Metabolic network reconstruction providing insights into the metabolism relevant for resource recovery in Rhodobacter sphaeroides

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Abstract
Rhodobacter sphaeroides is a versatile purple non-sulfur bacteria that can be used in resource recovery to produce hydrogen, polyhydroxyalkanoates, single-cell protein and other value-added products. We developed a genome-scale metabolic model representing the primary metabolism of a denitrifying strain *R. sphaeroides* ATCC 17025. This metabolic model predicted growth, production of hydrogen and polyhydroxybutyrate, and was in better agreement with literature-based experimental values as compared to predictions from existing models for *R. sphaeroides*. We evaluated the growth, production rates and product distribution under different metabolisms and using different organic substrates. In addition, it allowed to assess the metabolic mechanisms governing the preferential generation of different products. This model can be a valuable tool to optimize the generation of value-added products.

Keywords
Hydrogen production; metabolic modelling; polyhydroxybutyrate; resource recovery; *Rhodobacter sphaeroides*

INTRODUCTION

Purple phototrophic bacteria (PPB) can be used in the resource recovery of value-added products from waste streams. The versatility, high efficiency of removal of organics and the capability of PPB to utilize sunlight as its energy source for its growth and metabolism makes wastewater treatment using PPB economically feasible. *Rhodobacter sphaeroides* is one of the commonly observed PPB and was found dominant in mixed cultures cultivated outdoor (Hülsen, Stegman, et al., 2022; Hülsen, Züger, et al., 2022). A genome-scale metabolic model facilitates better understanding of the flexible metabolism of PPB and prediction of its varying physiological behaviour with different substrates and environmental conditions. Integration of this model with the bioprocess model would improve the reliability of the model and needs less recalibration with varying composition of wastewater feedstock or operational parameters.

To the best of our knowledge, there is no curated metabolic pathway database available for *Rhodobacter sphaeroides*, that would allow integration and visualization of the metabolic pathways, reactions, enzymes, proteins and genes and other evolving information related to transcript, protein and metabolite profiling. There are genome-scale metabolic models representing the photoheterotrophic growth of *Rhodobacter sphaeroides* strain 2.4.1 (Imam, Yilmaz, et al., 2011; Imam, Noguera, et al., 2013), *Rhodopseudomonas palustris* (Alsiyabi, Immethun, et al., 2019) that were applied to study the aspects of photosynthesis, hydrogen production, different substrate uptake and redox state. However, more study is needed to understand the substrate preferences of cells for growth and production of value-products and their distribution towards different products that are relevant to resource recovery. In the current study, a curated metabolic pathway database and a genome-scale metabolic model of *Rhodobacter sphaeroides* ATCC 17025 is developed, showing better predictions than that of existing models. The model was validated with experimental data under different trophic conditions like photoautotrophic, aerobic heterotrophic and anaerobic

photoheterotrophic conditions. The metabolic model was employed to study the product distribution of hydrogen, polyhydroxybutyrate, CO_2 fixation and growth under different light uptake rates.

MATERIALS AND METHODS

Development of pathway database & metabolic model

A genome-pathway database of *R. sphaeroides* ATCC 17025 was retrieved from the biochemical database, BioCyc. Pathways and reactions involved in the central carbon and nitrogen metabolism were curated by adding 111 modifications that were based on studies gathered from 42 scientific articles/reviews and genome-based evidence. The curation of the database was guided by analysis using the Metaflux module of Pathway Tools software (Latendresse, Krummenacker, et al., 2012) and thus a draft metabolic model was developed from the database. Compartments of cytoplasm, membrane space, periplasmic space, and extracellular space were also included in the Rba_sphCyc database. The metabolic model was validated qualitatively and quantitatively by comparing the predictions of the model under various trophic conditions with the experimental observations or conclusions published in 17 articles.

RESULTS AND DISCUSSION

Curation and validation of the metabolic model

The details of the curated pathway-database, RbaSphCyc, which will be published in MetaCyc, are given in Table 2. A genome-scale metabolic model was generated from the curated pathway database. As a part of the qualitative assessment of the metabolic model, growth of *R. sphaeroides* was simulated under photoautotrophic, aerobic heterotrophic, anaerobic heterotrophic, anaerobic photoheterotrophic condition. The validation of the model was carried out by conducting quantitative assessment of flux under photoautotrophic, aerobic heterotrophic and anaerobic photoheterotrophic conditions. The predicted values along with experimental data (gathered from literature) used for validation of the model under these conditions are shown in Figure 1, Figure 2 and Figure 3, respectively.

The photoheterotrophic growth of *R. sphaeroides* on different substrates (succinate-glutamate, succinate-ammonia, glucose-glutamate and glutamate only) were simulated using the uptake rates reported by Imam et al. (2011). In the first step, fluxes were optimized to produce maximum biomass and the specific growth rates predicted by RbaSphCyc model were in agreement with that observed in the reported experiments (Imam, Yilmaz, et al., 2011)(Imam, Noguera, et al., 2013). In the second step, a light constraint was applied and the production rates for polyhydroxybutyrate, hydrogen and CO_2 were predicted, which were in good agreement with the experimental values obtained by Imam et al. (2011).

Under photoheterotrophic and NH_3 limited conditions, when the light uptake rate is above the minimum rate that is needed for maximum growth, concomitant H_2 and PHB production was predicted (shown in Figure 4). An increase in light uptake rate increases ATP production and thus increases the rate of H_2 production, thereby affecting other electron sink pathways such as PHB production and CO_2 fixation inversely. This pattern was found similar in all the four cases mentioned, but the range of light uptake rate differs based on substrate. When the activity of nitrogenase is not limited by ammonia, the main factors that affect H_2 production were found to be light and redox condition of substrate.

CONCLUSION

We developed a genome-scale metabolic model representing primary metabolism of *R. sphaeroides*

ATCC 17025, that can be used to simulate photoautotrophic, anaerobic heterotrophic, anaerobic photoheterotrophic growth and predict flux distribution towards production. Under photoheterotrophic and ammonia-limited condition, a light uptake rate higher than that needed for growth, is required to produce hydrogen. Under this condition, light uptake rate plays a major role in defining the flux distribution through electron sink pathways like production of hydrogen, PHB and CO2 fixation.

Table 1: Details of genome-pathway database, RbaSphCyc

Model Field	Values
Pathways	252
Enzymatic reactions	1587
Transport reactions	91
Enzymes	1237
Compounds	1220
Genes	1020
Compartments	4

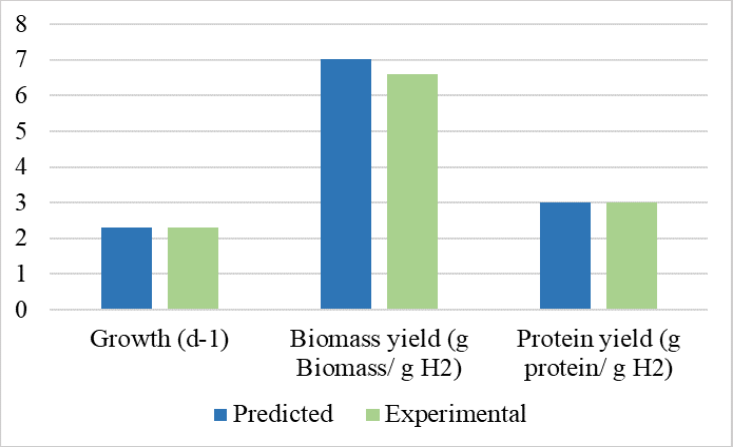

Figure 1:Validation of metabolic model of *R. sphaeroides* for photoautotrophic growth. Experimental data is taken from (Spanoghe, Vermeir, et al., 2021).

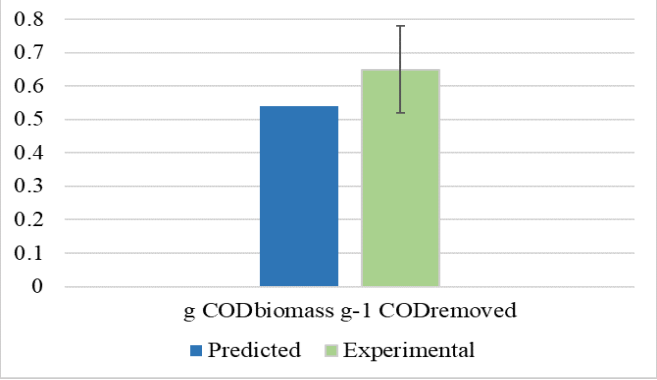

Figure 2: Validation of metabolic model of R. sphaeroides for photoautotrophic growth. Experimental data is taken from (Alloul, Muys, et al., 2021).

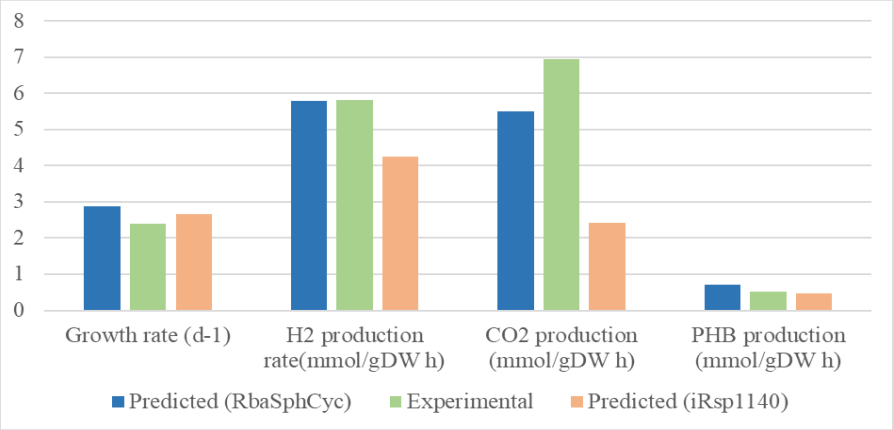

Figure 3:Validation of RbaSphCyc model for photoheterotrophic growth of *R. sphaeroides* growing on succinate as carbon source and glutamate as nitrogen source. iRsp1140 is a model developed for *R. sphaeroides* strain 2.4.1. by Imam et al. (2013).

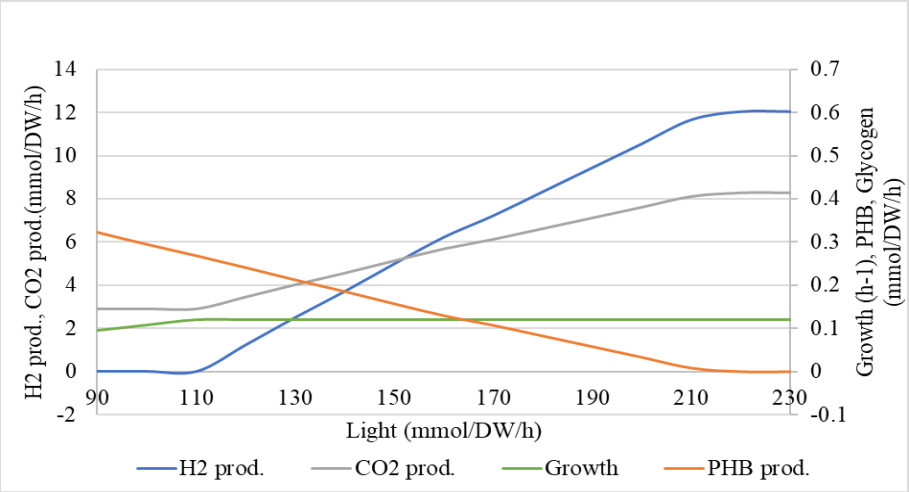

Figure 4: Growth of *R. sphaeroides* along with production of hydrogen, polyhydroxybutyrate (PHB) and CO2 with varying light uptake rate under photoheterotrophic condition. Succinate and glutamate were used as carbon source and nitrogen source.

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