



Idea I-2021-01895

Purple-B: Hydrogen production from immobilized cells in photo-bioreactors



Subcontractors:











Final public presentation

Photobioreactor Prototype

Final Report

Final Presentation slides

Executive Summary

3 min Video

Illustration of the activity in one self standing image



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Number	Description
WP 1	Selection of the most efficient PNSB strains for the photo-evolution of H ₂ from organic substrates
Task 1.1	Organic Substrate optimization and Strains selection
Task 1.2	Growth optimization of immobilized biomass culture and evaluation of the H2 productivity and yield
Task 1.3	Analysis and evaluation of the H ₂ evolution efficiency with continuous or pulsed light
WP 2	Evaluation of organic/synthetic matrices for the PNSB immobilization
Task 2.1	Chemical-physical and biological (toxicity) characterization of 3 organic and inorganic matrices at least (e.g. agar agar and metacrilate) potentially suitable for the immobilization of PNSB
	Protocol for the management of the PNSB harvesting and immobilization process in a hollow tubular immobilization
Task 2.2	support.
Task 2.3	Evaluation of H2 evolution efficiency with the selected geometry for the Purple-B reactor (tubular)
WP3	Design and realization of the photobioreactor (PBR) prototype Purple-B
Task 3.1	Design and rendering of the structural and functional components of the Purple-B reactor
Task 3.2	Realisation of the reactor and of the H ₂ capture system
WP4	Evaluation of the photoconversion process on the prototype Purple-B
Task 4.1	H ₂ conversion efficiency
Task 4.2	Permeability, transparency and resistance of the matrix during the photo-evolution of H_2

(as reported in MoM)











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Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
WP 1																		
Task 1.1																		
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WP1 Selection of the most efficient PNSB strains for the photo-evolution of H₂ from organic substrates

- T 1.1 --> Organic Substrate optimization and Strains selection
- T 1.2 --> Growth optimization of immobilized biomass culture and evaluation of the H₂ productivity and yield
- T 1.3 --> Analysis and evaluation of the H2 evolution efficiency with continuous or pulsed light











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This Task is divided into two sub-tasks:

- organic substrate dark fermentation process optimization in which the selected organic substrates were co-fermented applying different organic loading and evaluating the quality and quantity of produced metabolite as well as gas production and composition;
- <u>strain selection</u> in which the effluent was applied as organic source in culture medium in order to see the effect on purple non sulphur bacteria growth (suspended biomass).



F: Fermentation | PF: Photo-Fermentation | PNSB: Purple Non-Sulfur Bacteria | VFA: Volatile Fatty Acids

















	Total Solids (TS)	Total Volatile Solids (TVS)	TVS/TS ratio	COD			
	$g_{TS} kg_{FM}^{-1}$	$\boldsymbol{g}_{TVS} \boldsymbol{k} \boldsymbol{g}_{FM}^{-1}$	%	$g_{O2} kg_{FM}^{-1}$			
Lettuce	88.74 ± 3.15	74.29 ± 7.17	83.6 ± 5.12	80.06 ± 3.57			
Red beet	118.58 ± 1.10	111.13 ± 0.54	93.7 ± 0.41	113.97 ± 8.26			
Toilet paper	992.75 ± 10.25	958.45 ± 43.72	96.5 ± 3.41	1051.27 ± 40.32			
Sewage	24.57 ± 0.25	19.38 ± 0.24	78.9 ± 0.20	25.70 ± 1.56			
Mixture	83.50 ± 0.89	75.89 ± 0.75	90.9 ± 0.06	-			
	Total Solids (TS) Total Solids (TS) Fresh Matter						





















Reactors configuration: Batch condition, 4 L of working volume, 37 °C No initial inoculum, considering the WAS as a biologically active substrate Organic waste mixture: Lettuce + Red beets + Toilet paper (ratio 6:6:1 on Total Solid basis)

Co-substrate (inoculum): WAS



- ctrl (0 kg_{TVS(mixture)} m⁻³)
- OL 5 (5 kg_{TVS(mixture)} m⁻³)
- OL 10 (10 kg_{TVS(mixture)} m⁻³)
- OL 15 (15 kg_{TVS(mixture)} m⁻³)
- OL 20 (20 kg_{TVS(mixture)} m⁻³)
- OL 25 (25 kg_{TVS(mixture)} m⁻³)



Different organic loadings were obtained increasing progressively the amount of organic waste mixture and decreasing the amount of WAS

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OL higher than 15 $kg_{TVS(mixture)}$ m⁻³, caused a drop in pH below 4 after 24 h, due to the accumulation of acetic/lactic acid

	Solid Content (TS)	Organic Load (OL _{TVS} solid substrate)	Organic Load (OL _{COD} solid substrate)	Organic Load (OL _{TVS} WAS)	MIXTURE (soild substrates)	WAS
	g _{TS} L-1	kg _{™s} m-³	kg _{coD} m⁻³	kg _{™s} m⁻³	Total g _{FM} used	%VS/VS _(tot)
CONTROL (WAS)	24.57	0.00	0.00	20	0.00	100
TEST @OL5	27.98	4.41	6.45	18.25	204.80	81
TEST @OL10	31.73	9.45	14.65	17.9	498.10	65
TEST @OL15	35.46	14.52	22.62	15.48	787.70	52
TEST @OL20	39.09	19.36	30.16	14.14	1050.50	42
TEST @OL25	42.77	24.24	37.70	12.76	1315.40	34







End-test values	Tested condition	рН	VFAs (g _{VFA(COD)} L ⁻¹)	NH ₄ + (mg _{NH4+} L ⁻¹)	Acidification yield $(g_{VFA(COD)} kg_{TVS}^{-1})$
	ctrl	7.46 ± 0.08	3.7 ± 0.3	285 ± 14	-
	OL 5	5.39 ± 0.08	7.7 ± 0.1	701 ± 35	331.1
	OL 10	5.05 ± 0.01	11.0 ± 0.5	253 ± 13	410.0
	OL 15	4.90 ± 0.01	11.6 ± 0.6	107 ± 5	381.1
	OL 20	5.07 ± 0.04	11.4 ± 0.5	136 ± 7	334.1
	OL 25	4.97 ± 0.03	11.6 ± 0.6	260 ± 14	306.8

Gas production		OL sub+WAS	Specific gas production
		g _{TVS} L ⁻¹	NL g _{TVS} -1
	CTRL (WAS)	20,0	0
	OL 5	22,7	0
	OL 10	27,4	0,05
	OL 15	30,0	0,09
	OL 20	33,5	0,12
	OL 25	37,0	0,08



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Conclusion

The increasing of organic load due to increased mixed organic solid substrates addition, demonstrate a different impact on VFA (or organic acids) production and characterization.

The best acidification yields on total COD and TVS conversion are those obtained at OL 10 and 15, where the WAS amount was able to buffer the system, avoiding the initial pH drop as at higher OL.

Also considering the necessity to minimize gas production (even if most of the gas produced with pH below 4 is carbon dioxide), the OL that could fit with further VFAs production test is OL 10 with a VFAs concentration of 11 g L⁻¹ and an ammonia content of 252 mg L⁻¹.

Considering the necessity to minimize the fermentation broth dilution for PNSB (max 1:5), OL10 is the most suitable condition to be further investigated (ammonia concentration for PNSB test is below 60 mg L⁻¹). The OL15, with the lowest ammonia content could be also considered in case of PNSB inhibition.

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Rhodospirillum rubrum



• Strain selection

Rhodopseudomonas palustris



From the early tests on bacterial propagations, *Rsp. rubrum* resulted poorly active compared to *Rps. palustris*, facing difficulties in growing both on solid plates and on liquid medium.

Both strains were periodically reinoculated on solid plates as well as on liquid media, to maintain pure strains and to qualitatively verify their activity. Only during month 6, *Rsp. rubrum* showed a slight improvement in its quality and concentration



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WP1 Selection of the most efficient PNSB strains for the photo-evolution of H₂from organic substrates





Tested PNSB strains:

- Rhodopseudomonas palustris
- Rhodospirillum rubrum

Tested media:

- RPP standard growth medium
- FB Fermentative Broth medium



Reactors configuration: Batch condition, 250 mL of working volume, 30 °C suspended cultures





• TEST SET UP



Tested media:

- RPP standard growth medium
- FB Fermentative Broth medium

		RPP	FB
рН		6.8	6.8
NH4 ⁺	mg L ⁻¹	-	50.4
ORGANIC ACID	g L ⁻¹	4.0	2.2
NH₄Cl	g L ⁻¹	-	-
Na glutamate	g L ⁻¹	-	-
K ₂ HPO ₄	g L ⁻¹	0.5	0.5
KH ₂ PO ₄	g L ⁻¹	0.3	0.3
MgSO ₄ ·7H ₂ O	g L ⁻¹	0.4	0.4
NaCl	g L ⁻¹	0.4	0.4
$CaCl_2 \cdot 2H_2O$	g L ⁻¹	0.075	0.075
Ferric citrate	g L ⁻¹	0.005	0.005
Yeast extract	g L ⁻¹	0.4	0.4
Trace elements solution ^a	mL L ⁻¹	1	1
Vitamins solution ^b	mL L ⁻¹	1	1



• TEST SET UP



Reactors configuration: Batch condition 250 mL of working volume 30 °C, suspended cultures Tested PNSB strains:

- Rhodopseudomonas palustris
- Rhodospirillum rubrum

Tested media:

- RPP standard growth medium¹
- FB Fermentative Broth medium





Rhodopseudomonans palustris optical microscope, 100x magnification



Rhodospirillum rubrum optical microscope, 100x magnification



After 10 days

R. palustris R. palustrisVFA medium R. rubrum VFA medium standard standard medium medium

R. palustris vs. *R. rubrum* Growth kinetics on VFA-enriched medium

Start up of the test



















- Both strains showed a consistent boost in growth yields when fed with FB medium rather than standard RPP medium
- Both PNSB strains reached a higher biomass concentration when fed with the FB medium
- However, no hydrogen production was detected on the FB medium, attesting the presence of some inhibiting compound, most likely the ammonia concentration of the medium (50–80 mg_{NH4+} L⁻¹)

PNSB strain	Medium	biomass (g _{PNSB} L ⁻¹)	Pmax (mg L ⁻¹ d ⁻¹)	µmax <i>(d</i> -¹)
Rps. palustris	RPP FB	x2 0.54 1.08	×2 84.31 159.53	14% 4.43 5.05
Rsp. rubrum	RPP FB	× 8 0.13 1.04	×5 28.09 128.32	74% 2.16 3.76









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Test on agar immobilized Rps. palustris (test in serum bottles)

The immobilized culture was prepared using 0.5 % w/v agar. The immobilization protocol was the same as reported in WP2 (Task 2.1): after cooling down the agar solution at around 35-40 °C, it was poured directly into the test bottles containing the PNSB inoculum, and it was let to solidify before adding the RPP medium.

M&Ms were the same used for the suspended biomass tests





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Test on agar immobilized Rps. palustris (test in serum bottles)



The gas production rate obtained on immobilized culture was 151.4 NmL $L_{reactorWV}^{-1}$.

Immobilized culture resulted in a lower SGP (37.3 NmL $g_{(malic acid)}^{-1}$) compared to suspended biomass test with 50 NmL $g_{(malic acid)}^{-1}$).

(Light as limiting factor?)













Test on agar immobilized *Rps. palustris* (test in serum bottles)

After preliminary test on immobilized culture using serum bottles, a pre-pilot plant was set up to test the immobilized PNSB in a tubular hollow reactor configuration to optimize light distribution.













Elements of the pre-pilot plant for test on immobilized bacteria



- 1) Mold for agar cylinder
- 2) Reactor
- 3) Trap for gas / hydrogen
- 4) Light source
- 5) Temperature control

The reactor, consists of a borosilicate glass cylinder with an internal diameter of 37mm and a length of 550mm and contains the solidified agar column and the nutrient medium for the immobilized bacteria. The closing caps are made in plastic material. The glass cylinder is kept in vertical position by laboratory support stand and on upper cap there is insertion of a silicon tube for the collection of gas produced by bacteria.



1) Mold for agar cylinder

The mold, in methacrylate, was designed and built because it is necessary for the shaping of the agar column. It consists of a cylinder with the same dimensions as the reactor.

Moreover, by means of a cannula, also in methacrylate, placed centrally, with 10mm diameter, it allows to obtain a central empty volume; this volume serves two purposes:

- It offers a greater surface area of the agar cylinder in contact with the bacterial growth medium;

 It promotes the elimination of hydrogen.
At the ends two rubber closing caps are inserted, to retain the liquid agar during the solidification phase and offer a central housing for the methacrylate cannula too.







Reactor

- 3) Trap for gas / hydrogen
- 4) Light approximately 3000 lux
- 5) Temperaturec- 30°C



The trap to collect the produced gas consists of a glass cylinder, kept in vertical position, is open at the bottom and closed at the top. The cylinder is filled with water and the bottom is immersed in water contained in a beaker so that the atmospheric pressure prevents it from emptying. A silicone tube, with an internal diameter of 37.2 mm, which exits from the top of the reactor, conveys into the trap the gas produced by the bacteria immobilized in agar. The gas can therefore be measured, in volume, thanks to the graduated scale placed on the column (10 mm correspond to 10.75 ml of gas). The value of gas produced is recorded every day at 12:00.



first Hydrogen production test

Technical data of the gas production on immobilized PNSB in tubular reactor

R. palustris concentration	0.2 g L ⁻¹
Agar cylinder volume	460 mL
Agar cylinder dimension	diameter 3,7 mm and height 550 mm
Growing medium	RP0 (without Na glutamate)
Growing medium volume	131 mL
Light	Osram L18W / 827 fluorescent tube
Temperature	30°C
Duration of the test	12 days



The gas production rate, calculated as the ratio between the cumulative gas production and the reactor working volume was 440.2 NmL $L_{reactor}^{-1}$, considerably higher compared to the 200.0 and 151.4 NmL $L_{reactor}^{-1}$ obtained in the previous batch test for suspended and immobilized culture, respectively. The specific gas production as well results considerably higher than those obtained in previous test, with a SGP of 110 NmL $g_{(malic acid)}^{-1}$ compared to 49.3 and 37.3 NmL $g_{(malic acid)}^{-1}$ for suspended and immobilized culture, respectively.













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Conlclusions

Preliminary test on gas production were carried out on serum bottle and tubular pre-pilot plant using RPP and RP0 (without Na-glutamate). It was possible to observe that in tubular configuration the yields obtained in term of gas production were higher than those obtained with serum bottle, and this is mainly due to a better light distribution in a tubular reactor. Results must has been further improved considering the dark fermentation as liquid medium in batch system and with tubular reactor with optimized light supply.













In this Task 1.3 the research focus is the evaluation of the light supply and the effect of light in hydrogen production, applied to the tubular, immobilized reactor fed with standard medium in a batch mode.

This Task is divided into 2 subtask:

- i) three types of light were tested for the pre-prototype (and then for the final reactor), applying a continuous lighting approach (24h), abandoning the idea of pulsed light;
- i) the continuous LED light applied was optimized (internal light).













3 TYPE LIGHT SOURCES HAVE BEEN TESTED:

1) FLUORESCENT TUBES (neon) (out of production) (18 watt).

2) OPTICAL FIBERS: two emitters were purchased and tested: 3 and 9 watt. Various tests has been carried out but the light energy output was too low, with no hydrogen production, so the optical fibers option has been discarded.

















The main reason, in addition to the fact that fluorescent tubes are no longer available on the market, is that **COB LEDs** allow:

- energy saving,
- greater flexibility of use,
- a highly compact, small size design,
- a simpler single circuit design,
- a high luminous efficacy, greater intensity, particularly at close distances,
- uniform diffusion of light, even when at close working distances.



The "chip on board" technology allows the use of panels that have a single circuit and two simple contacts for the whole chip, with a potentially unlimited number of diodes with low energy requirement



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• (C)



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COB LED gave excellent results, such as a stable hydrogen production without damaging the photosystem I of the bacteria. Thanks to the COB LED the temperature monitored was uniformely distributed (28-33 °C).















COB LED gave excellent results, such as a stable hydrogen production without damaging the photosystem I of the bacteria. Thanks to the COB LED the temperature monitored was uniformely distributed (28-33 °C).

Light Position		External	External		
Light Source	Light Source		COB LED		
Medium		RPP	RPP	VFAs	
Carbon Source		Malic acid	Malic acid	VFAs	
OA concentration	g _{oa} L⁻¹	4.0	4.0	2.2	
Biogas Productivity	NmL d⁻¹	10	142	70	
H ₂ Productivity	$NmL_{H2} d^{-1}$	8	114	56	
	NmL g _{oA} -1	88	373.6	632.8	
SHP	NmL mmol _{oA} -1	11.8	50.1	55.7	
	mmol _{H2} mmol _{OA} ⁻¹	0.53	2.24	2.49	
	$NmL_{H2} L^{-1} d^{-1}$	61.8	867.2	427.5	
HPR *	$NmL_{H2} L^{-1} h^{-1}$	2.58	36.13	17.81	
	$mmol_{H2} L^{-1} h^{-1}$	0.11	1.61	0.80	
* on medium volume					

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To get a more efficient light, at a technological level and for aesthetic reasons too, a new lighting system was designed and built. Veritas has developed a special light bulb, in glass, composed of two LED strips, the light bulb has been positioned inside the reactor and inserted exactly in the center of the agar column in order to guarantee almost a 360° lighting.







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The light placed inside should allow to:

- Fully exploit to the light radiation: every single photon reaches the bacterial biomass;
- Make the distribution of heat produced by the LED strip homogeneous;



The LEDs are controlled by a power supply, with a dimmer with a maximum power of 8 Watts (with the two LED strips). The regulation of the light emission is essential when starting up the reactor: it is important to maintain a low lux flux which can be increased as the bacterial concentration increases.













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Evolution of lighting source: efficiency with continuous light

Spectrum. The LEDs chosen for the tests have a colour temperature of 4000° K. The emission of this type of light can be seen in the table. This light supplies to the bacteria the most complete radiation, when compared with the other spectra.



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WP1-T 1.3 --> Analysis and evaluation of the H2 evolution efficiency with continuous or pulsed light

Evolution of lighting source: efficiency with continuous light

• Colour Temperature of LED lighting

LED light sources are based on the <u>Kelvin system</u> of measure. A warm color temperature is typically 3,000 K or less. A "cool" white bulb commonly has a colour temperature of at least 4,000 K. This basic scale is a good indication of the range offered by LED sources.













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WP1-T 1.3 --> Analysis and evaluation of the H2 evolution efficiency with continuous or pulsed light



The luxmeter detects how much light comes out of the reactor. Obviously, the higher the cell concentration, the less the lux is detected by the device



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The prototype configuration was slightly changed over the experiment time, in order to facilitate the gas collection in the top of the column and in the gas trap, to facilitate the sampling and characterization.



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The test were carried out in batch mode adopting the tubular column as described in Tasks 2.1 and 2.2 and adopting the experimental set up as here below listed:

Internal LEDs system

























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Probably photoinhibition due to the too high initial lightning (75% directly)

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					RUN 1	RUN 2	RUN 3	RUN 4	RUN 5
		malic 4.0 g L ⁻¹	malic 4.0 g L ⁻¹	VFA 2.2 g L ⁻¹	malic 4.0 g L ⁻¹	¹ VFA 3.0 g L ⁻¹	VFA 3.1 g L ⁻¹	VFA 3.1 g L ⁻¹	VFA 3.1 g L ⁻¹
		RPP	RPP	VFA	RPP	VFA	VFA	VFA	VFA
		Fluorescent Lamp	COB LED-ext	COB LED-ext	COB LED-int	COB LED-int	COB LED-int	COB LED-int	COB LED-int
Biogas Productivity	NmL d ⁻¹	10	142	70	51	71	43	20	-
H2 Productivity	NmL d⁻¹	8	114	56	41	57	34	16	-
SHP	NmL g _{OA} -1	88	373,6	632,8	430,9	330,3	163,7	50,5	-
SHP	NmL mmol _{OA} -1	11,8	50,1	55,7	57,8	27,6	13,5	4,2	-
SHP	mmol mmol _{oA} -1	0,53	2,24	2,49	2,58	1,23	0,60	0,19	
HPR*	NmL L ⁻¹ d ⁻¹	61,8	867,2	427,5	410	570	340	160	-
HPR*	NmL L ⁻¹ h ⁻¹	2,58	36,13	17,81	17,08	23,75	14,17	6,67	-
HPR*	mmol L ⁻¹ h ⁻¹	0,11	1,61	0,80	0,76	1,06	0,63	0,30	-
* on medium volume									

Table 4 – Comp						
Culture type	Bacteria	Carbon source	Reactor type and volume	Conditions	Productivity (mmol/L/h)	References
Suspended	R. capsulatus YO3	Dark fer. Eff. of Thick Juice (acetate)	Panel (4 L)	Outdoor	1.36	[37]
Suspended	R. capsulatus YO3	Dark fer. Eff. of Thick Juice (acetate)	Panel (4 L)	Indoor	1.05	[16]
Suspended	R. capsulatus DSM 1710	Dark fer. Eff. of Thick Juice (acetate)	Panel (4 L)	Indoor	1.01	[16]
Suspended	R. capsulatus YO3	Dark fer. Eff. of molasses (acetate)	Panel (4 L)	Outdoor	0.67	[38]
Suspended	R. capsulatus YO3	Acetate	Panel (4 L)	Outdoor	0.51	[19]
Suspended	R. capsulatus YO3	Acetate	Panel (8 L)	Indoor	0.66	[35]
Suspended	R. capsulatus DSM 1710	Acetate	Tubular (80 L)	Outdoor	0.31	[18]
Immobilized	R. capsulatus DSM 1710	Acetate	Roux bottle (0.25)	Indoor	0.75	[23]
Immobilized	R. capsulatus YO3	Acetate	Roux bottle (0.25)	Indoor	2.04	[23]
Immobilized	R. capsulatus DSM 1710	Acetate	Panel (1.4 L)	Indoor	0.75	This study
Immobilized	R. capsulatus YO3	Acetate	Panel (1.4 L)	Indoor	1.3	This study



		RUN 1	RUN 2	RUN 3	RUN 4	RUN 5
		malic4.0gL ¹	VFA 3.0 g L ^{:1}	VFA 3.1 g L ^{'1}	VFA 3.1 g L ^{'1}	VFA 3.1 g L ^{'1}
		RPP	VFA	VFA	VFA	VFA
		COB LED-int	COB LED-int	COB LED-int	COB LED-int	COB LED-int
	NmL $\boldsymbol{g}_{\scriptscriptstyle 0}$, ''	430.9	330.3	163.7	50.5	-
SHP	NmL mmol _{0 A} ⁻¹	57.8	27.6	13.5	4.2	-
	$mmol_{H_2} \ mmol_{O_A}^{-1}$	2.58	1.23	0.60	0.19	-
	$NmL_{s,2} L^{1} d^{1}$	410	570	340	160	-
HPR *	$NmL_{s,2}$ L^{-1} h^{-1}	17.08	23.75	14.17	6.67	-
	$mmol_{H2} L^{1} h^{1}$	0.76	1.06	0.63	0.30	-

Table 5

Comparison of the photo-hydrogen production studies of photosynthetic suspension and immobilized cultures with the highest hydrogen production rates and yields on various carbon sources.

Substrate	Concentration (g/L)	Reactor type	Reactor volume (mL)	Bacteria	Light source	Light intensity	Culture type	Type of process	Hydrogen production rate (mL/L/h)	H ₂ yield	Reference
Acetic acid	1.0 ^b	CSTR	800	Rhodopseudomonas palustris WP3-5	Halogen/ Tungsten lamps	95 W/m ²	Suspension	Continuous HRT, 48 h	38.2	3.15	[134]
Acetic acid	3.6	Roux flask	200	Rhodobacter capsulatus YO3	Tungsten	4000 lux	Immobilized	Fed-batch	48.9 ^ª	3.08	[41]
Malic acid	0.12	Glass tube	38	Rhodobacter	Tungsten	7000-8000 lux	Suspension	Batch	65.9 ^ª	4.3ª	[135]
Malic acid	2	Flat plate	200	Rhodobacter sphaeroides O.U.001	Vitalux	64 W/m ²	Immobilized	Semi- continuous	59.0	4.2	[62]
Glucose	3.5	Auto chemostat	350	Rhodobacter capsulatus JP91	Incandescent	50 W bulbs	Suspension	Continuous HRT, 48 h	19.8ª	9.0	[136]
Glucose	9	Glass bottle	100	Rhodopseudomonas faecalis RLD-53	Incandescent	2000 <mark>l</mark> ux	Immobilized	Batch	9.1	6.32	[49]
Glucose	4	PMMA vessel	1200	Rhodopseudomonas palustris CQK 01	LED	5000 Lux	Immobilized	Batch	38.9	0.2	[54]
Sucrose	2	Glass bottle	125	Rhodobacter capsulatus JP91	Halogen lamps	200 W/m^2	Suspension	Batch	22.6 ^ª	14.9	[33]
Sucrose	1.7	Plexiglass	1400	Rhodobacter capsulatus YO3	Tungsten lamps	200 W/m^2	Immobilized	Fed-batch	17.8ª	19	[19]

^a Values was converted from original data.

^b g COD/L.



WP2 Evaluation of organic/synthetic matrices for the PNSB immobilization

Task 2.1 --> Chemical-physical and biological (toxicity) characterization of 3 organic and inorganic matrices at least potentially suitable for the immobilization of PNSB

Task 2.2 Protocol for the management of the PNSB harvesting and immobilization process in a hollow tubular immobilization support.

Task 2.3 DEvaluation of H2 evolution efficiency with the selected geometry for the Purple-B reactor (tubular)











Task 2.1 --> Chemical-physical and biological (toxicity) characterization of 3 organic and inorganic matrices at least potentially suitable for the immobilization of PNSB



The immobilization system has been tested by entrapment in solid gel matrices (polyacrylamide gels, alginate gels and agar gels).

The cells immobilized in **polyacrylamide gel** NN CH_{3-} acrylamide 3–(dimetylamino) propionitrile/K₂S₂O₈ (catal.) were not fully active due to the inhibitory effect probably caused by the release of toxic monomers.







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Task 2.1 --> Chemical-physical and biological (toxicity) characterization of 3 organic and inorganic matrices at least potentially suitable for the immobilization of PNSB





The **alginate gel structure** results not stable in fact it appeared that the **sulfate anion of the nutrient medium** has a strongly negative effect on the structural stability of the Ca⁺² -alginate matrix.





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The preparation of the matrix based on agar was carried out starting from a was carried out by trying two different ways:

1. The agar column gelled was immersed in a cylinder of liquid medium containing R. palustris, but in this case few bacteria managed to settle in the agar;

1. The bacterial cells were pre-concentrated by centrifugation, 10 minutes at 8500 rpm, and added to the gelling solution of agar and RP2 medium before it completes the solidification process; this turned out to be the optimal solution.

suspension of washed agar in liquid nutrient RPP. The cell trapping method

Task 2.1 --> Chemical-physical and biological (toxicity) characterization of 3 organic and inorganic matrices at least potentially suitable for the immobilization of PNSB



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Final meeting 14/09/2023





Task 2.1 --> Chemical-physical and biological (toxicity) characterization of 3 organic and inorganic matrices at least potentially suitable for the immobilization of PNSB

The agar-based matrix has been demonstrated to be the optimal solution.

• In order to identify the optimal operating process temperature, i.e. the one capable of guaranteeing both good fluidity for full homogenization of the cell agar suspension and sufficient integrity of the matrix, immobilization tests were performed at different temperatures, but close to the solidification point. of the agar solution. The results indicated the optimal value of 40 ° C at 4.0 g / I of agar.







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First step is the immobilization cells in agar (chosen matrix), the cell immobilization process requires three steps:

- 1. Preparation of porous matrix: dissolve 4 g of agar powder in 1 L of distilled water, and sterilize..
- 1. Immobilization: PNSB biomass is inserted when the temperature of the agar is approximately 37 40°C (0.2g of cells per 460 ml of solution).







3. when a homogenous distribution of PNSB cells in the agar is achieved, before the liquid matrix solidification, pour the medium immediately into the cylindrical methacrylate mold.

3. squeeze out the air bubbles by tapping the mold lightly

3. Leave the agar medium to solidify for 24h at room temperature.

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Reactor startup

8. the following day the agar cylinder is slid into the reactor. After solidification, the agar cylinder was inserted into the glass pre-pilot plant in which the growing medium RPO was also poured;

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9. pour the medium until the reactor is filled;

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10. turn on the light and start the test;





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Test startup

11. the test with RPO and VFAs mediums are ready to start (T1.3), with the following parameters: temperature 30°C and light source 4000 K.

12. H₂ production begins after 24 hours to the start of the test, this can be seen from the level of gas accumulated in the trap. The tests carried out in this period show a certain constancy: the level of the trap drops by an average of 4 cm per day (44 ml/day)/ reactor. The gas value produced is recorded every day at 12:00.





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13. Every day the head space of the trap, for safety reasons, is emptied to avoid the accumulation of too much hydrogen: the operation is carried out by taking with a syringe through the silicone septum present in the cap (from above) which is the same point used for taking samples for the gas chromatograph





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14. The replacement of the "exhausted" growth medium is carried out making the new medium flow inside each reactor with a peristaltic pump to avoid the risk of collapse of the agar column, in case it is left without a fluid to support it.

15. During the medium replacement operation 2 samples for reactor always be taken: the first with the "old" medium exhausted of organic acids and the second at the end of the filling. The samples will then be used for the HPLC analysis of the real composition of organic acids of each test cycle.





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• system to evaluate the behaviour of biomass:

The prototype is equipped with tree different sensors to oversee the process:

- temperature;
- pH;

- Light; a luximeter sensors is placed near the reactor, opposite to the LED light source, to consistently monitor and log the level of illumination.

All of these sensors transmit signals to the PLC.

• the immobilized bacteria maintain their vitality unaltered for each renewal of the medium with organic acids.





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WP3 Design and realization of the photobioreactor (PBR) prototype Purple-B

T 3.1 --> Design and rendering of the structural and functional components of the Purple-B reactor





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First hypothesys for the prototype: 9 columns (h 1500mm) and central light



WP3 T 3.1 \rightarrow design and rendering of the structural and functional components of the Purple-B reactor



Hydrogen circuit













WP3 T 3.1 → design and rendering of the structural and functional components of eesa the Purple-B reactor Hydrogen circuit Medium circuit The prototype will have dimensions of approximately **Reactors holders** 25x35 cm and h100 cm. Reactors It will be powered with 220V 50Hz electric current with a nominal power of less than 100W (~15W for leds, ~60W heating resistance, ~6W peristaltic pump and ~10W for PLC). Support structure









WP3 T 3.1 →design and rendering of the structural and functional components of the Purple-B reactor















WP3 T 3.1 →design and rendering of the structural and functional components of the Purple-B reactor

The reactor holders are 3 semi-circular aluminium sheets (internal diameter of ~27mm) and each of them supports magnetically one reactor. Moreover, each of them had a led strip for the illumination of the reactor. The semi-circular shape is intended to direct the light to the reactor without dispersion.



Led strip























WP3 Design and realization of the photobioreactor (PBR) prototype Purple-B

T 3.2 --> Realization of the reactor and of the H2 capture system







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The reactor















The light sources

















The light sources













The support for three reactors and the gas collector










Some components of phobioreactor were realized with 3D printer

















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The aluminum support structure

















The electrical cabinet











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The electrical cabinet

















The electrical cabinet



















Assembly of Purple B pilot















Finally the PLC was programmed to read the analog inputs and control the output.











Conclusions

The Purple B reactor was successfully realized!











WP4 Evaluation of the photoconversion process on the prototype Purple-B



T 4.1 --> H2 conversion efficiency











Fluid-dynamic model and simulation

Ansys Fluent software has been used to simulate the system. The first simulations were aimed at reproducing the system operation and finding the main important boundary conditions for the water motion. The velocity and pressure trends were found.



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The reactor



From WP3 T 3.2 Realisation of the reactor and of the H2 capture system













To simplify the problem, firstly a 2-D geometry has been implemented, while the last simulations were made with a 3-D one.

The water motion in the reactor was mainly studied, but first attempts with the addition of the H_2 bubble were also made.













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A zoom on the two-dimensional mesh















Parts of three-dimensional mesh. Right hand side represents the upper section of the reactor; left hand side represents the lower part.







Some results

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Static pressure field for g=9,81 m/s²

The effect of the weight of the water column is predominant

The presence of a stagnation point is highlighted by the 3D simulation

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Some preliminary results

- The total energy losses inside the reactor are about 2 Pa.
- The total pressure variation inside the reactor is about 5525 Pa.
- To ensure the flow in the current geometry and boundary conditions, a difference of pressure between the water and the hydrogen outlets of 124 Pa (12,5 mm H2O) must be guaranteed.

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First preliminary results

• The effects are evident on the pressure field, but also on the velocity one.

Future development

• A deeper analysis on the gravity effects must be performed.









Thermo fluid dynamic model

- The presence of the lamp has been considered.
- The water enters at 30°C.

Static temperature field

The effect on the temperatures of the lamp is negligible











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Some preliminary results

- The results from the simulation are confirmed by the experimental ones.
- There is a very small drop in temperature.
- The inlet temperature of water should be controlled.



















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VERITAS

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WP4 Evaluation of the photoconversion process on the prototype Purple-B

T 4.2 --> Permeability, transparency and resistance of the matrix during the photoevolution of H2











• Resistance of the matrix

The resistance of the matrix was evaluated with a compression test performed with Gabaldini Quasar 25 Machine











• Resistance of the matrix



F=14N A=1734 mm² σ_R =8.0 kPa



6N A=1734 mm² $\sigma_R \rightarrow 3.5 \text{ kPa}$











• Permeability of the matrix

The test was performed by preparing a solution of volatile fatty acids, then agar cubes of about 9.5 grams each were immersed (in 50 ml falcons), which were left in contact with the AGVs for various times: 1, 3 and 6 hours.

The tests were performed in duplicate.

the T0 is represented by the agar not immersed in the AGV solution. AGV absorption was evaluated by COD analysis.









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• Permeability of the matrix



DATE	Sample	Weight	Bicromate concentration	Bicromate Volume	FAS baseline	FAS measure	FAS sample	COD	AVERAGE	st. dev.	err%
		g	N	mL	mL	mL	mL	dry	gO2/kgTQ	gO2/kgTQ	%
07/06/2023	TQ	0.7216	0.25	15	18	18	13.2	11.09	11.24	0.212249	1.89%
07/06/2023	TQ	0.7904	0.25	15	18	18	12.6	11.39			
07/06/2023	1h	0.7824	0.25	15	18	18	12.5	11.72	11.66	0.077009	0.66%
07/06/2023	1h	0.8041	0.25	15	18	18	12.4	11.61			
07/06/2023	3h	0.6559	0.25	15	18	18	12.4	14.23	14.05	0.257972	1.84%
07/06/2023	3h	0.6972	0.25	15	18	18	12.2	13.86			
07/06/2023	6h	0.7632	0.25	15	18	18	11.6	13.98	14.26	0 106095	2 9 5 0/
07/06/2023	6h	0.6872	0.25	15	18	18	12.0	14.55	14.20	0.400985	2.65%



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• Transparency of the matrix



The transparency of the agar was tested analyzing the Lutron LM-8000A instrument.



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Conclusions:

- **the resistance** of the matrix is comparable of that of agar but is decreased by the presence of the bacteria (3.5kPa compression)
- the permeability of the matrix to nutrients is high: complete diffusion across 1cm of agar in approx. 3 h.
- **the transparency** of the matrix also showed a significant decrease of the over time (25% after one week) due to the fact that the amount of bacteria increases.



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Master Tesis: Giulio Barchielli, Valutazione delle rese produttive di un processo di Dark Fermentation e valorizzazione dell'effluente tramite fermentazione fotosintetica.

Scientific Paper: Biasiolo M., Barchielli G., Tassinato G., Turatello M., Cavinato C. (2023) Coupling mixed culture fermentation and photo fermentation for bio H2 recovery: preliminary assessment of the fermentation yields and PNSB growth on fermentative broth. Chemical Engineering Transactions Journal (CET)

Submitted Project: ASI call on «Esperimenti Scientifici per la Luna» BioMoon: low gravity biorefinery platform

ESA Purple B project has been properly Aknowledge









NEXT STEP

FROM PURPLE-B TO...

GREEN**P**ROPULSION**L**AB





BIOMOON PROJECT LOW GRAVITY BIOREFINERY PLATFORM

and



TOPICAL TEAM-LIFE SCIENCE Sustainable Low-Gravity Biorefinery Models for Energy, Food and Chemical Production in the Space Missions











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Biomooni projec

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TOWARDS...

Confronta più foto insieme Seleziona più anteprime per confrontarle insieme.

×
Purple-B Project



European Space Agency





THANKS FOR WATCHING



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