Highlight

New waves underneath the purple strain

Marta Tortajada*

BIOPOLIS S.L., Parc Científic Universitat de València. C/Catedrático Agustín Escardino, 9, Paterna, Valencia 46980, Spain.

Summary

Successful merging of chemical and biotechnological operations is essential to achieve cost-efficient industrialization of bio-based processes. The demonstration of the use of syngas, derived from microwave assisted pyrolysis of municipal solid waste, for the improved growth and poly-3-hydroxybutyrate production in *Rhodospirillium rubrum*, stands out as an example of the synergistic contribution of chemical engineering and applied microbiology to sustainable biomaterial manufacturing, paving the way to similar applications for other syngas derived bioproducts.

Great efforts have been devoted in the past decades to develop biotechnology-based factories, so called biorefineries, which convert renewable raw materials into valuable chemicals. In a fine combination of chemical engineering and applied microbiology, Revelles *et al.* (2016) validate the use of syngas, derived from microwave assisted pyrolysis of municipal solid waste (MSW), for *Rhodospirillium rubrum* growth and production of poly-3-hydroxybutyrate (PHB), a reference bio-based and biodegradable plastic (Zinn *et al.*, 2001).

In contrast to plant oils, grains and sugars used as fermentation substrates in first generation refineries, residual biomass-rich matrixes, such as MSW or sewage sludge, bear great potential as feedstocks for second generation plants, thanks to their generalized availability, vast surplus and food-chain independent origin. However,

Received 4 August, 2016; accepted 5 August, 2016. *For correspondence. E-mail: marta.tortajada@biopolis.es; Tel. +34 963 160 299; Fax +34 963 160 367. *Microbial Biotechnology* (2017) 10(6), 1297–1299 doi:10.1111/1751-7915.12409 **Funding Information**

The author acknowledges the support to the project SYNPOL, funded by the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 311815.

direct processing is hindered by their intrinsic complex composition, reduced carbon content and recalcitrance to hydrolysis. Syngas (CO+H₂) obtained by thermochemical conversion of these wastes, offers the advantage of yielding homogeneous composition and higher carbon source concentration (Drzyzga *et al.*, 2015).

Previous contributions have shown how microwave assisted pyrolysis (MIP) for syngas production results in reduced waste volumes, rapid and selective heating and improved quality of the final products in comparison to conventional pyrolysis, together with portable and cost-efficient processes for *in situ* treatment of waste (Beneroso *et al.*, 2014, 2015). Revelles *et al.* (2016) effectively utilize this stream for the first time with *R. rubrum* cultures for biomass and biodegradable plastic synthesis. From the present results, noteworthy is the fact that MIP syngas is not only consumed faster than synthetic syngas (1.3 times more in light, and twice as fast in darkness) but also provides increased yields on biomass, demonstrating a more efficient assimilation of the carbon fraction in the gas effluent.

Rhodospirillium rubrum is a versatile non-sulphur bacterium that can grow in aerobic or anaerobic conditions, the latter allowing the expression of photosynthetic pathways resulting in its distinct purple-red colour. Anaerobic growth also enables CO and CO₂ assimilation, both in light and darkness. The ability of *R. rubrum* for producing PHB when grown on syngas has been described (Do *et al.*, 2007; Choi *et al.*, 2010), and makes this singular bacterium, together with other syngas-converters such as *Clostridium ljungdahlii* (Köpke *et al.*, 2010; Schiel-Bengelsdorf and Dürre, 2012), a valuable biocatalyst for third generation, gas based biorefineries.

Results from Revelles *et al.* (2016) demonstrate that *R. rubrum* can readily use synthetic or MIP syngas, in a broad range of compositions and operational conditions, light or darkness. In this way, syngas streams with lower CO and CO₂ content can also be assimilated into biomass and long-chain hydrocarbons. Interestingly, low molecular weight hydrocarbons, found as minor impurities in MIP syngas, do not adversely affect bacterial growth either. The adaptability to variations in feedstock composition, in terms of lower carbon substrate concentrations and resistance to impurities, is shared by other gas-fed systems, such as the conversion of methane from biogas streams

© 2016 The Authors. *Microbial Biotechnology* published by John Wiley & Sons Ltd and Society for Applied Microbiology. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

1298 M. Tortajada

by methanotrophic cultures, again for biomass and PHB synthesis (del Cerro *et al.*, 2012; Rostkowski *et al.*, 2012). In comparison to the chemically catalysed syngas conversion into higher molecular weight compounds, as in the Fischer-Tropsch process, and apart from obvious lower energy requirements, the amenable and resilient character of *R. rubrum* as a biocatalyst is the basis of a major competitive advantage of the overall process.

The creation of PHB out of cheap and widely available renewable resources, such as syngas, is a major and critical step to ultimately launch these ever-promising materials towards massive commercial production (Chen, 2009). Indeed, it is well known that raw materials contribution accounts for more than a third of operational costs in PHB manufacturing (Solaiman et al., 2006). Syngas fermentation stands out as a breakthrough technology for the costeffective generation of chemicals and fuels from a wide range of low-cost feedstocks, including MSW, but also industrial waste, agricultural biomass and industrial offgases. Syngas fermentation has already been demonstrated at commercial scale for ethanol, and is underway for other building blocks, such as 2,3-butanediol (Köpke et al., 2011). In this context, the fact that Revelles et al. (2016) achieve production of PHB from microwave syngas is particularly relevant: since microwave assisted pyrolysis overcomes limitations of conventional pyrolysis (Fernández et al., 2011), it seems reasonable to expect that MIP syngas fermentation benefits from an enhanced overall process efficiency. Most probably, MIP syngas is also a worthy substrate for other relevant syngas-based bioprocesses, as the ones involving the Wood-Ljungdahl pathway (Köpke et al., 2010). Therefore, the validation by Revelles et al. (2016) of the usefulness of MIP syngas as a fermentation substrate for PHB production opens the door to similar applications for other syngas derived bioproducts.

Undoubtedly, both strain and process development strategies will further promote this approach. Synthetic biology and omics tools are being intensively exploited to expand the knowledge base on *R. rubrum* and to increase PHB productivity by remodelling bacterial metabolism (Jin and Nikolau, 2012; Heinrich *et al.*, 2015; Klask *et al.*, 2015; Revelles *et al.*, 2016). An intrinsic limitation of the process, substrate solubility, may be overcome with novel reactor configurations and feeding strategies (Munasinghe and Khanal, 2010). In this sense, the Revelles *et al.* (2016) contribution paves the way for improved PHB production using syngas from MSW, as an example of sustainable biomaterial manufacturing through biotechnology.

In conclusion, novel chemical and biotechnological processes are required to bring cost-efficient bio-based plants to industrial reality. The success of such hybrid biorefineries will stand on the synergistic contribution of both chemical and fermentative processes. Revelles *et al.* (2016) demonstrate in this article that emerging

chemical processing technologies, such as microwave assisted pyrolysis, can supply valuable substrates for existing biorefinery platforms. At least as importantly, versatile *R. rubrum* is shown to integrate the fluctuations in syngas composition to constant conversion, becoming a tuning-filter for biomass and biomaterial production. May there be many more such new applications for this and other (old) bacteria.

References

- Beneroso, D., Bermúdez, J.M., Arenillas, A., and Menéndez, J.A. (2014) Integrated microwave drying, pyrolysis and gasification for valorisation of organic wastes to syngas. *Fuel* **132**: 20–26.
- Beneroso, D., Bermúdez, J.M., Arenillas, A. and Menéndez, J.A. (2015) Microwave pyrolysis of organic wastes for syngas-derived biopolymers production. In *Production of Biofuels and Chemicals with Microwave*. Z., Fang, R.L., Smith Jr, X., Qi (Eds). Netherlands. Dordrecht: Springer DOI 10.1007/978-94-017-9612-5-6
- del Cerro, C., García, J.M., Rojas, A., Tortajada, M., Ramón, D., Galán, B., *et al.* (2012) Genome sequence of the methanotrophic poly-β-hydroxybutyrate producer *Methylocystis parvus* OBBP. *J Bacteriol* **194**: 5709–5710.
- Chen, G.Q. (2009) A microbial polyhydroxyalkanoates (PHA) based bio-and materials industry. *Chem Soc Rev* **38:** 2434–2446.
- Choi, D., Chipman, D.C., Bents, S.C., and Brown, R.C. (2010) A techno-economic analysis of polyhydroxyalkanoate and hydrogen production from syngas fermentation of gasified biomass. *Appl Biochem Biotechnol* **160**: 1032– 1046.
- Do, Y.S., Smeenk, J., Broer, K.M., Kisting, C.J., Brown, R., Heindel, T.J., *et al.* (2007) Growth of *Rhodospirillum rubrum* on synthesis gas: conversion of CO to H₂ and poly-b-hydroxyalkanoate. *Biotechnol Bioeng* **97**: 279–286.
- Drzyzga, O., Revelles, O., Durante-Rodríguez, G., Díaz, E., García, J.L., and Prieto, A. (2015) New challenges for syngas fermentation: towards production of biopolymers. *J Chem Technol Biotechnol* **90**: 1735–1751.
- Fernández, Y., Arenillas, A. and Menéndez, J.Á. (2011) Microwave heating applied to pyrolysis. In Advances in Induction and Microwave Heating of Mineral and Organic Materials. Grundas, S. (Ed.). Rijeka, Croatia: InTech, pp. 723–752.
- Heinrich, D., Raberg, M., and Steinbüchel, A. (2015) Synthesis of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) from unrelated carbon sources in engineered *Rhodospirillum rubrum. FEMS Microbiol Lett* **362**: fnv038.
- Jin, H., and Nikolau, B.J. (2012) Role of genetic redundancy in polyhydroxyalkanoate (PHA) polymerases in PHA biosynthesis in *Rhodospirillum rubrum*. J Bacteriol **194**: 5522–5529.
- Klask, C., Raberg, M., Heinrich, D., and Steinbüchel, A. (2015) Heterologous expression of various PHA synthase genes in *Rhodospirillum rubrum*. *Chem Biochem Eng Q* 29: 75–85.
- Köpke, M., Held, C., Hujer, S., Liesegang, H., Wiezer, A., Wollherr, A., *et al.* (2010) *Clostridium ljungdahlii*

^{© 2016} The Authors. *Microbial Biotechnology* published by John Wiley & Sons Ltd and Society for Applied Microbiology., *Microbial Biotechnology*, **10**, 1297–1299

represents a microbial production platform based on syngas. *Proc Natl Acad Sci USA* **107**: 13087–13092.

- Köpke, M., Mihalcea, C., Liew, F., Tizard, J.H., Ali, M.S., Conolly, J.J., *et al.* (2011) 2,3-Butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. *Appl Environ Microbiol* **77**: 5467–5475.
- Munasinghe, P.C., and Khanal, S.K. (2010) Biomassderived syngas fermentation into biofuels: opportunities and challenges. *Bioresour Technol* **101**: 5013–5022.
- Revelles, O., Tarazona, N., García, J.L., and Prieto, M.A. (2016) Carbon roadmap from syngas to polyhydroxyalkanoates in *Rhodospirillum rubrum*. *Environ Microbiol* **18**: 708–720.
- Rostkowski, K.H., Criddle, C.S., and Lepech, M.D. (2012) Cradle-to-gate life cycle assessment for a cradle-to-cradle cycle: biogas-to-bioplastic (and back). *Environ Sci Technol* **46:** 9822–9829.
- Schiel-Bengelsdorf, B., and Dürre, P. (2012) Pathway engineering and synthetic biology using acetogens. *FEBS Lett* 586: 2191–2198.
- Solaiman, D.K., Ashby, R.D., Foglia, T.A., and Marmer, W.N. (2006) Conversion of agricultural feedstock and coproducts into poly (hydroxyalkanoates). *Appl Microbiol Biotechnol* **71**: 783–789.
- Zinn, M., Witholt, B., and Egli, T. (2001) Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. *Adv Drug Deliv Rev* **53:** 5–21.