# Chapter 2
# Industrial Microbiology

# Outline:

- 2.1 Introduction
- 2.2 The microorganisms
- 2.3 The fermentation medium
- 2.4 The fermentation
- 2.5 Fermenter
- 2.6 Product developement steps
- 2.7 Product of Industrial Microbiology
- 2.8 Environmental role of microorganisms

# Learning outcomes:

- Explain basic concept of industrial microbiology.
- Describe application of microorganism in industry.
- Recognise products of industrial microbiology.
- Explain industrial important enzymes and their applications

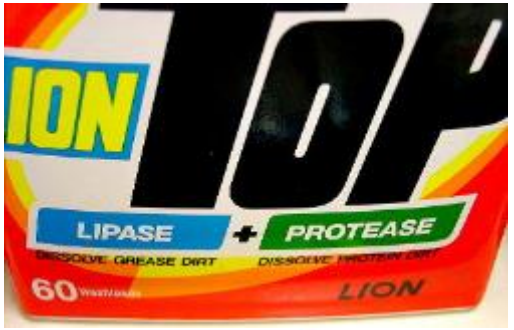
# Learning outcomes:

- Compare and contrast extracellular and intracellular enzyme
- Describe biofuel from various sources.
- Explain the environmental role of microorganisms.

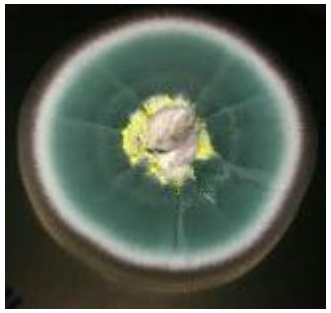
# LIST SOME PRODUCTS OF INDUSTRIAL MICROBIOLOGY IN YOUR DAILY LIFE.

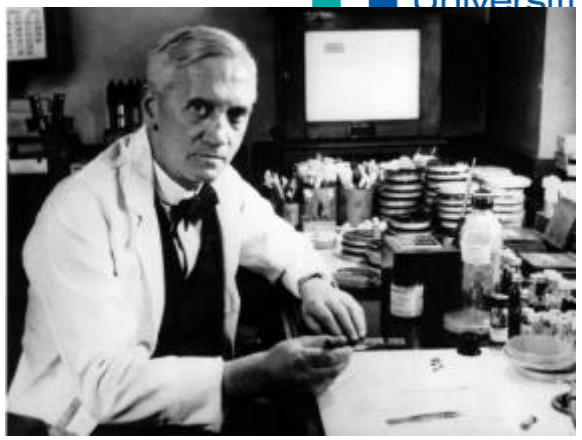


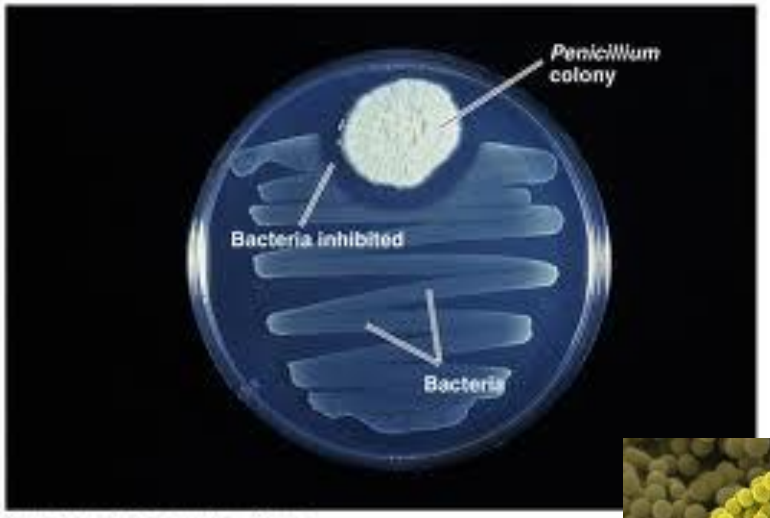


- Penicillin
- Alexandra Fleming
- *Penicillium*






# 2.1 Introduction

- **Uses microorganism, grown in large scale, to produce valuable commercial products or maintain and improve the environment.**

Figure 26-13 photo (f) Microbiology, 7/e
Courtesy of Carolina Brewing Company

Figure 26-9a Microbiology, 7/e
John Colwell/Grant Heilman Photography

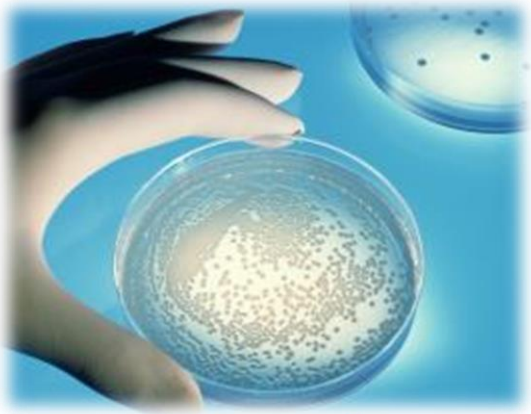
# 2.1 Introduction

**Important components :**

- The microorganisms
- The fermentation medium
- The fermentation
- The fermenter

# 2.2 The microorganisms

- Desired characteristics:
  - Grow and multiply rapidly
  - Produce products  rapidly
  - Pure culture
  - Able to grow in inexpensive culture medium
  - Genetically stable



# 2.2 The microorganisms

- Desired characteristics:
  - Should not be pathogenic (harmful) to humans or economically important animal or plants
  - Amenable to genetic manipulations
  - Can be stored for years without any effect on their productivity/desired character

# 2.3 The fermentation medium

- Growth medium (solid or liquid) for microorganism to grow or multiply
- Must be cost effective
- Waste products from the industry- molasses, whey, sawdust.

# 2.4 The fermentation

**Fermentation**

- In the context of industrial microbiology, the term fermentation refers to the growth of large quantities of cells under aerobic or anaerobic conditions, within a vessel referred to as a fermenter or bioreactor.

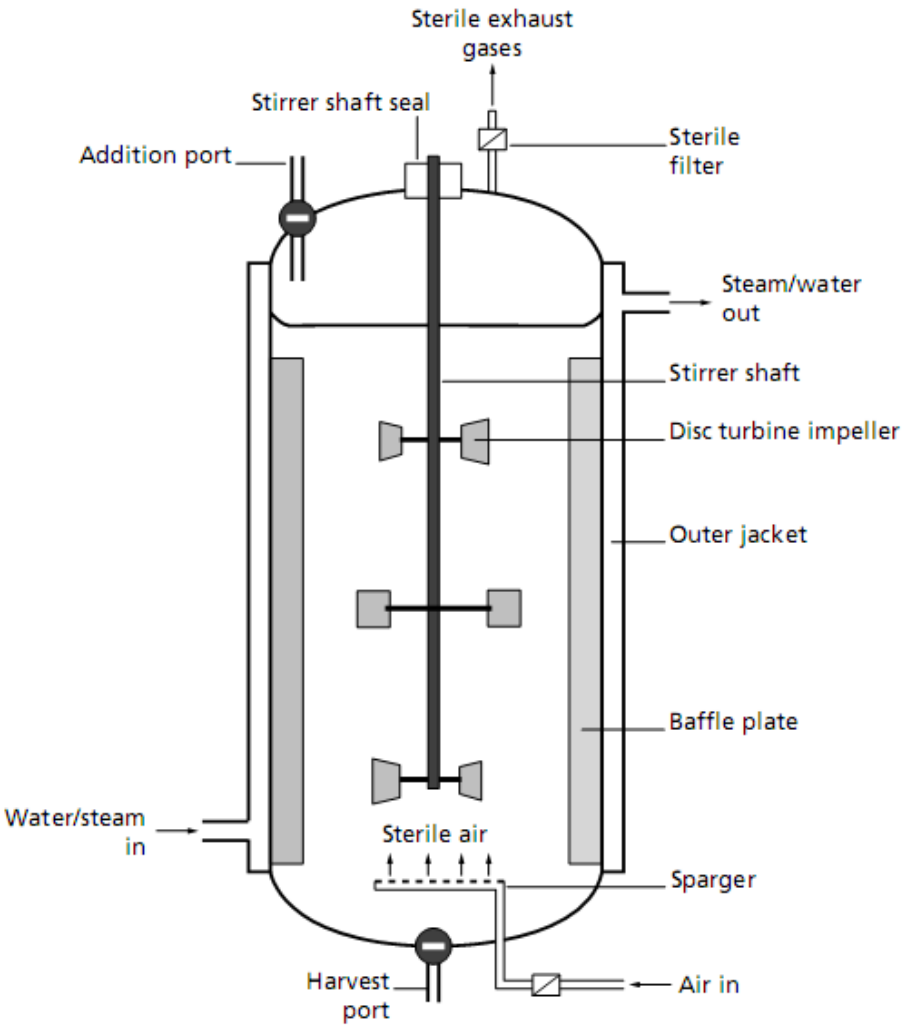
# 2.4 The fermentation

- carried out under controlled condition with optimized physical (pH, temperature, aeration etc.) and chemical (carbon, nitrogen, mineral sources etc.) composition of the fermentation medium.

# 2.5 Fermenter

- the microbe are cultivated under controlled conditions in a fermenter
- Vary in size (5L, 10L, 500000L)

# 2.5 Fermenter

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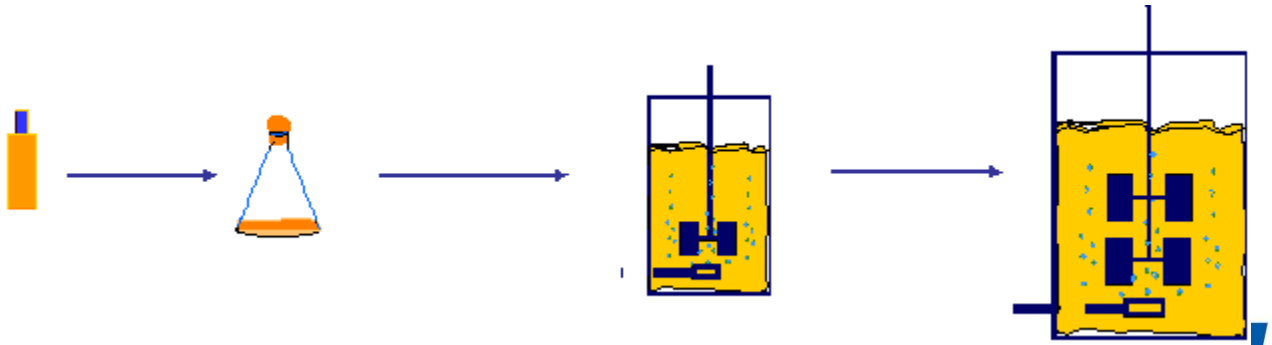
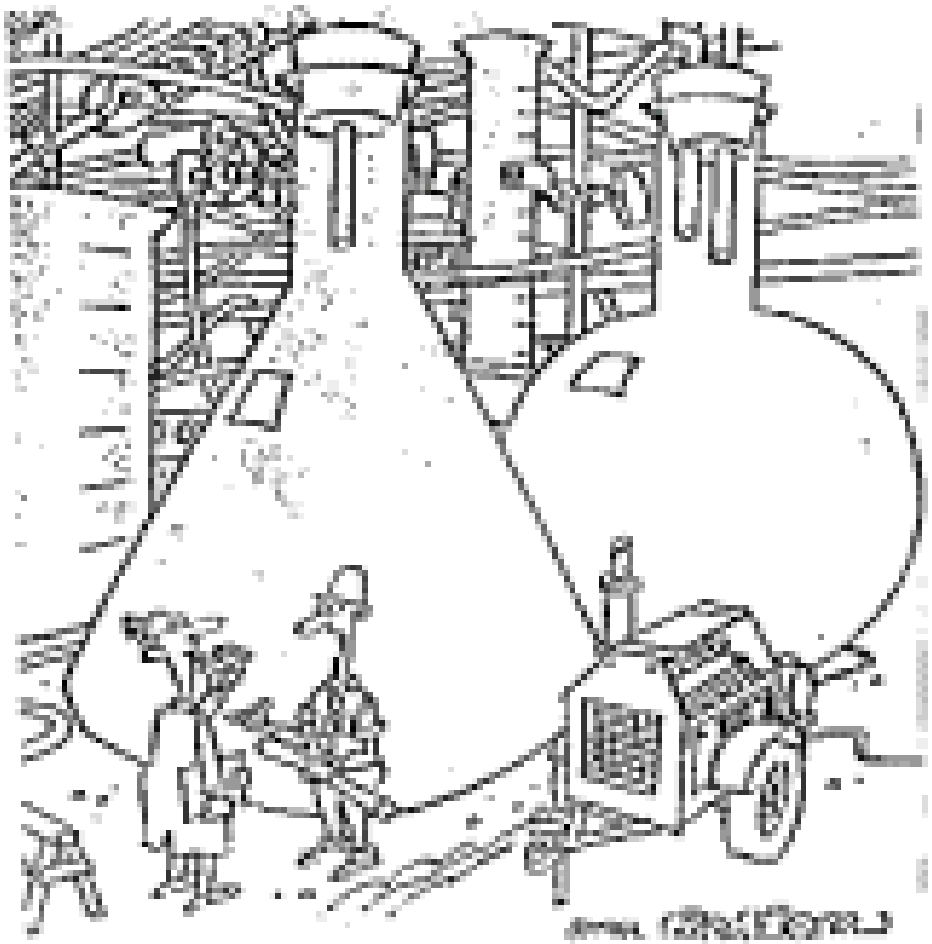
# 2.6 Product development steps

- Isolate of microbes that produce product of interest
- Screening for best producing strain: naturally or mutation or genetic engeneering
- Optimise production condition
- Scale up from lab scale (up to 10 L) to industrial scale (>10,000 L)



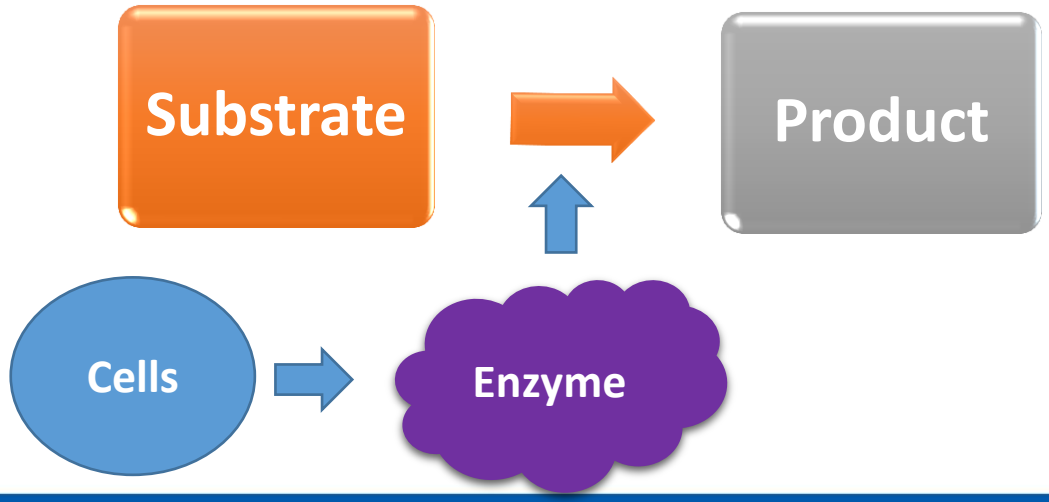


"Got a few problems going from lab scale up to full-scale commercial."

# 2.7 Product of Industrial Microbiology

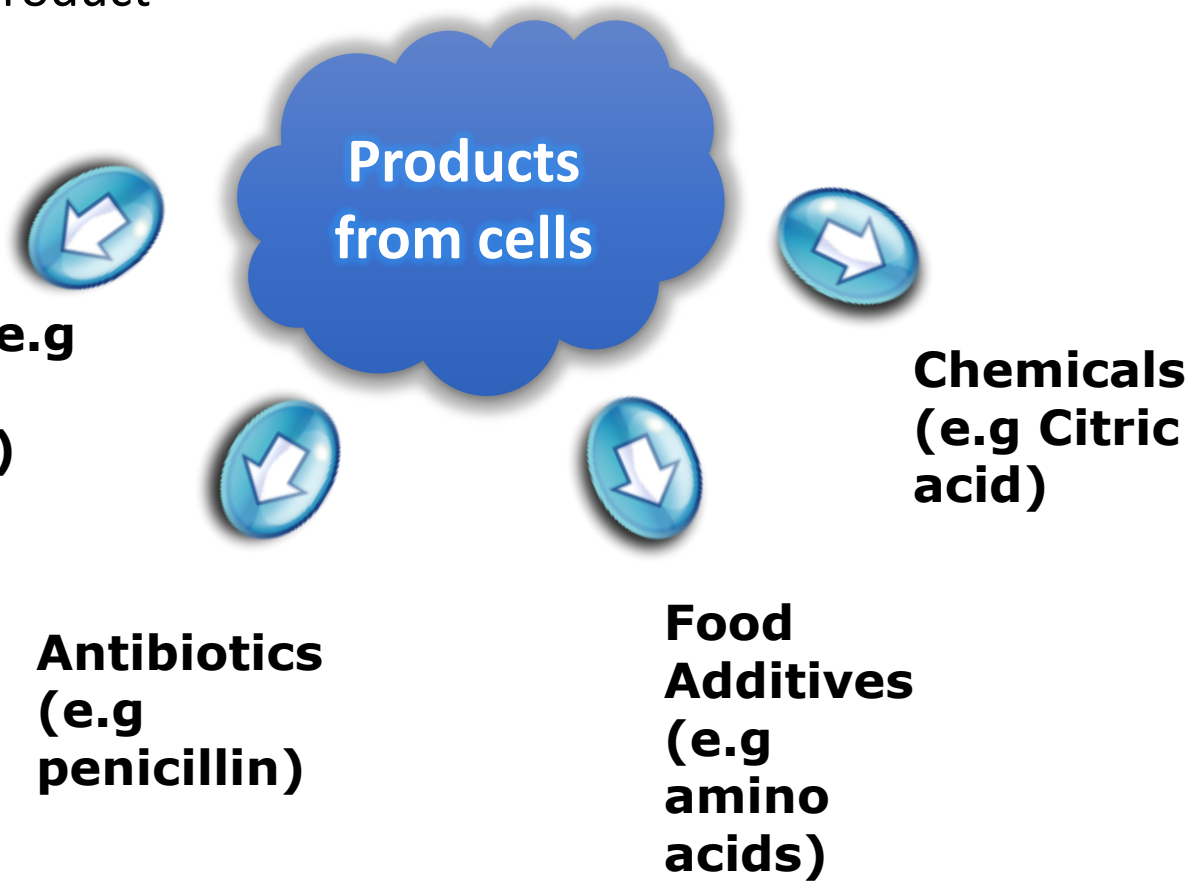
- Biomass


- Bioconverstion/Biotransformation



# 2.7 Product of Industrial Microbiology

- Metabolite product



# 2.7 Product of Industrial Microbiology

- **<u>Food and beverages</u>**
- **Wine , beer – brewer yeast *Saccharomyces cerevisiae***

# 2.7 Product of Industrial Microbiology

- **Food and beverages**
- **Cheese**
- **Coagulation of milk by rennin enzyme/chymosin or acids precipitate casein**
  - **From calves's stomachs, engineered bacteria**
  - **Acid production by lactic acid bacteria**

# 2.7 Product of Industrial Microbiology

- **Food and beverages**
- **Yogurt- Lactic acid bacteria e.g lactobacilli and bifidobacteria**


# 2.7 Product of Industrial Microbiology

- **<u>Food and beverages</u>**
- **Vineger - *Acetobacter* and *Gluconobacter*.**

# 2.7 Product of Industrial Microbiology

- **<u>Food and beverages</u>**
- **Soy sauce - *Aspergillus oryzae***


# 2.7 Product of Industrial Microbiology

- **Food and beverages**
- **Fermented meat**
  - **Use to preserve food E.g. salami, summer sausage, cured ham**
  - **Main organism –*Pediococcus serevisiae, Lactobacillus plantarum,* other lactic acid bacteria**

# 2.7 Product of Industrial Microbiology

- **<u>Food and beverages</u>**
- **Baker's yeast**

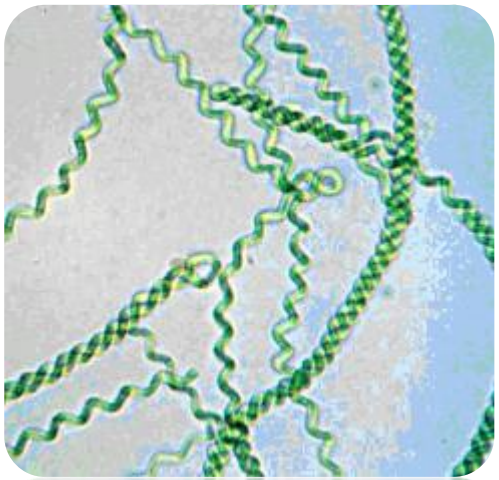



# 2.7 Product of Industrial Microbiology

- **Food and beverages**
- **Yeast as single cell protein**
- **Alternative food sources – used as supplementary diet e.g. yeast is a source for B Vitamins.**


# 2.7 Product of Industrial Microbiology

- **<u>Food and beverages</u>**
- ***Spirulina* as single cell protein**
- **excellent source of protein, iron, calcium, magnesium, and a range of vitamin Bs.**




# 2.7 Product of Industrial Microbiology

- **Food and beverages**
- **Mushroom: *Agaricus bisporus*(botton mushroom), *Lentinus edulus* (Shitake mushroom)**

# Discussion

- Categorised the following products into biomass or bioconversion or metabolite products.
- Cheese
- Mushroom
- Spirulina
- Yogurt
- Baker's yeast
- Beer (brewer yeast)

# 2.7 Product of Industrial Microbiology

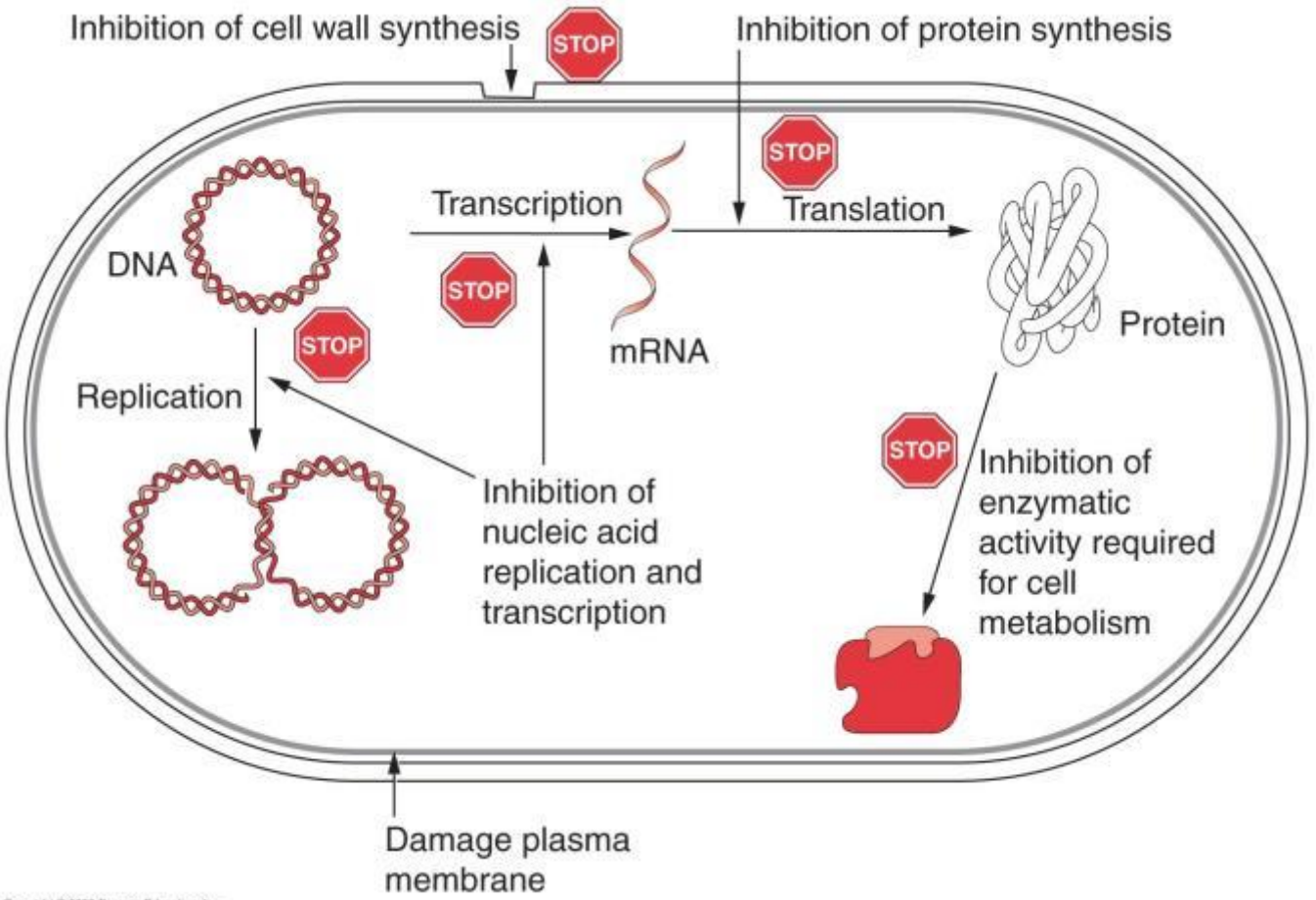
- **Health-care product**
- **antibiotics -the most important compounds produced by industrial microorganism**

**Table 5.2 COMMON ANTIBIOTICS**

| Antibiotic | Source Microbe | Common Uses of Antibiotic |
|---|---|---|
| Bacitracin | *Bacillus subtilis* (bacterium) | First aid ointment and skin creams |
| Erythromycin | *Streptomyces erythraeus* (bacterium) | Broad uses to treat bacterial infections especially in children |
| Neomycin | *Streptomyces fradiae* (bacterium) | Skin ointments and other topical creams |
| Penicillin | *Penicillium notatum* (fungus) | Injected or oral antibiotic used in humans and farm animals (cattle and poultry) |
| Streptomycin | *Streptomyces griseus* (bacterium) | Oral antibiotic used to treat many bacterial infections in children |
| Tetracycline | *Streptomyces aureofaciens*(bacterium) | Used to treat infections of the urinary tract in humans; commonly used in animal feed to reduce infections and stimulate weight gain |

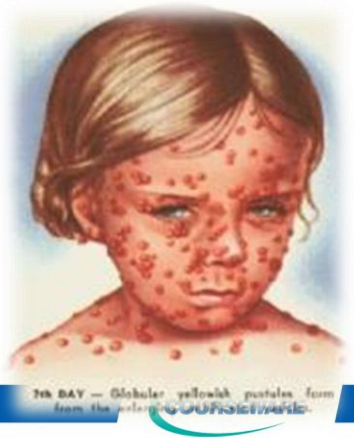
# 2.7 Product of Industrial Microbiology

- **Health-care product**



# 2.7 Product of Industrial Microbiology

- **<u>Health-care product</u>**
- **Vaccine- parts of a pathogen or whole organisms that can be given to humans or animals by mouth or by injection to stimulate the immune system against infection by those pathogens**
- **First vaccine developed in 1796 by Edward Jenner**
  - **Used live cowpox virus to vaccinate against smallpox**



# 2.7 Product of Industrial Microbiology

- **Health-care product**
- **Vaccine**
- **Three Major Strategies to Make Vaccines:**
  - **Subunit/recombinant vaccines are made by injecting portions of viral or bacterial structures**
  - **Attenuated vaccines use live bacteria or viruses that have been weakened through aging or by altering their growth conditions to prevent replication**
  - **Inactivated (killed) vaccines are made by killing the pathogen and using the dead or inactivated microorganism for the vaccine**

# 2.7 Product of Industrial Microbiology

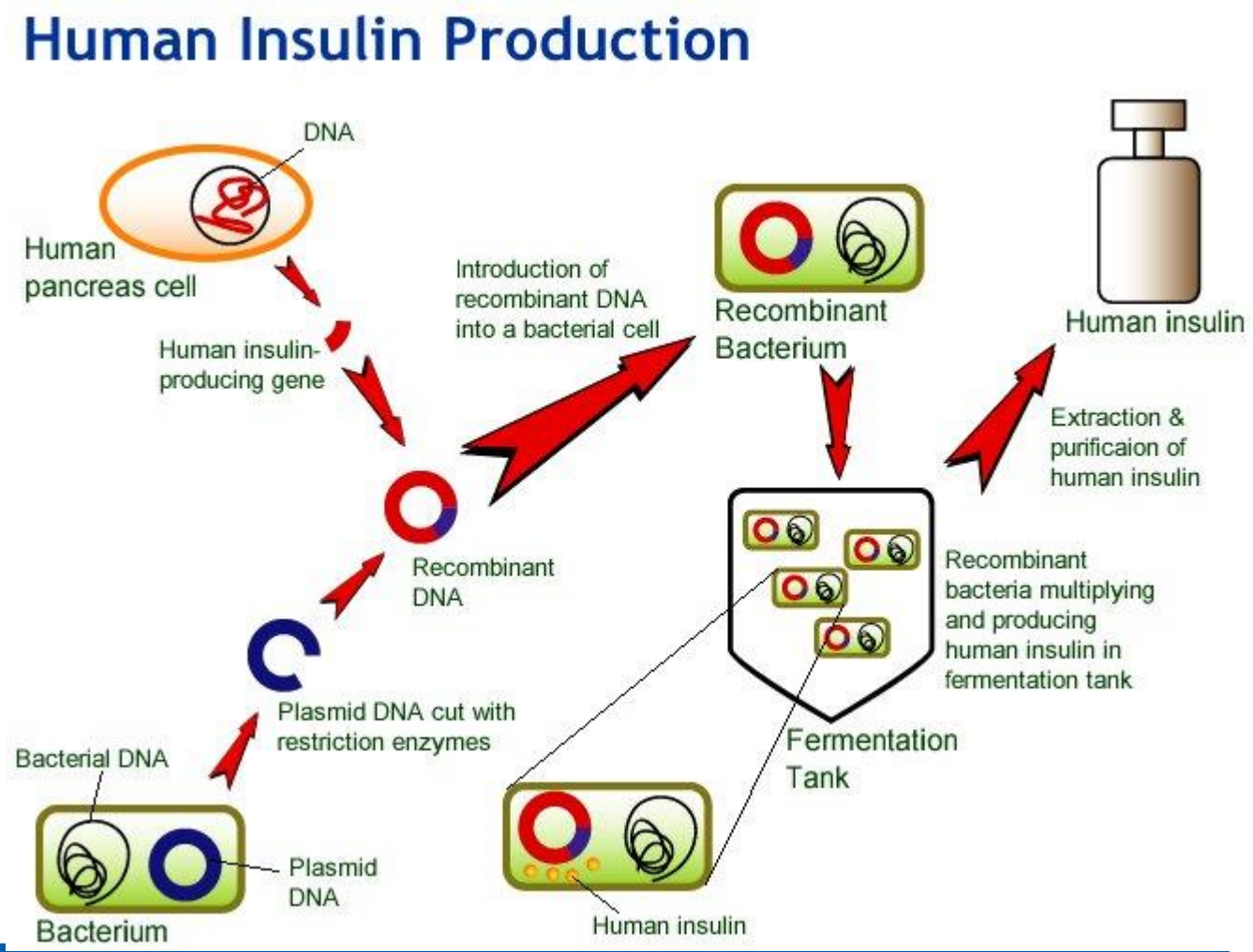
- **Health-care product**

**Table 5.1 THERAPEUTIC PROTEINS FROM RECOMBINANT BACTERIA**

| Protein | Function | Medical Application(s) |
|---|---|---|
| DNase | DNA-digesting enzyme | Treatment of cystic fibrosis patients |
| Erythropoietin | Stimulates production of red blood cells | Used to treat patients with anemia (low number of red blood cells) |
| Factor VIII | Blood clotting factor | Used to treat certain types of hemophilia (bleeding diseases due to deficiencies in blood clotting factors) |
| Granulocyte colony-stimulating factor | Stimulates growth of white blood cells | Used to increase production of certain types of white blood cells; stimulate blood cell production following bone marrow transplants |
| Growth hormone (human, bovine, porcine) | Hormone stimulates bone and muscle tissue growth | In humans used to treat individuals with dwarfism. Improves weight gain in pigs and cows; stimulates milk production in cows. |
| Insulin | Hormone required for glucose uptake by body cells | Used to control blood sugar levels in patients with diabetes |
| Interferons and interleukins | Growth factors that stimulate blood cell growth and production | Used to treat blood cell cancers such as leukemia; improve platelet counts; some used to treat different cancers |
| Superoxide dismutase | An antioxidant that binds and destroys harmful free radicals | Minimizes tissue damage during and after a heart attack |
| Tissue plasminogen activator (tPA) | Dissolves blood clots | Used to treat heart attack patients and stroke victims |
| Vaccines (e.g., Hepatitis B vaccine) | Stimulate immune system to prevent bacterial and viral infections | Used to immunize humans and animals against a variety of pathogens; also used in some cancer tumor treatments |

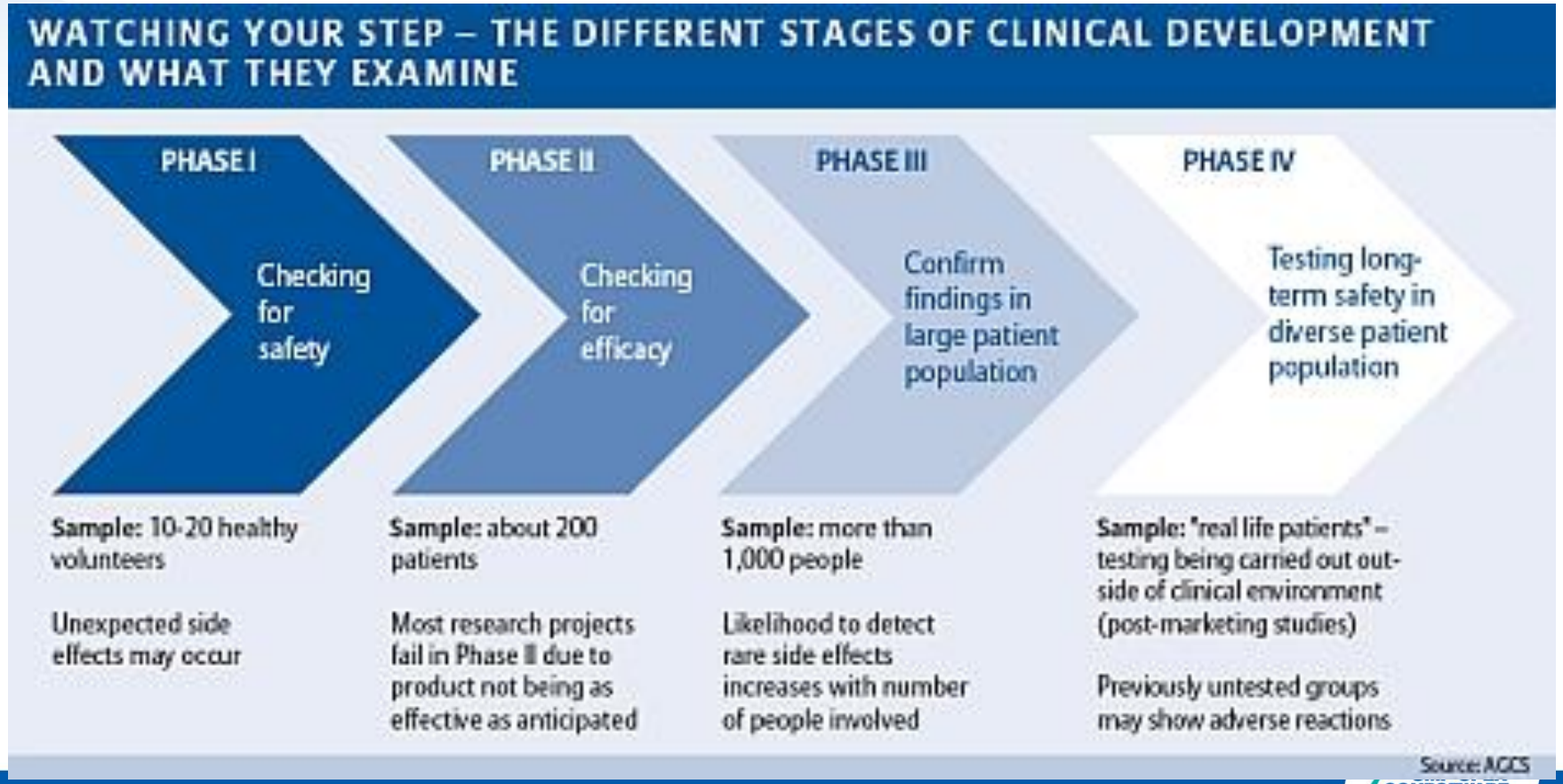
# 2.7 Product of Industrial Microbiology

- **Health-care product**



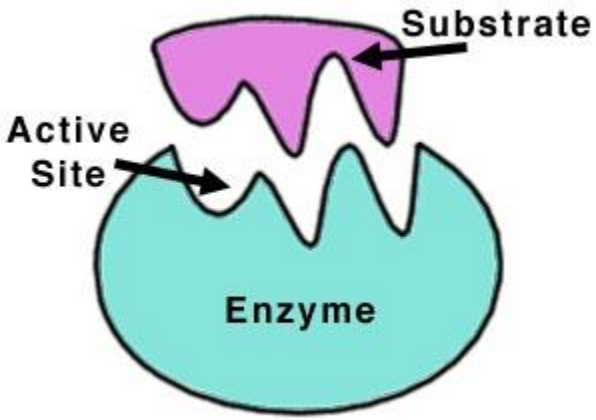
# 2.7 Product of Industrial Microbiology

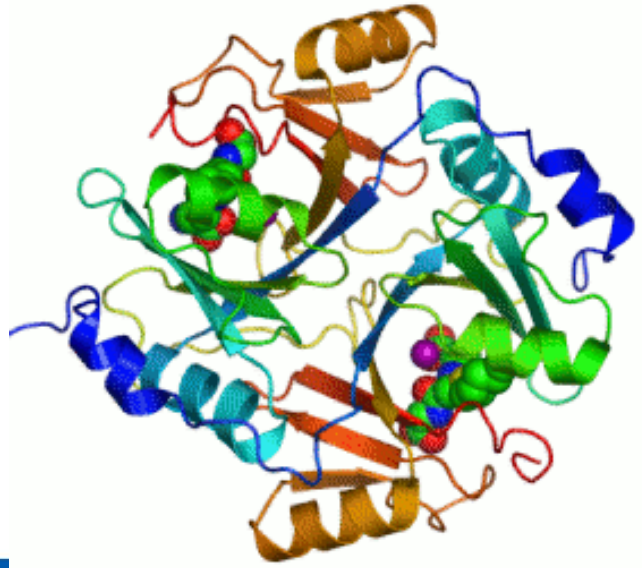
- **Health-care product**



# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**
- **Enzyme or Biocatalyst – biological molecule that activate or accelerate a biochemical reaction, and are naturally produced in living organisms.**



# 2.7 Product of Industrial Microbiology

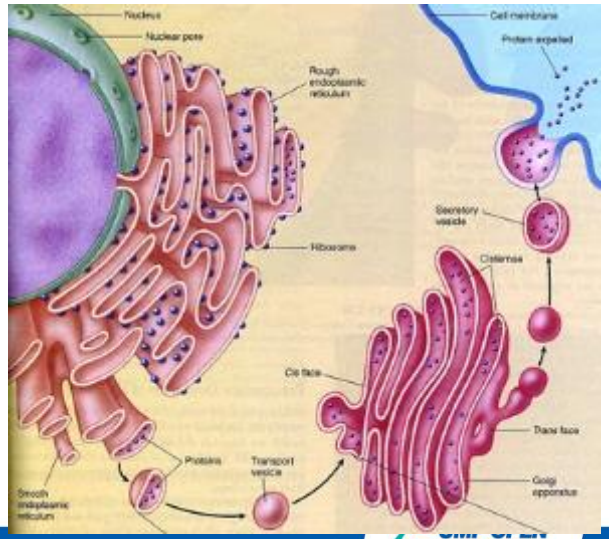
- **Why Microbial enzyme ??**

Many of the reactions catalysed by enzymes have commercial uses. Previously, these reactions used heat and/or strong acids but enzymes offer the following advantages:

| |
|---|
| They are **specific** in their action and therefore **produce a pure product**. |
| They are extremely **efficient**, so a little enzyme **quickly** makes **a lot** of product |
| They are **biodegradable** and so cause **less environmental pollution** (£!) |
| **Safer,** since any contamination with an enzyme or known microbe is harmless |
| **They work in mild conditions** *i.e. low temperatures, neutral pH and normal atmospheric pressure*, and are **therefore energy saving**. |
| Some products (wine, cheese) are **virtually impossible to create** using chemicals alone |
| Some foods **rely on microbial by-products** to create/enhance flavour and **so add value**. |

# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**
- **Intracellular enzyme: work inside the cell, in a stable environment (cytoplasm)**
- **Extracellular enzyme: secreted outside the cell and work in the surrounding environment**



# 2.7 Product of Industrial Microbiology

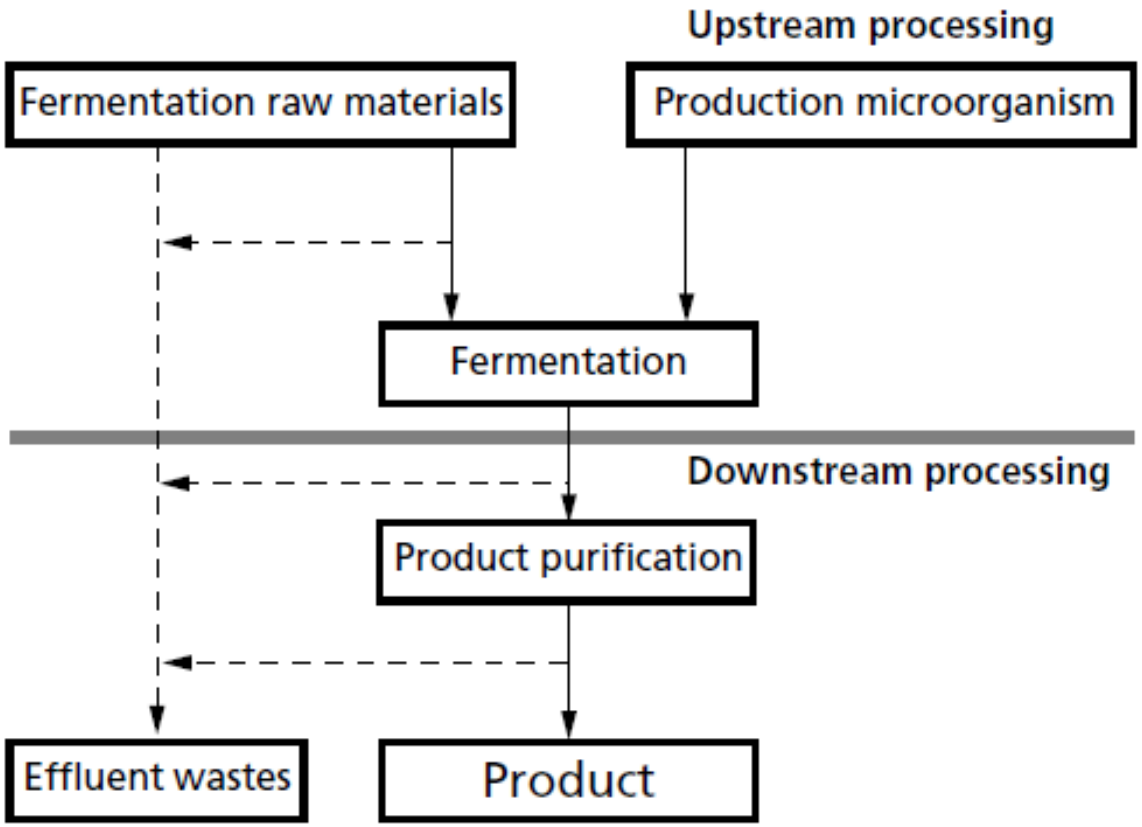



Fig. i Outline of a fermentation process.

# 2.7 Product of Industrial Microbiology

- **Extracting the enzyme**

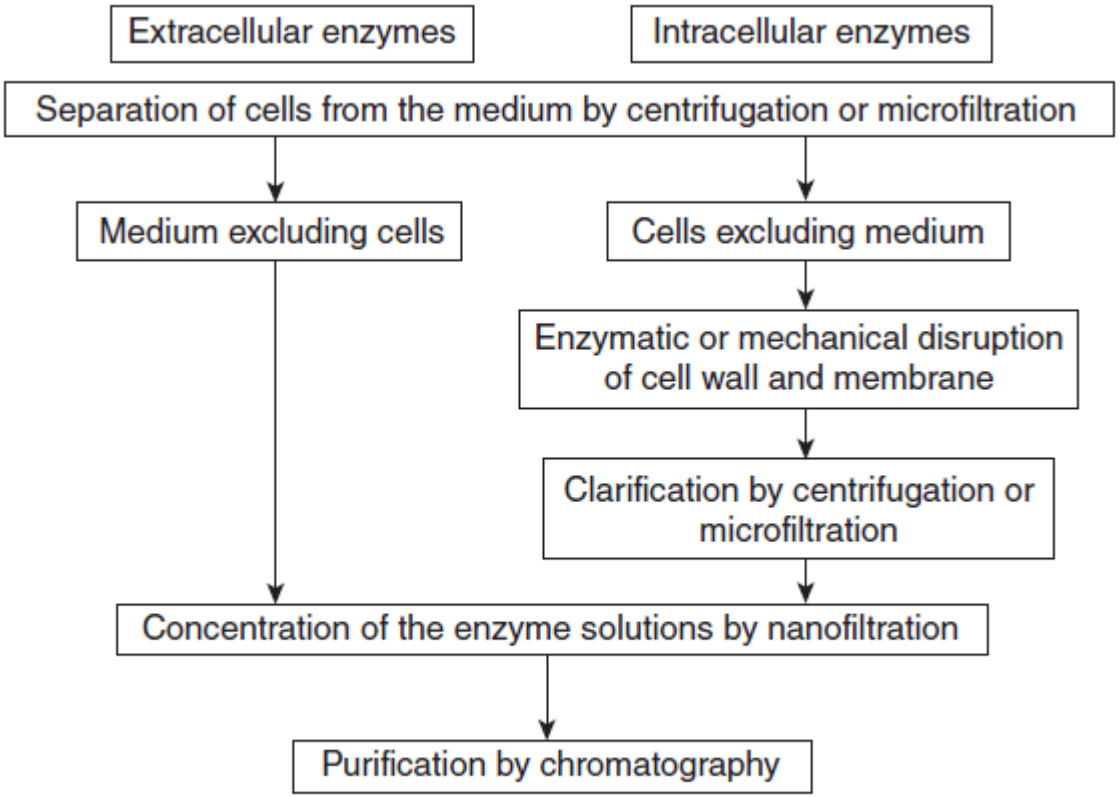


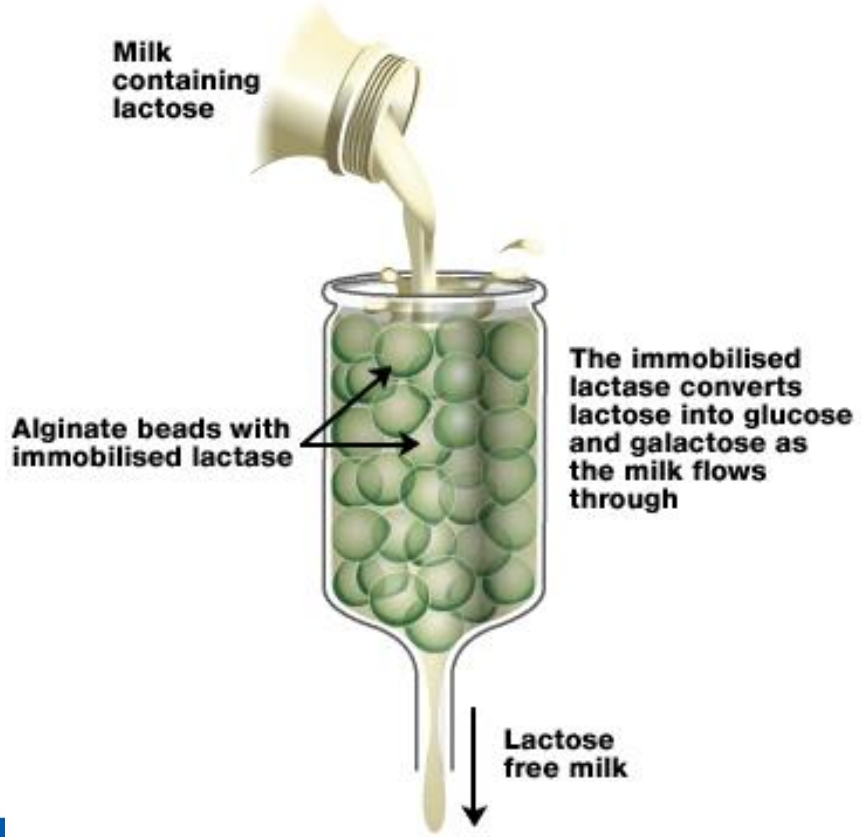

**Figure 5.2** Preliminary steps involved in downstream processing of enzymes.

# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**
- **Intracellular enzyme VS Extracellular enzyme**
- **Which one is better for large scale production?**

# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**
- **immobilised enzyme-enzymes are fix in a bead or a porous solid.**



# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**
- **immobilised enzyme**
  - **widely used in industry because it allows the reaction to flow continuously and the product will not be contaminated with the enzyme so will not need to be purified.**
  - **Enzyme can be recover and recycle**
  - **Enzyme are protected in the beads and therefore remain functional for a longer time.**

# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**

**Table 5.1** Application of various enzymes in important industrial sectors.

| Industry | Enzyme | Application/function/role |
|---|---|---|
| Detergent | Protease | Removing protein stains by degrading them |
| | Cellulase | Loosening of cellulose fibers to easily remove dirt and color brightening |
| | Lipase | Removing fat stains by degrading them |
| Paper and pulp | Xylanase | Biobleaching |
| | Cellulase | De-inking of paper for recycling |
| | Laccases and peroxidase | Polymerizing materials with wood-based fibers |

# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**

| Industry | Enzyme | Application/function/role |
|---|---|---|
| Textile | Cellulase | Bio stonewashing denim, biopolishing |
| | Amylase | Desizing of textiles |
| | Catalase | Bleach clean-up |
| Leather | Protease, lipase | Soaking, bating, and de-hairing of animal skin |
| Animal feed | Phytase | Release of phosphate |
| | Xylanase | Fiber solubility |

# 2.7 Product of Industrial Microbiology

- **Microbial enzyme**

| Industry | Enzyme | Application/function/role |
|---|---|---|
| Food industry | | |
| Starch | α- and β-Amylase, pullulanase, invertase, glucose isomerase | Production of various types of syrups from starch and sucrose |
| | Glucose oxidase | Enhancing the storability of food by removing oxygen and glucose from the food stuff |
| Fruit juice | Cellulase, xylanase, pectinase | Juice clarification and juice extraction |
| Bakery | Xylanase | Dough conditioning |
| | α-Amylase | Loaf volume, shelf-life |
| | Glucose oxidase | Dough quality |
| Dairy | Renin | Protein coagulation |
| | Lactase | Lactose hydrolysis |
| | Protease and lipase | Ripening of cheese |

# 2.7 Product of Industrial Microbiology

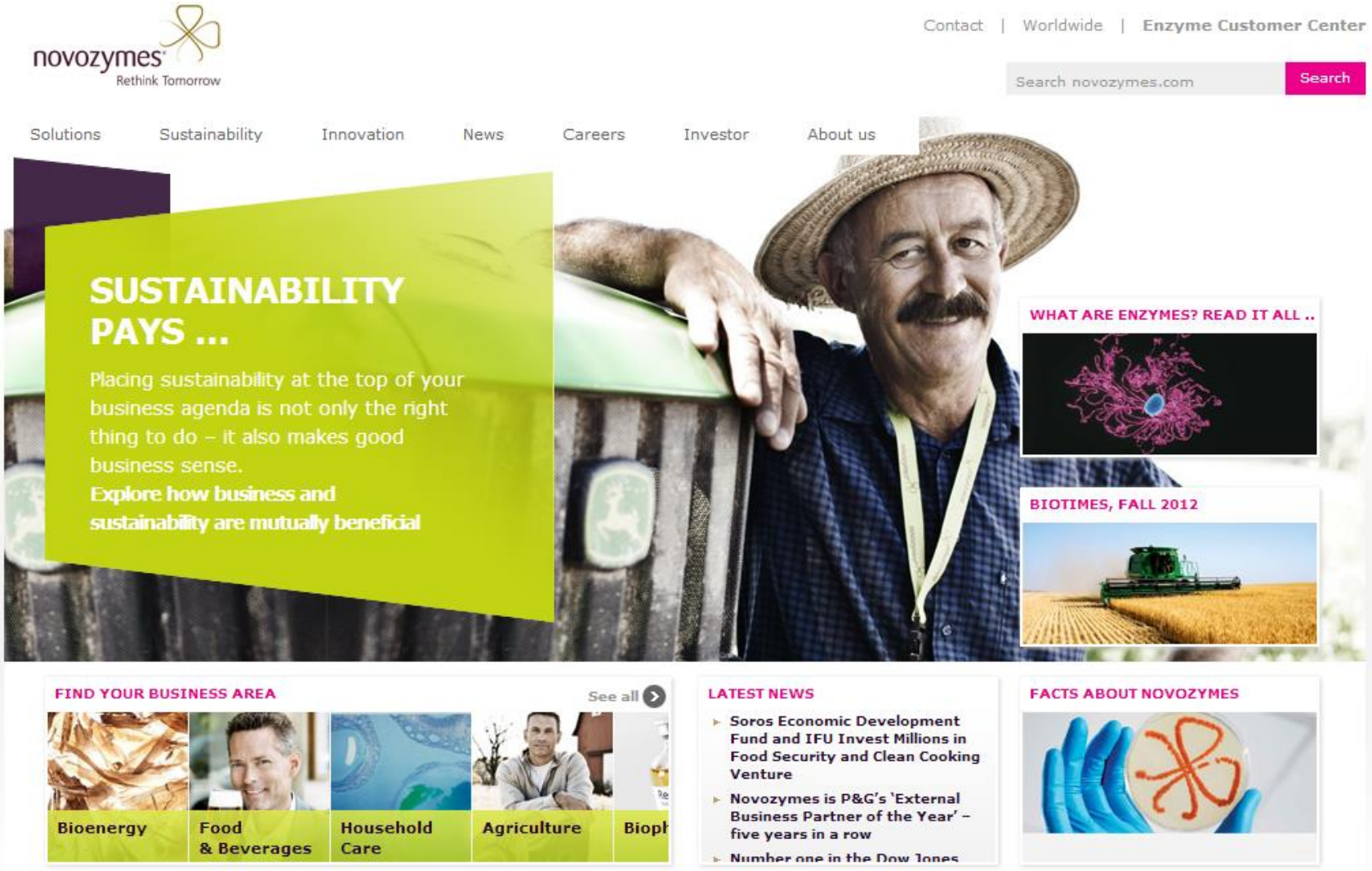
- **Microbial enzyme**

| Industry | Enzyme | Application/function/role |
|---|---|---|
| Biofuel | Cellulase and β-glucosidase | Hydrolyzing cellulosic biomass to generate glucose |
| | Xylanase | Hydrolyzing hemicelluloses to generate pentoses |
| Personal care products | Proteinase and lipase | Contact lens cleaning |
| | Glucose amylase | Liberating glucose from starch-based oligomers |
| | Glucose oxidase | In toothpaste to convert glucose into gluconic acid and hydrogen peroxide as both act as disinfectant |

# 2.7 Product of Industrial Microbiology

- **Enzyme improvement**
- **Recombinant DNA technology - cloning the genes encoding these enzymes and heterologously expressing them in commonly used industrial strains**
- **Protein engineering - allows structural alteration of enzymes**
- **Enzyme engineering - allows enzymes to operate in a variety of microenvironments**

# Case study - Novozyme



# Case study - Novozyme

- Novozymes is the largest producer of enzymes worldwide, capturing 47% of the global market share in industrial use enzymes.
- Malaysia is a net importer of enzymes, where total imports doubled over the past five years to reach USD 14.3 million (RM 50 million) in 2008.
- As a technopeuner in Malaysia, you would like to venture into the enzyme industry, what is your plan to ensure you will be succesful in your business?

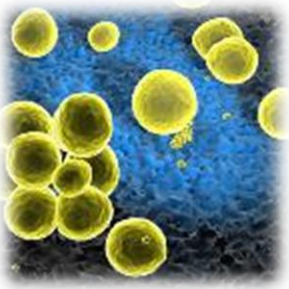
http://www.novozymes.tv/video/6318097/creating-the-foundation-for

# 2.7 Product of Industrial Microbiology

- **Industrial chemicals and fuels**
- **Industrial feedstock chemicals : various alcohols, solvents such as acetone, organic acids, polysaccharides, lipids and raw materials for the production of plastics.**
- **Acetone, butanol, butyric acid and isopropanol – *Clostridium***
- **Organic acid**
  - **Citric acid – *Aspergillus niger***
  - **Lactic acid – *Lactobacillus***

# 2.7 Product of Industrial Microbiology

- **Discussion**
- **Why bioprocess is adopted to produce chemicals?**



# 2.7 Product of Industrial Microbiology

- **Industrial chemicals and fuels**
- **Biofuel - a type of fuel whose energy is derived from biological carbon fixation.**
- **Bioethanol, biodiesel, methane, hydrogen**
- **"First generation" biofuels: food-based biofuels that are currently commercially available.**
- **"Second generation" biofuels: cellulosic biofuels of the future**

# 2.7 Product of Industrial Microbiology

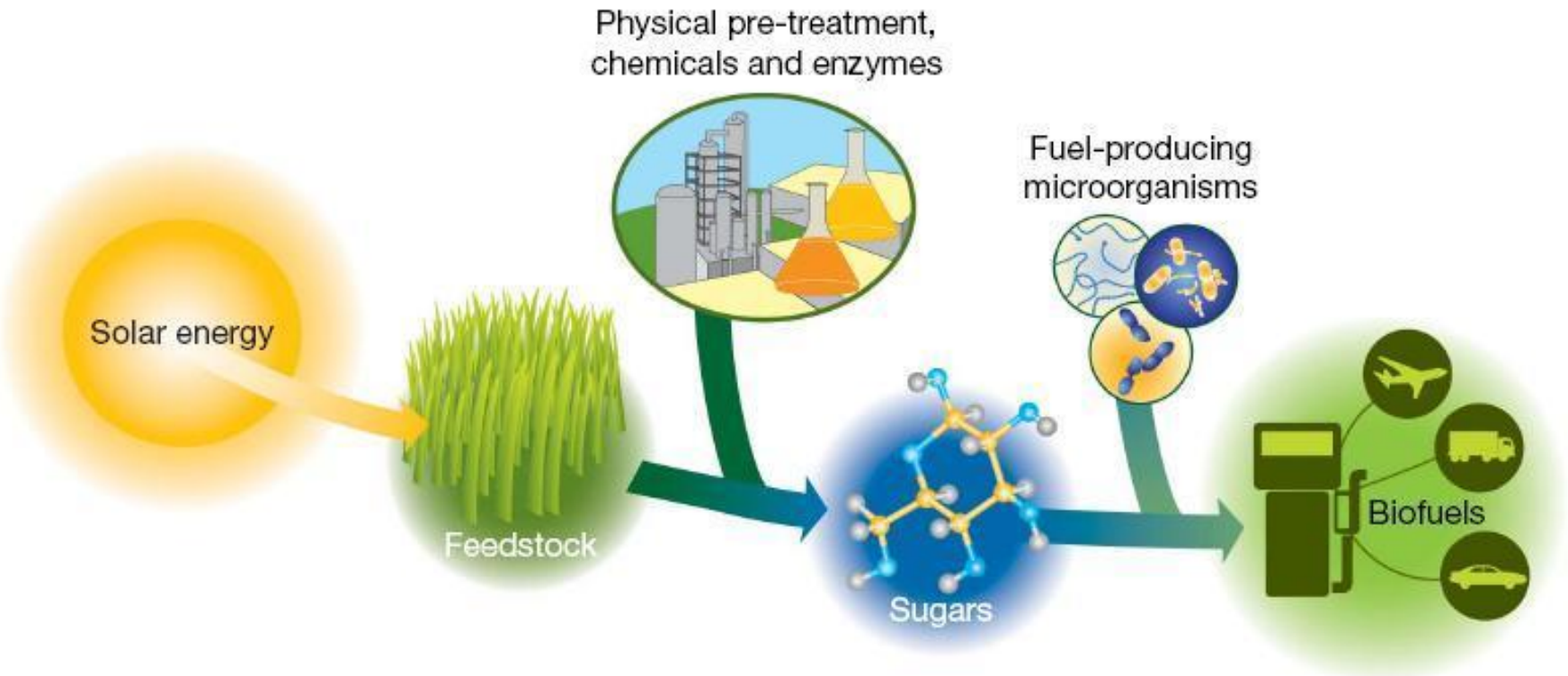
- **Discussion**

**First generation biofuels** **VS** **Second generation biofuels**


http://www.engadget.com/2012/08/14/researchers-efficient-microbial-fuel-cell-sell-electricity/

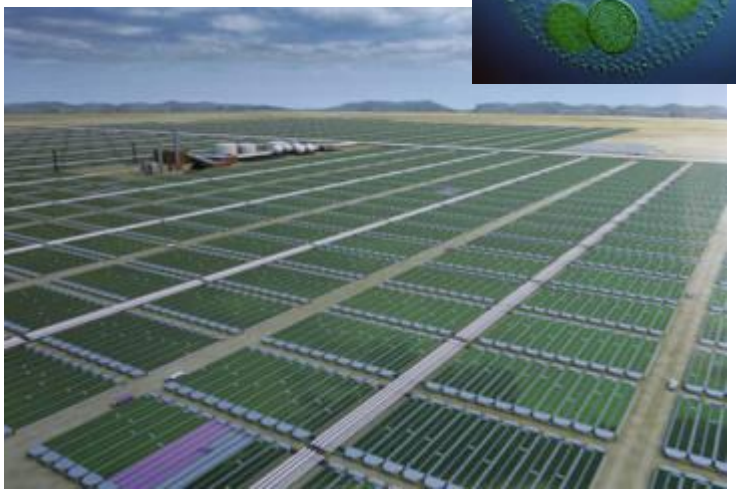
# 2.7 Product of Industrial Microbiology

- **Industrial chemicals and fuels**
- **Second generation biofuels**



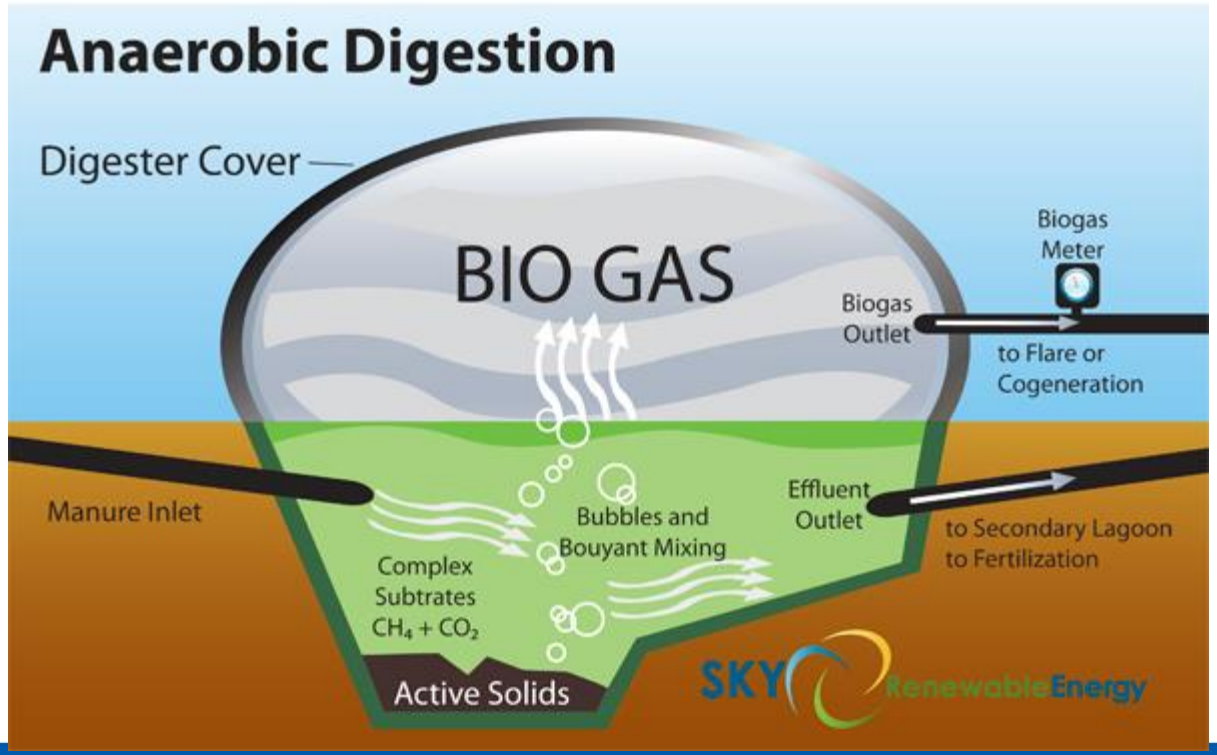
# 2.7 Product of Industrial Microbiology

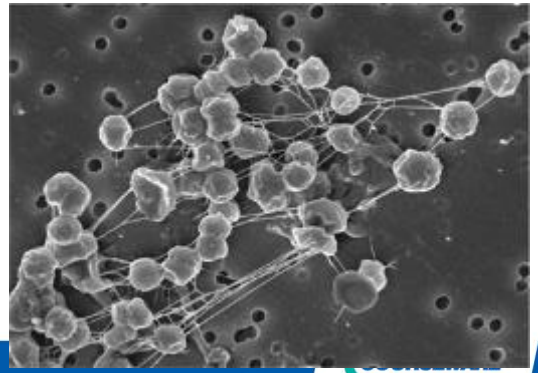
- **Industrial chemicals and fuels**
- **Biofuels from algae**




# 2.7 Product of Industrial Microbiology

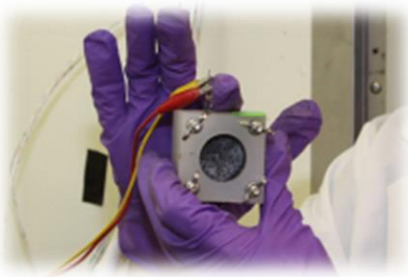
- **Industrial chemicals and fuels**
- **Biogas – methane from methanogens**

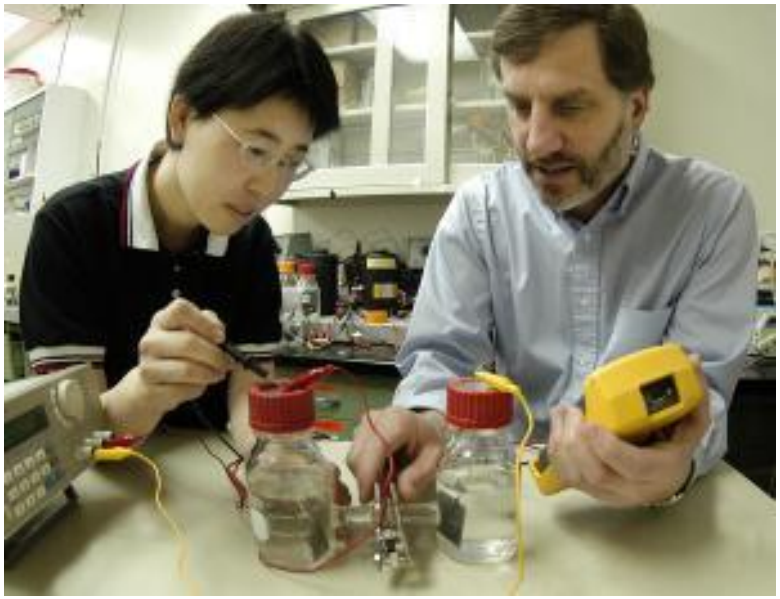


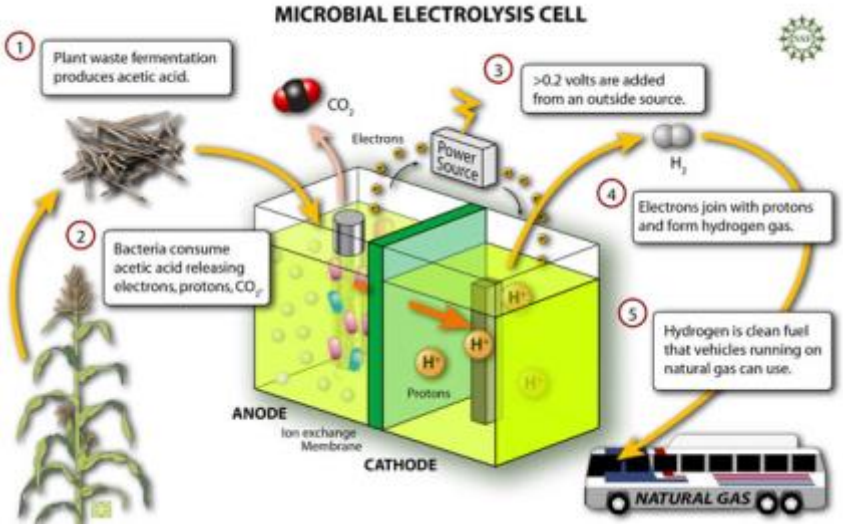



# 2.7 Product of Industrial Microbiology

- **Industrial chemicals and fuels**
- **Microbial fuel cell- generate electricity or Hydrogen**



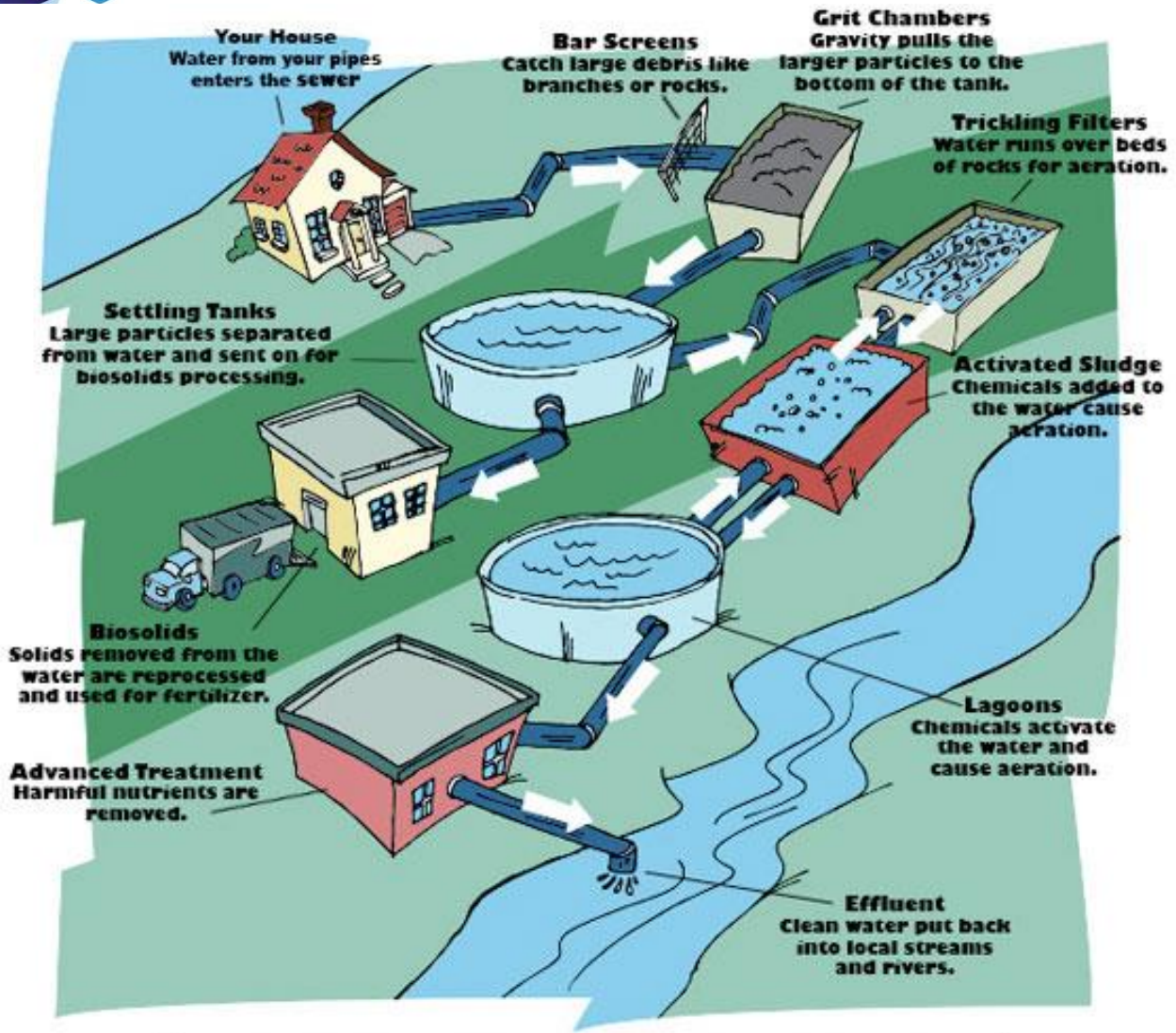





http://www.engadget.com/2012/08/14/researchers-efficient-microbial-fuel-cell-sell-electricity/

# 2.8 Environmental role of microorganisms

- **waste water treatment - utilizes the metabolic activities of diverse mixed microbial populations capable of degrading any compound that may be presented to them.**




| Primary Treatment | Secondary Treatment | Advanced Treatment |
|---|---|---|
| Bar Screens | Trickling Filters | Advanced Treatment |
| Grit Chambers | Activated Sludge | Effluent |
| Settling Tanks | Lagoons | |
| Biosolids | | |

# 2.8 Environmental role of microorganisms

- **Bioremediation – the use of microbes to transform toxic molecules to nontoxic degradation products**
- **regarded as green and safe, with cost and efficiency advantages compared to other methods of remediation as it capitalises on naturally occurring processes, minimises disturbance to the environment, reduces waste, removes or neutralises contaminants**
- **help reduce the carbon footprint and environmental impact**

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# THANK YOU