



SEMPRE-BIO

Cow manure digestate treatment with purple phototrophic bacteria and biomass valorization

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Problematic



Anaerobic digestion (AD) is a key technology for sustainable waste management

- ✓ Contributes to clean energy production and climate change mitigation
- ✓ AD as an alternative aligned with the Sustainable Development Goals(SDG)

However, the management and disposal of **digestate** are becoming a growing challenge.

Projected population will reach to 9 billion by 2050 poses significant **challenges to global food security** -> land demand, livestock expansion and need for waste management

- ✓ Efficient treatment of digestate
- ✓ The need for sustainable fertilizers



Opportunity



- ✓ Cow manure digestate is a valuable source of nutrients.



- ✓ Direct use → variability + leaching risks.

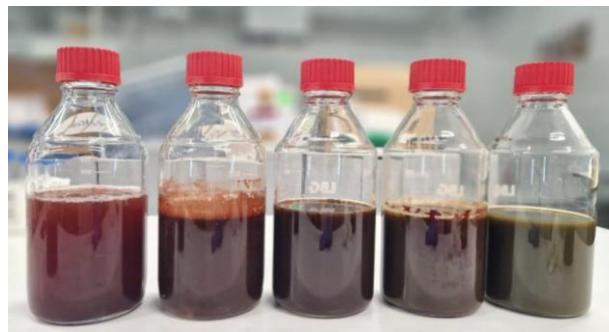


Direct use in agriculture

- ✓ Valorization → opportunity for circular economy & high-value by-product

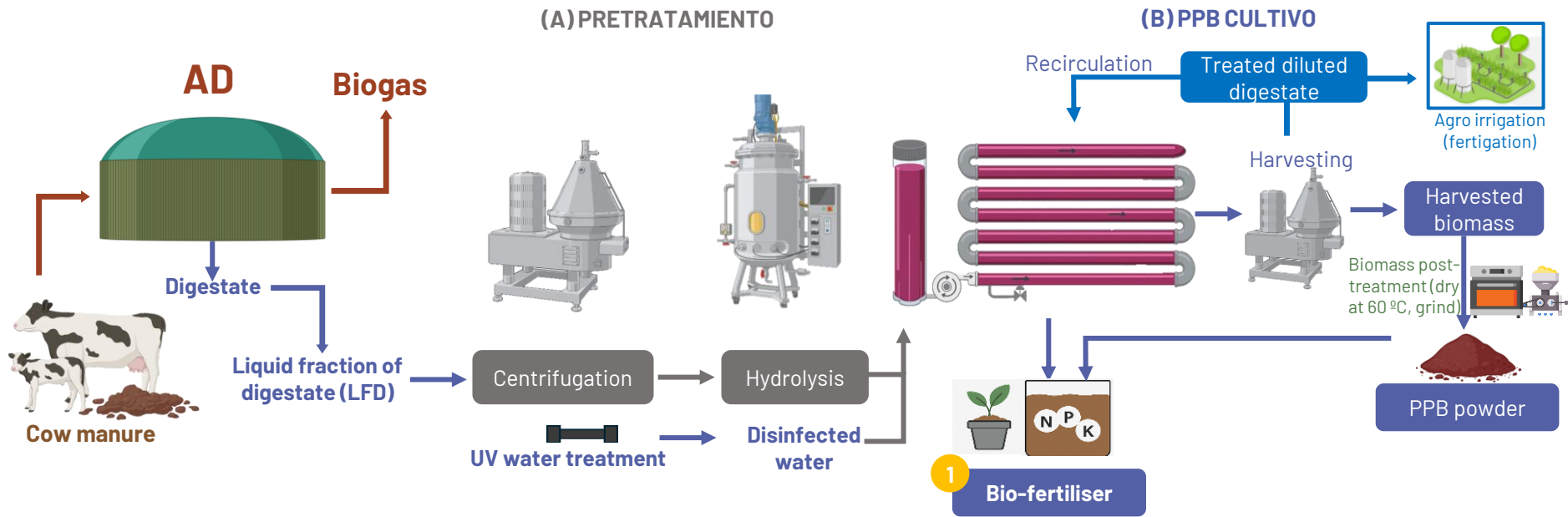


- ✓ **Purple phototrophic bacteria (PPB) cultivation** using digestate could a powerful technology to produce valuable biomass.
- ✓ PBP have shown greater tolerance to high levels of turbidity and high concentrations of N, up to 4.5 g·L⁻¹ of N-NH₄⁺.



PPB cultivation

Our Solution





Experimental design



A) PRETREATMENT: HYDROLYSIS



Experimental series

- 1 Chemical
 - alkaline NaOH
 - acidic HCl

- 2 NaOH dose (g/g ST)
 - 0.02
 - 0.05
 - 0.1
 - 0.2

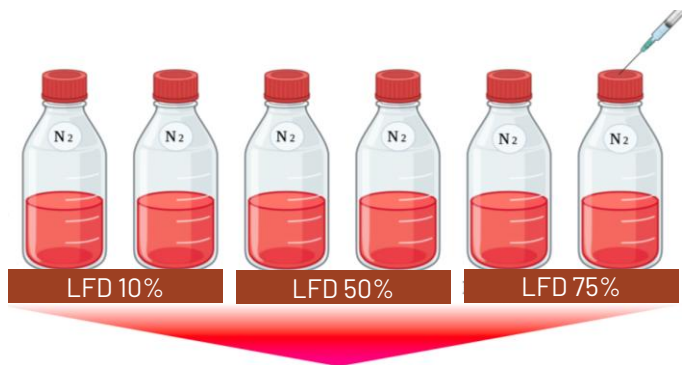
- 3 Initial Total Solid (TS) concentration (g/L)
 - 48
 - 30
 - 28
 - 20



Experimental design



B) PPB CULTIVATION



Operational conditions

Anaerobic
Volume = 0.9 L
pH: 8.0 ± 0.1

Led NIR λ = 660/850 nm
Inoculum = 10%
Ta = $28^\circ\text{C} \pm 2$

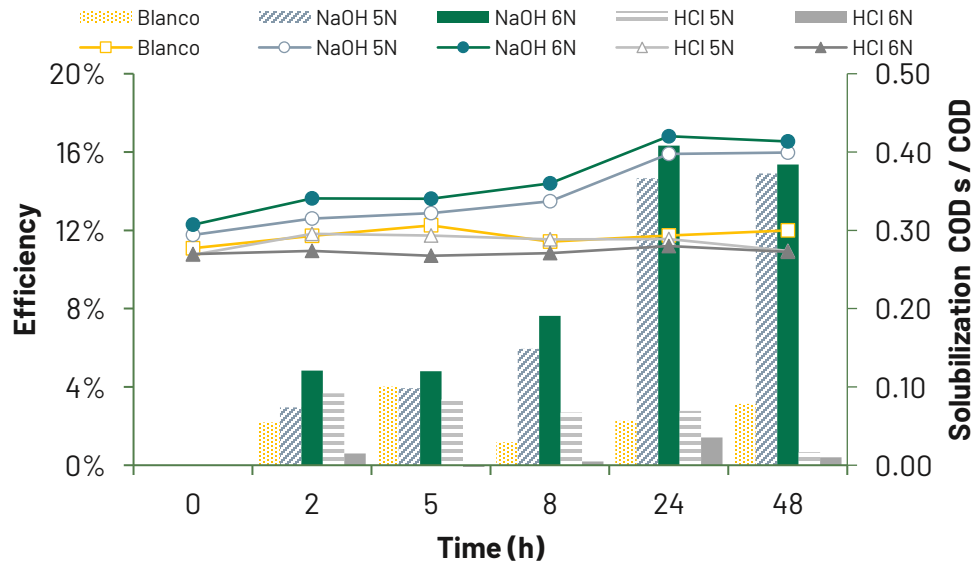
Rhodospirillum rubrum
3 dilution 10, 50, 75%
5 days cultivation time

- 10% : 10% LFD + 90% water
- 50% : 50% LFD + 50% water
- 75% : 75% LFD + 25% water

Results



A) HYDROLYSIS: Chemical effect



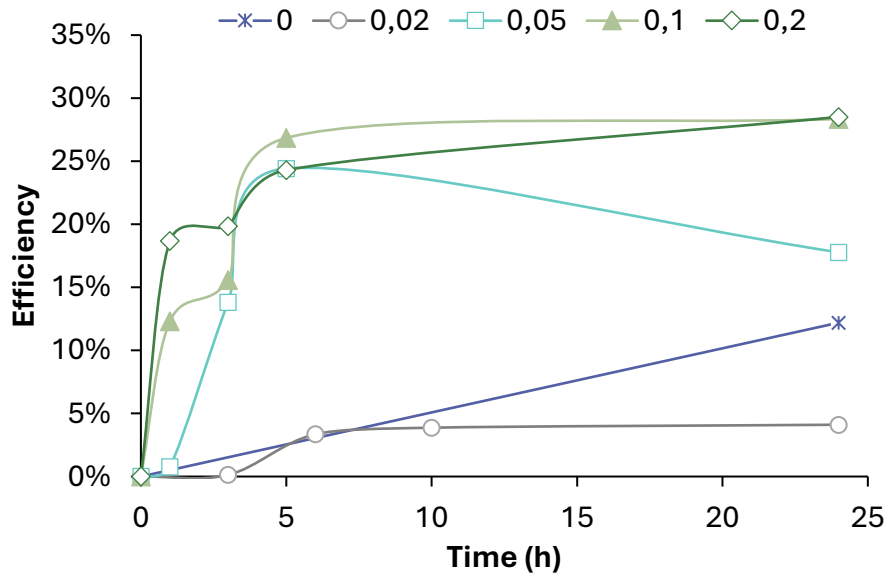
Key results

- ✓ Alkaline hydrolysis is 39 times higher than acid hydrolysis
- ✓ Efficiency 15.4% vs. 0.4%
- ✓ Solubilization grade 0.414 vs. 0.272.

Results



A) HYDROLYSIS: Effect of NaOH Dose



Key results

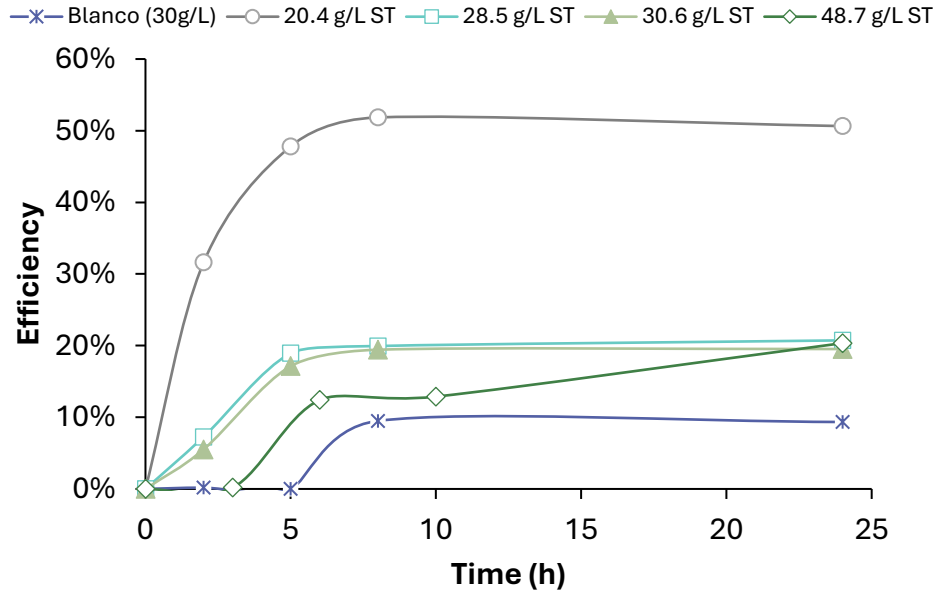
- ✓ A statistically significant difference was observed between low (0 - 0.05 g NaOH·g ST⁻¹) and high (0.1- 0.2 g NaOH·g ST⁻¹) doses.

NaOH Dose (g·g ⁻¹ ST)	$\frac{COD_S}{COD}$	Efficiency %
0.02	0.17	4
0.05	0.36	16
0.10	0.45	30
0.20	0.45	34

Results



A) HYDROLYSIS: Effect of initial ST concentration



Key results

- ✓ A Higher solubilization (0.817; 50.6% efficiency) was achieved at $\sim 20 \text{ g L}^{-1}$ ST.

Total Solid (g-L-1 ST)	$\frac{\text{COD}_S}{\text{COD}}$	Efficiency %
48.4	0.284	17
30.6	0.536	22
28.5	0.501	25
20.4	0.817	51



Results

A) HYDROLYSIS: OPTIMAL

Parameter	Unit	LFD	LFDH
pH	-	7.58	10.2
Conductivity	$\mu\text{S}\cdot\text{cm}^{-1}$	17360	18320
Alkalinity	$\text{mg CaCO}_3\cdot\text{L}^{-1}$	7750	8187
Alkalinity	VFA	2563	3875
VFA Ind.	-	0.3	0.5
COD	$\text{mg}\cdot\text{L}^{-1}$	20580	19460
COD s	$\text{mg}\cdot\text{L}^{-1}$	12830	16350
NKT	$\text{g}\cdot\text{L}^{-1}$	1.77	1.58
N-NH ₄	$\text{g}\cdot\text{L}^{-1}$	1.72	1.47
TP	$\text{mg}\cdot\text{L}^{-1}$	673	659
P-PO ₄	$\text{mg}\cdot\text{L}^{-1}$	205	320
COD/N/P	m/m/m	100:8.35:1	100:7.6:1.6
CODS/COD		0.62	0.84

LFD: Liquid fraction of digestate

LFDH: Liquid fraction digestate with hydrolysis



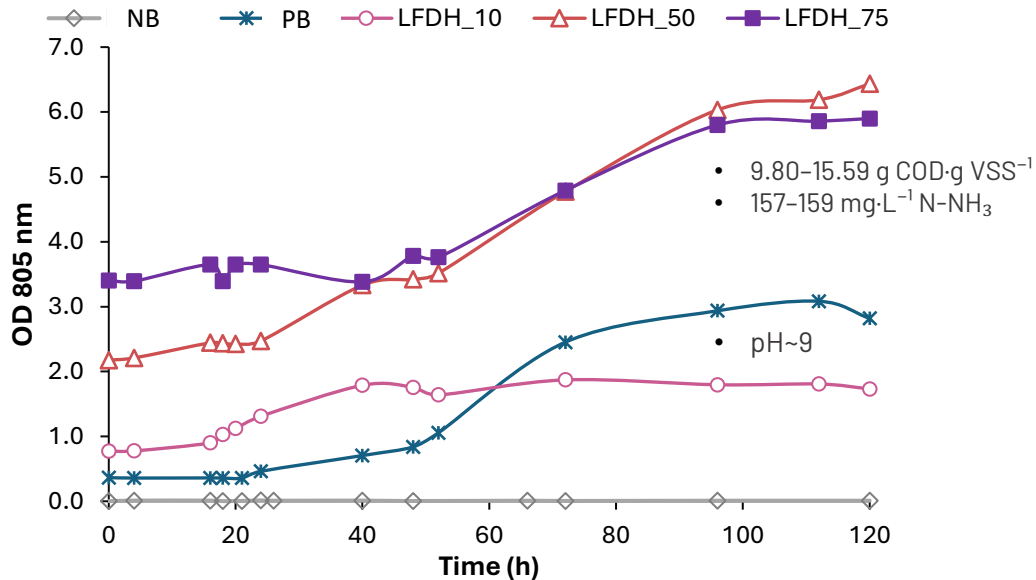
Key results

The optimal experimental setup for LFD is anaerobic **alkaline** hydrolysis using **0.1 g NaOH·g⁻¹ ST** in media with approximately **~20 g·L⁻¹ ST**.

Results



B) PPB CULTIVATION: PPB growth curve



Key results

TEST	Y	μ (d-1)	HRT (máx. prod.)
PB	0.80	1.4	1.4
LFDH_10	0.73	1.8	1.1
LFDH_50	0.89	0.7	2.9
LFDH_75	0.23	0.4	4.9

- LFDH_10: 10% LFD + 90% water
- LFDH_50: 50% LFD + 50% water
- LFDH_75: 75% LFD + 25% water

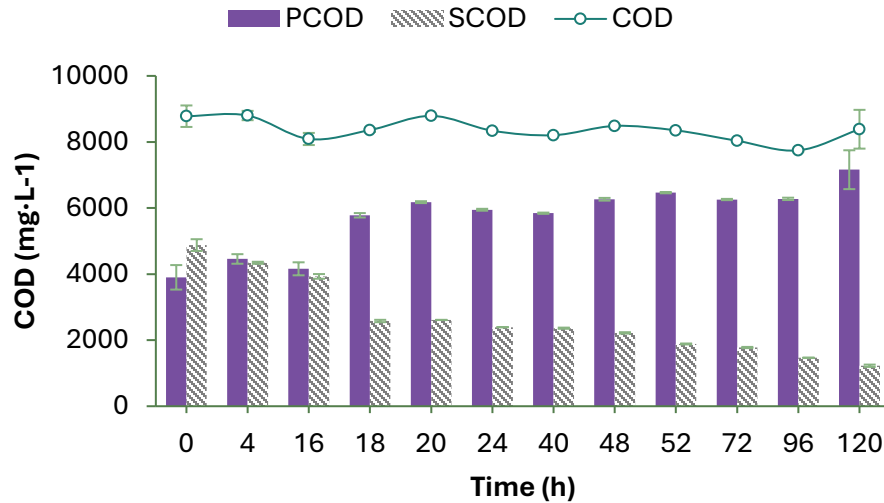
Results



B) PPB CULTIVATION: Removal



Key results



Time evolution of COD during PPB growth in LFDH_50

TEST	Initial COD (mg·L ⁻¹)	Initial SCOD (mg·L ⁻¹)	SCOD %	N-NH ₄ %	P-P0 ₄ %
BP	3360	3240	52	53	51
LFDH_10	1932	1178	68	48	90
LFDH_50	8780	4878	75	17	50
LFDH_75	13250	7300	35	11	31



Results

PPB BIOMASS COMPOSITION

Protein content (% dw)

PPB biomass (this study)

PB	40±2 %
10%_LFDH	36±3 %
50% LFDH	30±2 %

 **Soybean** 40-50 % dw

 **Sunflower Seed** 20-30 % dw

 **Microalgae biomass** 40-50 % dw

Biofertilizer potential

Regulation (EU) 2019/1009 : Solid organic fertilizer (PFC 1A) must meet the following criteria

	% N	% P ₂ O ₅	% K ₂ O	N + P ₂ O ₅ + K ₂ O	% C org	CFU/ml E.Coli
PB	6.4	1.0	2.8	10.2	33	~0
10%	5.7	1.0	2.7	9.4	39	~0
50%	4.8	0.9	5.7	10.4	27	~0
<i>Regulation (EU) 2019/1009</i>	>1	>1	>1	>4%	>15%	0-200

1,00E-03 dilution



Take home messages



Necessity to treat **digestate**, subproduct from biogas plants. **PPB** as a candidate technology for this treatment, surpassing other biotechnologies.



PPB can **tolerate up to 75%** digestate concentration, with maximum growth rate at 10% dilution, achieving 1.8 d^{-1}

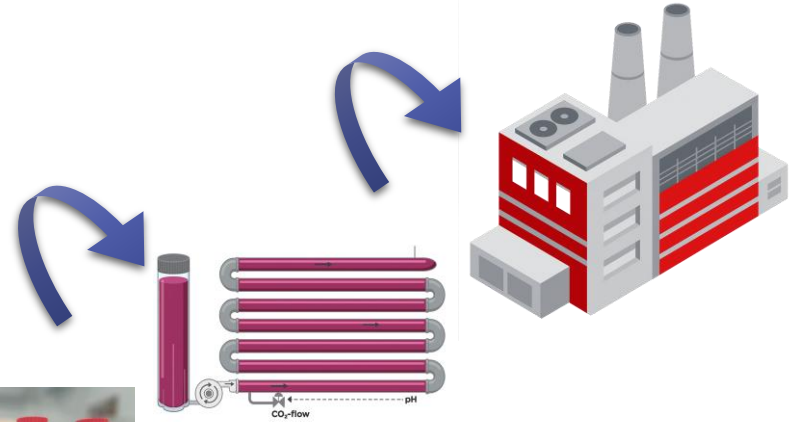
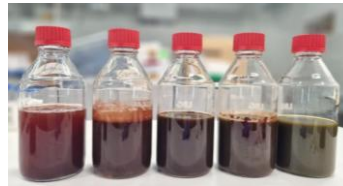


The generated **PPB biomass** produced through this process has potential to be used as a **biofertilizer** and as an **alternative protein source** for livestock feeding.

75% Composition: ($932 \pm 11.3 \text{ mg} \cdot \text{L}^{-1} \text{ N-NH}_4$, $15 \pm 1 \text{ g DQO-SSV-1}$, $13250 \pm 400 \text{ mg} \cdot \text{L}^{-1}$)

Future work

- ✓ Process upscaling (outdoor)
- ✓ Testing fertilizing capacity of PPB
- ✓ Legislative barriers of digestate
- ✓ Patent analysis
- ✓ Market analysis





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