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Microbial biodegradable plastic production from a wheat-based biorefining strategy

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ABSTRACT

Restructuring the current fermentation and recovery practices employed for the production of polyhydroxyalkanoates is essential for the commercialisation of environmentally benign and cost competitive biodegradable plastics. This study presents the potential of a wheat-based biorefinery for the production of poly(3-hydroxybutyrate) (PHB). Fed-batch bioconversions using *Wautersia eutropha* growing on wheat-derived media led to the production of 162.8 g/l PHB. A high PHB to total dry weight (TDW) yield of 93% (w/w) was achieved due to microbial autolysis at the end of fermentation. Images of bacterial cells taken with a Transmission Electron Microscope (TEM) indicated the potential of bacterial autolysis as a mean to shorten downstream processing for PHB purification. The consumption of amino acids and peptides derived from wheat gluten hydrolysis resulted in a high glucose to PHB conversion yield of 0.47 g/g. The respective yield regarding the amount of wheat used for the production of enzymes and PHB was around 0.3 g PHB/g wheat, which corresponds to 82.8% of the maximum theoretical conversion yield. The productivity achieved was around 0.9 g/l h. Fermentations carried out on wheat-derived media and media formulated with various commercial sources of nutrients (glucose, yeast extract, soy-protein acid hydrolysate, casein hydrolysates, corn steep liquor and various inorganic chemicals) showed that the proposed wheat-based biorefinery strategy enhanced PHB production.

1. Introduction

Polyhydroxybutyrate (PHB) belongs to the general group of polyhydroxyalkanoates (PHAs) and has numerous potential uses, such as a biodegradable substitute for petroleum-derived plastics, biocomposite production, speciality biopolymer for medical applications and a source of the platform molecule 3-hydroxybutyric acid [1–4]. However, industrial PHB production is hindered by unfavourable PHB production costs [5,6]. Low cost and environmentally friendly PHB production is dependent on the restructuring of traditional fermentation and recovery technologies [7,8].

Reduction of microbial PHB production costs could be achieved by utilising renewable raw materials, such as potato processing waste, pulp fiber sludge, whey, green grass, silage, and cereals [9–13]. Solaiman et al. [14] presented a review on microbial PHA production from agricultural feedstocks and co-products, such as intact triacylglycerols (vegetable oils and animal fats), dairy whey, molasses, and meat-and-bone meal. Targeting the substitution of purified carbon sources (i.e. glucose, sucrose), commercial pure starch is one of the carbon sources used in many publications for microbial PHB production [15–19]. Yu et al. [16] showed that PHB fermentations on starch hydrolysates using a recombinant *Escherichia coli* strain resulted in higher PHB concentration and PHB content as compared to fermentations carried out on glucose. A PHB concentration of 167.6 g/l at a productivity of 3.05 g/l h has been reported by Yu et al. [16] when a semi-defined medium with starch hydrolysate as carbon source was used. Quillaguaman et al. [17] reported the utilisation of starch hydrolysates as carbon source in fermentations carried out with *Halomonas boliviensis* to produce a PHB content of 35%. Huang et al. [18] used extruded rice bran as carbon source in a fed-batch fermentation carried out by *Haloferax mediterranei* to produce a total dry weight of 140 g/l with a PHA content of 55.6%. Commercial protein hydrolysates (i.e. yeast extract, casein hydrolysates) and by-products of agro-industrial origin (i.e. corn steep liquor) have been employed as sources of nitrogen. Several studies have stressed the potential of amino acids and peptides to enhance microbial PHB production [20–25]. Bormann et al. [23] reported the production of 65 g/l total dry weight with a PHB content of 60–80% when 20–30 g/1 casein peptone or casamino acids were used as sole sources of nitrogen. In the majority of these studies, mineral supplementation is achieved by the addition of inorganic chemicals.

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The establishment of bioprocesses as a viable alternative to (petro)chemical routes for chemical production is dependent on the development of integrated biomass-based biorefineries. A wheat-based biorefining strategy has been proposed for the production of a spectrum of chemicals with significant economic improvements [13,26–32]. Koutinas et al. [33] presented various alternative schemes of the generic biorefining concept, depending on the targeted product (i.e. bioethanol, PHB, succinic acid). Fig. 1 presents the proposed process in the case of microbial PHB production. On-site fungal bioconversions by *Aspergillus awamori* is used for the production of wheat hydrolysates by exploiting the enzymatic consortia (rich mainly in amylolytic enzymes) produced by the fungus to hydrolyse mainly the starch content in wheat (gluten and phytic acid are also partly hydrolysed) [34]. Fungal autolysis is used for the production of nutrient supplements [35] that can be mixed with wheat hydrolysates to formulate nutrient-complete media for a range of microbial bioconversions. The optimised combination of gluten hydrolysis and fungal autolysis would generate mixtures of amino acids and peptides that would not only provide a nitrogen source but also an additional carbon source. In conventional fermentation practices, the protein fraction in cereal grains is rarely hydrolysed to enrich fermentation media in amino acids and peptides, which could improve microbial fermentations significantly. Fouache et al. [36] demonstrated that the use of gluten hydrolysates as nutrient supplements for lactic acid bioconversions resulted in higher productivities when compared against yeast extracts and corn steep liquor. Wheat hydrolysates and fungal autolysates would also contribute vitamins, minerals and trace elements.

The scheme presented in Fig. 1 offers many advantages as compared to conventional fermentation and recovery strategies for PHB production. Wheat components (starch, gluten, bran) are used for the production of at least three end-products (i.e. PHB, recombinant proteins from surplus gluten and various value-added products from bran-rich pearlings). Wheat fractionation steps generate streams with optimum quantities of starch, gluten and bran. A stream containing bran-rich pearlings could be used for the production of various products such as ferulic acid or arabinoxylans. Starch- and gluten-rich streams are used for the production of hydrolysates that are subsequently used for the production of PHB and recombinant proteins. Shake flask cultures of recombinant *E. coli* carried out in both enriched LB and gluten hydrolysates containing 2 g/l of glucose resulted in the production of similar intracellular SUMO-EAK$_{16}$ fusion protein (30–35% of the total bacterial protein) [37]. Future research should focus on the identification and optimisation of recombinant protein production from gluten hydrolysates. The combined production of commodity (i.e. biodegradable plastics) and speciality (i.e. pharmaceuticals) products in the same biorefinery would improve process economics and add value to the initial raw material.

Fungal fermentations by *Aspergillus oryzae* provide crude enzyme-containing solutions (rich mainly in proteolytic enzymes) that could enhance gluten hydrolysis and *W. eutropha* cell disruption to facilitate PHB purification. It should be stressed that *A. oryzae* is more efficient in protein hydrolysis, whereas *A. awamori* in starch hydrolysis. In addition, preliminary results (not published yet) showed that crude enzyme-containing solutions from *A. oryzae* solid state fermentations led to the disruption of 70% (w/w) of residual cell weight (non-PHB cell mass) increasing the purity of PHB to 96% (w/w). These steps facilitate the creation of an integrated biorefining concept that leads to the production of many added-value products, targets diversified market outlets, reduces waste production through recycling of nutrients, and exploits all the components of a renewable resource.

Koutinas et al. [13] showed in shake flask fermentations that wheat could be used as the sole source of nutrients for microbial PHB production. The main aim of this study was to demonstrate that efficient PHB production could be achieved in a bioreactor by using the wheat-based biorefinery presented in Fig. 1. The nutrient composition (glucose, free amino nitrogen, total nitrogen, phosphorus) in the wheat-derived media varied by mixing different amounts of wheat hydrolysates and fungal autolysates. The superiority of the wheat-derived feedstock against conventional

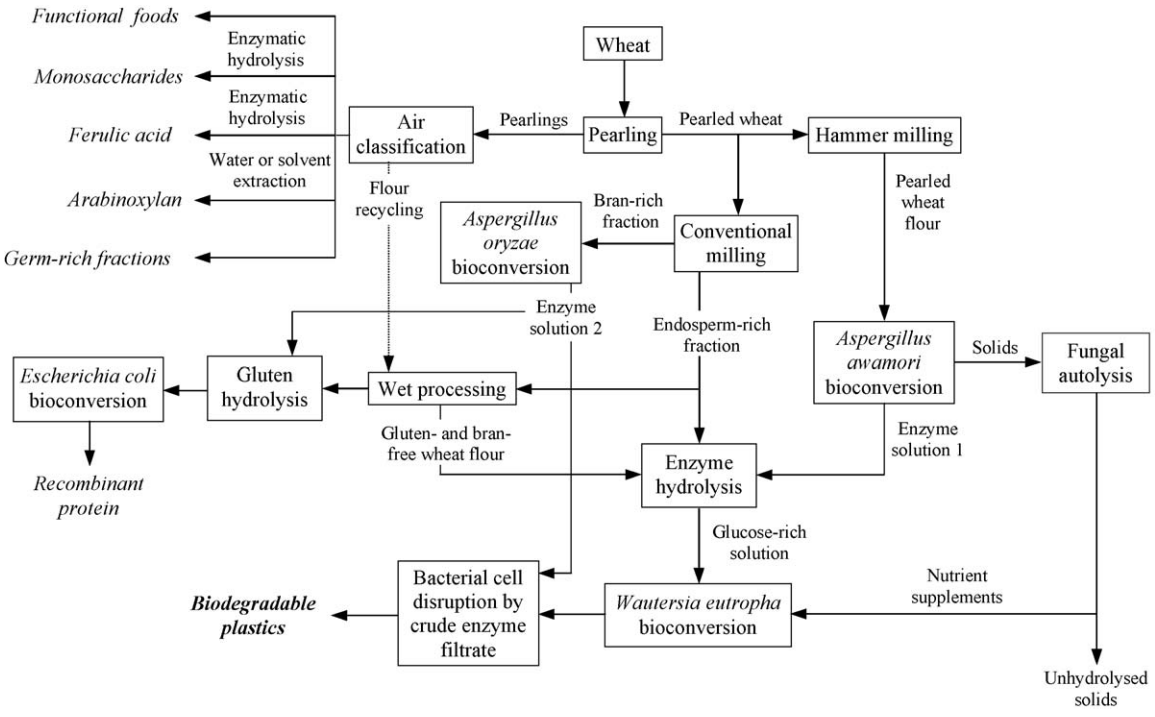

Fig. 1. Proposed wheat-based biorefinery for PHB production.