Metabolic modelling of purple non sulfur bacteria for resource recovery

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Summary of key findings

Purple non sulfur bacteria (PNSB) have been used for resource recovery of hydrogen, polyhydroxyalkanoates, single-cell protein and other value-added products. Understanding their physiological behaviour and response to environmental conditions would benefit optimization of its growth and production of value-added products. In this work, we developed a pathway-based genome-scale metabolic model for a PNSB, *Rhodobacter sphaeroides* ATCC 17025. The model was validated with literature based experimental values under conditions of various trophic conditions and the predictions of the model were in better agreement with the experimental values as compared to predictions from existing models for *R. sphaeroides*. The genome-scale metabolic models of two PNSB that are used in resource recovery, *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris,* were analyzed in order to study the growth and their response to change in environmental conditions under anaerobic photoheterotrophic conditions. Under nitrogen-limited condition, *R. palustris* was predicted to show a higher hydrogen production at a rate double of that predicted in *R. sphaeroides*. In *R. sphaeroides,* a high C/N ratio favours poly(3-hydroxybutyrate) production whereas in *R. palustris*, a higher C/N ratio favours production of both hydrogen and poly(3-hydroxybutyrate) when the substrates were succinate and glutamate. Analysis of this models can reveal how operational conditions can influence the population dynamics, leading to strategies that favour the production of targeted resources of interest.

Background and relevance

Since 2000, various strains of purple non-sulfur bacteria (PNSB) and their enriched cultures have been studied and used for wastewater treatment and resource recovery (Capson-Tojo et al., 2020). If this technology is to be successfully implemented, value-added products (e.g., polyhydroxyalkanoates or single-cell proteins) must be generated. To do so efficiently, our understanding on how PNSB can generate different products depending on environmental conditions must be increased, as well as our knowledge on the interactions between different PNSB in mixed cultures (Lu et al., 2021). Metabolic modelling is a powerful tool that can allow us to do so.

Rhodobacter sphaeroides and *Rhodopseudomonas palustris* are two well-studied PNSB species for resource recovery from wastewater. These two species are commonly dominant in the microbial community of mixed PNSB cultures that have been studied for such applications (Alloul et al., 2021). There are genome-scale metabolic models representing the central carbon metabolism of *Rhodobacter sphaeroides* strain 2.4.1 (Imam, Yilmaz, et al., 2011; Imam, Noguera, et al., 2013) and *Rhodopseudomonas palustris* (Alsiyabi, Immethun, et al., 2019). These have been applied to study different aspects of photosynthesis, hydrogen production, different substrate uptake and redox states. However, more research is needed to understand the physiological behaviour of the cells in terms of growth, production of different value-added products, and their flux distribution in changing environmental conditions. In this study, we present a novel pathway-based genome-scale metabolic model for *Rhodobacter sphaeroides* ATCC 17025 that provides many important extensions on previous models based on genome and experimental studies available from recent literature publications. Furthermore, we have conducted analyses of genome-scale metabolic models for two species, *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris,* to study and compare the metabolic behaviour of these strains. We have analysed parameters related to growth and to the production of hydrogen, poly(3-hydroxybutyrate) (PHB) and glycogen for both strains when growing individually. As C/N ratio is one of the main factors that can impact the production rates of PHB and hydrogen in PNSB, the response of these organisms to the varying C/N ratio was also analysed. Understanding the response

of various strains to this and other operational conditions (to be covered in the conference) would help us to implement better process control of phototrophic processes for resource recovery.

Materials and Methods

A genome-pathway database of *R. sphaeroides* ATCC 17025 was retrieved from BioCyc. Pathways and reactions involved in the central carbon and nitrogen metabolism were curated by adding 111 modifications that were based on data gathered from 42 scientific articles/reviews and genome-based evidence. A metabolic model was thus curated and developed using the Pathway Tools software (Karp et al., 2010). The metabolic model of *R. palustris* CGA009 that was developed by Alsiyabi et al. (2019) was curated to simulate the growth of bacteria in photoautotrophic, aerobic heterotrophic and photoheterotrophic conditions. Using the COBRA toolbox (Schellenberger et al., 2011), flux balance analysis of individual models were conducted to study the growth of strains under anaerobic photoheterotrophic conditions. In addition to the inputs of light intensity, sulfate, phosphate and micronutrients, succinate (along with aspartate) and glutamate were used as carbon source and nitrogen source, respectively. The objective function for flux balance analysis (FBA) was maximizing growth rate in both cases. A C/N ratio of 12 was defined for comparison of growth and other parameters of two species as this C/N ratio has been previously applied experimentally by Imam et al. (2011) for the growth of *R. sphaeroides*. The C/N ratio was varied by adjusting the uptake rate of succinate (carbon source) when the glutamate uptake rate was kept constant at 1.08 mmol/dry weight/h.

Results

A well-curated, pathway-based, genome-scale metabolic model was developed for *R. sphaeroides* ATCC 17025 (see Table 1). The metabolic model was validated using experimental values available in the literature for photoautotrophic, aerobic heterotrophic and anaerobic photoheterotrophic growth of *R. sphaeroides*. The predicted values are shown in Figure 1 (photoautotrophic), Figure 2 (aerobic heterotrophic) and Figure 3 (photoheterotrophic). The growth of *R. sphaeroides* and *R. palustris* under anaerobic photoheterotrophic conditions was simulated using the developed model of *R. sphaeroides* and the refined model of *R. palustris* CGA009 (Alsiyabi et al., 2019). The predicted values for growth rates and for production rates of hydrogen, PHB and glycogen of *R. sphaeroides* and *R. palustris* were compared (see Table 2). The influence of varying C/N ratios on the values of these parameters for both strains are shown in Figure 4 and Figure 5.

Discussion

We developed a genome-scale metabolic model representing the primary metabolism of *R. sphaeroides* ATCC 17025 and the predictions were closer to the experimental values when compared with the predictions of other existing model (see Figure 3). When growth rates and production rates of *R. sphaeroides* and *R. palustris* were compared, the production rate of hydrogen of *R. sphaeroides* was predicted to be higher than that of *R. palustris* (Table 2). *R. sphaeroides* seems to divert the reducing equivalents from growth towards hydrogen production and growth was limited by glutamate availability (nitrogen source). Individual growth rates of *R. sphaeroides* and *R. palustris* were predicted to be constant with varying C/N ratio for both strains, as growth was limited by glutamate. As expected, the production rates of hydrogen and PHB for the two strains varied with the C/N ratio, however, the variation patterns were different (see Figure 4 and Figure 5). An inverse relationship was predicted between the production rates of hydrogen and PHB in *R. sphaeroides*, whereas the production of hydrogen and PHB increased simultaneously with the C/N ratio (at the lower range of C/N ratios) in *R. palustris*. A similar pattern of hydrogen production of *R. sphaeroides* was observed by Al-Mohammedawi et al. (2018). A simultaneous production of hydrogen and PHB was also reported by Demiriz et al. (2019) for *R. capsulatus* that was similar to the pattern predicted for hydrogen and PHB production rate in *R. palustris*. In *R. sphaeroides*, the flux distribution of hydrogen and PHB production was affected by glycogen production. This may be due to differences in the carbon flux distributions caused by pathways and reactions that were exclusive to one of these strains, such as the oxidative phase of the pentose phosphate pathway, phosphoenol pyruvate carboxylase, pyruvate carboxylase and the Entner-Doudoroff pathway. However, results reveal that, under glutamate limited conditions, *R.*

sphaeroides and *R. palustris* are better candidates for the production of PHB and hydrogen, respectively. The production rates for both products are favoured at high C/N ratios.

The flux distribution involved in both the models will be detailed in the presentation. Future work will involve the mixed metabolic modelling of *R. sphaeroides* and *R. palustris* to study the growth and the metabolic capabilities of mixed cultures, and to understand how other operational factors and the substrate composition impact the population dynamics.

Table 1 Details of the genome-scale metabolic model developed for *R. sphaeroides* ATCC 17025

Model Field	Values
Pathways	252
Enzymatic reactions	1,587
Transport reactions	91
Enzymes	1,237
Compounds	1,220
Genes	1,020
Compartments	4

Table 2 Growth and production rates of key value-added compounds predicted by the metabolic models

	R. sphaeroides	*R. palustris*
Growth rate (h^{-1})	0.12	0.14
Hydrogen (mmol/dW/h)	5.38	4.13
PHB (mmol/dW/h)	0.15	0.15
Glycogen (mmol/dW/h)	0	0
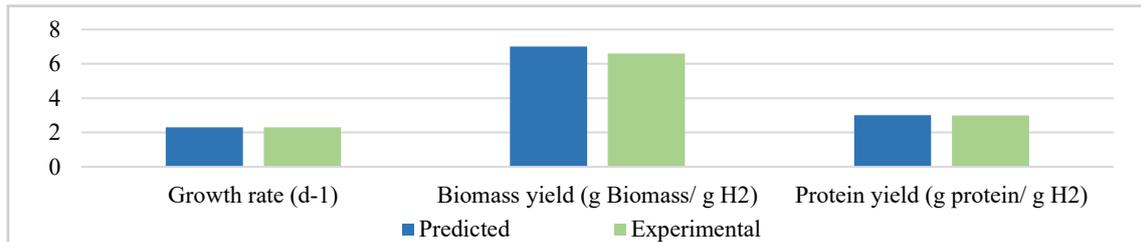
Figure 1. Validation of the metabolic model for *R. sphaeroides* for photoautotrophic growth. Experimental data was taken from Spanoghe et al. (2021).
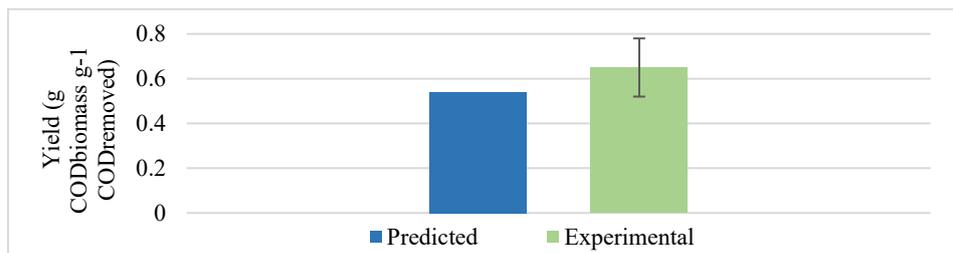
Figure 2. Validation of the metabolic model for *R. sphaeroides* for aerobic heterotrophic growth. Experimental data was taken from Alloul et al. (2021).
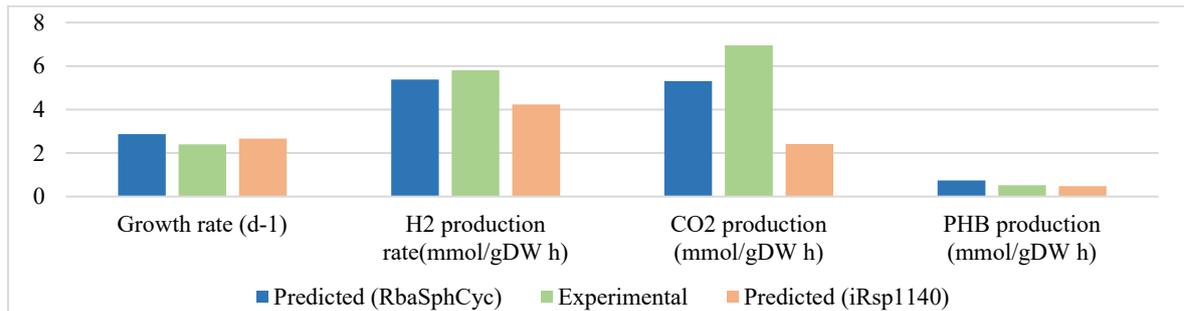
Figure 3. Validation of the model for *R. sphaeroides* for photoheterotrophic growth. iRsp1140 is the model developed for *R. sphaeroides* strain 2.4.1. by Imam et al. (2013).
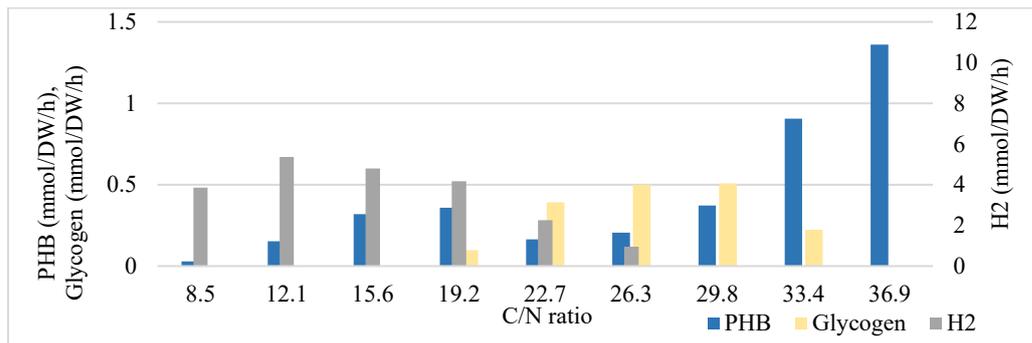
Figure 4. Prediction of PHB, hydrogen and glycogen production rates for *R. sphaeroides* under photoheterotrophic conditions.
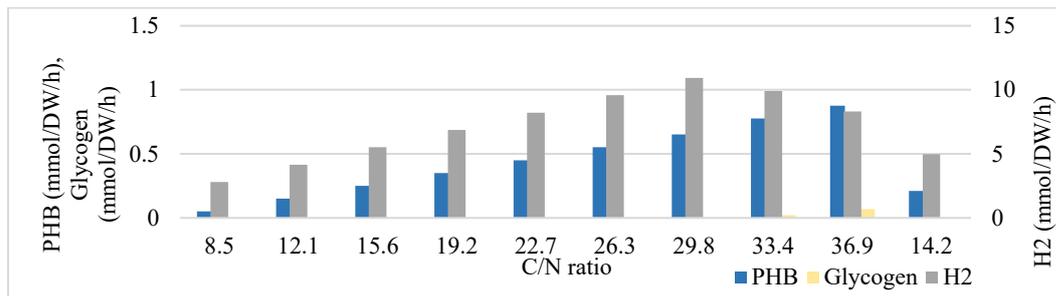
Figure 5. Prediction of PHB, hydrogen and glycogen production rates for *R. palustris* under photoheterotrophic conditions.

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