



waag society

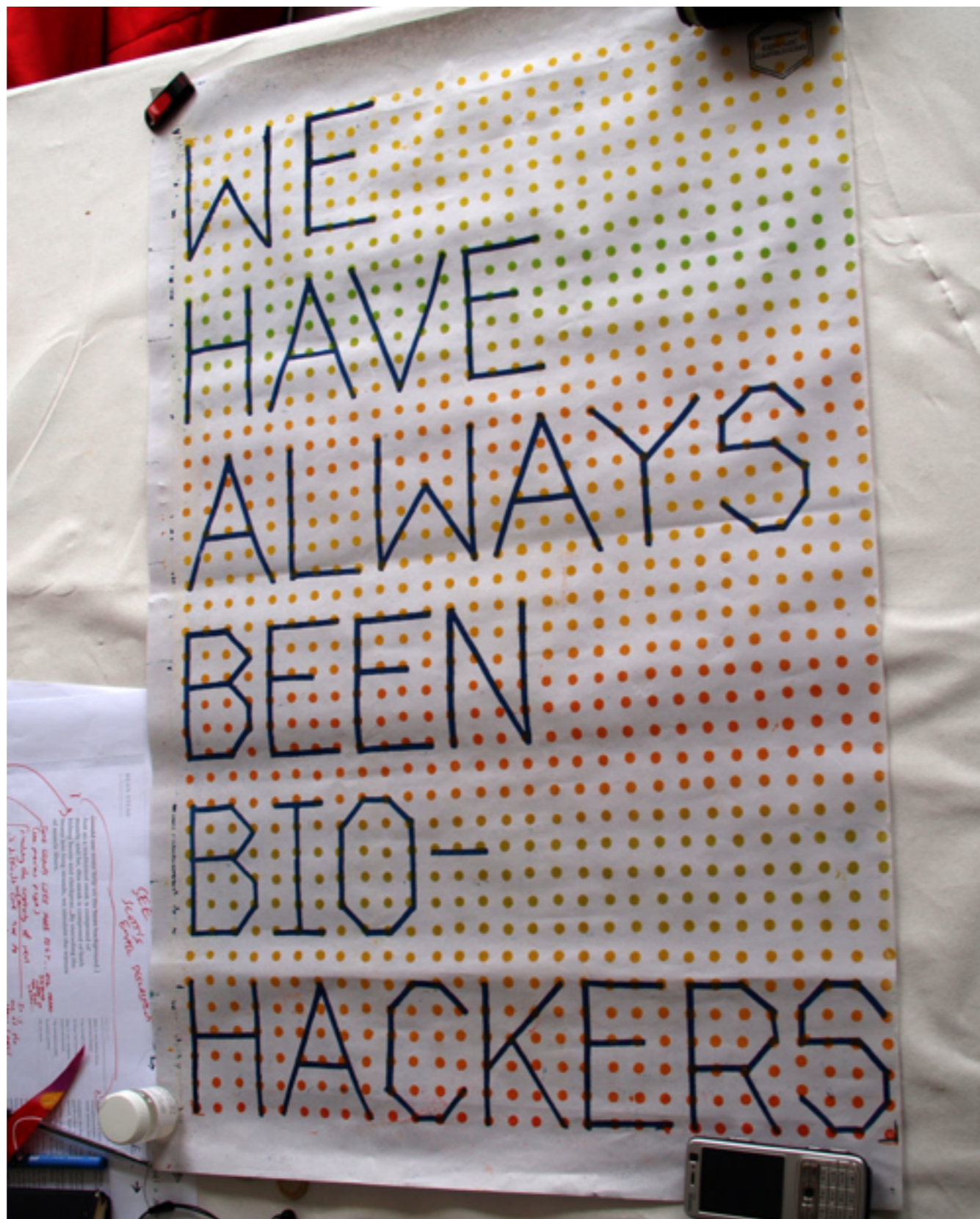
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BioHack Academy Bioreactor Design



We have always been biohackers



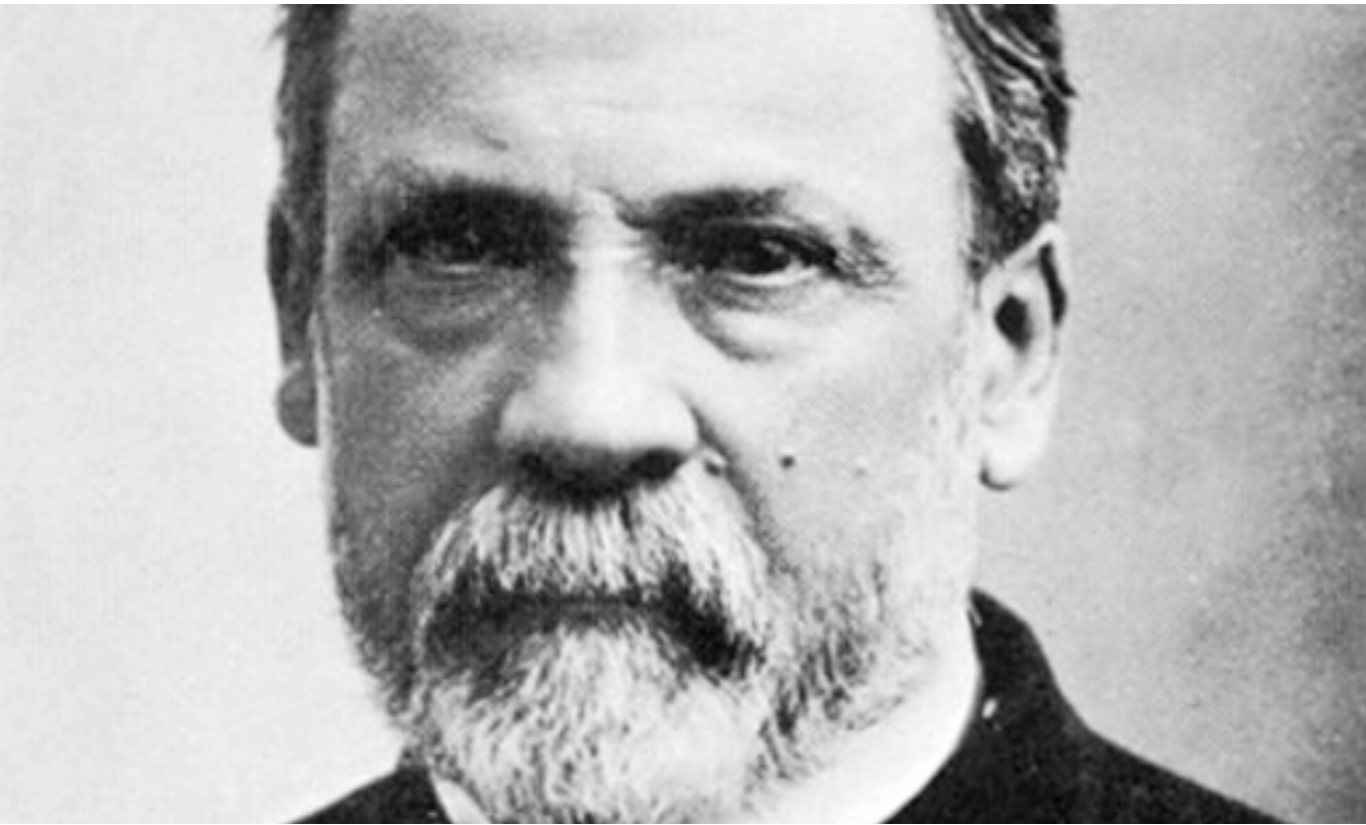


We have always been biohackers

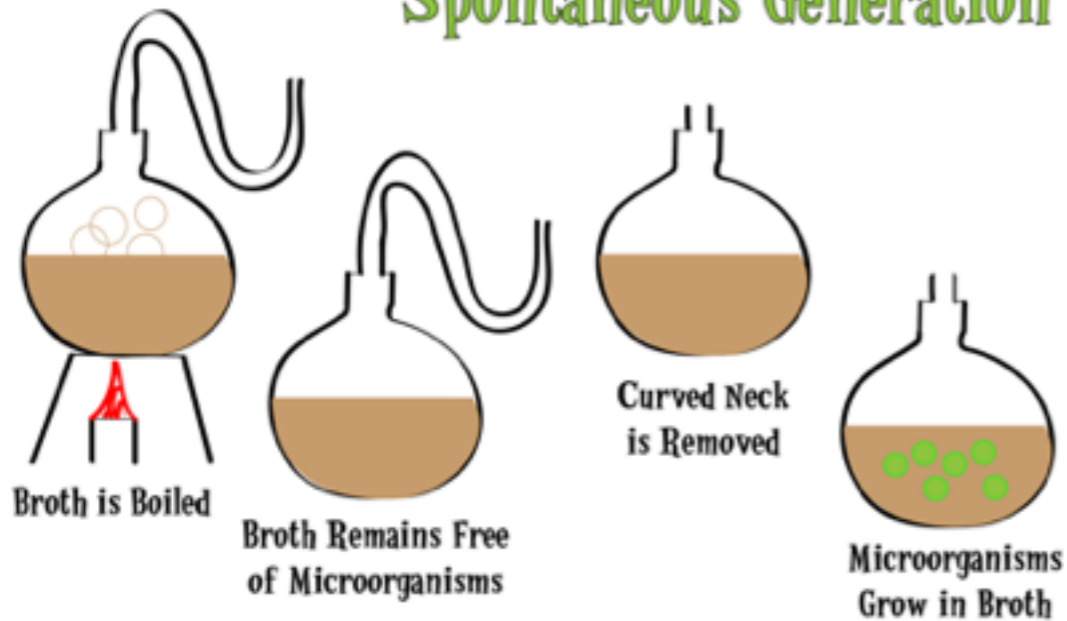




Louis Pasteur



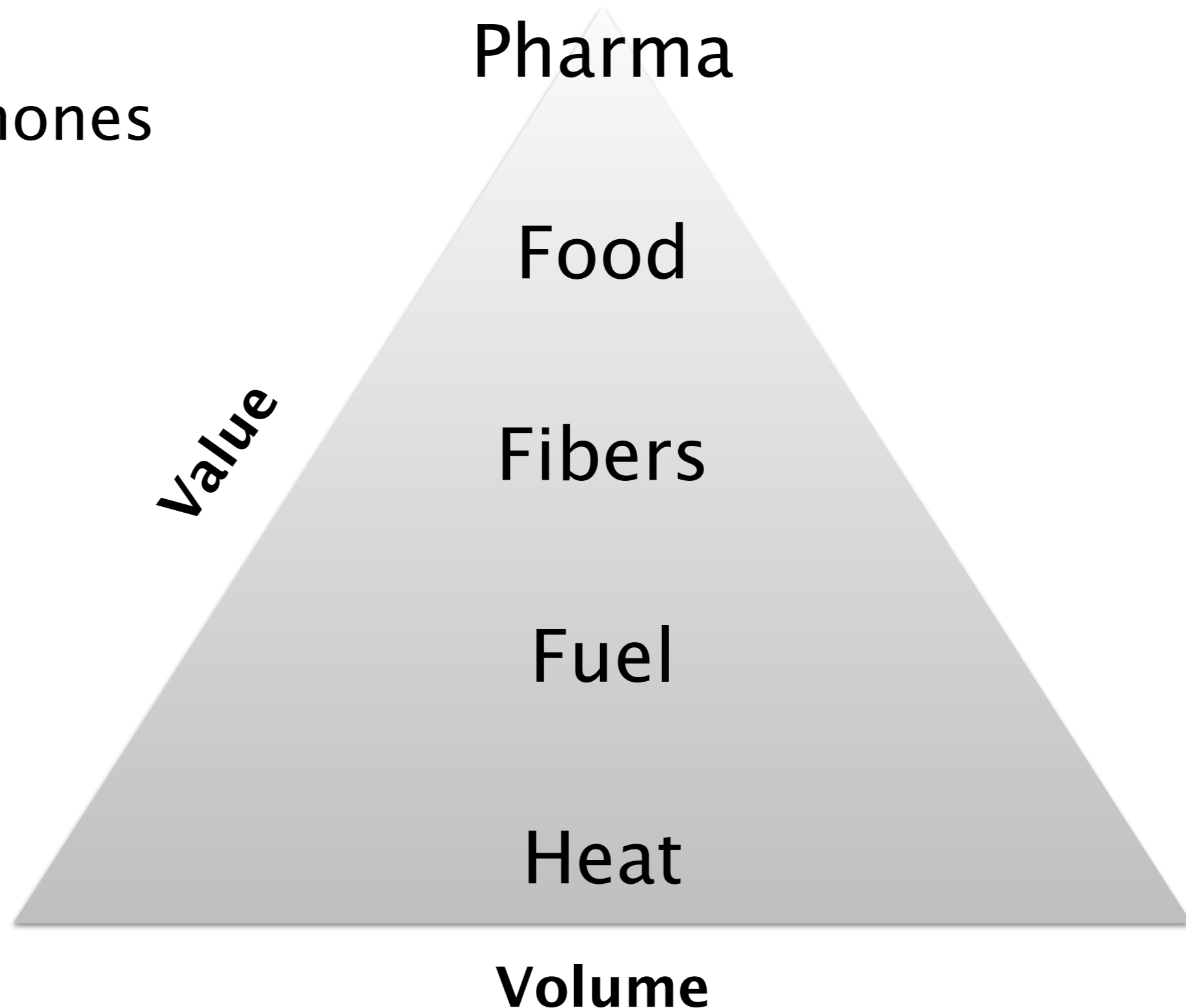
Pasteur's Test of Spontaneous Generation





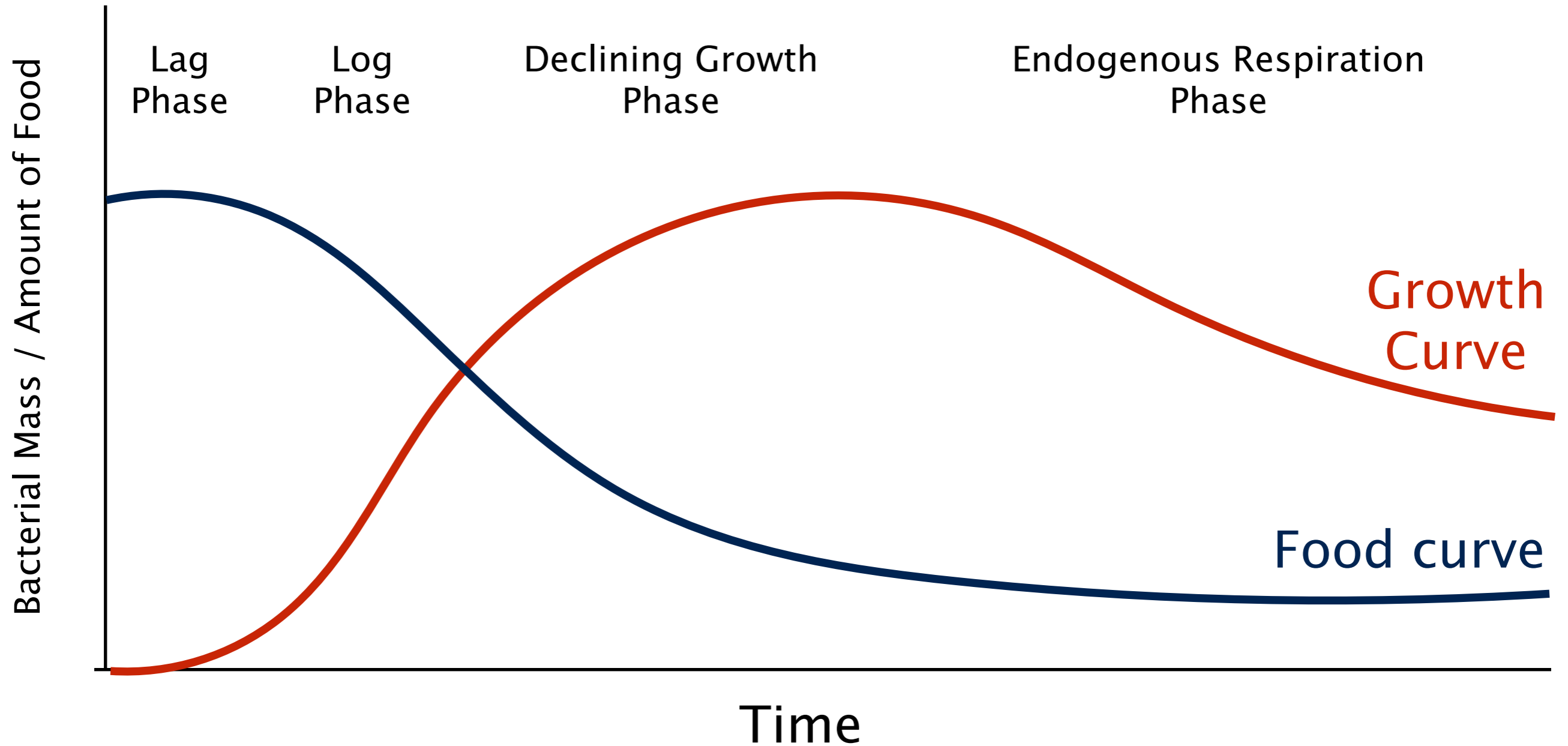
Bioreactor value pyramid

- Antibiotics
- Steroids / hormones
- Vitamins
- Proteins
- Sugars
- Acids



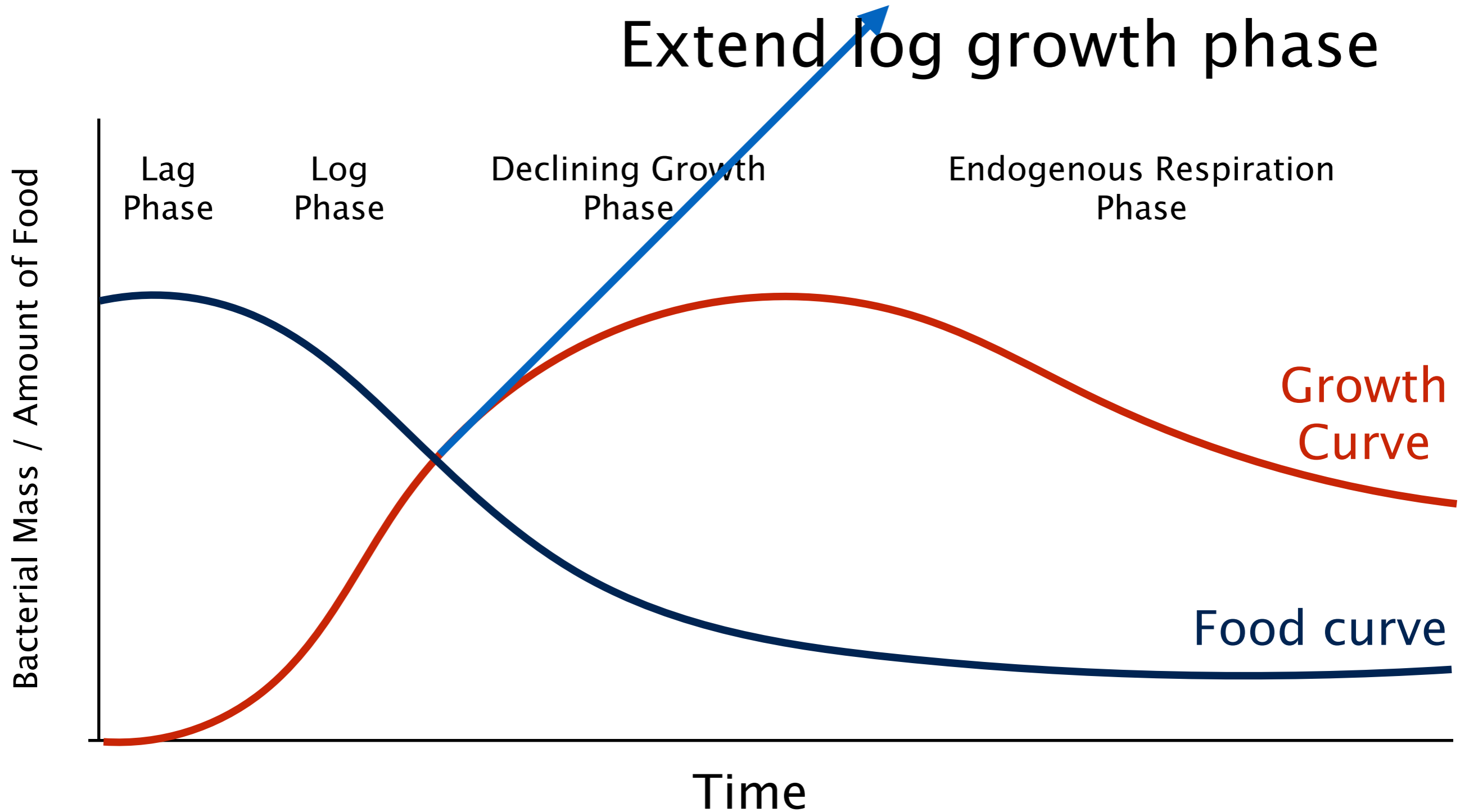


Bacterial growth curve – Batch



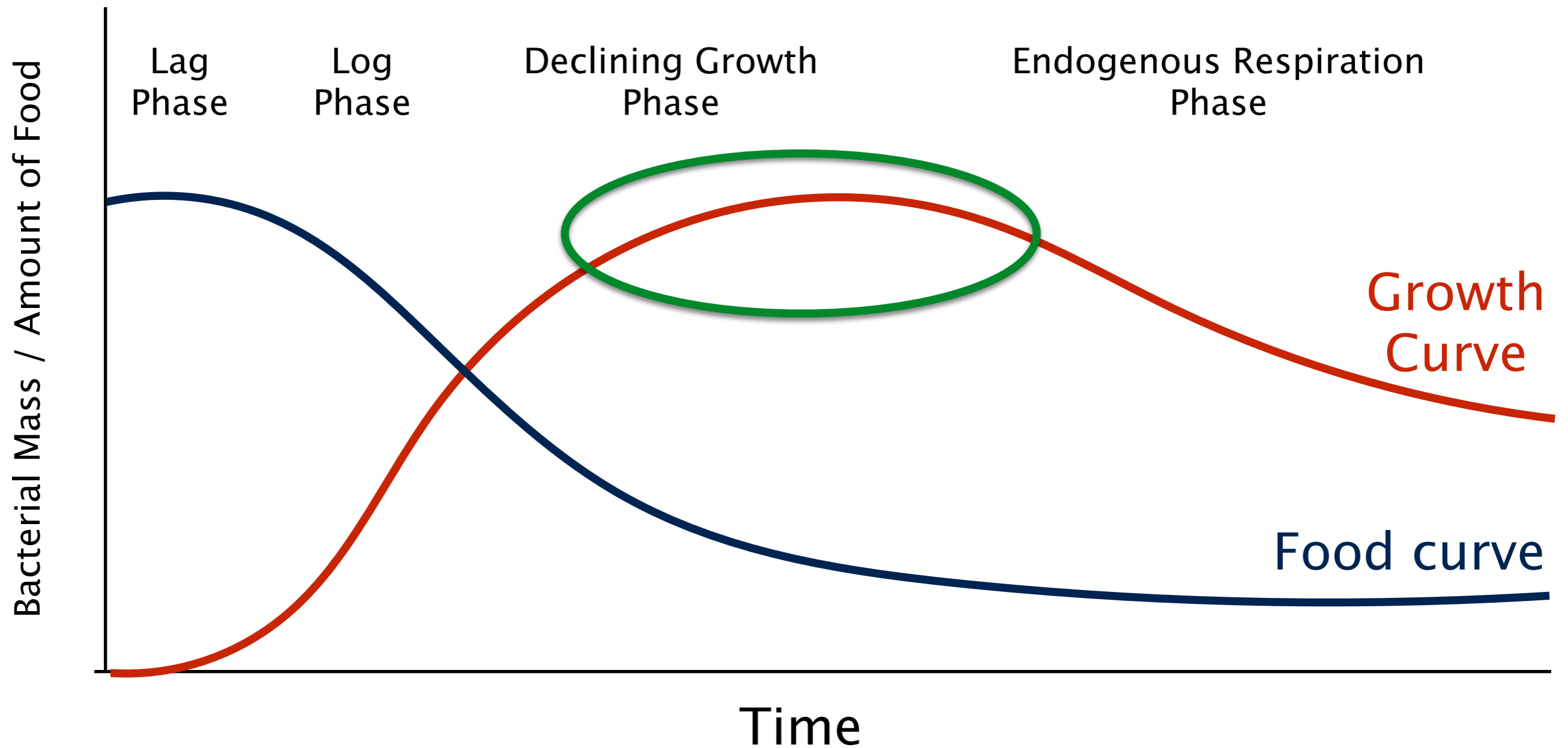


Primary products



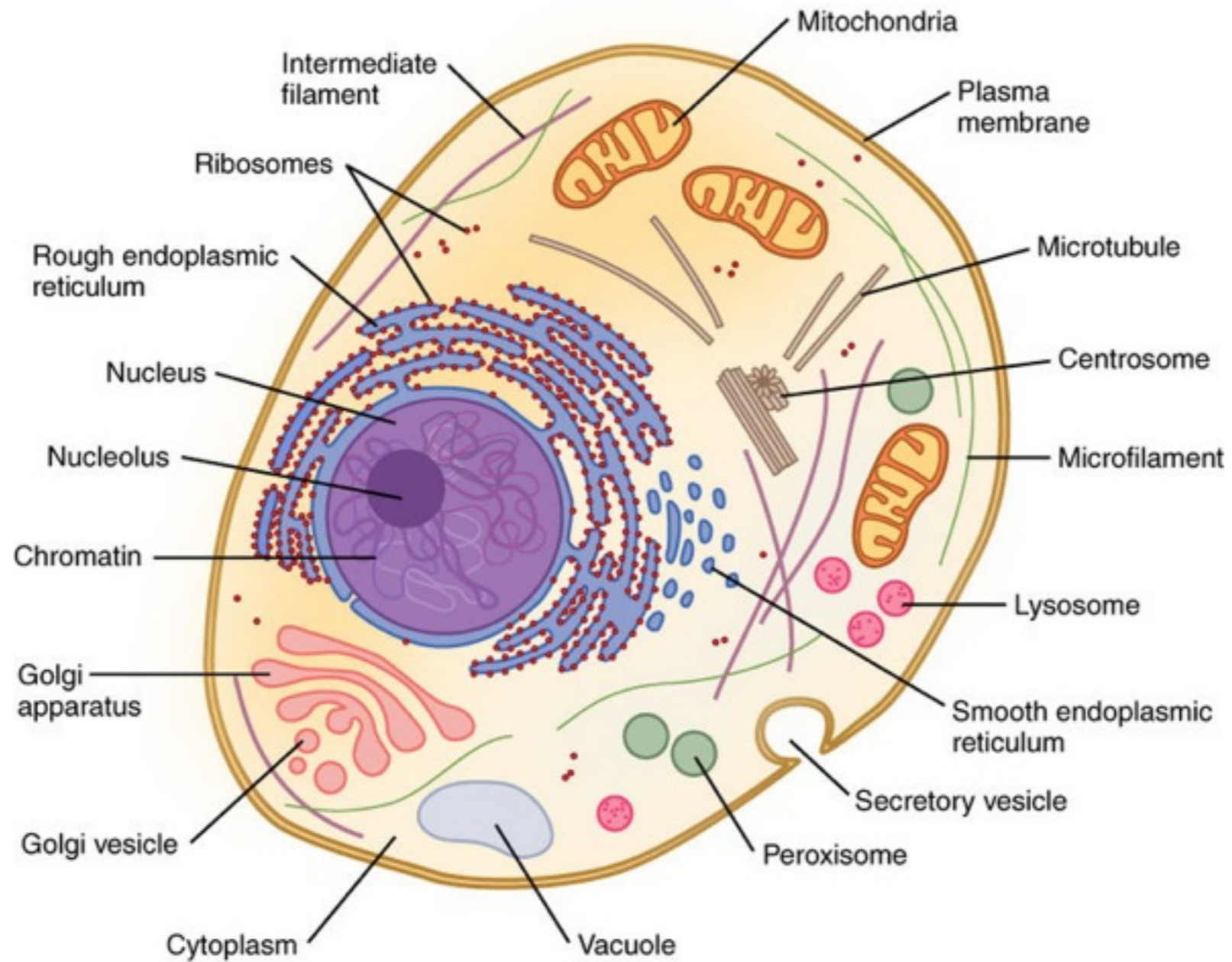


Secondary products



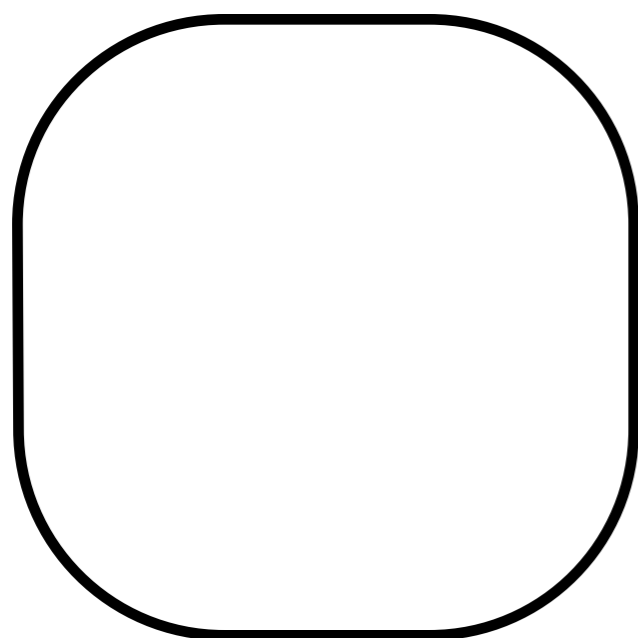


Intra vs extra cellular

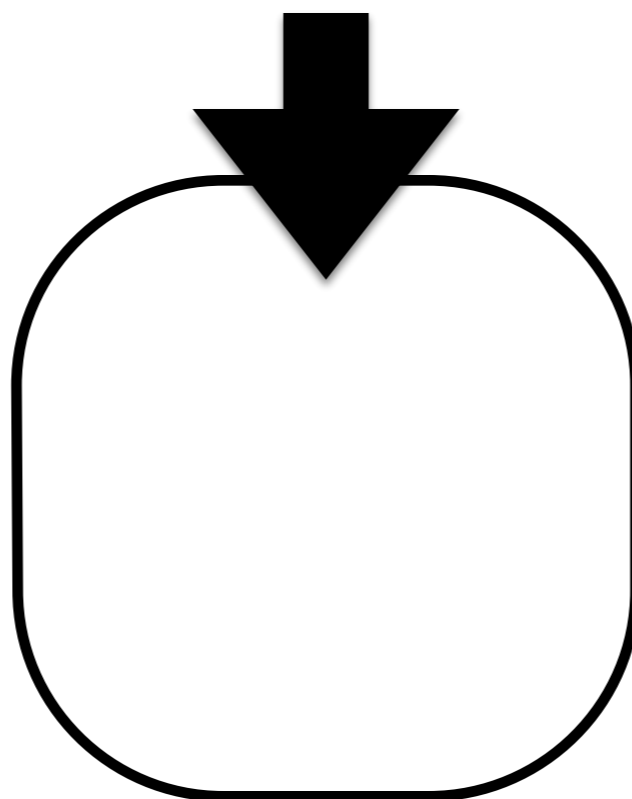




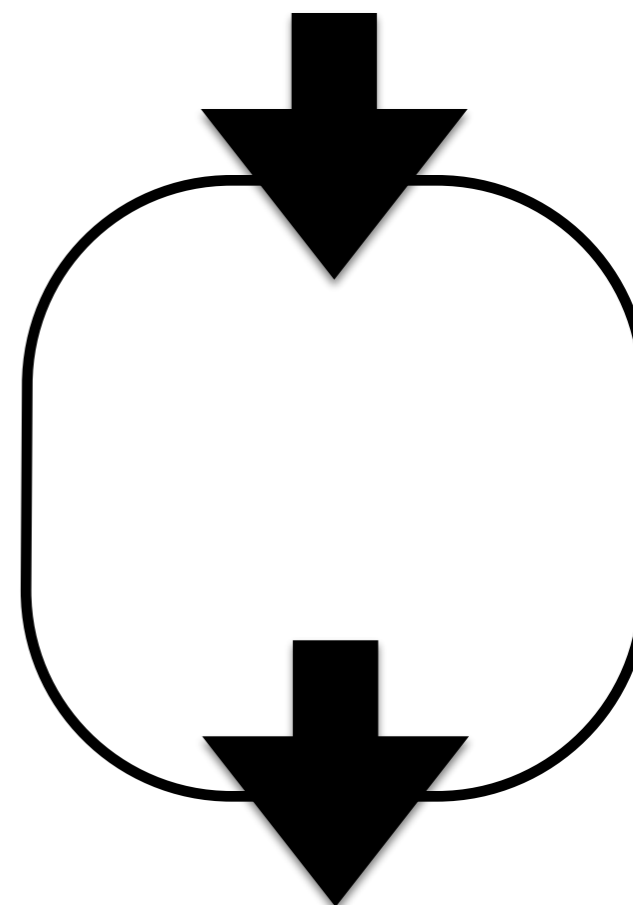
Growth strategies



Batch



Fed Batch



Chemostat



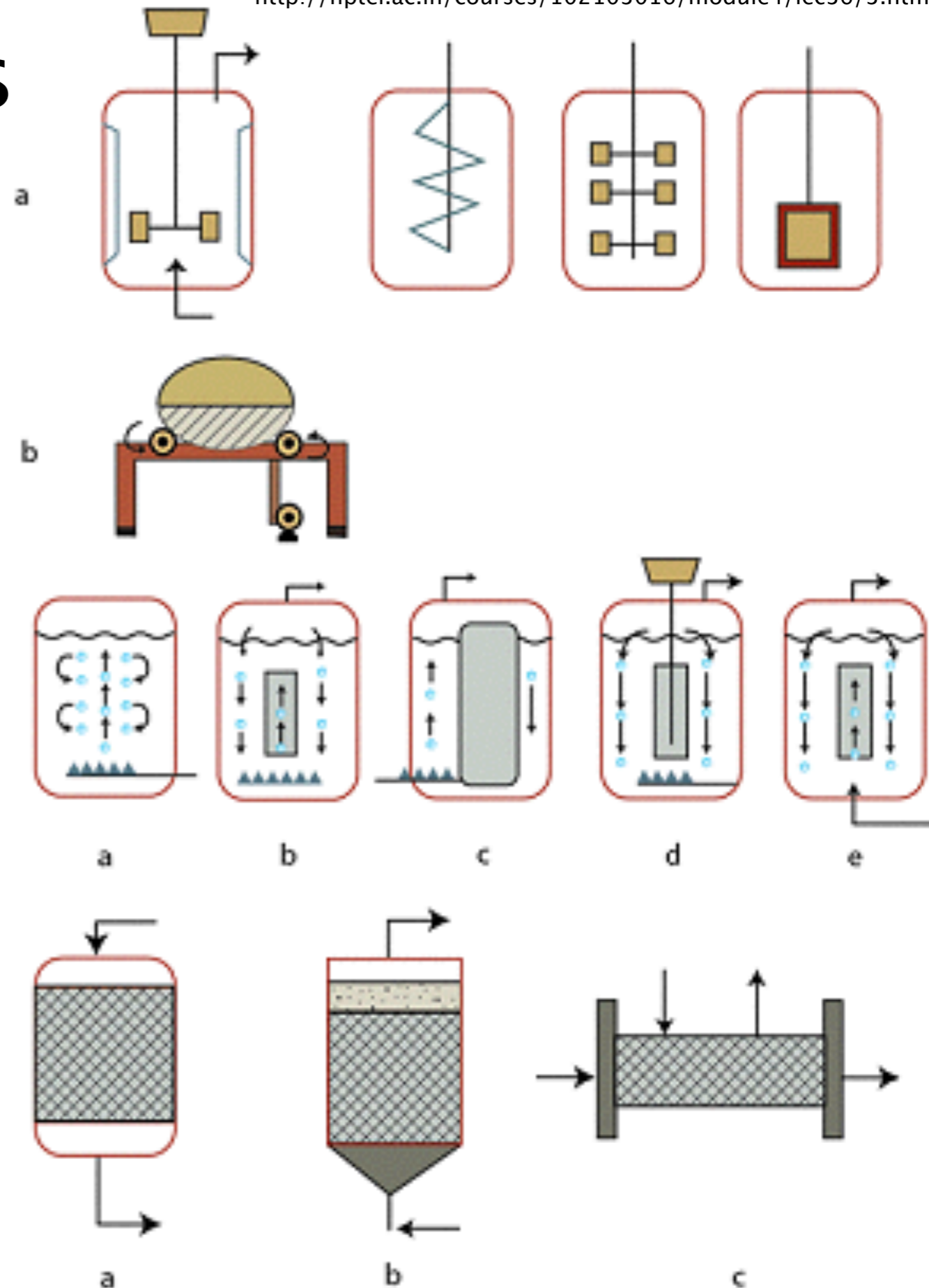
Bioreactor Wishlist

- Simple design
- Many cells per volume
- Uniform distribution
- Simple oxygen supply
- Low energy use



Bioreactor Types

- Stirrer tank
- Air-lift
- Membrane
- Immobilized cells
- Cell culture
- Solid state
- Photobioreactor
- Microbioreactors
- Animals



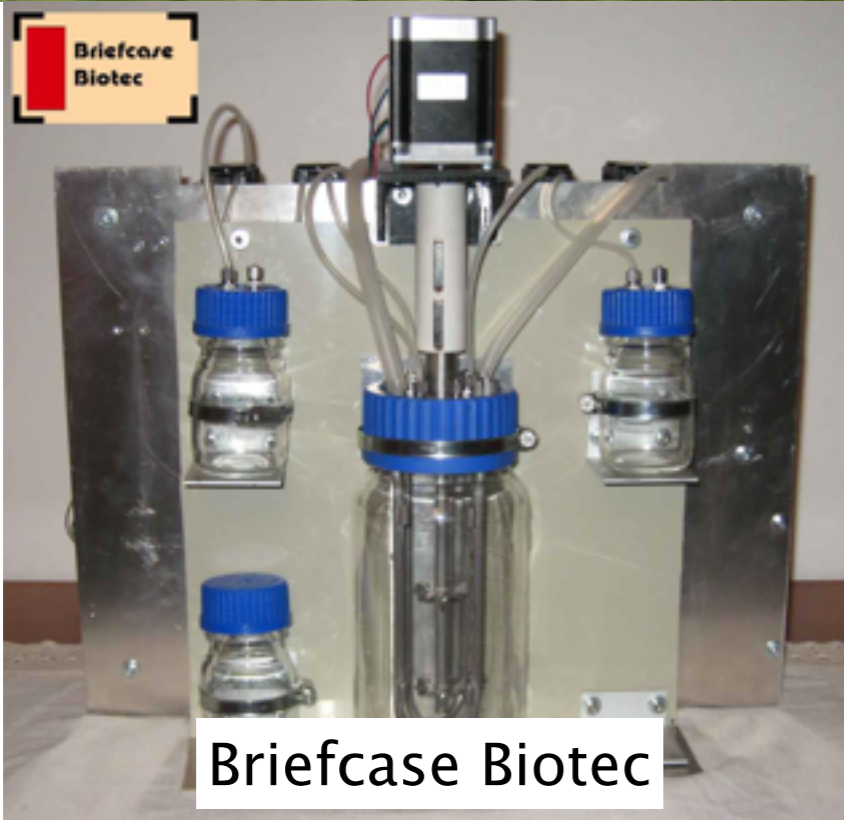


Bioreactor hacks

<http://www.instructables.com/id/An-Algae-Bioreactor-from-Recycled-Water-Bottles/>



<http://www.instructables.com/id/Biogas-at-home-Cheap-and-Easy/>



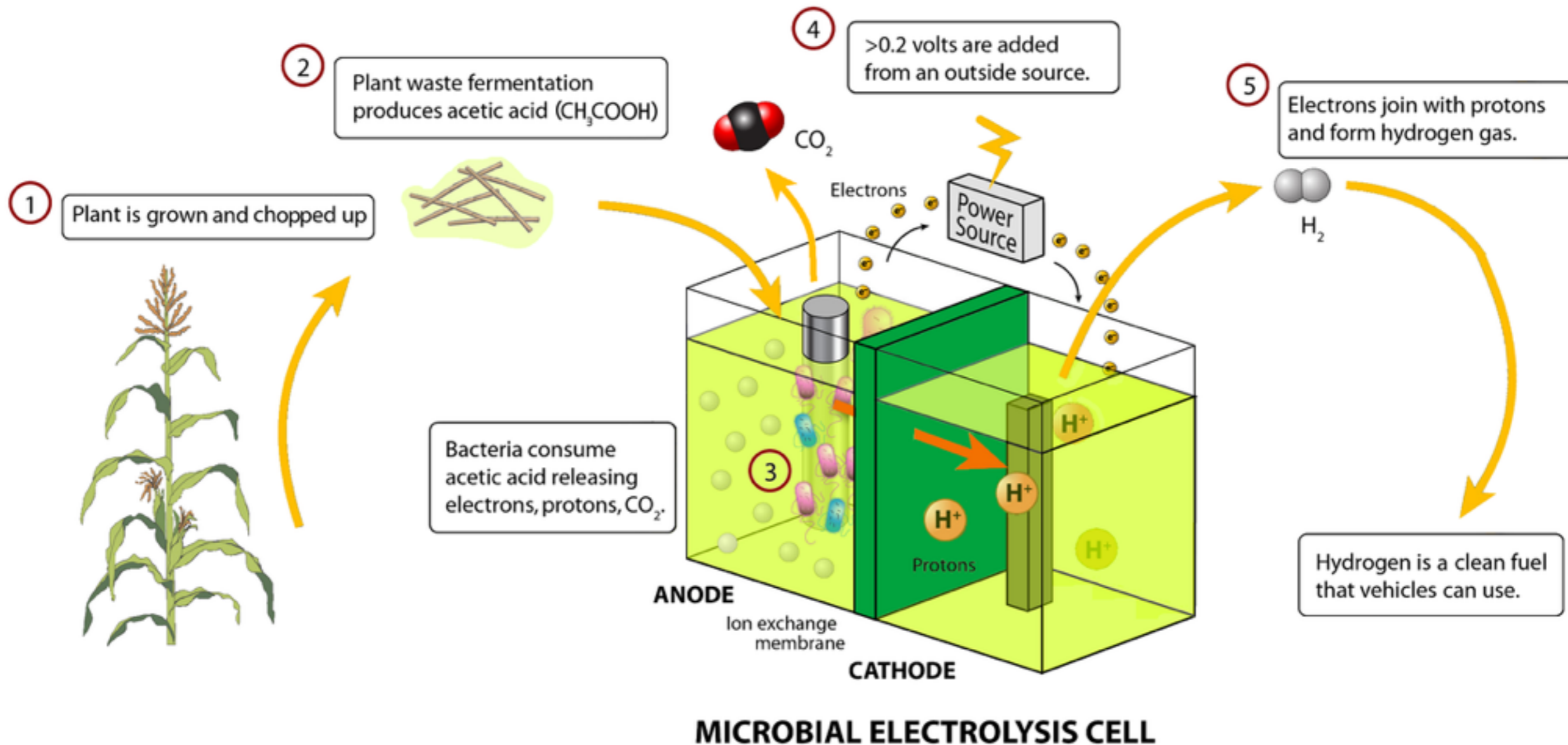
Briefcase Biotec



<https://www.youtube.com/watch?v=yDarDR4vi1A>

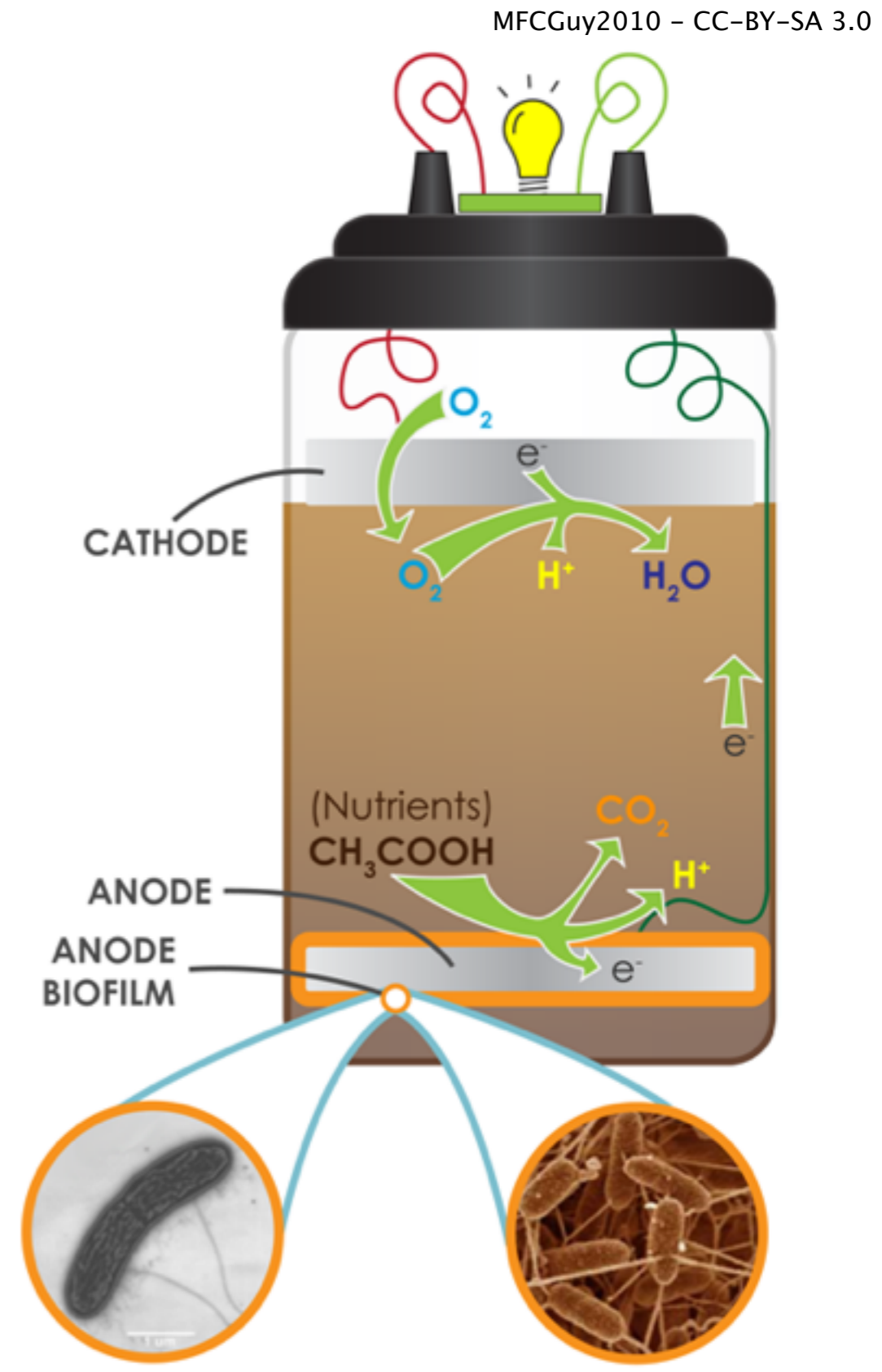
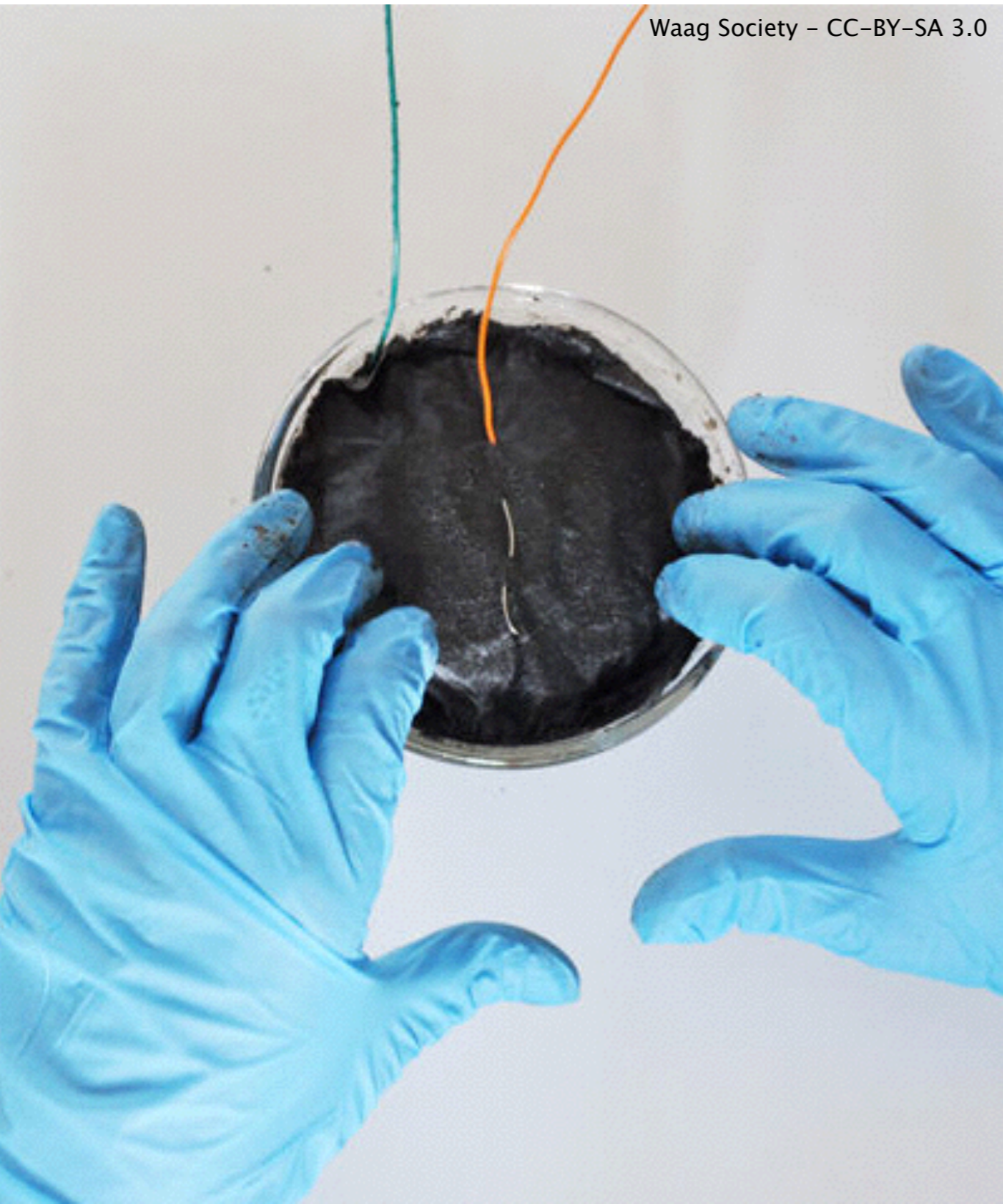


Membrane reactor: Fuel Cells



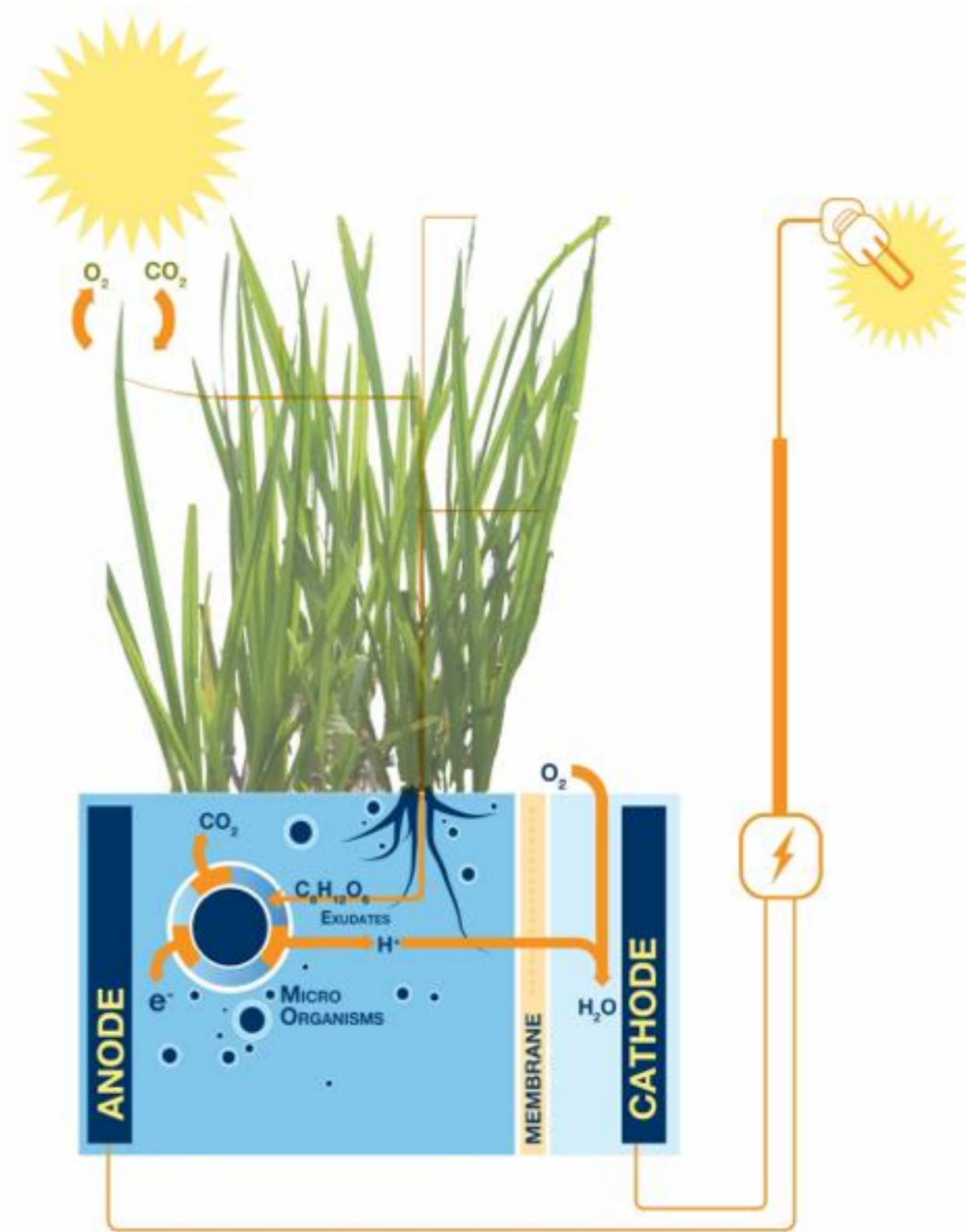


Soil based fuel cells





Plant-e





Bio mud battery

<http://www.instructables.com/id/Create-your-own-mud-battery-Bouw-je-eigen-modderba/>





BIOMONSTAAAR

<http://biomonstaaar.com>





Flu vaccine production in eggs



How flu vaccine is prepared

Each year's influenza vaccine is 85% effective against the dominant flu strain.

- 1 Human flu virus injected into fertilized hen's eggs
- 2 Flu virus reproduces in hen's eggs
- 3 Formaldehyde solution injected to kill virus
- 4 Killed virus extracted; vaccine solution prepared

Who should be vaccinated

People with:

- ▶ Immune system disorders
- ▶ Heart or lung disease
- ▶ Diabetes
- ▶ Chronic kidney disease
- ▶ Anemia

People over age 65

Health care workers

Home care-givers

Influenza vaccine myths

Myth Vaccine can cause flu	Myth Body builds up immunity against flu	Myth Vaccine causes side effects
Truth Vaccine viruses cannot invade healthy tissue	Truth Different strains of flu appear each year	Truth May cause temporary redness, soreness in inoculated area

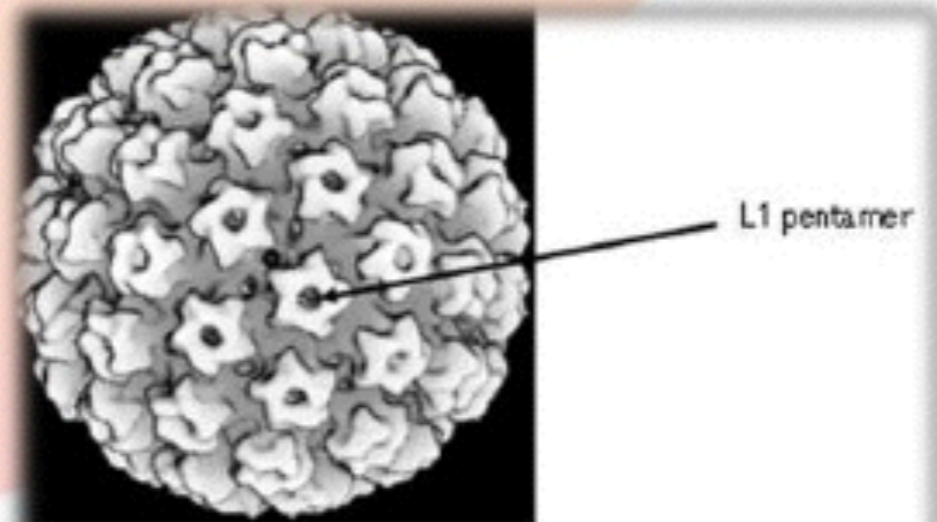
SOURCES: Baylor College of Medicine, World Book Health & Medical Annual, research by BRENNIA SINK



Insect cell line

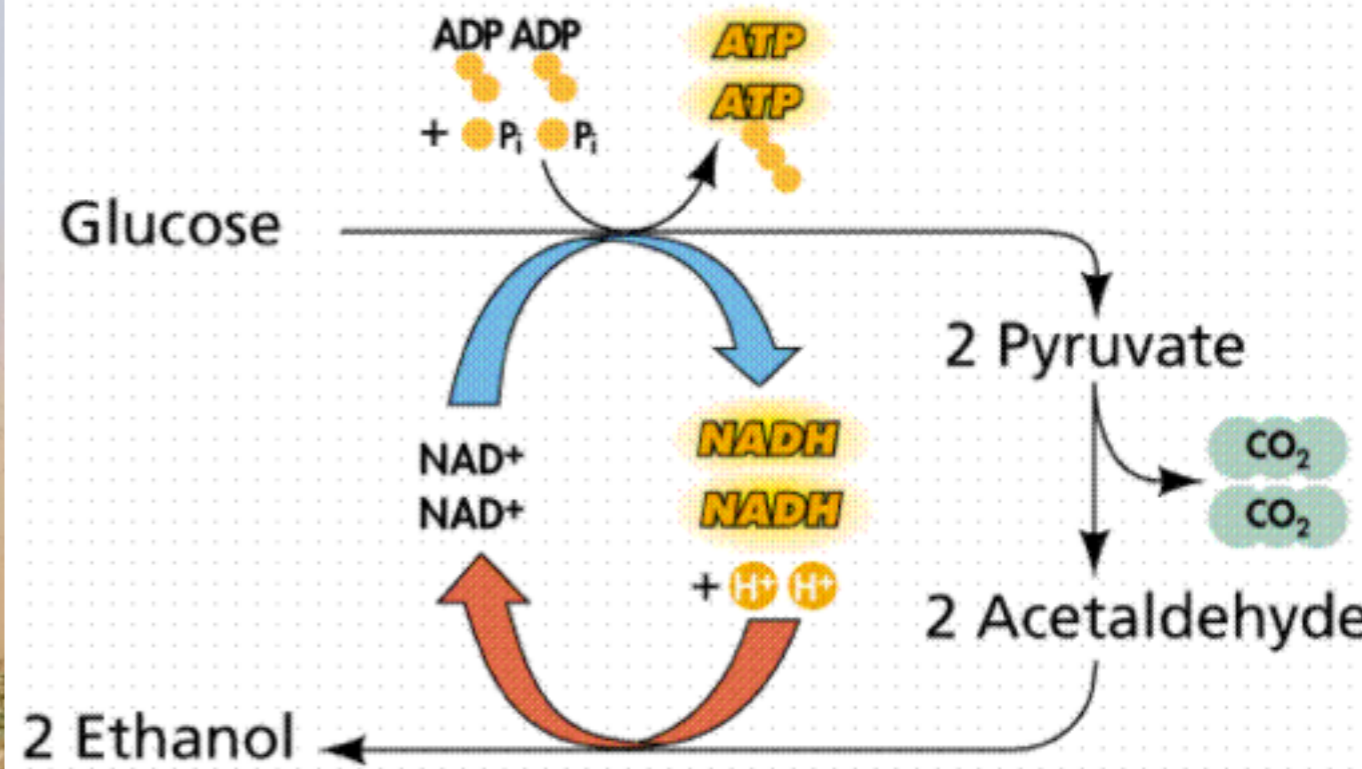
Cervarix Active Ingredient

- Cervarix, HPV-16/18 L1 AS04 vaccine contains recombinant C-terminally truncated major capsid L1 proteins of HPV types 16 and 18 as active ingredients
- The first vaccines for humans from Baculovirus Expression System



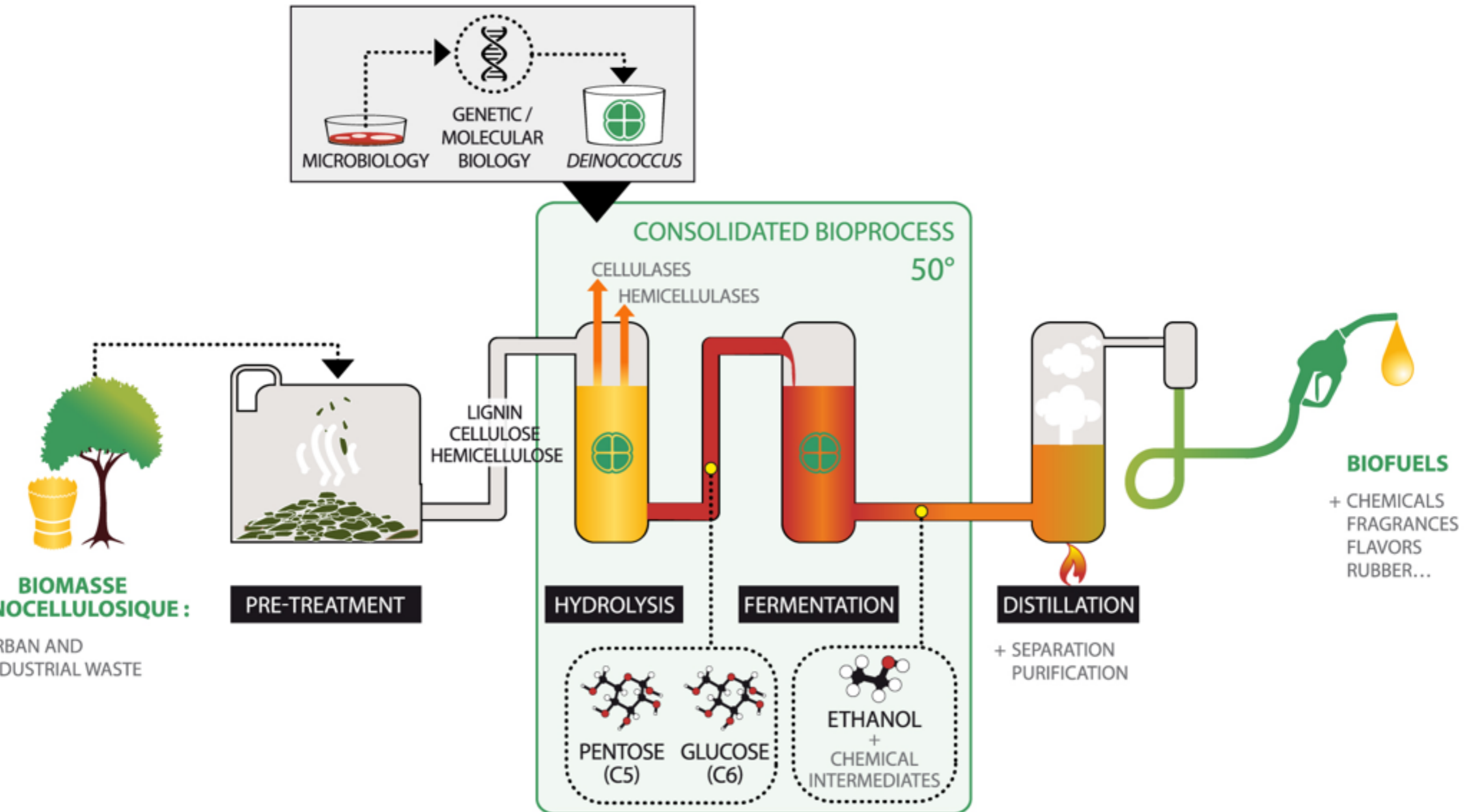


Sugar cane to ethanol



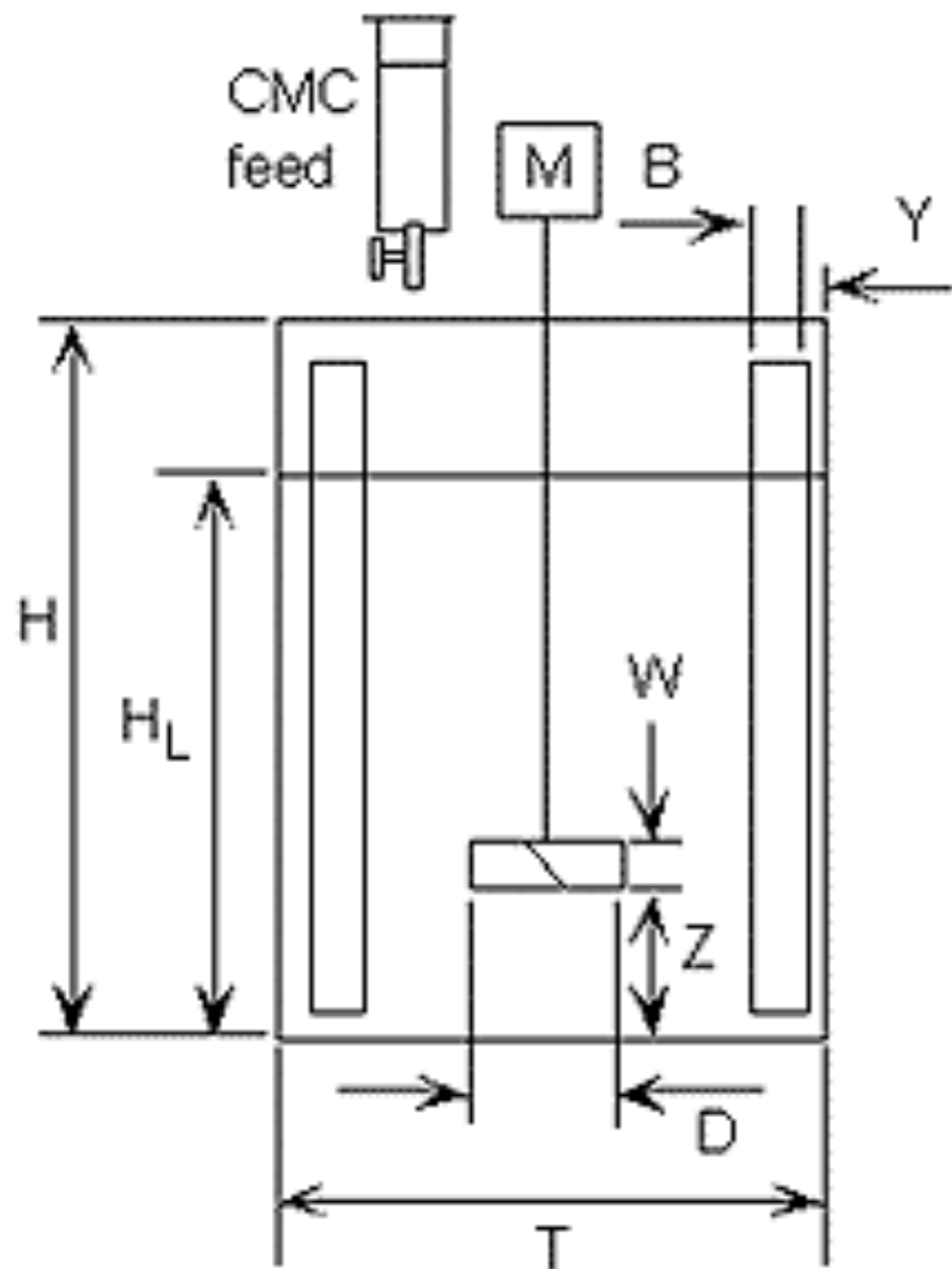


Second generation





Geometry of standard stirred tank for aerobic reactions



Volume = 800 mL

Reactor Configuration		
Tank diameter	T	105 mm
Baffles		4 number
Baffle width	B	$T/12$
Baffle spacing	Y	$T/60$
Impeller diameter	D	$T/3$
Bottom clearance	Z	$T/3$
Liquid depth	H_L	T
Number of blades	n	4
Blade width	w	$D/5$
Blade angle	α	45°



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Example design

Violacein production



My search for *J. lividum*

- „*Janthinobacterium lividum*” +
 - „growth conditions”
 - „violacein pathway”
 - „violacein genes”
 - „patent”
 - „yield”
 - „inhibition”
 - „extraction”





Violacein pricing

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[Netherlands Home](#) > [V9389 - Violacein from *Janthinobacterium lividum*](#)



V9389 SIGMA

Violacein from *Janthinobacterium lividum*

>98% (violacein (minimum 85% violacein) and deoxyviolacein, HPLC)

MSDS

SIMILAR PRODUCTS

CAS Number [548-54-9](#) | Empirical Formula (Hill Notation) $C_{20}H_{13}N_3O_3$ | Molecular Weight 343.34

POPULAR DOCUMENTS: [DATASHEET \(PDF\)](#) | [SPECIFICATION SHEET \(PDF\)](#)

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Safety & Documentation

Peer-Reviewed Papers **33**

Properties

Related Categories	Apoptosis Inducers , Apoptosis and Cell Cycle , Bioactive Small Molecule Alphabetical Index , Bioactive Small Molecules , Cell Biology , More...
assay	>98% (violacein (minimum 85% violacein) and deoxyviolacein, HPLC)
solubility	H ₂ O: insoluble
	acetone: soluble
	ethanol: soluble

Price and Availability

SKU-Pack Size	Availability	Price (EUR)	Quantity
V9389-1MG	1 left in stock. Order soon. - FROM	308.00	<input type="text" value="0"/>

[Bulk orders?](#)

[ADD TO CART](#)

Protein-Protein Interaction Webinar Series

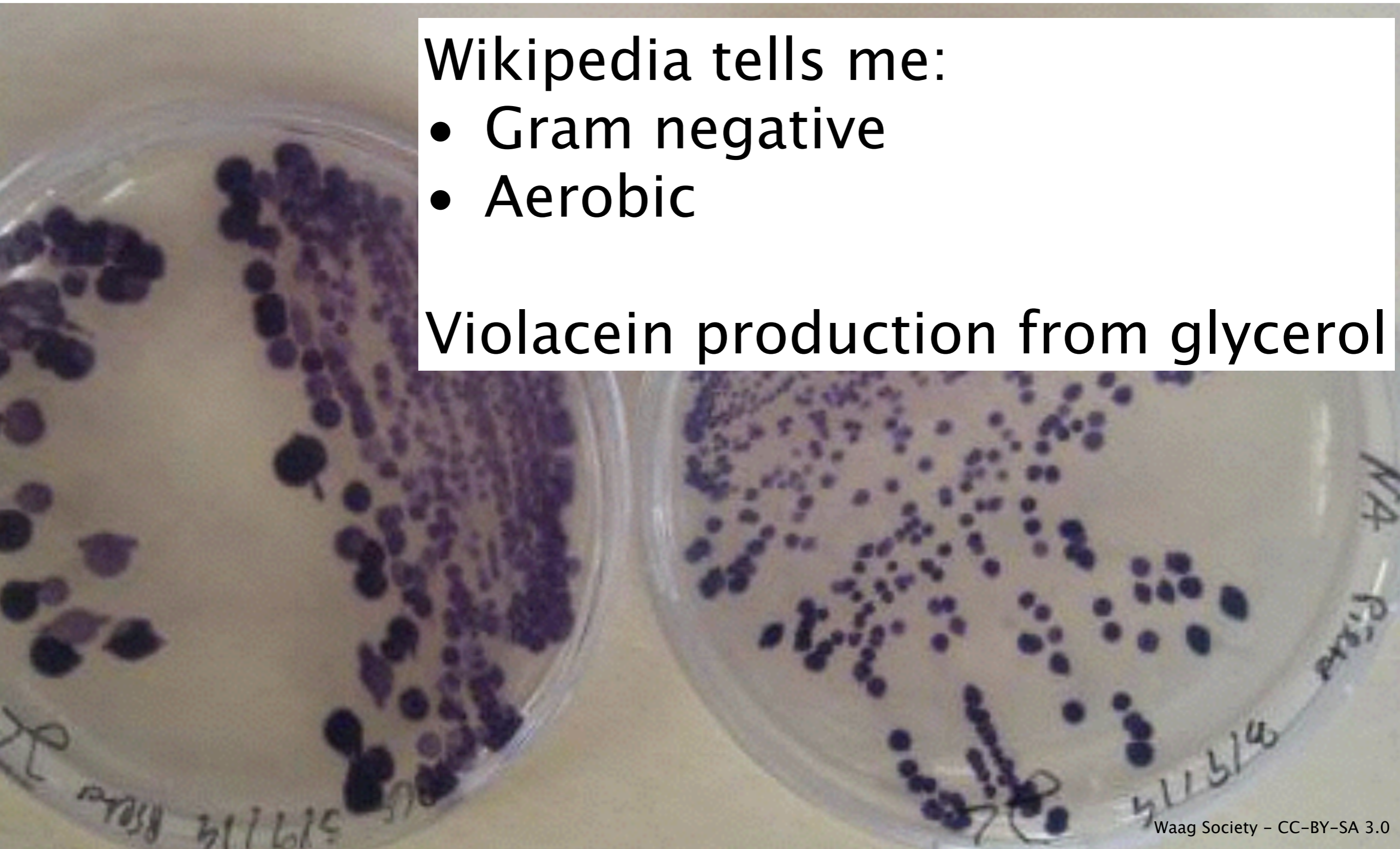


Janthinobacterium lividum

Wikipedia tells me:

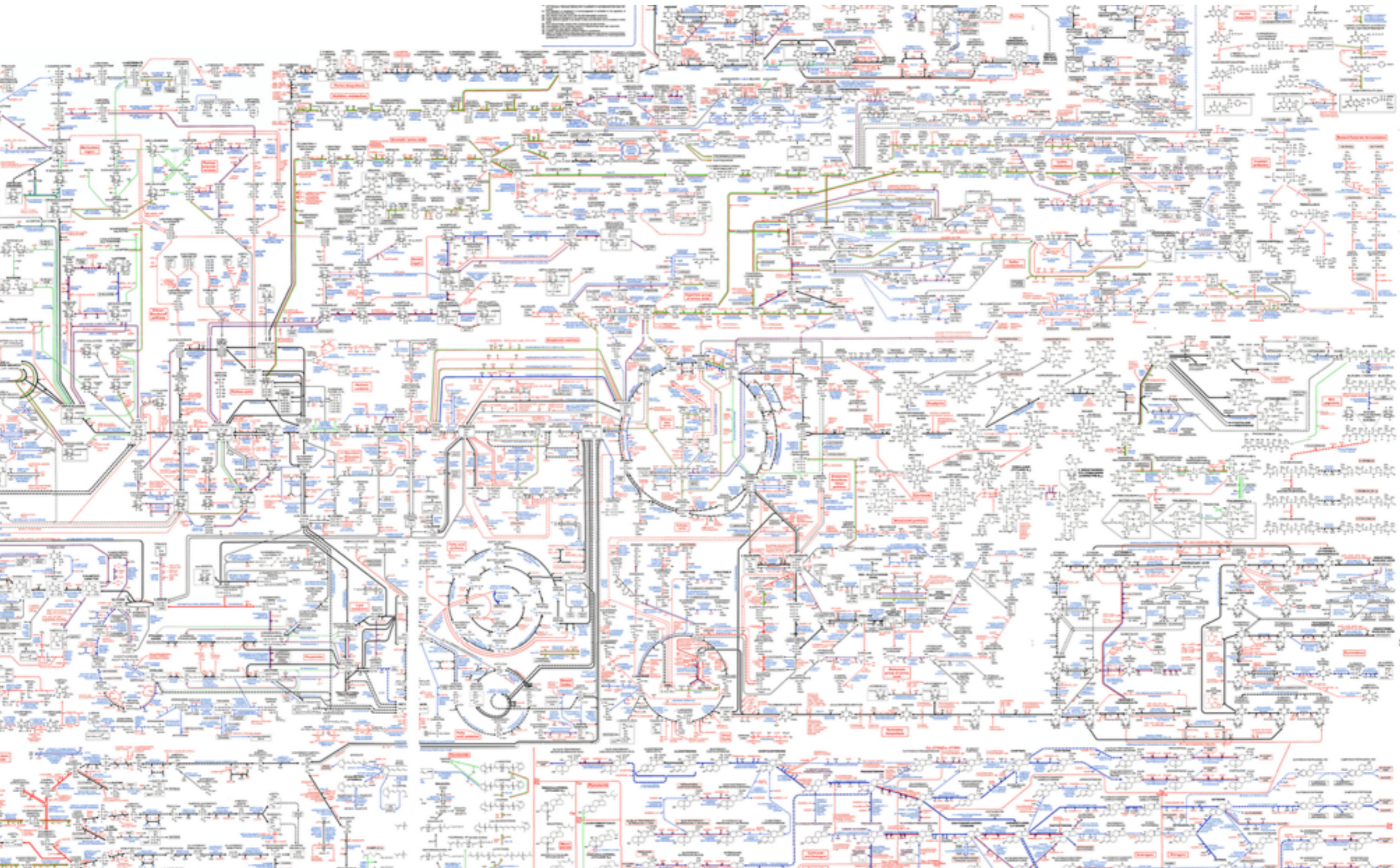
- Gram negative
- Aerobic

Violacein production from glycerol



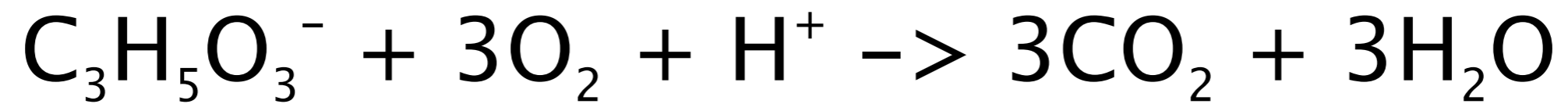


Production pathway?





P. Roqueforti eating lactate



Acid is consumed



Violacein genes

Hornung et al. – The *Janthinobacterium* sp. HH01 Genome Encodes a Homologue of the *V. cholerae* CqsA and *L. pneumophila* LqsA Autoinducer Synthases (2013)

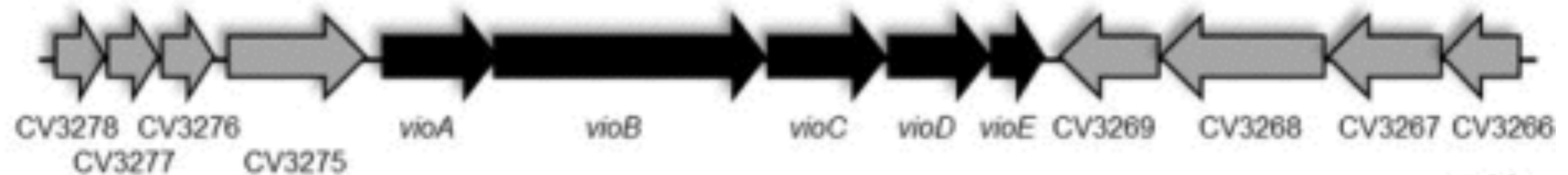
Janthinobacterium sp. HH01



Pseudoalteromonas tunicata D2



Chromobacterium violaceum ATCC 12472

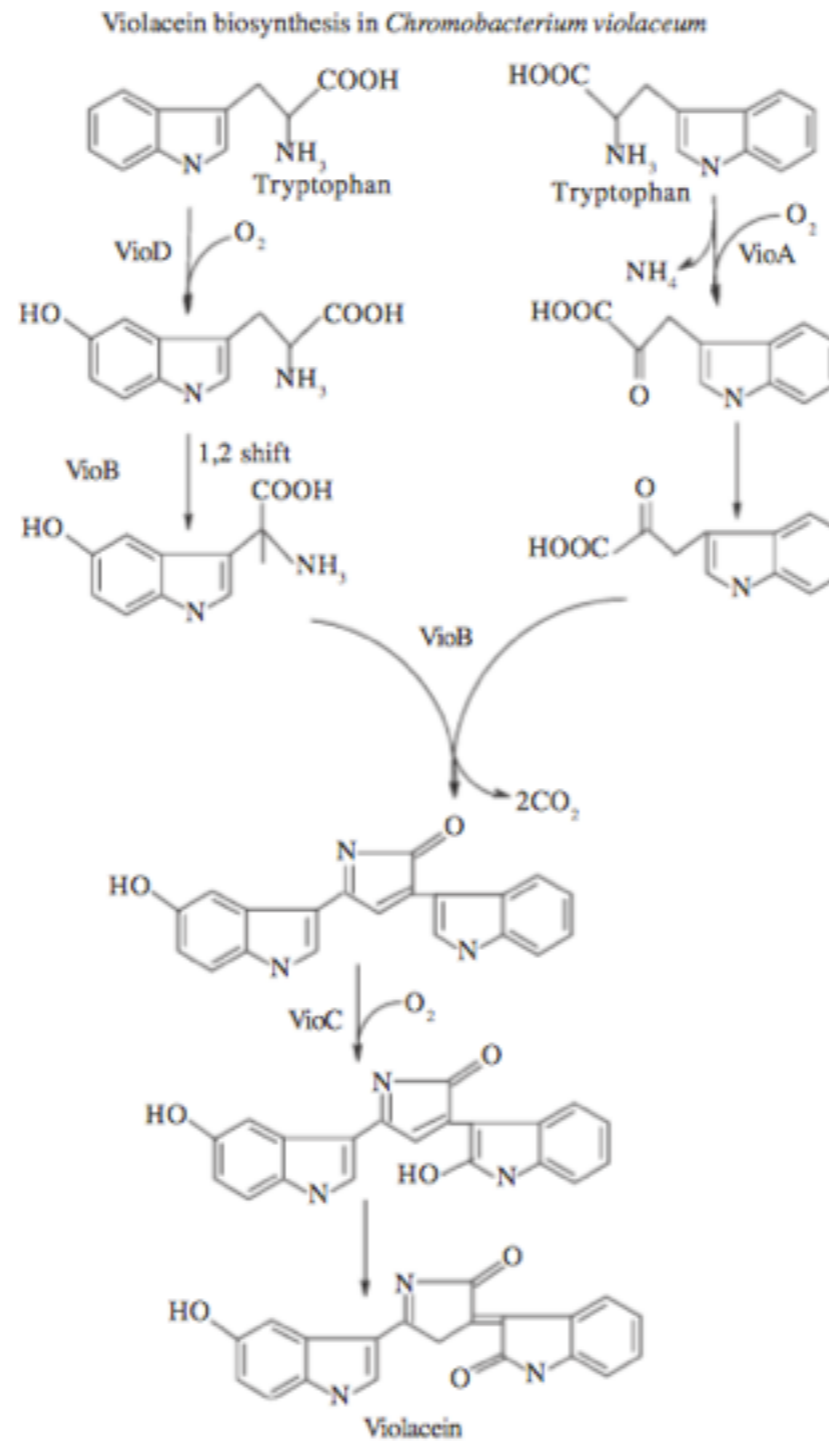


1 kb



Production pathway?

Tryptophan



89

Figure 2. Violacein biosynthesis, as proposed by August et al., 2000. VioA, VioB, VioC, and VioD are the gene products of the biosynthesis operon, encoding nucleotide-dependent monooxygenases and a protein similar to a polyketide synthase (VioB).



Other interesting things:

- *J. lividum* produces a metallo- β -lactamase conferring resistance to several β -lactam antibiotics

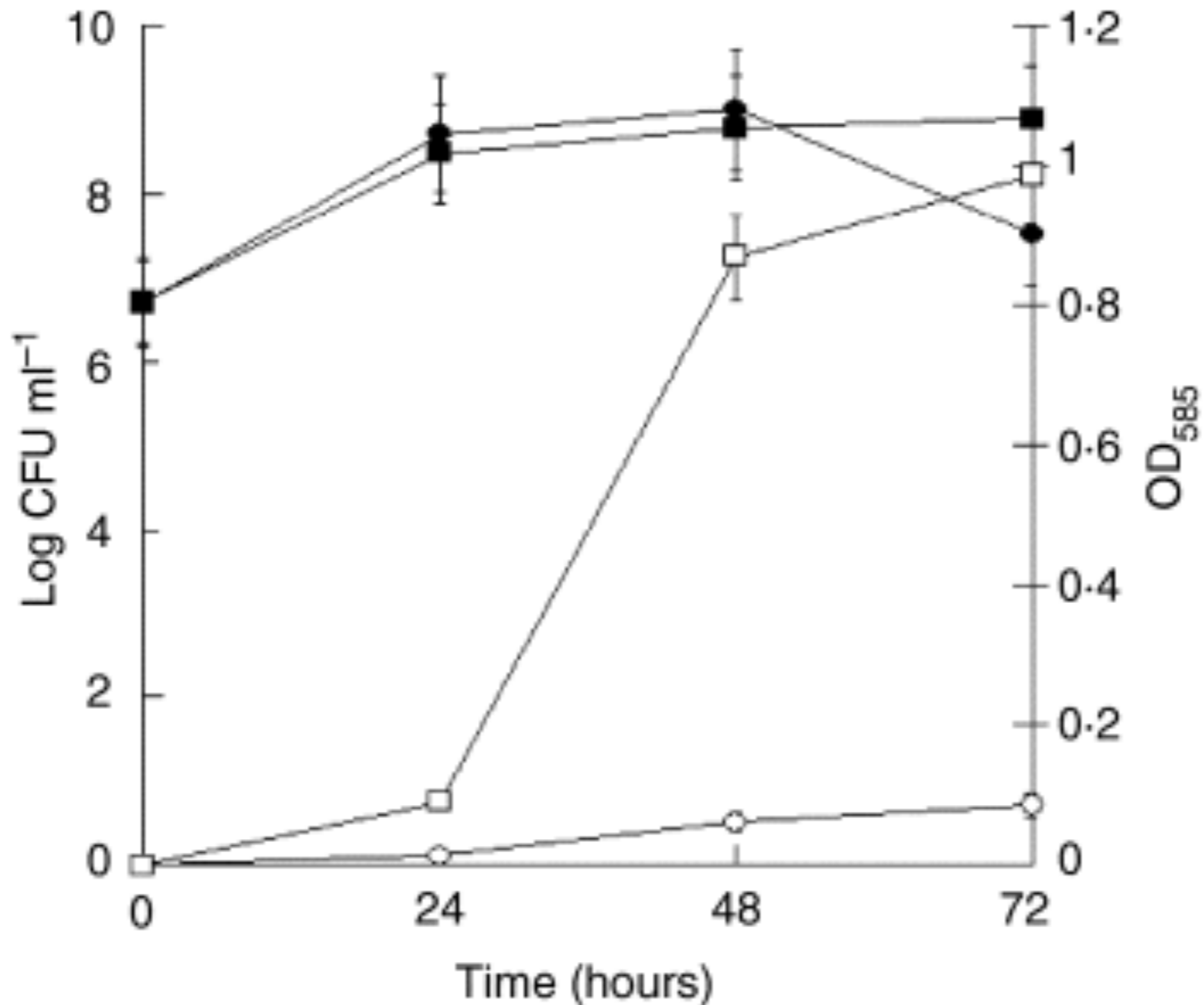
Rossolini, G.M., Condemi, M.A., Pantanella, F., Docquier, J.D., Amicosante, G. and Thaller, M.C. (2001) Metallo- β -lactamase producers in environmental microbiota: new molecular class B enzyme in *Janthinobacterium lividum*. *Antimicrob Agents Chemother* 45, 837-844.

- Violacein:
 - $C_{20}-H_{13}-N_3-O_3$
 - molecular weight of 343.33
 - insoluble in water
 - soluble in alcohols as methanol, ethanol and acetone
 - maximal absorption in a solution of methanol is at 585 nm

Blosser, R.S. and Gray, K.M. (2000) Extraction of violacein from *Chromobacterium violaceum* provides a new quantitative bioassay for N-acyl homoserine lactone autoinducers. *J Microbiol Methods* 40, 47-55.



Production inhibition





Production conditions

Growing the bacteria in culture took 5 days before the culture would turn purple due to *J. lividum* forming a biofilm in the media. Large culture growth by embedding sterile cotton mats in sterile 2L bottles with nutrient media with the added glycerol and L-tryptophan (**fig. 2**) that showed purple coloring after 48 hour incubation [9]. The mats were extracted after 5 days to harvest the violacein. Yield of violacein from after crude methanol extraction and low was about 10mg.



Figure 2: Violacein optimization. 1% Glycerol and 250 μ M L-tryptophan were added to the nutrient broth media to enhance pigment development. Cotton mats were used to allow bacteria to become sessile and produce violacein faster than with liquid cultures.



Patent – USPTO

Process for the production of violacein and its derivative deoxyviolacein containing bioactive pigment from *Chromobacterium* sp. (MTCC5522)

EXAMPLE 1

PRODUCTION AND EXTRACTION OF THE BIOACTIVE PIGMENT FROM THE CULTURE OF CHROMOBACTERIUM SP. NIIST-CKK-01

A loopful of 24 hrs old pure culture *Chromobacterium* sp. NIIST-CKK-01 from solid agar medium (LB agar or Nutrient agar) was inoculated with 50 ml of the growth medium (0.5% Yeast extract and 1.5% Peptone) taken in a 250 ml Erlenmeyer flask. Alternatively, 10% (v/v) of 24 hour old pure culture of *Chromobacterium* sp. NIIST-CKK-01 in LB broth was also used as inoculum. The pH of the medium was 7. The flasks inoculated with *Chromobacterium* sp. NIIST-CKK-01 were subsequently incubated in a rotary shaker at ambient temperature (30 °C) and 200 rpm for 24 hours. The deep purple purple-blue pigment starts appearing in the medium by about 6 hours of incubation and continued beyond biomass increase (Fig 1).

After 24 hrs of incubation, the bacterial biomass with pigment was centrifuged at 9676.8 x g and 4 °C for 10 minutes. After centrifugation, the clear supernatant was removed. The pellet containing biomass and pigment was mixed thoroughly with 5 ml of extra pure methanol. The mixture was centrifuged again at 9676.8 x g and 4 °C for 10 minutes to separate the cell pellet from the solvent-pigment mixture. The pigment extraction was repeated twice using fresh solvent as described. All the pigment extracted solvent pooled together and the pigment was concentrated by normal vacuum drying in a desiccator. The quantity of biomass and pigment produced could be accounted by measuring optical density at 600 nm and 575 nm respectively. The yield of pigment by this method was about 1.0 g pigment/g of dry biomass in 24 hrs.

HPLC analysis is carried out for checking the purity of the pigment produced using an ODS column (Lichrospher-100; Merck) with acetonitrile (40%) at 1ml/min as mobile phase and using UV-VIS detector at 575 nm (Figure 2). UV-VIS absorption spectra indicated maximum absorption at 575 nm, typical of violacein and its derivatives (Figure 3).

EXAMPLE 2



J. Lividum canvas

BioFactory
canvas



input

330 ml

volume



species



time



48h

stirrer



No

Yes

oxygen

O₂

light



No

temp.



15

absorbance



585

pH



?

mass



1 mL?



observations

day #

day #

day #

day #

day #



material

Nutrient

Broth

Glycerol

tryptoph.

C

N

P

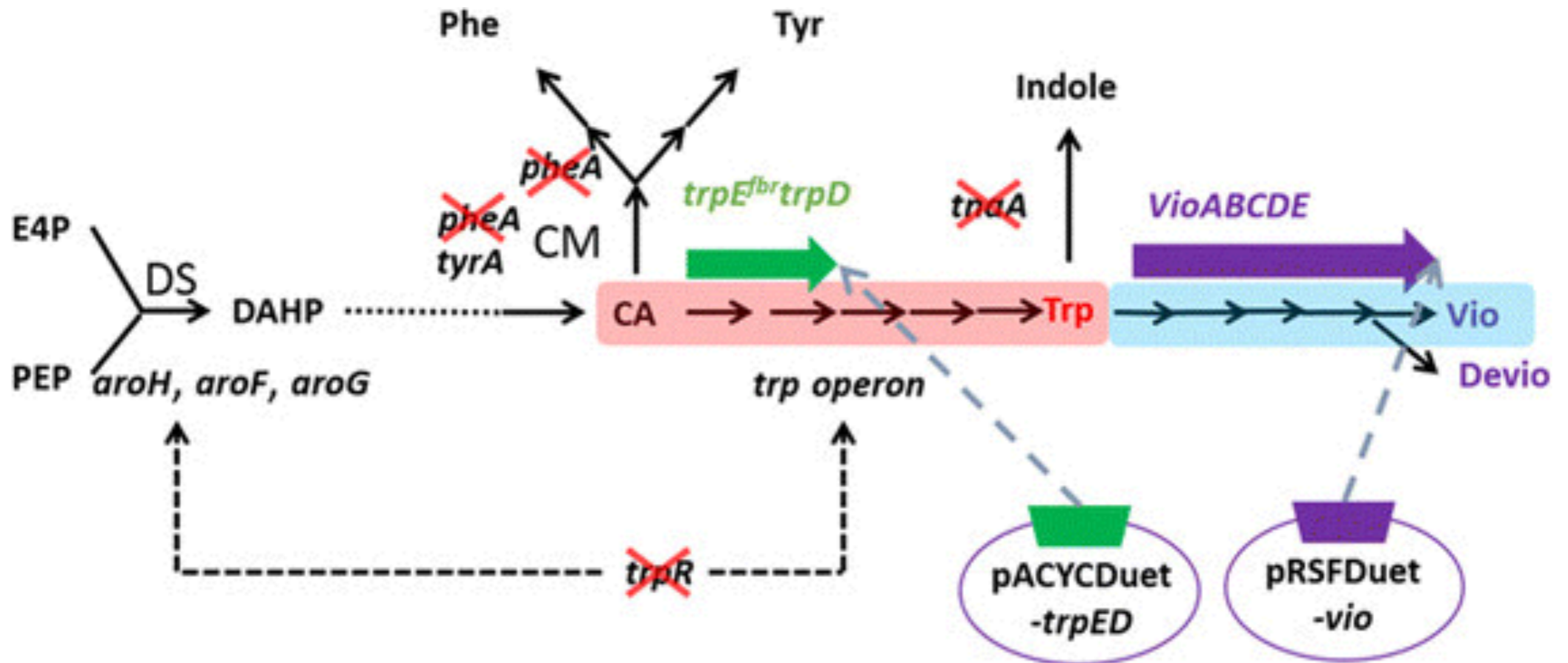
O₂

S





Genetic construct for E. coli





Synbiota – ScienceHack



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Transport Phenomena



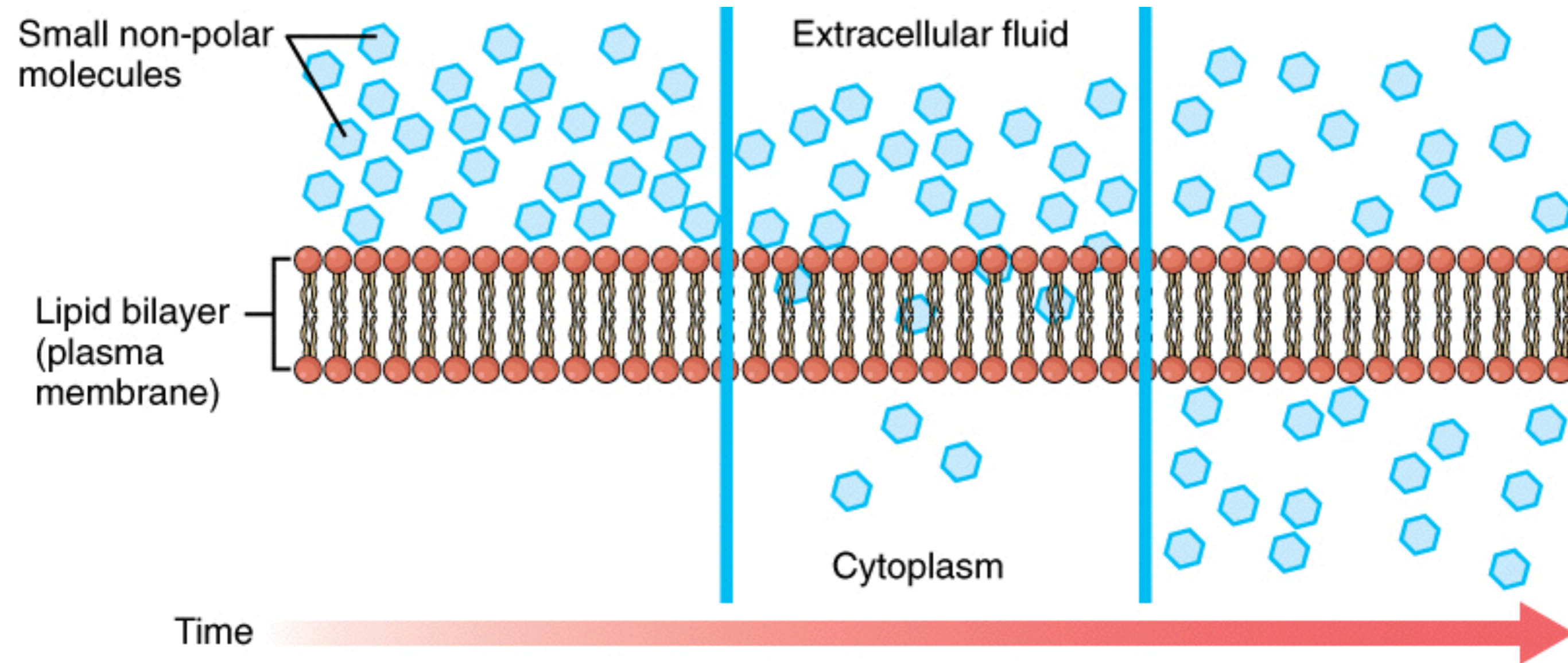
Transport Phenomena

- Mass transfer
 - Nutrients
 - Oxygen
- Energy transfer
 - Heat
 - (electrons)

$\varphi \leftrightarrow \theta_l$			
Type 1 MSC		$\theta_l = g_\varphi(\varphi)$	$\theta_l =$
Type 3 MSC		$\theta_l = g_{\varphi,T}(\varphi, T)$	$\theta_l =$
Partial derivatives			
	$\left. \frac{\partial \varphi}{\partial \theta} \right _T$	$\left. \frac{\partial \varphi}{\partial T} \right _\theta$	$\left. \frac{\partial p_c}{\partial \theta} \right _T$
Equivalency	$\left(\left. \frac{\partial \theta}{\partial \varphi} \right _T \right)^{-1} = \frac{1}{\xi_{\varphi\varphi}}$	$-\left. \frac{\partial \varphi}{\partial \theta} \right _T \cdot \left. \frac{\partial \theta}{\partial T} \right _\varphi = -\frac{\xi_{\varphi T}}{\xi_{\varphi\varphi}}$	$\left(\left. \frac{\partial p_c}{\partial \varphi} \frac{\partial \varphi}{\partial \theta} \right) \right _T = \frac{\partial f(\varphi, T)}{\partial \varphi} \bigg _T \frac{1}{\xi_{\varphi\varphi}}$
Type 1 MSC	$\left(\frac{dg_\varphi}{d\varphi} \right)^{-1}$	0	
Type 3 MSC	$\left(\left. \frac{\partial g_{\varphi,T}}{\partial \varphi} \right _T \right)^{-1}$	$-\left. \frac{\partial g_{\varphi,T}}{\partial T} \right _\varphi \cdot \left(\left. \frac{\partial g_{\varphi,T}}{\partial \varphi} \right _T \right)^{-1}$	$-\frac{R_v \rho_l T}{\varphi \xi_{\varphi\varphi}}$
Balance equation			
$\frac{\partial \theta}{\partial t} = \nabla \cdot [(D_{\theta T}^l + D_{\theta T}^v) \nabla T + (D_{\theta\theta}^l + D_{\theta\theta}^v) \nabla \theta]$			
Secondary moisture transport functions			
	Vapor transport		
	Isothermal	Non-isothermal	Liqu Isothermal
	$D_{\theta\theta}^v = \frac{\delta_v p_{sat}}{\xi_{\varphi\varphi} \rho_l}$	$D_{\theta\theta}^v = \frac{\delta_v}{\rho_l} \left(\varphi \frac{\partial p_{sat}}{\partial T} - \frac{\xi_{\varphi T} p_{sat}}{\xi_{\varphi\varphi}} \right)$	$D_{\theta\theta}^l = \frac{K_l R_v T}{\varphi \xi_{\varphi\varphi}}$



Diffusion





Transport Phenomena

- Oxygen Uptake Rate (OUR)
- Oxygen Transfer Rate (OTR)
 - 100 mmol/L-h: low
 - 150 mmol/L-h: medium
 - 300 mmol/L-h: high



Shaken or stirred?

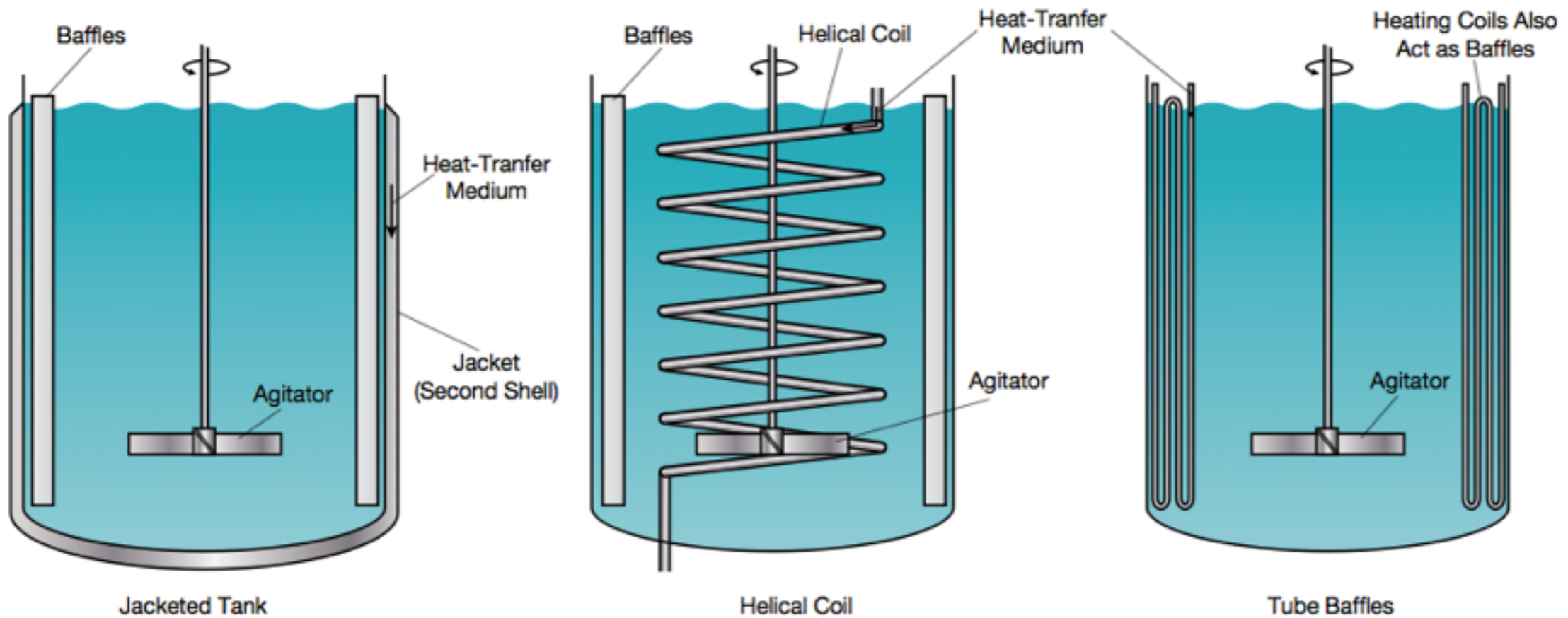
- Agitation by stirring
- Mixing by air bubbles

- Shear force
 - Fine for some bacteria and yeast
 - More delicate: animal, fish, insect and plant cells



Heat transfer

Back to Basics



▲ **Figure 5.** Many fermenters remove heat using tank jackets.

▲ **Figure 6.** Helical coils are often installed in fermenters to remove heat.

▲ **Figure 7.** Vertical tube bundles, which double as tank baffles, are another type of internal structure that can remove heat from fermenters.



Reactor dimensions

Height / Diameter

Tall thin vessels for aerobic reactions:

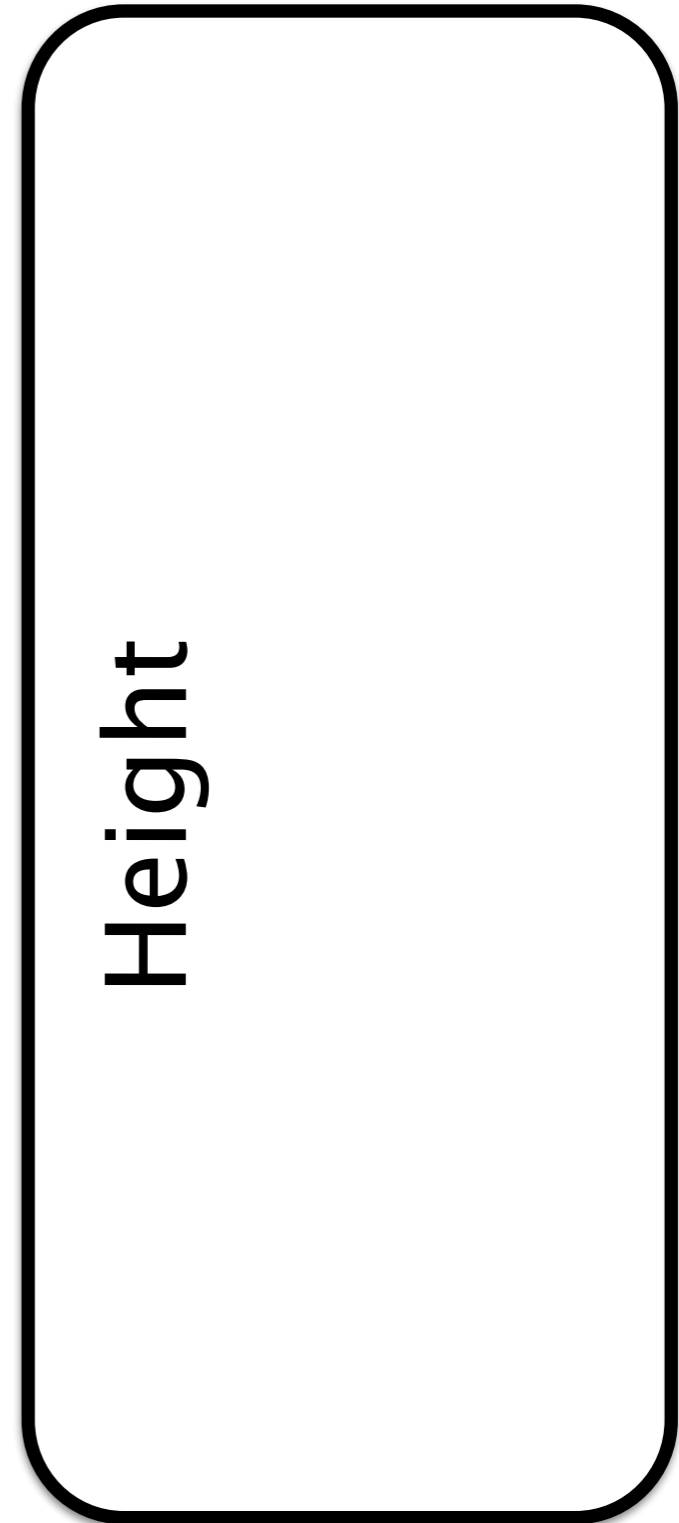
- higher pressure
- better mass-transfer
- better surface/volume ratio

Anaerobic vessels:

1 / 1

Diameter

Height





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