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Community modelling of growth and metabolism of purple phototrophic bacteria for resource recovery

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Introduction

Purple phototrophic bacteria (PPB) are a promising candidate for phototrophic production of value-added products from wastewater treatment. Its products are found to be the biomass (as fertilizer and single-cell protein for animal feed), polyhydroxyalkanoates (as bioplastic precursors), H₂, pigments like carotenoids or bacteriochlorophylls, coenzyme Q10 and 5 aminolaevulinic acid (Capson-Tojo et al., 2020). In most cases, PPB mixed cultures are used due to advantages of technical feasibility and ease of operation. However, there is no clear understanding of how operational parameters affect the competition between different microbes in the system, and on how this competition affects the final products obtained. In this work, using genome-scale metabolic modelling, we studied the co-cultivation of two well-known PPB species that commonly dominate mixed culture systems applied in resource recovery: *Cereibacter sphaeroides* and *Rhodopseudomonas palustris* (Alloul et al., 2021). We aim to understand the performance of each bacterium (in terms of growth, polyhydroxybutyrate (PHB) synthesis and H₂ production) when co-cultivated, also understanding how metabolic mechanisms are affected by operational parameters (e.g. C/N (carbon-to-nitrogen) ratio) and the implications for performance indicators. This understanding would help bioprocess engineers to adjust the operational parameters to maintain the environmental conditions that favor the production of desired products in mixed PPB cultures treating wastewater.

Materials and Methods

A pathway-genome database of *C. sphaeroides* ATCC 17025 (formerly known as *R. sphaeroides*) was retrieved from BioCyc portal using Pathway Tools software (Karp et al., 2010) and was curated. Curation involved more than 200 modifications based on the evidence gathered from 40 scientific articles/reviews and genome-based evidence. A genome-scale metabolic model (RsphCyc model) was generated from the curated database using COBRA Toolbox (Schellenberger et al., 2011). For *R. palustris* CGA009, the metabolic model was taken from (Alsiyabi et al., 2019) and was curated to simulate growth under photoautotrophic, aerobic heterotrophic and photoheterotrophic conditions. Using COBRA Toolbox, the co-cultivation of bacteria was simulated by merging models of the two species and conducting flux balance analysis (FBA), with an objective function of maximizing growth of both species together. In all simulations, the inputs were light, succinate (along with aspartate), ammonia, sulfate, phosphate and micronutrients, where succinate and ammonia were used as carbon source and nitrogen source, respectively. The C/N ratio was varied by adjusting the uptake rate of succinate when the ammonium uptake rate was kept constant.

Results and discussion

The resulting RsphCyc model was composed of 2,341 reactions, 2,321 metabolites, 1,029 genes and four compartments, whereas the curated *R. palustris* model was composed of 1,455 reactions, 1,543 metabolites, 563 genes and four compartments. The growth prediction of both the metabolic models of *C. sphaeroides* and *R. palustris* were validated under trophic conditions like photoautotrophic, aerobic heterotrophic, anaerobic heterotrophic, and photoheterotrophic conditions. The prediction of different parameters related to growth, protein, PHB synthesis and H₂ production using the *C. sphaeroides* model, was validated with the experimental data from (Imam et al., 2013; Spanoghe et al., 2021; Alloul et al., 2021) under photoautotrophic, aerobic heterotrophic and anaerobic photoheterotrophic conditions respectively. Similarly, the prediction using the *R. palustris* model was also validated with experimental data from (Spanoghe et al., 2021) for photoautotrophic growth and that from (McKinlay & Harwood, 2011; Touloupakis et al., 2021) for photoheterotrophic production of biomass, PHB and H₂.

Using our refined models, the effect of varying C/N (carbon-to-nitrogen) ratio on the production rate of H₂ and PHB from both species when grown individually and co-cultivated were compared (Fig.1.). Individual growth rates of both species were predicted to be constant with varying C/N ratio, as growth was limited by ammonium. However, production of PHB and H₂ responded to the varying C/N ratio in both the species, though the response patterns were different. This may be due to differences in the carbon flux distributions caused by pathways and reactions that were exclusive to one of these species, such as the oxidative phase of the pentose phosphate pathway, phosphoenol pyruvate carboxylase, pyruvate carboxylase and the Entner-Doudoroff pathway. In co-cultivation, *R. palustris* tend to produce a higher amount of PHB and H₂ by consuming more C substrate at higher C/N ratio, even though they grow at the same rate. Results reveal that in pure culture cultivation, *C. sphaeroides* was a better candidate for the production of PHB and *R. palustris* was a better candidate for hydrogen production, at high C/N ratios. However, co-cultivation was predicted to be capable of producing products at a rate higher than that of pure culture cultivation and a high C/N ratio seemed to facilitate a higher production rate. The flux distribution involved in both models will be detailed in the presentation. Future work will involve the validation of co-cultivation results and the study of the effect of other operational parameters on mixed culture cultivation.

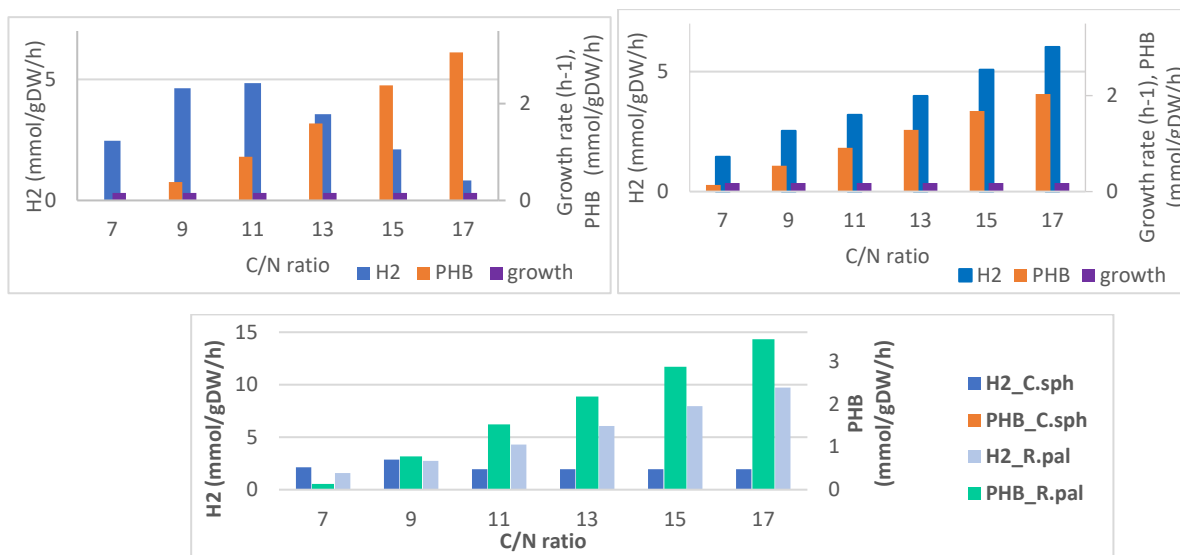


Fig. 1. Effect of C/N ratio on production rates of biomass (growth), H₂ and PHB in (a) *C. sphaeroides* when individually grown, (b) *R. palustris* when individually grown and (c) co-cultivation

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