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Full Length Research Paper

Production cellulase by different co-culture of *Aspergillus niger* and *Tricoderma viride* from waste paper

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In Iraq, there is attempts to transfer the various industrial carbon waste to veterinary proteins depend on microorganisms by using of chemical process. Five different co-culture combinations (1:1 ratio, 1 × 10^6 conidia)of *Aspergillus niger* and *Trichoderma viride*, mixing of *A. niger* and *T. viride*, in 24 and 48 h old monocultures of *Aspergillus* similar mixing of A. in 24 and 48 h old monoculture of *Trichoderma* and the monocultures of both were evaluated for their potential performance of cellulases production. The study indicates that the cellulases obtained from compatible mixed cultures simultaneous mixing of both fungi have more enzyme activity as compared to their pure cultures and other combinations. The fermentation experiments were performed in solid stat fermentation (SSF). Incubation time, carbon sources and initial pH of fermentation medium was optimized with simultaneous mixed culture. It was revealed that the newspaper at pH = 5 and 40°C was the best source of carbon for the enhanced production of cellulase in the compatible mixed culture experiments after 8 days of incubation with 5.70 U/ml. Based on the reported results, it may be concluded that industrial carbon waste can be a potential substrate for production of cellulase, incorporation of co-culturing *A. niger* and *T. viride.* The aim of this work is to produce of Cellulase from waste paper and reduce the pollution.

Key words: *Aspergillus niger*, *Tricoderma viride*, cellulase, culture.

INTRODUCTION

Plant cell walls are composed of various polysaccharides and lignin, forming a rigid and complex matrix recalcitrant to microbial degradation. This structure is strengthened by crosslinkages such as diferulic acid bridges between adjacent hemicelluloses chains (Oosterveld et al., 1997) or between lignin and hemicellulose (Lam et al., 1992), increasing its resistance to microbial invasion. The products of degradation constitute both nutrients for growth and regulators of the production of lignocellulolytic enzymes (de Vries et al., 1999; Peij et al., 1998). Among these microorganisms, filamentous fungi such as *Trichoderma* spp. and *Aspergillus* spp. are especially good secretors of lignocellulolytic enzymes (Archer and Peberdy, 1997). Grasses and cereals contain substantial amounts of cell wall-bound hydroxycinnamate esters linked to polysaccharides (Ishii, 1997). Particularly, ferulates play an important role in cross-linking cell wall polysaccharides (Ralph et al., 2004). The cotton strip assay (Latter and Howson, 1977; Howard and Howard, 1985) was put into practical use before it was properly evaluated and before the relationships, if any, between tensile strength change of cotton cloth and soil processes relevant to soil research programmes were examined. Perhaps the clearest expression of the reasons for using the assay is given by (Walton and Allsopp, 1977): (i) Cellulose is a major constituent of plant remains; (ii) the decomposition of dead plant remains is a major biological process, of great interest to many scientists studying soil processes; (iii) cellulose provides an important food source

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for a wide variety of organisms and (iv) a method is needed to compare rates of breakdown of cellulose in different soils. Cotton is a natural substrate and degradation of any material must begin with bond breaking, which leads to changes in tensile strength. Walton and Allsopp considered that, as long as this technique is used for comparative assessments of biological activity in different soils, it will remain a powerful tool for field research. Cellulose is a homopolymer, consisting of glucose moieties joined in 0 - 1, 4 linkages, the number of glucose moieties in a molecule being the degree of polymerization (DP). Native cellulose from higher plants has a DP of about 14000 and this value appears to be remarkably constant. X-ray and infra-red data suggest that the basic structure of cellulose involves the anhydrocellobiose unit ($C_{12}H_{20}O_{10}$) rather than the anhydroglucose unit (C61-11005), so that the shortest cellulose-like molecule would be cellotetrose (DP = 4):

$(C_6H_{11}O_5)$, $(C_{12}H_{20}O_{10})$, $(C_6H_{11}O_5)$

Here, (C61-11105) represents a glucose moiety with a free secondary hydroxyl group on C-4 and (C61-11106) is a glucose molecule with a reducing group. Cellobiose, cellotriose, and cellotetrose are water-soluble, cellopentose is very sparingly soluble and cellulose molecules with DP greater than 6 are insoluble (Ljungdahl and Eriksson, 1985). Because cellulose is a polymer, we have to think rather carefully about how we define cellulose decomposition. In nature, cellulose is degraded by a range of aerobic and anaerobic fungi and bacteria, many of which occur in extreme conditions of temperature and pH. A list of fungi which have been examined in cellulose decomposition studies is given in (Ljungdahl and Eriksson, 1985). Many fungi can utilize oligosaccharides and polysaccharides as carbon sources, but, in general, these molecules are too large to be transported directly into the cell and must first be hydrolyzed to their subunits. Cellulose represents an important potential carbon and energy source for fungi, many of which produce a series of enzymes, collectively called cellulase (Sagar, 1988), which facilitate the degradation of cellulose to glucose units. Cellulose-degrading enzyme systems have been studied in detail in 2 fungi, *Sporotrichum pulverulentum,* and the conidial state of the white-rot fungus *(Phanerochaeate chrysosporium)* (Eriksson, 1981) and the mould *Trichoderma reesei* (Ryu and Mandels, 1980). They have similar hydrolytic enzyme systems with at least 3 components: (i) Endo-β-1,4-glucanases, which split randomly β-1,4-glucosidic linkages within the cellulose polymer. (ii) Exo-β-1,4-glucanase, which split off either cellobiose or glucose from the non-reducing end of the cellulose polymer. (iii) β-glucosidase, which hydrolyze cellobiose and water-soluble cellodextrins to glucose. Cellulase is a synergistic enzyme that is used to break up cellulose into glucose or other oligosaccharide compounds (Chellapandi and Jani, 2008; Acharya et al., 2008). Cellulases have a wide range of applications. Potential applications are in food, animal feed, textile, fuel, chemical industries, paper and pulp industry, waste management, medical/ pharmaceutical industry, protoplast production, genetic engineering and pollution treatment (Tarek and Nagwa, 2007; Beguin and Anbert, 1993; Mandels, 1985).

MATERIALS AND METHODS

General

All chemical used were of reagent grade (supplied by either Merck or Fluka) and used as supplied.

Substrates

Waste paper was obtained from Alnajaf factory for producing paper in Alnajaf city in Iraq.

Microorganisms

A. niger procured from biotechnology division were maintained on Potato dextrose ager (PDA) slants ager and *T. viride* from biotechnology division was maintained on PDA slants. All cultures were subcultured every 4 weeks, incubated at 30°C for 7 days and subsequently stored at 4°C for inoculums preparations.

Conidial count

The conidial count was made on a Haemacytometer slide bridge (Sharma, 1989).

Culture media

A suspension containing *A. niger* and *T. viride* were used to initiate growth in 250 ml Erlenmeyer conical flask supplemented with $K_2H_2PO_4$ (0.15 g/L), KH_2PO_4 (0.20 g/L), $NaHPO_4$ (1.50 g/L), $NaHPO_4$ (2.00 g/L), $NaN0_3$ (3.80 g/L), H_3BO_3 (0.057×10^{-3}g/L), $MnSO_4.H_2O$ (5.5×10^{-6} g/L), $CuSO_4.7H_2O$ (2.5×10^{-6} g/L), $ZnSO_4.7H_2O$ (0.5×10^{-3} g/L), $Fe(SO_4)_3.6H_2O$ (4.5×10^{-6} g/L), $MgSO_4.7H_2O$ (5.5×10^{-6} g/L), NH4NO3 (0.60 g/L), $(NH_4)_6Mo_7O_{24}$ (0.025×10^{-3} g/L).

Fermentation procedure

Using for cellulase production in 250 ml Erlenmeyer conical flask (and addition of 2 ml of media culture with 2 gm of carbon source (Paper). After the inoculation, the flasks were sterilized by using of autoclave at 121°C for 15 min. After cooling at room temperature was then added 10^6 per ml of fungi and incubated for 6 days. The supernatant was estimated for Whatman filter papers (No. 1) saccharifying activity of cellulases.

Determination of the optimum co-cultured media (*A. niger* and *T. viride*)

We use five co-cultured medium:

(A + T, 24 A + T, 24 T + A, 48 A + T and 48 T + A) in addition of monoculture for each fungi by using of Sunders culture medium at

30℃ for 6 days.

Determination of optimum carbon source

To determine the optimum carbon source, 2 gm of (wood fibers, paper waste and cotton waste) act as culture medium at 30℃ for 6 days.

Effect of pH

The working pH of co-cultured media (*A. niger* and *T. viride*) was from 3 to 6 using phosphate buffer. The optimum pH was evaluated.

Effect of temperature

The working temperature of the co-cultured medium was varied from 20 to 50℃ (+5).

Determination of enzyme activity

The determination of enzyme activity was done by using of Mandels method. Incubation of 0.9 mL of substrate solution with enzyme extract at 45℃ for 1 h, then added 1 mL of DNS solution. The mixture was then heated at water bath for 5 min. then let it to cool and then added 10 mL of distilled water. The equivalent solution was prepared by added 1 mL of DNS to 0.9 mL of substrate then added 0.1 mL of enzyme solution. The determination of reduction saccharides was done by using of Mandels method and then calculates the enzyme activity (Mandels et al., 1976).

RESULTS AND DISCUSSION

Figure 1 represent the highest enzyme activity by using of co-culture media (*A. niger* and *T. virid*) and it raised 2.37 unit per mL and it was very high as compared with another co-culture medias and that because of Antagonism between *A. niger* and *T. virid* (Haq et al., 2005).

The three carbon sources wood fibers, paper waste and cotton waste were optimized. Among them, cotton waste was proved to be the best for cellulase production and it was 3.40 unit per mL and it was batter from the other wastes (that use in this paper, Figure 2) due to high percentage of cellulose production and the easy of braking bonds of waste paper, but wood and cotton waste have other substances like lignin and that is difficult to analyzed by enzyme.

The effect of the H^+ concentration on the activity and stability of the cellulase is shown in Figure 3. Hydrolysis of was confined to acid media. The enzyme activity being maximal (4.54) at pH 5. In contrast with the sharp pH optimum seen in the activity profile, the cellulase was stable in the absence of substrate, over a wide range of pH values. Initial pH has a direct effect on the uptake of mineral nutrients, which are present in the fermentation medium. So, the effect of different pH (3.0 - 6.0) of fermentation medium on the enzymes production was

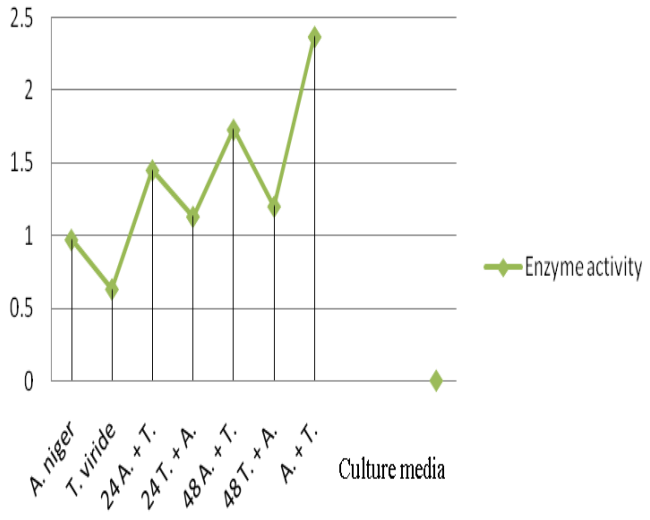

Figure 1. Enzyme activity vs culture medias.

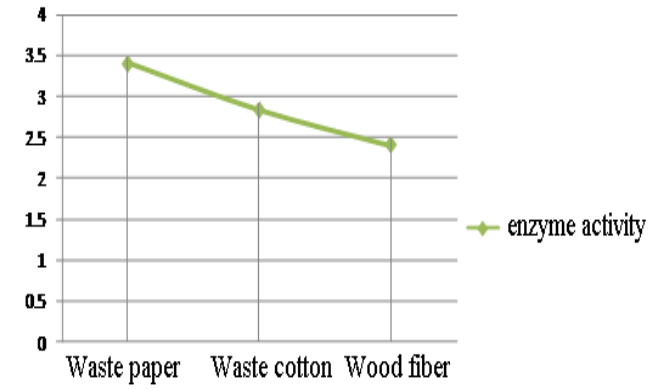

Figure 2. Enzyme activity vs. carbon source.

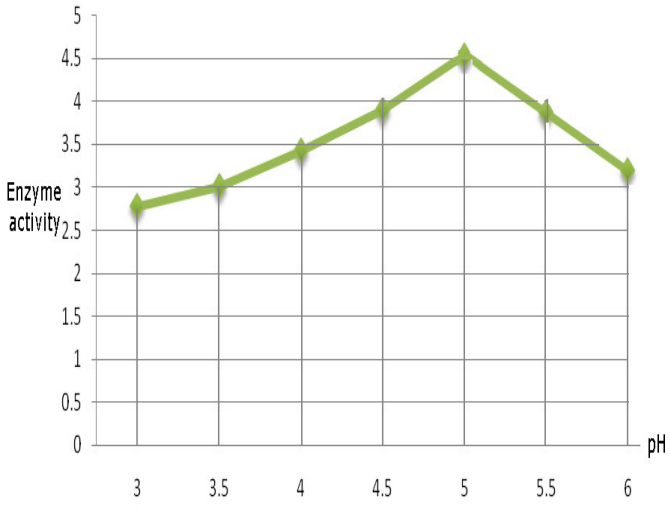

Figure 3. Enzyme activity vs pH.

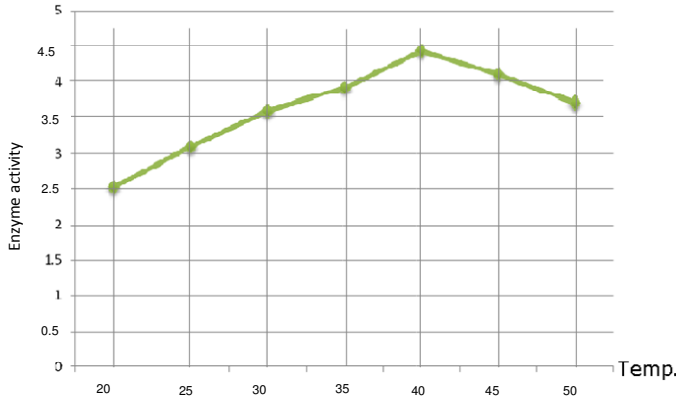

Figure 4. The enzyme activity vs temperature.

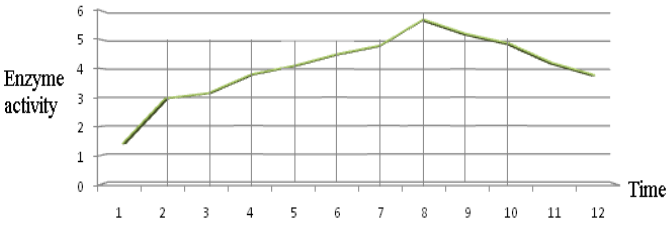

Figure 5. Enzyme activity vs time in days.

also investigated (Figure 3) with simultaneous co-culturing of *A. niger* and *T. viride*. High acidic and high basic pH, both showed negative effects, but a medium with low acidic pH 5 was ideal for enzyme fungal cultures require slightly acidic pH for their growth and enzyme biosynthesis (Haltrich et al., 1996). In the present study, a temperature of 20 - 50°C (+5°C) was used in the standard assay; however, the temperature of maximum activity under these conditions proved to be 40°C (Figure 4). Incubation temperature plays an important role in the metabolic activities of microorganism. Figure 5 represent the maximum enzyme activity at the 8th day and it was 5.70 units per mL.

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