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High cell density cultivation of six fungal strains efficient in azo dye bioremediation

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ABSTRACT

This work aims at optimizing the high cell density fungal cultivation for producing large quantities of fungal biomass to be used in azo dye residues bioremediation. In our previous studies the efficacy of using certain fungal strains to decolorize a range of commercial textile dyes of different structures (azo, disazo) were investigated. Several promising fungal strains belonging to *Aspergillus tubigenesis*,*Aspergillus niger*, *Aspergillus terreus*, and *Aspergillus fumigates* demonstrated high capacity in decolorizing various azo dyes. This study focuses on the high cell density cultivation of the fungal strains identified as potential bioremediation agents. The study includes the optimization of all parameters involved in bioprocess development for high cell density cultivation of six promising fungal strains. The growth of the fungal strains was tested on the sucrose medium in 7 l-fermenter. The growth of these fungal strains having the capacity to accumulate large quantities of biomass was also tested in medium containing molasses as a cheap substrate. The residual molasses, biomass dry weight and protein content of the six fungal strains showed that the strains 20 and 2 were marked by the highest protein content. In this study a comparative analysis between the results of dry weight, residual molasses and protein content of geowth of the strains 20, 5 and 2 under uncontrolled and controlled pH of media in batch fermentation was studied to follow the accumulation of biomass and protein production in the growth media. The results indicate that the dry weight accumulated by strains No. 20, 5 and 2 grown on molasses was better than those of strains grown on sucrose. Fungal strain No. 5 had the highest biomass dry weight accumulation. The study shows that the molasses as cheaper sugar sources were better than sucrose for growing fungal biomass.

1. Introduction

The filamentous fungi are naturally excellent protein secretors and can produce industrial enzymes in sufficient amounts [14]. Abd-El Rahim [20] assessed the textile dyes bioremediation using different fungal biomass as compared with some abiotic agents. Abd-El Rahim et al. [19] studied enhancing the bioremoval of textile dyes by biomass of fungal strains from media supplemented with gelatine wastes and sucrose. In another work Abd-El Rahim [19] studied the enhancment of fungal growth and biomass accumulation by several promising strains. The biomass was tested for rapid dye removal. Filamentous fungi represent attractive bioflocculating agents because of their self-pelletization capacities. Fungal self-pelletization has been observed for numerous filamentous strains and can be explained by coagulative and non-coagulative mechanisms [7,24,10,23]. The coagulative mechanism observed in representatives of *Aspergillus* spp., *Basidiomycete* spp., *Phanerochaete* spp. that involves spore coagulation leading to developments of aggregates/pellets. As a result fungi produce dense spherical aggregates [24,7]. The non-coagulative mechanism involves spores germinating into hyphae, which then intertwine into pellets. Representatives of several fungal species: *Rhizopus* spp., *Mucor* spp. and *Penicillium* spp. display the non-coagulative mechanism [24,7] which play role in reducing the cost of biomass harvesting that requires additional energy input. Such property can play significant role in high cell density cultivation of fungi particularly for easy harvesting of the fungal biomass. The aim of this study is to grow several selected fungal strains having the potential of high cell density accumulation to accumulate large biomass to be used for textile azo dyes bioremediation.

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2. Material and methods

2.1. Fungal strain and inocula preparation

The six highly efficient fungal strains identified in our previous laboratory testing as promising bioremediation agents were included in this study for further high biomass accumulation in the growth media. The six fungal strains previously isolated from an industry effluent damping site and tested for azo dyes bioremediation were maintained in potatoes agar slants medium and kept in the refrigerator. The strains were sub-cultured every 4 months. The composition of the culture medium used for inoculum preparation and biomass cultivation was: 10 g/l sucrose, 0.5 g/l H_2PO_4, 0.2 g/l $MgSO_4$ $7H_2O$, 0.1 g/l NaCl [19].

A spore suspension was used for inoculation of the flask cultures. Agar plates with the sporulation medium (24 g L^{-1} potato dextrose broth with 20 g L^{-1} agar) were plated out with spores from a refrigerated stock and incubated for 6 days at 28 °C. Spore suspensions were harvested by adding 10 ml of sterilized water onto the surface of agar plates to release spore from the aerial mycelium.

2.2. Batch fermentation

Biomass production experiments were performed in a 7.5L BioFlo 310 bench top Fermenter/Bioreactor (New Brunswick scientific Co., Inc.) with a working volume of 5L. The fermenter and media were sterilized by autoclaving at 121 °C for 20 min. The spore suspensions were used to inoculate the fermenter.

Samples were regularly withdrawn from the fermenter every 24 h. and analyzed for residual sugar (using hand Refractometer), accumulated biomass and total protein. The pH was recorded throughout the course of batch process. Foam was controlled by adding silicon oil antifoam (Fluka). The fermenter was aerated at an air flow rate of 2.5 l/min, corresponding to 0.5 v/v/min. The temperature was controlled at 28 °C. The process continued for 5 days (120 h).

2.3. Evaluation of biomass accumulation

Samples were collected from fermenter at certain intervals of incubation (24, 48, 72, 96 and 120 h, either was mentioned). The samples were passed through filter Watman paper No. 1. and the biomass was measured by drying of the biomass collected from the volume taken to constant weight at 65 °C.

2.4. Residual sugar readings

Sucrose consumption was monitored as the intake percentage of sucrose were quantified by Atago hand Refractometer.

2.5. Growth of fungal strains for biomass accumulation on sucrose and molasses

The fungal biomass accumulation was evaluated at intervals of incubation. Accumulation of biomass by six fungal strains were assessed in sucrose and molasses media. The changes in pH, biomass dry weight, residual sugars and protein content in the culture media were determined at all sampling intervals. Then specific growth, specific substrate consumption and specific protein production rates were calculated.

3. Results

3.1. Performance of the potential fungal strains in relation to sucrose consumption, biomass accumulation, and protein production

Change in sucrose containing media inoculated with the 6 fungal strains were tracked in the culture media throughout the experimental time. The selection of the strains no. 2, 5, 6, 11, 20, and 21 was based on the efficiency of such strains in bioremediation of textile dye residues either by bio-removal and/or biodegradation.

In the medium containing 1% sucrose, strain 2 gradually consumed sucrose up to 50% after 72 h of incubation. Sampling after 96 h showed that, 80% of sucrose was used from the medium. The pH decreased from 3.5 to 2.2 at the end of the incubation. The biomass accumulation increased as the time of incubation increased and reached 8.05 g/l after 96 h. The protein content in the growth medium reached the maximum after 96 h of incubation being 13.01 μg/ml (Fig. 1).

In Fig. 2, growth of fungal strain No. 5 in the sucrose medium was increased as the time of incubation increased. At 48 h of incubation the biomass accumulation by strain 5 was less than that of strain 2 being 5.55 g/l compared with 7.25 g/l in case of strain 2. At the end of incubation period strain 5 had more biomass accumulation (8.55 g/l) than strain 2 (8.05 g/l). The pH of the growth media decreased at the end of incubation period. Increase in protein production in the growth media was noted with strain 5 being the highest producer at 72 h and amounted 13.86 μg/ml (Fig. 1).

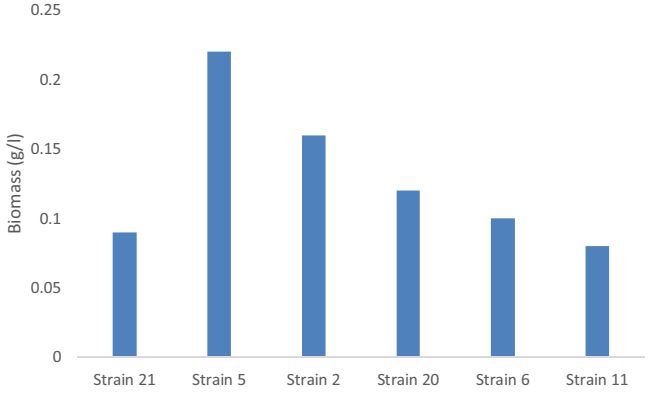

Fig. 1. The cell mass produced by the six fungal strains grown on sucrose after 96 h incubation period.

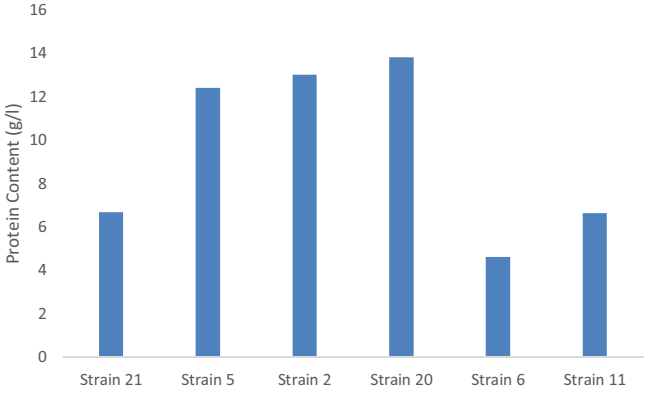

Fig. 2. The protein content produced by the six fungal strains grown on sucrose after 96 h incubation period.