# Production of polyhydroxyalkanoates by purple phototrophic bacteria using wastewater treatment products

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## Introduction

*Rhodospirillum rubrum* is a purple non-sulphur bacterium (PNSB) belonging to the a-proteobacteria group well-known for its metabolic versatility. This metabolic versatility leads *Rs. rubrum* to the forefront of the stage in the biotechnological field and as a reference organism for the study of photosynthesis and assimilation of different carbon sources. Among those carbon sources, due to their cheapness, volatile fatty acids (VFAs) are extensively studied in the biotechnology field especially in PHA production.

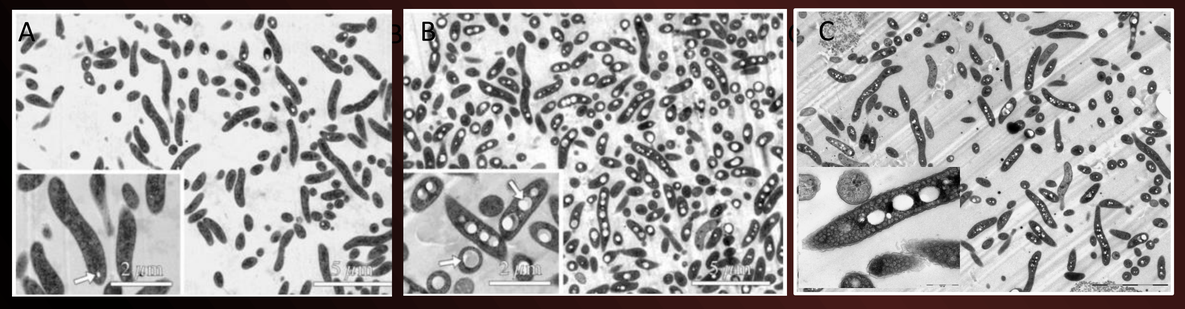


Figure 1 : Transmission Electron Microscopy (TEM) pictures of *Rhodospirillum rubrum* in presence of different carbon sources showing PHA granules. A) succinate. B) acetate. C) butyrate

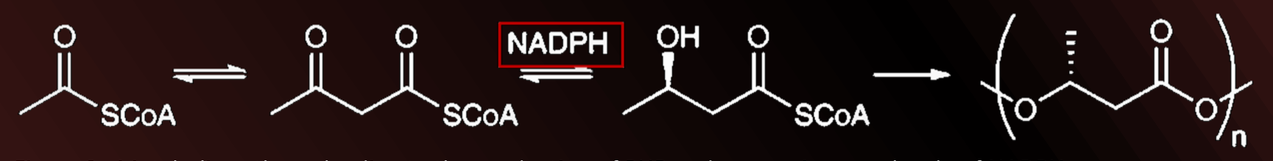


Figure 2 : Metabolic pathway leading to the production of PHB and consuming a molecule of NADPH

## Impact of carbon source

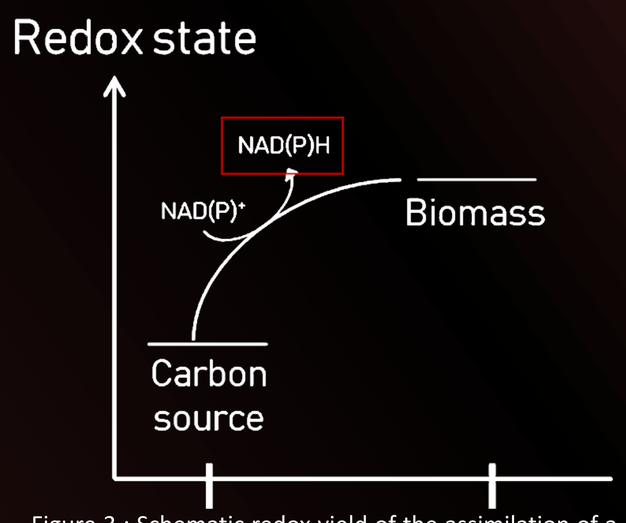


Figure 3 : Schematic redox yield of the assimilation of a reduced carbon source

The use of reduced carbon sources, such as VFAs, induces the production of reduced cofactors (Fig. 3). Valeric acid constitutes one of the most reduced VFAs and its assimilation by *Rhodospirillum rubrum* is strictly associated to the presence of $HCO_3^-$ (Fig. 4). $HCO_3^-$ could be used as electron sink and are thus competing with PHA production. PHA quantitation revealed higher PHA content when $HCO_3^-$ are added progressively.

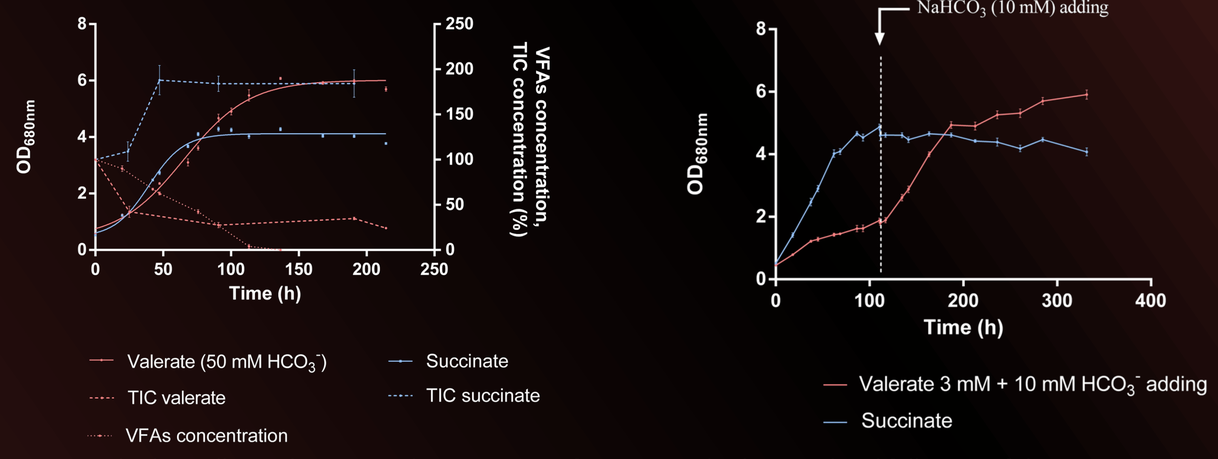


Figure 4 : *Rhodospirillum rubrum* growth in presence of succinic and valeric acid and following of the VFAs concentration and Total Inorganic Carbon (TIC)

Table 1 : PHA synthesis related proteins highlighted by proteomic analysis comparing valerate to succinate

| Accession number | Locus Tag | Identified peptided | Description | *p*-value | fold change |
|---|---|---|---|---|---|
| Q2RQI1 | Rru_A2817 | 24 | Phasin | 0.0004 | 48.24 |
| Q2RP67 | Rru_A3283 | 80 | Phasin | 0.0278 | 2.06 |
| Q2RNZ5 | Rru_A3356 | 3 | Polyhydroxyalkanoate depolymerase | 0.0041 | 0.58 |

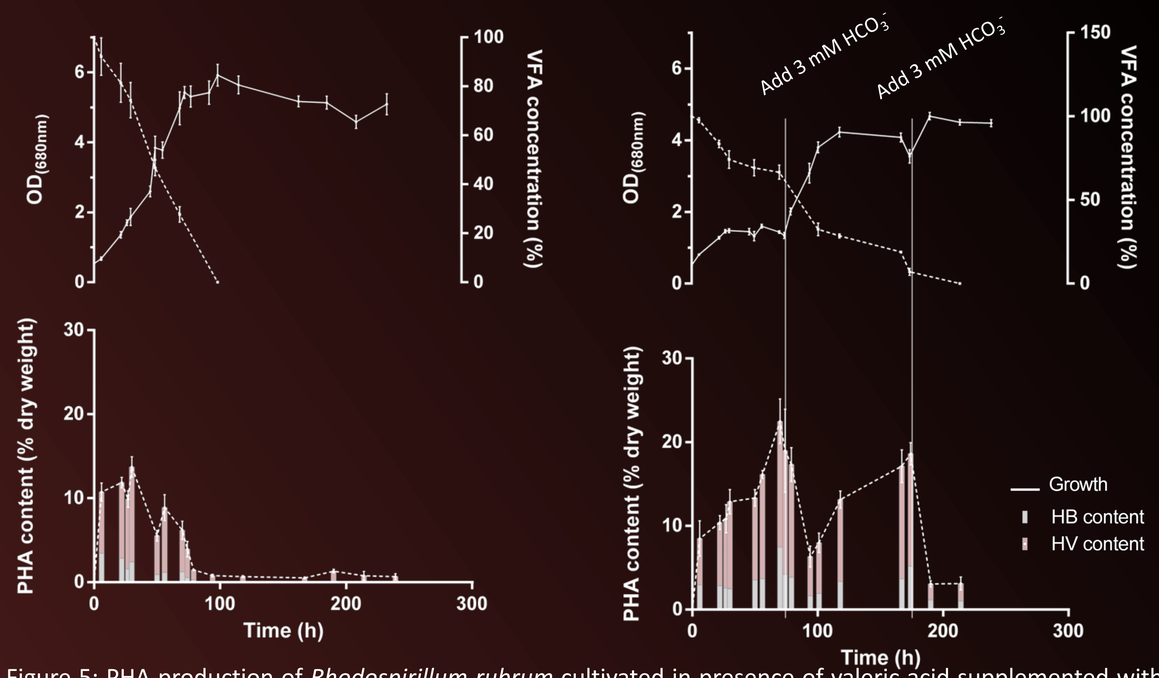


Figure 5: PHA production of *Rhodospirillum rubrum* cultivated in presence of valeric acid supplemented with 50 mM $NaHCO_3^-$ or with progressive adding of 3mM $NaHCO_3^-$. Dotted lines represent addition of bicarbonates ions

## Impact of light induced stress

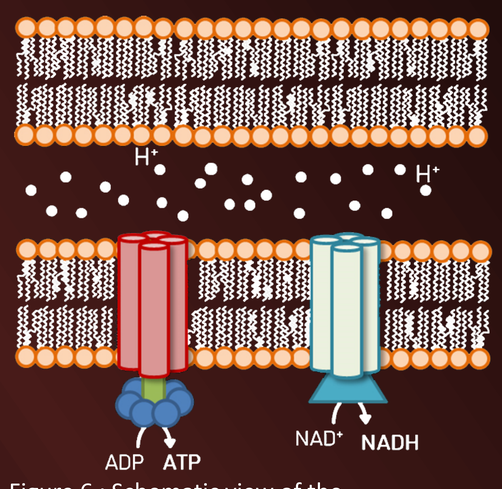


Figure 6 : Schematic view of the photoreduction of $NAD^+$

High light intensity induces a deregulation of the cofactor redox state via the reverse activity of the NADH dehydrogenase (Fig. 6). Our results showed that high light intensity switch induces a stop of the WT strain in presence of acetic acid as well as a stop of acetic acid assimilation (Fig. 7). Proteomic analysis revealed the development of a redox stress response (Fig. 8). This was accompanied by the expected higher PHA content (Table 2).

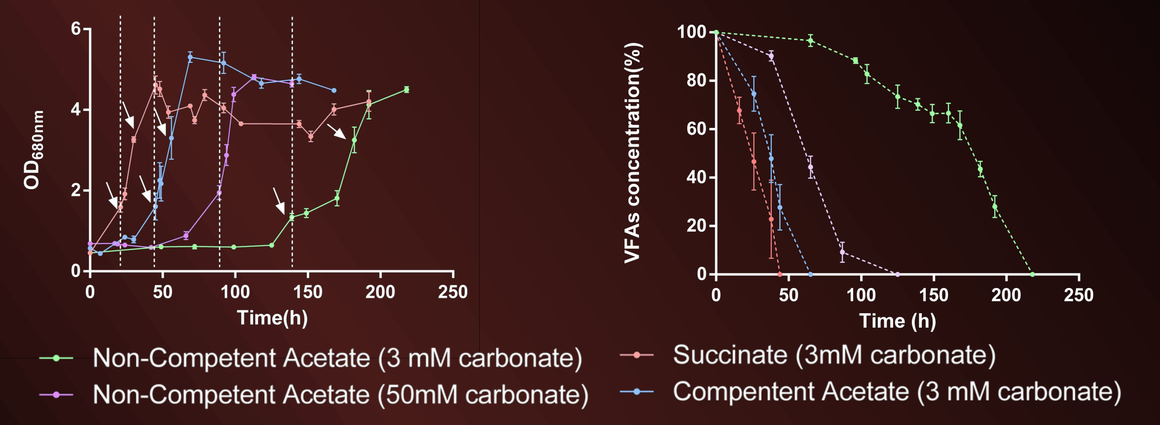


Figure 7 : Growth of *Rhodospirillum rubrum* WT and acetate competent strain in presence of acetic acid and response to high light intensity switch

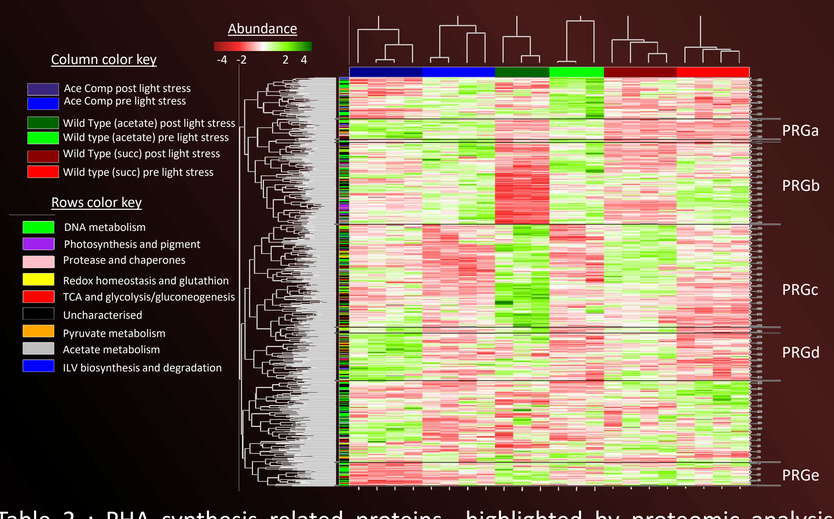


Figure 8 : Heatmap representation of differential abundance of protein considered for the analysis. Columns were separated by using Forced Euclidian Hierarchical Clustering based on conditions. Rows were separated by Unforced Euclidian hierarchical clustering. Protein Response Groups (PRGs) were established based on colour pattern and supported by Monte-Carlo simulation.

Table 2 : PHA synthesis related proteins highlighted by proteomic analysis before and after light intensity increase

| Accession number | Locus tag | Identified peptides | Description | Ace Comp post vs Ace Comp pre | | Ace non comp post vs Ace non comp pre | | Succ post vs Succ pre | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | p-value | fold change | p-value | fold change | p-value | fold change |
| Q2RP67 | Rru_A3283 | 5 | Phasin | 0.632 | 1.108 | 0.030 | 0.157 | 0.236 | 0.880 |
| Q2RNZ5 | Rru_A3356 | 1 | Polyhydroxyalkanoate depolymerase | 0.008 | 3.279 | 0.010 | 0.267 | 0.061 | 2.404 |
| Q2RXR4 | Rru_A0276 | 5 | Polyhydroxyalkanoate synthesis repressor PhaR | 0.474 | 0.947 | 0.013 | 0.436 | 0.497 | 0.932 |

## Conclusion

Our researh indicates that PHA production in *Rhodosprillum rubrum* is **driven by intracellular redox state**. The resulting strategy could be the mimicking of the carbon **feast and famine process** actually used in industry. The understanding of this phenomenon is mandatory to increase the yield of the PHA production and make it **economically profitable**.

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