# Supplementary information

Table S 1. List of scientific papers, published in the last 5 years, mentioning PHB production from microalgae or cyanobacteria without considering microalgae-bacteria association.

| Title | DOI | Year | Journal | Axenic Culture? |
|---|---|---|---|---|
| Optimization of Polyhydroxybutyrate Production by Amazonian Microalga *Stigeoclonium* sp. B23 | https://doi.org/10.3390/biom10121628 [1] | 2020 | Biomolecules | No |
| Production of polyhydroxybutyrate by the cyanobacterium cf. *Anabaena* sp. | https://dx.doi.org/10.1016/j.ijbiomac.2021.09.054 [2] | 2021 | International Journal of Biological Macromolecules | No |
| Nutritional factors influence polyhydroxybutyrate in microalgae growing on palm oil mill effluent | https://doi.org/10.1007/s10811-021-02654-2 [3] | 2021 | Journal of Applied Phycology | No |
| Screening, optimization and characterization of polyhydroxy butyrate from freshwater microalgal isolates | https://doi.org/10.1080/24759651.2021.1926621 [4] | 2021 | International Journal of Biobased Plastics | Yes (no molecular analyses carried out) |
| Photoautotrophic and Mixotrophic Cultivation of Polyhydroxyalkanoate-Accumulating Microalgae Consortia Selected under Nitrogen and Phosphate Limitation | https://doi.org/10.3390/molecules26247613 [5] | 2021 | Molecules | No |
| Newly isolated native microalgal strains producing polyhydroxybutyrate and energy storage precursors simultaneously: Targeting microalgal biorefinery | https://doi.org/10.1016/j.algal.2021.102625 [6] | 2022 | Algal Research | No |
| Production of polyhydroxyalkanoates from microalgae- a review. | https://doi.org/10.51847/NeYIasA2Ix [7] | 2022 | Journal of Biochemical Technology | / |
| Polyhydroxybutyrate production by *Chlorella sorokiniana* SVMIICT8 under Nutrient-deprived mixotrophy | https://doi.org/10.1016/j.biortech.2022.127135 [8] | 2022 | Bioresource Technology | No |
| Biopolymers production from microalgae and cyanobacteria cultivated in wastewater: Recent advances | https://doi.org/10.1016/j.biotechadv.2022.107999 [9] | 2022 | Biotechnology Advances | / |
| Use of exogenous substrate in Chlorella cultivation: Strategy for biomass and polyhydroxybutyrate production | https://doi.org/10.1016/j.ijbiomac.2023.123193 [10] | 2023 | International Journal of Biological Macromolecules | No |
| Semi-continuous production of polyhydroxybutyrate (PHB) in the Chlorophyta *Desmodesmus communis* | https://dx.doi.org/10.1016/j.algal.2023.103196 [11] | 2023 | Algal Research | No |

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|---|---|---|---|---|
| Drying strategies for maximizing polyhydroxybutyrate recovery from microalgae cultivated in a raceway pond: a comparative study | https://doi.org/10.1016/j.envpol.2024.124821 [12] | 2024 | Environmental Pollution | No |
| Unveiling the potential of microalgae for bioplastic production from wastewater – current trends, innovations, and future prospects | https://doi.org/10.1186/s44316-024-00010-1 [13] | 2024 | Biotechnology for Sustainable Materials | / |
| Comparative study of the synthesis of polyhydroxyalkanoates by cyanobacteria *Spirulina platensis* and green microalga *Chlorella vulgaris* | https://doi.org/10.1016/j.algal.2024.103826 [14] | 2025 | Algal Research | No |
| Integrated biorefinery approach for sustainable production of biodiesel, bioplastics and high value bioproducts from a bloom forming alga, *Botryococcus braunii* | https://doi.org/10.1016/j.scitotenv.2025.178599 [15] | 2025 | Science of The Total Environment | No |
| Exploring media optimization and extraction methods to enhance poly-β-hydroxybutyrate (PHB) yield in *Synechocystis pevaleikii*: Scale-up from shake flask to 10 L photobioreactor | https://doi.org/10.1016/j.ijbiomac.2025.147862 [16] | 2025 | International Journal of Biological Macromolecules | No |

Table S 2. Rarefied average relative abundances (%) of most abundant (cut off: 0.5%) bacterial genera identified through 16S rRNA gene metabarcoding analysis at four time points (d0, d12, d21, and d25) in mixotrophic, semicontinuous *D. communis* cultures. Gram staining features are listed for each bacterial genus.

| **Genera** | **d0** | **St. Dev** | **d12** | **St. Dev** | **d21** | **St. Dev** | **d25** | **St. Dev** | **Gram staining** |
|---|---|---|---|---|---|---|---|---|---|
| *Other* | 18.75 | 1.25 | 8.31 | 0.56 | 8.04 | 0.16 | 8.72 | 1.13 | NA |
| *Hydrogenophaga* | 18.69 | 2.82 | 30.80 | 3.05 | 32.64 | 11.14 | 33.15 | 6.68 | Negative |
| *Limnospira* | 7.47 | 0.66 | 0.10 | 0.09 | 0.19 | 0.20 | 0.36 | 0.02 | Negative |
| *Nostoc* | 5.44 | 2.11 | 0.12 | 0.07 | 0.16 | 0.13 | 0.24 | 0.41 | Negative |
| *Rhizobium* | 4.68 | 0.66 | 3.11 | 0.09 | 3.17 | 0.18 | 3.98 | 0.23 | Negative |
| *Agrobacterium* | 4.39 | 0.88 | 3.01 | 1.69 | 2.84 | 2.56 | 3.59 | 3.34 | Negative |
| *Streptococcus* | 3.58 | 0.33 | 0.04 | 0.09 | 0.12 | 0.13 | 0.21 | 0.01 | Positive |
| *Thermanaerothrix* | 3.19 | 0.99 | 0.07 | 1.74 | 0.10 | 2.32 | 0.03 | 0.18 | Negative |
| *Gloeocapsopsis* | 2.69 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | NA |
| *Rhodobacter* | 2.36 | 0.25 | 0.75 | 0.02 | 0.61 | 0.10 | 0.76 | 0.05 | Negative |
| *Annamia* | 2.20 | 0.45 | 0.04 | 0.23 | 0.07 | 0.52 | 0.08 | 0.00 | Negative |
| *Erythrobacter* | 2.05 | 0.19 | 0.21 | 0.27 | 0.64 | 0.93 | 0.88 | 0.00 | Negative |
| *Bosea* | 1.73 | 0.56 | 0.42 | 0.07 | 0.88 | 0.76 | 1.95 | 1.19 | Negative |
| *Comamonas* | 1.42 | 0.27 | 3.39 | 0.21 | 3.49 | 1.01 | 3.34 | 0.25 | Negative |
| *Anaerolinea* | 1.41 | 0.28 | 0.00 | 0.24 | 0.03 | 0.81 | 0.18 | 0.04 | Negative |
| *Sinocapsa* | 1.32 | 0.18 | 0.02 | 0.00 | 0.01 | 0.00 | 0.03 | 0.00 | NA |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Dapis* | 1.26 | 0.10 | 0.03 | 0.03 | 0.04 | 0.05 | 0.05 | 0.04 | NA |
| *Phreatobacter* | 1.25 | 0.12 | 0.16 | 0.01 | 0.15 | 0.02 | 0.21 | 0.10 | Negative |
| *Cymatolege* | 1.24 | 0.16 | 0.02 | 0.01 | 0.03 | 0.04 | 0.05 | 0.06 | NA |
| *Gemmobacter* | 1.23 | 0.12 | 0.43 | 0.00 | 0.34 | 0.05 | 0.39 | 0.00 | Negative |
| *Potamolinea* | 1.21 | 0.12 | 0.00 | 0.32 | 0.00 | 0.61 | 0.00 | 0.00 | NA |
| *Acidovorax* | 1.09 | 0.30 | 2.18 | 0.04 | 2.19 | 0.10 | 2.16 | 3.03 | Negative |
| *Selenomonas* | 1.03 | 0.27 | 0.01 | 0.16 | 0.01 | 0.25 | 0.02 | 0.03 | Negative |
| *Polaromonas* | 1.00 | 0.16 | 2.32 | 0.12 | 2.29 | 0.17 | 2.30 | 0.05 | Negative |
| *Paracoccus* | 0.98 | 0.08 | 0.35 | 0.01 | 0.24 | 0.02 | 0.27 | 0.43 | Negative |
| *Komarekiella* | 0.68 | 0.39 | 0.01 | 0.04 | 0.02 | 0.00 | 0.03 | 0.00 | Negative |
| *Entomoplasma* | 0.67 | 0.08 | 0.01 | 0.01 | 0.02 | 0.03 | 0.03 | 0.03 | Negative |
| *Aerococcus* | 0.60 | 0.17 | 0.02 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | Positive |
| *Pseudorhizobium* | 0.58 | 0.02 | 0.37 | 0.00 | 0.38 | 0.01 | 0.49 | 0.47 | Negative |
| *Thainema* | 0.57 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 | 0.02 | 0.00 | NA |
| *Shinella* | 0.56 | 0.06 | 0.11 | 0.15 | 0.07 | 0.60 | 0.09 | 0.10 | Negative |
| *Alkalibacter* | 0.56 | 0.09 | 0.01 | 0.06 | 0.01 | 0.03 | 0.02 | 0.03 | Positive |
| *Chthonobacter* | 0.51 | 1.00 | 0.16 | 0.01 | 0.07 | 0.01 | 0.18 | 0.01 | Negative |
| *Sediminibacterium* | 0.51 | 0.14 | 0.24 | 0.17 | 1.24 | 0.30 | 2.75 | 0.91 | Negative |
| *Altererythrobacter* | 0.49 | 0.13 | 0.07 | 0.02 | 0.14 | 0.17 | 0.15 | 0.01 | Negative |
| *Rodentibacter* | 0.46 | 0.31 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | Negative |
| *Malikia* | 0.37 | 0.02 | 0.81 | 0.01 | 0.83 | 0.01 | 0.96 | 2.56 | Negative |
| *Limnobacter* | 0.36 | 0.03 | 34.92 | 0.03 | 31.62 | 0.22 | 25.00 | 0.43 | Negative |
| *Ottowia* | 0.27 | 0.03 | 0.59 | 0.00 | 0.60 | 0.01 | 0.56 | 0.02 | Negative |
| *Brevundimonas* | 0.24 | 0.00 | 0.16 | 0.05 | 0.31 | 0.18 | 1.00 | 0.02 | Negative |
| *Achromobacter* | 0.23 | 0.00 | 1.33 | 0.10 | 1.32 | 0.22 | 1.15 | 0.09 | Negative |
| *Rhodoferax* | 0.21 | 0.02 | 0.44 | 0.00 | 0.48 | 0.01 | 0.43 | 0.31 | Negative |
| *Pandoraea* | 0.19 | 0.03 | 1.05 | 0.07 | 0.93 | 0.07 | 0.82 | 0.52 | Negative |
| *Paraburkholderia* | 0.13 | 0.02 | 2.00 | 0.04 | 1.81 | 0.08 | 1.50 | 0.00 | Negative |
| *Caballeronia* | 0.06 | 0.01 | 0.53 | 0.00 | 0.50 | 0.00 | 0.44 | 0.05 | Negative |
| *Cupriavidus* | 0.05 | 0.02 | 0.75 | 0.02 | 0.72 | 0.02 | 0.50 | 0.44 | Negative |
| *Burkholderia* | 0.03 | 0.01 | 0.51 | 0.00 | 0.42 | 0.00 | 0.37 | 0.54 | Negative |
| *Phenylobacterium* | 0.01 | 0.01 | 0.02 | 0.00 | 0.17 | 0.00 | 0.51 | 0.01 | Negative |

Table S 3. Results of PERMANOVA conducted between the different time points (Ti) on bacterial communities identified through metabarcoding analysis at four time points (d0, d12, d21, and d25) in mixotrophic, semicontinuous *D. communis* cultures.

| Source | df | SS | MS | Pseudo-F | P(perm) |
|---|---|---|---|---|---|
| Ti | 3 | 6179.4 | 2059.8 | 8.8011 | 0.011 |
| Res | 8 | 1872.3 | 234.04 | | |
| Total | 11 | 8051.7 | | | |
| | Time points | | | | |
| Pairwise | d0≠d12 | | | | |
| | d0≠d21 | | | | |
| | d0≠d21 | | | | |
| | d12=d21 | | | | |
| | d12=d25 | | | | |
| | d21=d25 | | | | |

Table S 4. SIMPER analysis on bacterial communities identified through metabarcoding analysis at four time points (d0, d12, d21, and d25) in mixotrophic, semicontinuous *D. communis* cultures. AVG diss.: average dissimilarity; Contr. %: contribution %.

| Contrast | AVG diss. | Taxa contribution | Contr. % | Contrast | AVG diss. | Taxa contribution | Contr. % |
|---|---|---|---|---|---|---|---|
| d0 vs d12 | 54.24 | *Limnobacter* | 4.85 | d12 vs d21 | 19.33 | *Limnobacter* | 3.56 |
| | | *Limnospira* | 2.23 | | | *Hydrogenophaga* | 2.84 |
| | | *Nostoc* | 1.84 | | | *Agrobacterium* | 2.66 |
| | | *Streptococcus* | 1.59 | | | *Rhizobium* | 2.61 |
| | | *Gloeocapsopsis* | 1.5 | | | *Sediminibacterium* | 2.28 |
| | | *Thermanaerothrix* | 1.44 | | | *Bosea* | 1.27 |
| | | *Annamia* | 1.17 | | | *Erythrobacter* | 1.07 |
| | | *Hydrogenophaga* | 1.12 | | | *Rhodobacter* | 1.05 |
| | | *Anaerolinea* | 1.09 | d12 vs d25 | 21.64 | *Sediminibacterium* | 3.54 |
| | | *Potamolinea* | 1 | | | *Limnobacter* | 3.51 |
| d0 vs d21 | 54.5 | *Limnobacter* | 4.51 | | | *Rhizobium* | 2.76 |
| | | *Limnospira* | 2.13 | | | *Agrobacterium* | 2.58 |
| | | *Nostoc* | 1.8 | | | *Bosea* | 1.85 |
| | | *Gloeocapsopsis* | 1.5 | | | *Brevundimonas* | 1.49 |
| | | *Thermanaerothrix* | 1.42 | | | *Hydrogenophaga* | 1.42 |
| | | *Streptococcus* | 1.41 | | | *Rhodobacter* | 1.32 |
| | | *Hydrogenophaga* | 1.24 | | | *Phenylobacterium* | 1.24 |
| | | *Annamia* | 1.15 | | | *Erythrobacter* | 1.18 |
| | | *Potamolinea* | 1 | | | *Pseudorhizobium* | 1.02 |
| | | *Anaerolinea* | 1 | | | *Streptococcus* | 1.02 |
| d0 vs d25 | 52.17 | *Limnobacter* | 4.07 | d21 vs d25 | 21.97 | *Limnobacter* | 3.43 |
| | | *Limnospira* | 2.07 | | | *Rhizobium* | 2.85 |
| | | *Nostoc* | 1.76 | | | *Agrobacterium* | 2.74 |
| | | *Thermanaerothrix* | 1.57 | | | *Hydrogenophaga* | 2.57 |
| | | *Gloeocapsopsis* | 1.53 | | | *Bosea* | 2.08 |
| | | *Streptococcus* | 1.36 | | | *Sediminibacterium* | 1.76 |
| | | *Hydrogenophaga* | 1.34 | | | *Erythrobacter* | 1.41 |
| | | *Annamia* | 1.15 | | | *Phenylobacterium* | 1.27 |

|  |  |  |  |
|---|---|---|---|
| *Potamolinea* | 1.03 | *Brevundimonas* | 1.23 |
|  |  | *Rhodobacter* | 1.17 |
|  |  | *Pseudorhizobium* | 1.04 |

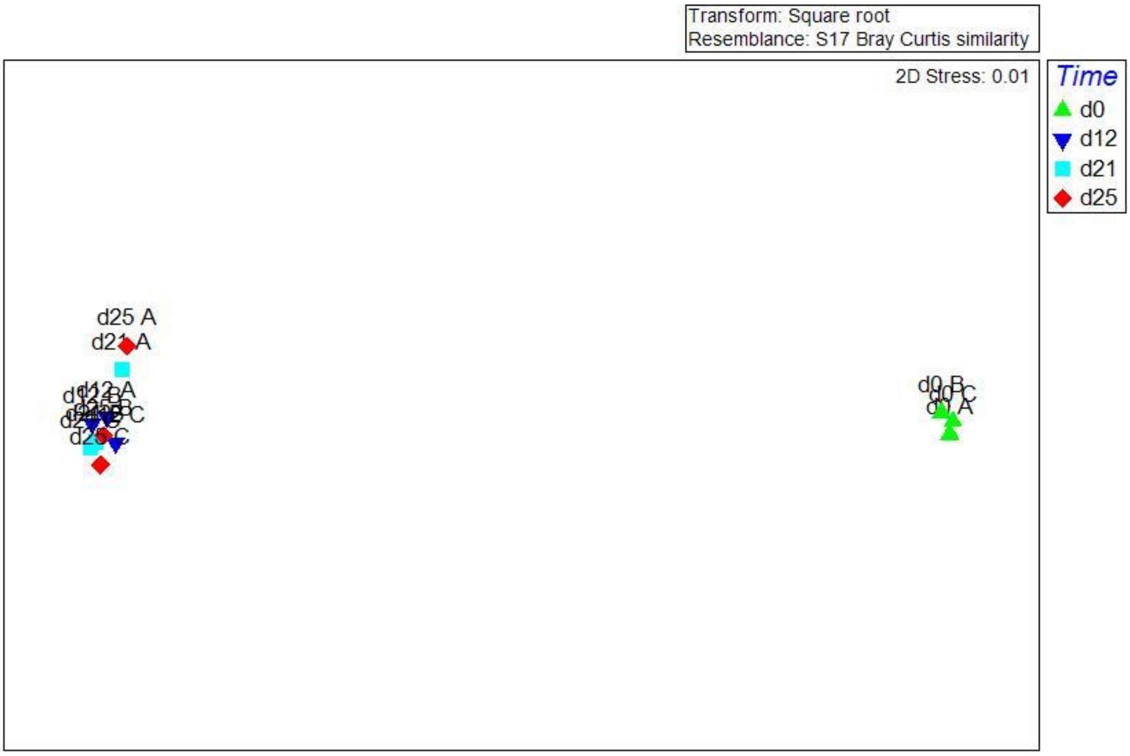

Fig. S 1. nMDS ordination of bacterial community identified by 16S rRNA gene metabarcoding based on square-root transformed abundance and Bray-Curtis similarity, at four time points (d0, d12, d21, and d25) of mixotrophic, semicontinuous growth of *D. communis*. Three biological replicates (A, B, C) were evaluated for each time point.

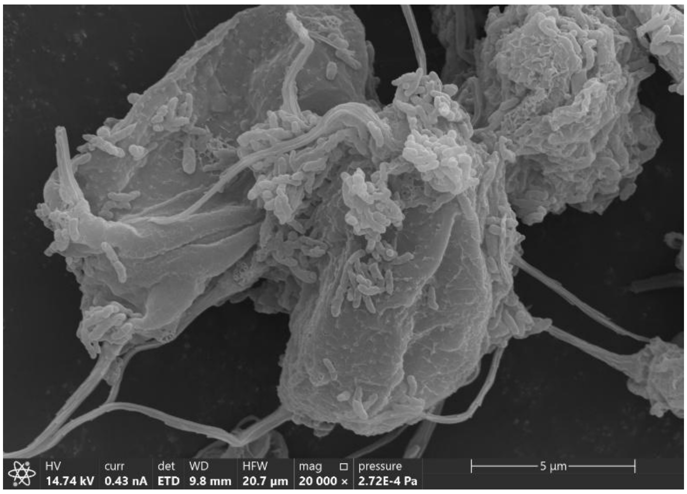

Fig. S 2. ESEM micrograph of *D. communis* cells after 4 days of PHB-inducing mixotrophic growth, with resident bacteria visible on the surface of microalgal cells.

## References

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