A Short History of Biomanufacturing

Microorganisms (bacteria and fungi) have been used for thousands of years to make products for human use:

Bread, beer, wine, cheese, pickles, yogurt, kimchee, soy sauce……..

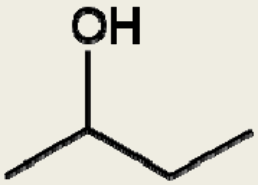
Production relied on the use of “wild” microorganisms that were naturally found associated with the raw material

- Attempts at understanding these processes stimulated the work of Pasteur and other early microbiologists

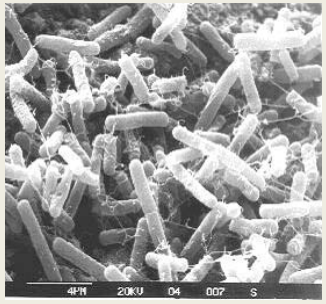
The Industrial (microbiology) Revolution 1900 – 1941

Scarcity of natural rubber stimulated search for synthetic rubber

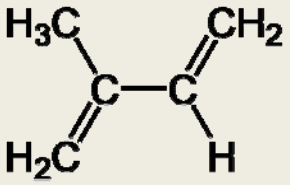
Butanol



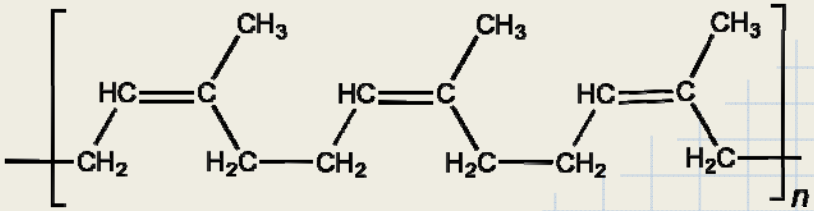
Microbial Fermentation



Isoprene



polymerization



Rubber !

ABE (acetone, butanol, ethanol) Fermentation

Chaim Weizmann- Chemist working at Univ. of Manchester

isolated a strain of bacteria called BY (later *Clostridia acetylbutylicum)*

- Could ferment a wide variety of substrates (potatoes, maize, etc) and produced higher yield of butanol and acetone then earlier isolates
- Referred to as ABE fermentation or Weizmann process

The Industrial (microbiology) Revolution

WAR

Acetone needed to make cordite used in smokeless gunpowder

- Usual method of making acetone unavailable due to lack of raw materials

Weizmann worked with the British admiralty to produce acetone using the Weizmann (ABE) process

- Several large plants built in England, Canada, India, and the US
- During the war acetone was the product of interest and butanol stored and disposed of.

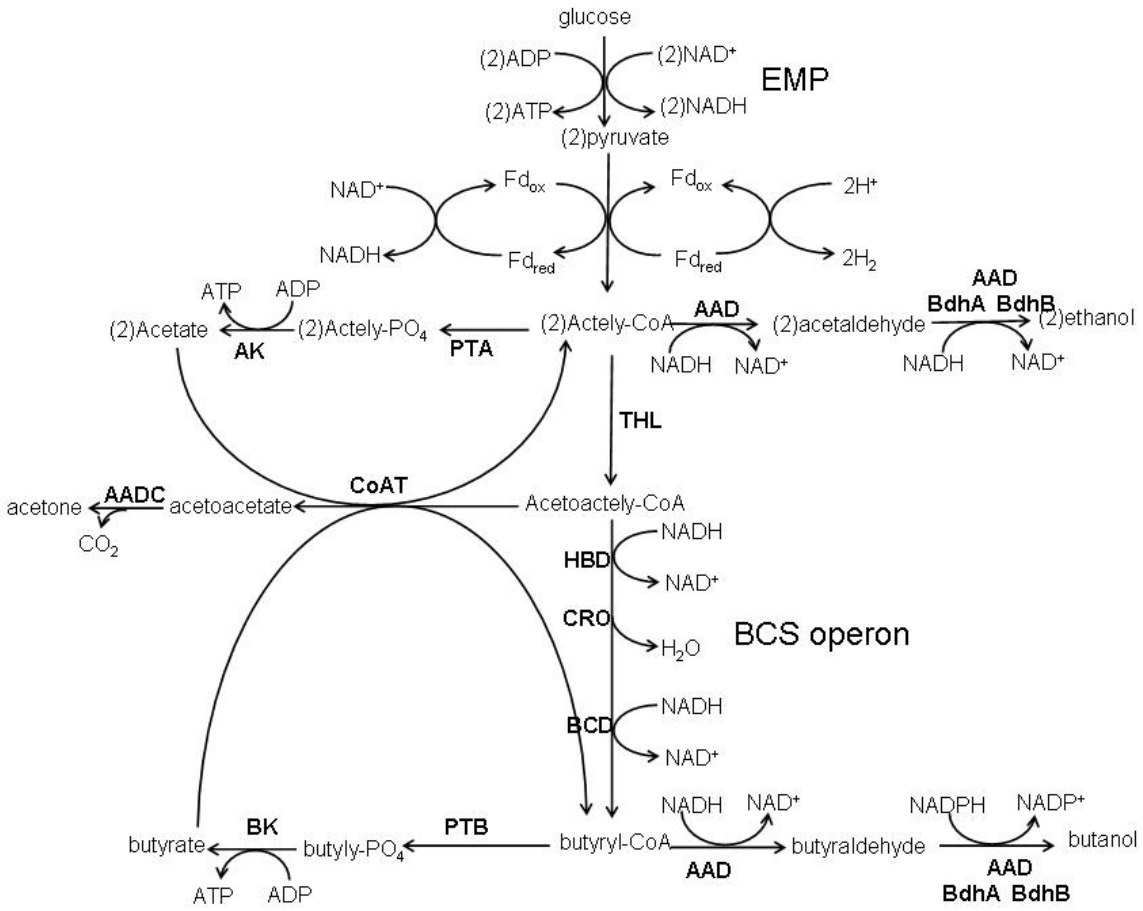
The Industrial (microbiology) Revolution

Butanol was an ideal solvent for paint lacquers that were being developed for the rapidly expanding automobile industry:

- Commercial Solvents Corporation of Maryland built large facilities in Terra Haute, IN & Peoria, IL
- 50 50,000 gallon (189,250 liter) fermenters produced 100 tons of solvent/day!

Industrial microbiology development continued after war producing products such as amino acids, vitamins, organic acids, solvents, and polysaccharides

- Many of these products are still produced using microbes with annual markets over 10^8 $/yr
- These products are all primary (I^0) metabolites required for cell growth and reproduction

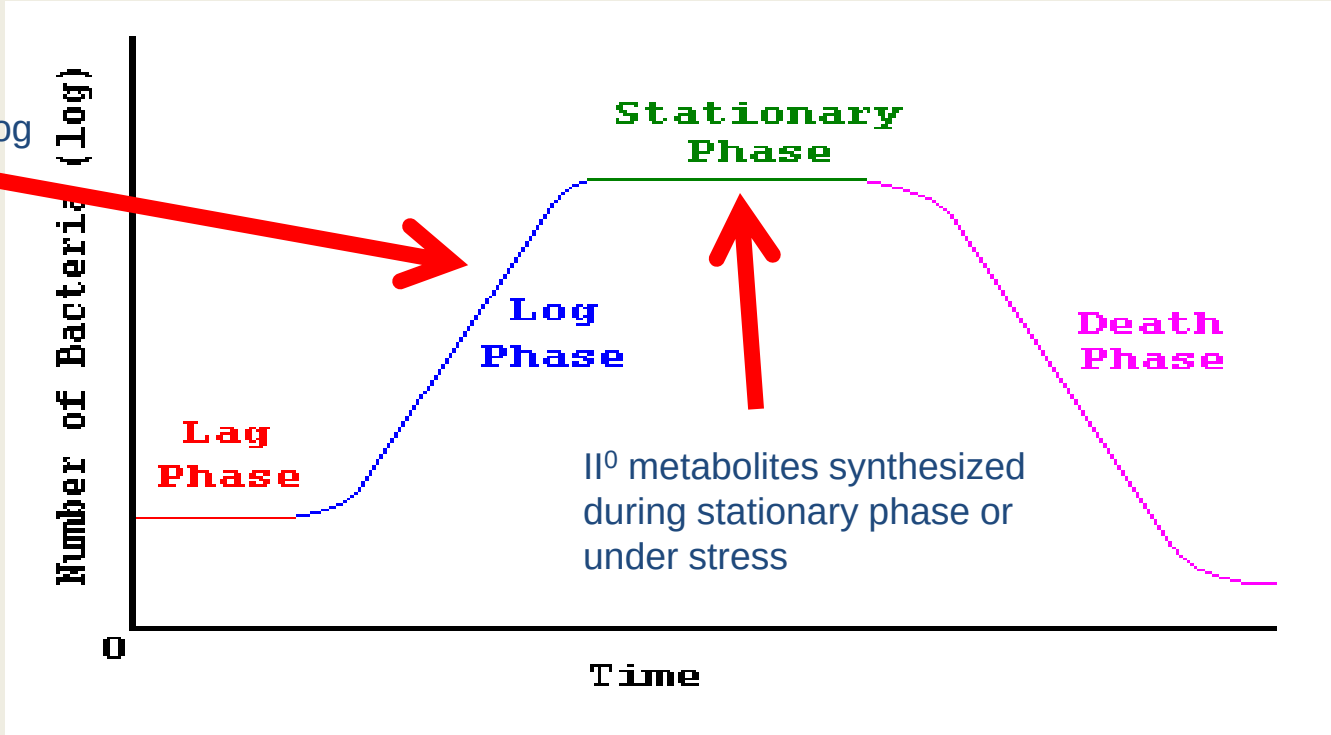


Liu, H., G. Wang, et al. (2013). The Promising Fuel-Biobutanol

Renewed interest in ABE fermentation pathway for production of butanol as a 3rd generation biofuel

Solvents & Other small molecules examples of I^0 metabolites

I^0 metabolites synthesized during log phase



Examples of I^0 metabolites currently biomanufactured

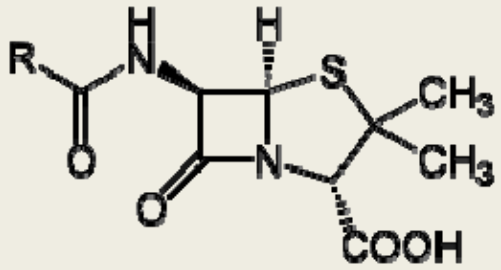
Product	Growth Requirement mg/L	Production level mg/L	Organism
Lysine	250	100,000	
Glutamic Acid	300	125,000	
Inosinic acid	25	30,000	
Riboflavin	0.5	25,000	
Vitamin B12	0.0001	150	

Demain,A.L. 2010 Production of industrial and pharmaceutical products by microbes and cell cultures. *SIM News* July/August 2010

The Second Wave-Antibiotics and other II^0 metabolites

In 1941 H. Florey showed that penicillin (originally discovered by A. Fleming in 1920's) could be used to cure bacterial infections.

- However, the yield of penicillin produced was too low (µg/L) to be economically viable
- Research on culture conditions, media, and identification of a "super-producer" strain of *Penicillium* increased yield considerably



Penicillin core structure

Penicillin mold on mandarin oranges

The Second Wave-Antibiotics and other II^0 metabolites

Medical and commercial success of Penicillin spawned intensive efforts to identify new antibiotics by screening natural isolates (primarily soil microbes) for antibiotics

- Resulted in discovery of streptomycin's, cephalosporin's and many other antibiotics/ antitumor compounds
- Antibiotics are a class of compounds known as secondary metabolites
- II^o metabolites are not required for growth and reproduction of cells – typically synthesized during stationary phase

The Third Wave- Recombinant DNA

1973-S. Cohen and H. Boyer created first “in vitro” recombinant DNA molecules

- New ability to “reprogram” microorganisms using recombinant DNA opened door to produce new products-proteins
  - -Process of introducing DNA from other species into microbial cells
  - -Universal process of information flow (Transcription & Translation – central dogma) allows “other DNA” to be used as a template for protein synthesis
  - -The cell is now capable of making a “new protein (heterologous, or recombinant)” which it was never capable of making before
- Initial focus on high value biopharmaceutical proteins

The third wave- Recombinant DNA

Proteins are large, complex molecules that cannot be economically synthesized via conventional chemical synthesis

- All proteins are polymers of amino acid -20 naturally occurring amino acids
- Order of amino acids in a protein specified by DNA sequence of gene
- Because a “template” is used protein synthesis is very high fidelity
- Protein Function dependent on order of amino acids, folding of protein (3-D shape), modifications, and co-factors

Microbial cells become the “factories” for producing the protein product

“New” products made possible with recombinant DNA

Many medical ailments caused by deficiencies in particular proteins:

Insulin

hGH

interferon

Prior to recombinant DNA technology those proteins were purified from animals and/or cadavers

Problems with supply, adverse reactions, adventitious agents

Coupling of rDNA technology with industrial microbiology techniques expanded the types of products that could be produced

Enzymes used in research and diagnostics (Taq, R.E., etc)

Enzymes used in processing foods (rennin, amylases, lipases, etc)

Enzymes used in processing textiles (cellulases)

Enzymes used in cleaning / industrial processes (amylases, lipases, proteases)

Enzymes used in fine chemical synthesis (racimases, reductases, etc)

The fourth wave- synthetic biology

(moving from chance to design)

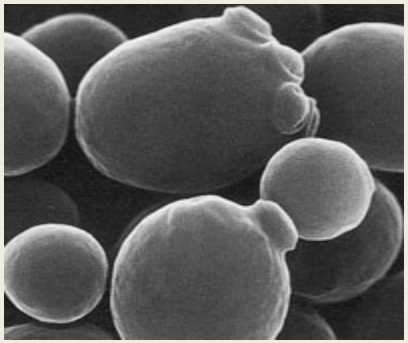
Prior phases of biotechnology relied on naturally occurring microbes that produced a product of interest (grind & find approach)

The present phase of biotechnology (1970- onward) characterized by having microbes make products they never before had the capacity to make.

Instead of chance now rely on design.

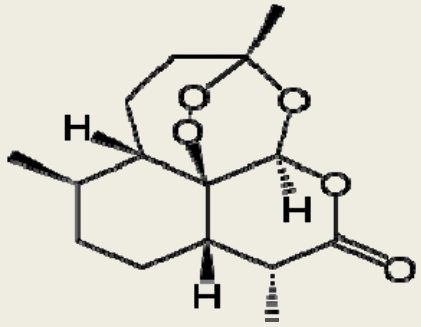
Initially, single protein products encoded by single genes. Now small molecule products synthesized by complex metabolic pathways.

In 2006 a team from Berkeley published an article claiming that they had engineered *Saccharomyces cerevisiae*, *a* yeast, to produce artemisinic acid. The synthesized artemisinic acid can then be purified and turned into an antimalarial drug (artimisinin and derivatives) that they claim will cost roughly 0.25 cents per dose.



Ro DK, Paradise EM et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature. (2006) 440: 940-943.

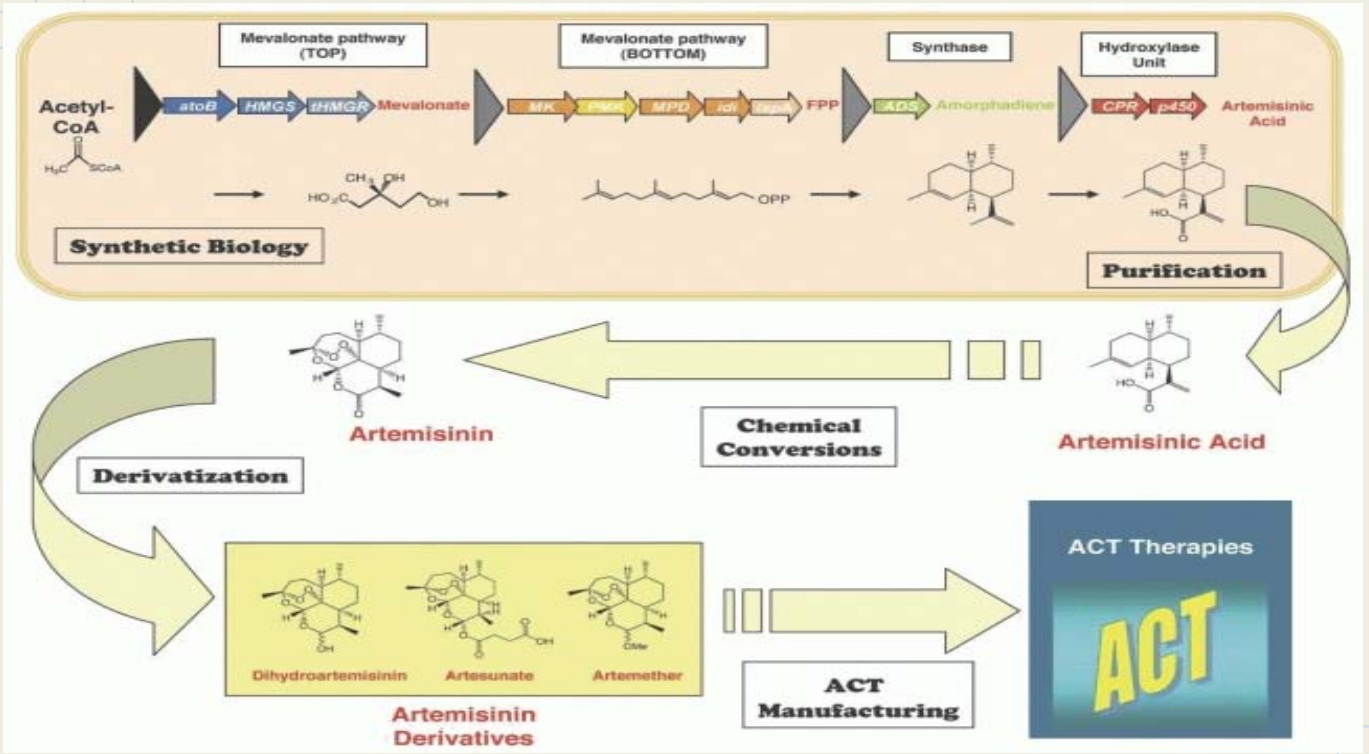
Artemisinin



Artemisinin is a drug used to treat multi-drug resistant strains of falciparum malaria. The compound (a sesquiterpene lactone) is isolated from the plant *Artemisia annua*.

Limited availability of artemisinin hampered implementation

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



Microbially Derived Artemisinin: A Biotechnology Solution to the Global Problem of Access to Affordable Antimalarial Drugs

Victoria Hale,* Jay D. Keasling, Neil Renninger, and Thierry T. Diagana

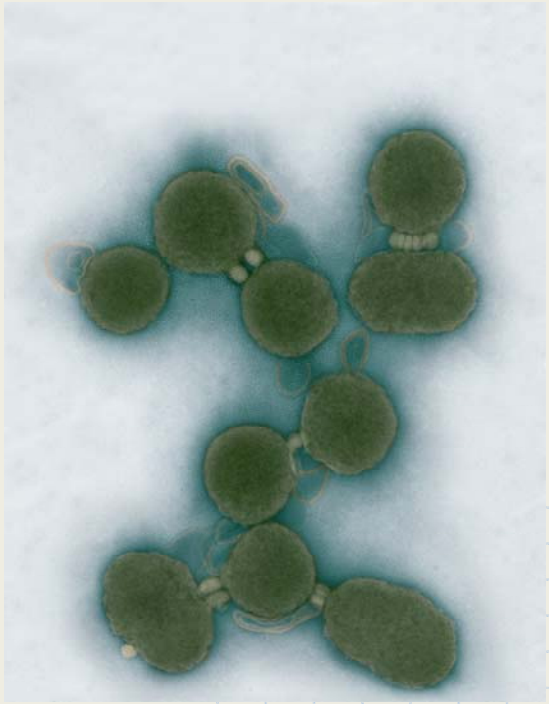
In 2010 the same team announced that they had successfully engineered *E. coli* cells to produce fatty esters for the production of biodiesel, fatty alcohols, and waxes directly from simple sugars

Steen, E.J. *et al* 2010 Microbial production of fatty acid derived fuels and chemicals from plant biomass. Nature 463 p 559.

A synthetic cell as a platform for synthetic biology

In 2010 scientist at JCVI (J. Craig Venter Institute) reported on the construction of a viable cell (*Mycoplasma mycoides JCVI syn 1.0)* created by transplanting an artificially constructed genome into a genome-less cell of *Mycoplasma mycoides.*

Goal of research at JCVI and other institutes is the creation of a minimal cell into which novel metabolic pathways can be inserted

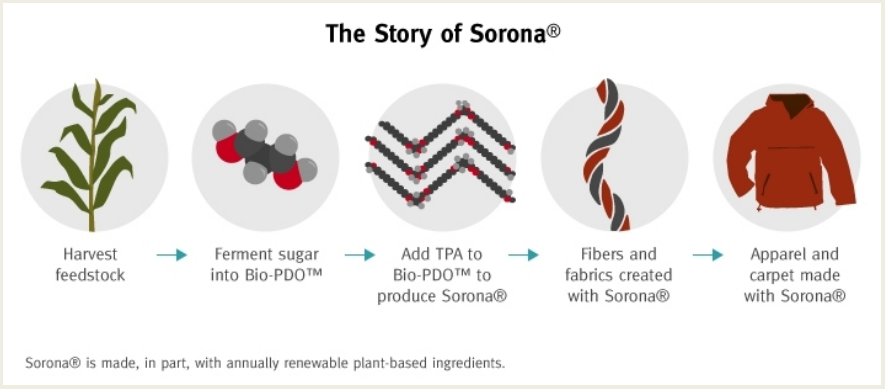


Today more and more products that were produced from petrochemical feedstocks are now being produced or being developed for biological production

Lactic acid (PLA plastics -Bioplastics)

Biodiesel

Ethanol/Butanol



5 reasons microbes are so good as a manufacturing platform

A high surface to volume ratio

- Facilitates the rapid uptake of nutrients which can support high rates of metabolism and biosynthesis

Diversity of metabolic reactions

- Can utilize a number of different carbon, nitrogen, and energy sources and produce many different compounds

Ability to adapt to different environmental conditions

- Natural isolates can be grown in laboratory flasks or large fermenters

Ease of genetic manipulations

- Microbes can be treated with mutagens and mutants that overproduce isolated
- Microbes can be reprogrammed with recombinant DNA

Microbial enzymes make very specific compounds

- Only R sugars and L amino acids
- Conventional chemical synthesis is not stereo-specific

Increasing yield : The key problem in biomanufacturing

Increasing the yield or productivity of the product is necessary to make biomanufacturing economically viable

Takes a combined approach of:

- Increasing the yield per cell – identifying “super producer” strains
- Increasing the number of cells producing product
- Efficient purification strategies

The Conventional Strain Improvement (CSI) approach to increasing yield

Microorganisms identified that made low levels of product of interest

- Strains mutagenized randomly (radiation, chemicals)
- Screen for higher productivity

Repeat!

Repeated rounds of mutagenesis and selection/screen yielded *Penicillium* strains that produced >50 times the initial amount. Advances in fermentation technology and purification provided enough of an improvement to make the process of producing Penicillin economically feasible

Today, strains make over 50 gm penicillin per Liter (a >10,000 fold improvement)

Production level vs Cost of Penicillin		
Year	Amount Produced	Cost
1945	2,300 kg	$11,000/kg
1963	3×10^6 Kg	$150 / Kg
1978	15×10^6 Kg	$18/ Kg
1995	31×10^6Kg	$ 4.5 / Kg

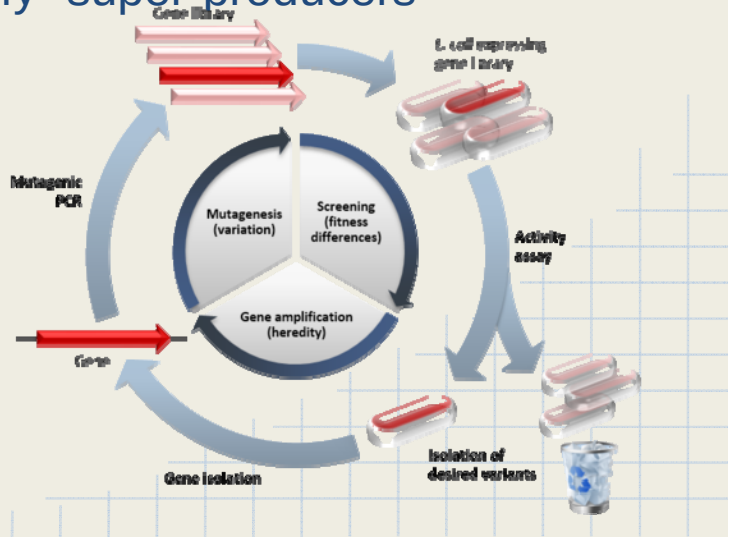
Demain, A.L. 2010 Production of industrial and pharmaceutical products by microbes and cell cultures. *SIM News* July/August 2010

Directed evolution-an extension of CSI

Similar goal of CSI approach-increasing yield per cell

Combination of random mutagenesis as well as targeted mutagenesis

Devise a selection / screen that can identify “super-producers”



Biomanufacturing involves three core processes:

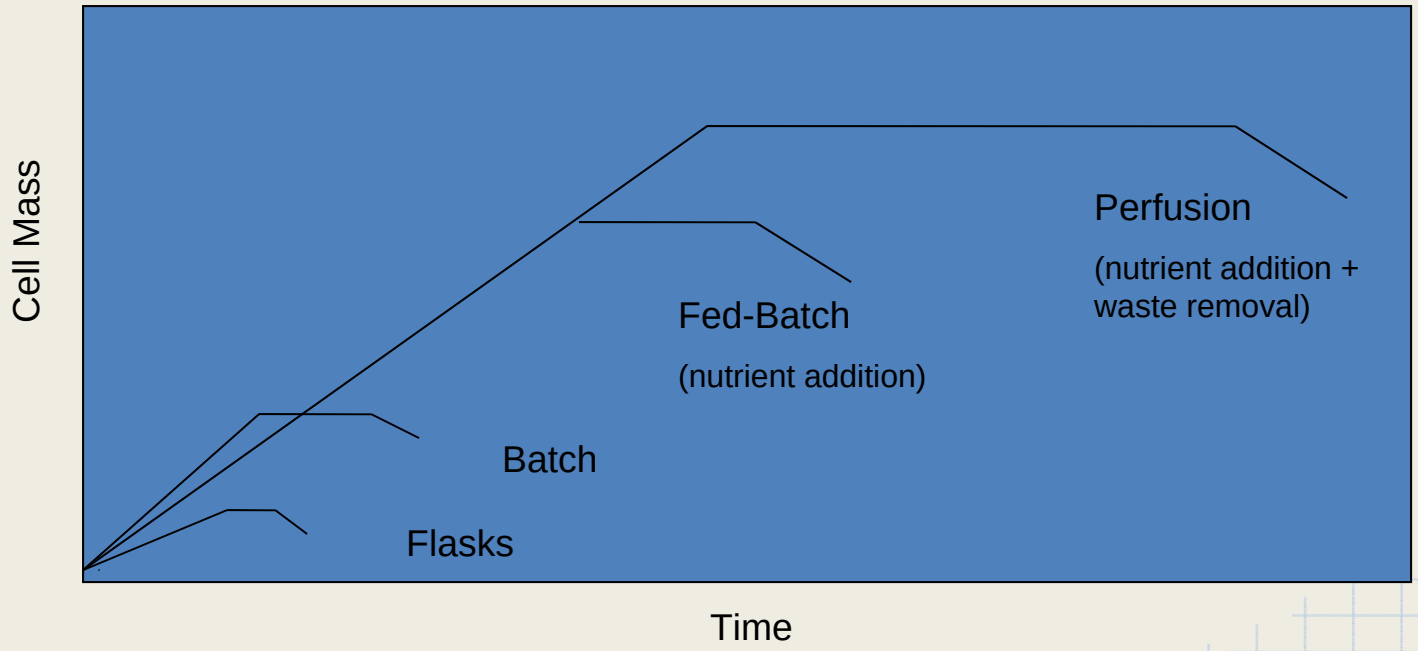
1. Controlled growth of microorganisms, cells, tissues or organisms

2. Conversion of simple raw materials or complex molecules to desired product

3. Isolation & purification of the product from complex mixtures

Scale-Up

Research scale equipment can’t produce commercial quantities cost effectively
Product yield is (usually) proportional to cell mass
Shake flasks and spinners generate low product yields (mg/L or less) due to lack of process control (nutrient limitation, waste accumulat
Cost effective production requires 1000-fold higher product
mass)


Cell Mass
Perfusion
(nutrient addition + waste removal)
Fed-Batch
(nutrient addition)
Batch
Flasks
Time