

Elucidating metabolic tuning of mixed purple phototrophic bacteria biofilms in photoheterotrophic conditions through microbial photo-electrosynthesis

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SUPPLEMENTARY INFORMATION



Figure S1. Full-growth PPB mixed biofilm developed for photo-bioelectrochemical and Open Circuit Control experiments.

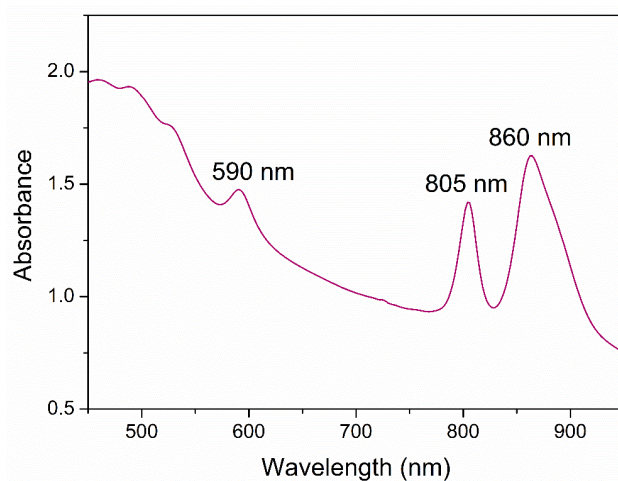


Figure S2. Absorbance screening of PPB mixed biofilm presented at the beginning of the experiment carried out at -0.8V.

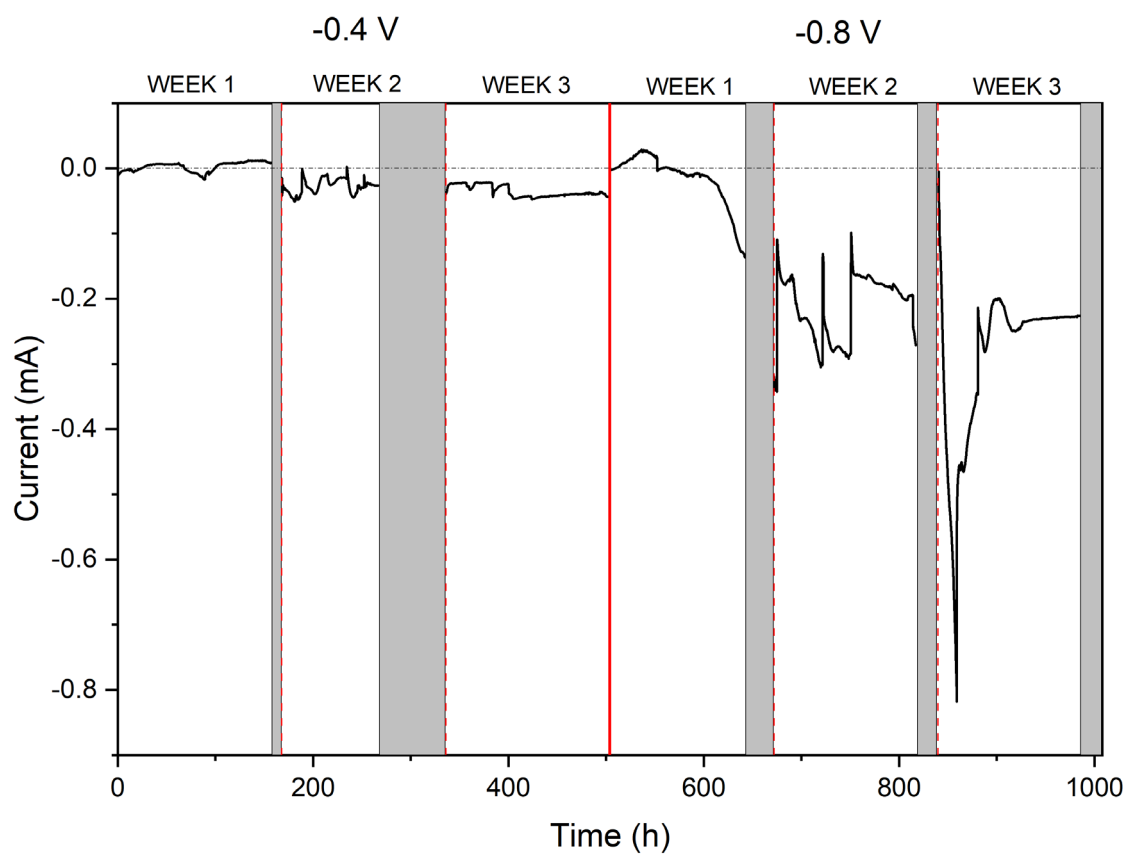


Figure S3. Full chronoamperometry data of the successive experiments at -0.4 V and -0.8V (vs Ag/AgCl). Dashed regions indicate arbitrary fails in the computer data saving during weekends.

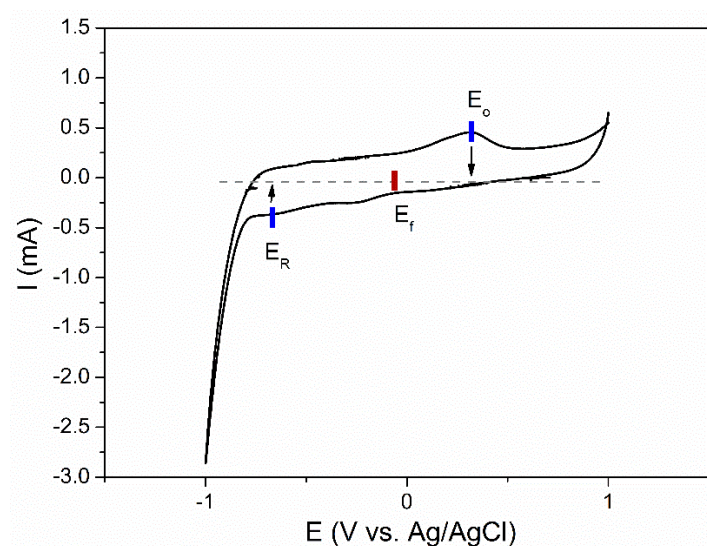


Figure S4. Example of apparent midpoint potential (E_f) from a Cyclic voltamperometry obtained from the day 2 of the photo-bioelectrochemical experiment at -0.8V

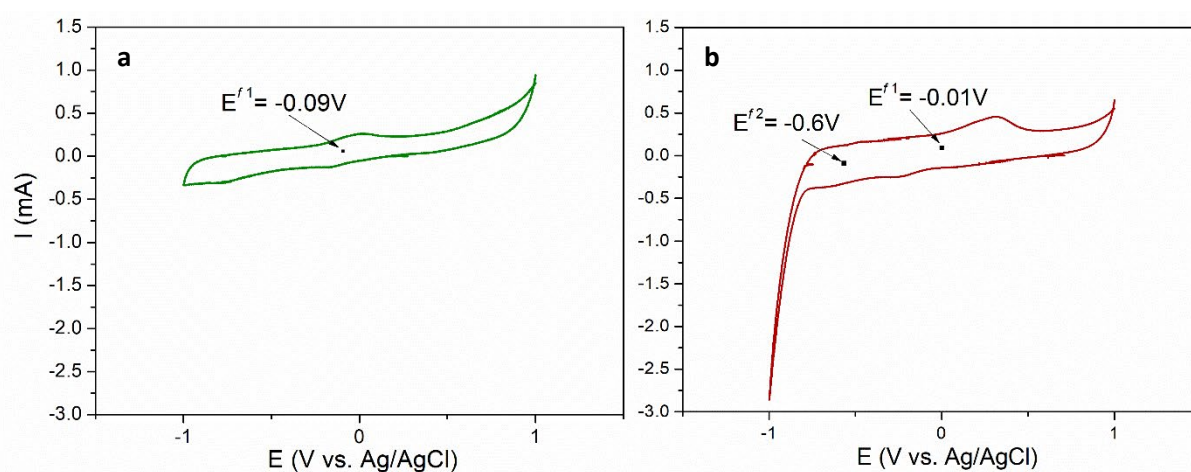
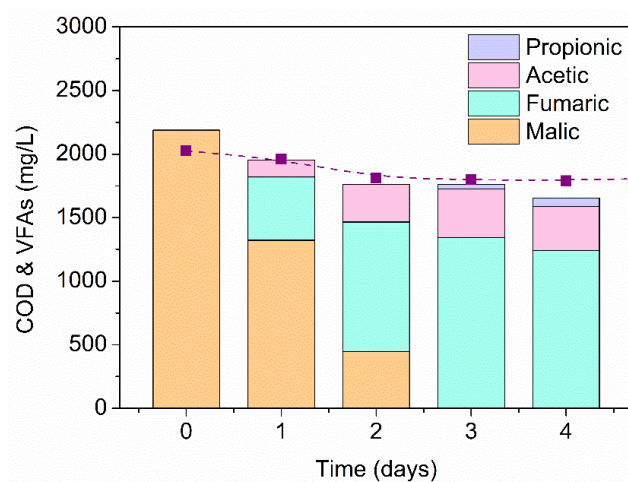


Figure S5. Cyclic Voltamperometry of photo-bioelectrochemical test on day 2 and formal potential (E_f) of each experiment (a) -0.4V and (b) -0.8V.

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36 **Figure S6.** Chemical Oxygen Demand (COD) and short-chain carboxylic acids (SCCA) time-course of
37 dark control: Malic (orange). fumaric (green), acetic (pink) and propionic (purple).

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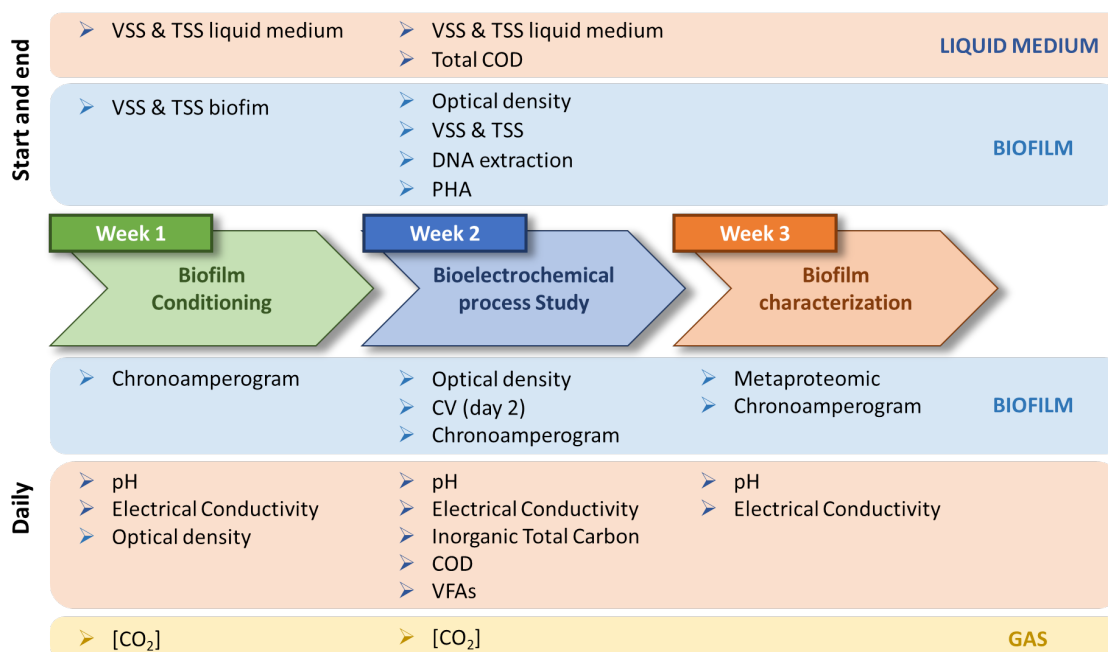


Figure S7. Detailed experimental procedure.

Table S1. pH values of the cathode chamber liquid medium

Voltage applied	Days				
	0	1	2	3	4
-0,4V	6.79	7.12	7.55	7.99	7.98
-0,8V	6.78	7.3	8.52	8.57	8.43

Table S2 Enzymes involved in different metabolic pathways and their acronym used in **Figure 7**

	ACRONYM	ENZYMES
Tricarboxilic Acid Cycle (TCA)	MD	Malate dehydrogenase
	CS	Citrate synthase
	DRS	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex
	SCoAL (sub α)	Succinate-CoA ligase subunit α
	SCoAL (sub β)	Succinate-CoA ligase subunit β

	SD	Succinate dehydrogenase
	MS	Malate synthase
	Is	Isocitrase
	ID	Isocitrate dehydrogenase
Calvin–Benson–Bassham (CBB) Cycle	RuBisCo	Ribulose biphosphate carboxylase
	PGK	Phosphoglycerate kinase
	F1,6BA	Fructose-1,6-bisphosphate aldolase
	F1,6B	Fructose 1,6-biphosphatase
Polyhydroxyalkanoate synthesis	ACoAat	Acetyl-CoA acetyltransferase
	3O[acp]R	3-oxoacyl-[acyl-carrier-protein] reductase
	ACoAD	Acyl-CoA dehydrogenase
	ECoAH	Enoyl-CoA hydratase
Anaplerotic CO₂ fixation	PC	Pyruvate carboxylase
	PEPC	Phosphoenolpyruvate carboxykinase
	En	Enolase
	PK	Pyruvate Kinase
	PD	Pyruvate dehydrogenase
	P-f	Pyruvate-ferredoxin/flavodoxin oxidoreductase
	PEPS	Phosphoenolpyruvate synthetase
	Podk	Pyruvate, orthophosphate dikinase
	CS	Citrate synthase

Table S3 Molecules involved in different metabolic pathways and their acronym used in **Figure 7**.

ROUTE	ACRONYM	MOLECULES
Tricarboxylic Acid Cycle (TCA)	Mal	Malate
	Ox	Oxalacetate
	Cit	Citrate
	Cis-Ac	Cis-aconitate
	DTI	D-theo-isocitrate
	2Ox	2-oxoglutarate
	SCoA	Succinyl-CoA
	Succ	Succinate
	Fum	Fumarate

Calvin–Benson–Bassham (CBB) Cycle	DR1,5B	D-ribulosa-1,5-biphosphate
	3PDG	3-phospho-D-glycerate
	3PDGP	3-phospho-D-glyceroyl phosphate
	DG3P	D-glyceraldehyde 3-phosphate
	GP	Glycerone phosphate
	βDF1,6B	β-D-fructofuranose 1,6-biphosphate
	βDF6P	β-D-fructofuranose 6-phosphate
	DX5P	D-xylulose 5-phosphate
	DR5P	D-ribulose 5-phosphate
Polyhydroxyalkanoate synthesis	ACoA	Acetyl-CoA
	AaCoA	Acetoacetyl-CoA
	PCoA	Propionyl-CoA
	KCoA	Ketovaleryl-CoA
	HCoA	Hexanol-CoA
	H2ECOA	Hex-2-enoyl-CoA
	3HBCoA	3-Hydroxybutyryl-CoA
	3HVCOA	3-Hydroxyvaleryl-CoA
	3HHCOA	3-Hydroxyhexanoyl-CoA
Anaplerotic CO₂ fixation	PEP	Phosphoenolpyruvate
	Pyr	Pyruvate
	ACoA	Acetyl-CoA

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56 **SUPPLEMENTARY REFERENCES**

- 57 1. Carlozzi, P. & Sacchi, A. Biomass production and studies on
58 Rhodopseudomonas palustris grown in an outdoor, temperature controlled,
59 underwater tubular photobioreactor. *J Biotechnol* **88**, 239–249 (2001).
- 60 2. Batstone, D. J. *et al.* The IWA Anaerobic Digestion Model No 1 (ADM1).
61 *Water Science and Technology* **45**, 65–73 (2002).