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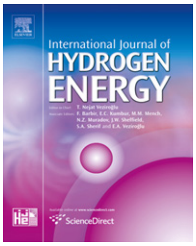

Photosynthetic bacterial growth and productivity under continuous illumination or diurnal cycles with olive mill wastewater as feedstock

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ABSTRACT

Photofermentative hydrogen production from olive mill wastewater by *Rhodobacter sphaeroides* O.U.001 was investigated under different regimes of illumination. The analysis included measurements of biomass accumulation, H_2-production, high-value bio-product accumulation (polyhydroxybutyrate and carotenoid) and measurements of the medium pH as a function of growth and productivity. Batch cultures were grown under continuous light (CL) or 12 h light/12 h dark (12L/12D) diurnal cycles. Growth under CL or 12L/12D cycles yielded about the same amount of biomass (0.5 g dry cell weight per L culture) and volume of H_2 gas (50 ml H_2 per L culture). On the other hand, 12L/12D cultures showed a pronounced lag in biomass and H_2 accumulation. Advances described in the work would find application in lowering operational costs for hydrogen production by better management of the energy source and cheap feedstock utilization. Compare to CL, equivalent amount of hydrogen gas accumulation within shorter time interval denoted to have two times higher hydrogen production rate and light conversion efficiencies via diurnal cycles, which can yield 50% savings on consumed energy source.

1. Introduction

Alternative solution to overcome the economic restrictions of biological hydrogen production by photosynthetic bacteria is to couple this process with wastewater utilization. Another approach for enhancing the premise of the H_2-production bioprocess is to couple it with the simultaneous production of higher value bio-products, which can bring added value to the overall process.

The olive-oil production process generates a dark colored wastewater after the extraction of oil from the pulp of olive fruit. This wastewater consists of (i) olive fruit extract, (ii) waters from the washing and cooling processes, and (iii) some olive-derived solid particles. This dark colored wastewater is known to be a considerable pollutant due to its high organic matter content and recalcitrant compounds such as polyphenols, and also because of its high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values reaching up to 200 g/L and 100 g/L, respectively [1,2]. As a consequence, disposal of such a waste material becomes an important economic and environmental problem that needs to be solved urgently. Modification of the technology used in

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Nomenclature

BOD	Biochemical oxygen demand
CL	Continuous light
COD	Chemical oxygen demand
k_c	Apparent specific growth rate, h^{-1}
L/D	Light/Dark cycles
OMW	Olive mill wastewater
PHB	Polyhydroxybutyrate
R^2	Dispersion of the distribution from the mean, the coefficient quantifying goodness-of-fit
r_{H2}	Maximum H_2 productivity based on culture volume, L L^{-1} h^{-1}
$r_{H2}{}^*$	Maximum H_2 productivity based on biomass, L g^{-1} h^{-1}
Δt_{H2}	Duration of hydrogen production, h
VFA	Volatile fatty acids
X_{max}	Maximum cell concentration (Dry weight), g/L
X_o	Initial cell concentration (Dry weight), g/L
μ	Specific growth rate, h^{-1}
η	Light conversion efficiency, %

oil extraction is one way of reducing the hazardous effects of OMW disposal. However, most of the treatment methods are focused on bioremediation, i.e., reducing the polluting effect of OMW, or biotransformation of OMW into alternative valuable products. In our previous studies, it was shown that olive mill wastewater could be utilized as feedstock for photobiological hydrogen production, serving as a sole substrate source [3]. OMW was found to have a high C/N ratio, in addition to its high organic contents such as sugars, organic acids (mainly acetic acid), polyalcohols and triglycerides, all of which are known to be utilized in photofermentative hydrogen production by purple non-sulphur phototrophic bacteria [4].

Even though their values are not yet very high for commercialization, valuable bio-products such as polyhydroxybutyrate (PHB) and carotenoid pigments were also produced throughout this photosynthetic process in addition to hydrogen [3]. PHB has become commercially attractive for biotechnological applications as an alternative for petrochemical plastics, due to its thermoplastic properties and its biodegradability. It is mostly synthesized during stress conditions as an intracellular carbon and energy storage material and accumulates as granules within the bacterial cytoplasm [5–9]. PHB was also reported to be useful as a buffer system for regulating the intracellular redox balance [10]. It is completely biodegradable under ambient conditions in the environment and has similar properties to polypropylene [11]. Due to its chemical properties, PHB has important industrial applications, particularly in the construction of biodegradable carriers either in agriculture for herbicides and insecticides or in the medical field for drugs [12]. However, compared to synthetic plastics, the production costs of PHB are substantially higher since they involve the production of biomass from expensive carbon feedstock sources [13]. Thus, a combination of the photosynthetic process and a source of carbon from waste material can open a new avenue for renewable PHB production. PHB accumulation inside the cells of photosynthetic bacteria depends on carbon and nitrogen availability, as well as the pH of the medium. One of the highest levels of PHB production by *Rhodobacter sphaeroides* was obtained with acetate as a source of carbon [14] under both ammonium and nitrogen deprived conditions. A better understanding of PHB biosynthesis with organic waste substrates as feedstock would permit commercial exploitation of photosynthetic bacteria for such a high-value product generation. Carotenoid pigments are another valuable bio-product, which is essential for photosynthesis, since they absorb and transfer light energy to bacteriochlorophyll, and are to such an extent functional as light harvesting pigments. Moreover, carotenoids can be used commercially as vitamins, food colorants, natural antioxidants, and for cancer chemoprevention [15].

There is limited-only information of photosynthetic bacterial hydrogen production, bio-product and biomass accumulation upon fermentation with waste material under diurnal light–dark cycle conditions [16]. Most recent and past work with photosynthetic bacteria was performed under continuous illumination and upon use of artificial growth media. Thus, there is a need to investigate the performance and productivity of a culture grown with untreated OMW media under light–dark cycles of illumination that simulate the diurnal cycle that would be experienced by a scale-up culture under ambient conditions. Accordingly, this work investigated the effect of *R. sphaeroides* photofermentative growth and productivity under continuous illumination (CL) and a 12 h light/12 h dark (12L/12D) diurnal cycle on the transformation of OMW feedstock into hydrogen, as well as other bio-products, notably PHB and carotenoid.

2. Materials and methods

R. sphaeroides O.U.001 (DSM 5864) was used in this study as previously described [17]. Olive mill wastewater (OMW) was obtained from a centrifugal olive-oil mill in Balikesir - Edremit district (Western Anatolia, Turkey). Its physicochemical properties were reported by Eroglu et al. (2009) [4]. Hydrogen production media contained 2% OMW (only water was used for dilution), as this was found to yield optimal production rates [3]. Indoor hydrogen production experiments were performed in jacketed glass-column photobioreactors (400 ml of total volume). Experimental procedures were previously described in detail [3]. 12 h light - 12 h dark (12L/12D) cycles were applied with an incandescent light source (tungsten lamp), automatically switched on for 12 h and off for 12 h, respectively. The temperature was maintained at 32 °C by the circulation of a thermostated water stream through the water jacket surrounding the reactors. Flow of water through water jacket was obtained by a circulatory water bath (Hetofrig CB-11 Cooling Bath), which was kept at a constant temperature by a thermostat (Hetotherm DT). Barometric pressure is taken as 690 mmHg which is an average value for Ankara. The light intensity at the surface of the reactor was maintained at 200 W/m^2. Results from the light–dark cycles were compared with a control culture grown under continuous illumination (CL).