Enhancement of starch hydrolysis bioprocesses via immobilization of *Aspergillus niger* and *Aspergillus awamori* on carbonaceous materials

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1. Introduction

Potato (*Solanum tuberosum*) consists the third largest food crop worldwide following cereal crops (rice, wheat), while its production accounts for 360 million t per year (FAO, 2022). The low requirements for its cultivation and the high starch content entailed renders potato a promising alternative raw material for saccharification and fermentation for the production of biofuels (Lareo and Ferrari, 2019). Saccharification of starchy substrates commonly requires application of acid and/or enzyme hydrolysis, where the enzymatic process can be performed under mild conditions, as compared to acid hydrolysis, resulting in high rate of biomass conversion to glucose (Bansal *et al* 2021).

Enzyme hydrolysis of starchy materials is performed in the presence of amylolytic enzymes, such as α-amylases and glucoamylases, which comprise the main highly efficient starch-degrading enzymes (Hua and Yang, 2016). Over the past decades, various researchers have assessed fungi such as *Aspergillus niger*, *Aspergillus awamori* or *Aspergillus oryzae* towards *in situ* production of amylolytic enzymes (Wang *et al* 2008, Haque *et al* 2016). Furthermore, *Aspergilli* hold the capacity to produce proteases enabling simultaneous hydrolysis of proteins, which constitutes an activity needed in a range of biotechnological applications (Silva-Lopez *et al* 2022).

Whole cell immobilization has attracted increasing interest for application in industrial biotechnology. Different carriers such as magnetic (Hermida and Agustian, 2022) and carbonaceous materials (Kyriakou *et al* 2019) have been assessed as cell immobilization matrices to improve biological processes including enzyme hydrolysis and ethanol fermentations. Specifically, carbonaceous materials (e.g. activated carbon, biochar) hold the capacity to assist interspecies electron transfer, buffering capacity and nutrient adsorption into their surface, improving cell activity and growth (Kyriakou *et al* 2019).

Herein, different types of carbonaceous materials were assessed as immobilization carriers to enhance starch hydrolysis. Specifically, *Aspergillus niger* and *Aspergillus awamori* were applied for amylolytic enzyme production, which was subsequently immobilized on the carriers selected to enhance the saccharification yield of the process.

2. Materials and Methods

Potato (Spunta) was applied as starchy substrate, which was obtained from a local potato producer (Nicosia, Cyprus) and directly used in experiments upon collection. Characterization of potato was performed employing standard analytical methods including Kjeldahl nitrogen analysis, as well as ash, starch and fibre (cellulose, hemicellulose) content.

Aspergillus niger MUCL 28817 and *Aspergillus awamori* MUCL 28815 were employed in the enzyme hydrolysis process, while carbonaceous materials, char and biochar obtained via pyrolysis of recycled car tyres and pistachio shells respectively, were used as immobilization carriers.

Potato starch saccharification was determined during enzyme hydrolysis via analysis of reducing sugars' concentration through DNS method.

3. Results and Discussion

Potato was initially characterized constituting 16.55% dry matter, 0.82% total nitrogen, 5.10% protein, 7.56% ash, 12.57% starch, 12.92% hemicellulose and 1.30% cellulose. The varying composition reported for potato (current study, Kita 2002, Sato *et al* 2017) could be attributed to a wide range of parameters associated to crop cultivation.

Potato infusion was used for *Aspergillus niger* and *Aspergillus awamori* cultivation, while 20% of potato solids was employed for saccharification. A preliminary study was conducted to determine the most efficient conditions for cultivation and enzyme production using each microorganism. Specifically, freely suspended and immobilized cells as well as free and immobilized enzymes were assessed for their capacity to hydrolyze potato starch using char as immobilization material. The release of reducing sugars in each trial indicated that immobilized *Aspergillus niger* cells and enzymes performed elevated saccharification rates as compared to the use of freely suspended cells and enzymes. Lower increase in reducing sugar yields were also observed in trials performed by immobilized *Aspergillus awamori* cells and enzymes as compared to the use of freely suspended cells and enzymes. Moreover, the two fungi strains were immobilized using different contents of char (1.6%, 3.2%, 6.4%, 12.8% and 25.6%) demonstrating that 3.2% of the material enhanced the release of glucose by both microorganisms applied. Char and biochar were additionally employed in different particle sizes as immobilization carriers for potato starch hydrolysis. Results demonstrated that the particles incorporating diameter between 0.3-0.5 cm enabled higher production of reducing sugars (20-25 g L^{-1} final concentration) as compared to the use of smaller particles (15 g L^{-1}) using char as material for immobilization of both fungi. Moreover, the co-cultures of the two strains assessed exhibited elevated glucose yields. The study will additionally include determination of enzyme activity during the process.

4. Conclusions

The current work constitutes a preliminary study demonstrating the potential for substantial enhancement of *Aspergillus niger* and *Aspergillus awamori* amylolytic activity using carbonaceous materials as immobilization carriers.

5. References

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