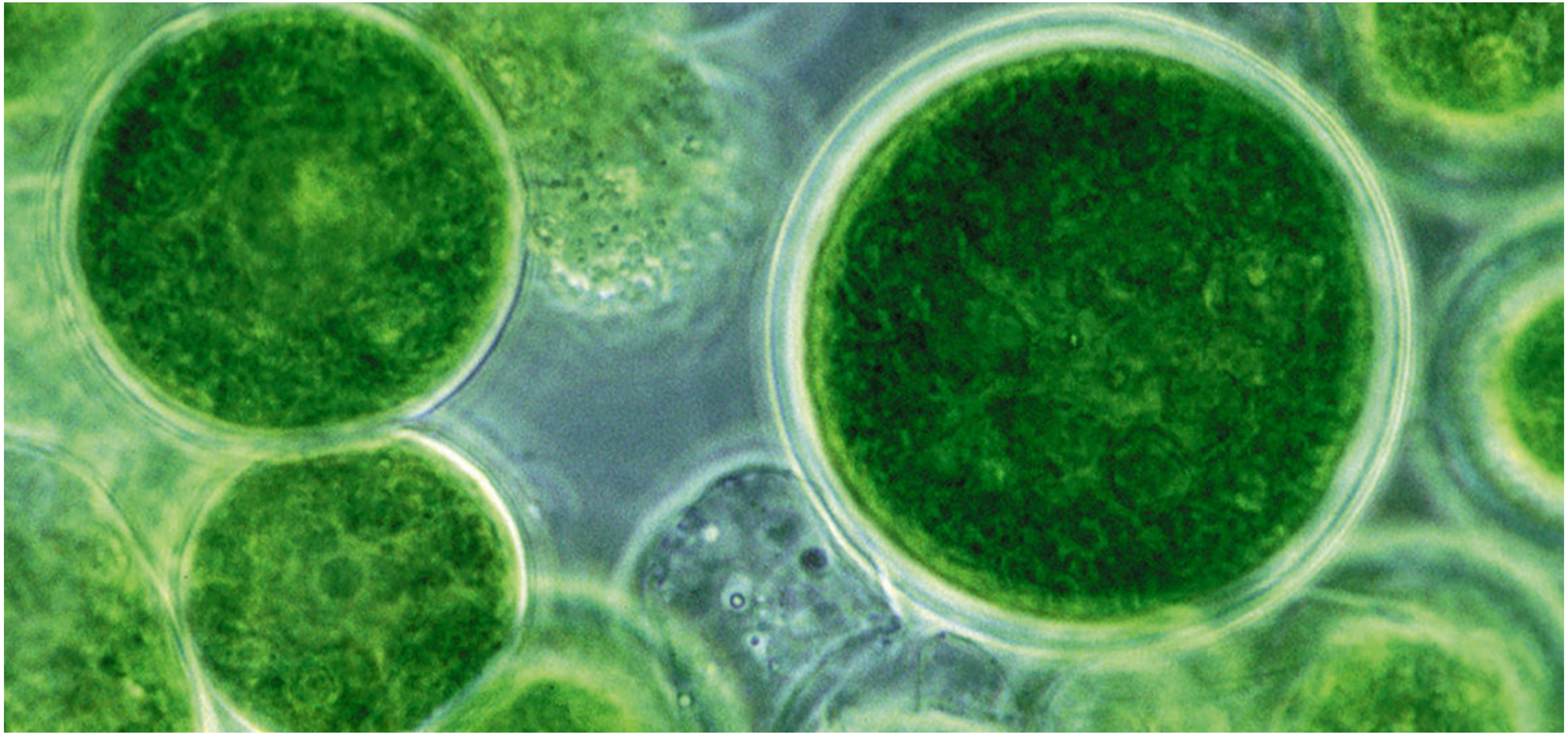
VALORIZATION OF GAS FERMENTATION ACETATE-RICH STREAM INTO VALUABLE MICROALGAL BIOMASS

G.Proietti Tocca, F. Regis, S. Fraterrigo Garofalo, V. Agostino, B. Menin, T. Tommasi, D. Fino



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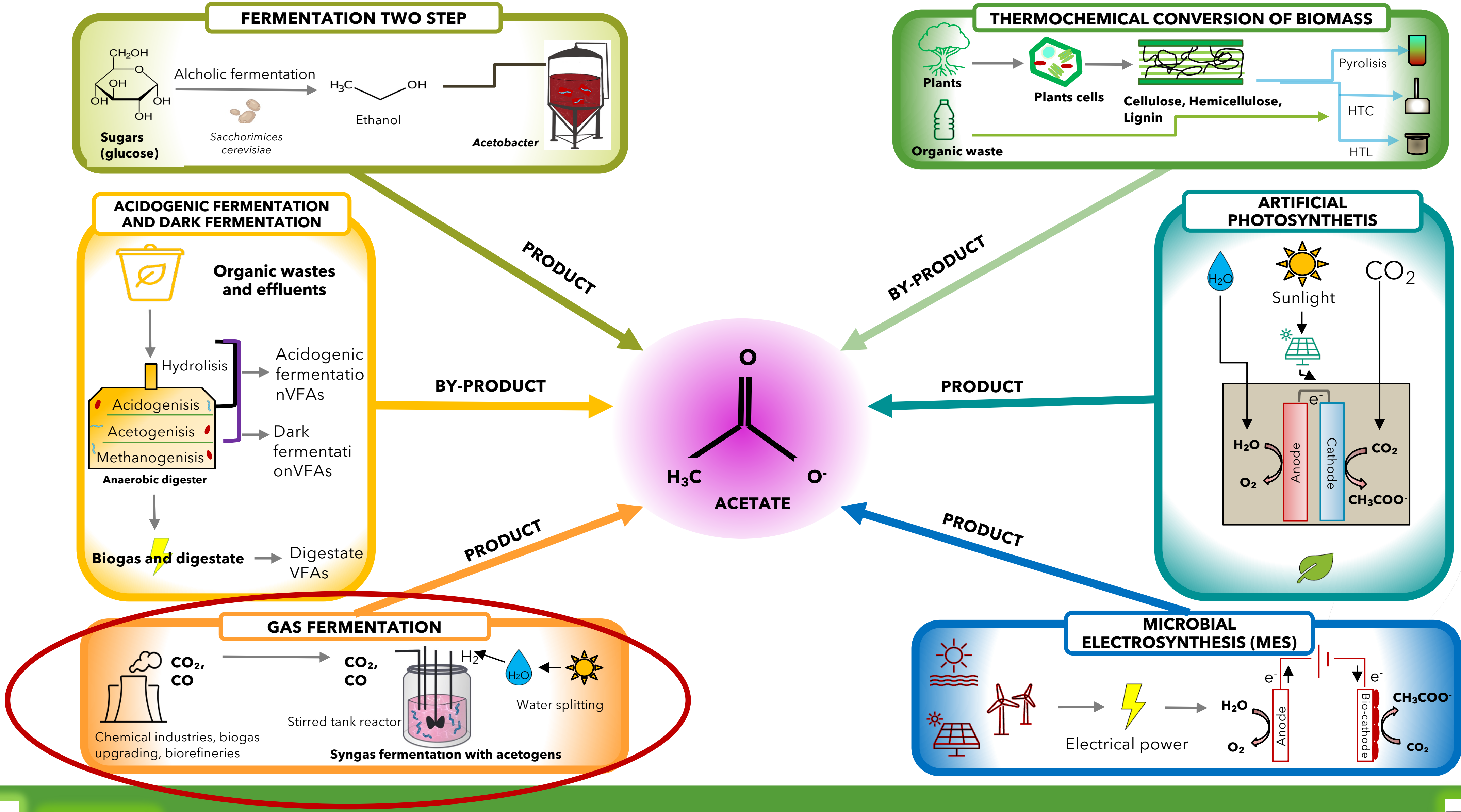
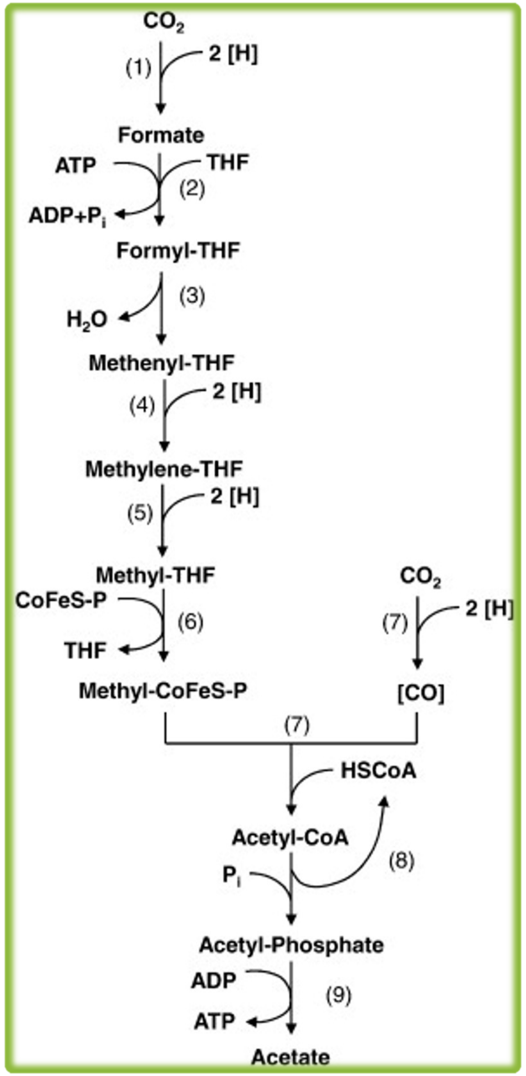
ACETATE IS A PRODUCT OR BY-PRODUCT OF DIFFERENT BIOTECHNOLOGICAL PROCESSES



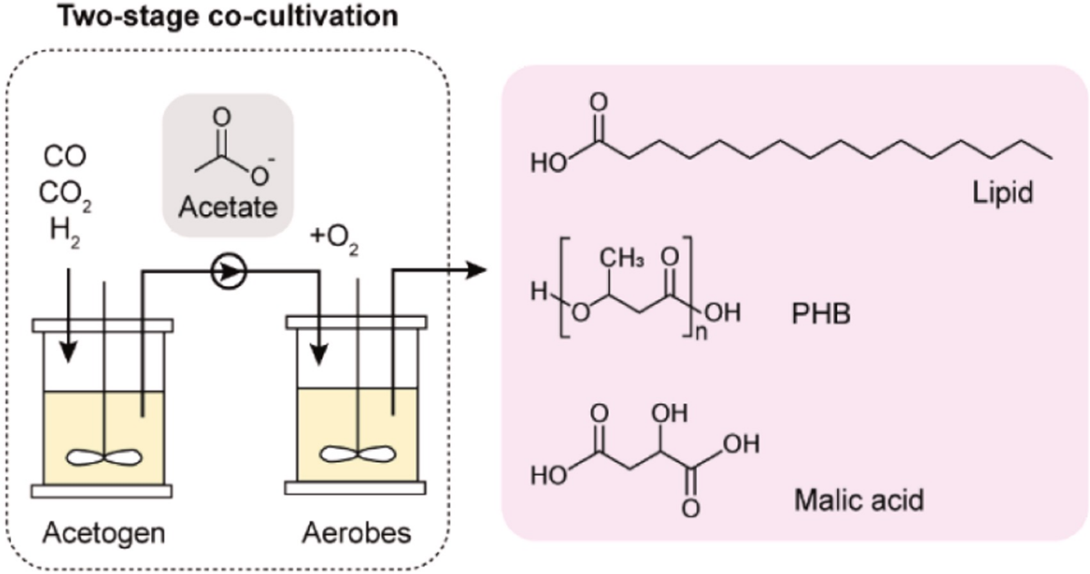
GAS FERMENTATION WITH ACETOGENS – CHALLANGES AND LIMITS



Wood–Ljungdahl pathway of CO_2 fixation

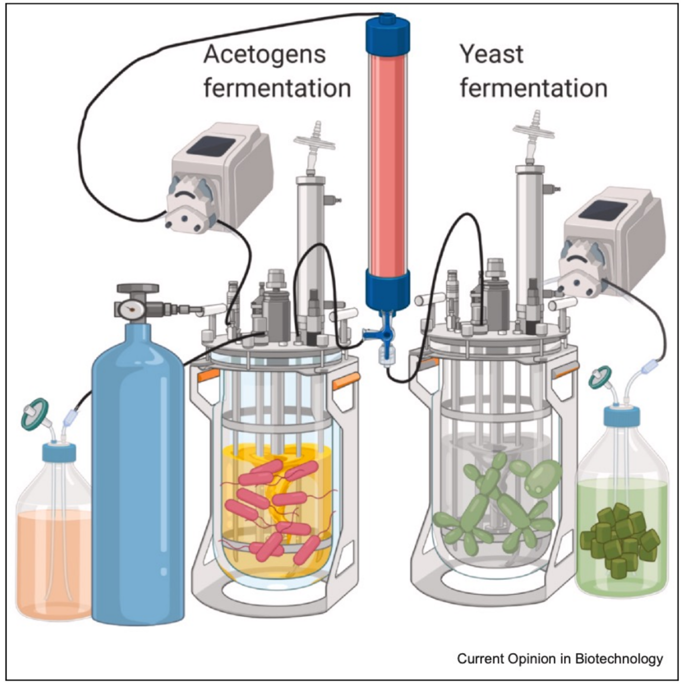
Limits in using acetogens as biocatalysts:

1. PRODUCTS VARIETY;   2. LOW ECONOMIC VALUE OF ACETATE.



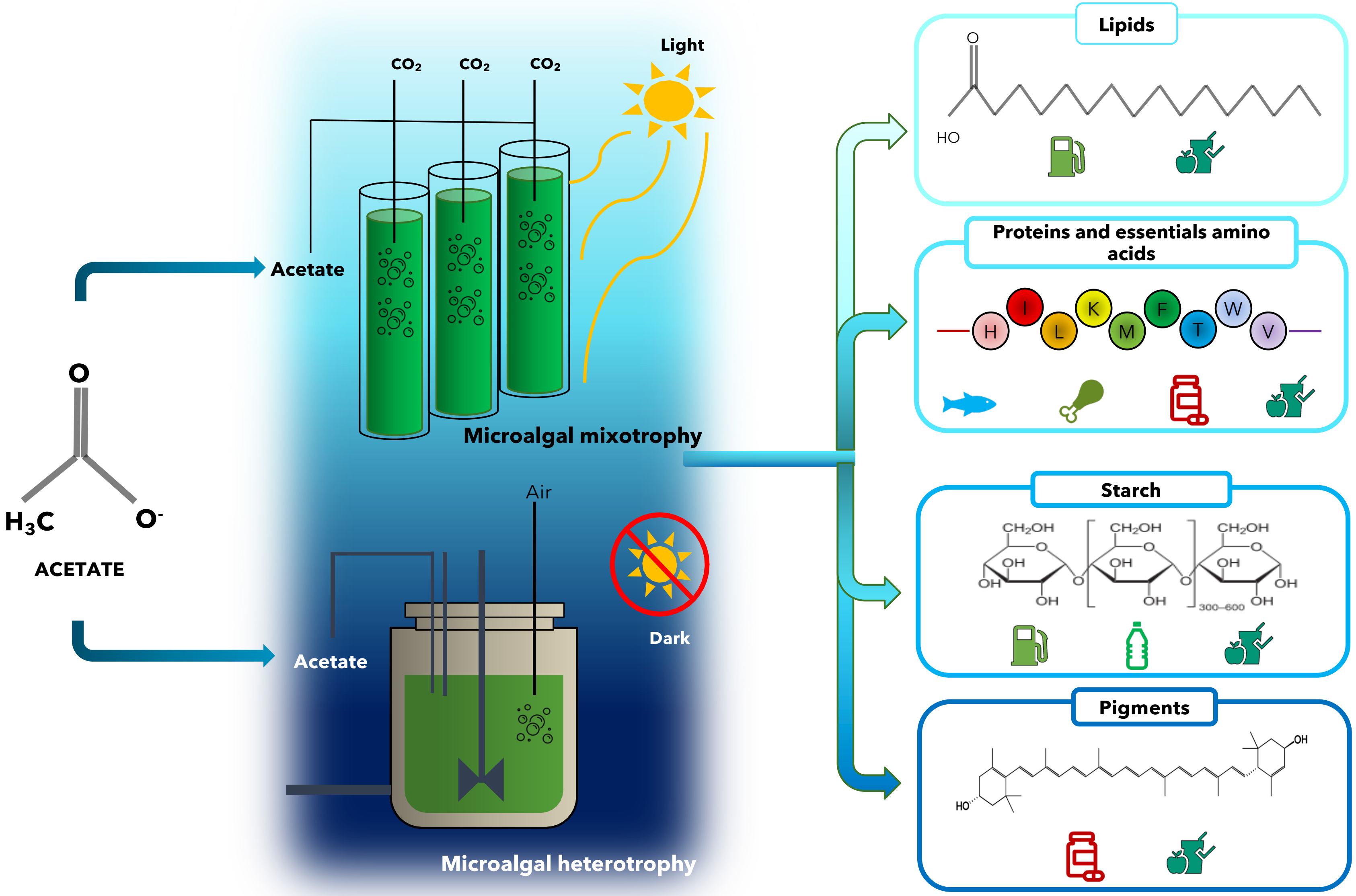
- Two fermenters in series (different culture conditions)
- Acetate from C1 gases has been utilized by *Saccharomyces cerevisiae*, *Yarrovia lipolytica*, *Ralstonia eutropha* etc.

Valorization of C1 gases to value-added chemicals using acetogenic biocatalysts
Jiyun Bae, Yoseb Song, Hyeonsik Lee, Jongoh Shin, Sangrak Jin, Seulgi Kang, Byung-Kwan Cho

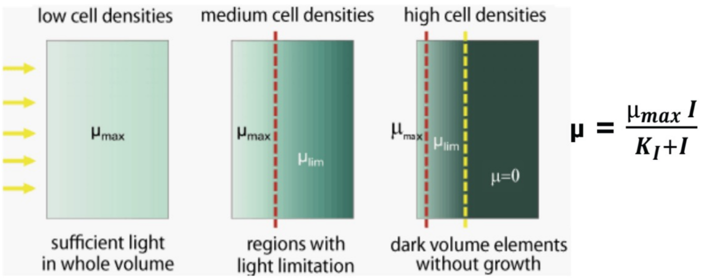


Recycling carbon for sustainable protein production using gas fermentation - Esteban Marcellin, Largus T Angenent, Lars K Nielsen and Bastian Molitor

MIXOTROPHIC AND HETEROTROPHIC MICROALGAL GROWTH USING ACETATE AS CARBON SOURCE



- Advantage compared to photoautrophic cultivation: **higher biomass productivity and economic sustainability!**



- **Heterotrophy**:
  - complete independence of light;
  - easy sterilization of fermenters (hard using tubular photobioreactor in mixotrophy);
  - valorization of waste and effluent as carbon feedstock;
  - high productivities

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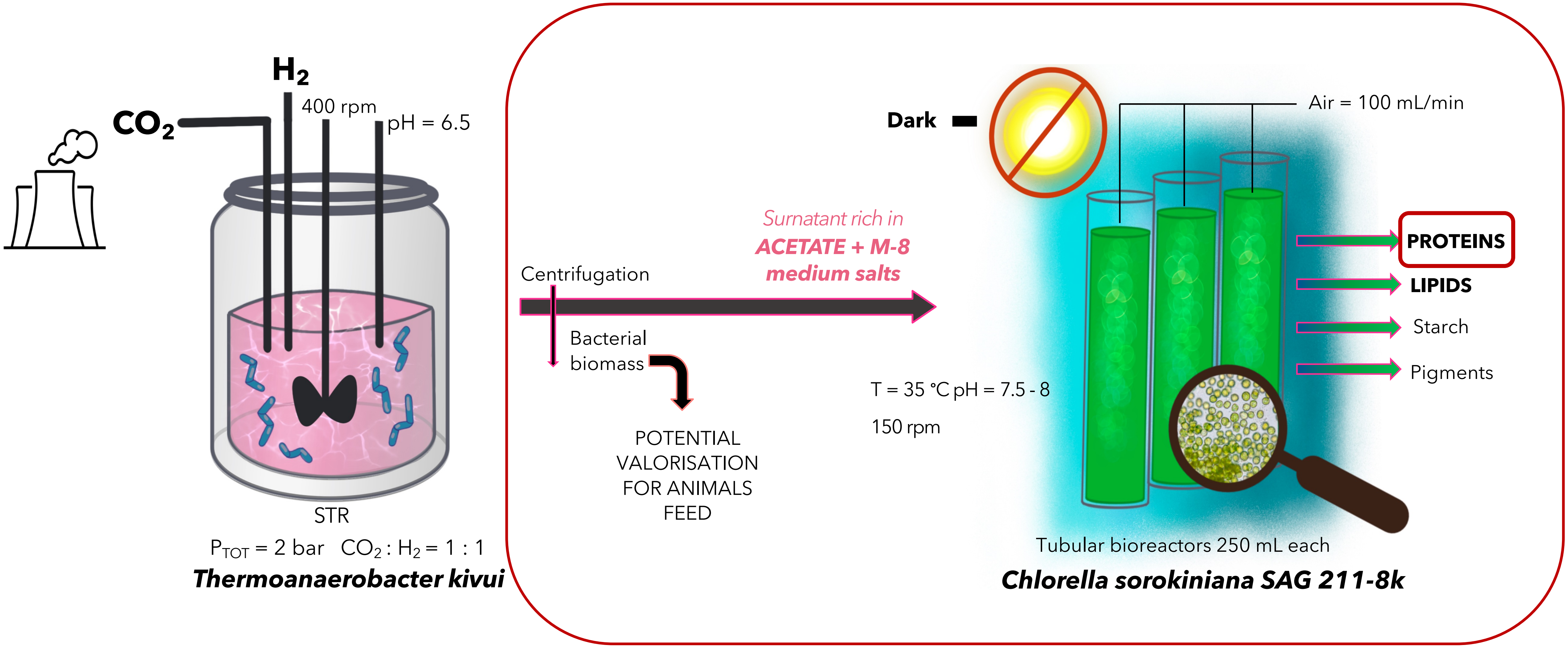
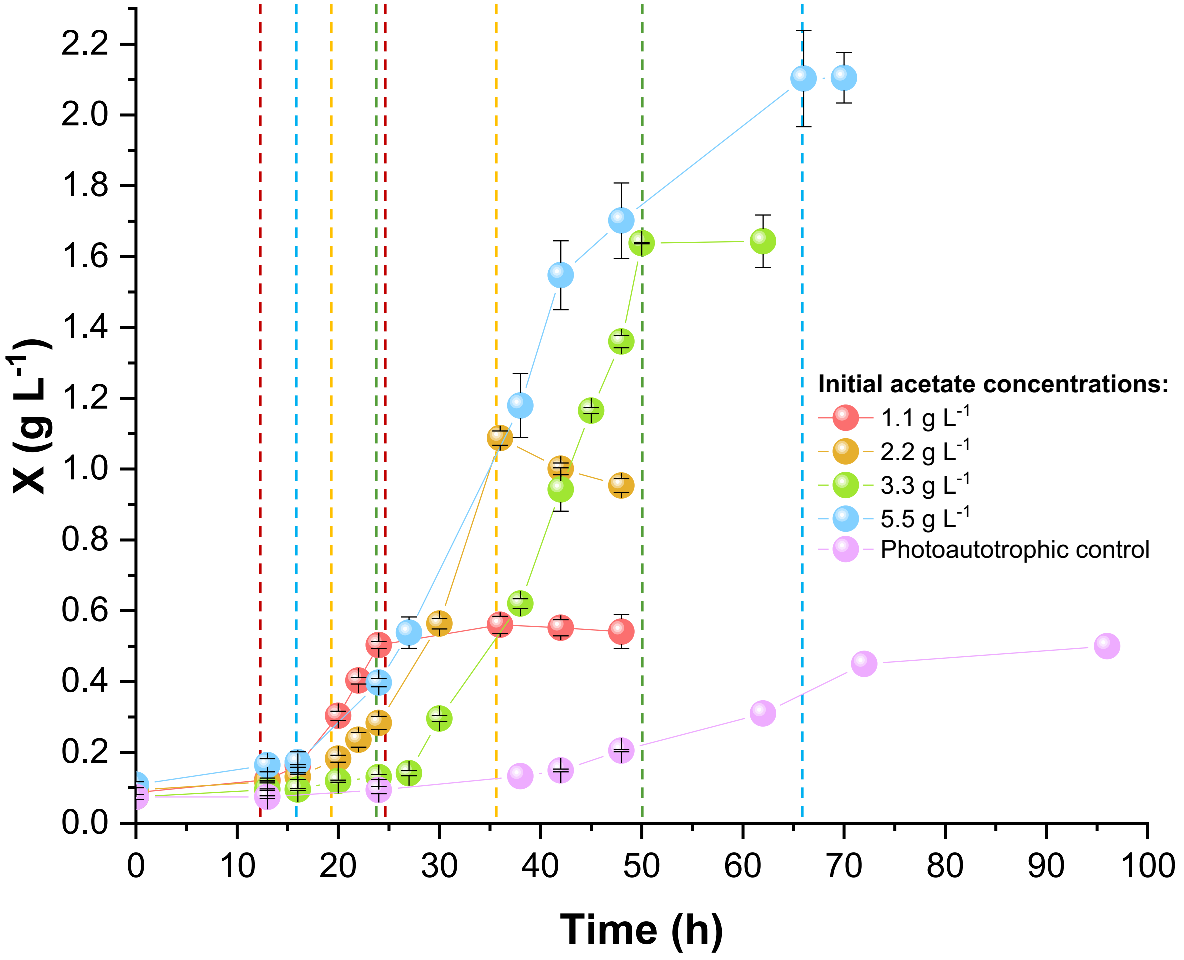
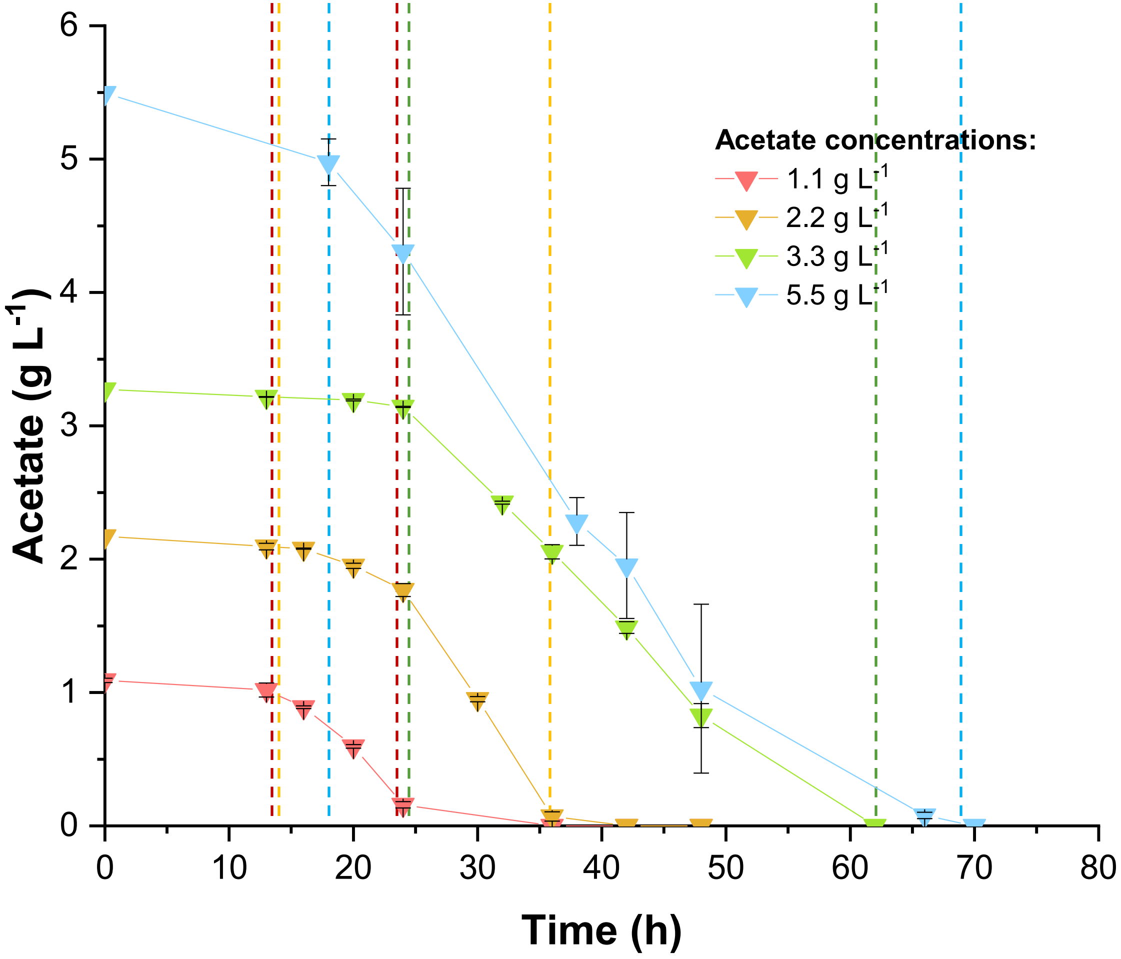
VALORIZATION OF GAS FERMENTATION ACETATE-RICH OUTFLOW INTO VALUABLE MICROALGAL BIOMASS



CHLORELLA HETEROTROPHIC GROWTH ON GAS FERMENTATION ACETATE-RICH EFFLUENT

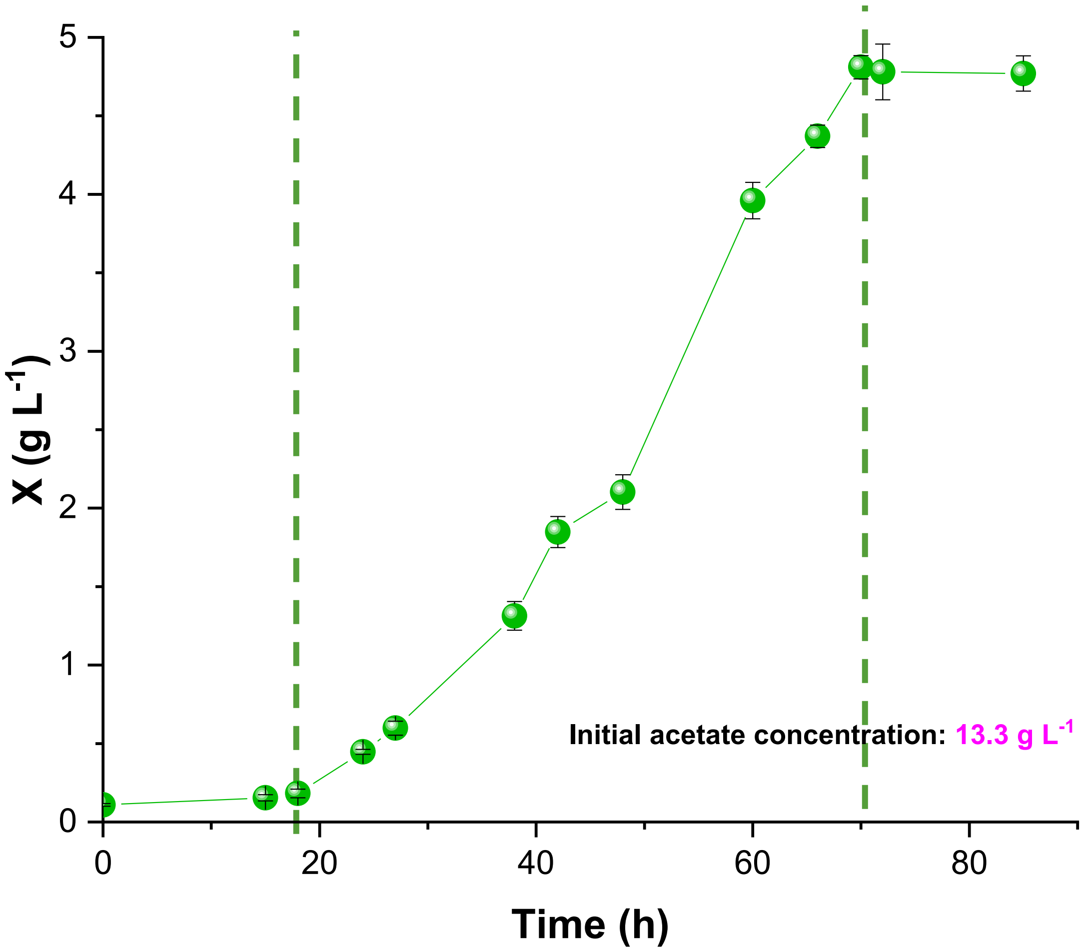
To evaluate microalgal growth, 4 acetate concentrations were tested, diluting the gas fermentation effluent in the M-8 medium.

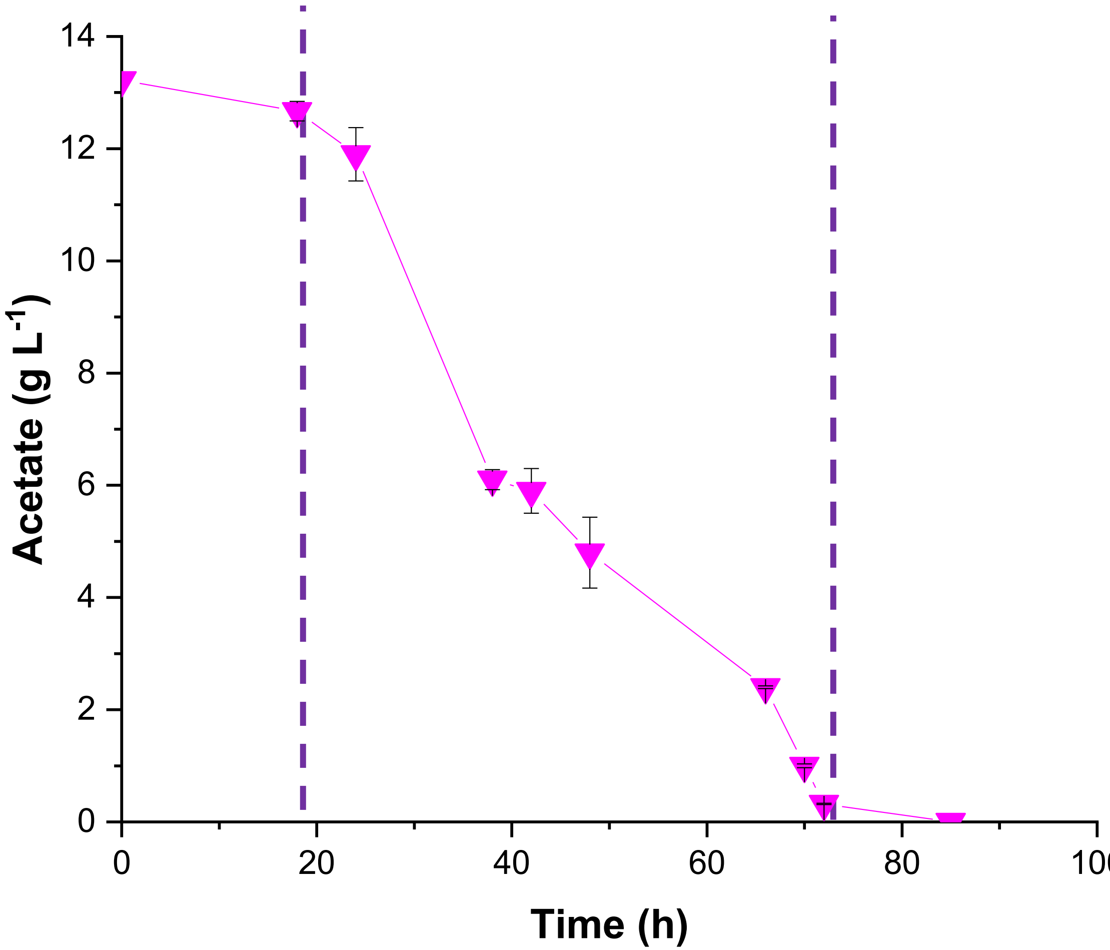




CHLORELLA HETEROTROPHIC GROWTH ON GAS FERMENTATION ACETATE-RICH EFFLUENT

Test using gas fermentation effluent without any dilution (13.3 g L^{-1} of acetate), adding M-8 medium salts directly in the effluent.





PROCESS PARAMETERS AND BIOMASS CHARACTERIZATION

- **Process parameters**

Specific growth rate and duplication time

	$\mu_{MAX}\ (h^{-1})$	$t_d\ (h)$
Heterotrophy	0.1153 ± 0.0187	6.12 ± 0.86
Photoautotrophy	0.0558 ± 0.005	12.4 ± 0.9

Biomass productivity:

	$r_{END}\ (g\,L^{-1}day^{-1})$	$r_{MAX}\ (g\,L^{-1}day^{-1})$
Heterotrophy (1.1 gL^{-1})	0.227 ± 0.023	0.417 ± 0.009
Heterotrophy (2.2 gL^{-1})	0.429 ± 0.009	0.662 ± 0.013
Heterotrophy (3.3 gL^{-1})	0.607 ± 0.029	0.750 ± 0.001
Heterotrophy (5.5 gL^{-1})	0.684 ± 0.023	0.882 ± 0.052
Heterotrophy (no dilution – 13.3 gL^{-1})	1.32 ± 0.11	1.61 ± 0.12
Photoautotrophy	0.101 ± 0.005	0.189 ± 0.008

- **Biomass characterization:** no differences between the different heterotrophic conditions.

	Proteins (%)	Lipids (%)	Carbohydrates (%)
Heterotrophy	**53.1 ± 1.1**	21.8 ± 3.2	14 ± 2.1
Photoautotrophy	**45.4 ± 2.8**	21.1 ± 1.9	13 ± 3.5

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PROTEINS CHARACTERIZATION: AMINO ACIDS PROFILE

	$(mgAA\ mgProt.^{-1})(\%)$			
	Heterotrophy	Photoautotrophy	Soy[a]	Beef[a]
Histidine*	1.54 ± 0.34	1.63 ± 0.20	1.05	0.6
Isoleucine*	3.47 ± 0.38	3.67 ± 0.45	1.89	0.85
Leucine*	8.22 ± 1.65	8.93 ± 1.91	3.23	1.44
Lysine*	4.15 ± 0.83	2.72 ± 1.4	2.60	1.57
Methionine*	1.28 ± 0.29	1.73 ± 0.41	0.53	0.48
Cysteine	0.2 ± 0.03	0.1 ± 0.03	0.55	0.23
Phenylalanine*	4.01 ± 0.58	5.30 ± 1.0	2.06	0.78
Tyrosine	3.32 ± 0.21	3.65 ± 0.44	1.31	0.64
Valine*	5.45 ± 1.32	6.01 ± 1.11	2.01	0.89
Threonine*	4.24 ± 0.31		1.62	0.81
Aspartic acid	7.67 ± 0.25	7.77 ± 0.31	4.86	1.59
Glutamic acid	9.55 ± 0.61	9.54 ± 1.12	7.77	2.71
Arginine	5.25 ± 0.56	5.56 ± 0.77	3.02	1.12
Glycine	4.68 ± 0.32	5.38 ± 0.69	1.74	0.86
Alanine	7.11 ± 0.23	7.18 ± 0.27	1.77	1.03
Serine	4.19 ± 0.24	4.84 ± 0.33	2.13	0.71

- No significative differences between the microalgal biomass obtained in heterotrophy and photoautotrophy;
- The content of essential amino acids is higher compared to the content of the essential amino acids of common vegetable proteins (soy) and animals proteins (beef).

*Essential amino acids
[a]FAO 1970

PROTEINS CHARACTERIZATION: ESSENTIAL AMINO ACIDS PROFILE

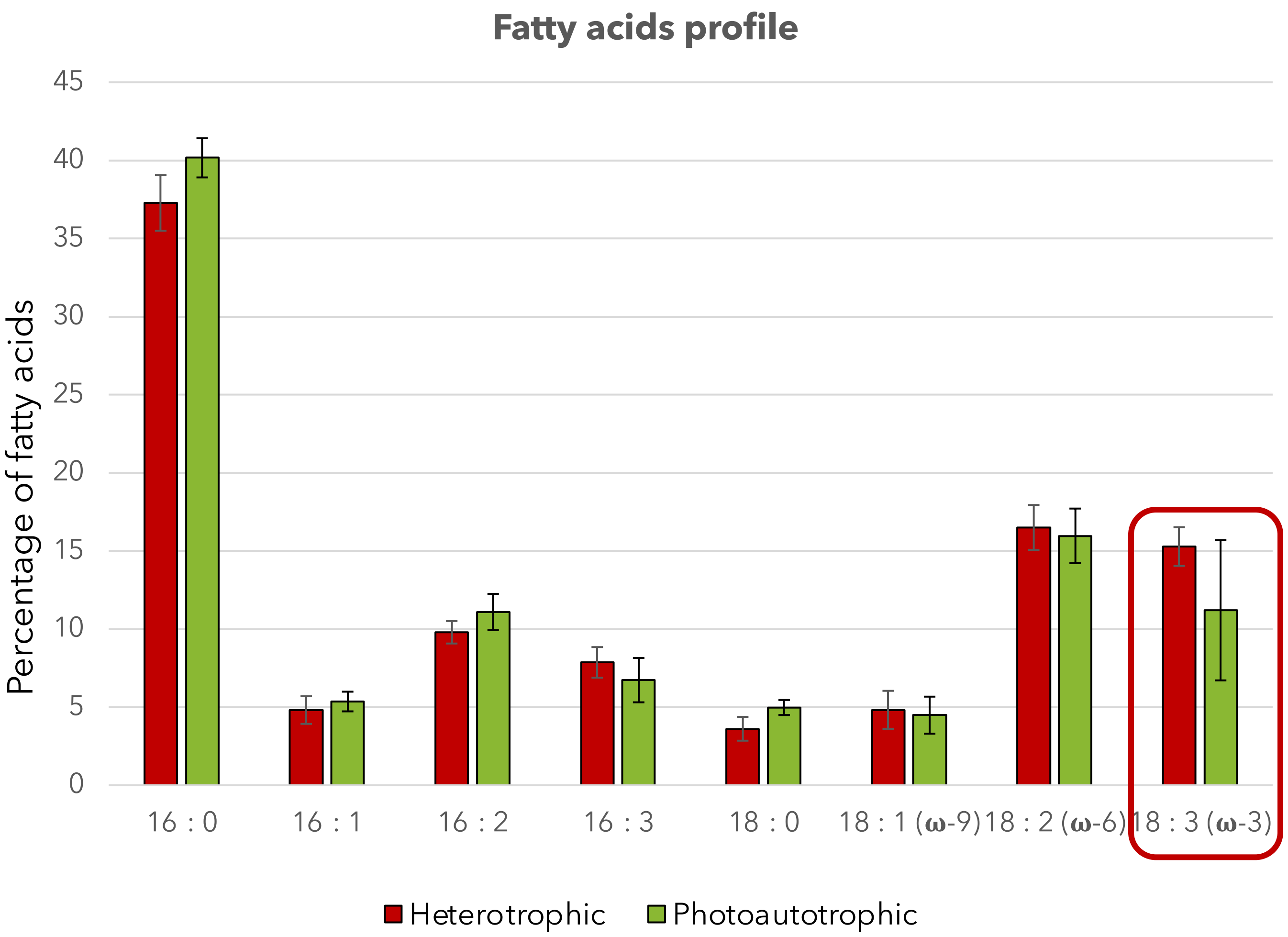
	$mgAA\ gProt.^{-1}$			
	Heterotrophic *Chlorella* (this study)	FAO requirements for human food*	*Spirulina*[a]	Commercial *Chlorella*[a]
Hystidine	**15.4 ± 3.4**	15	10.4 ± 2.4	10.4 ± 2.1
Isolecuine	**34.9 ± 3.8**	30	57.3 ± 5.1	35 ± 5.4
Leucine	**82.2 ± 15.8**	59	92.2 ± 9.1	83.4 ± 8.3
Lysine	**45.5 ± 8.3**	45	53.3 ± 6	45.1 ± 7.4
Valine	**54.5 ± 13.1**	39	56.7 ± 5.3	50.4 ± 5
Threonine	**42.4 ± 3.2**	23	34.1 ± 3.8	29.5 ± 2.8
Sulfuric containing amino acids	**15.4 ± 2.9**	22	16.9 ± 5.3	12.2 ± 4.3
Aromatic amino acids	**53.3 ± 4.4**	38	59.3 ± 13.6	56.9 ± 3.7

Sulfuric containing amino acids: methionine + cysteine
Aromatic amino acids: phenilalanine + tyrosine

***** *Joint WHO/FAO/UNU Expert Consultation, Protein and amino acid requirements in human nutrition, World Health Organ. Tech. Rep. Ser. 35 (2007) 1–265.*

[a] M. Muys, Y. Sui, B. Schwaiger, C. Lesueur, D. Vandenheuvel, P. Vermeir, S. E. Vlaeminck, High variability in nutritional value and safety of commercially available Chlorella and Spirulina biomass indicates the need for smart production strategies, Bioresour. Technol. 275 (2019) 247–257

LIPIDS CHARACTERIZATION: FATTY ACIDS PROFILE



	Heterotrophy	Photoautotrophy
Total lipid (%)	21.8 ± 3.2	21.1 ± 1.9
Saturated fatty acids (%)	40.9 ± 2.5	45.2 ± 1.8
Unsaturated fatty acids (%)	59.1 ± 1.4	54.8 ± 2.7
Polyunsaturated fatty acids (%)	49.5 ± 1.2	45.1 ± 1.1

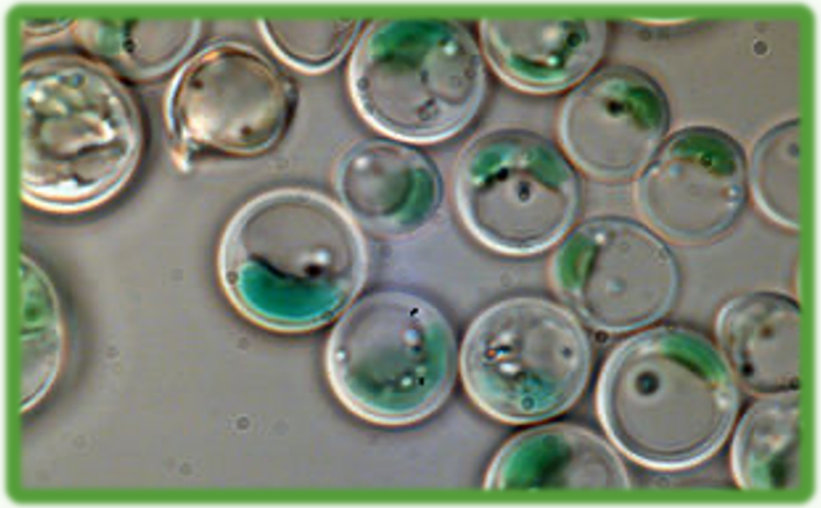
CONCLUSIONS

- *Chlorella sorokiniana* **can grow heterotrophically on real gas fermentation effluent** using acetate as carbon source also at high concentrations, observing **the complete conversion of acetate into valuable microalgal biomass;**
- The heterotrophic **specific growth rate and the biomass productivity are high and satisfactory**, especially when compared to the photoautotrophic ones;
- The heterotrophic biomass is **rich in proteins and essential amino acids**: the amount of them **overcome the FAO requirments**;
- The heterotrophic biomass is **rich in polyinsaturated fatty acids**, including good percentage of *alpha-linolenic acid* omega-3;
- The high heterotrophic biomass quality is confirmed by the **absence of significative differences with the photoautotrophic biomass**; this results indicates also an absence of contamination.

NEXT STEPS

Mixotrophy using acetic acid

Continuing the acetic acid valorisation for mixotrophic cultivation of *Galdieria sulphuraria*



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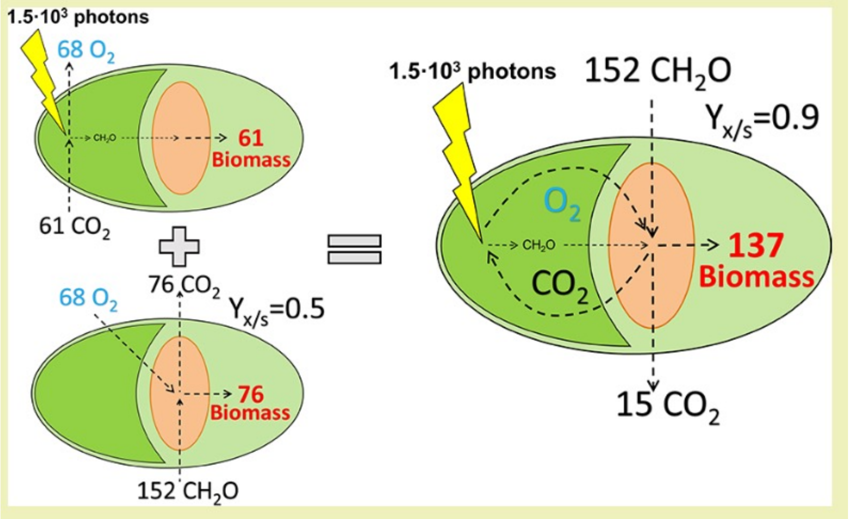

Doubling of Microalgae Productivity by Oxygen Balanced Mixotrophy
Fabian Abiusi, Rene H. Wijffels, and Marcel Janssen

Mixotrophy guarantees higher biomass yields: all (or almost) the carbon in the organic acids is converted into microalgal biomass, without CO_2 losses.

G. Sulphuraria is the perfect strain to grow mixotrophically:

- It grows at very acid pH (1-2) → avoiding the contamination from competitive or predatory bacteria…

…the sterilization of the photobioreactor is not necessary.

THANK YOU FOR YOUR KIND ATTENTION

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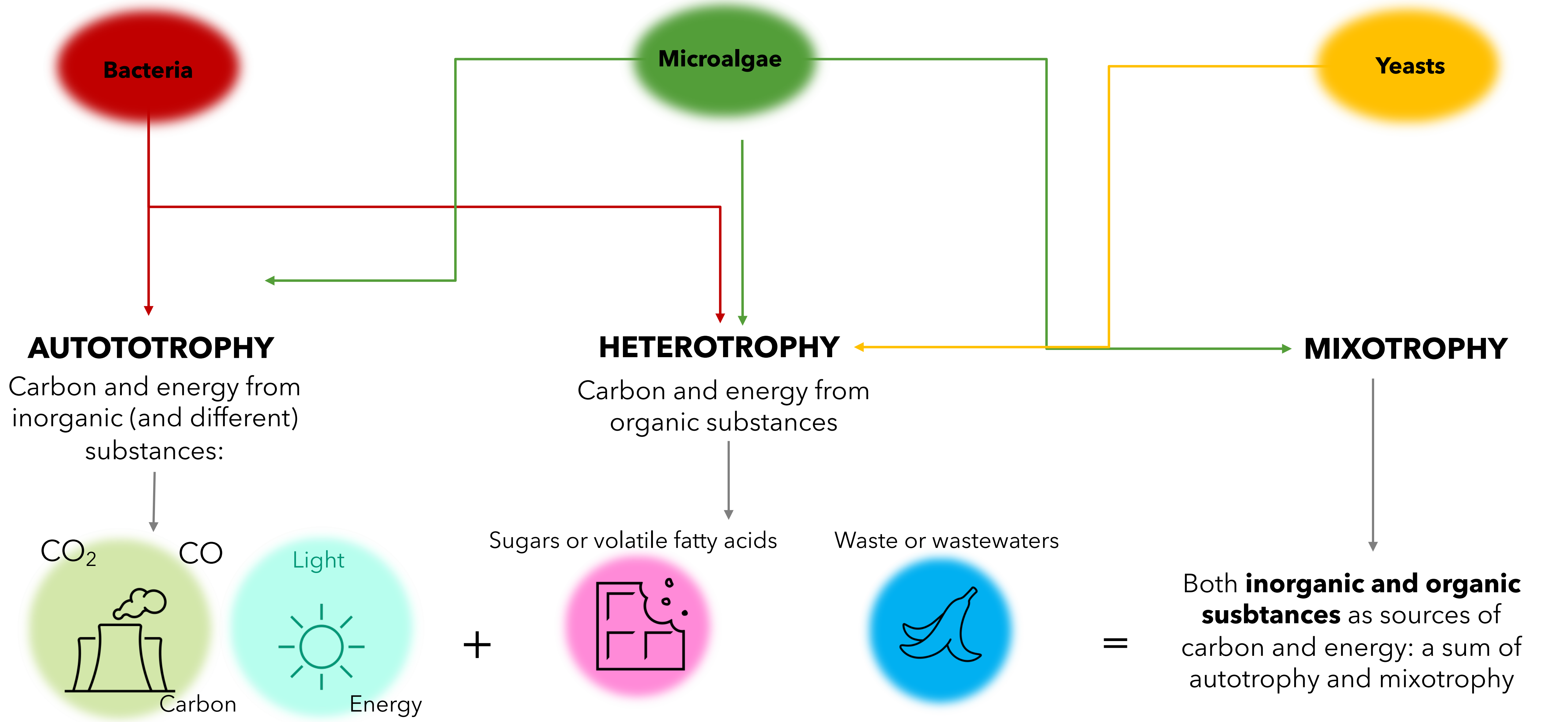
APPENDAGES

BIOTECHNOLOGICAL PROCESSES EXPLOIT MICROORGANISMS AS BIOCATALYSTS



ACETATE-RICH STREAMS FROM DIFFERENT PROCESSES

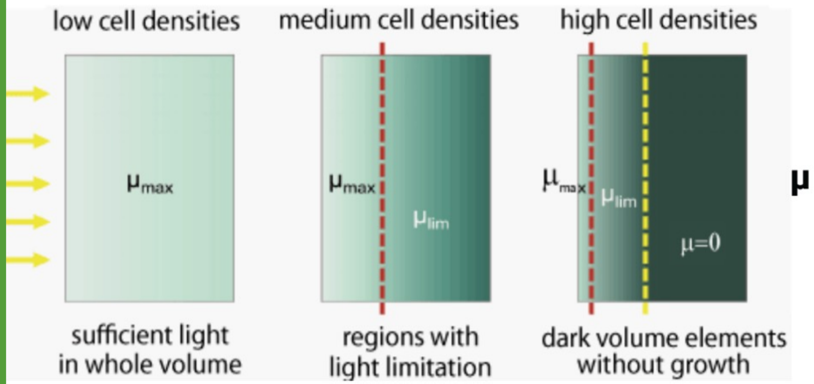
Process	Acetate - product type	Chemical composition	Production cost (€/kG)	References
Methanol carbonylation	Main Product	Pure acetic acid	0.32	https://doi.org/10.1021/acs.est.6b02101
Aerobic fermentation	Main Product	Acetic acid 66 - 106 g L^{-1} Other potential compounds: propanoic acid, butyric acid, butanone, ethyl acetate, formic acid, microbial biomass, inorganic salts, colloids, CO_2, organic noneloctrolytes	0.85 - 1.45	https://doi.org/10.3311/PPch.11004; DOI: 10.1080/15422119.2016.1185017
Pirolysis	By-product (acqueous phase of bio-oil)	Acetic acid: 5 - 157 g L^{-1} Other potential compounds: glycoaldheyde 10-137 g L-1, acetol 26-86 g L-1, levoglucosan 30 - 65 g L-1, other minor organic compounds (propanoic acid, nonaromatic aldehydes, furans, formic acid, acetone, formaldhheide)	//	DOI: 10.1002/bbb.2273; doi:10.1016/j.fuproc.2007.05.002
Hidrotermal treatments (HTC and HTL)	By-product: residual process water	Acetic acid: 0.7 - 33 g L^{-1} Other potential compounds: formic acid 0.13-2.45 g L-1, 1 - 4.5 g L^{-1} lactic acid, propionic acid 0.14 - 0.42 g L-1, glycolic acid 1.57 - 6.82 g L^{-1}, levulinic acid 0.37 - 1.44 g L^{-1}, phenols 1.5 g - 4.5 g L-1,	//	http://dx.doi.org/10.1016/j.biombioe.2015.01.011; https://doi.org/10.1016/j.rser.2018.09.008; http://dx.doi.org/10.1016/j.biortech.2013.05.098
Acidogenic fermentation (AF)	By - product: AF effluents	Acetic acid: 0.3 - 29 g L^{-1} Other potential compunds: butyric acid: 0.33 - 32 g L^{-1} ; propionic acid: 0.2 - 11.7 g L^{-1}; valeric acid: 0.26 - 5.66 g L^{-1}; iso-valeric acid: 0.16 - 21.8 g L^{-1}	//	https://doi.org/10.1007/s11157-021-09566-0
Dark fermentation (DF)	By - product: DF effluent	Acetic acid: 0.06 - 12.2 g L^{-1}. Other potential compunds: butyric acid 0.05 - 14.8 g L-1; propionici acid 0 - 2.8 g L^{-1}; lactic acid 0.1 - 0.9, ethanol 0 - 0.9 g L-1 Other potential nutrients: TN (mainly ammonium) 0.1 - 3.46 g L-1; TP (mainly ortophosphate) 0.02 - 0.38 g L-1	//	doi:10.1016/j.ijhydene.2008.07.057 ; http://dx.doi.org/10.1016/j.procbio.2016.03.018; http://dx.doi.org/10.1016/j.rser.2012.11.030
Anaerobic digestion (AD)	By - product of Liquid Digestate	VFAs: 0.1 - 1.0 g L^{-1} Other potential nutrients: NH4 - N (ammonia nitrogen) up to 12 g L^{-1}; P (phosphorus) up to 5.8 g L^{-1}	//	https://doi.org/10.3390/app11031056; doi:10.1039/c5ee01633a
Gas fermentation (for acetate production)	Main Product	Acetic acid: 1.9 - 59.2 g L^{-1} Other potential compunds: formic acid 1.12 - 4.8 g L^{-1}; ethanol 0.03 - 0-17 g L-1; butyrate 0.05 - 0.14 g L-1; Ions that can be present: Mg^{2+}, Cu2+, Ca2+, Mn2+, Zn2+, Ni2+, Co2+, Fe2+	4.68 (starting from CO)	http://dx.doi.org/10.1016/j.jbiotec.2016.04.032; https://doi.org/10.1016/j.cej.2023.141555; https://doi.org/10.1021/acs.est.6b02101
Bio-electrosynthesis	Main Product	Acetic acid: 0.002 - 11 g L-1. Other potential compounds: 2-oxobutyrate 0.001 - 0.0051 g L-1 ; formic acid 0.00023 - 0.59 g L-1; butyrate 0.046 - 0.74; propionate 0.007 - 0.16; ethanol in traces	1.76	Referenze 6-7-8-13-16 di Bioelectrosynthesis of acetate
Artificial photosyntesis	Main Product	Acetic acid: 0.05 - 45 g L^{-1}. Other by-products in minor concentrations: propionate, n-propanol, ethanol, ehtyilene	?	https://doi.org/10.1038/s43016-022-00530-x

ADVANTAGES AND LIMITS OF HETEROTROPHIC CULTIVATION OF MICROALGAE

Commonly cultivated in:

- Open pounds

- Photobioreactor



$$\mu = \frac{\mu_{max}\, I}{K_I + I}$$

- Heterotrophic fermenters:

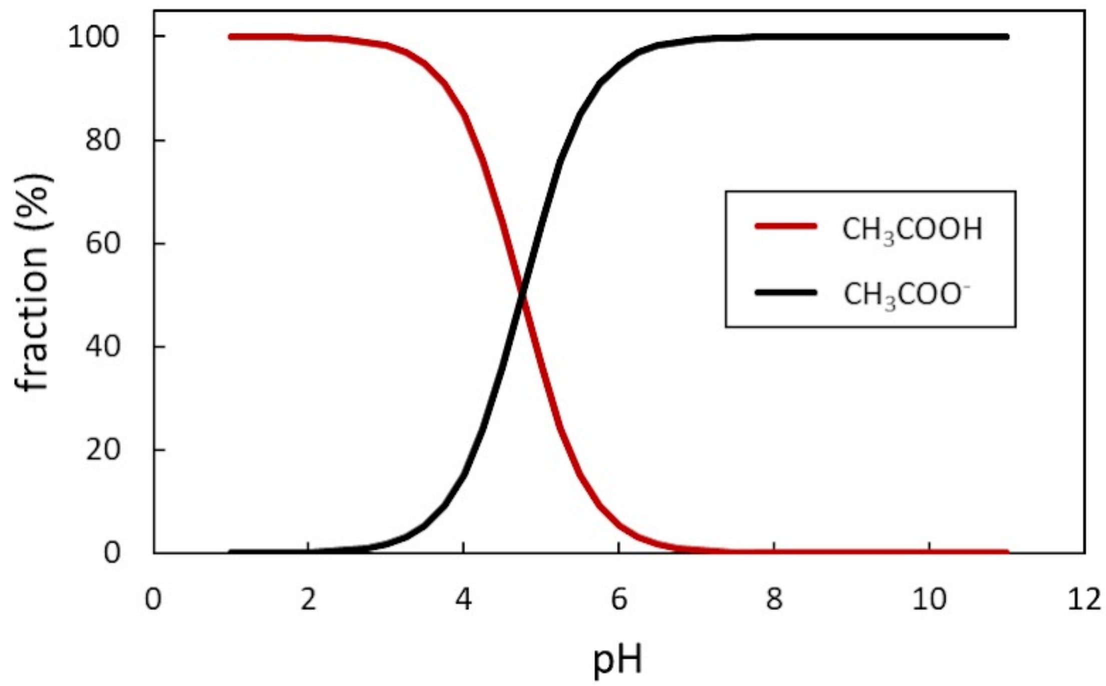
Advantages:

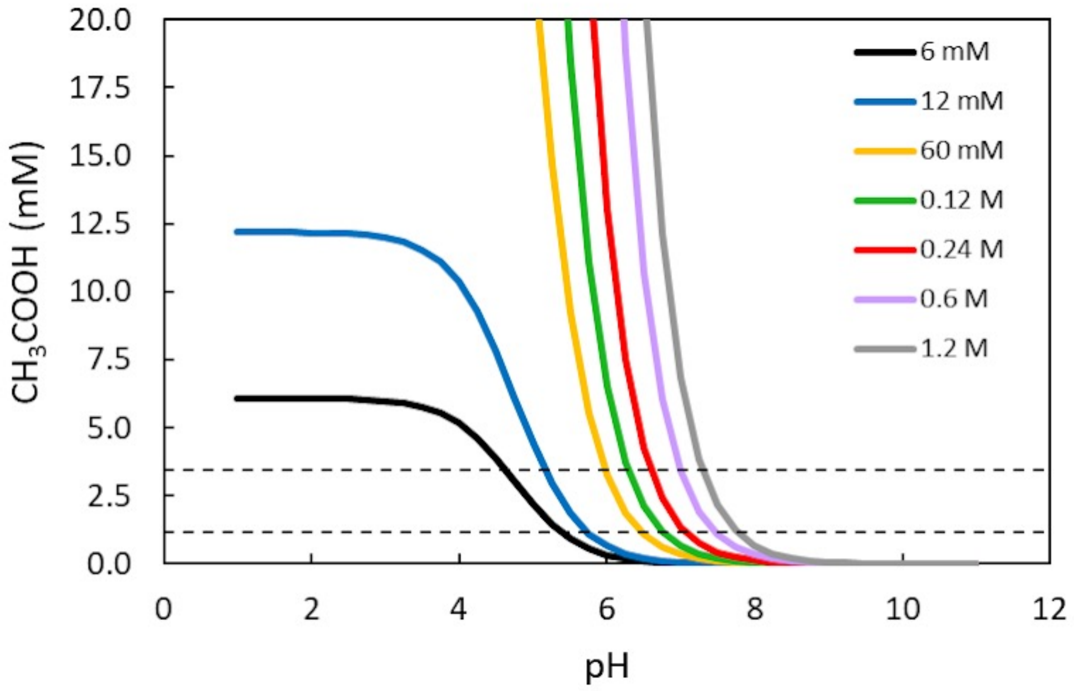
- Indipendent of light;
- Costs of feedstock exploiting agro-industrial waste;
- Control;
- Major growth, yields and productivity

Limit:

- Cost of carbon feedstock

ACETATE AND pH





Heterotrophic vs autotrophic production of microalgae: Bringing some light into the everlasting cost controversy

Jesús Ruiz [a,b,*], Rene H. Wijffels [c,d], Manuel Dominguez [a,b], Maria J. Barbosa [c]

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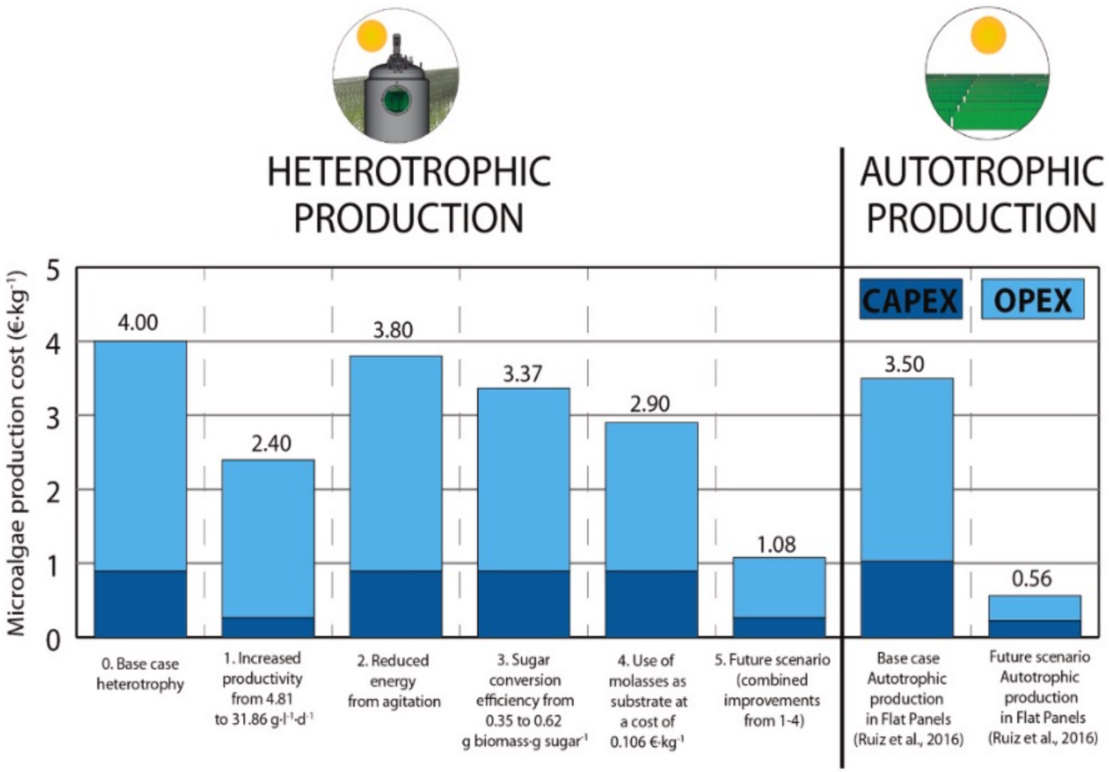

Total investment costs (6094 tons/years):

- Het: 76M € --> Fermenters
- Auto: 90.6M € →Flat panels – Tubular photobioreactor

Biomass production costs

- Het : 4.00 €/Kg
- Auto: 3.50 – 5 €/Kg

In Heterotrophy, the operational costs are very higher compared to autotrophy (especially for the carbon feedstock costs)

BUT

Higher productivities and the integration with waste or wastewaters treatment (zero or negative cost feedstock) can strongly decrease the biomass production costs: in a potential scenario, 1.08 €/Kg

PROCESS PARAMETERS

- **Specific growth rate and duplication time:**

$$\mu_{MAX} = \frac{ln\left(X_f\right) - \ln(X_{0,Exp})}{t_f - t_0}$$

$$t_{DUPL.} = \frac{ln2}{\mu_{MAX}}$$

- **Biomass productivity:**

$$r_X = \frac{(X(t) - X_0)}{t}$$

- **Biomass yield:**

$$Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{(X(t) - X_0)}{\left(S_0 - S(t)\right)}$$

PROTEIN CONVERSION FACTOR

Proteins = 16% nitrogen → 100g of proteins = 16g of nitrogen → 100/16 = 6.25

$$K_A = {(\sum E_i)}/{(\sum D_i)}$$

E_i = sum of the amino acids residues;
D_i = sum of %N found in these amino acids (including N lost during hydrolosys)

K_A is an upper bound

$$K_P = {\sum E_i}/{N}$$

N = Total nitrogen, including non-protein nitrogen (NPN)

K_P is a lower bounds

Ideal K for real samples: Average between K_A and K_P

ACETATE METABOLISM IN ALGAE