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Moving towards a realistic application of purple phototrophic bacteria for resource recovery

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Abstract: Purple phototrophic bacteria are receiving increasing attention due to their unique capability of growing photoheterotrophically, using energy from light to simultaneously recover carbon and nutrients in the form of a different value-added products. In this work, PPB-based applications and potential products are reviewed to identify major challenges and opportunities. A comprehensive analysis of data has shown that, despite the potential of this technology, most of the research on PPB applications has been carried out using pure cultures, axenic conditions and artificial illumination. If a real application of this technology is to be developed, research on PPB should be performed using enriched non-axenic cultures and natural light, aiming at producing results that can be extrapolated to economically-feasible, full-scale systems. Amongst the products obtained from PPB, using the biomass as fish feed represents the most profitable approach, with a potential revenue of 1.14 \$ \cdot kg_{biomass}⁻¹.

Keywords: biological wastewater treatment; carbon and nutrient recovery; anoxygenic photosynthesis

INTRODUCTION

Purple phototrophic bacteria (PPB) can use energy from light to grow photoheterotrophically. This allows PPB to assimilate carbon and nutrients simultaneously, facilitating their recovery in the form of biomass. PPB have been efficiently used for carbon and nutrient recovery from different wastewater streams, with average removals of 63 ± 32 %, 37 ± 31 % and 36 ± 38 % in terms of chemical oxygen demand (COD), nitrogen and phosphorous (calculated from literature values).

When compared with recovery processes based on other growth modes (*i.e.* autotrophic growth, respiration or anaerobic fermentation) anaerobic phototrophic energy generation offers several advantages, including: higher biomass yields (close to unity in COD basis), effective selection and enrichment in non-sterile environments and no aeration requirements (Hädicke et al., 2011; Hülsen et al., 2016).

Despite these advantages, the light requirement is a major drawback of any phototrophic process, hindering the economic feasibility of industrial applications. In addition, other major costs such as substrate used (if waste streams are not utilized) and capital investment for the reactors and lamps, further challenge the implementation of phototrophic systems. Microalgae are a perfect example of this issues, with few industrial applications despite decades of research (Luque, 2010).

Therefore, if realistic PPB applications are to be developed, research should address the main challenges faced by this technology and innovate towards developing an economically feasible process. In this work, crucial factors for feasible PPB-based applications are assessed, comparing also potential products (*i.e.* biomass as feed, biomass as fertilizer, proteins, polyhydroxyalkanoates (PHA), carotenoids and hydrogen) that can be generated.

METHODOLOGY

This work utilises quantitative information to identify performance limits and viability across the broad set of literature that was evaluated. Quantitative and qualitative data



were collected from 177 studies, obtaining a database consisting of 1,487 independent data points. To produce a coherent/normalized database, different categories were defined for reactor type, substrates, inocula/PPB and light source. Statistical analyses were performed using the software R 3.5.0 (2019).

RESULTS AND DISCUSSION

Starting with the substrate used, the utilization of synthetic axenic media for PPB growth is an interesting and relevant approach for fundamental research. Nevertheless, these conditions do not represent a realistic approach, mainly due to the costs of sterilization and the substrate itself. A simple calculation using malic acid for hydrogen production by PPB (most commonly used substrate) exemplifies this, with a monetary recovery of less than 4 % considering only the substrate costs.

Besides these limitations, 73 % of the research focused on PPB biomass production or wastewater treatment has used pure cultures and sterile substrates (see Figure 1). When considering hydrogen production, the numbers are even worse, with 84 % of the studies using pure cultures. Only 27 % of the studies on biomass growth have applied enriched PPB cultures, under conditions where the selection of PPB is favoured (Hülsen et al., 2014). The latter represents an approach that can be applied for the treatment of complex non-sterile waste streams, achieving biomass yields comparable to those achieved using pure cultures (Figure 2). Using waste streams is not only cost-free, but can also increase the benefits by lowering discharge costs.

The type of light source applied also limits the potential application of the current research on PPB processes. In most cases, light sources were either fluorescent, halogen or tungsten lamps or non-specific LEDs, with only 2 studies so far (3 % of the articles reviewed) using natural light (Carlozzi et al., 2006; Carlozzi and Sacchi, 2001). Although the use of LEDs has increased considerably the biomass yields in terms of energy input (Figure 3), the highest yields still result in costs of 1.9 \$·kg_{biomass}⁻¹, a value that confirms the need of using natural light as energy source.

Amongst the potential products within the PPB biomass (Figure 4), its application as feed appears as a viable option, based on the high protein contents. The high prices of carotenoids and coenzyme Q10 might also open the door for their potential application from PPB biomass. Nevertheless, extraction processes (that will surely impact the process economics) will be needed. The combined presence of proteins, PHA and carotenoids facilitates the application of PPB biomass as fish feed substitute, where proteins would serve as feed, PHA as biocontrol agent and carotenoids as immune system enhancer and as meat colourant. The utilization of PPB as fish feed substitute has been recently been proven to be feasible (Delamare-Deboutteville et al., 2019) and could represent a revenue of 1.14 \$\cdot kgbiomass^{-1}.

CONCLUSIONS

PPB-based processes appear as a novel, promising approach for wastewater treatment and simultaneous carbon and nutrient recovery with several advantages when compared to other options. Nevertheless, most of the research efforts to date have been carried out using pure cultures, axenic conditions and artificial illumination. If a realistic application of PPB is to be developed, research should focus on using enriched mixed cultures and natural light illumination, obtaining results that can be extrapolated to large-scale processes. Amongst the potential utilizations of the produced PPB, its application as bulk fish feed seems feasible.



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Figure 1. Inocula used for biomass production and/or wastewater treatment using purple phototrophic bacteria. The numbers represent the percentages of a total of 70 studies. The data from "enriched PPB cultures" corresponds to enriched cultures grown on non-sterile, complex media.





Figure 2. Biomass yields reported in the literature for axenic, pure inocula (white) and non-sterile, enriched cultures (red). The blue dots represent the arithmetic means. Only the inocula with three or more independent values ($n \ge 3$) are presented.



Figure 3. Evolution of the purple phototrophic bacteria biomass energy yields obtained in processes illuminated with different light sources: not specified (\blacksquare), fluorescent (\diamondsuit), halogen (\bigcirc), tungsten (\bigcirc) and light-emitting diode (LED; \triangle).



Figure 4. Contents of different potential products within the purple phototrophic bacteria biomass. Methane refers to the methane yields produced via anaerobic digestion. The coloured squares represent the arithmetic means achieved with different reactor configurations: batch (\blacksquare), sequencing batch reactor (SBR; \blacksquare), fed-batch (\blacksquare), fed-batch (\blacksquare), overflow photobioreactor (PBR; \blacksquare), tubular PBR (\blacksquare), photo-anaerobic membrane bioreactor (PAnMBR; \blacksquare) and continuous stirred-tank reactor (CSTR; \blacksquare). PHB stands for Poly(3-hydroxybutyrate).