A holistic approach for the utilization of an industrial sugary wastewater towards biofuels and bioplastics production

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Abstract
The biotechnological exploitation of an industrial sugary wastewaters towards biohydrogen, methane and polyhydroxyalkanoates (PHAs) was investigated in a three stage system. Initially, the diluted wastewater was acidified via mixed acidogenic consortia in a column bioreactor, for the generation of biohydrogen and the effect of organic loading rate and supporting media for the attachment of microorganisms on the yields of the metabolic products was studied. The acidified effluents were then used as carbon source, by an enriched mixed bacterial culture, for the production of PHAs, which were extracted from the cells via solvent extraction and characterized. Finally, the biochemical methane potential of the residual biomass after PHAs extraction was evaluated, closing the loop for the holistic exploitation of the wastes to biofuels send bioplastics.

Introduction
Biohydrogen and methane are biofuels that represent clean and green energy carriers, since they can be generated from renewable sources and their production for energy release is accompanied by minimal by-products i.e. carbon dioxide and/or water. Methane production through wastes is a mature technology, already broadly applied. Hydrogen is an intermediary product of the process that, however, is not available since it is rapidly taken up and converted into methane by methane-producing microorganisms. In biological hydrogen production processes, hydrogen formation and consumption are uncoupled, so that hydrogen is available as the final product (Reith et al., 2003). During that process, volatile fatty acids are also produced which can be exploiting serving as precursors of polyhydroxyalkanoates (PHAs) i.e. microbial bioplastics. PHAs bear similar properties to fossil plastics, being also fully biocompatible, biodegradable and non-toxics.
The current study aspires to provide a sustainable biotechnological solution for the holistic exploitation of industrial food wastes i.e. of confectionery industry wastes, towards energy and bioplastics in a three stage system.

Materials and Methods

Wastes
Food wastes were obtained from a large confectionary industry of Western Greece. Specifically, the wastes were residues that remain on the cauldrons during the production process of fruit jellies syrups and which are removed by rinsing with hot water, namely jelly rinsing (JR) and syrup rinsing (SR). The composition of JR was: Sugars, %, 13.71 ± 1.72; COD, %, 17.44 ± 1.15; TKN, ‰,0.29 ± 0.00; pH, 4.15 ± 0.01. The composition of SR was: Sugars, %, 7.65 ± 0.03; COD, %, 11.38 ± 0.04; TKN, negligible; pH, 5.50 ± 0.01.

Biohydrogen production
Biohydrogen production was studied in two 500 mL active volume mesophilic (35°C) continuous up-flow column reactors (UFCR), with different supporting media, i.e. ceramic and plastic beads, for the attachment of microorganisms. A mixture of JR and SR after proper dilution with tap water was used as feed, and the indigenous microorganisms of the wastes served as start-up culture. Start-up was performed with initial sugars concentration (C_s) 30 g/L and with hydraulic retention time 12 h. The pH was adjusted at 5-5.5. Upon achievement of steady state the C_s was increased and the effect of the increase organic loading was studied for the different supporting media. Hydrogen production rate was estimated daily, whereas sugars, volatile fatty acids, ethanol and lactate were quantified in the liquid phase daily. and after reaching steady state recirculation of the effluent into the reactor was introduced at ratio 1:1.

Polyhydroxyalkanoates production
Effluents of the UFCB were further exploited for towards bioplastics via a PHAs accumulating consortium acclimated to VFAs was used. PHAs production was assessed in batch mode, in 1L Erlenmeyer flasks with

initial substrate concentration 10g/L COD, carbon to nitrogen ratio 200mg COD/mg $N\text{-}NH_4^+$, (adjusted with ammonium sulphate), 30°C± 1 ^oC, and contact agitation at 150rpm. The concentrations of COD, carbon sources and ammonium were followed versus time and PHAs yields were estimated for 48h cultures gravimetrically after solvent extraction.

BMP tests
The Biochemical Methane Potential (BMP) of the residual microbial biomass from PHAs production after solvent extraction was assessed via BMP tests. Experiments were carried out in duplicate at 35 ^oC in serum bottles of 160 mL, according to Owen and Chynoweth (1993).

Analytical methods
Volatile suspended solids (VSS), chemical oxygen demand (COD) and Total Kjeldahl Nitrogen (TKN) were quantified according to Standard Methods (APHA, 1995). For the quantification of the VFAs, sugars, ethanol, as well as for the analysis of produced gas composition in hydrogen and methane, the methods described in Ntaikou et al (2020) were used. Lactate was quantified using a D-L lactate Megazyme kit.

Results
Effect of substrate concentration and recirculation on biohydrogen production process
It was shown that C_s and the type of supporting media significantly affected both the bioconversion efficiency of sugars, which show a decreasing tendency for increased C_s indicating the occurrence of kinetic limitations for higher substrate concentrations, as previously reported for other types of wastes (Antonopoulou et al 2011). Hydrogen yield reached 0.57 ± 0.1 mol/mol consumed sugars being maximum for the lower C_s, during which the main metabolic product was butyrate. For higher C_s lactate was also detected at high concentrations at both reactorsin the reactor.

Assessement of PHAs production yields and properties
The persentage of PHAs in the microbial cells after culturing for 48h in the different effluents (i.e. effluents of UFCR fed with C_s 20g/L (E20), with C_s 30g/L (E30) and with C_s 30g/L with recirucation (ER)), varied significantly, indicating that the accumulation capacity of the consortium is affected by the type of carbon sources. Indeed, E20, in which minimal residual sugar were present led to the highest PHAs content, up to 40%, whereas E30 which had the maximum lactate cocentration led to the lowest accumipation of PHAs. The produced plastics were in all cases copolymers of hydroxyburyrate and hydroxyvalerate (PHBV) with HV content, ~2% approximately.

BMP of the residual biomass
The BMP of the different residual biomasses did not differenciate significantly. Methane generation rate was similar to that of the control (glucose) and also of the non-extracted biomass culture, indicating that microbial cells are easily and quicky biodegradable, with high methane production potencial whereas the extraction with solvents does not inhibit AD.

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