



# waag society

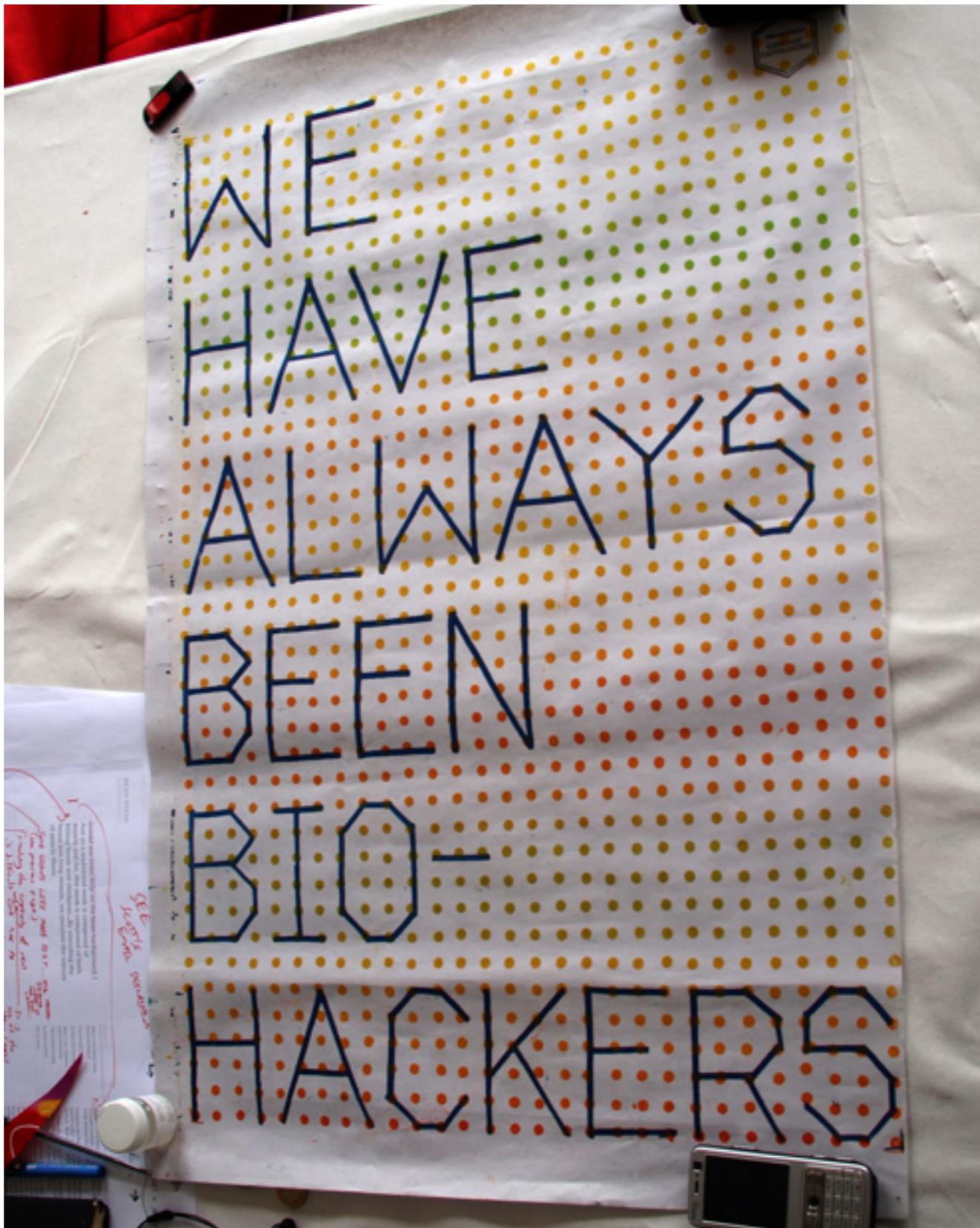
institute for art, science and technology



BioHack Academy  
Bioreactor Design



# We have always been biohackers





We have always been biohackers

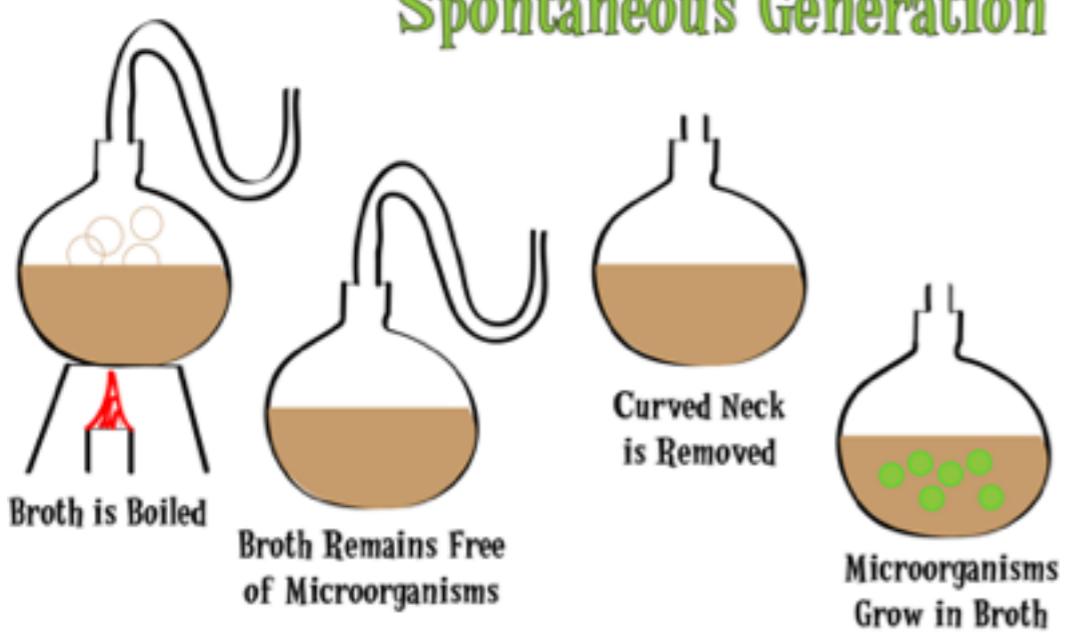




# Louis Pasteur



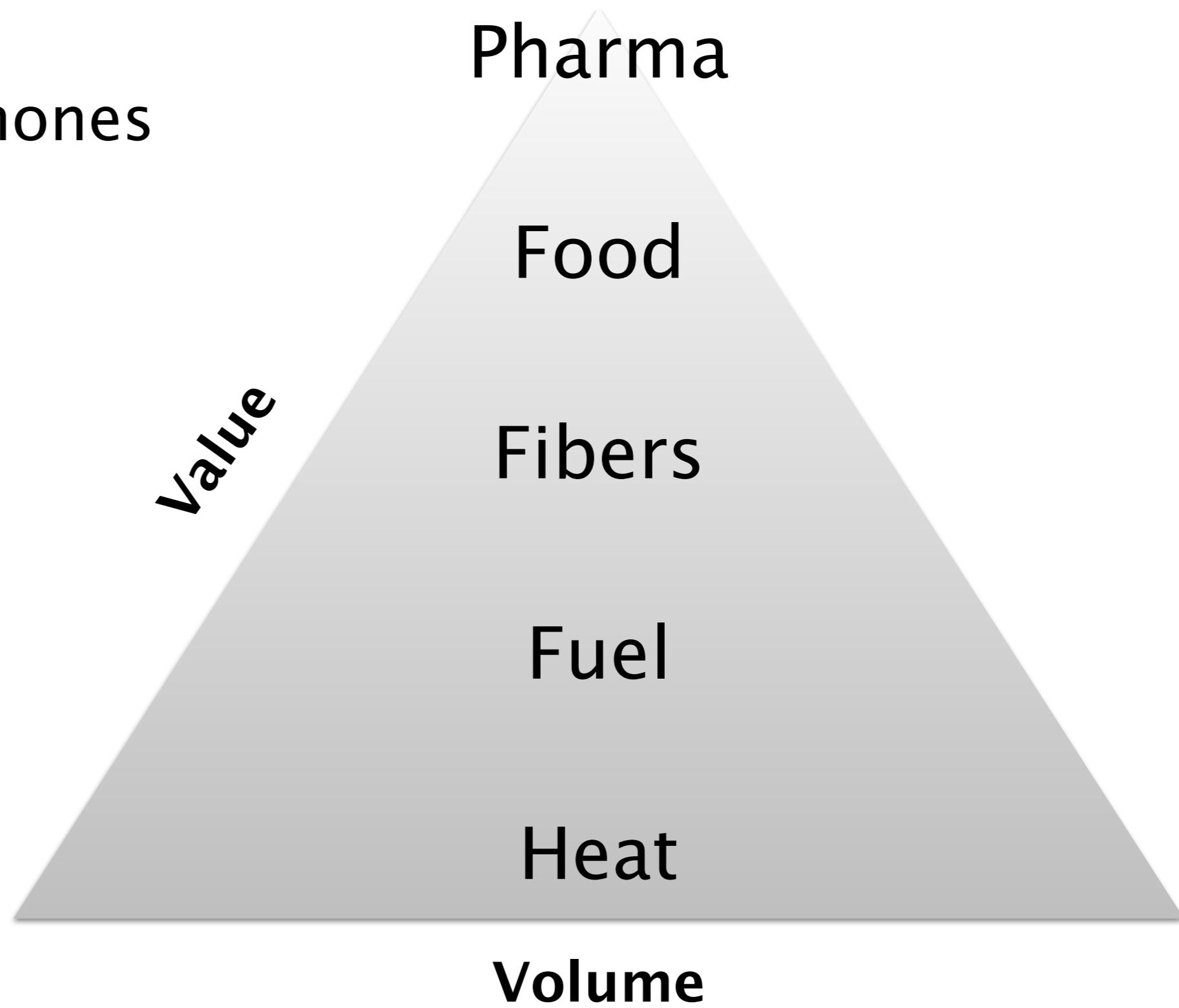
## Pasteur's Test of Spontaneous Generation





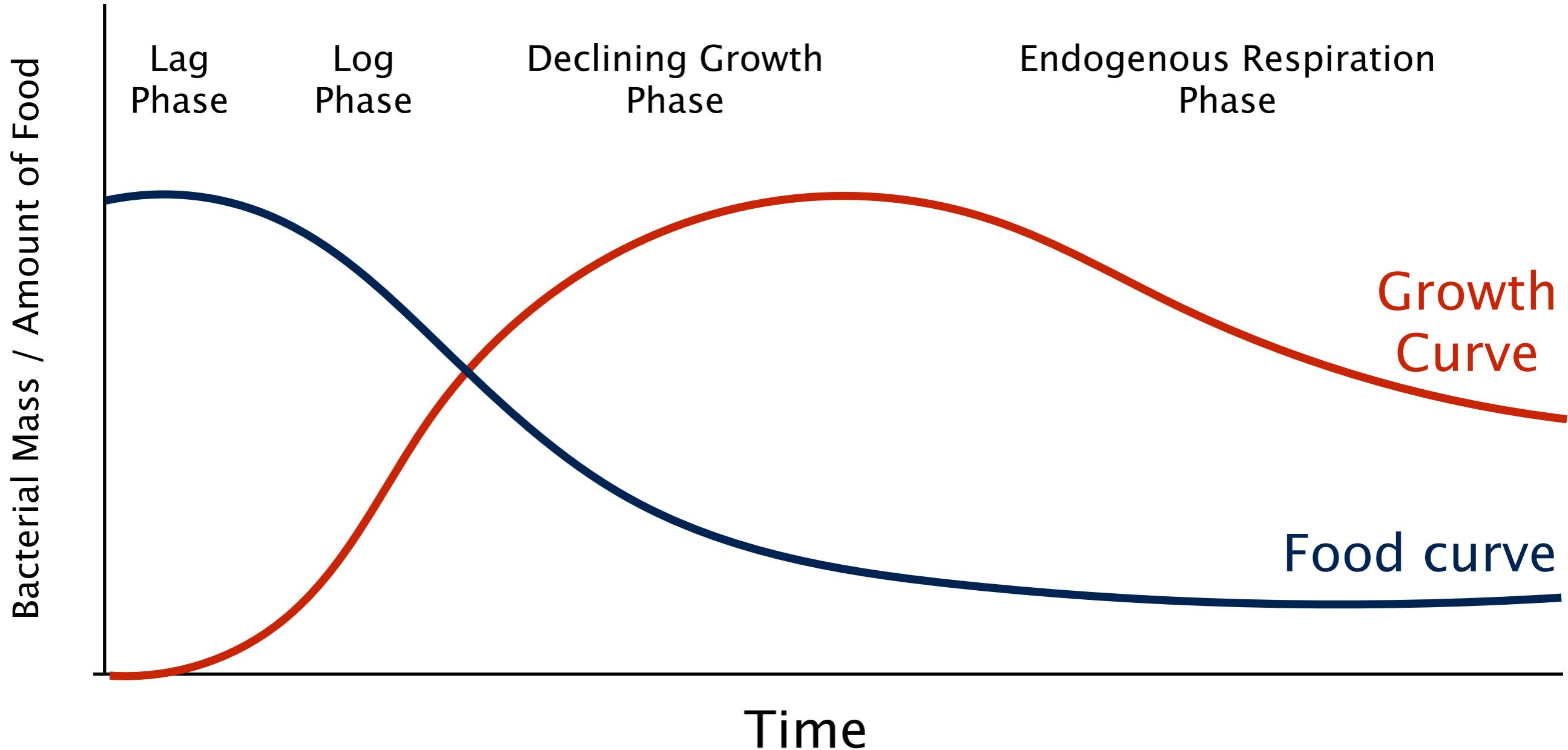
# Bioreactor value pyramid

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



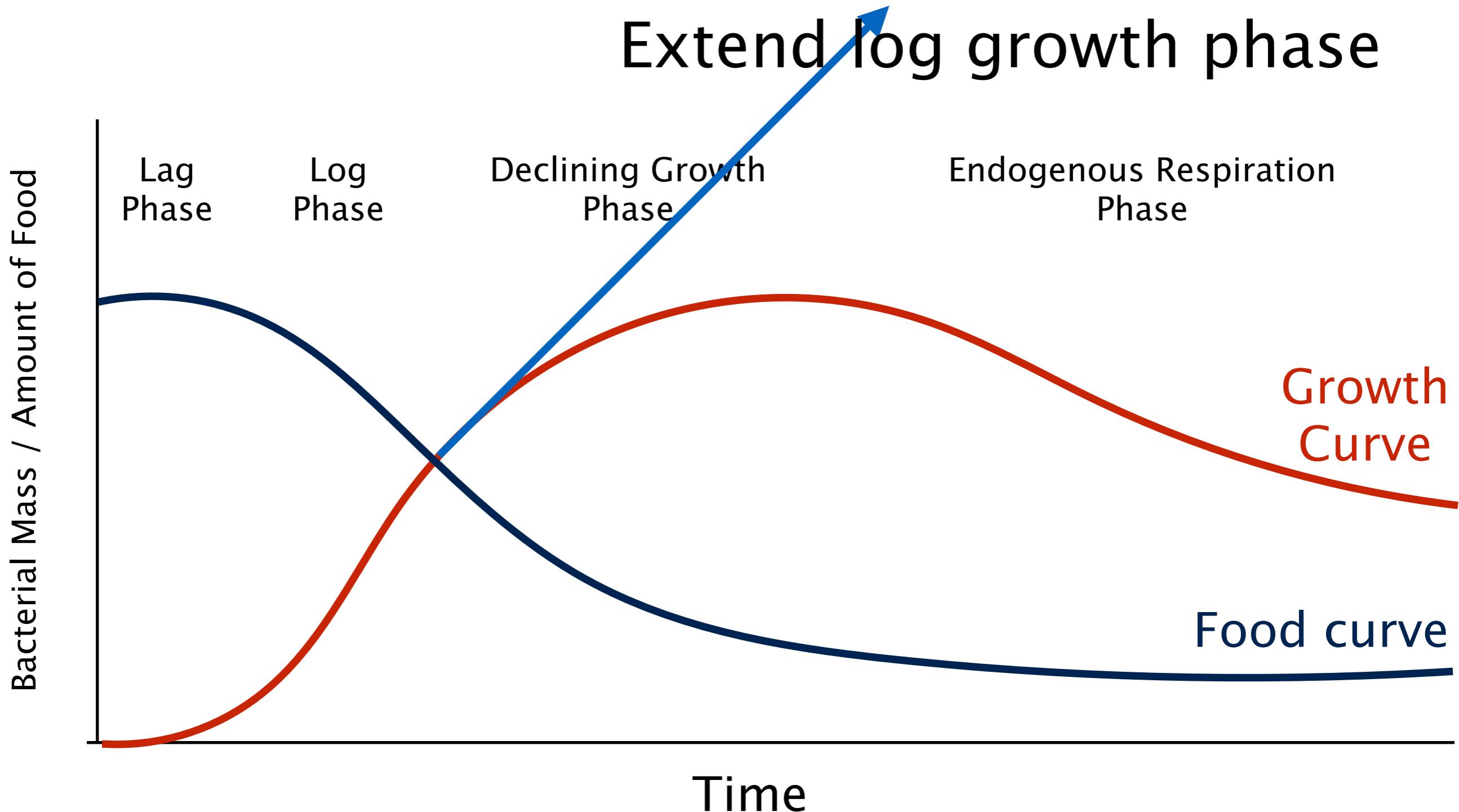


# Bacterial growth curve – Batch



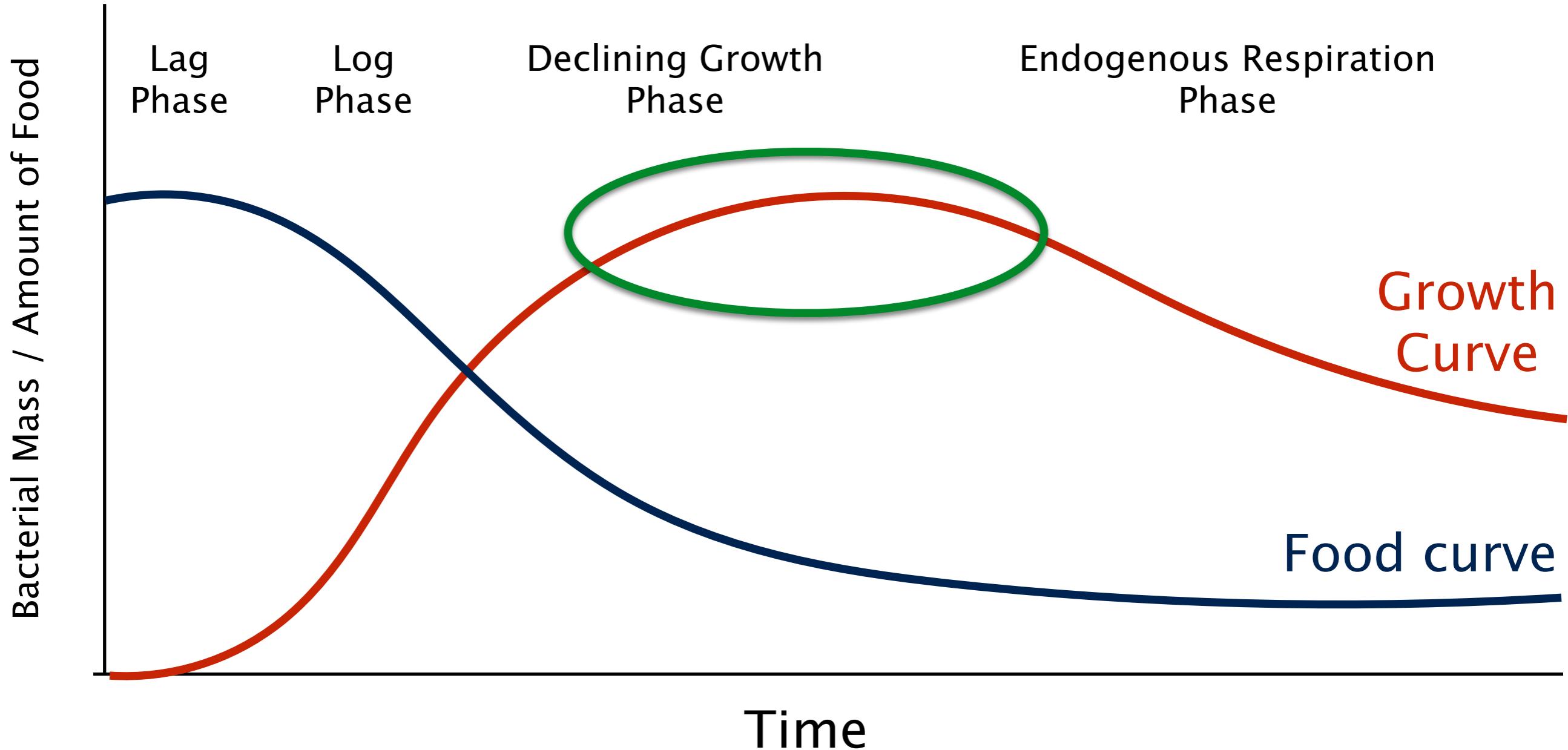


# Primary products



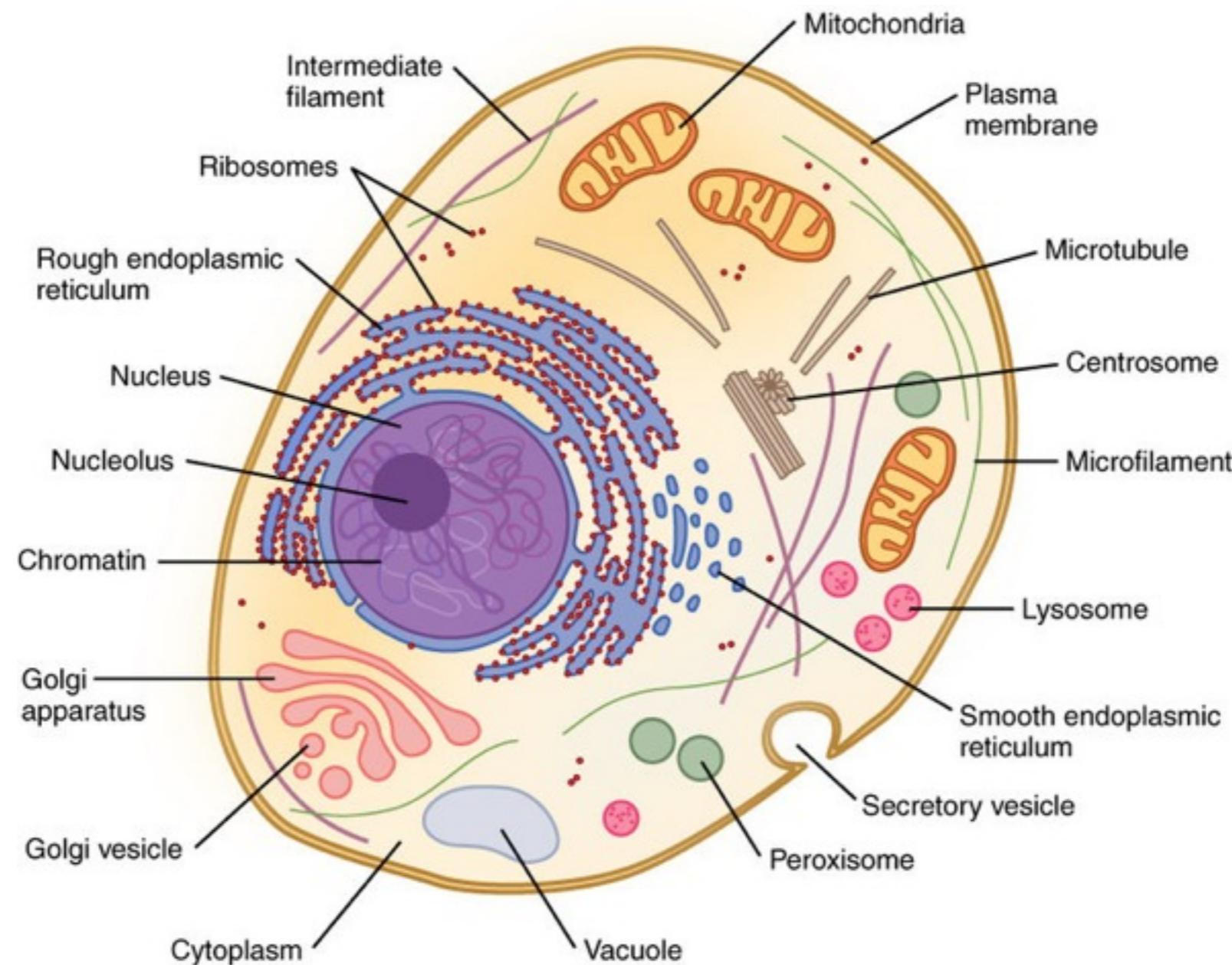


# Secondary products



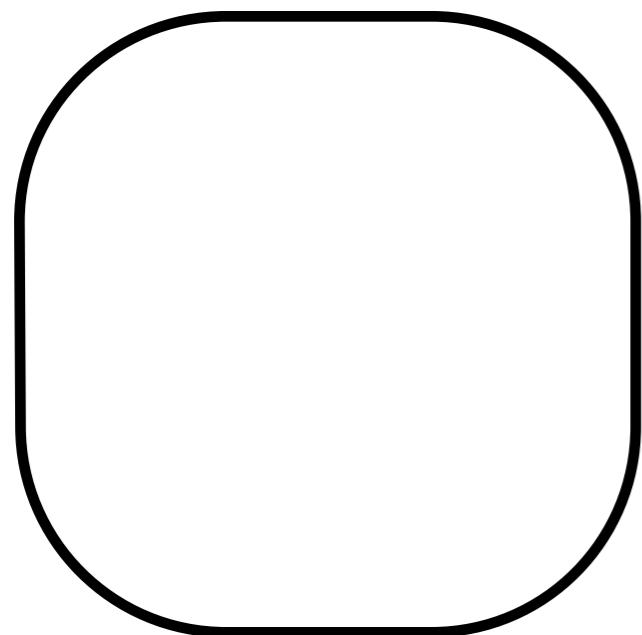


# Intra vs extra cellular

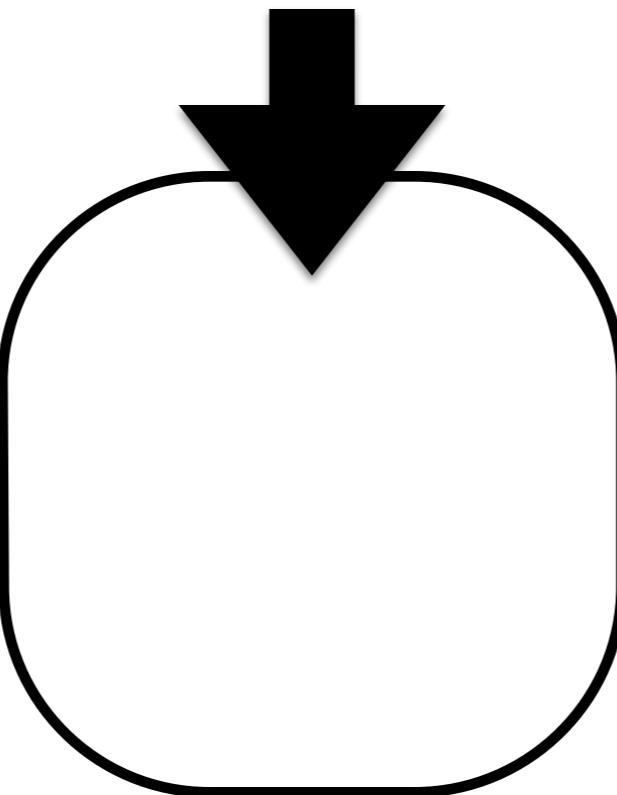




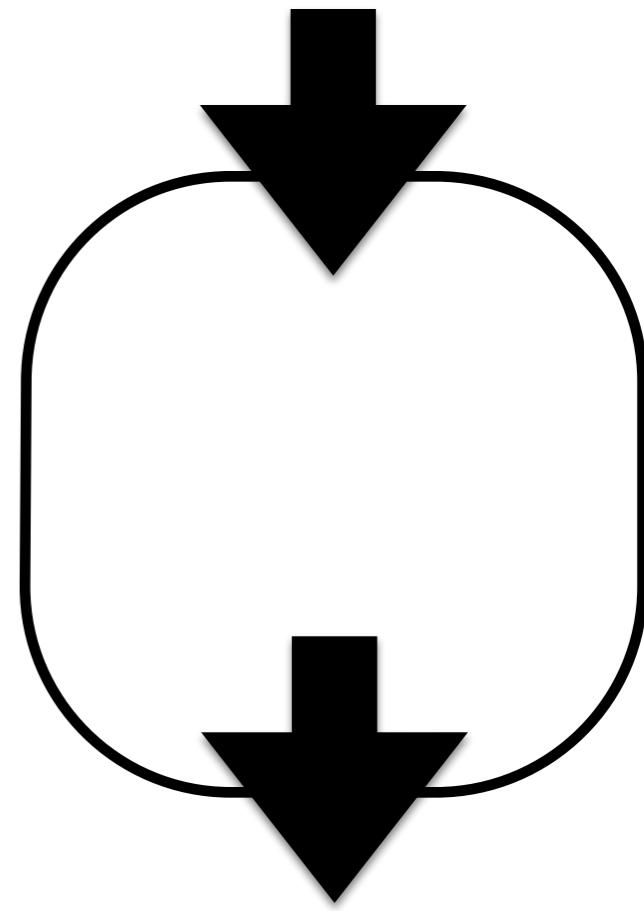
# Growth strategies



Batch



Fed Batch



Chemosstat



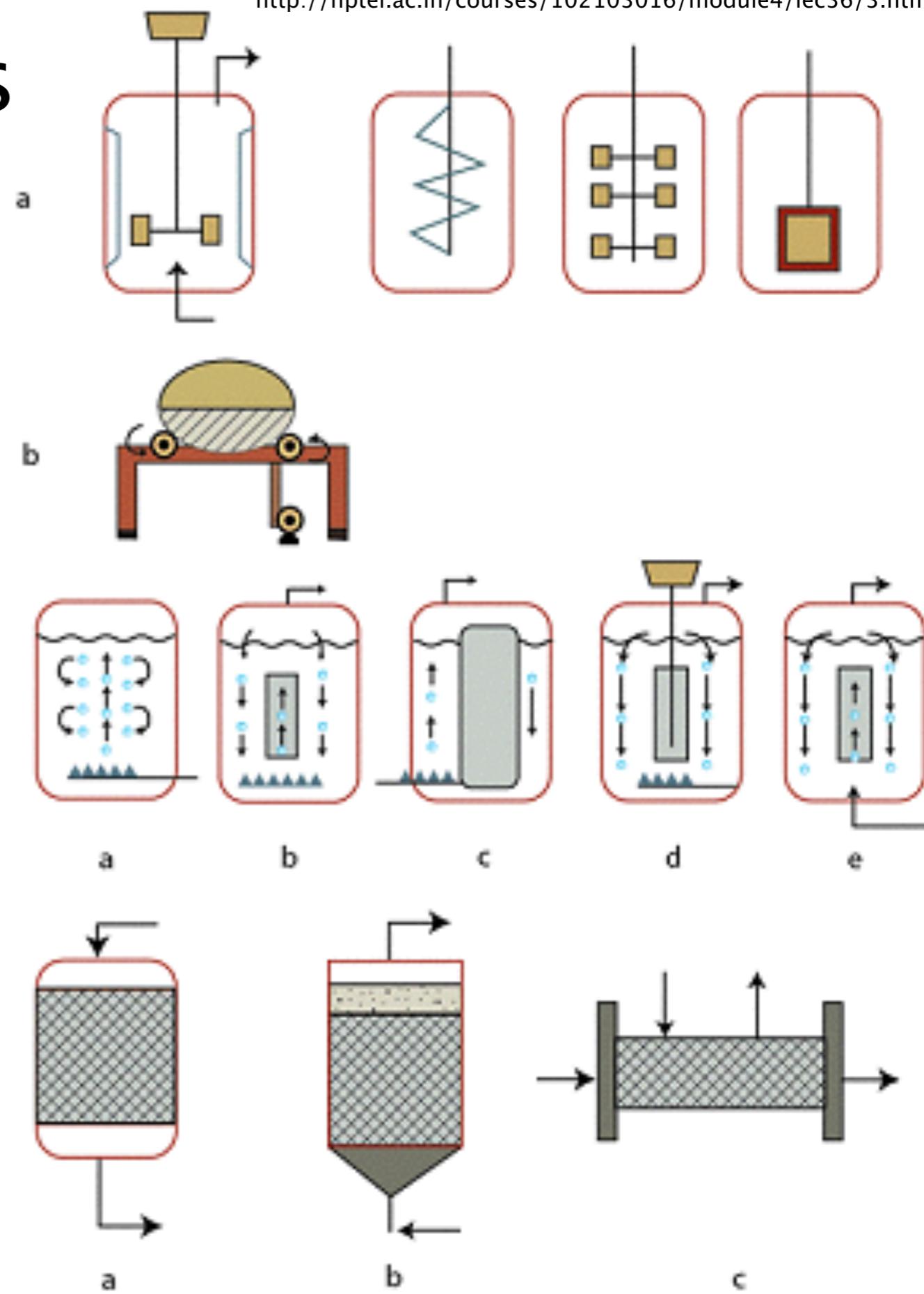
# Bioreactor Wishlist

- 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/>

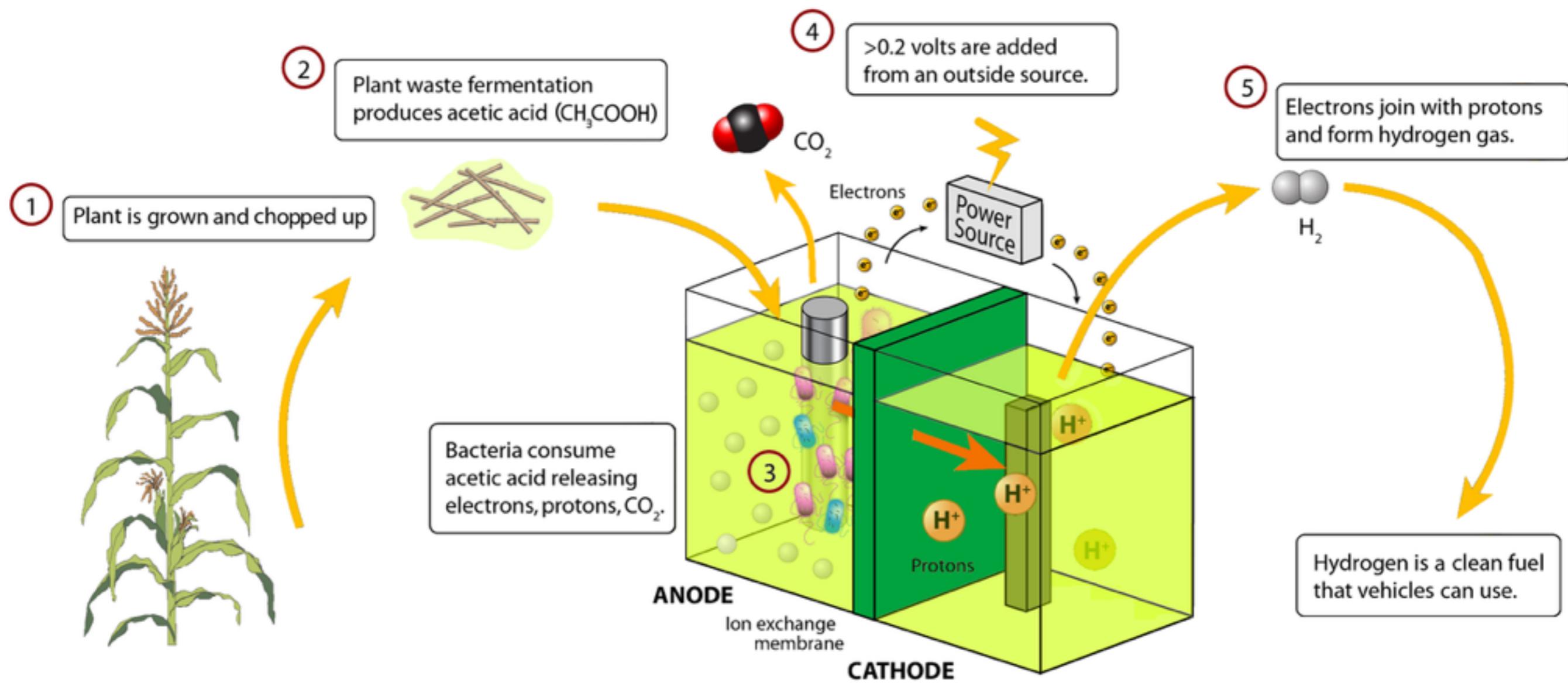


<http://www.instructables.com/id/Biogas-at-home-Cheap-and-Easy/>



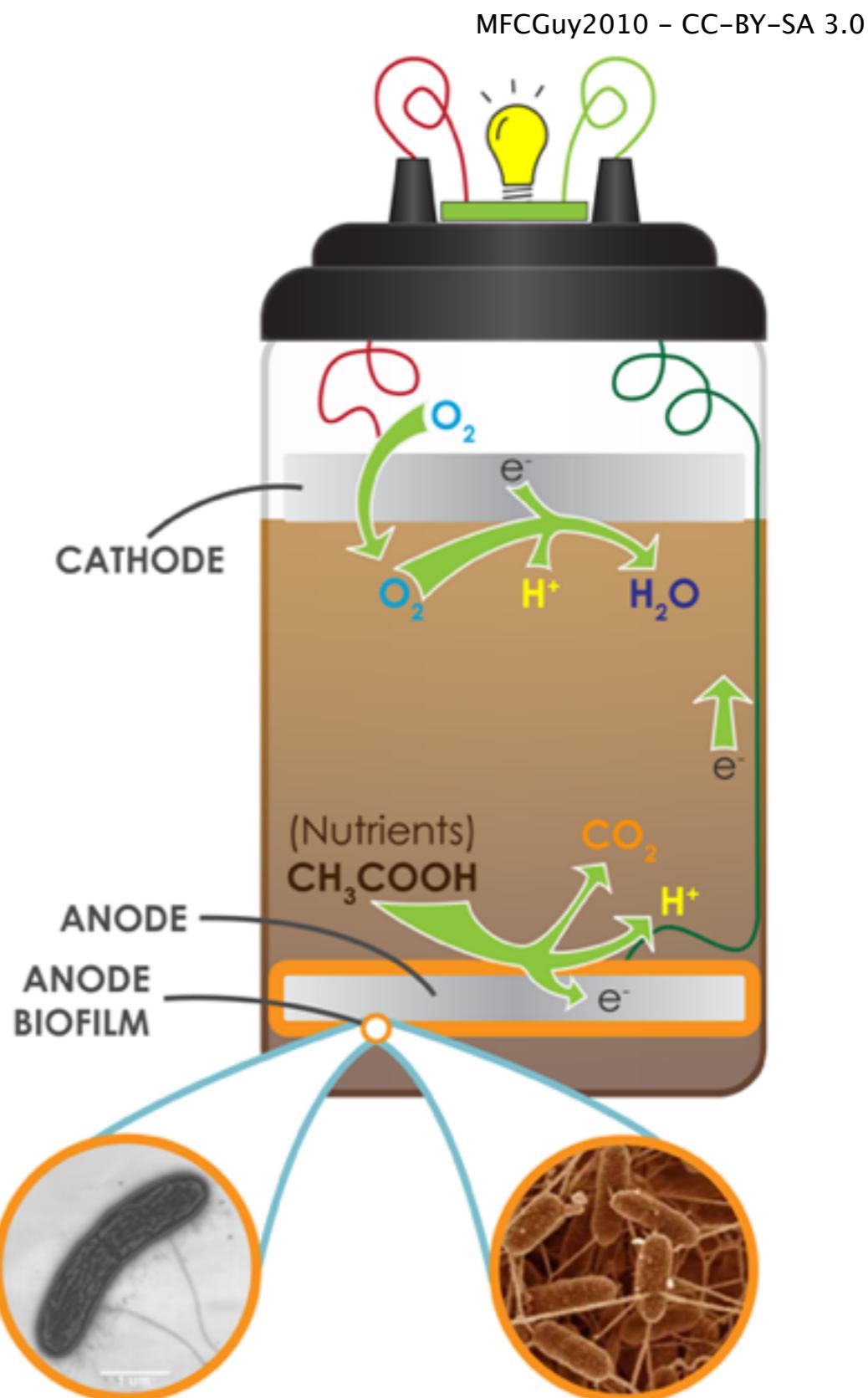
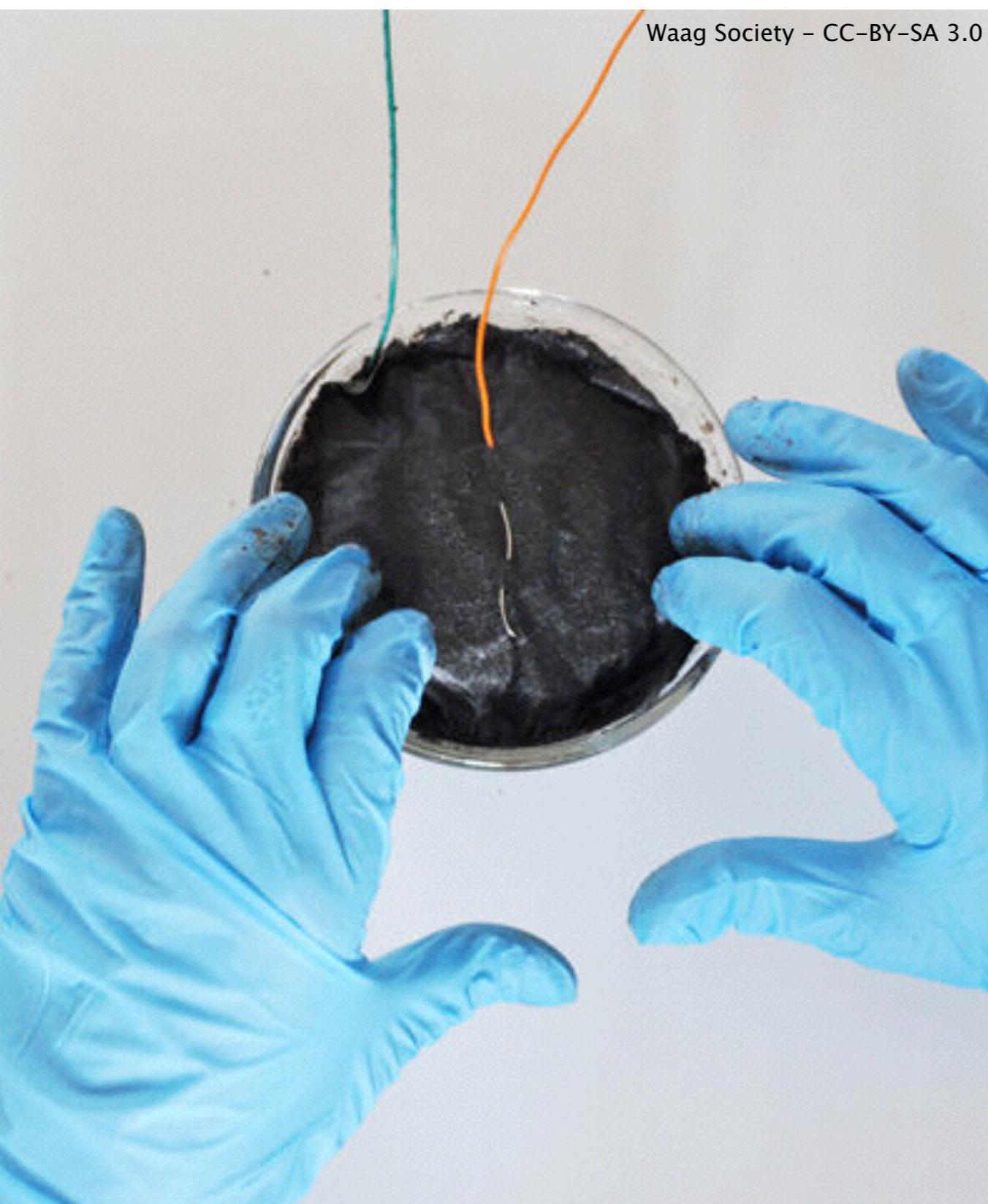


# Membrane reactor: Fuel Cells



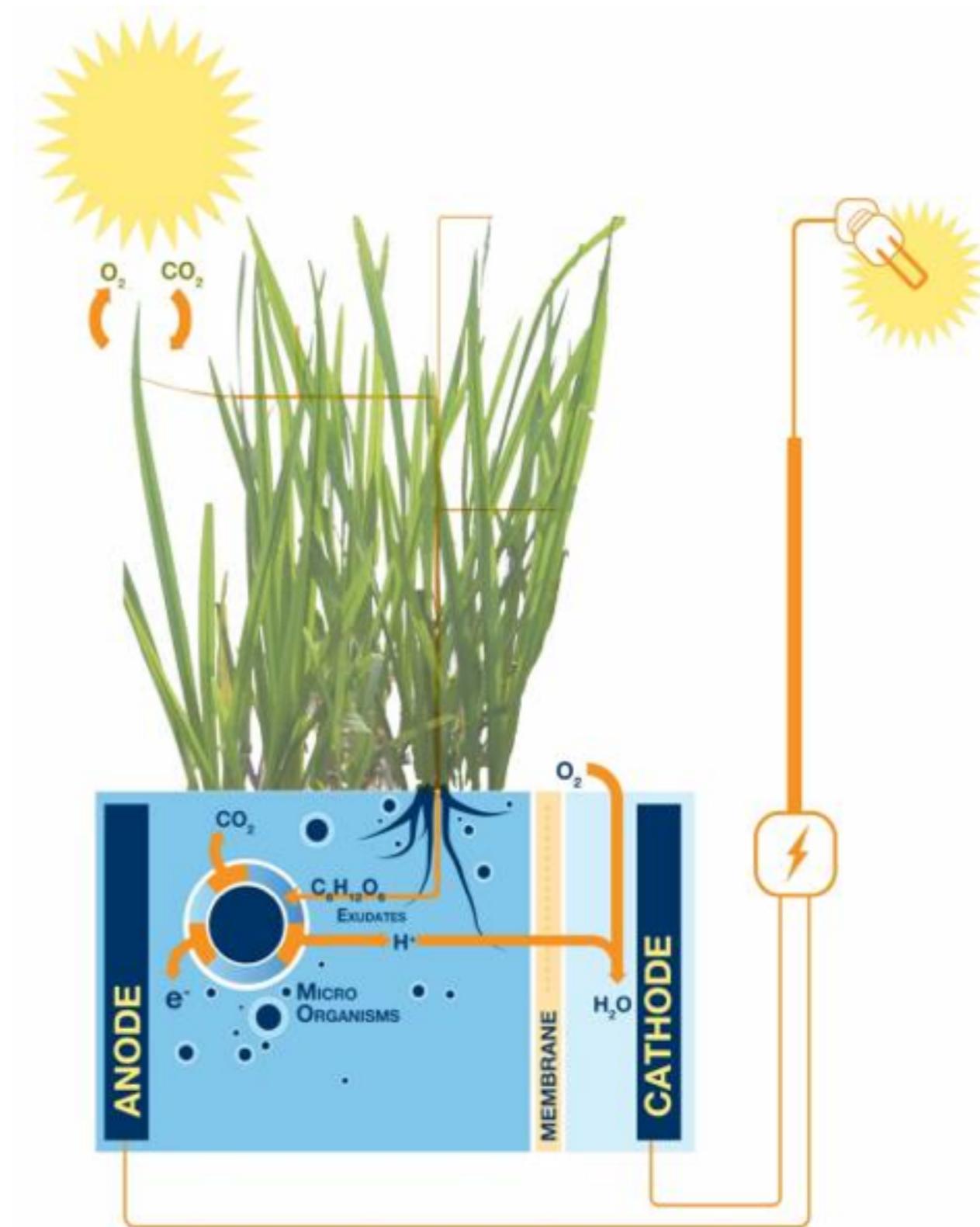


# Soil based fuel cells





# Plant-e





# Bio mud battery

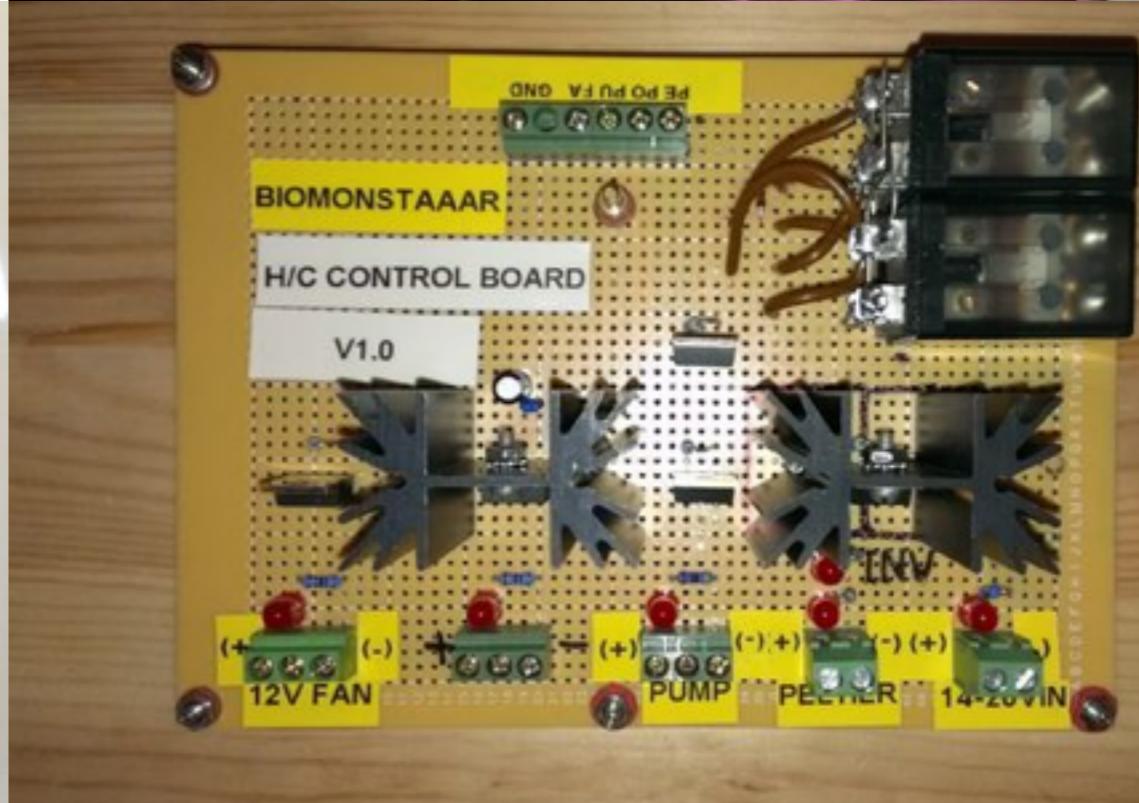
<http://www.instructables.com/id/Create-your-own-mud-battery-Bouw-je-eigen-modderba/>





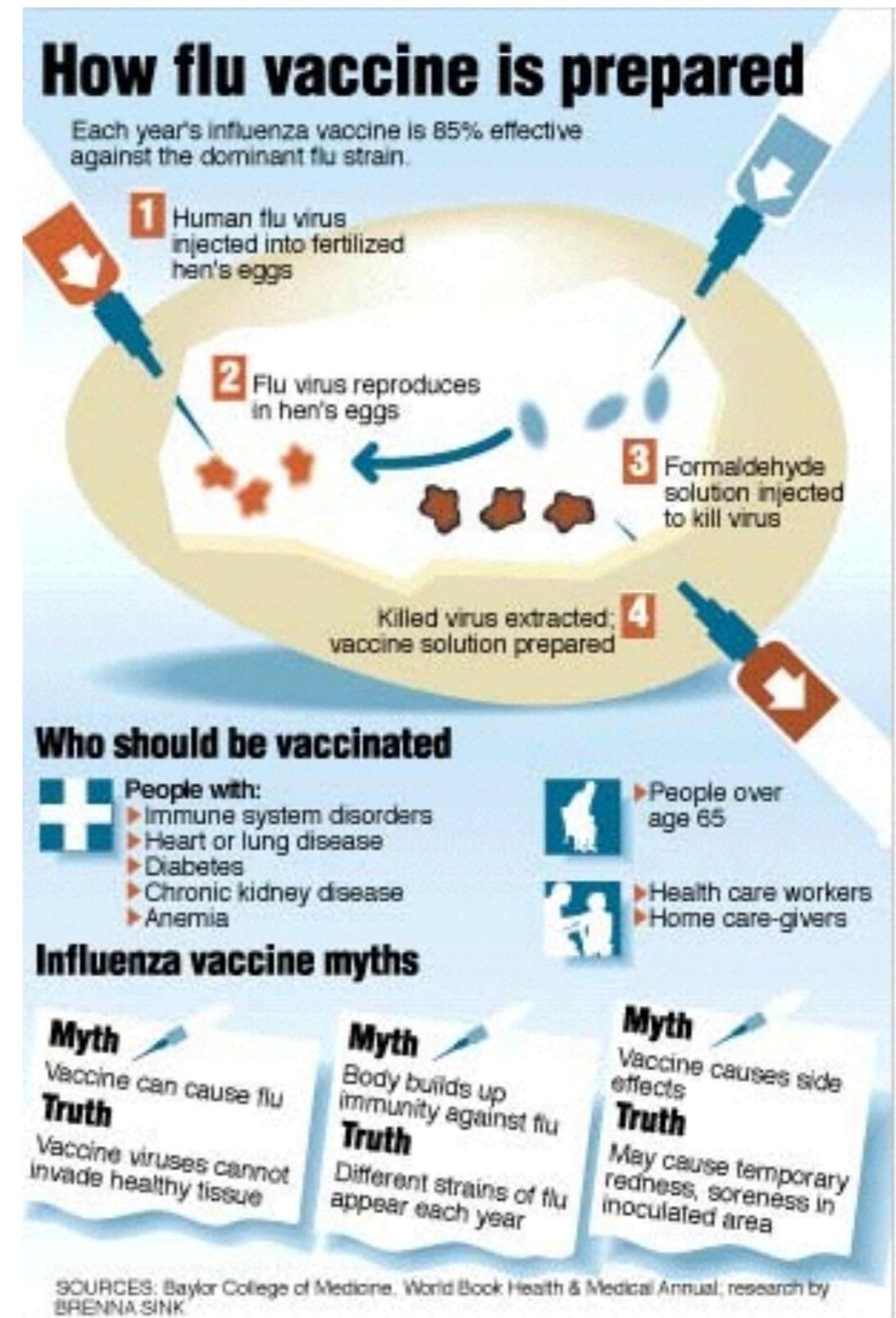
# BIOMONSTAAAR

<http://biomonstaaar.com>





# Flu vaccine production in eggs

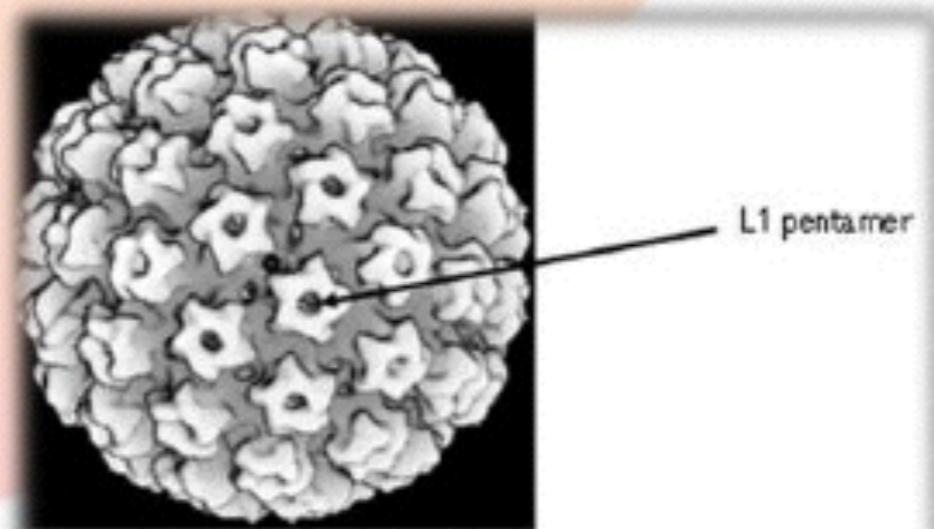




# Insect cell line

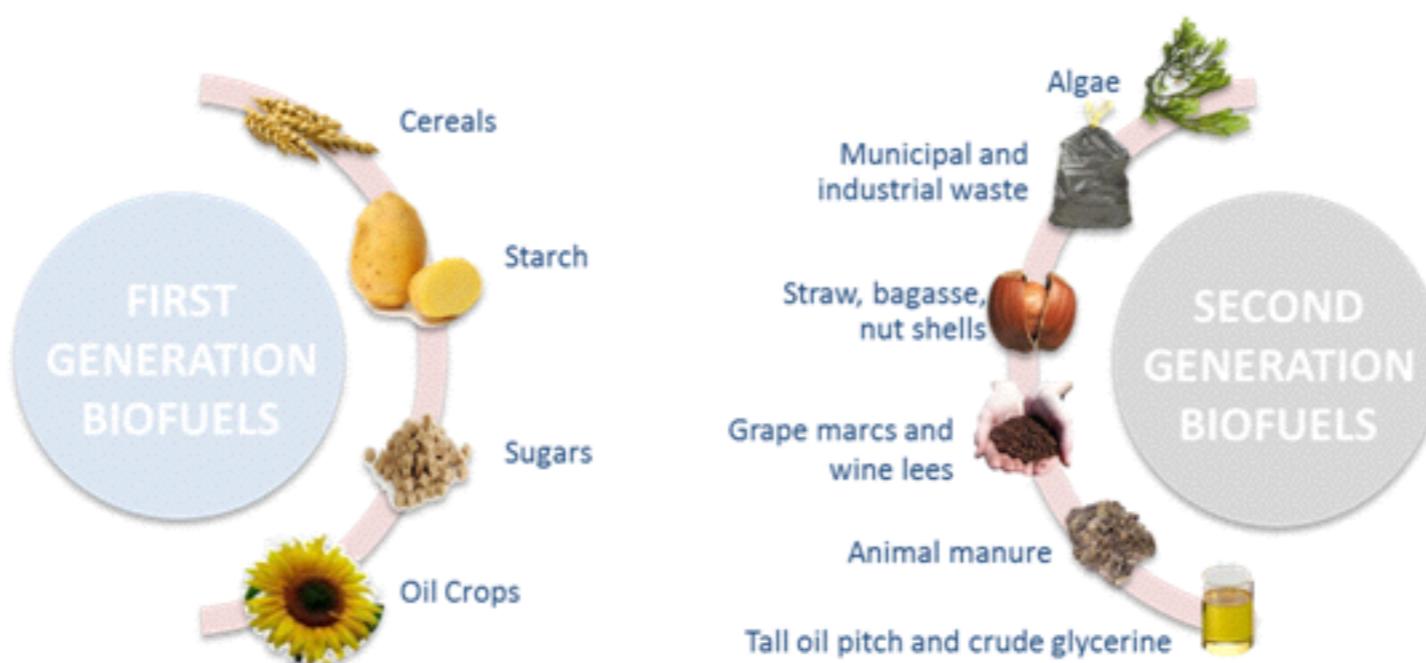
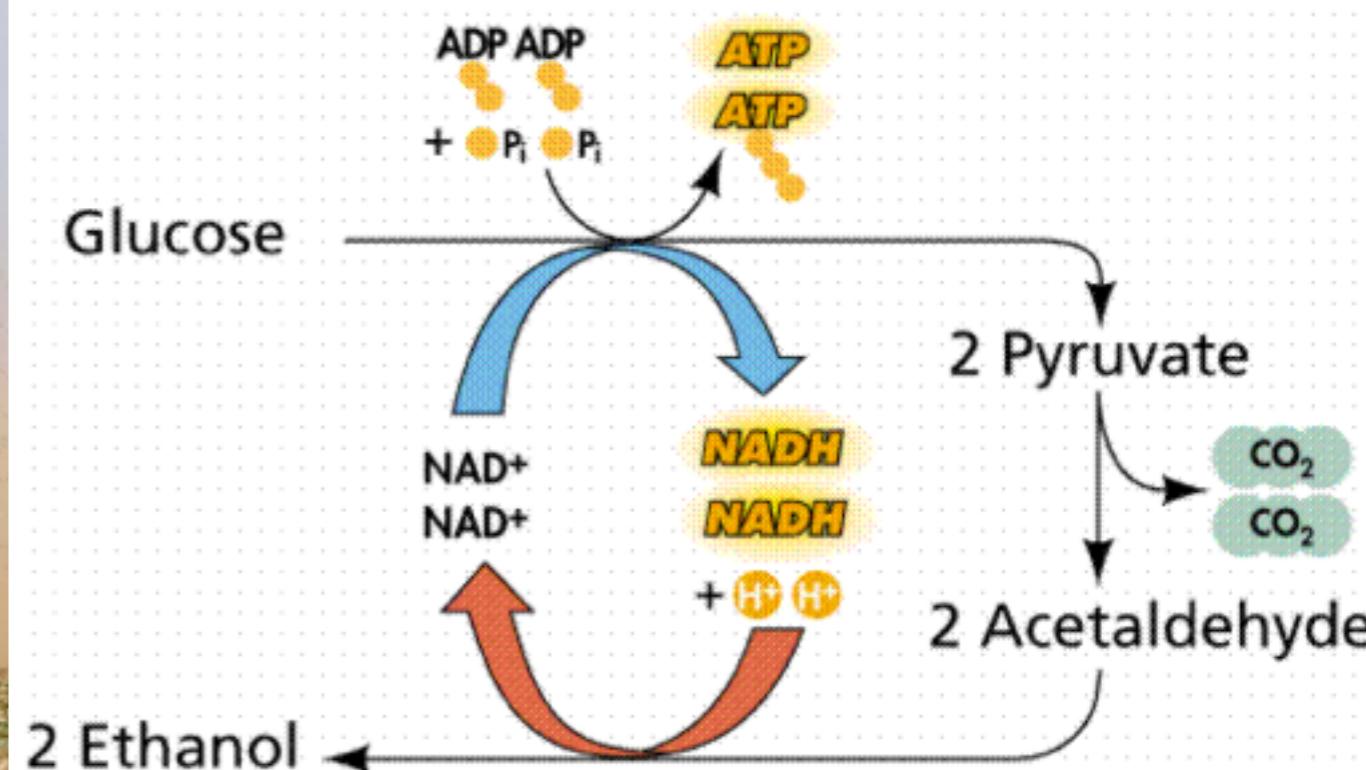
## Cervarix Active Ingredient

- 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



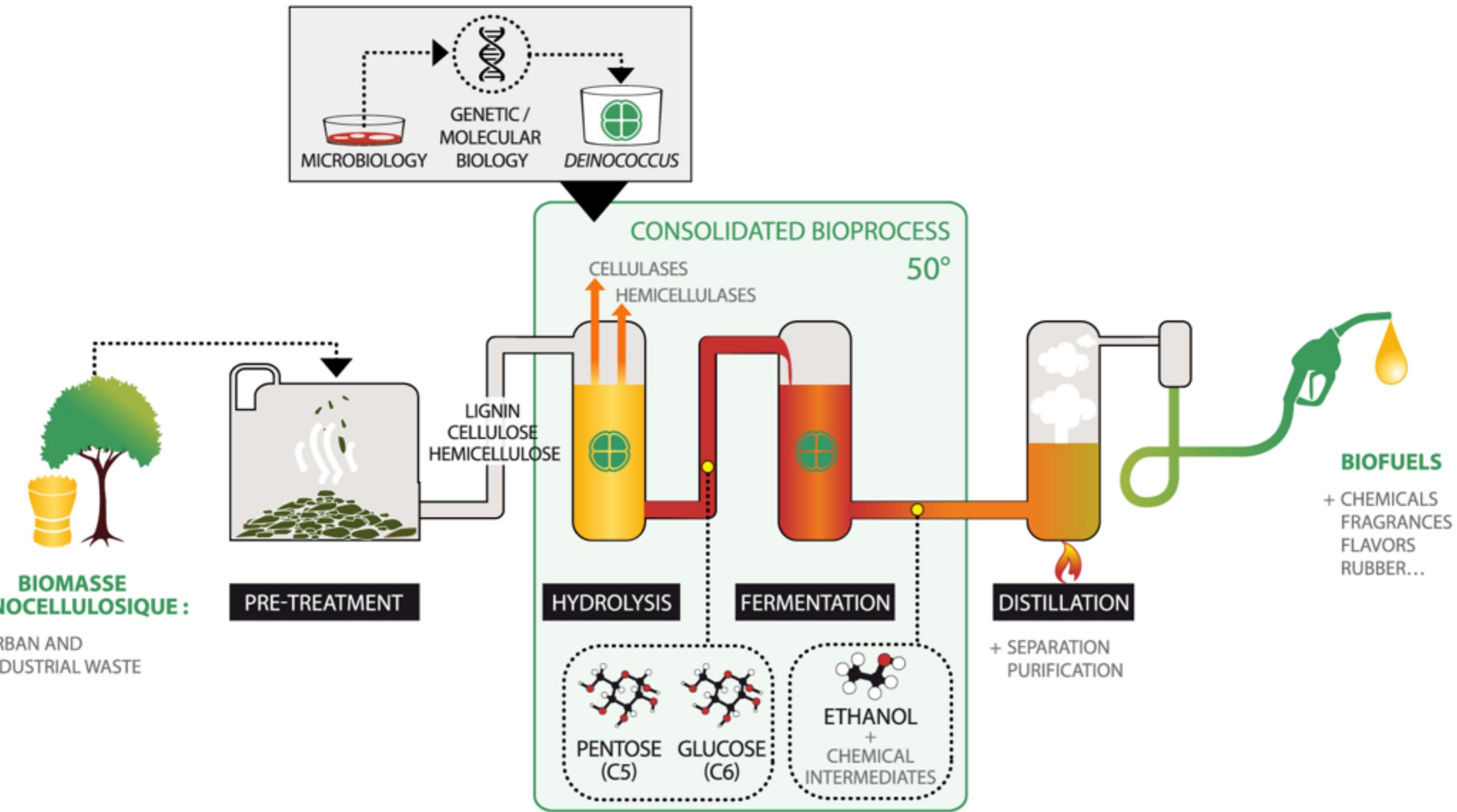


# Sugar cane to ethanol



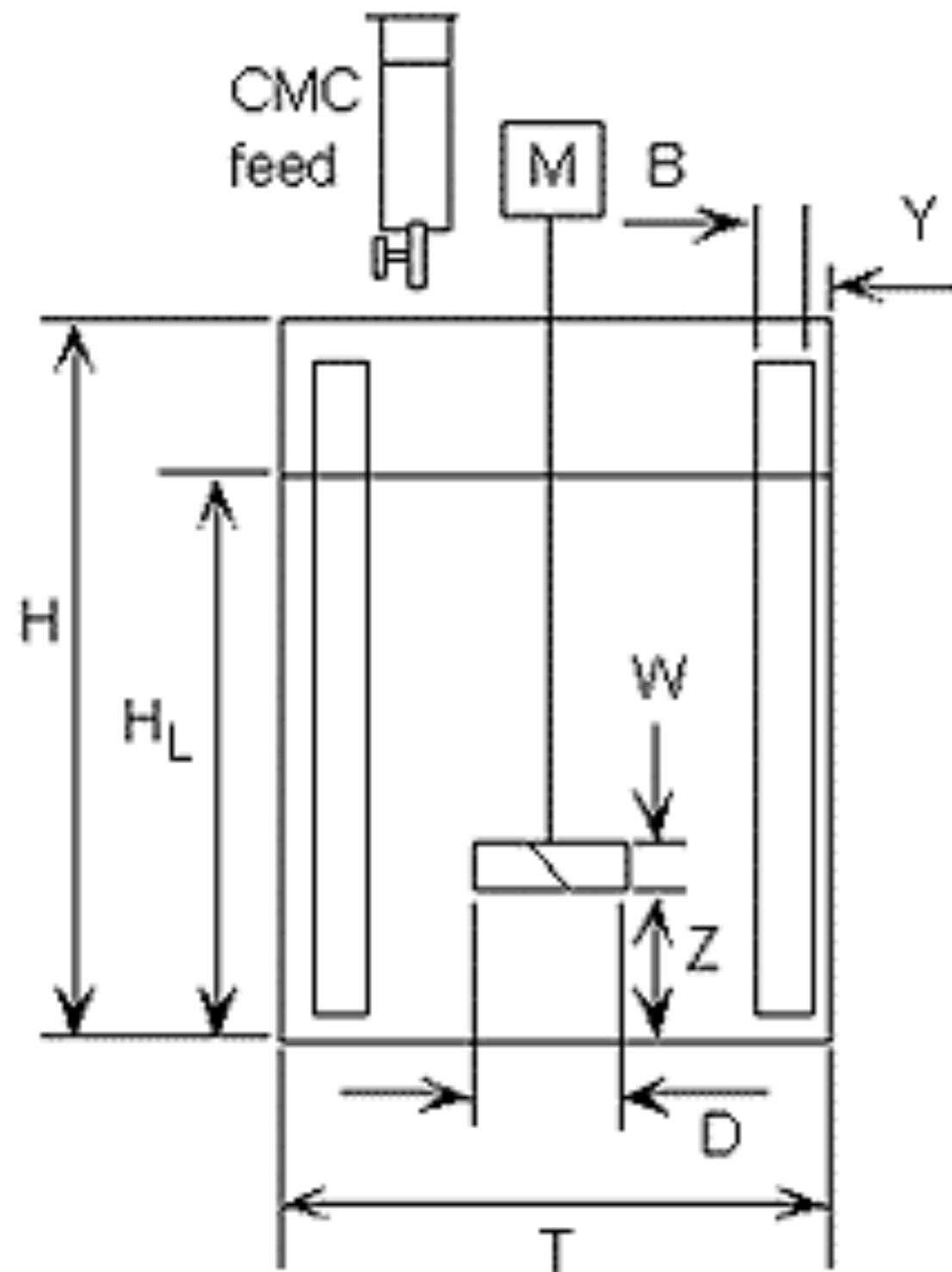


# Second generation





# 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 <sub>L</sub>	T
Number of blades	n	4
Blade width	w	D/5
Blade angle	$\alpha$	45 °



**waag society**

institute for art, science and technology

# Example design

Violacein production



# My search for *J. lividum*

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





# Violacein pricing

SIGMA-ALDRICH®



200,000+  
PRODUCTS

500+  
SERVICES

Featured  
INDUSTRIES

Hello. Sign in.  
ACCOUNT

24/7  
SUPPORT

0 Items  
ORDER

[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<sub>20</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub> | Molecular Weight 343.34

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

Purchase

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 <sub>2</sub> 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

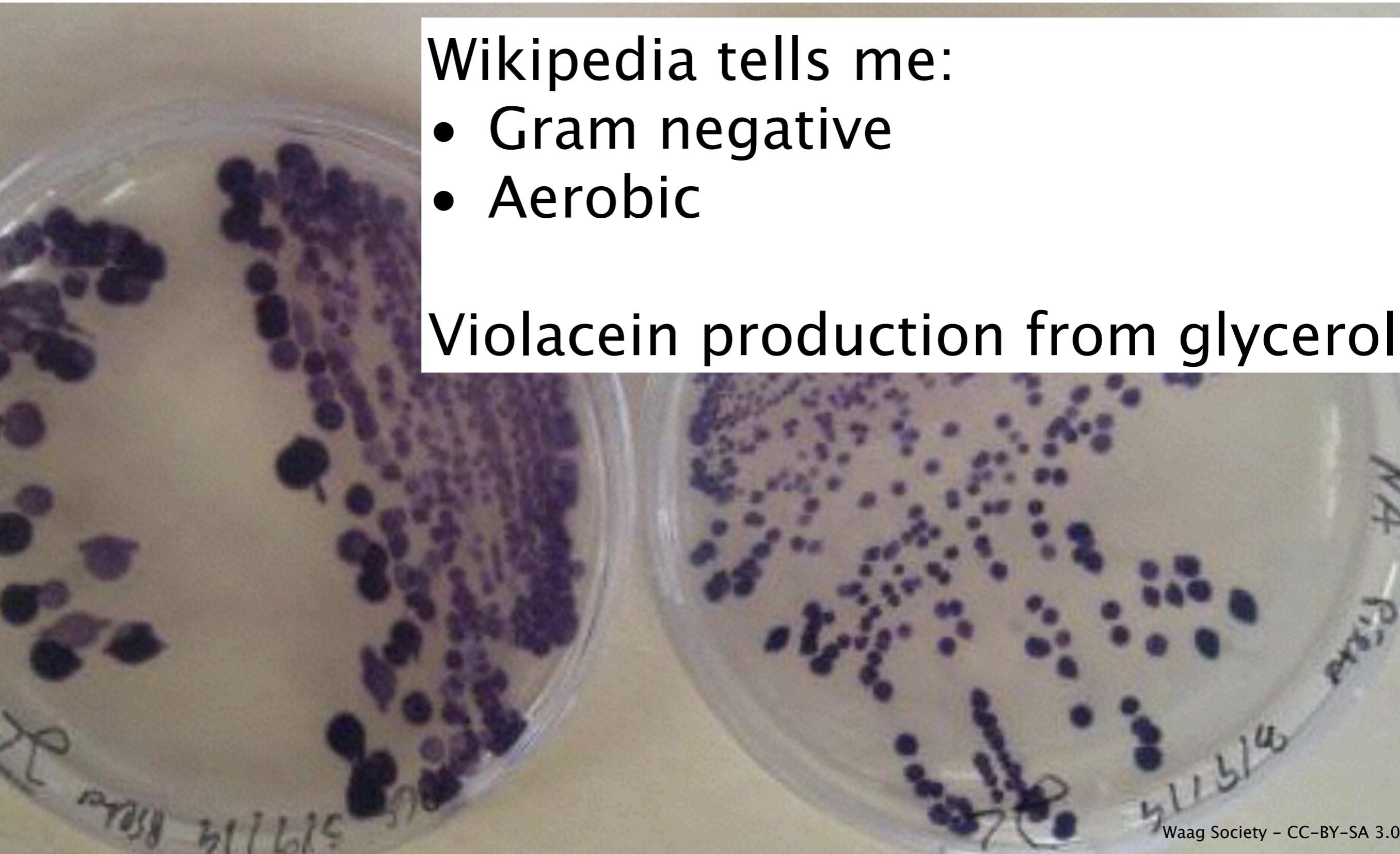


# *Janthinobacterium lividum*

Wikipedia tells me:

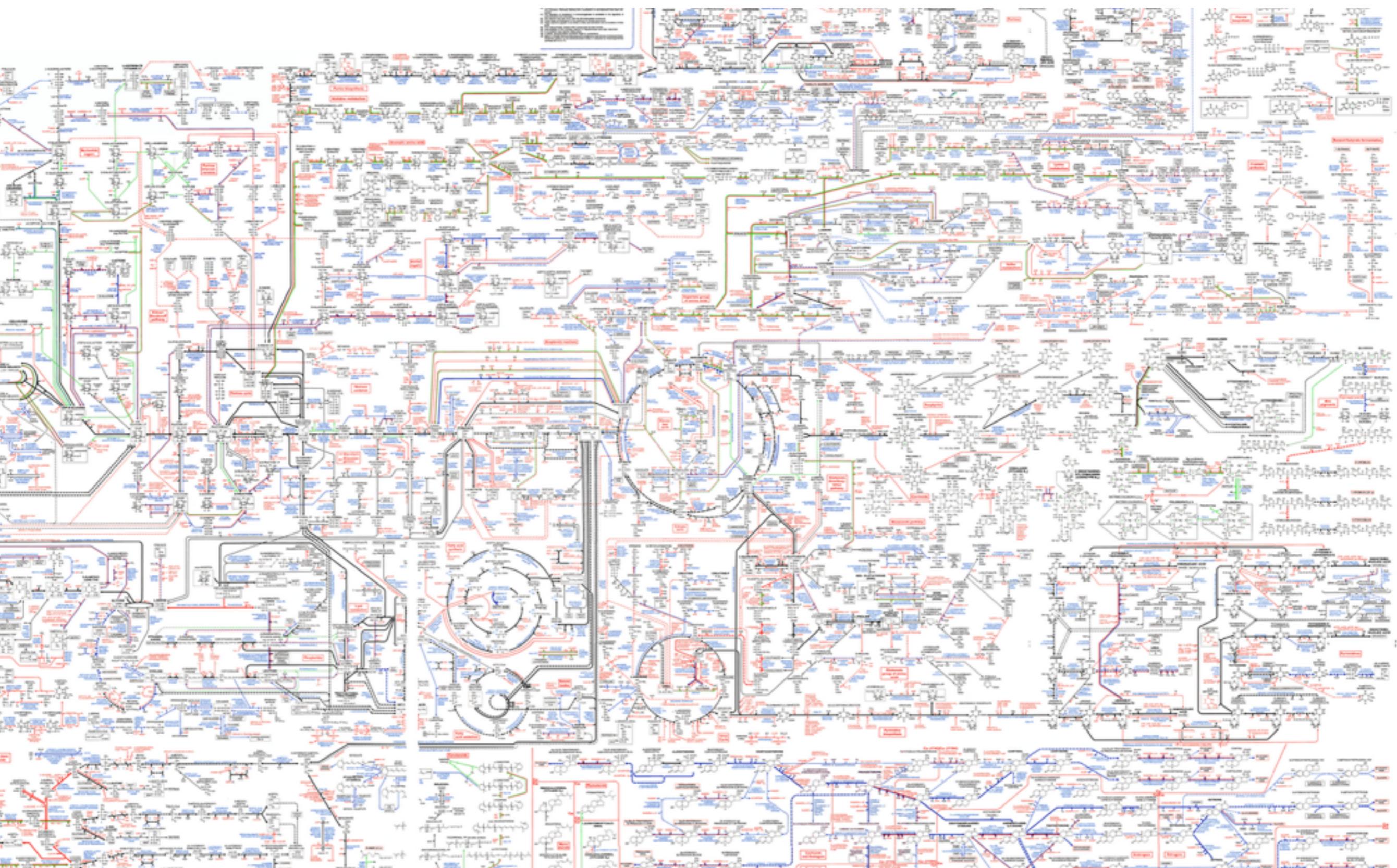
- Gram negative
- Aerobic

Violacein production from glycerol



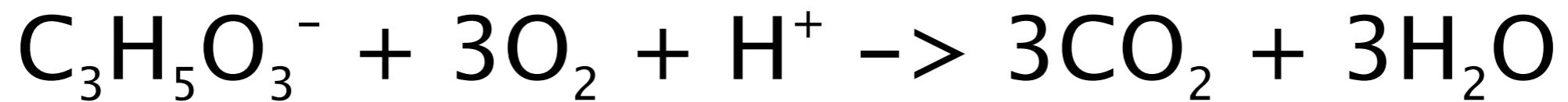


# Production pathway?





# P. Roqueforti eating lactate



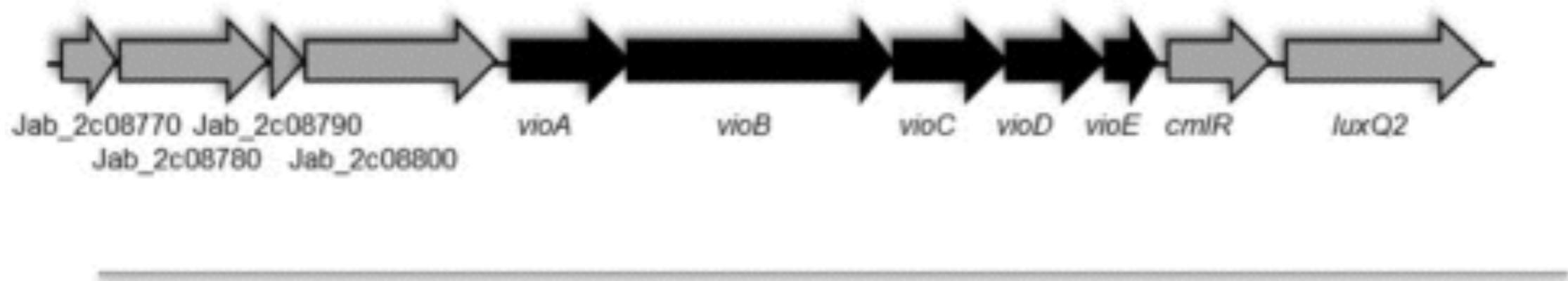
Acid is consumed



# 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)

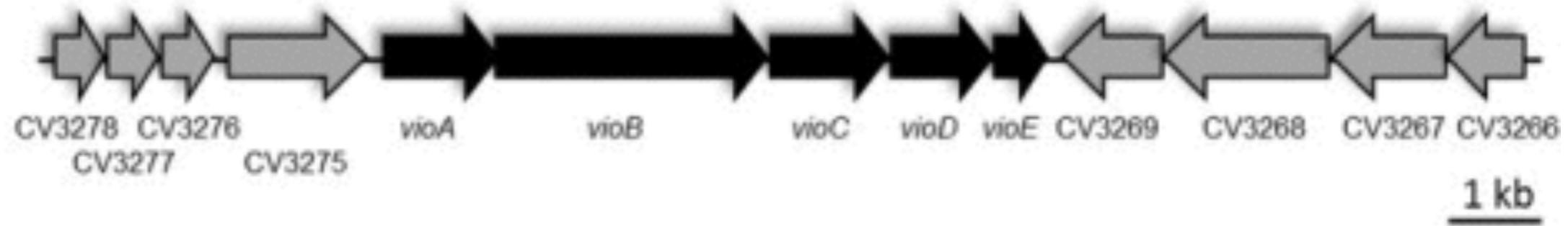
## *Janthinobacterium* sp. HH01



## *Pseudoalteromonas tunicata* D2



## *Chromobacterium violaceum* ATCC 12472





# Production pathway?

## Tryptophan

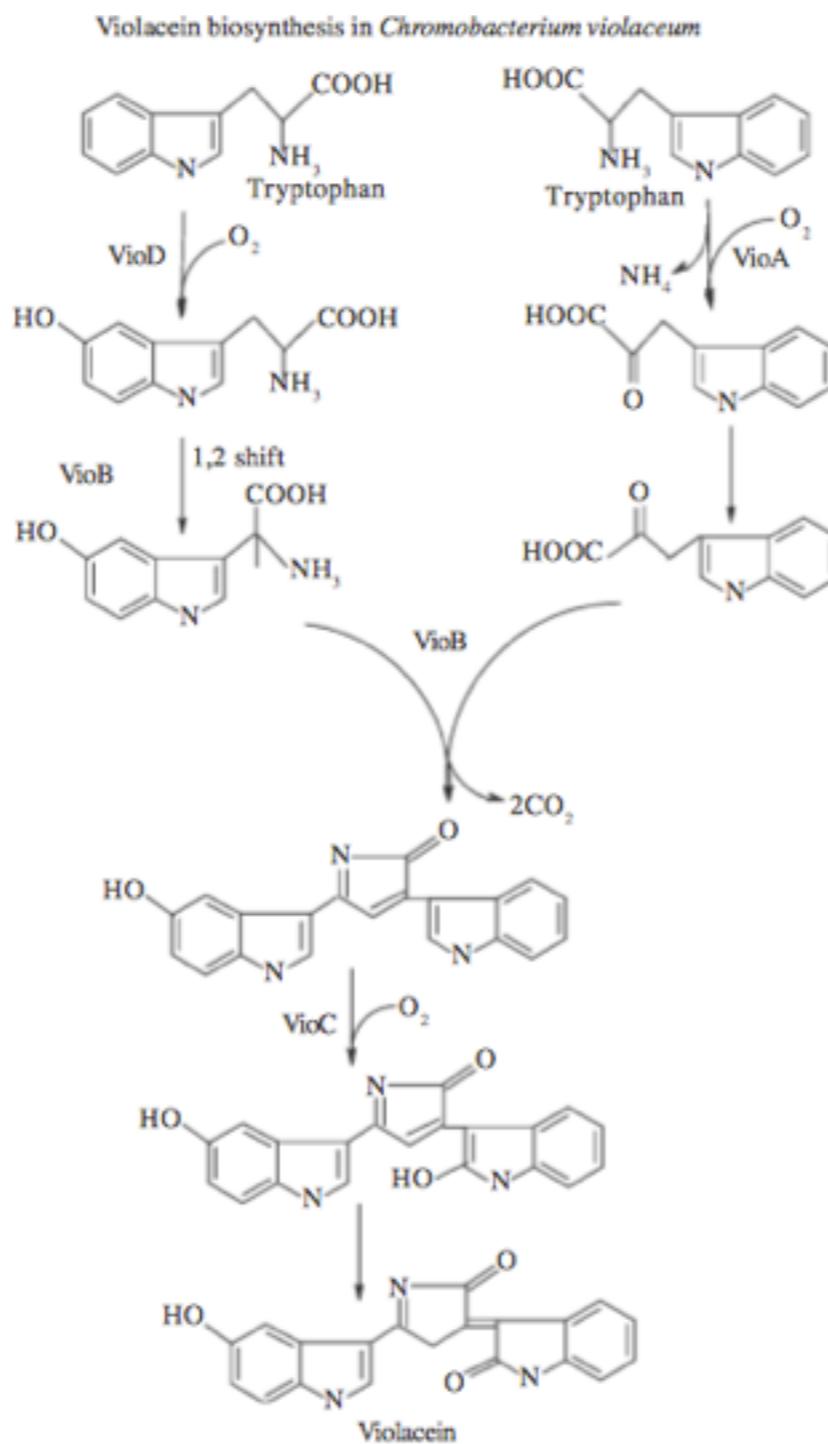


Figure 2. Violacein biosynthesis, as proposed by August et al., 2000. VioA, VioB, VioC, and VioD are the gene products of the biosynthesis operon, encoding nucleotide-dependent monooxygenases and a protein similar to a polyketide synthase (VioB).



# Other interesting things:

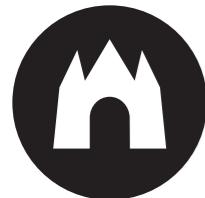
- *J. lividum* produces a metallo- $\beta$ -lactamase conferring resistance to several  $\beta$ -lactam antibiotics

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

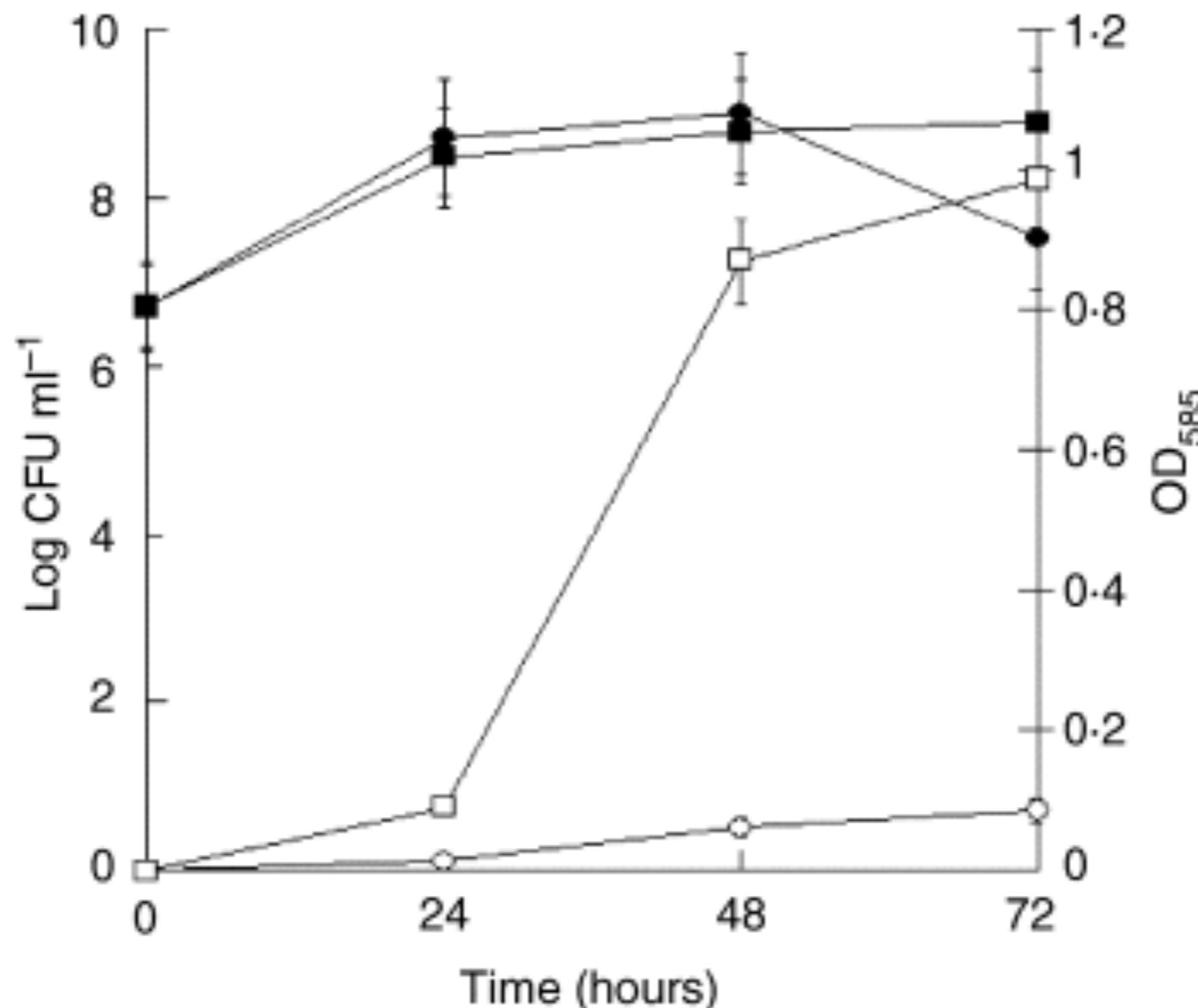
- Violacein:

- $C_{20}-H_{13}-N_3-O_3$
- 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 $\mu$ 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



330 mL



Nutrient  
Broth  
Glycerol  
Tryptoph.  
C  
N  
P  
O<sub>2</sub>  
S



48h



Yes

O<sub>2</sub>



volume



15



temp.



585



pH



mass

1 mL?

No



observations

day #

day #

day #

day #

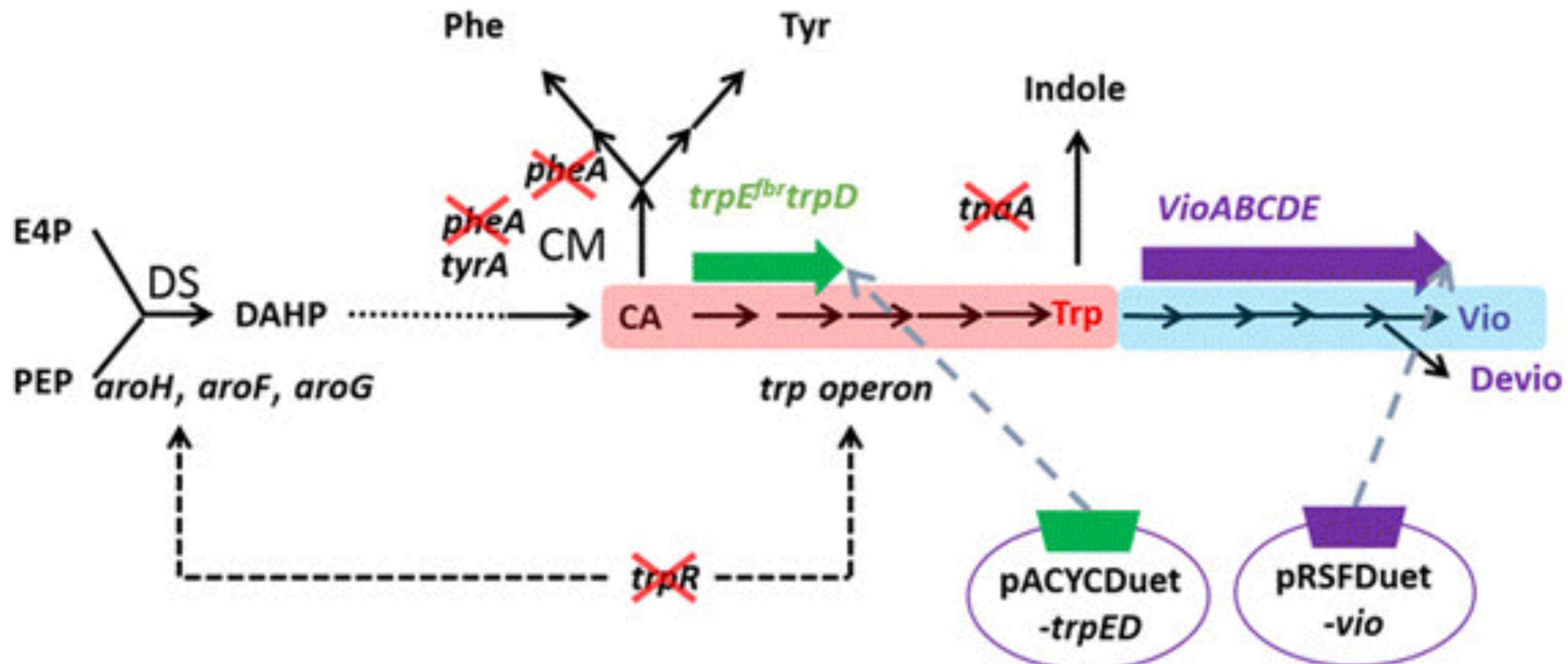
day #



material



# Genetic construct for E. coli





# Synbiota – ScienceHack

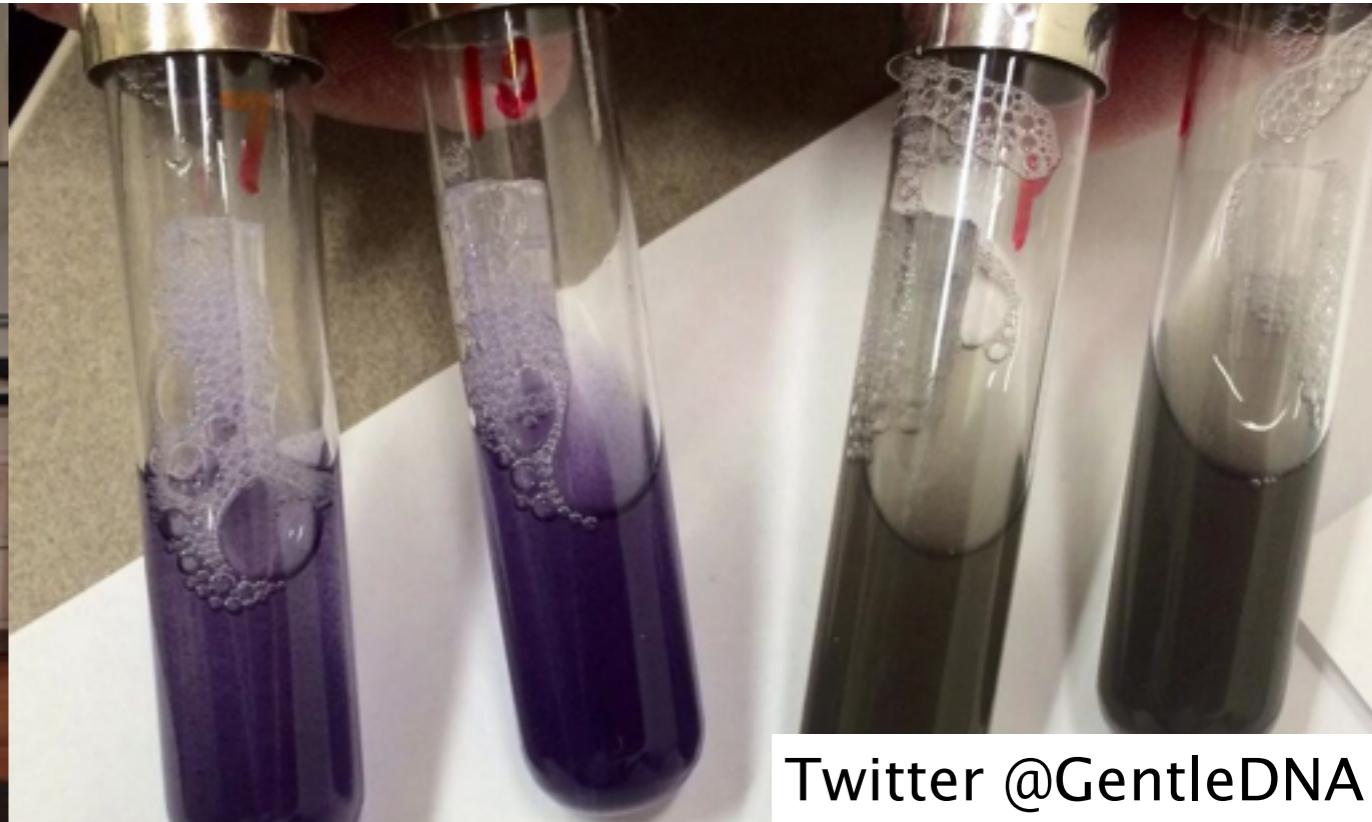


OpenTrons  
#ScienceHack  
@Genspace

4/8/14



Twitter @synbiota



Twitter @GentleDNA



**waag society**

institute for art, science and technology

# Transport Phenomena



# Transport Phenomena

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

	$\varphi \leftrightarrow \theta_l$	$\theta_l = g_\varphi(\varphi)$	$\theta_l = g_{\varphi,T}(\varphi, T)$	$\theta_l = \dots$
Type 1 MSC				
Type 3 MSC				

	Partial derivatives		
	$\frac{\partial \varphi}{\partial \theta} \Big _T$	$\frac{\partial \varphi}{\partial T} \Big _\theta$	$\frac{\partial p_c}{\partial \theta} \Big _T$
Equivalency	$\left( \frac{\partial \theta}{\partial \varphi} \Big _T \right)^{-1} = \frac{1}{\xi_{\varphi\varphi}}$	$- \frac{\partial \varphi}{\partial \theta} \Big _T \cdot \frac{\partial \theta}{\partial T} \Big _\varphi$ $= - \frac{\xi_{\varphi T}}{\xi_{\varphi\varphi}}$	$\left( \frac{\partial p_c}{\partial \varphi} \frac{\partial \varphi}{\partial \theta} \right) \Big _T$ $= \frac{\partial f(\varphi, T)}{\partial \varphi} \Big _T \frac{1}{\xi_{\varphi\varphi}}$

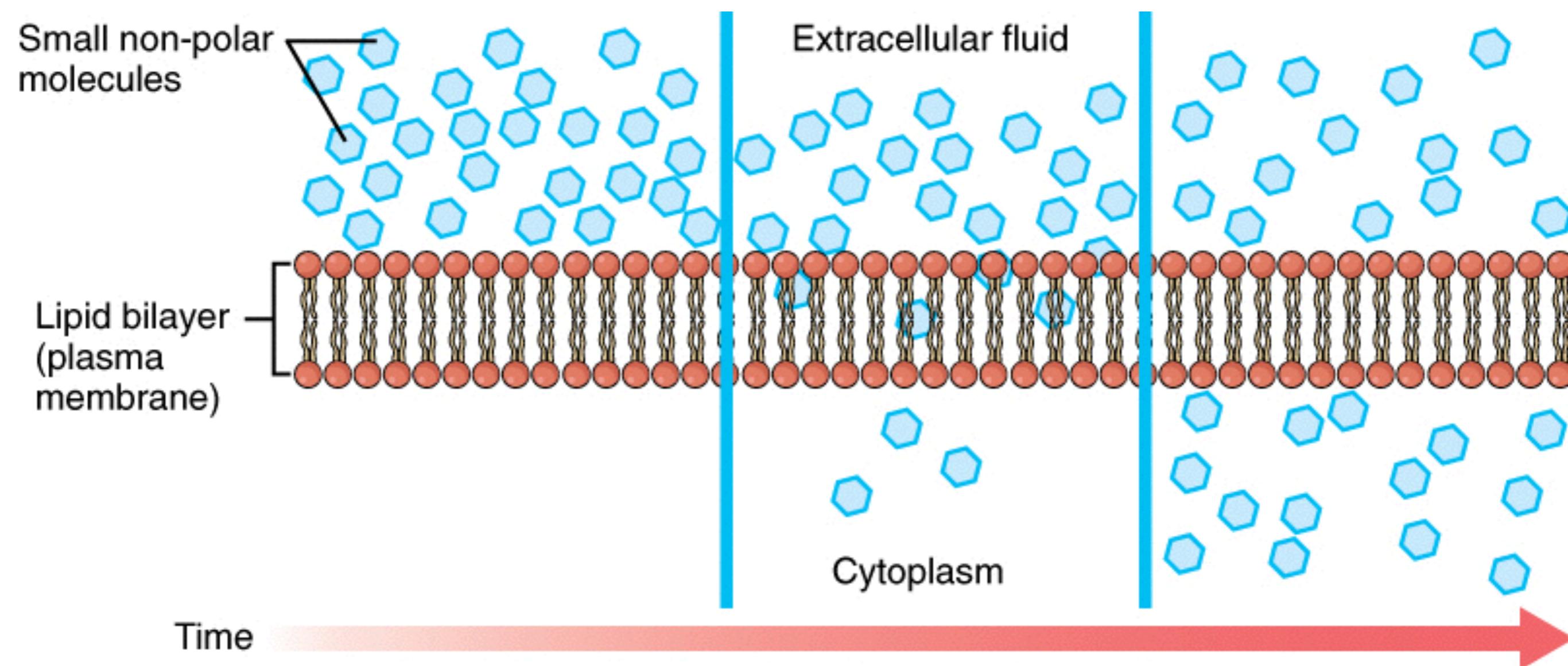
	Type 1 MSC	Type 3 MSC	
	$\left( \frac{dg_\varphi}{d\varphi} \right)^{-1}$	$\left( \frac{\partial g_{\varphi,T}}{\partial \varphi} \Big _T \right)^{-1}$	$0$ $- \frac{\partial g_{\varphi,T}}{\partial T} \Big _\varphi \cdot \left( \frac{\partial g_{\varphi,T}}{\partial \varphi} \Big _T \right)^{-1}$ $- \frac{R_v \rho_l T}{\varphi \xi_{\varphi\varphi}}$

	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	Liquid transport	
Isothermal	$D_{\theta\theta}^v = \frac{\delta_v p_{sat}}{\xi_{\varphi\varphi} \rho_l}$	$D_{\theta\theta}^l = \frac{D_{\theta T}^v}{\delta_v} \left( \varphi \frac{\partial p_{sat}}{\partial T} - \frac{\xi_{\varphi T} p_{sat}}{\xi_{\varphi\varphi}} \right)$	$D_{\theta\theta}^l = \frac{K_l R_v T}{\varphi \xi_{\varphi\varphi}}$
Non-isothermal			



# 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



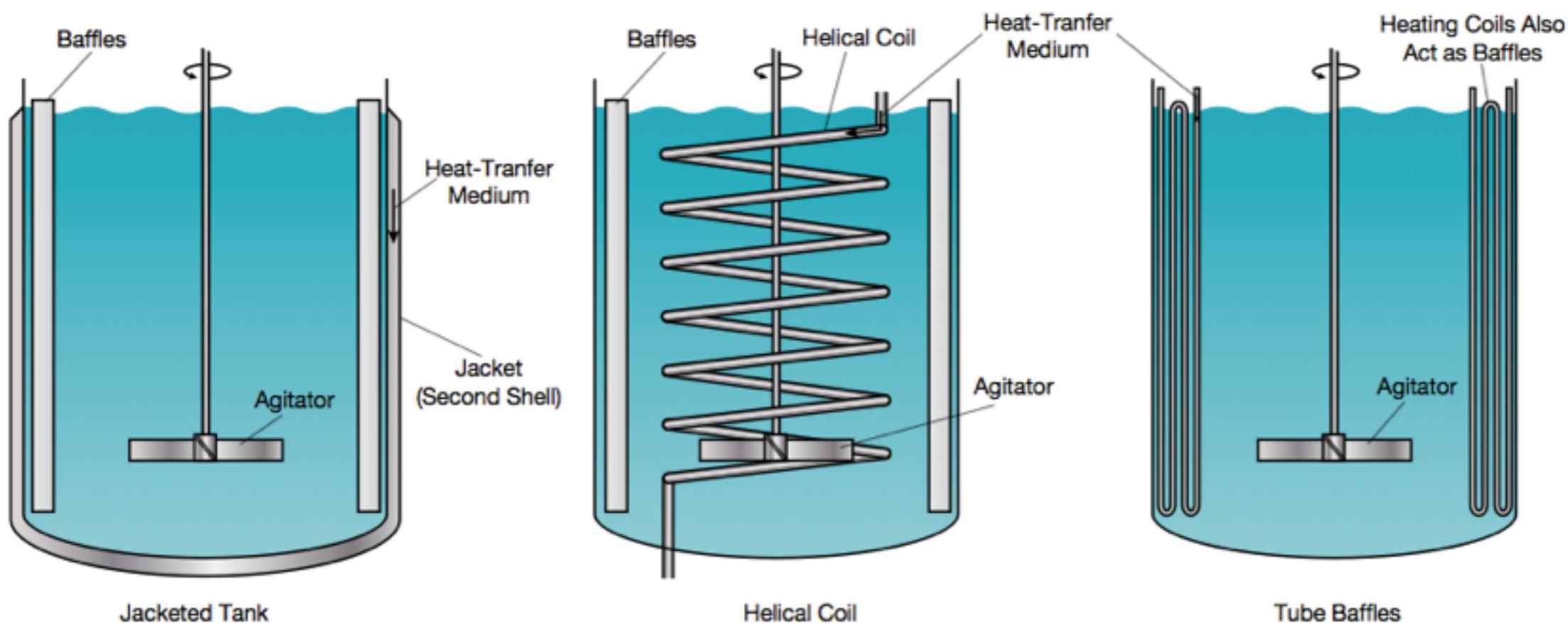
# Shaken or stirred?

- 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



**some  
rights  
reserved**