„Two-stage biodegradation of contaminating wastes linked to bioenergy generation using genomic approaches”

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NIBIO

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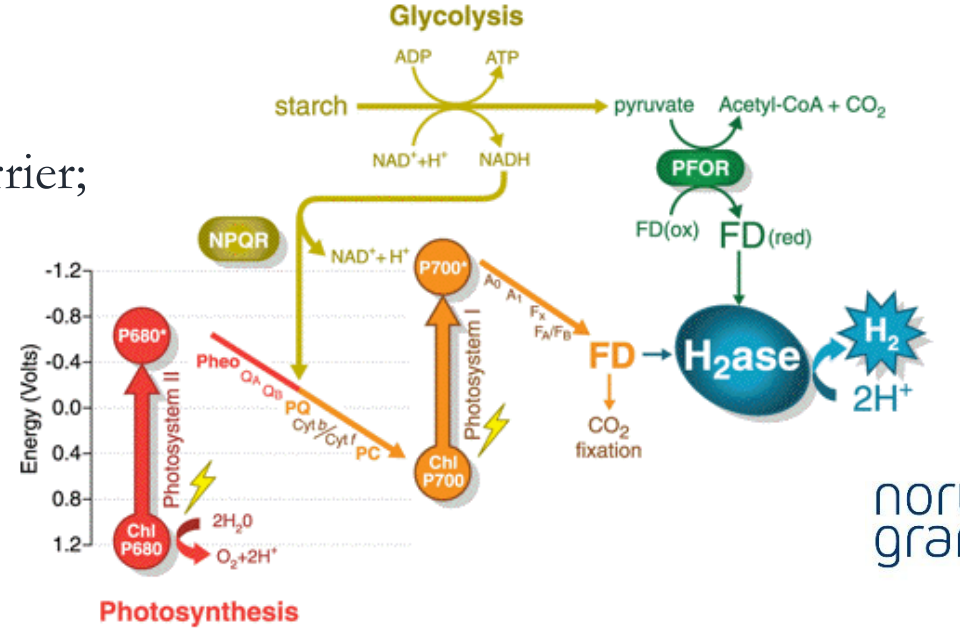
SCIENTIFIC-SOCIETAL CONTEXT

- Major issues to be addressed:
  - protecting the **fresh-water** resources;
  - **organic wastes** management;
  - **rising** energy demands;
  - energy **independence**.

- Biohydrogen production:
  - **versatile**;
  - **environmentally** friendly, **renewable** energy carrier;
  - highest **energy potential** (142 kJ/g);
  - biohydrogen production methods → carbon **neutral** or even **negative.**



General objectives, strategy

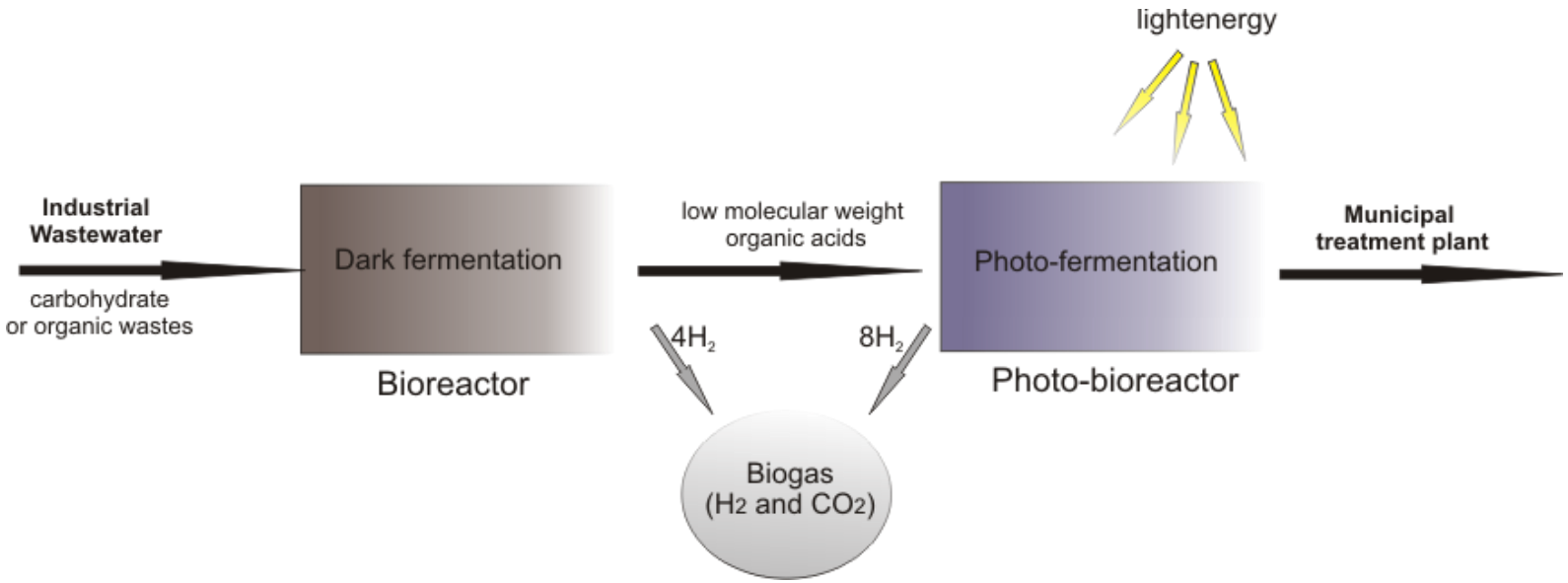
Hybrid system using fermentative and photosynthetic microorganisms

- **Stage I**. Dark fermentation (**strictly or facultative anaerobes**):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

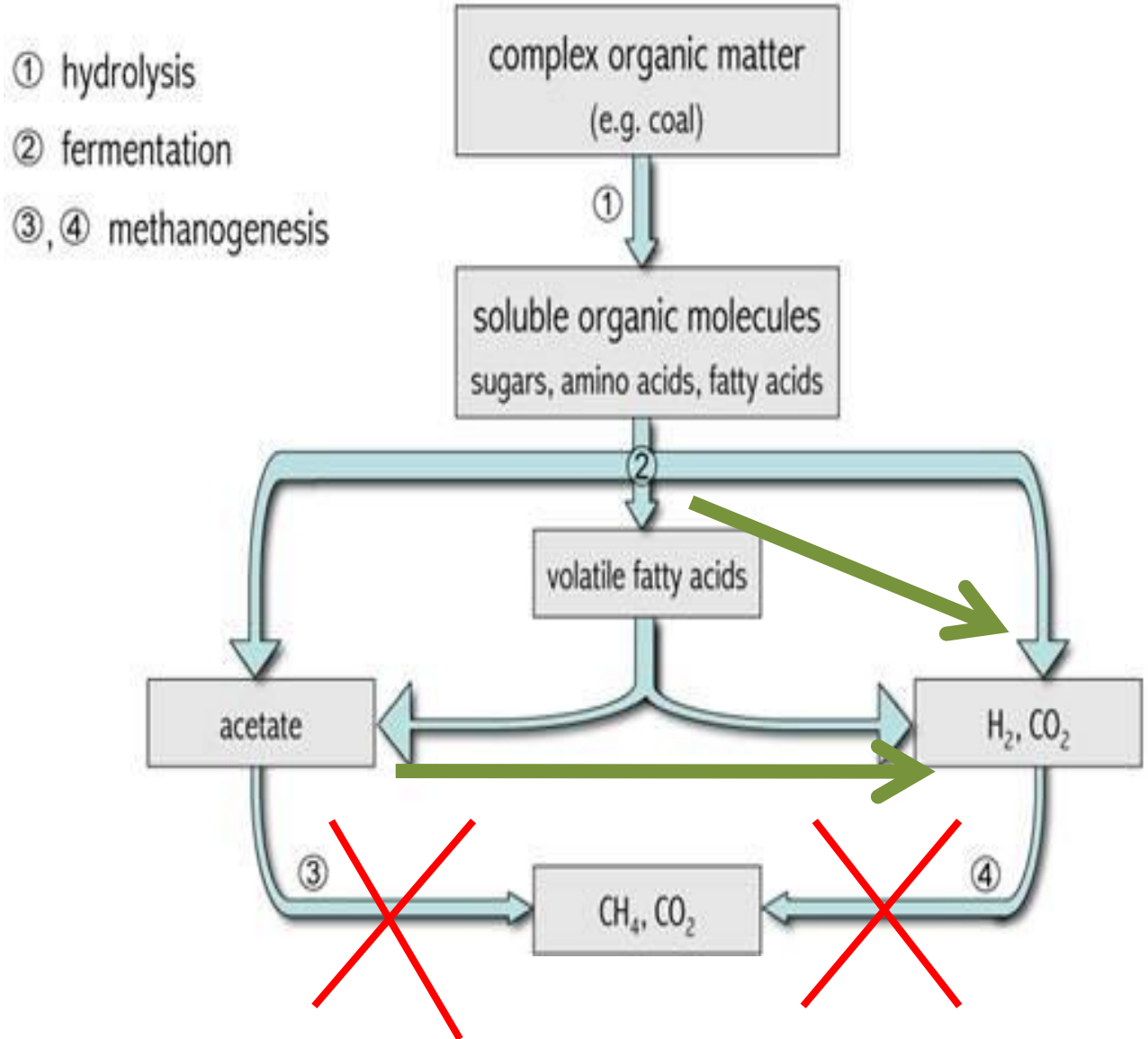
- **Stage II**. Photo-fermentation (**photosynthetic bacteria** and **algae**):

$$2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2$$



1st stage: Anaerobic wastewater treatment and combined bioenergy generation

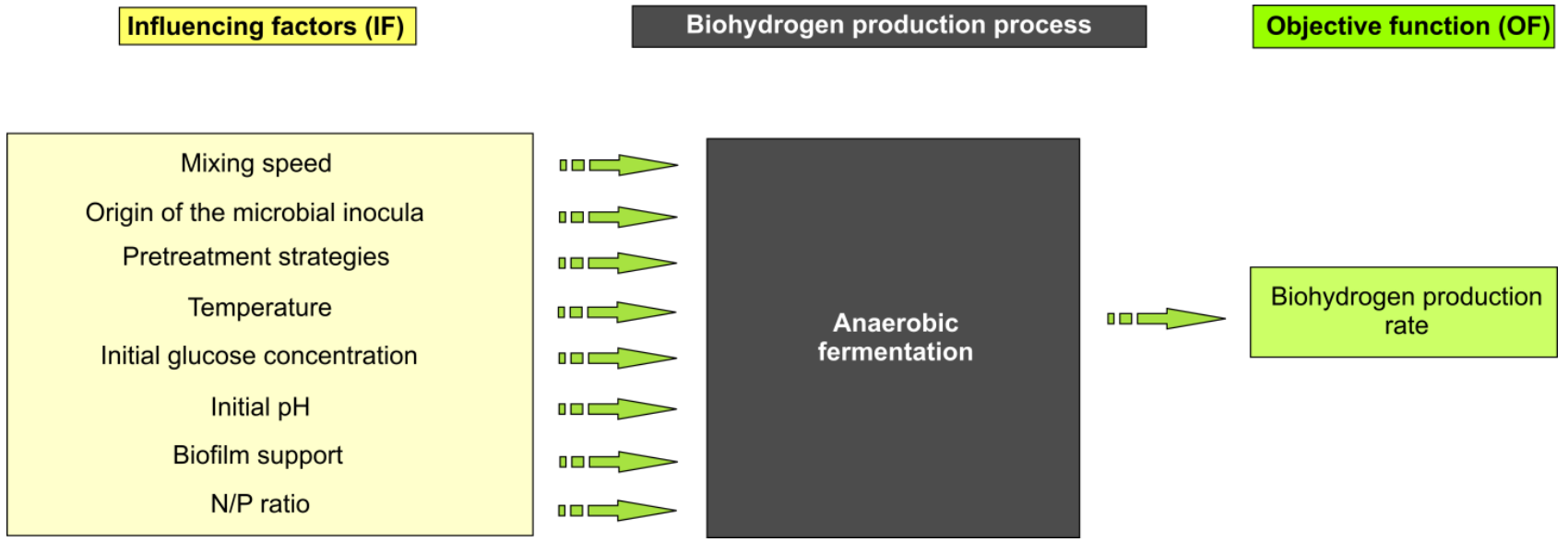
Goal: to engineer the Anaerobic Degradation (AD) of wastes for selective biohydrogen generation



1st stage: Anaerobic wastewater treatment and combined bioenergy generation

APPROACH

1. Design of synthetic wastewater types (sterile)
2. Enrichment of the active inoculum (active sludge)
   - Heat treatment
   - Acid treatment
   - Ultrasonication
3. Anaerobic degradation experiments (in controlled small scale batch bioreactors)
   - Identification of influencing factors, interactions among them
4. Gas and Sludge analytics and metagenomics
5. Correlations, conclusions, process optimization with real wastewater



1st stage: Anaerobic wastewater treatment and combined bioenergy generation

Development of a **design of experiments** (DOE) approach

- rank the different **influencing factors (IF)** according to their **relative importance**;
- identify the **directions of influence**;
- analysis of the effect of the **interactions** occurring among the **investigated variables**;
- process **optimization**.

Experiment example: 3 IF tested with 3 values each

Table: Coded values of the variables used in the Full and Central-Composite Factorial Experimental Design.

Coded symbol	Variables	Values of coded levels		
		(-1)	(0)	(+1)
X_1	Operating temperature (°C)	25	31	37
X_2	Initial value of fermentation pH	4.8	5.6	6.5
X_3	Glucose addition (g/L)	5	10	15

1st stage: Anaerobic wastewater treatment and combined bioenergy generation

Experiment matrices for 2 statistical models (with hydrogen yield results)

Table: Full Factorial (run no. 1-8) and Central-Composite (run no. 9-16) multifactorial experimental design matrix of the three investigated variables, with the total measured H_2 production for each of the experimental runs.

Run No.	Variables			Response
	X_1	X_2	X_3	Total hydrogen production mean (ml/L/day)
1	-1	-1	-1	8,66
2	-1	-1	1	9,62
3	-1	1	-1	22,34
4	-1	1	1	25,23
5	1	-1	-1	8,28
6	1	-1	1	8,35
7	1	1	-1	14,67
8	1	1	1	11,92
9	-1.28	0	0	17,67
10	1.28	0	0	10,43
11	0	-1.28	0	2,97
12	0	1.28	0	27,18
13	0	0	-1.28	14,95
14	0	0	1.28	25,92
15	0	0	0	21,08

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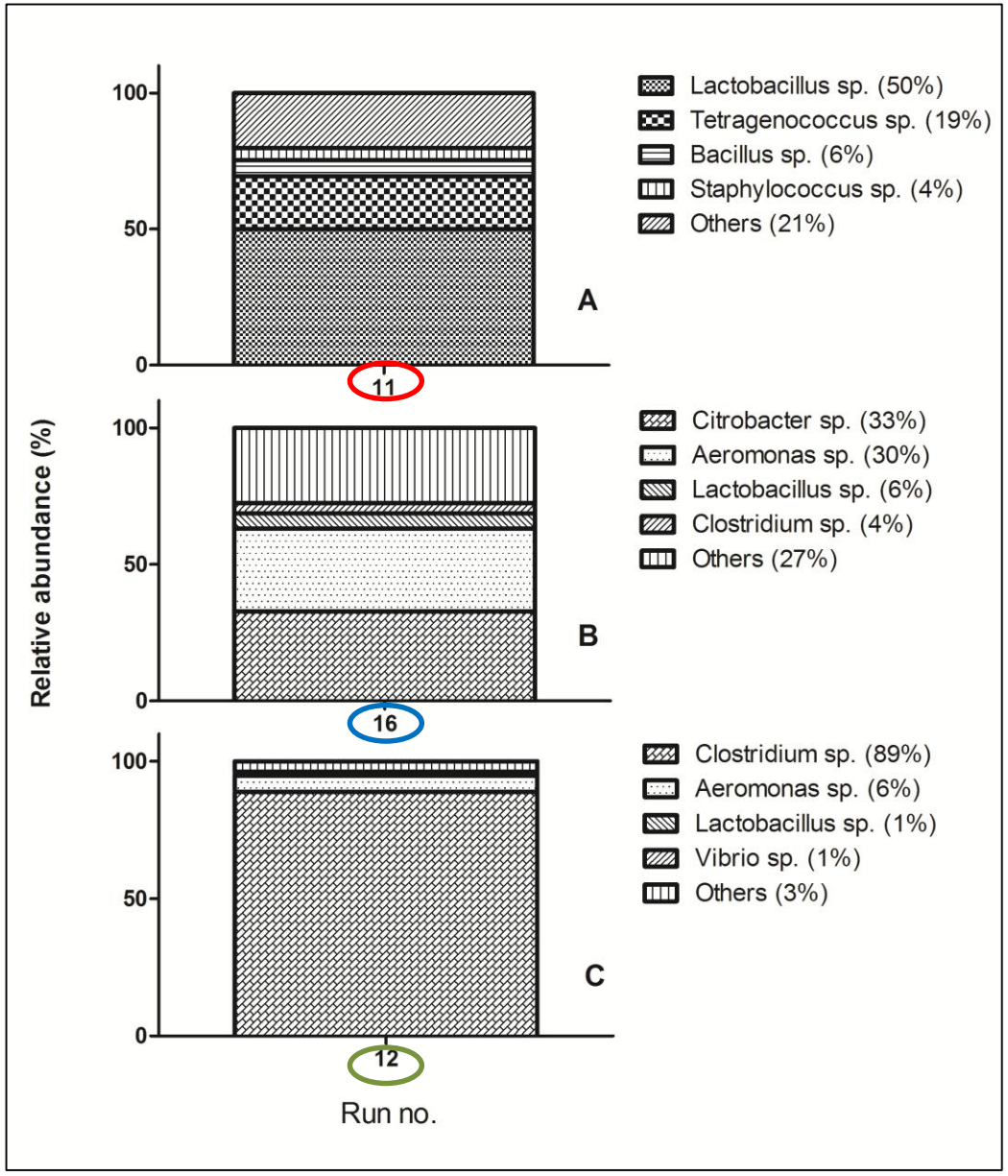
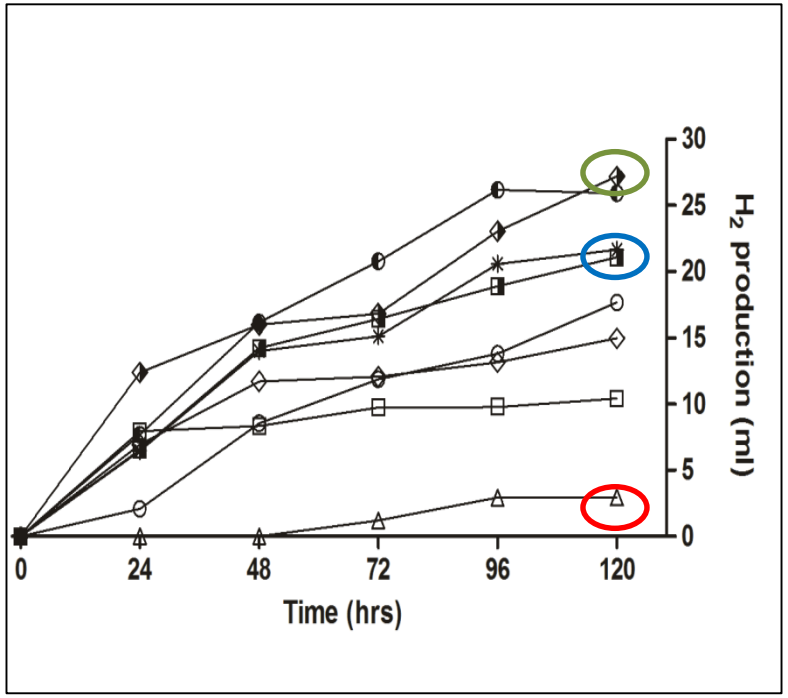
1st stage: Anaerobic wastewater treatment and combined bioenergy generation



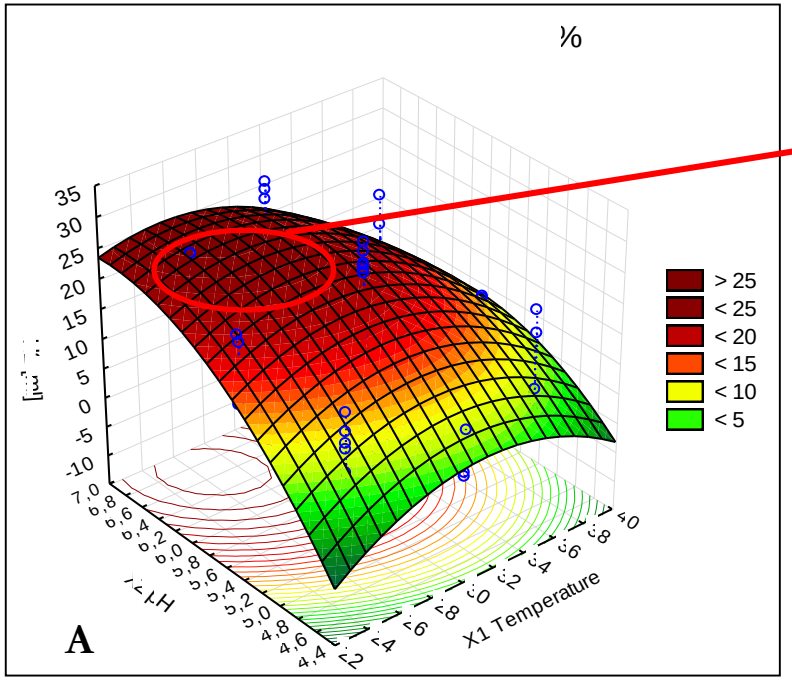
Microbial community composition assessment for the total metagenomes extracted from experimental run 11 (A), 16 (B) and 12 (C)

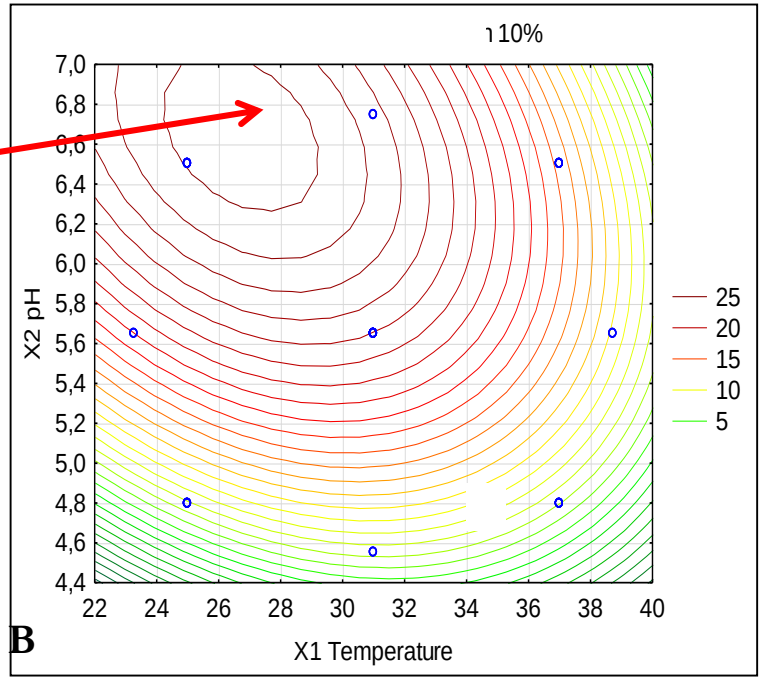


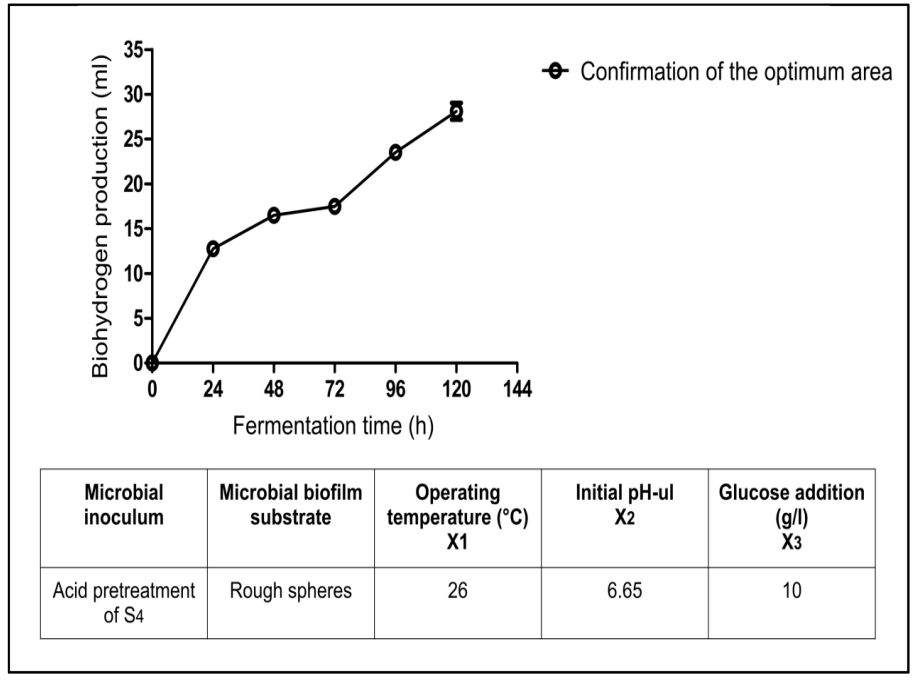
Biohydrogen yield is strongly correlated with the relative abundance of *Clostridia*

1st stage: Anaerobic wastewater treatment and combined bioenergy generation

Identification of the optimum area for each pair of influencing factor







Microbial inoculum	Microbial biofilm substrate	Operating temperature (°C) X1	Initial pH-ul X2	Glucose addition (g/l) X3
Acid pretreatment of S4	Rough spheres	26	6.65	10

1st stage: Anaerobic wastewater treatment and combined bioenergy generation

Process optimization for real wastewater

Beer Factory

Wastewater
Treatment Plant

Methane producing pilot
scale bioreactor

Polluted river

Substrate-dependent pretreatment approach for the possible highest AD biohydrogen yield

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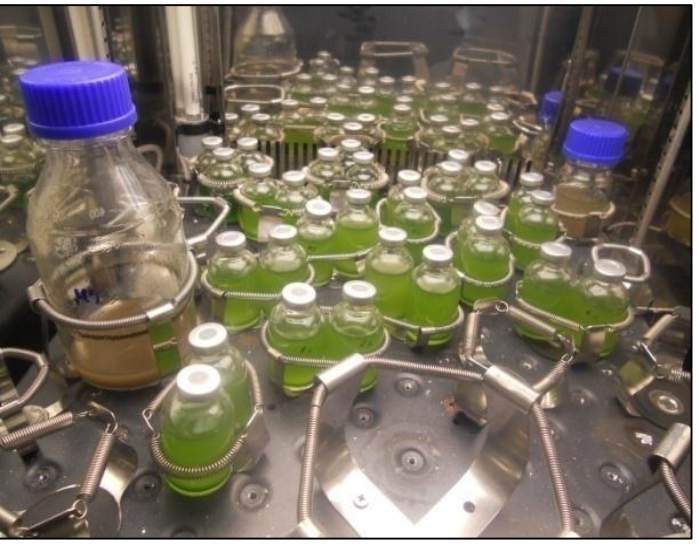
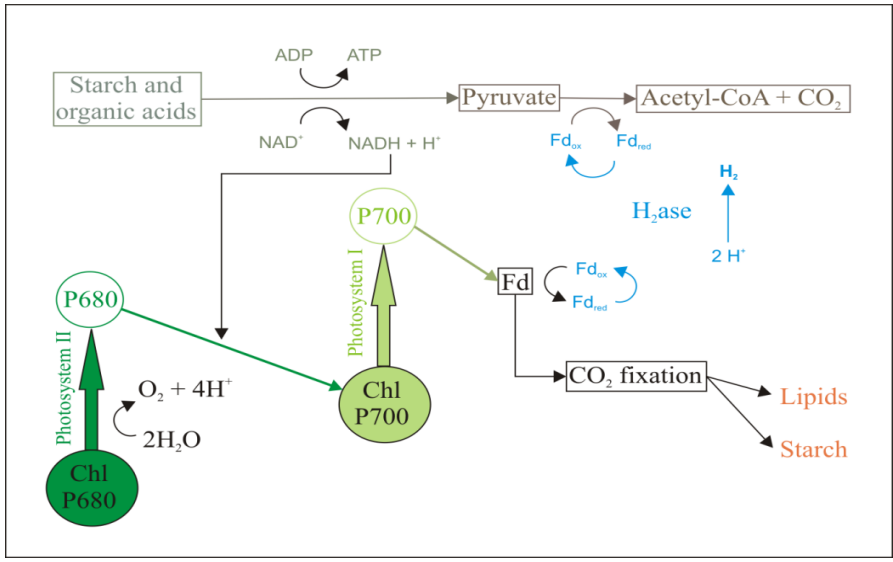
2nd stage: Algal biohydrogen production using the AD effluent

WAYS for ALGAL H_2 GENERATION



- **Direct biophotolysis of water** (green algae, cyanobacteria)
- **Photodecomposition of organic compounds** (indirect biophotolysis, green algae, photosynthetic bacteria)
- **Dark fermentative hydrogen production** (algae, bacteria, complex microbial comm.)



BOTTLENECKS OF PRESENT ALGAE TECHNIQUES

Sulfur deprivation in light and dark fermentation

- Two-phase systems (biomass and hydrogen production are separated)
- Sulfur deprivation is difficult (media exchange) and lethal for algae cells
- Low hydrogen yield

$ 2.5/Kg
(US Department of Energy)

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ALTERNATIVE SOLUTION

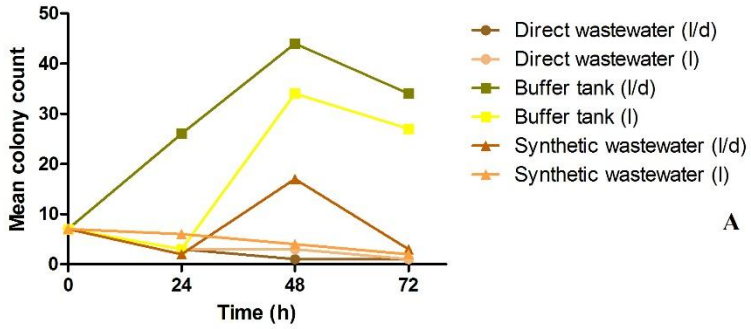
ALGAL-BACTERIAL ASSOCIATIONS under illumination

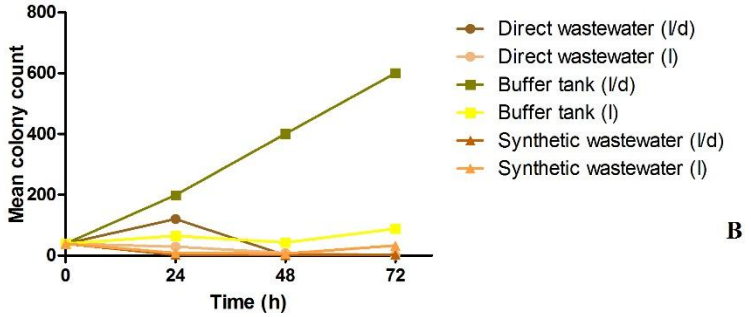
- Efficient bacterial respiration consumes the hydrogenase-inhibiting oxygen
- Bacterial partners enhance algal growth
- Active photosynthesis and biomass generation maintained throughout hydrogen production

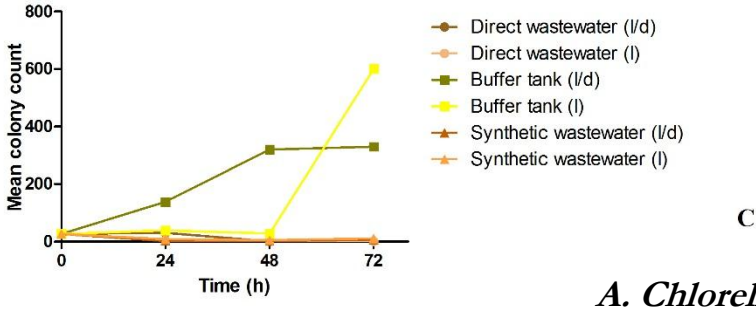
2nd stage: Algal biohydrogen production using the AD effluent

Applied Strains	**Relevant genotype or phenotype**
Green Algae	
Chlamydomonas reinhardtii cc124	Wild type
Chlorella sp. MACC 360	Wild type
Chlorella sp. MACC 411	Wild type
Chlamydomonas sp. MACC 530	Wild type
Chlamydomonas sp. MACC 549	Wild type
Chlamydomonas sp. MACC 772	Wild type
Chlamydomonas sp. MACC 775	Wild type
Chlorella sorokiniana	Own isolate
Micractinium sp.	Own isolate
Monoraphidium neglectum	Own isolate
Applied bacterial partners	
Escherichia coli JW5433	BW25113, Δ*hypF*::*kan*
Enterobacter sp.	Own isolate

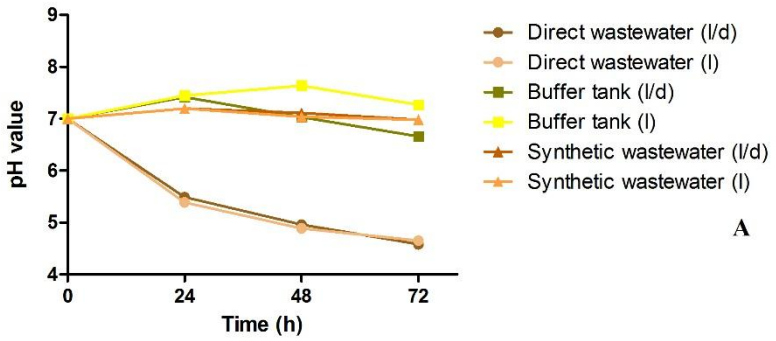
2nd stage: Algal biohydrogen production using the AD effluent

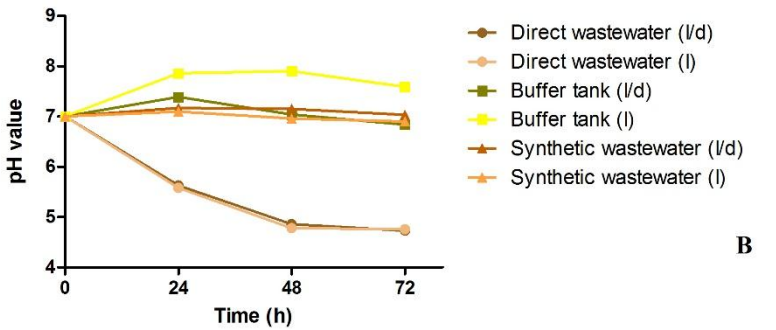


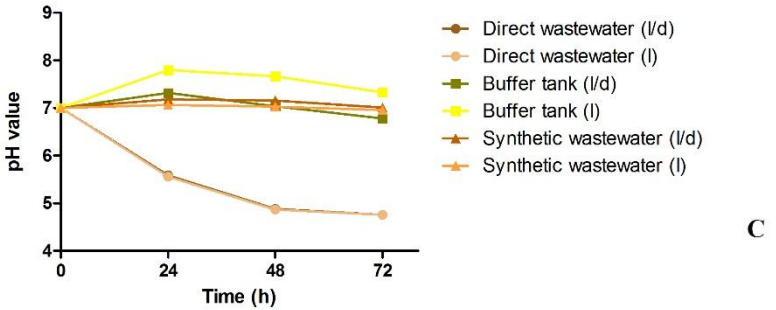




Algae growth



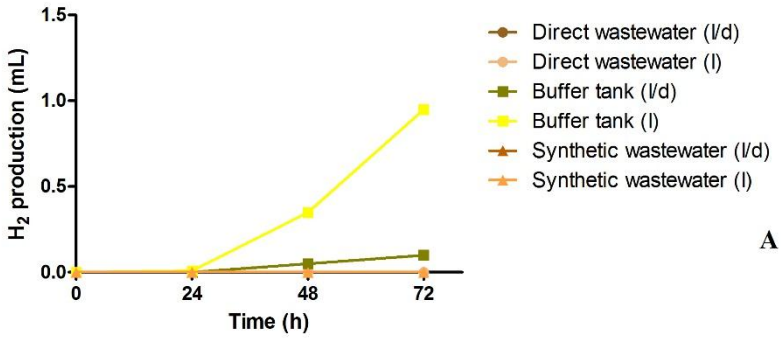




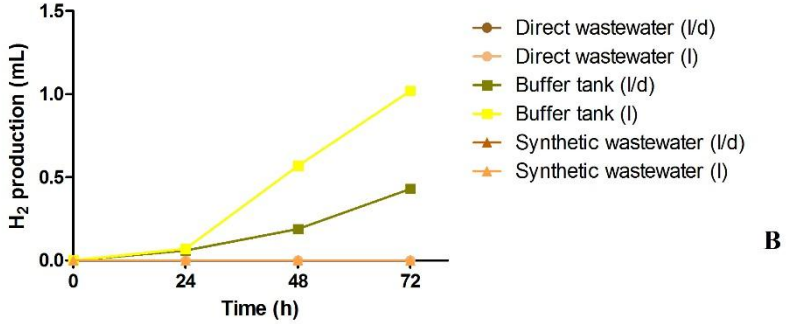
pH

A. Chlorella MACC360
B. Ch. reinhardtii cc124
C. Chlamydomonas sp. MACC549

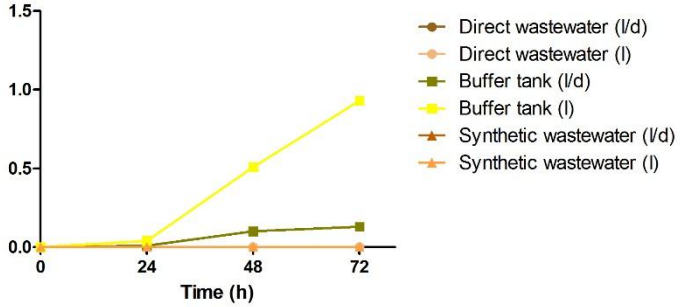
2nd stage: Algal biohydrogen production using the AD effluent



A



B



C

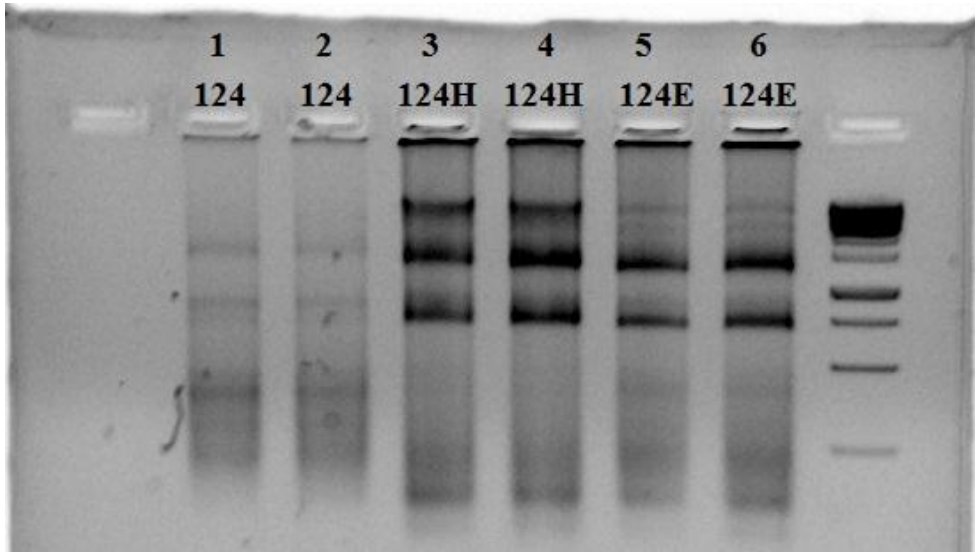
A. Chlorella **MACC360**
B. Ch. reinhardtii **cc124**
C. Chlamydomonas **sp. MACC549**

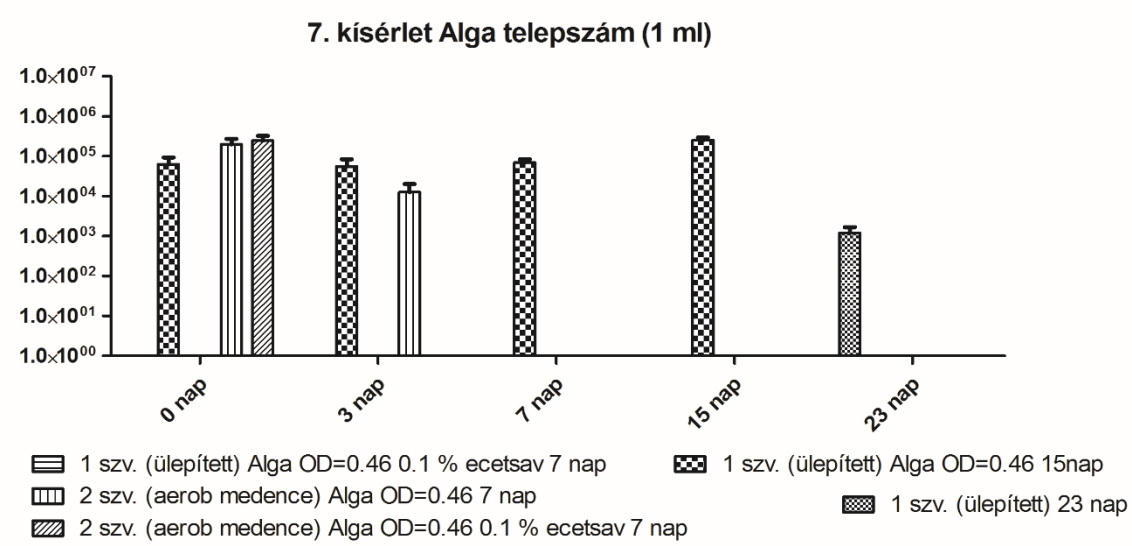
Hydrogen production

2nd stage: Algal biohydrogen production using the AD effluent

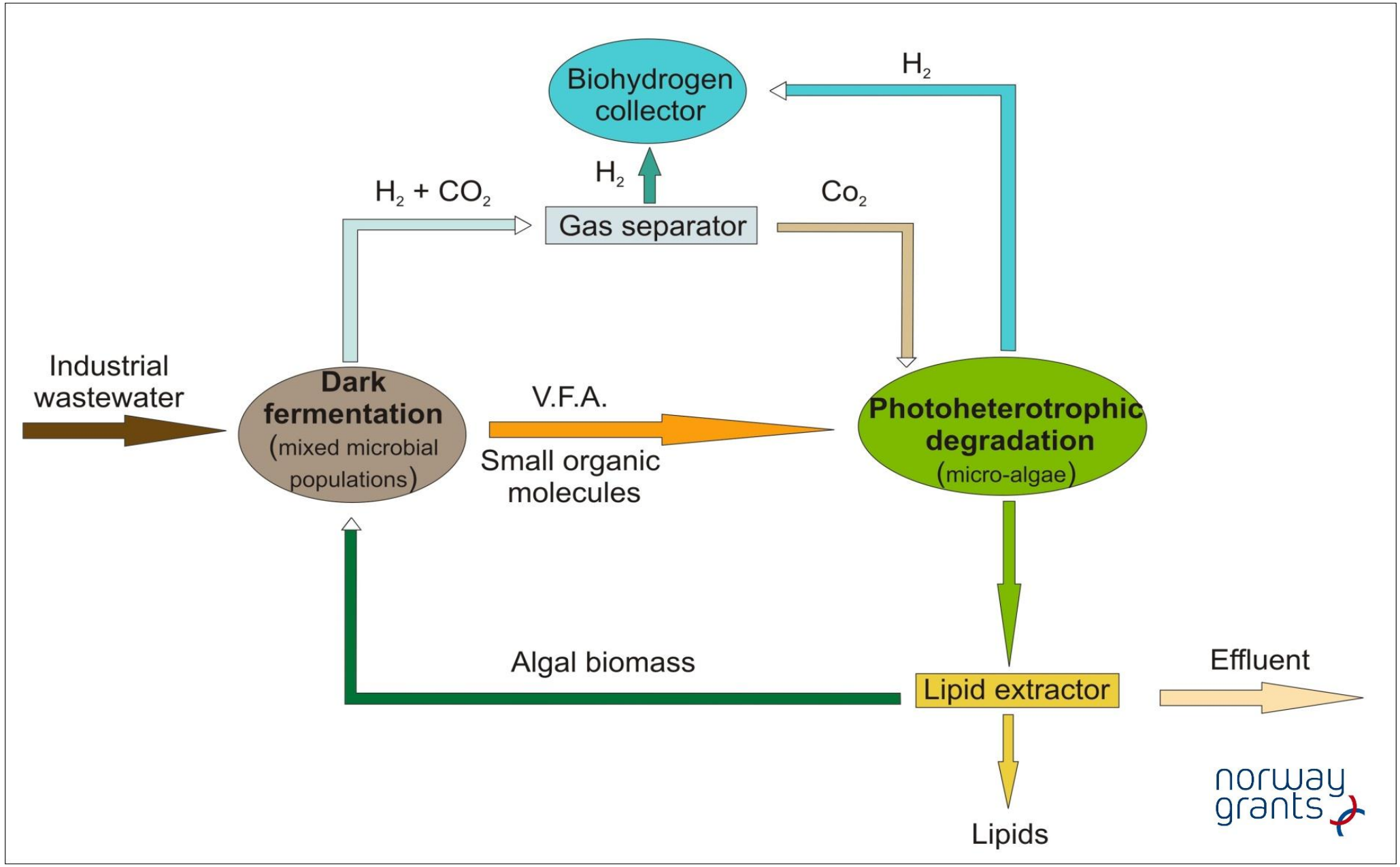
Molecular investigations of the algal-bacterial interaction in wastewater:

- miRNA Seq of *Ch. reinhardtii* under different conditions
- De novo genome sequencing of 10 selected strains, draft genomes assembled
- RNA-Seq of the 10 investigated algae strains under 4 conditions each
- key genes identified in algal hydrogen production in wastewater (PFR and 4 genes encoding hyptothetical proteins)





Overview of the technology



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