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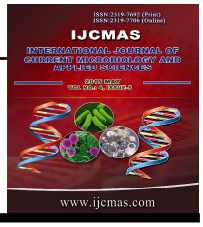

Original Research Article

Production of Carboxy Methyl Cellulase by *Aspergillus niger* using plant wastes as substrate

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A B S T R A C T

Keywords

CMCases, wheat bran, *Aspergillus niger*

CMCase production by immobilized *Aspergillus niger* in submerged fermentation was studied. Studies were carried out on different parameters like different substrates, substrates concentrations and comparison with standard CMC. The maximum CMCase activity of 0.499 U/ml was achieved with 4% wheat bran concentration. Immobilized cells gave almost same result with wheat bran as compared with standard CMC with a slight decrease in activities.

Introduction

Agriculture being the main occupation in India, leads to production of large amount of agro-wastes like wheat bran, sugarcane baggase, groundnut shell, sawdust etc. which may be generated from either forest, agricultural practices or from agro based industries. Because of the use of machinery in agriculture ,the livestock population with farmer decreases and resulted in the accumulation of these agro-wastes in the biosphere leading to environmental pollution in the form of carbon dioxide, methane etc. The major part of agro waste comprises of cellulose and hemicellulose, as they are the main component of plant cell wall.(4) Cellulose most abundant polymer in the biosphere and is considered to be an almost inexhaustible source of raw material for different products (12). Production of enzymes seems to be a good strategy for the efficient utilization of lignocellulosic biomass, as it is renewable, easily available and is non-competitive with food crops.(5) Degradation of cellulose is very important in several agricultural and waste treatment processes. Cellulases are gaining more interest due its wide applications in food and feed ,brewery, textile, juice extraction etc.(7,14).The utilization of cheaper and indigenous substrates for cellulase production has contributed somewhat to the economic recovery(2,15).

Material and Methods

Experimental Micro-organism

A mold strain A*spergillus niger*, isolated and identified. The fungal culture was raised

on potato starch-dextrose-agar (PDA) slants. The medium was prepared and pH 6 was adjusted with 1M HCl/1M NaOH. It was autoclaved for 15 minutes at 121°C (1.1 kg/cm pressure) and then incubated aerobically at 37°C for 5 days. (3). It was maintained on potato dextrose agar slant at 4^{o}C with a periodic regeneration.

Inoculum preparation

The inoculum medium was also prepared, adjusted to pH 5.5 and autoclaved for 15 minutes at 121°C (1.1 kg/cm pressure) and cooled. Fungal inoculum was prepared by scrapping 5 days old slant with an inoculation loop into 5ml sterile distilled water. The suspension was filtered through sterile glass wool to form uniform suspension. The number of spores was counted in the medium with the help of haemocytometer. The spore concentration was adjusted at$1*10^{6}$ spores/ml in the homogenous spore suspension. To each fermentation flask this inoculum was added to optimize different fermentation parameters (pH, temperature) for carboxymethyl cellulase production (10).

Immobilization of cells

20ml of 4%sodium alginate and 5ml spore suspension were mixed and added to 0.2M $CaCl_2.H_2O$ solution. The beads formed were kept at 4^{0}C for 1hr and then washed with sterile distilled water.

Fermentation conditions and Enzyme assay

Beads prepared by above methods was introduced in standardized media, incubating at 37°C for production of CMCase and then enzyme activity of each batch was determined spectrophotometrically at 540nm periodically in every 24hrs upto 120 hrs.

One unit of enzyme activity is defined as the amount of glucose (µMol) released per mL enzyme solution**.**

Optimization

Effect of different substrates on enzyme production

Four different substrates i.e..wheatbran, sugarcane baggase, groundnut shell and sawdust were compared for the production of CMCase.

Effect of various concentrations of substrates on the production of CMCase

Different concentrations of substrates (3% , 4%,5% and 6%) were used and CMCase activities were estimated.

Effect of various concentrations of wheat bran on the production of CMCase.

Different wheatbran concentrations (1-6%) were studied.

Comparison of wheat bran and standard CMC

Substrate wheat bran is further compared with standard CMC for the production of CMCase.

Results and Discussion

Effect of various substrates on the production of CMCase

Different agricultural byproducts such as wheat bran, sugarcane baggase, groundnut shell and sawdust were tested for the production of enzyme . Of all the substrates tested, wheat bran was found to be the best substrates for the production of CMCases. The other substrates gave comparatively less production of CMCase.

Effect of various concentrations of substrates on the production of CMCase

Substrate concentrations of 3-6% were considered. Enzyme activity increases gradually for all substrate concentration, in which 4%concentration of wheatbran show maximum activity as compared to sugarcane baggase, groundnut shell and sawdust. A decrease in enzyme activity beyond maximum 4 % substrate concentration may be due to inhibitors. The decrease may also be due to depletion of other nutrients or due to specific binding of the enzyme with the substrate. This is supported by the findings of Gbekeloluwa and Moo-young (6) who reported the inhibitory effect of accumulated cellobiose and cellodextrin of low degree of polymerization. The decrease may also be due to depletion of the other nutrients (mineral-salt) other than the energy source or due to the specific binding of the enzymes with the substrate (8, 11,13).

Effect of various concentrations of wheat bran on the production of CMCase

Out of various concentrations 1-6% of wheat bran, CMCases production in fermentation medium was found to be maximal when 4.0% of wheat bran was used.Further increase in amount of wheat bran resulted decrease in the production of enzyme.

Fig.1 Effect of various substrates on the production of CMCase by immobilized *A.niger* cells in IU/ml

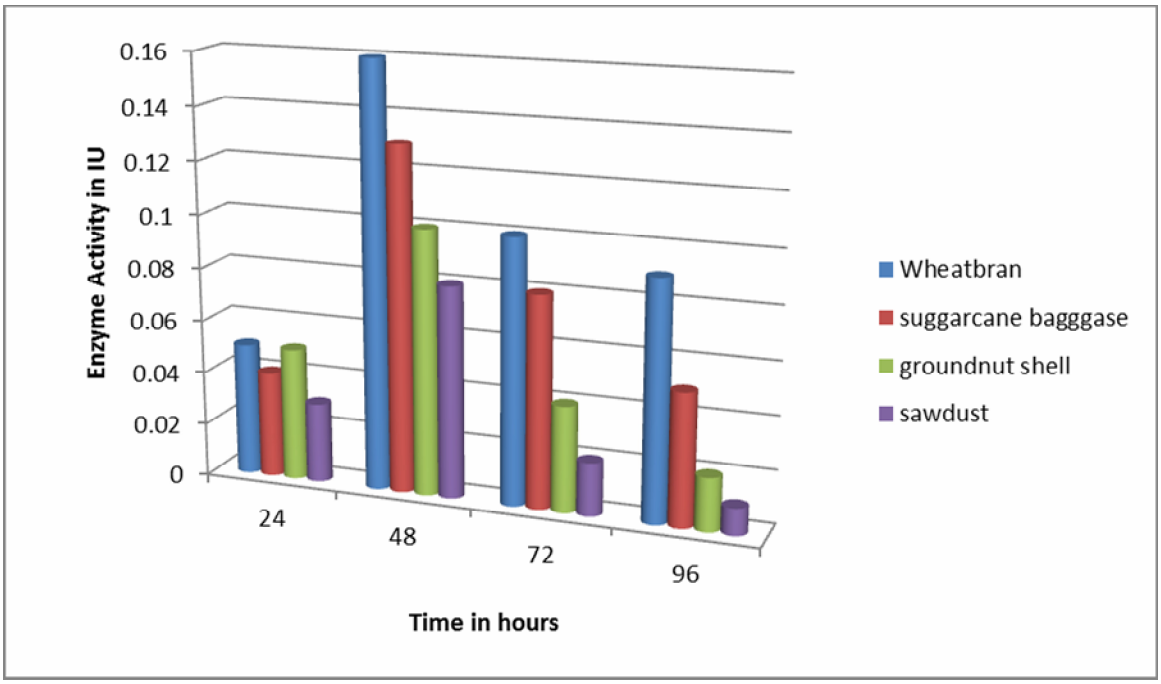
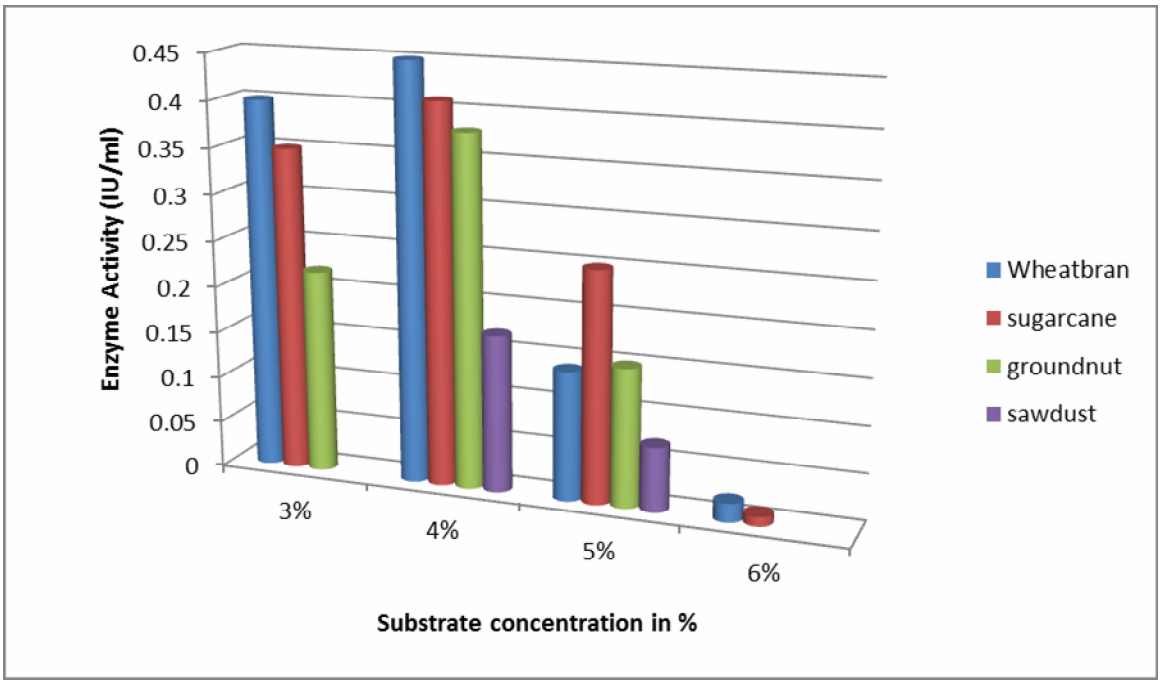


Fig.2 Effect of substrate concentration on CMCase production



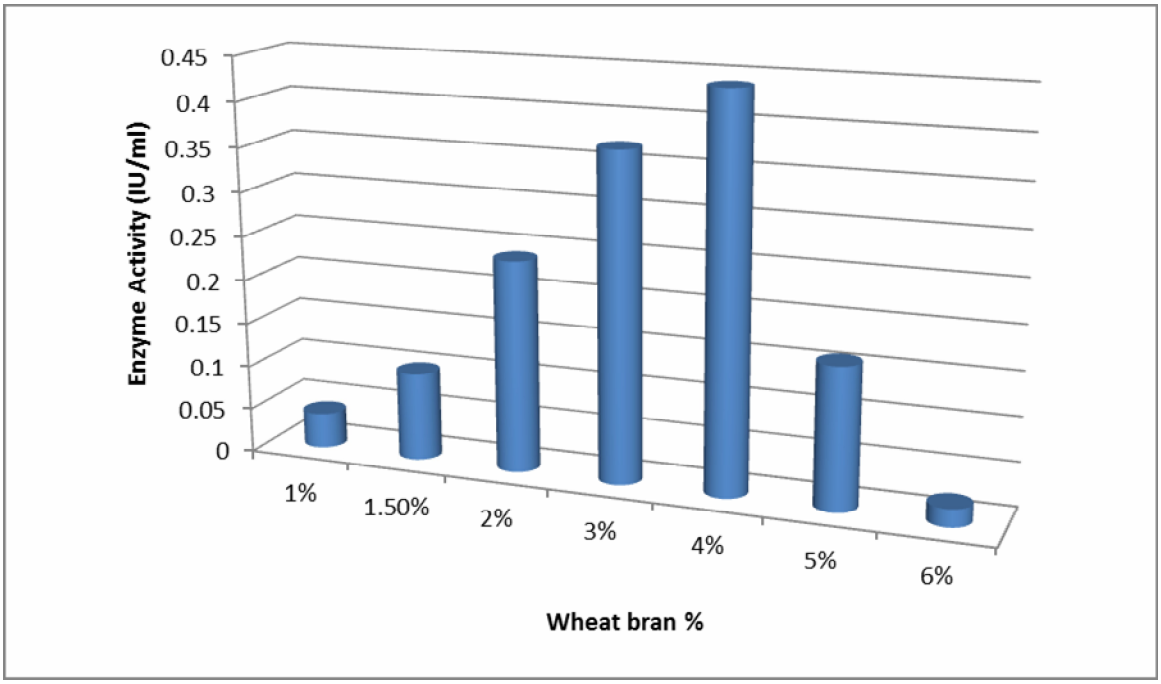
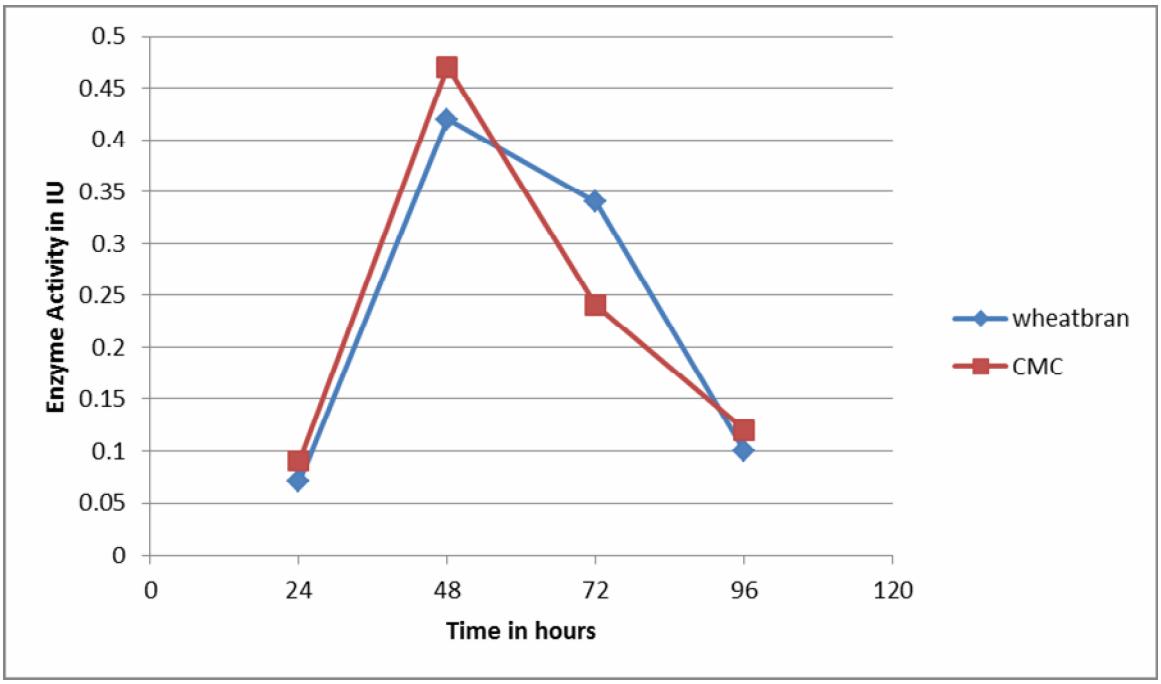


Fig.4 Comparison of wheatbran and standard CMC for the production of CMCase



Comparison of wheatbran and standard CMC

On considering different substrates ie. Wheat bran, Sugarcane baggase, Groundnut shell and Sawdust, WB gave maximum production of cellulase enzyme from *Aspergillus niger*. This substrate wheat bran is further compared with standard CMC .On comparison it was revealed that wheat bran gave nearly same enzyme activity as standard CMC but due to high cost and low availability of standard CMC ,use of wheat bran as substrate is more feasible ,easily, economical and also environmental friendly.

Wheat bran shows the highest cellulase activity as compared to sugarcane baggase, groundnut shell and sawdust at 48 hrs. The decrease in activity after a fermentation period of highest activity may be attributed to cumulative effect of cellobiose (14)which is a dimer of glucose and is known to inhibit endoglucanase. It may also suggest that delignification produces aromatic water soluble proteins that repress the cellulolytic action of enzyme (2).

Substrate concentrations of 3%-6% were considered. Enzyme activity increases for all substrate concentration, in which 4%concentration of wheat bran show maximum activity as compared to sugarcane bagasse, groundnut shell and sawdust. A decrease in enzyme activity beyond maximum 4 % substrate concentration may be due to inhibitors. The decrease may also be due to depletion of other nutrients or due to specific binding of the enzyme with the substrate.

The result highlights that wheat bran has potential of to be an indigenous source of cellulase production and also the industrial potential of these substrates as possible raw materials for cellulase production. Haq et al.(9) and Abo-State et al.(1) also reported the wheat bran as best source of carbon and nitrogen for cellulase production.

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