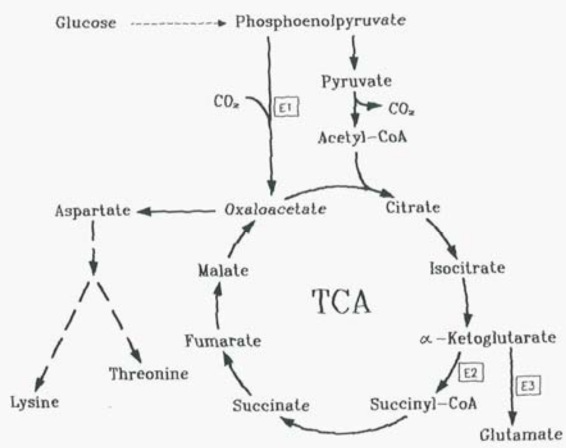


Fig. 2. Metabolic pathways in glutamic acid-producing corynebacteria. Abbreviations: E1, phosphoenolpyruvate carboxylase; E2, α-ketoglutarate dehydrogenase; E3, glutamate dehydrogenase.

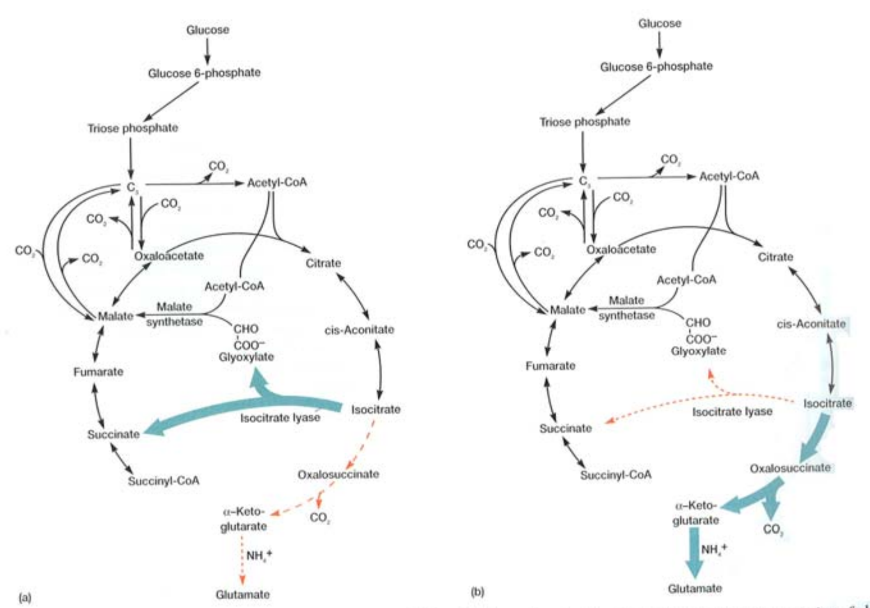
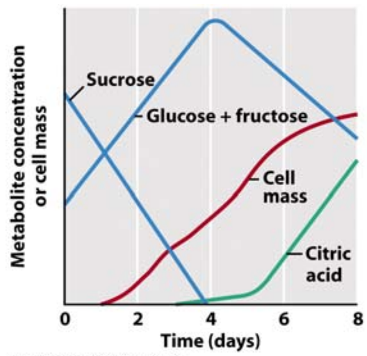


Figure 42.10 Glutamic Acid Production. The sequence of biosynthetic reactions leading from glucose to the accumulation of gl[illegible]tamate by *Corynebacterium glutamicum.* Major carbon flows are noted by bold arrows. **(a)** Growth with use of the glyoxylate bypass [illegible] provide critical intermediates in the TCA cycle. **(b)** After growth is completed, most of the substrate carbon is processed to glutamat[illegible] (note shifted bold arrows). The dashed lines indicate reactions that are being used to a lesser extent.

Citric acid

- over 130,000 tons produced worldwide each year
- used in foods and beverages
- iron citrate as a source of iron
  preservative for stored blood, tablets, ointments,…
  in detergents as a replacement for polyphosphates
- a microbial fermentation for production of citric acid developed in 1923
- today >99% of the world's output produced microbially
  Aspergillus niger
- submerged fermentation in large fermenters
- sucrose as substrate, and citric acid produced during idiophase
  - during trophophase mycelium produced and CO_2 released
  - during idiophase glucose and fructose are metabolized directly to citric acid



Antibiotics

- Antibiotics are small molecular weight compounds that inhibit or kill microorganisms at low concentrations
- often products of secondary metabolism
- the significance of antibiotic production is unclear, may be of ecological significance for the organism in nature
- antibiotics produced by various bacteria, actinomycetes & fungi
  Bacillus
  Streptomyces
  Penicillium

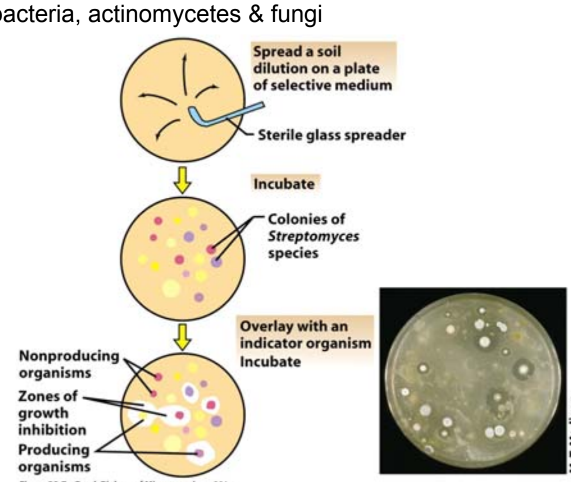


Figure 30-7a Brock Biology of Microorganisms 11/e
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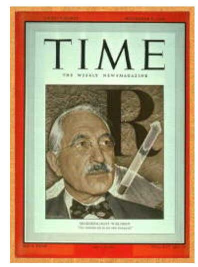
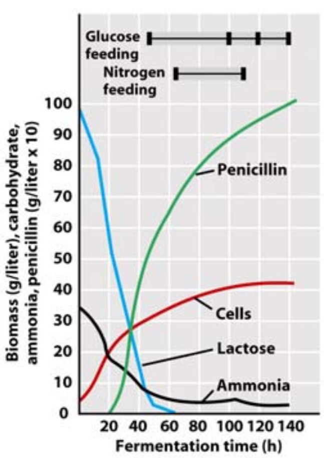
Table 20.13 Important antibiotics produced by *Streptomyces* species

Antibiotic Group	Species	Common Name	Effective Against
Chloramphenicol	*S. venezuelae*	Chloramphenicol	Broad spectrum
Tetracycline	*S. aureofaciens*	Tetracycline	Broad spectrum
Chlortetracycline	*S. aureofaciens*	Chlortetracycline	Broad spectrum
Polyenes	*S. noursei*	Nystatin	Fungi
Macrolides	*S. erythreus*	Erythromcin	Most gram-positives
Legionella	*S. lincolnensis*	Clindamycin	Obligate anaerobes
Aminoglycosides	*S. griseus*	Streptomycin	Most gram-negative
	S. fradiae	Neomycin	Broad spectrum





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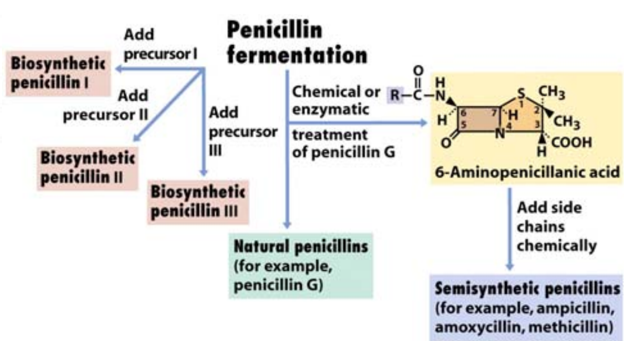

Figure 30-9 Brock Biology of Microorganisms 11/e
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Enzymes

Commercially produced enzymes:

- enzymes used in ***industry***, such as amylases, proteases, catalases, isomerases
- enzymes used for ***analytical*** purposes, such as glucose oxidase, alcohol dehydrogenase, hexokinase, cholesterol oxidase
- enzymes used in ***medicine***, such as asparaginase, proteases, lipases
- different levels of quantity and quality

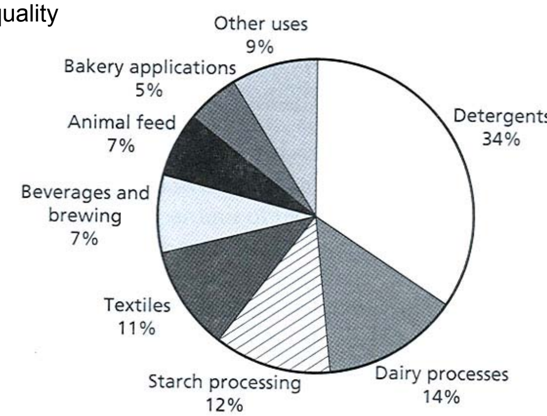


Fig. 9.1 Applications of bulk microbial enzymes.

Table 31.7 Microbial enzymes and their commercial application

Enzyme	Genus of Producer	Use
Bacterial proteases	*Bacillus, Streptomyces*	Detergents
Asparaginase	*Escherichia, Serratia*	Antitumor agent
Glucoamylase	*Aspergillus*	Fructose syrup production
Bacterial amylases	*Bacillus*	Starch liquefaction, brewing, baking, feed, detergents
Glucose isomerase	*Bacillus, Streptomyces*	Sweeteners
Rennin	*Alcaligenes, Aspergillus, Candida*	Cheese manufacture
Pectinase	*Aspergillus*	Clarify fruit juice
Lipases	*Micrococcus*	Cheese production
Penicillin acylase	*Escherichia*	Semisynthetic penicillins
Taq polymerase	*Thermus aquaticus*	Polymerase chain reaction

Table 30.4 Microbial enzymes and their applications

Enzyme	Source	Application	Industry
Amylase (starch-digesting)	Fungi	Bread	Baking
	Bacteria	Starch coatings	Paper
	Fungi	Syrup and glucose manufacture	Food
	Bacteria	Cold-swelling laundry starch	Starch
	Fungi	Digestive aid	Pharmaceutical
	Bacteria	Removal of coatings (desizing)	Textile
	Bacteria	Removal of stains; detergents	Laundry
Protease (protein-digesting)	Fungi	Bread	Baking
	Bacteria	Spot removal	Dry cleaning
	Bacteria	Meat tenderizing	Meat
	Bacteria	Wound cleansing	Medicine
	Bacteria	Desizing	Textile
	Bacteria	Household detergent	Laundry
Invertase (sucrose-digesting)	Yeast	Soft-center candies	Candy
Glucose oxidase	Fungi	Glucose removal, oxygen removal	Food
		Test paper for diabetes	Pharmaceutical
Glucose isomerase	*Bacteria*	High-fructose corn syrup	Soft drink
Pectinase	Fungi	Pressing, clarification	Wine, fruit juice
Rennin	Fungi	Coagulation of milk	Cheese
Cellulase	*Bacteria*	Fabric softening, brightening; detergent	Laundry
Lipase	Fungi	Breaks down fat	Dairy, laundry
Lactase	Fungi	Breaks down lactose to glucose and galactose	Dairy, health foods
DNA polymerase	*Bacteria* *Archaea*	DNA replication in polymerase chain reaction (PCR) technique (Section 7.9)	Biological research; forensics

Table 30-4 Brock Biology of Microorganisms 11/e

KAISERLICHES PATENTAMT.

AUSGEGEBEN DEN 4. MAI 1915.

PATENTSCHRIFT

— № 283923 —

KLASSE 8*i*. GRUPPE 5.

DR. OTTO RÖHM IN DARMSTADT.

Verfahren zum Reinigen von Wäschestücken aller Art.

Patentiert im Deutschen Reiche vom 12. Dezember 1913 ab.

Die tryptischen Enzyme haben bekanntlich die Eigenschaft, Eiweiß und Fett abzubauen. Von der Erwägung ausgehend, daß der Schmutz der menschlichen Kleidungsstücke aller Art zu einem großen Teil aus Fett- und Eiweißresten besteht, werden der Waschbrühe tryptische Enzyme zugesetzt. Es zeigte sich, daß die Wäsche viel rascher, mit viel geringerer Kraftanstrengung und bei einer weit unter dem Siedepunkt des Wassers liegenden Temperatur rein wurde und ein viel schöneres Aussehen erhielt, als ohne Zusatz der Enzyme. Auch kommt man mit weniger Seife aus. Der Hauptvorteil der Verwendung von Enzymen gegenüber anderen, namentlich alkalischen Zusätzen beruht darin, daß sie das Gewebe nicht im allermindesten angreifen und auch für die Hände der Wäscherinnen vollkommen unschädlich sind.

Die benötigten Mengen Enzym sind äußerst gering. Für 100 l Waschbrühe genügen z. B. 2 g Pankreatin.

Weiter wurde gefunden, daß die tryptischen Enzyme auch äußerst wichtige Toilettenmittel sind. Denn bekanntlich scheidet der Körper durch die Hauptporen alle möglichen Eiweiß- und Fettreste ab, die sich teilweise in den Hauptporen festsetzen. Die tryptischen Enzyme sind nun hervorragend geeignet, diese Stoffe löslich zu machen. Zusatz von Enzymen zum Waschwasser macht das Wasser weich und übt auf die Haut einen außerordentlich wohltätigen Einfluß aus. Die Haut wird rein und auffallend weich und zart, und obendrein ist der Seifenverbrauch geringer.

Als Zusatz zum Wasch- und Badewasser genügt 0,5 bis 1 g Pankreatin auf 100 l Wasser.

Da derart kleine Mengen schlecht zu handhaben sind, empfiehlt es sich, die Enzyme für den praktischen Gebrauch, sei es als Wasch- oder Toilettenmittel, entsprechend zu verdünnen. Geeignet hierzu ist jedes indifferente, leicht lösliche Mittel, z. B. Kochsalz u. dgl.

PATENT-ANSPRÜCHE:

1. Verfahren zum Reinigen von Wäschestücken aller Art, gekennzeichnet durch den Zusatz tryptischer Enzyme, wie Pankreatin, zur Waschbrühe.
2. Anwendung tryptischer Enzyme, wie Pankreatin, zur Herstellung von Wasch- und Toilettenmitteln.

BERLIN. GEDRUCKT IN DER REICHSDRUCKEREI.

Amylases

- hydrolysis of starch (glucose polymer), one of the most readily available plant polysaccharides
- amylases are enzymes that hydrolyse starch
  production of sweeteners from starch: maltose or glucose syrups
  (further transformation to high fructose syrup with glucose isomerase)
- starch hydrolyates used as additives in the manufacture of candies, baked goods, canned goods, and frozen foods

Glucose Isomerase

- D-glucose ketoisomerase: causes the isomerization of glucose to fructose
- since reaction is reversible the ration of glucose and fructose depends on the enzyme and reaction conditions
- high fructose corn syrup
  fructose 2x sweeter than sucrose

Chymosin

- site-specific proteolysis by chymosin detaches hydrophilic "tails" of κ-casein resulting in coagulation (curlding)
- calf chymosin (prochymosin) cloned and expressed in E. coli (first genetically engineered protein approved for human consumption, 1990)

Proteases

- used in laundry detergents

Industrial enzymes produced by *Bacillus* species

Enzyme	Producer strains
α-Amylase	*B. amyloliquefaciens, B. circulans, B. licheniformis, B. stearothermophilus, B. subtilis*
β-Amylase	*B. polymyxa, B. cereus, B. megaterium*
Alkaline phosphatase	*B. licheniformis*
Cyclodextran glucanotransferase	*B. macerans, B. megaterium, Bacillus* sp.
β-Galactosidase	*B. stearothermophilus*
β-Glucanase	*B. subtilis, B. circulans*
β-Glucosidase	*Bacillus* sp.
Glucose isomerase	*B. coagulans*
Glucosyl transferase	*B. megaterium*
Glutaminase	*B. subtilis*
Galactomannase	*B. subtilis*
β-Lactamase	*B. licheniformis*
Lipase	*Bacillus* sp.
Metalloprotease	*B. lentus, B. polymyxa, B. subtilis, B. thermoproteolyticus*
Metalloprotease	*B. amyloliquefaciens*
Penicillin acylase	*Bacillus* sp.
Pullulanase	*Bacillus* sp., *B. acidopullulans*
Serine protease	*B. amyloliquefaciens, B. amylosaccharicus, B. Licheniformis, B. subtilis*
Urease	*Bacillus* sp.
Uricase	*Bacillus* sp.

TABLE 7.1 Microbial Enzymes with Industrial-Scale Applications and Some of Their Sources

Enzyme	*Source*	*Action*	*Applications*
α-Amylase	*Bacillus subtilis* *Bacillus licheniformis* *Aspergillus oryzae*	Endo-hydrolysis of α-1,4-glucosidic linkages	Starch processing
Glucoamylase	*Aspergillus oryzae* *Aspergillus niger* *Rhizopus oryzae*	Removes glucose from nonreducing end of starch, also splits α-1,6-linkages at branch points but more slowly	Starch processing; brewers' and distillers' mashes
Pullulanase	*Klebsiella aerogenes*	Splits α-1,6-glycosidic linkages in pullulan and amylopectin	Starch processing
Glucose isomerase	*Bacillus coagulans* *Streptomyces albus*	Converts D-glucose to D-fructose. This enzyme is actually a xylose isomerase that converts D-xylose to D-xylulose	Production of high-fructose syrups
β-Glucanase	*Bacillus subtilis* *Aspergillus niger* *Penicillium emersonii*	Degrades β-glucan by cleaving β-1,3 (4)-glucosidic linkages	Brewing
Invertase	*Saccharomyces cerevisiae*	Splits sucrose to glucose and fructose	Confectionery industry; baking
Lactase	*Saccharomyces lactis* *A. oryzae, A. niger,* *Rhizopus oryzae*	Splits lactose to glucose and galactose	Dairy industry (treatment of milk and whey)
Pectinase	*A. oryzae, A. niger,* *Rhizopus oryzae*	Degrades pectin, α-1,4-linked anhydrogalacturonic acid with some of the carboxyl groups esterified as the methyl esters	Clarification of fruit juices and wines
Neutral protease	*Bacillus subtilis* *Aspergillus oryzae*	Hydrolyzes peptide bonds in proteins	Flavoring of meat and cheese; baking
Alkaline protease	*Bacillus licheniformis*	Hydrolyzes peptide bonds in proteins	Laundry detergents
Rennin	*Mucor miehei* spp. Recombinant enzyme produced in *E. coli* and fungi	Hydrolyzes a specific bond in κ-casein, leading to coagulation of milk proteins	Cheesemaking
Lipase	*A. oryzae, A. niger,* *Rhizopus oryzae*	Hydrolyzes ester linkages in fats	Dairy industry; detergents

Single Cell Protein Production

- microbial protein for use as human food or animal feed
- source of low-cost protein?

Advantages:

1. rapid growth rate and high productivity
2. high protein content (30-80% of dw)
3. ability to utilize a wide range of cheap carbon sources
   methane, methanol, molasses, whey, lignocellulose waste, etc.
4. relatively easy selection of cells
5. little land area required
6. production independent of season and climate

- protein content and quality largely dependent on the specific microbe utilized and on the fermentation process
- fast growing aerobic microorganisms

Some problems:

- high nucleic acid content
- high protein content (elevated RNA levels - ribosomes)
  - digestion of nucleic acids results in elevated levels of uric acid
  - treatment with RNAses
- sensitivity or allergic reactions

Table 14.3 Microorganisms used for SCP production using various carbon sources

Carbon substrate	Microorganism
Carbon dioxide	*Spirulina* species *Chlorella* species
Liquid hydrocarbons (*n*-alkanes)	*Saccharomycopsis lipolytica* *Candida tropicalis*
Methane	*Methylomonas methanica* *Methylococcus capsulatus*
Methanol	*Methylophilus methylotrophus* *Hyphomicrobium* species *Candida boidinii* *Pichia angusta*
Ethanol	*Candida utilis*
Glucose (hydrolysed starch)	*Fusarium venenatum*
Inulin (a polyfructan)	*Candida* species *Kluyveromyces* species
Molasses	*Candida utilis* *Saccharomyces cerevisiae*
Spent sulphite waste liquor	*Paecilomyces variotii*
Whey	*Kluyveromyces marxianus* *Kluyveromyces lactis* *Penicillium cyclopium*
Lignocellulosic wastes (solid substrate)	*Chaetomium* species *Agaricus bisporus* *Cellulomonas* species

Single Cell Protein

Mushrooms

Pekilo prossess
- filamentous fungus **Paecilomyces variotii**
- use of waste from wood processing (monosaccharides + acetate)
- use as animal feed

Pruteen
- methanol (from methane - natural gas) as C1 carbon source
- methylotrophic bacteria (*Methylophilus methylotrophus*)
- feed protein

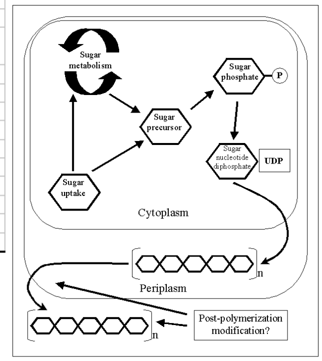
Quorn
- fungal mycelium, *Fusarium* (mycoprotein) for human consumption
- processing to resemble meat

Microbial polymers

- e.g. xanthan gum from *Xanthomonas campestris*

Polymer	Application
Xanthan	Emulsion stabilization and suspension agent in foods
	Foam stabilization in foods
	Crystallization inhibitor in foods
	Viscosity control in oil drilling mud and inkjet printing
Bacterial cellulose	Moisture retention in wound dressings
	High strength acoustic diaphragms in sound reproduction
Hyaluronic acid	Hydrating agent in cosmetics and pharmaceuticals
	Replacement for synovial fluid and vitreous humor in biomedicine
Emulsan	Emulsifier and vaccine adjuvant
Curdlan	Gelling agent in foods
Gellan	Gelling agent in foods
Pullulan	Food coatings
Various	Paper coating and water flocculant

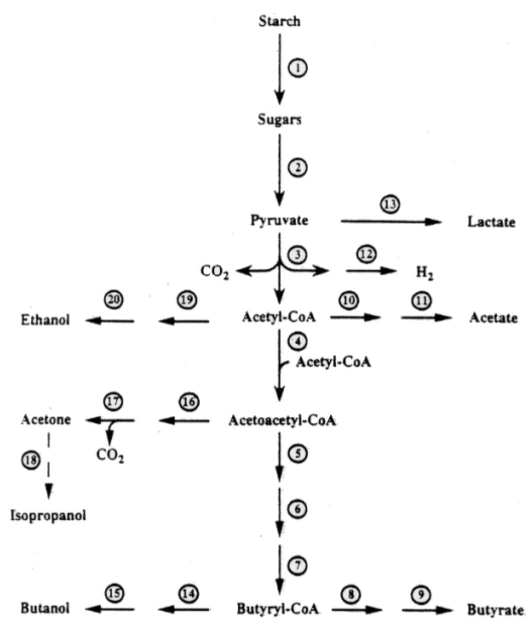
Adapted from Sutherland (1998).



Production of solvents: Acetone-butanol fermentation

Clostridium acetobutylicum

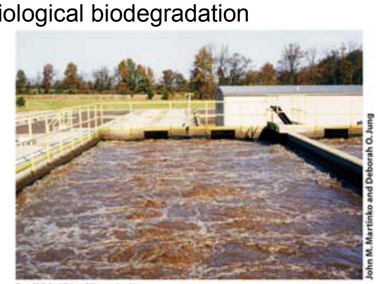
- utilizes EMP pathway for glucose catabolism with the formation of C3 and C4 products
- biphasic fermentation: during growth acetate and butyrate are formed (acidogenic phase)
- as pH drops the culture enters stationary phase and there is a metabolic shift to solvent phase (solvetogenic phase)
- acetyl-CoA as central intermediate, which can be:
  - a) reduced to butyrate and butanol
  - b) cleaved via decarboxylation to acetone



Waste-water and sewage treatment

Objectives of wastewater treatment:
- reduce the organic content of wastewater
  - BOD
  - trace (toxic) organics that are recalcitrant to biodegradation
- removal / reduction of nutrients to reduce pollution of receiving waters
  - nitrogen, phosphorous
- removal or inactivation of pathogenic microorganisms and parasites

Treatment methods:
- various types of bioreactors to produce effluent that can be discharged into the natural environment without adverse effects
- combinations of physical & chemical treatment and aerobic / anaerobic biological biodegradation



Activated Sludge Process

1. Preliminary treatment
- removal of debris and coarse materials that may clog equipment

2. **Primary** treatment
- screens, settling tanks and skimmers to remove suspended solids
- physical separation

3. **Secondary** treatment
- aerobic microbiological process: trickling filters, activated sludge
- effluent from primary treatment aerated, aerobic bacteria growing in flocs degrade organic material
- an important characteristic of the process is the recycling of a large proportion of the biomass
- removal of BOD and nutrients:
  1. oxidation of biodegradable organic matter
  (soluble organic matter converted to new cell mass)
  2. flocculation, separation of newly formed biomass from effluent

4. **Tertiary or advanced** treatment
- removal of ammonia and phosphate
- oxidation of ammonia to nitrate followed by denitrification
- accumulation of polyphosphate granules
- chemical treatments

5. **Disinfection** (when needed)
- chlorination

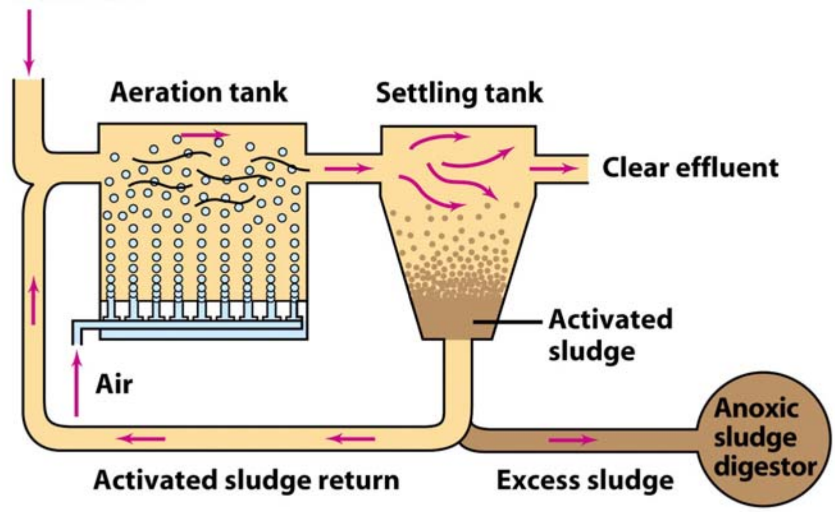


Figure 28-6c Brock Biology of Microorganisms 11/e

Microbiological mining - Sulfur and Iron-Oxidizing Bacteria

- *Thiobacillus*, *Acidithiobacillus*, *Beggiatoa*, and others
  - *Thiobacillus thiooxidans* (Jaffe and Waksman 1922)
- scattered in the Proteobacteria: α,β, γ subdivisions
- acidophiles
- chemolithotrophs: energy from oxidation of reduced sulfur compounds or iron
- used in bioleaching of ores
- problems with acid mine drainage

Figure 19-37a Brock Biology of Microorganisms 11/e

T. D. Brock

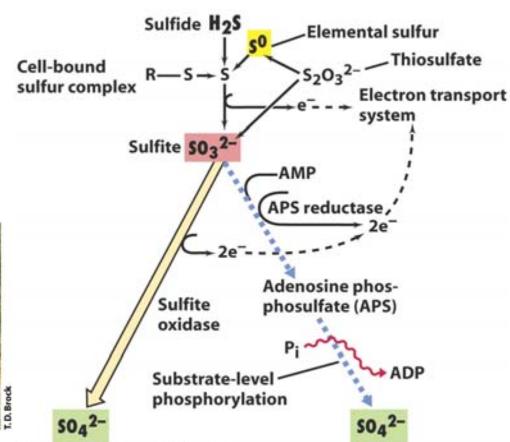


Figure 17-27a Brock Biology of Microorganisms 11/e

Microbiological mining - Leaching of metals with *Thiobacillus*

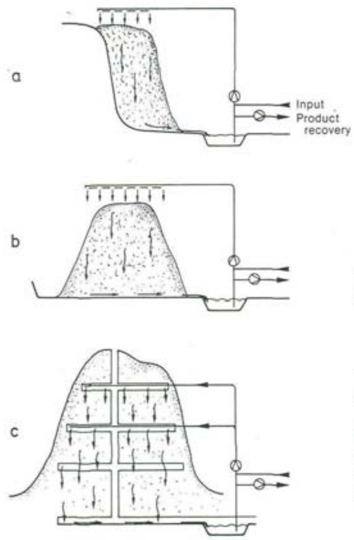


Figure 18.1 Diagram of (a) slope, (b) heap, and (c) in-situ leaching

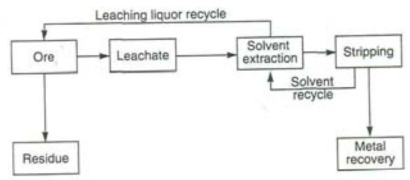


Figure 15.3
Flowchart of metal recovery from low-grade ores by bioleaching using the activity of *Thiobacillus ferrooxidans*. (Source: Torma 1977.)