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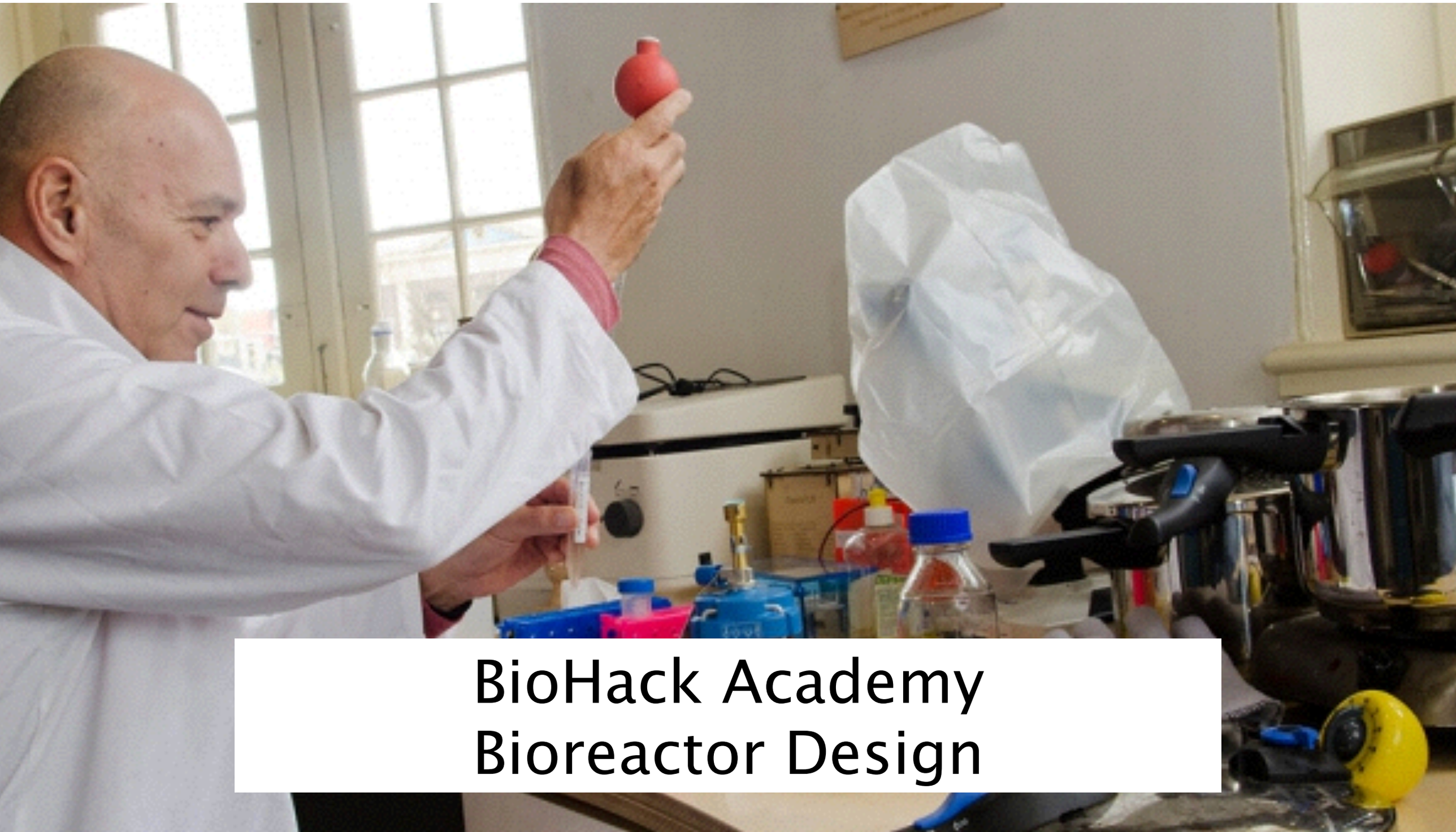
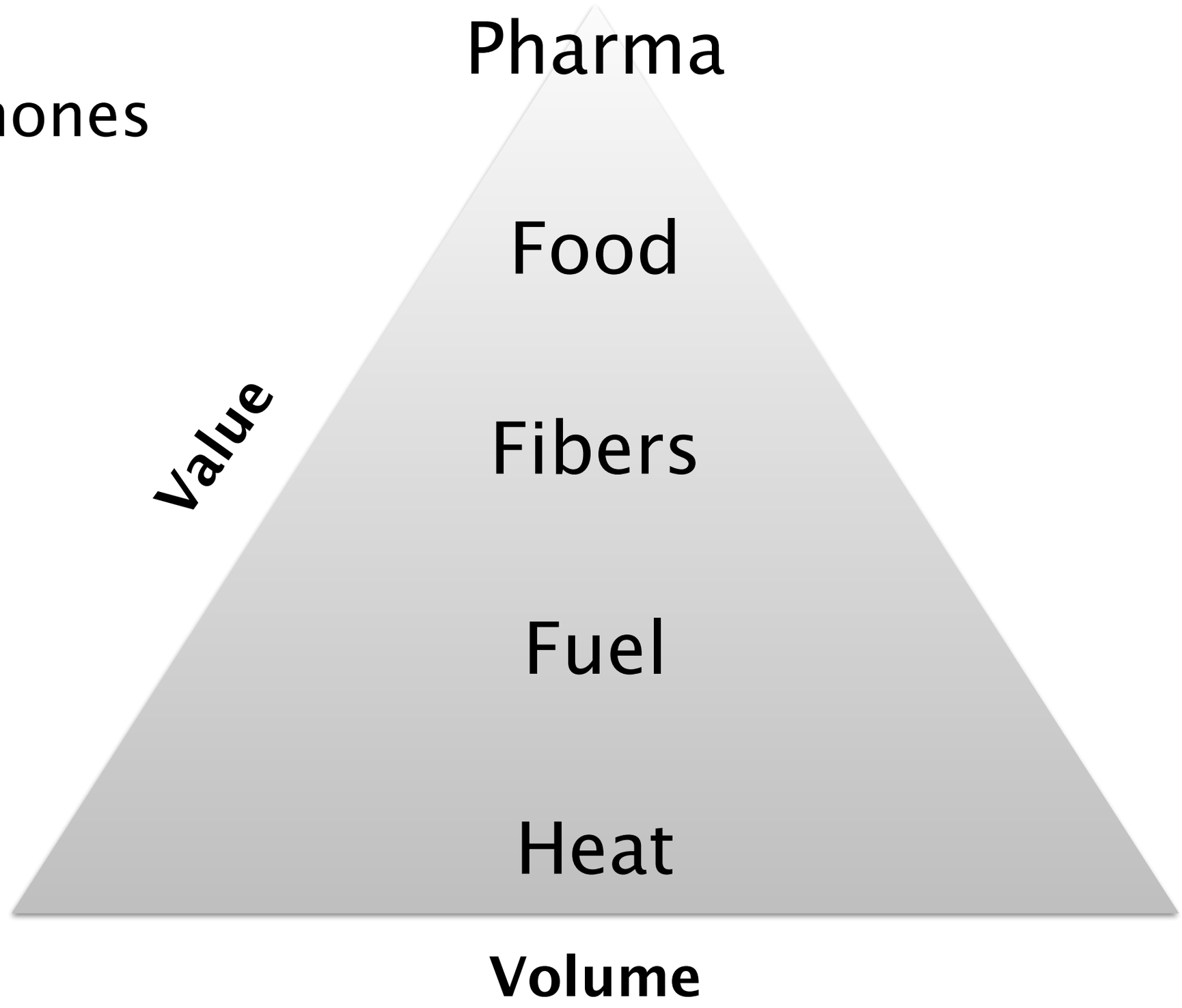


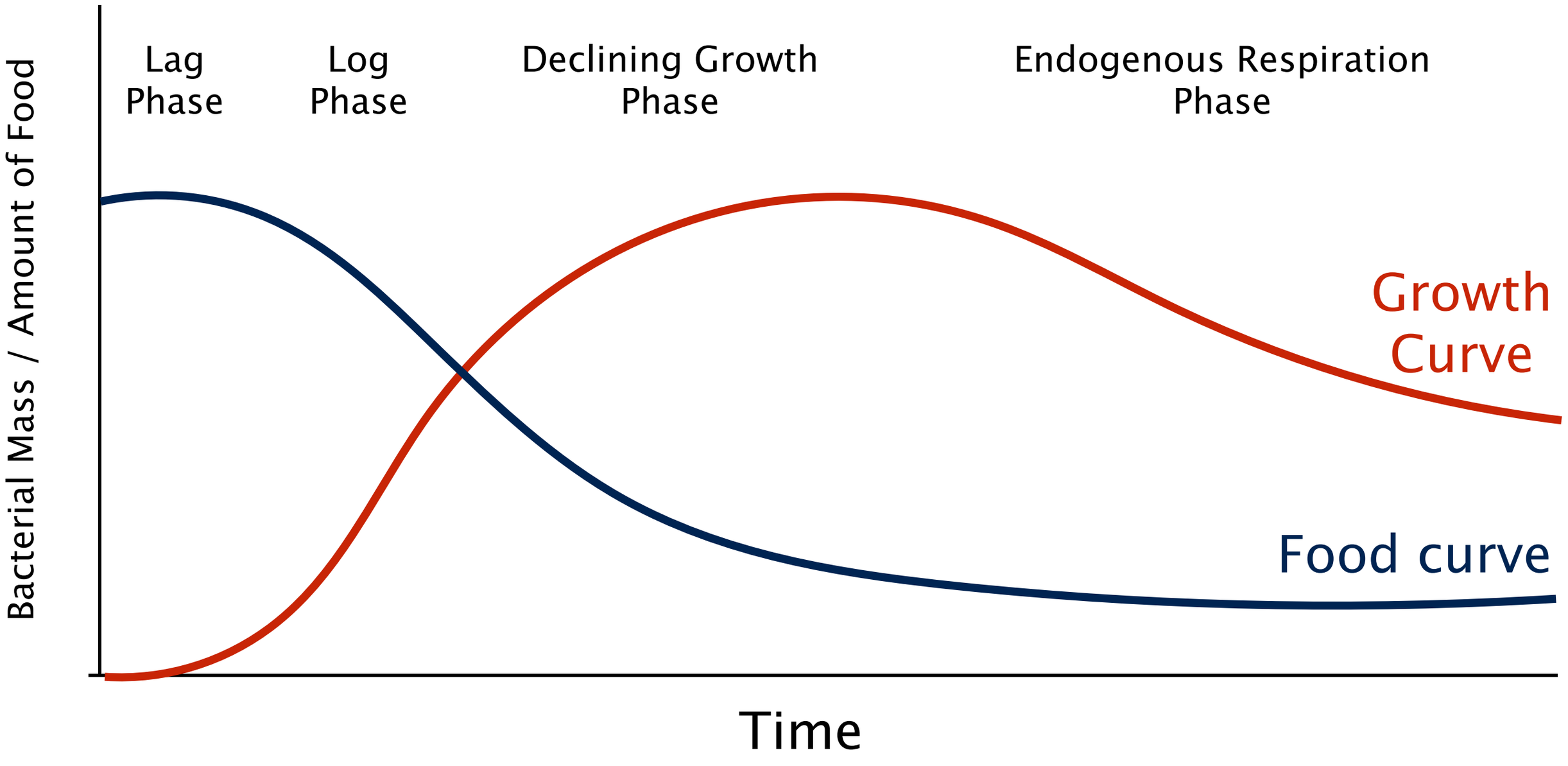
BioHack Academy
Bioreactor Design

Bioreactor value pyramid

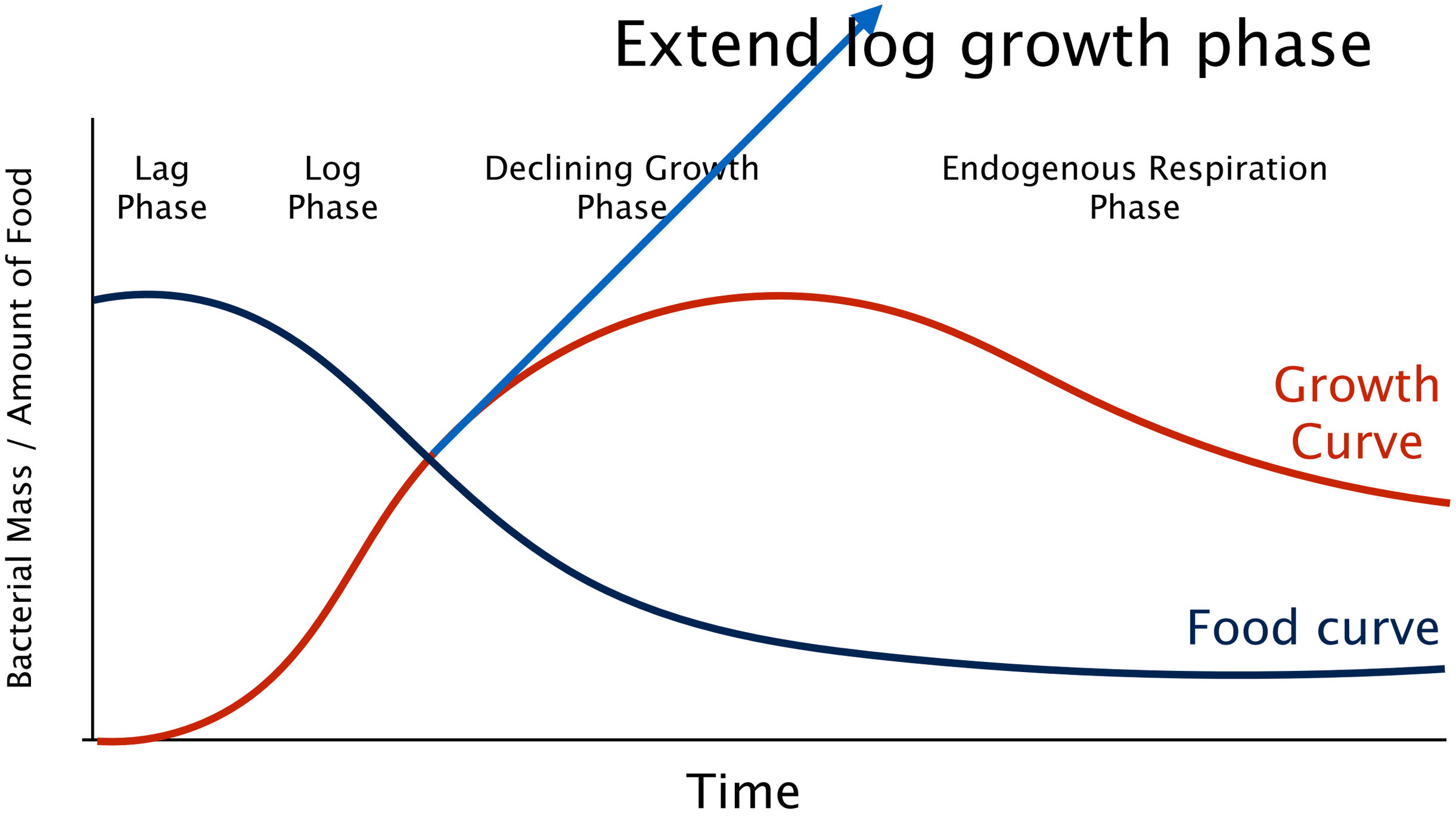
- Antibiotics
- Steroids / hormones
- Vitamins
- Proteins
- Sugars
- Acids



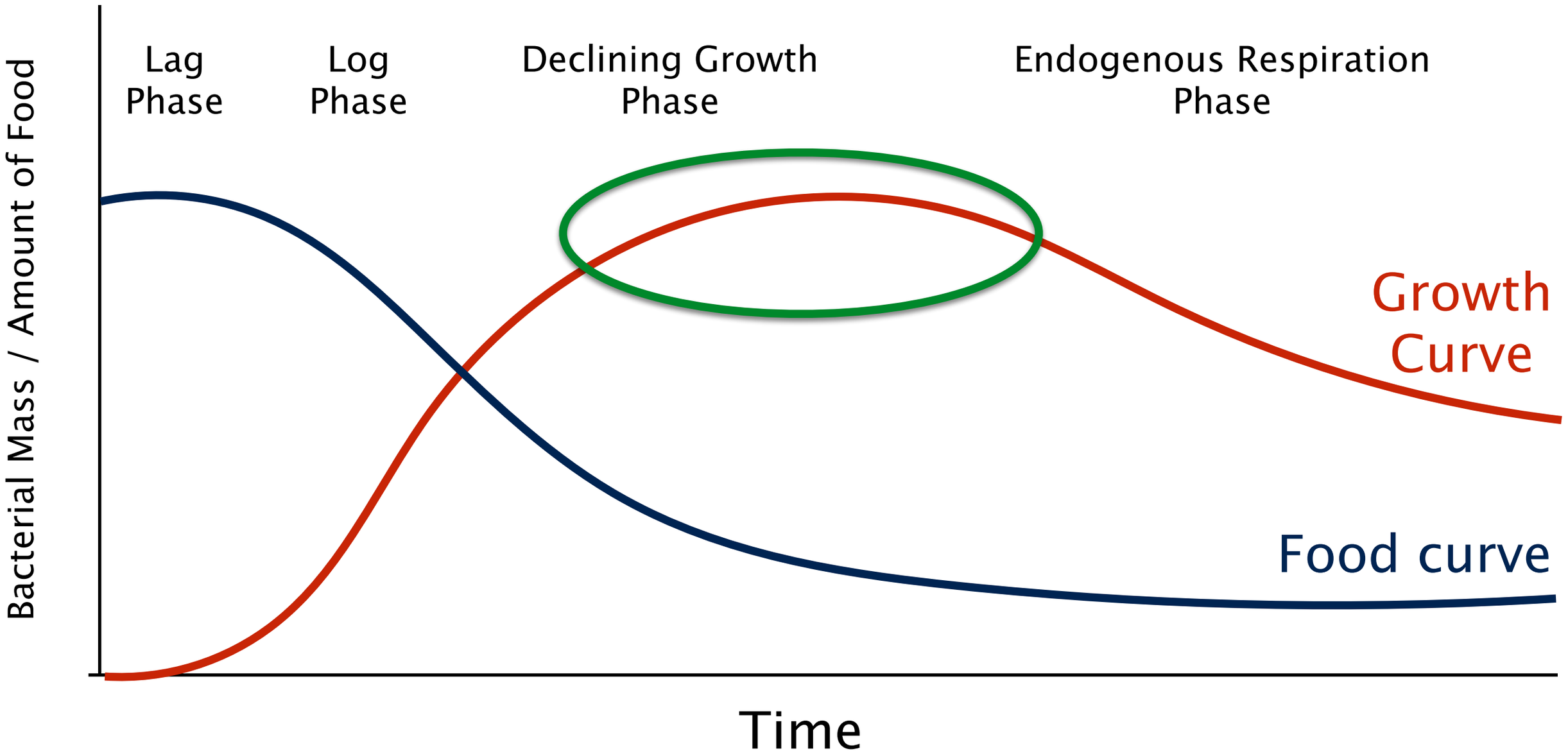
Bacterial growth curve – Batch



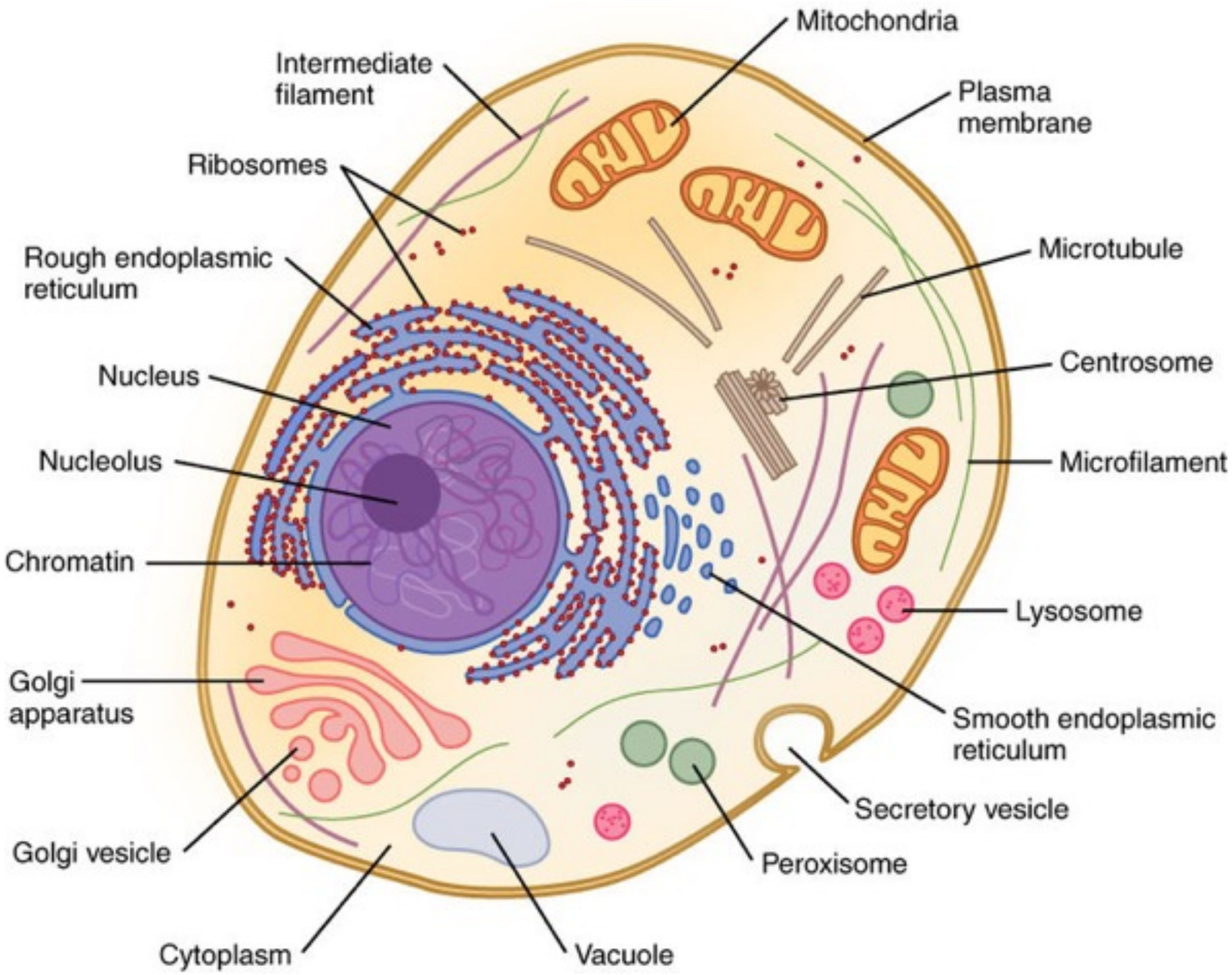
Primary products



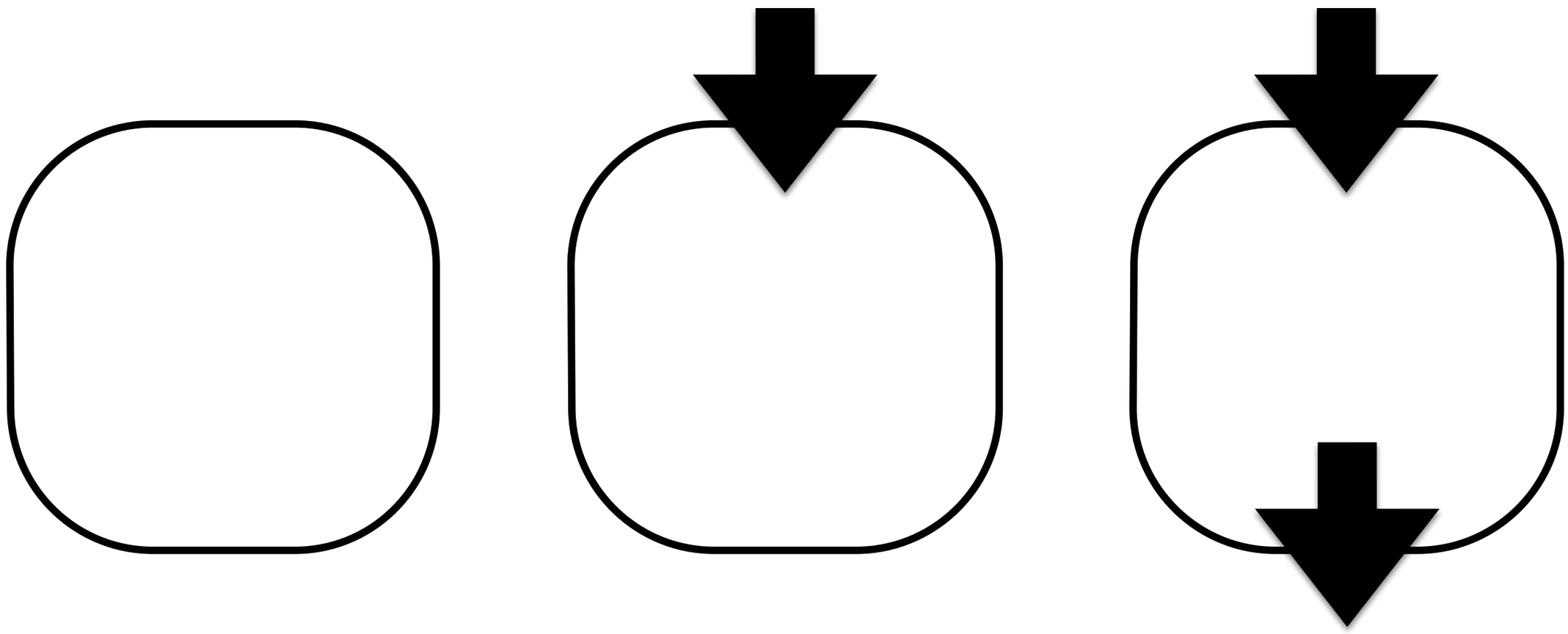
Secondary products



Intra vs extra cellular



Growth strategies



Batch

Fed Batch

Chemostat

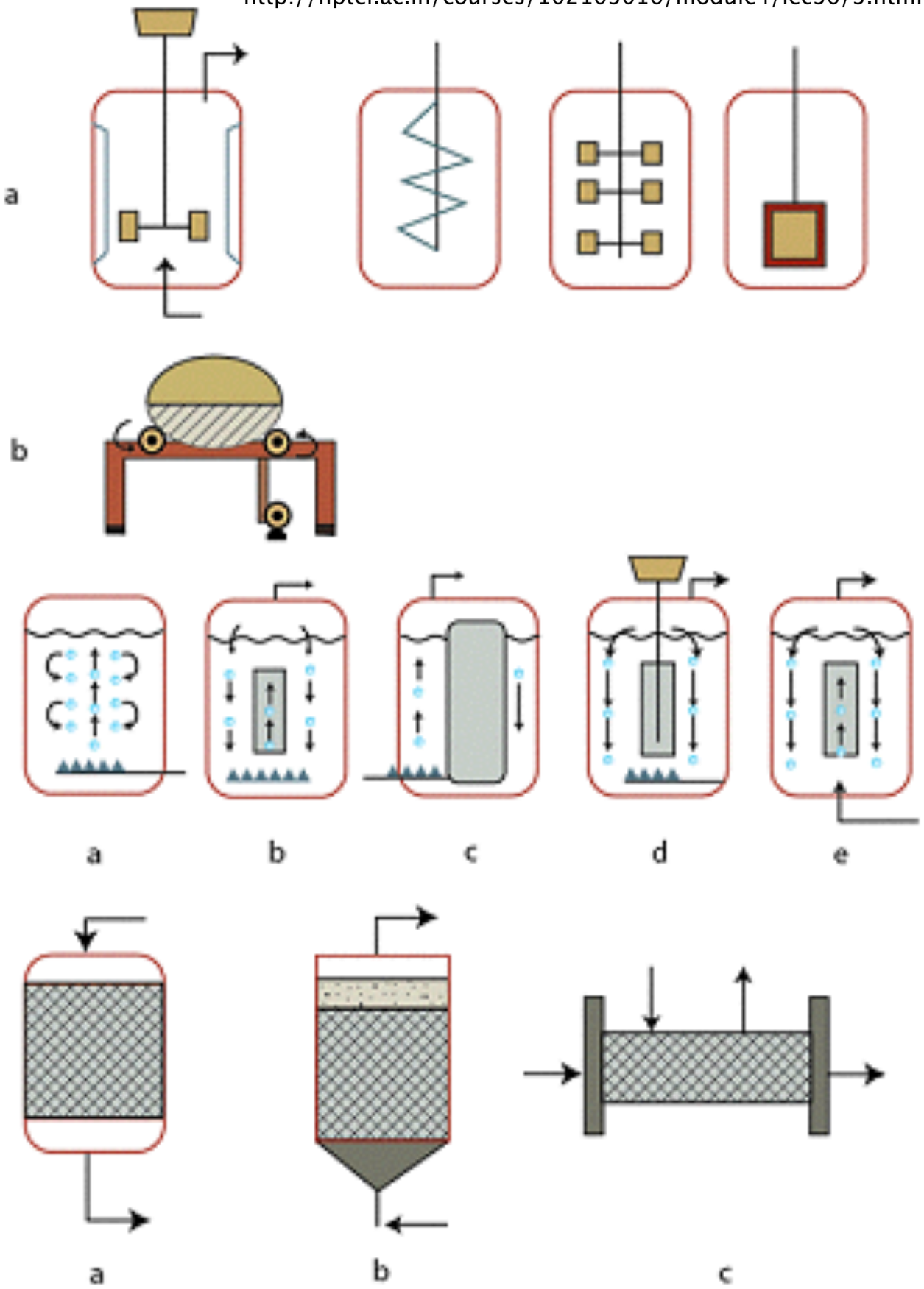
Bioreactor Wishlist

- 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

http://nptel.ac.in/courses/102103016/module4/lec36/3.html



Bioreactor hacks

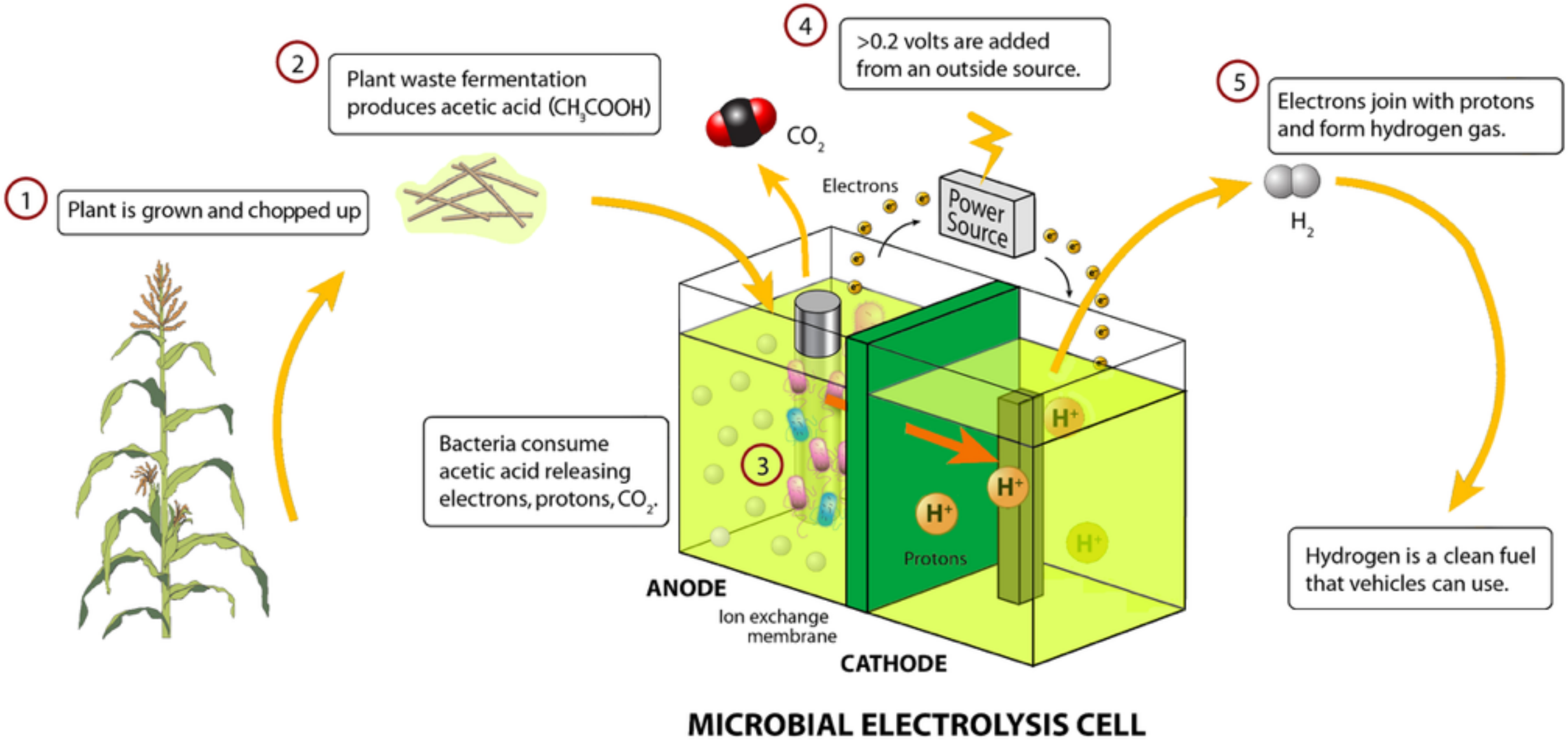
http://www.instructables.com/id/An-Algae-Bioreactor-from-Recycled-Water-Bottles/

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

Briefcase Biotec

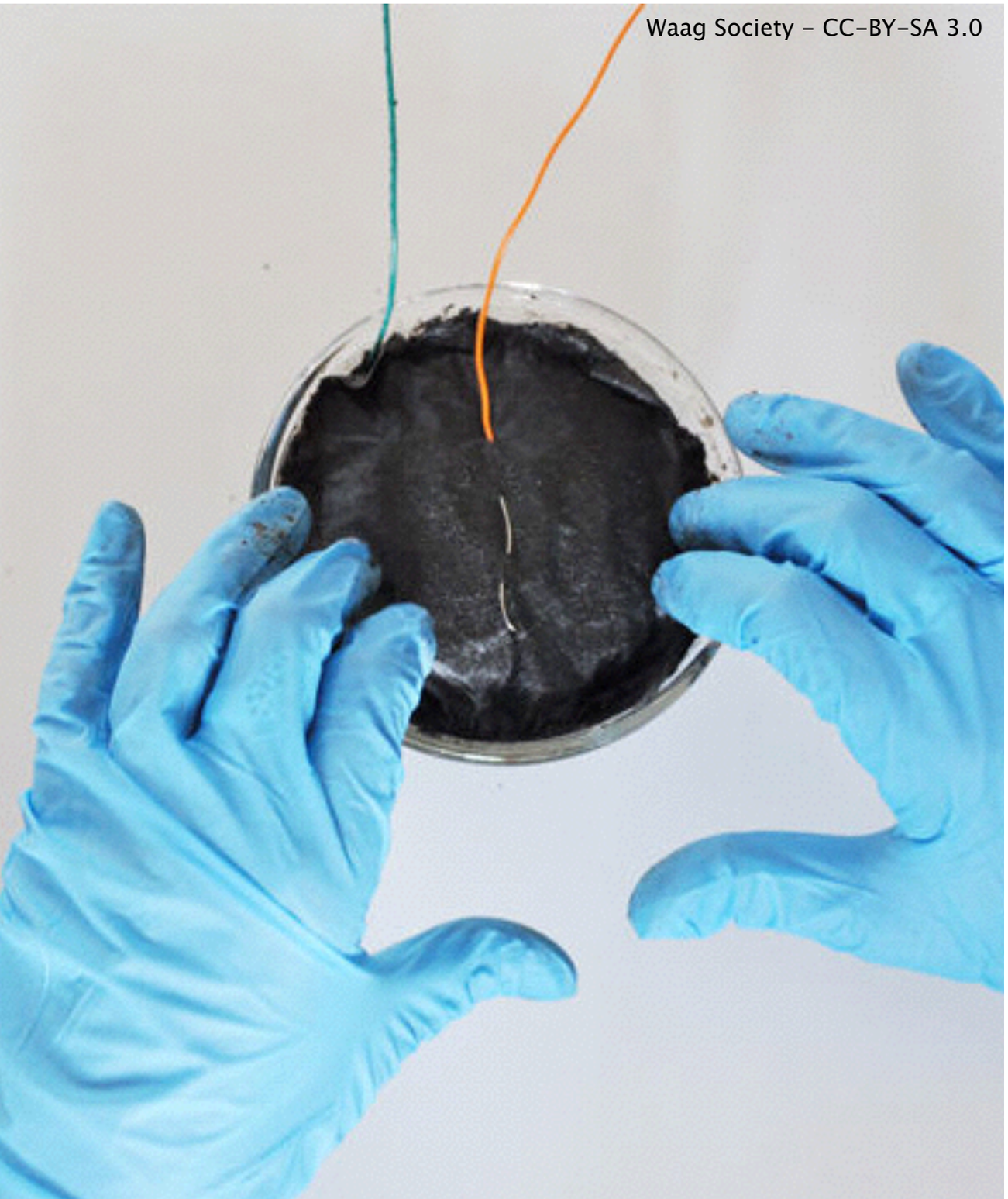
https://www.youtube.com/watch?v=yDarDR4vi1A

Membrane reactor: Fuel Cells

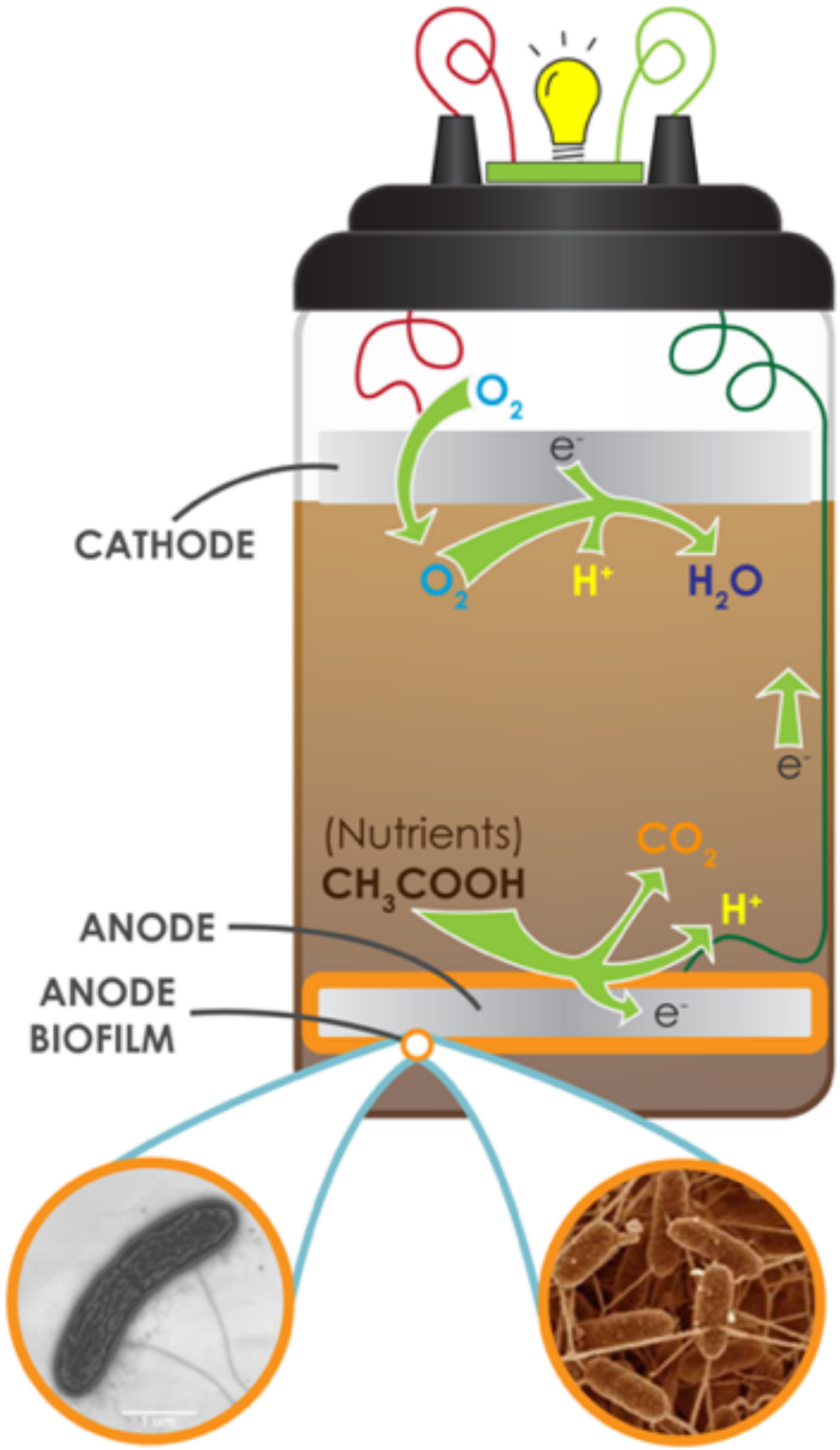


Soil based fuel cells

Waag Society - CC-BY-SA 3.0



MFCGuy2010 - CC-BY-SA 3.0



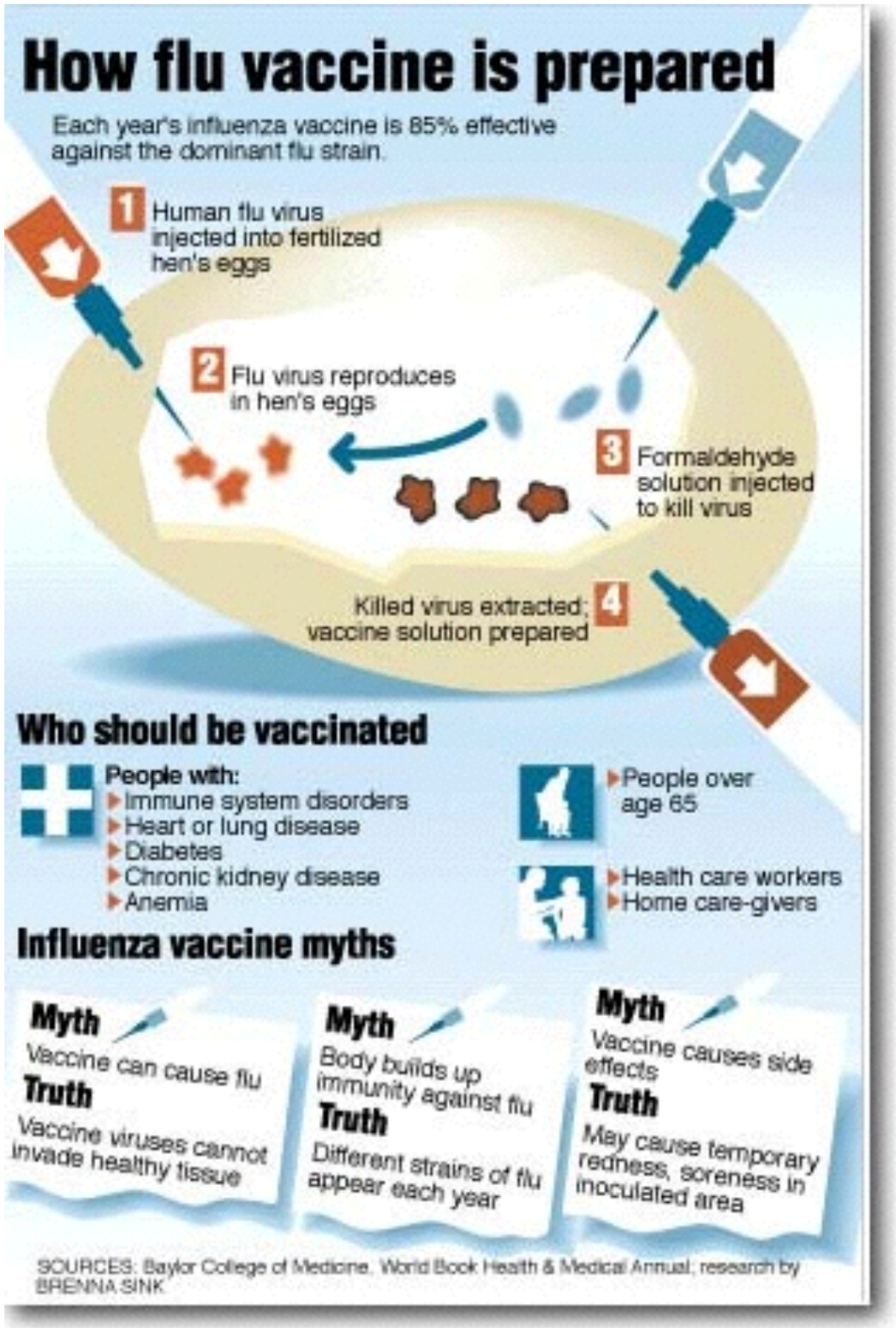
Bio mud battery

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

BIOMONSTAAAR

http://biomonstaaar.com

Flu vaccine production in eggs

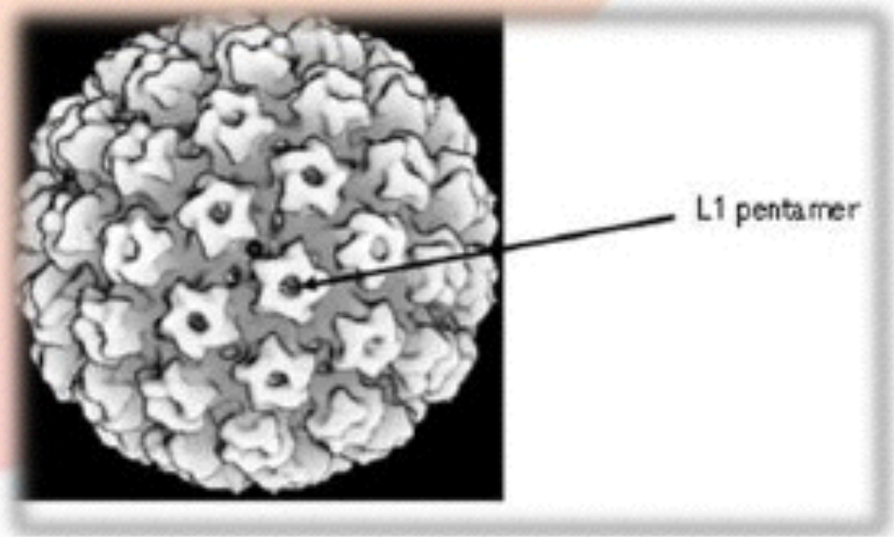


Insect cell line

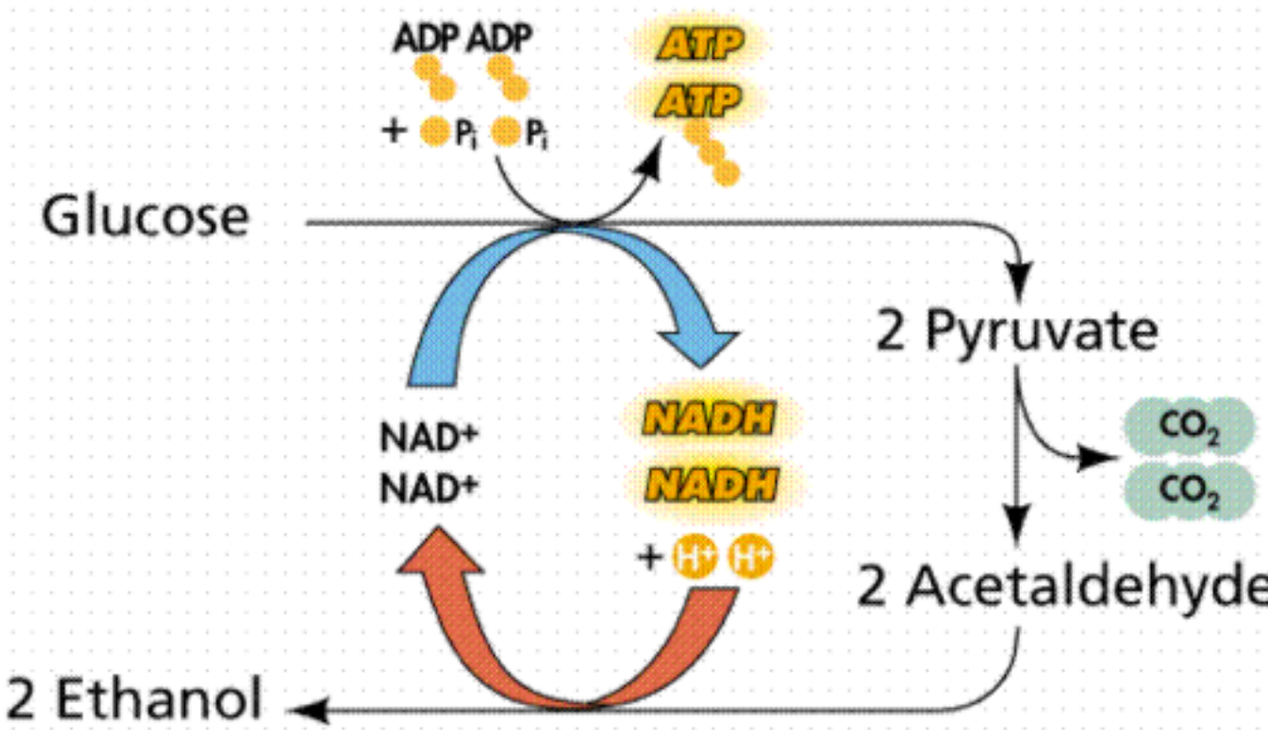
Cervarix Active Ingredient

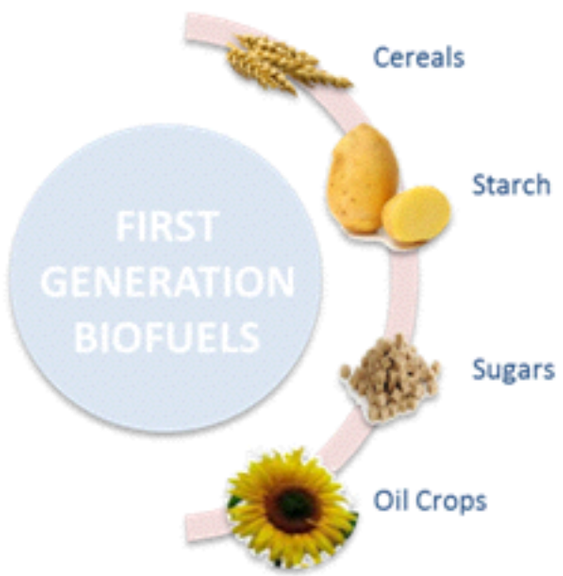
- Cervarix, HPV-16/18 L1 AS04 vaccine contains recombinant C-terminally truncated major capsid L1 proteins of HPV types 16 and 18 as active ingredients

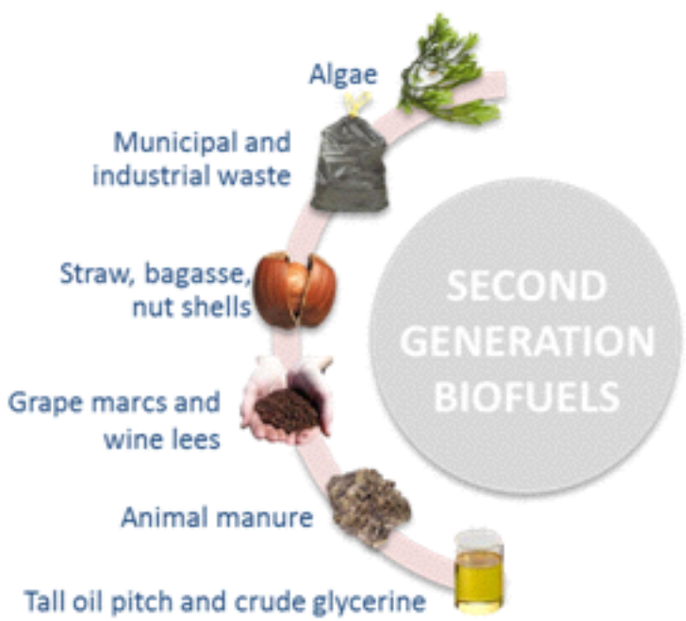
- The first vaccines for humans from Baculovirus Expression System



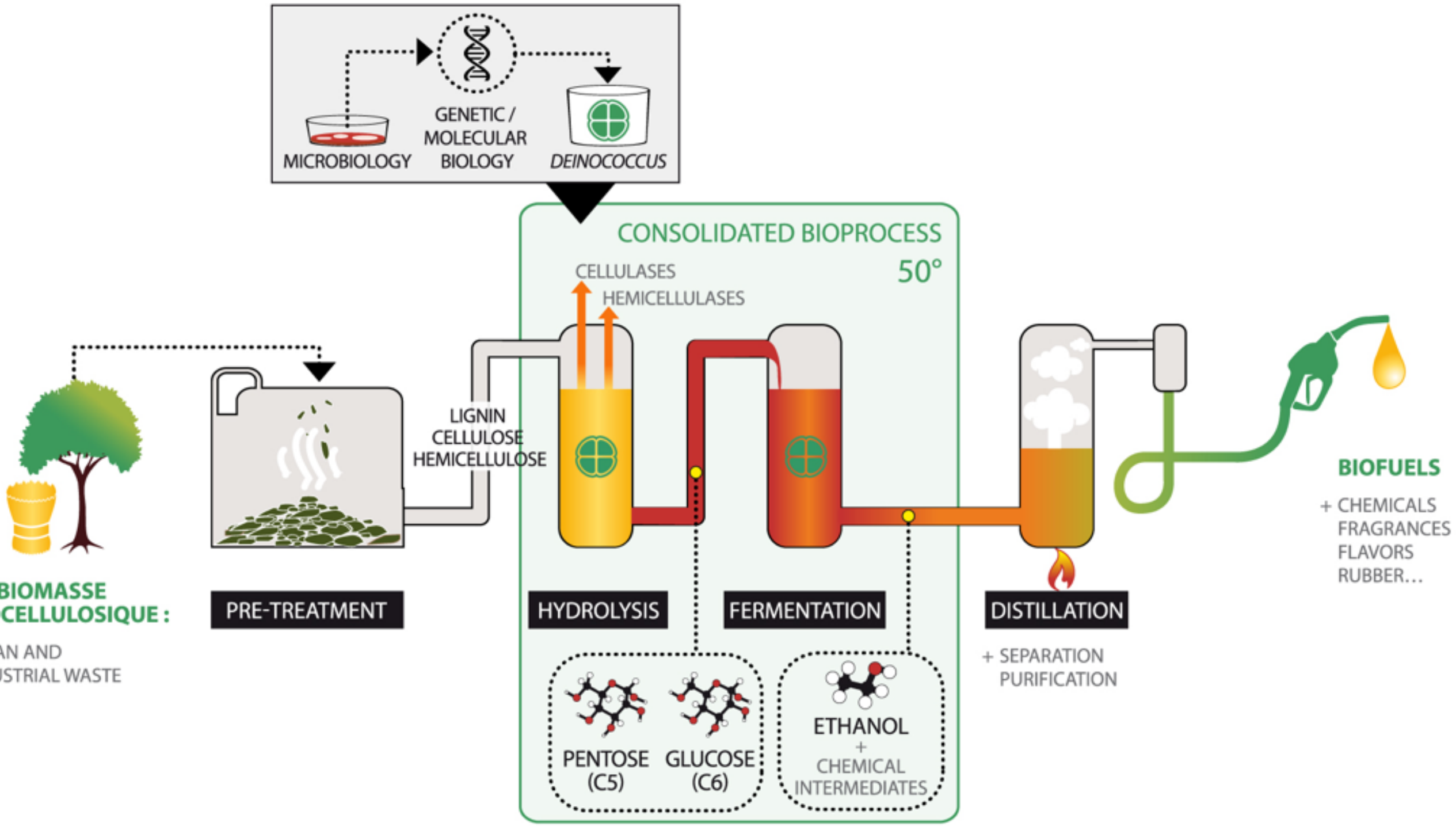
Sugar cane to ethanol





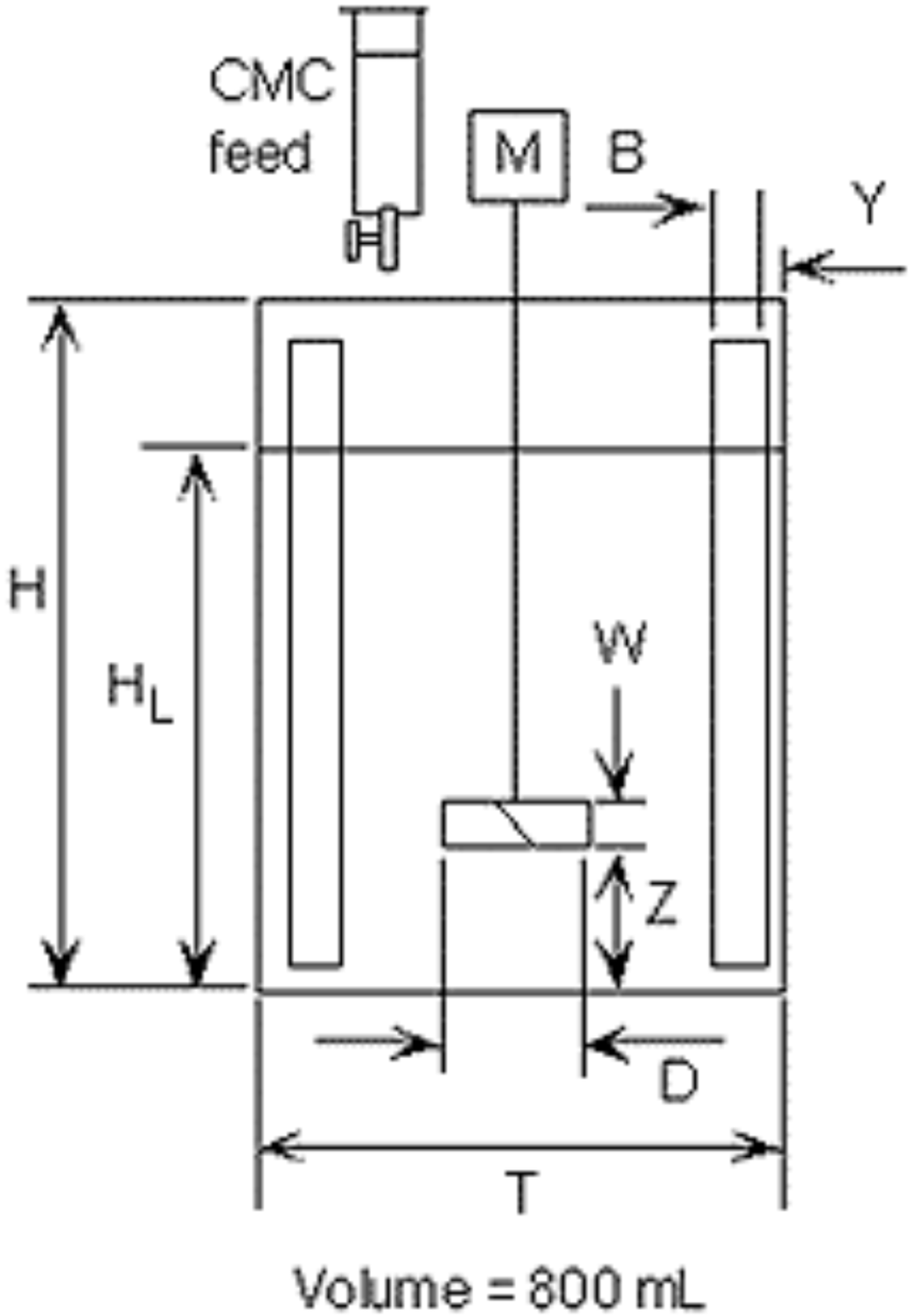


Second generation



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_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”

http://www.housatonicrods.com/rivrpics_2007.html

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) $C_{20}H_{13}N_3O_3$ | 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_2O: 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**	0

Bulk orders?

ADD TO CART

Protein-Protein Interaction Webinar Series

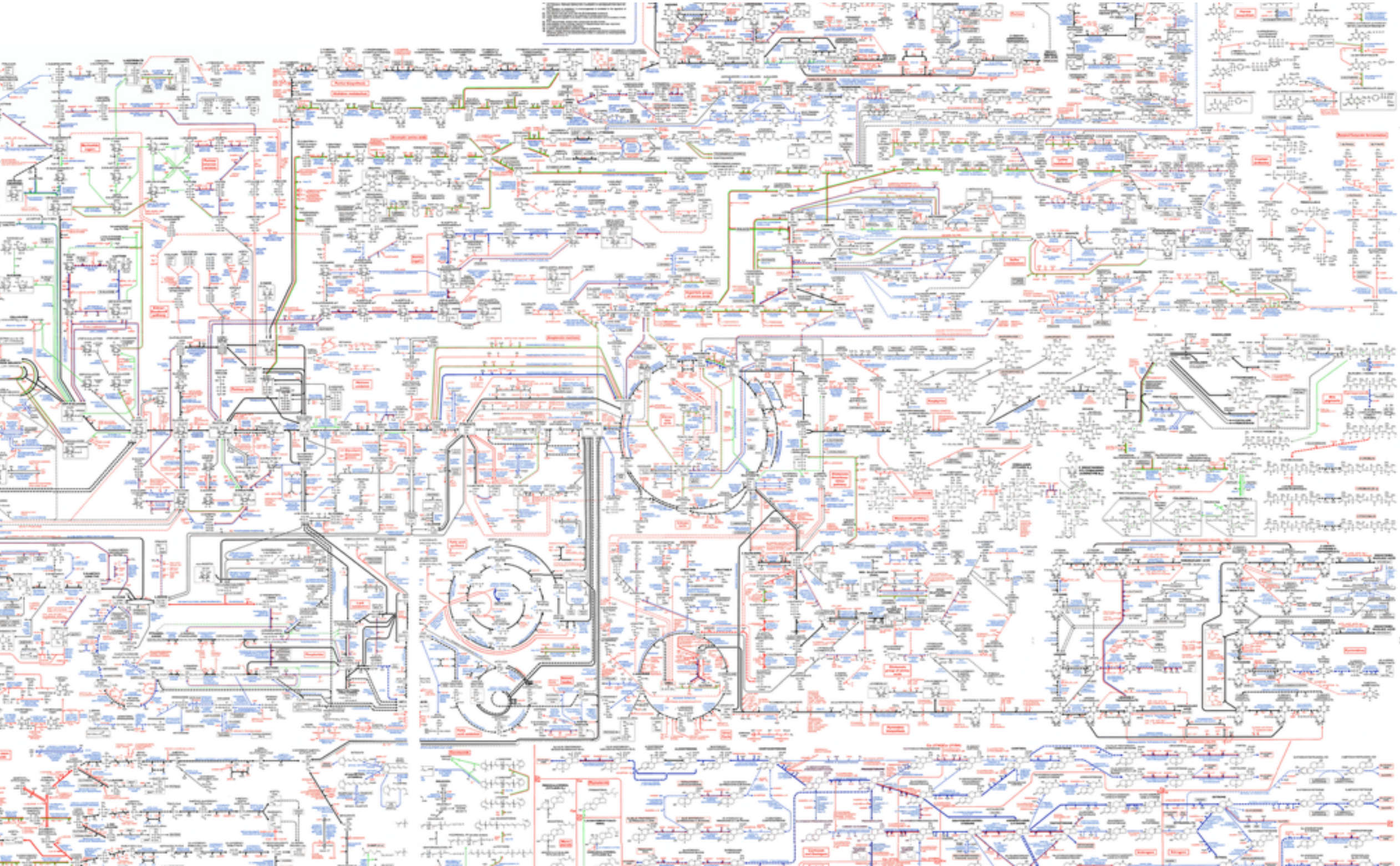
Janthinobacterium lividum

Wikipedia tells me:

- Gram negative
- Aerobic

Violacein production from glycerol

Production pathway?



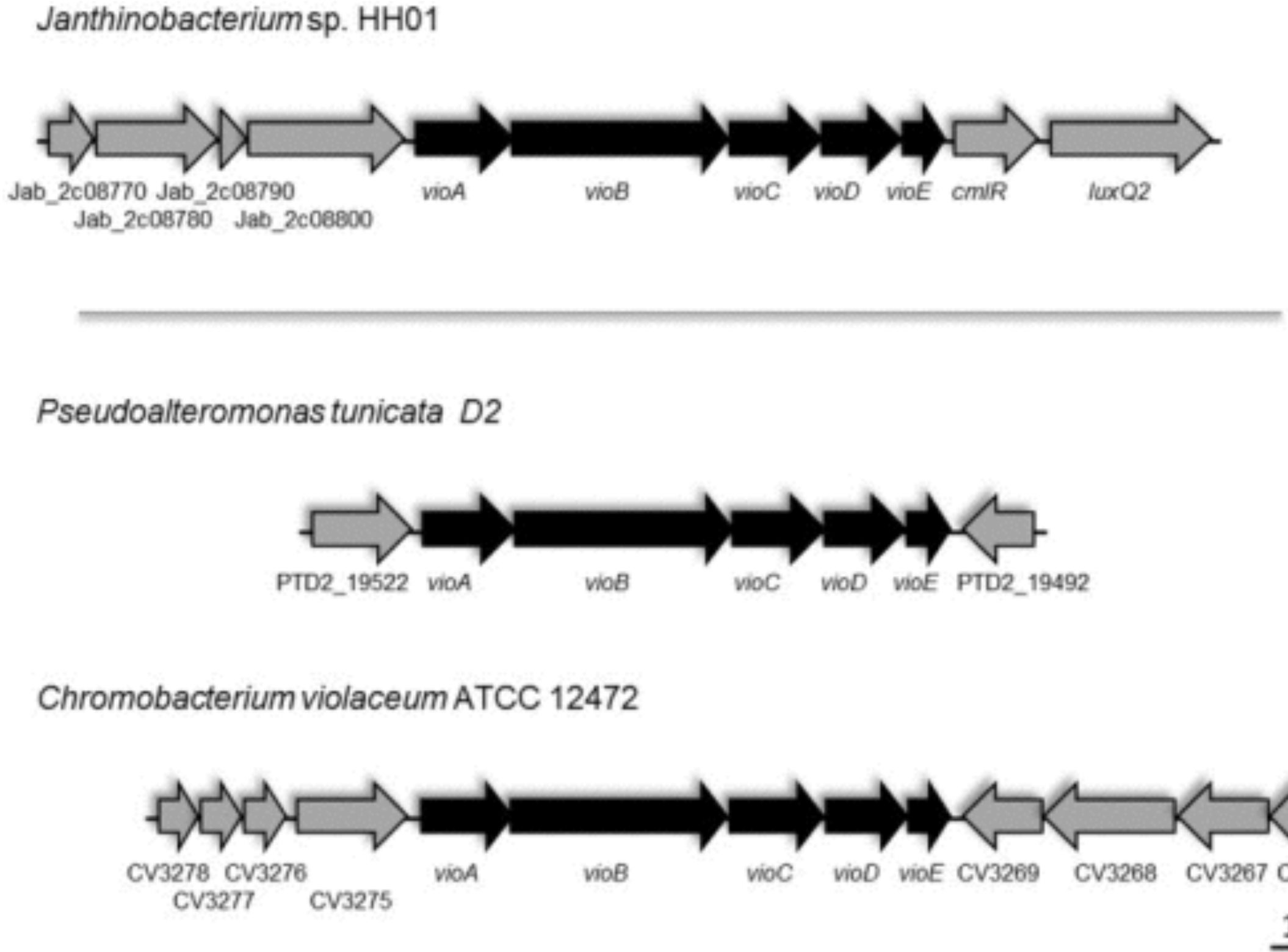
P. Roqueforti eating lactate

$$C_3H_5O_3^- + 3O_2 + H^+ -> 3CO_2 + 3H_2O$$

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)



Production pathway?

Tryptophan

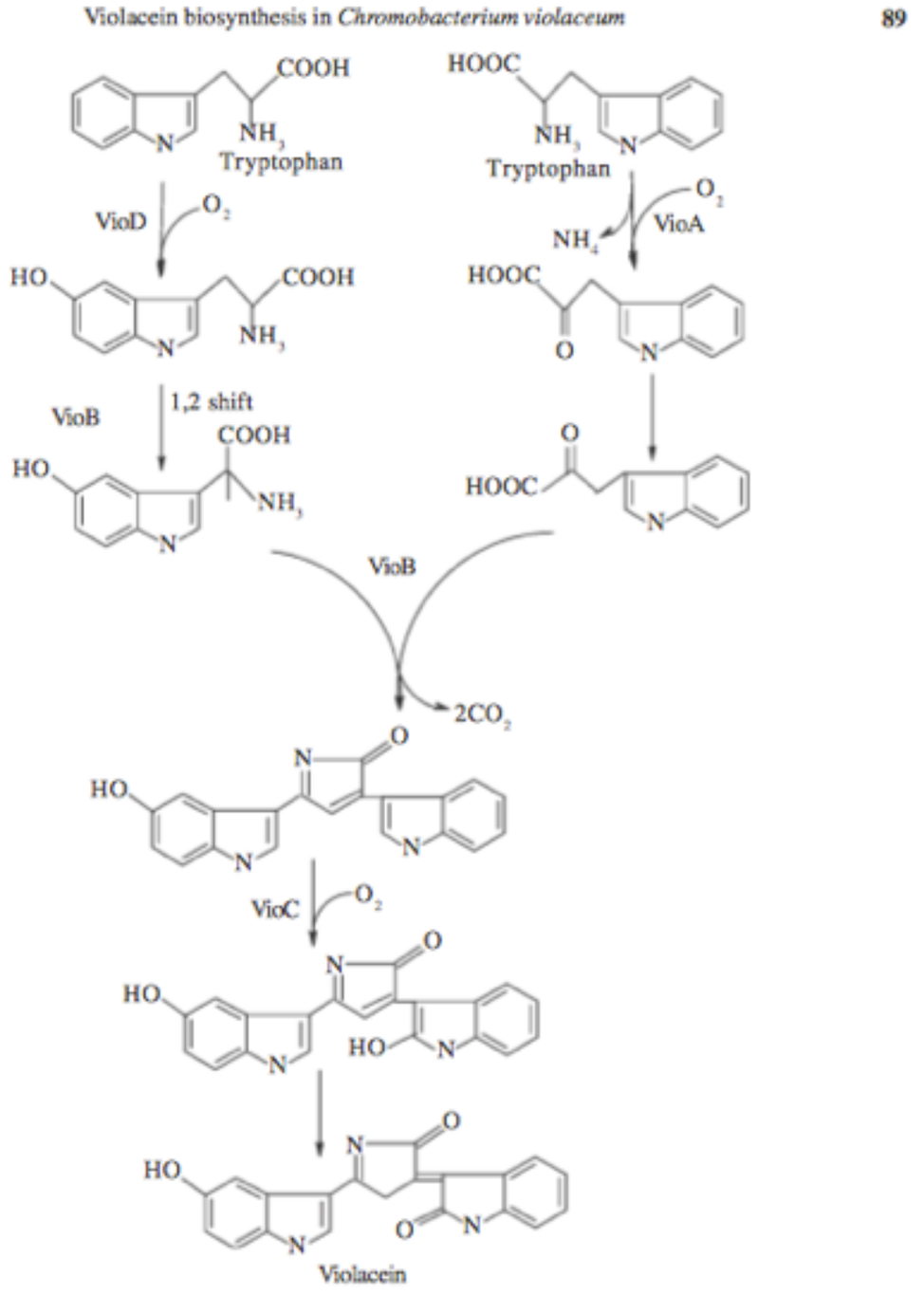


Figure 2. Violacein biosynthesis, as proposed by August et al., 2000. VioA, VioB, VioC, and VioD are the gene products of the biosynthesis operon, encoding nucleotide-dependent monooxygenases and a protein similar to a polyketide synthase (VioB).

Antonio, R.V. and Creczynski-Pasa, T.B. (2004) Genetics analysis of violacein biosynthesis by C. violaceum. Genet Mol Res 3, 85-91.

Other interesting things:

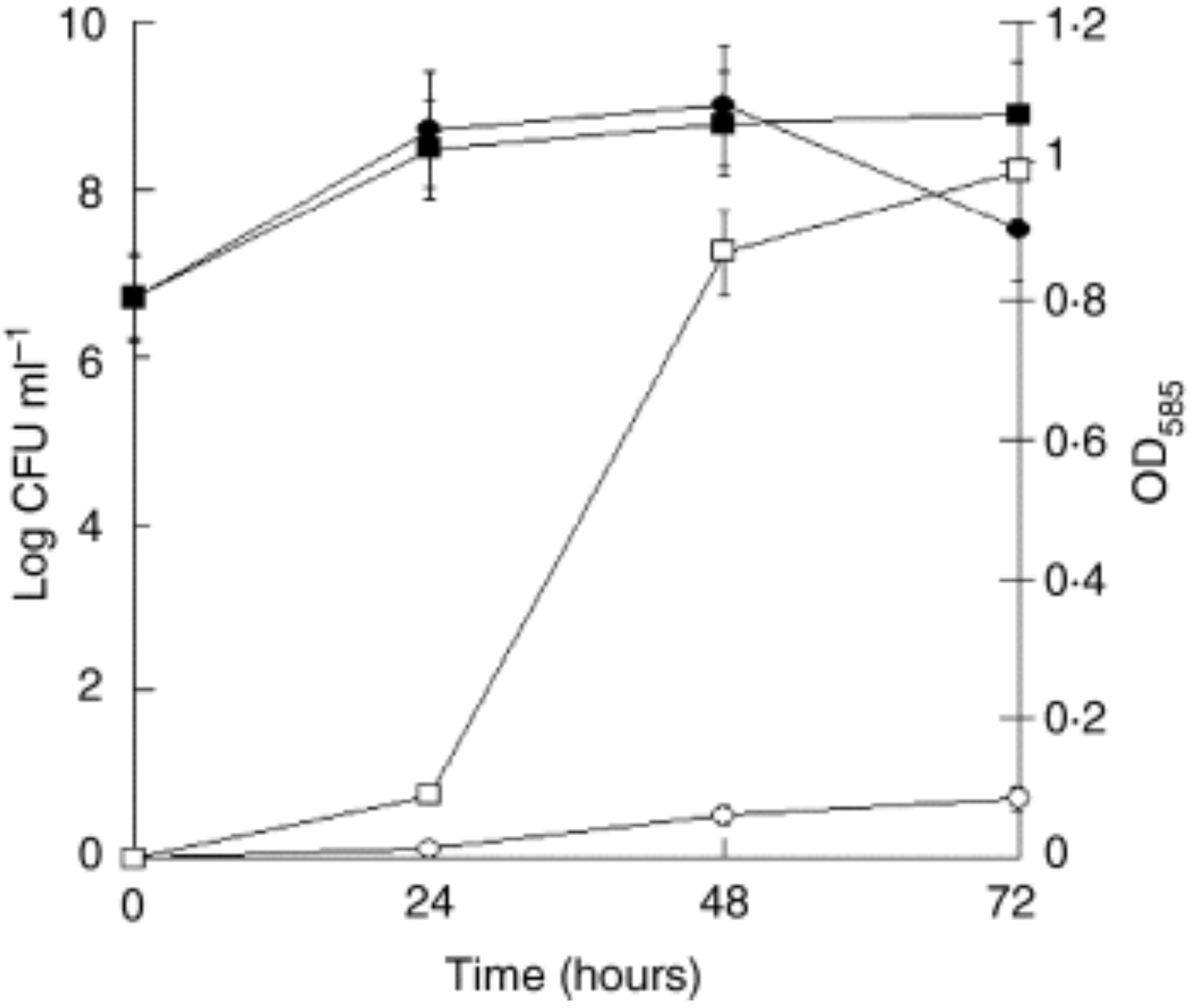
- *J. lividum* produces a metallo-*β*-lactamase conferring resistance to several *β*-lactam antibiotics

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 bioassey for N-acyl homoserine lactone autoinducers. J Microbiol Methods 40, 47–55.

Production inhibition



Pantanella, F., Berlutti, F., Passariello, C., Sarli, S., Morea, C. and Schippa, S. (2007), Violacein and biofilm production in *Janthinobacterium lividum*. Journal of Applied Microbiology, 102: 992–999. doi: 10.1111/j.1365-2672.2006.03155.x

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 250μM 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 χ 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 lml/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

open wetlab
waag society

Yes

oxigen
O_2

No light

temp.

15

volume

330 ml

absorbance

585

species

pH

?

time

48h

stirrer

No

mass

KG

1 mL?

input

C

N

P

O_2

S

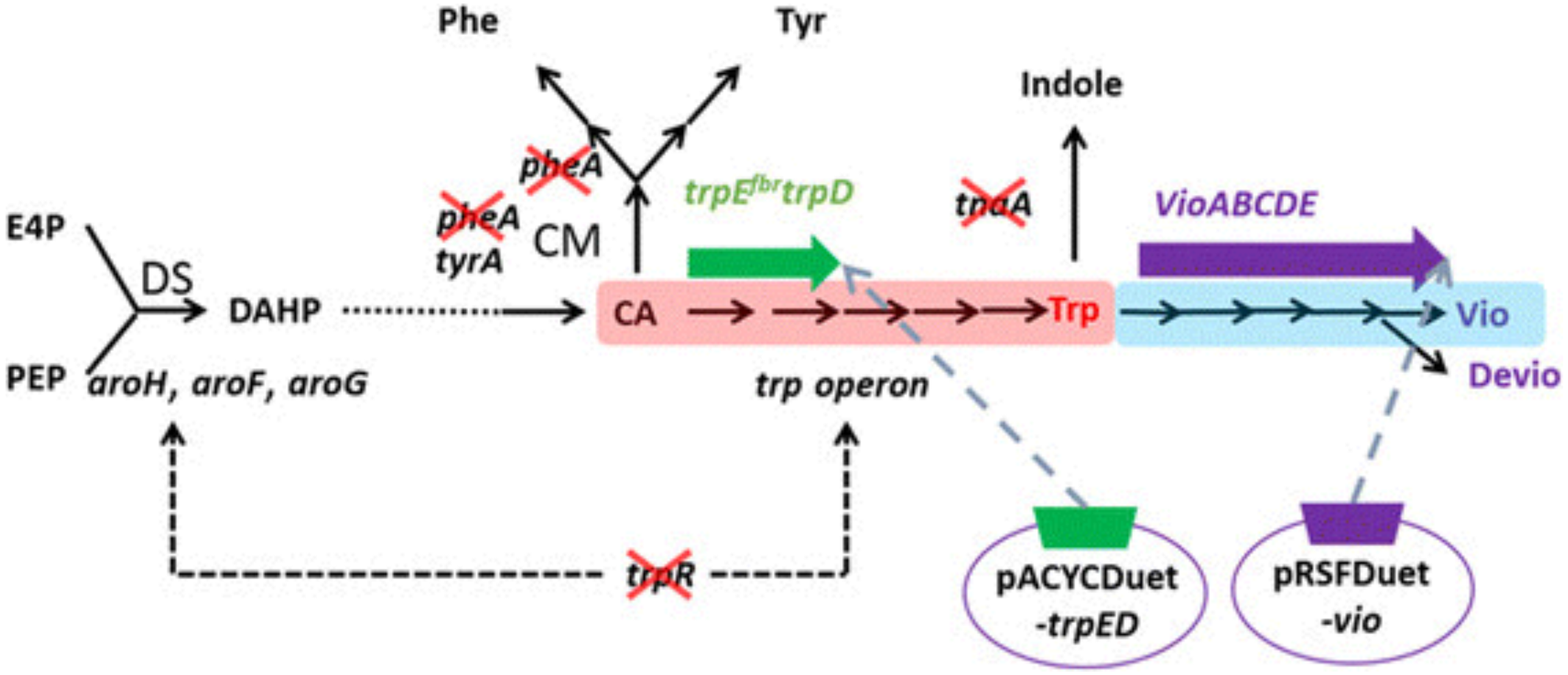
Nutrient Broth
Glycerol
Tryptoph.

observations

day #	
day #	
day #	
day #	

material

Genetic construct for E. coli



Fang et al. Microbial Cell Factories 2015 14:8 doi:10.1186/s12934-015-0192-x

Synbiota - ScienceHack

Synbiota™

violacein factory
Genomikon

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4/8/14

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