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Agroindustrial residues and energy crops for the production of hydrogen and poly-β-hydroxybutyrate via photofermentation

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HIGHLIGHTS

- Starch and sugars rich substrates led to higher H_2 yields than fibrous substrates.
- Dark fermentation effluent of wheat bran allowed to accumulate 649 mL H_2 L^{-1}.
- H_2 production began only after propionic acid was depleted from the media.
- Mg^{2+} and carbon deficiency can shift *R. palustris* from H_2 to poly-β-hydroxybutyrate.
- Dark fermented ensiled olive pomace led to high poly-β-hydroxybutyrate accumulation.

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ABSTRACT

The present study was aimed at assessing the biotransformation of dark fermented agroindustrial residues and energy crops for the production of hydrogen and poly-β-hydroxybutyrate (PHB), in lab-scale photofermentation. The investigation on novel substrates for photofermentation is needed in order to enlarge the range of sustainable feedstocks. Dark fermentation effluents of ensiled maize, ensiled giant reed, ensiled olive pomace, and wheat bran were inoculated with *Rhodopseudomonas palustris* CGA676, a mutant strain suitable for hydrogen production in ammonium-rich media. The highest hydrogen producing performances were observed in wheat bran and maize effluents (648.6 and 320.3 mL L^{-1}, respectively), both characterized by high initial volatile fatty acids (VFAs) concentrations. Giant reed and olive pomace effluents led to poor hydrogen production due to low initial VFAs concentrations, as the original substrates are rich in fiber. The highest PHB content was accumulated in olive pomace effluent (11.53% TS), ascribable to magnesium deficiency.

1. Introduction

Hydrogen is a promising future energy carrier, is the most abundant element in the universe and represents a clean and renewable biofuel, with high conversion efficiency (Holladay et al., 2009; Christopher and Dimitrios, 2012). Currently, about 96% of hydrogen is synthesized from fossil fuels, in particular from methane reforming, and the remaining percentage is produced by water electrolysis. It can be used directly in internal combustion engines or in fuel cells, after appropriate purification, without a direct combustion (Holladay et al., 2009; Christopher and Dimitrios, 2012). Biological systems have significant potential as sustainable means for producing hydrogen, in the developing hydrogen economy (Holladay et al., 2009; Redwood et al., 2009). Biohydrogen production processes can be classified into different groups, as follows: biophotolysis of water by microalgae and cyanobacteria, photodecomposition of organic compounds by photosynthetic bacteria, namely photofermentation (PF), dark fermentation (DF) of organic compounds by anaerobic or facultative anaerobic bacteria, and bioelectrohydrogenesis (Redwood et al., 2009; Argun and Kargi, 2011).

The combination of dark fermentation with photofermentation represents an interesting opportunity as a future plant-scale technology for biological hydrogen production and this system is

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extensively applied at lab-scale in order to investigate the biohydrogen potential of a diverse range of substrates, both synthetic and non-synthetic (Redwood et al., 2009; Keskin et al., 2011; Argun and Kargi, 2011; Adessi et al., 2012a; Basak et al., 2014; Hallenbeck and Liu, 2016). During dark fermentation, heterotrophic bacteria convert organic substrates, mainly carbohydrate-rich materials, into organic products, hydrogen and carbon dioxide. Subsequently, dark fermentation effluents can be used for the photofermentation performed by purple non sulfur bacteria (PNSB). These bacteria are able to produce hydrogen through nitrogenase enzyme using light as source of energy and simple organic acids of dark fermentation effluents as electron donors (Redwood et al., 2009; Adessi and De Philippis, 2014; Basak et al., 2014; Hallenbeck and Liu, 2016).

Dark fermentation effluents are typically characterized by high concentrations of ammonium that inhibits the synthesis and activity of nitrogenase (Keskin et al., 2011; Adessi et al., 2012a). Thus, some strategies have been explored in order to produce hydrogen even in the presence of ammonium, such as the dilution of the substrate or the use of microorganisms genetically manipulated (Kars and Gündüz, 2010; Heiniger et al., 2012; Keskin et al., 2011; Adessi et al., 2012b). Generally, media for photofermentation coming from the dark fermentation of biomasses need a dilution in order to let the light penetrate better, increasing the light conversion efficiency and the H_2 production rate (Keskin et al., 2011; Adessi et al., 2012a,b). Furthermore, with dilution, organic acids and ammonium concentrations decrease, reducing the time required to reach the not inhibitory ammonium threshold for the nitrogenase and the amount of organic acids consumed for growth before the H_2 production starts (Adessi et al., 2012b).

PNSB are able to biosynthesize and store poly-β-hydroxybutyrate (PHB), that is a member of polyhydroxyalkonate family and represents a biodegradable polymer, that can be used for the production of biodegradable plastics (Wu et al., 2012; Adessi and De Philippis, 2014). Bacteria produce PHB when they lack a range of nutrients required for cell development, like nitrogen, phosphate, sulfur or magnesium, and receive an abundant supply of carbon sources (Wu et al., 2012; Repaske and Repaske, 1976). In PNSB, PHB biosynthesis represents a competitive reductive reaction compared to the nitrogenase activity (Hustede et al., 1993; Vincenzini et al., 1997; Redwood et al., 2009; Wu et al., 2012).

The investigation of novel substrates suitable for photofermentation is needed in order to enlarge the range of sustainable feedstocks (Hallenbeck and Liu, 2016). Lignocellulosic biomasses (e.g. hardwood, softwood, grasses and agricultural residues) are abundant in nature and together with waste material (e.g. agroindustrial residues) they potentially represent low cost and renewable feedstocks for hydrogen production (Kapdan and Kargi, 2006; Keskin et al., 2011). In the present study, the photofermentation of DF effluents of wheat bran and of the ensiled biomass of giant reed (*Arundo donax* L.), maize (*Zea mays* L.) and olive pomace was investigated. Giant reed is a perennial rhizomatous grass, which represents a promising no-food energy crop, with high biomass yields and high adaptability to marginal land (Dragoni et al., 2015). Wheat bran and olive pomace are abundant and inexpensive agroindustrial residues in the Mediterranean area (Pan et al., 2008; Mateo and Maicas, 2015). Maize is a widespread energy and food crop, which is an annual species requiring relevant input and high fertile soils (Dragoni et al., 2015).

Amongst PNSB, *Rhodopseudomonas palustris* is a very versatile microorganism and it is ideal to study hydrogen production (Larimer et al., 2004; McKinlay and Harwood, 2011); indeed, its wide versatility gives it the opportunity to adapt to very different culture conditions, like growing on complex agroindustrial residues and energy crop effluents. In particular, *R. palustris* CGA676 was demonstrated to be a suitable strain to be used for the photofermentation of dark fermentation effluents of vegetable wastes (Adessi et al., 2012a,b).

Aim of the present work was to investigate the photofermentation process carried out by *R. palustris* strain CGA676 using DF effluents of wheat bran and of the ensiled biomass of giant reed, maize and olive pomace. The amount of PHB accumulated in the residual biomass of *R. palustris*, grown in the four DF effluents was investigated and the effect of magnesium deficiency on the PHB production was assessed.

2. Materials and methods

2.1. Growth media and inoculum

Dark fermentation of ensiled biomass of *A. donax* (eGR), ensiled biomass of *Z. mays* (eZM), ensiled olive pomace (eOP) and wheat bran (WB) was carried out in triplicate at lab-scale under mesophilic conditions, using heat-treated digestate of a biogas plant as inoculum, as described by Corneli et al. (2016). Each reactor was filled with biomass and 100 g of inoculum (substrate to inoculum ratio of 2:1, based on volatile solids and ultrapure water) was added up to a volume of 650 mL. The assay was kept for four days and the final DF effluents were used as substrates for the photofermentation. DF effluents were centrifuged 10 min at 2500*g*, in order to separate the gross solid fraction and each supernatant was used undiluted and diluted with distilled water (1:1) and in both cases supplemented with (g L^{-1}): ferric citrate, 0.005; sodium sulfate, 0.23; buffer solution (K_2HPO_4 0.5 and KH_2PO_4 0.3). These addictions were shown to enhance H_2 production of *R. palustris* strains in previous studies (Bianchi et al., 2010; Adessi et al., 2012b). The pH was adjusted to 6.8 with NaOH before and after autoclaving. Photofermentation was carried out using a pure culture of *R. palustris* strain CGA676. The strain, kindly provided by C. S. Harwood (Dept. Microbiology, School of Medicine, University of Washington, Seattle WA, USA), was constructed as described by McKinlay and Harwood (2010) and has constitutive nitrogenase activity allowing it to produce H_2 in the presence of NH_4^+ (McKinlay and Harwood, 2010). The strain was activated in a 200 mL bottle at 30 °C and 150 μmol (photons) m^{-2} s^{-1} using RPN medium (g L^{-1}): sodium acetate, 2.44; NH_4Cl, 0.5; K_2HPO_4, 0.5; KH_2PO_4, 0.3; $MgSO_4$ $7H_2O$, 0.4; NaCl, 0.4; $CaCl_2$ $2H_2O$, 0.075; ferric citrate, 0.005; yeast extract, 0.4. Trace elements were supplied by adding 10 mL per liter of a solution containing (mg L^{-1}): $ZnSO_4$ $7H_2O$, 10; $MnCl_2$ $4H_2O$, 3, H_3BO_3, 30; $CoCl_2$ $6H_2O$, 20; $CuCl_2$ $2H_2O$, 1; $NiCl_2$ $6H_2O$, 2; Na_2MoO_4 $2H_2O$, 30. The pH was adjusted to 6.8 with NaOH before and after autoclaving.

2.2. Phenotypic acclimation and batch assay

The phenotypic acclimation of the activated strain (OD660 > 1.5) was realized into glass tubes (20 mL) with 20 mL of autoclaved DF effluent both undiluted and diluted (1:1) of each substrate (eGR, eZM, eOP, WB, separately) and in triplicate, by suspending in each tube the pellet of 3 mL of culture (grown in RPN medium) centrifuged for 15 min at 5000 rpm. A total of 24 tubes were set up and put under the following growing conditions: temperature 30 °C and light intensity 150 μmol (photons) m^{-2} s^{-1}. After 60 days, in the undiluted samples no hydrogen formation was observed (data not shown), thus the following experiments were carried out with diluted substrates. The batch assay of photofermentation was carried out using photobioreactors (100 mL) under anaerobic conditions. Rubber-stoppered glass bottles equipped with syringes for the detection of hydrogen were filled with 100 mL of autoclaved DF effluent in triplicate and an