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Bread wastes to energy: Sequential lactic and photo-fermentation for hydrogen production

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ABSTRACT

One third of the World's entire food production is lost or wasted every year. Biohydrogen production offers a possibility to re-use this resource; in particular, bread and bakery products wastes, due to their composition (up to 70% of carbohydrates, mostly starch), represent an appropriate source of nutrients for microorganisms. The aim of this work was to convert bread wastes into hydrogen with a sequential system composed of lactic fermentation and photofermentation, with a minimum number of treatments to the substrate. The best results were provided by *Lactobacillus amylovorus* DSM 20532, followed by photofermentation by *Rhodopseudomonas palustris* 42OL, supplemented with ferric citrate and magnesium sulfate. The process led to 3.1 mol H_2 mol^{-1} glucose, among the highest yields obtained on starch containing substrates, with an energy recovery of 54 MJ t^{-1} dry waste. This study promotes the re-use of energy-containing food wastes for improving circular economy.

Introduction

Food wastes occur in every step of food supply chain, from production to processing, distribution, and final consumption. They are defined as "fractions of food and inedible parts of food removed from the food supply chain to be recovered or disposed" (FUSIONS [1]). According to Food and Agricultural Organization (FAO), 1.3 billion tons of food per year are globally lost or wasted, that is one third of the World's entire production [2]. In 2012, in the European Union (EU) about 90 million tons of food were wasted [3]. The EU set the target of halving *per capita* food waste at the retail and consumer level and reducing food losses along production and supply chain by 2030 [4]. Many EU member States have guidelines for addressing the food wastes problem.

In this connection, bioenergy production offers a possibility to use this resource. Indeed, an increasing number of scientific papers have been recently published on the conversion of food wastes into energy, and in particular into hydrogen [5]. Food wastes usually have a very high moisture content, which makes them poorly efficient for physical energy-recovery

Abbreviations: LAB, lactic acid bacteria; PABA, *p*-aminobenzoic acid; LCE, light conversion efficiency; HPY, hydrogen production yield; SC, substrate conversion.

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treatments, as pyrolysis or gasification, but makes them perfect candidates for being converted into energy by using microorganisms [6]. Moreover, food wastes usually contain low amount of lignin and in many cases very little or no pretreatments are required for their conversion to biofuel, compared to agricultural and forest residues [7]. Among biofuels, hydrogen is considered the most viable energy carrier for the future because of its non-polluting nature and its high energy content [8,9], hence a large part of research is dedicated to study its production from food wastes using microorganisms [10,11]. It is possible to set combined microbial systems, with a first dark fermentation stage either producing hydrogen and organic acids from sugars (as *Clostridia* or *Enterobacteria* [12]), or just organic acids at a high yield (as lactic acid bacteria (LAB)), followed by a second stage of photofermentation converting organic acids into hydrogen by purple non-sulfur bacteria [13–16]. Food wastes have a very heterogeneous nature and are depending on the food habits and industrial development of the place they are produced [17]. In Italy, it has been estimated that bread represents 19% of wastes only at household level [18]. Hence, bread and bakery products wastes represent a possible choice for biohydrogen production in Italy. Containing up to the 70% of carbohydrates, in particular starch [19–21], bakery wastes represent an appropriate source of nutrients for microorganisms. Starch-containing substrates require enzymatic or biological hydrolysis in order to increase the simple sugar content available for dark fermentation [22–24]. The enzymatic treatment is usually obtained by the addition of enzymes of the α-amylase family [25], while biological hydrolysis can occur directly during the dark fermentation. Relatively few LAB strains (mainly lactobacilli) have starch-degrading properties [22]. One of these, *Lactobacillus amylovorus*, was positively used to produce lactic acid from starch substrates, in sequential or combined dark-photo fermentation [12,26,27]. The lactate produced by LAB can be converted to hydrogen (up to 6 mol of H_2 can be theoretically produced from a mole of lactate) by photofermentation. *Rhodopseudomonas palustris* is a very versatile organism [28], able to transform reduced carbon sources into hydrogen through nitrogenase, with a high efficiency.

However, often the combination of two step processes has several technical hurdles, in terms of substrate treatments, preservation of axenicity and low hydrogen yield (especially when scaling up).

This research aims to prove that it is possible to produce hydrogen from bread wastes with a combined system that uses lactic fermentation in the first stage to obtain the organic acid to be photofermented in the second stage, at a high yield and with a minimum number of treatments to the starch-containing substrate.

Materials and methods

Microorganisms and enzymatic treatment

Different species and strains of *Lactobacillus* have been tested to obtain the best yield of lactic acid: *L. rossiae LRB9,* from the collection of GESAAF (University of Florence, Italy) and previously isolated from an Italian sourdough; *L. amylovorus* DSM 20531 and DSM 20532, purchased from DSMZ (*Deutsche Sammlung von Mikroorganismen und Zellkulturen*) collection. In figures and in tables the two *L. amylovorus* strains are referred as LA1 and LA2. The enzymatic treatment was carried out adding α-amylase (1820 U mg^{-1} supplied by Sigma-Aldrich) to a set inoculated with *L. rossiae* LRB9.

Rhodopseudomonas palustris 42OL, from the collection of DISPAA (University of Florence, Italy), has been chosen for photofermentation tests due to its high adaptability to different kinds of substrates [29].

Cultivation media

Before being used for bread waste fermentation, lactic acid bacteria (LAB) were grown for 24 h in MR3i, a medium containing: peptone 5 g L^{-1}; tryptone 5 g L^{-1}; lab-lemco 5 g L^{-1}; yeast extract 12 g L^{-1}; maltose 20 g L^{-1}; glucose 6 g L^{-1}; fructose 6 g L^{-1}; sodium gluconate 2 g L^{-1}; sodium acetate 2 g L^{-1}; ammonium citrate 2 g L^{-1}; dipotassium hydrogen phosphate 2 g L^{-1}; magnesium sulfate·$7H_2O$; 0.2 g L^{-1}; manganous sulfate · $10H_2O$; 0.05 g L^{-1}; cysteine 0.5 g L^{-1}; vitamin mix 1000 × 1 mL L^{-1}; Tween80 1 mL L^{-1}, fresh yeast extract 15 mL L^{-1}, distilled water. The pH of the medium was adjusted at 5.6. Stationary phase cells were harvested by centrifugation at 2500 × *g* for 10 min, washed with sterile physiological solution, and suspended in the medium for the tests. *R. palustris* cultures were maintained in RPN medium (composition as in Ref. [30]) (containing lactic acid as carbon source); they were then activated for hydrogen production in RPP medium (composition as in Ref. [30]) for ten days. Active cells were then centrifuged for 20 min at 4000 rpm, and resuspended in the medium for the tests.

The medium used for hydrogen production experiments was obtained by filtering the effluent of lactic fermentation (3 L experimental set, described in paragraph 2.3), for removing larger bread particles. A centrifugation step (2500 × *g* for 20 min) was performed only for the first experimental set. The filtered product was diluted with distilled water in 3:1 ratio (broth: water). The pH was adjusted at 6.8, buffer K_2HPO_4/KH_2PO_4 was added, and then the medium was autoclaved. After autoclaving, the pH was checked and adjusted again. For the hydrogen production experimental set, one or more elements were added to the medium as described in paragraph 2.4.

Bread wastes fermentation experimental sets

The bread used for the studies was composed as follows (g for 100 g of bread): water 27.0; proteins 8.5; lipids 0.4; total carbohydrates 64.1, of which: starch 56.3; soluble sugars 1.3; fiber 3.1.

In order to select the best conditions to obtain the highest lactic acid yield, various experimental sets were established. The strains, or a combination of them, were inoculated in a concentration of 1 × 10^9 CFU/mL into a medium composed by 10% bread/water w/v, α-amylase was used in a concentration of 50 U/mL together with *L. rossiae* LRB9; each set was carried out in duplicate. Bread wastes had been previously oven dried overnight at 60 °C, grinded, and stored at −20 °C 100 mL flasks

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