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

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## Introduction

Saccharification of starchy substrates such as potato 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).
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).

## Aim and Objectives

The aim of current study is the development of a starch hydrolysis bioprocess for the enhancement of saccharification yield through immobilization technology. The main objectives of the present work comprise:

- ❑ Characterization of starchy substrate (potato).
- ❑ Examination of *Aspergillus niger* and *Aspergillus awamori* for amylolytic enzyme production.
- ❑ Evaluation of different types of carbonaceous materials as immobilization carriers to enhance starch hydrolysis.

## 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 and HPLC analysis.

## Results and Discussion

### Characterization of raw material

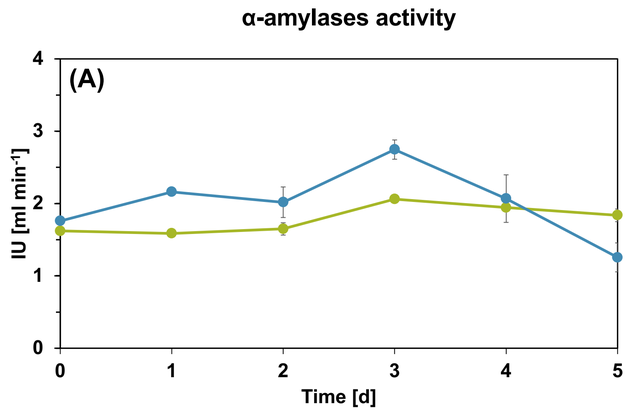
Potato composition was characterized as given in Table 1.

**Table 1**: Characterization of raw material (potato)

| | |
|---|---|
| Dry matter | 16.55 (±0.08) |
| Total nitrogen | 0.82 (±0.04) |
| Protein nitrogen | 5.10 |
| Ash | 7.56 (±0.09) |
| Starch | 12.57 (±0.08) |
| Hemicellulose | 12.92 (±0.03) |
| Cellulose | 1.30 (±0.01) |
| Lignin | 0 |

### Amylolytic enzyme production

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 sugars 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.
Enzyme production was further assessed demonstrating that α-amylases and glucoamylases performed maximum activity at 3 and 1 d for both fungi respectively (Fig.1).



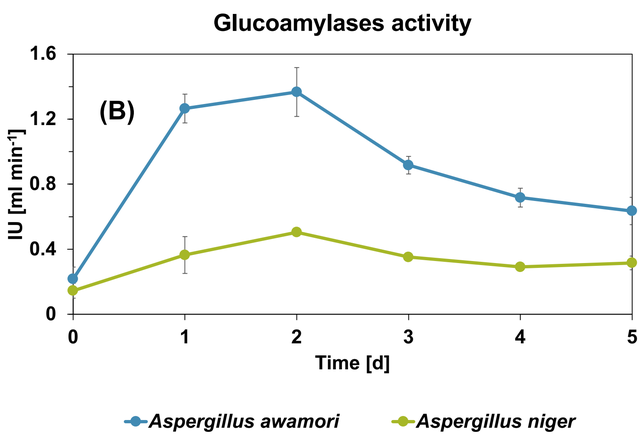




**Fig.1** Production of **(A)** α-amylase and **(B)** glucoamylase for *Aspergillus niger* and *Aspergillus awamori*

### Enhancement of starch hydrolysis

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 at 24 h (Fig. 2).

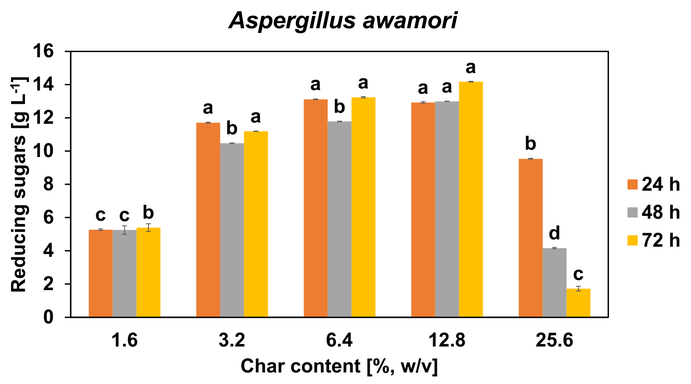



**Fig. 2** Production of reducing sugars from immobilized *Aspergillus awamori*

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 (Fig. 3).

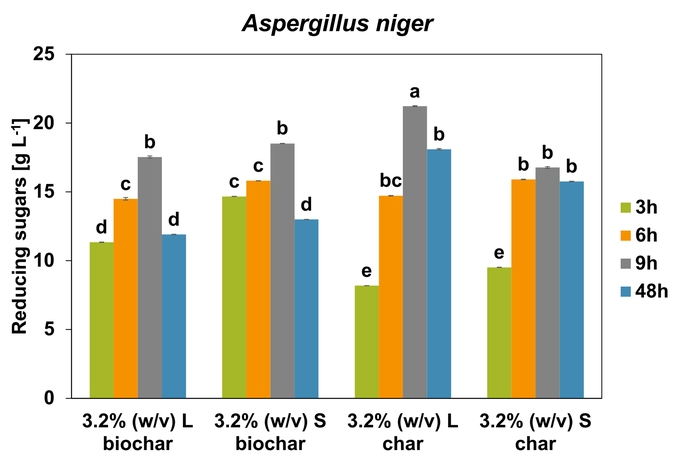



**Fig. 3** Production of reducing sugars from immobilized *Aspergillus niger* on char and biochar in different particle sizes (L biochar: particle size of biochar higher than 0.3 cm; S biochar: particle size of biochar lower than 0.3 cm; L char: particle size of char higher than 0.3 cm; S char: particle size of char lower than 0.3 cm)

Moreover, the co-culture of the two strains assessed exhibited elevated glucose yields. Therefore, 50% and 100% of potato solids were employed in saccharification. The maximum glucose yield was achieved for both microorganisms as well as the co-culture following 9 and 72 h of hydrolysis upon use of 50% and 100% of potato solid content respectively. However, higher production of glucose was observed at 72 h using 100% of potato solids. Specifically, glucose concentration reached 63 g $L^{-1}$, while the product yield exhibited 0.51 g $g_{starch}^{-1}$ (Fig. 4).

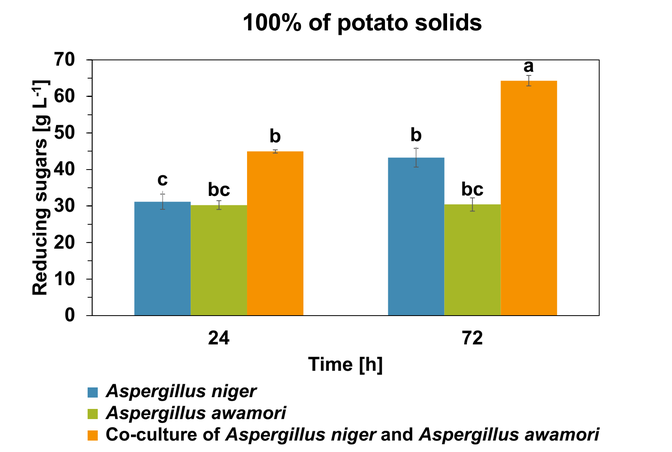



**Fig. 4** Production of glucose using 100% of potato solids in immobilized *Aspergillus niger, Aspergillus awamori* and co-cultivation of both fungi

## CONCLUDING REMARKS

- ➢ α-amylases and glucosidases performed maximum activity at 3 and 1 d for both *Aspergillus niger* and *Aspergillus awamori* cultures.
- ➢ Application of 3.2% (w/v) char was demonstrated as the most efficient content for fungi and enzymes immobilization.
- ➢ Char particles incorporating diameter between 0.3-0.5 cm enabled higher production of reducing sugars.
- ➢ Co-cultivation of fungi increased the glucose yield at 0.51 g $g^{-1}$ in hydrolysis of 100% potato solids.

## FUTURE WORK

- ➢ Future work involves optimizing the conditions of starch hydrolysis to enhance glucose yield.
- ➢ Development of a consolidated ethanol bioprocess performing starch hydrolysis and fermentation of the hydrolyzate formed in a single step aiming to enhance biofuel production.

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