Effect of Various Growth Factors and Nutrients On Growth and Elaboration of Enzymes from Neurospora crassa

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***Abstract* – Microorganisms, mainly fungi, are responsible for biodegradation of various cellulosic materials, like cotton textile, linen goods, paper & pulp products etc. The biodegradation occurs by accumulation of Cellulase Enzymes in their culture media. These cellulase enzymes aid in the biodegradation of cellulosic substances, either directly by providing cellulase breakdown products for nutrition of microorganisms or indirectly by the release of nutrients from disrupted cells. *Neurospora crassa* is a filamentous fungus, capable of secreting large quantities of cellulases from cellulosic substrates, such as, sugarcane bagase, wood cellulose etc. and has got the ability to ferment D-glucose, D-xylose and treated cellulosic substrates directly to ethanol in a single step process. The present work deals with the study of Vitamins and various growth factors responsible for growth and Cellulase Enzymes production by the microorganisms.**

***Index Terms* – Cellulose, Cellulase, filamentous.**

I. INTRODUCTION

Plant cell wall polymers which consist mainly of cellulose, hemicellulose and lignin are highly degraded by many microorganisms, especially filamentous fungi by secreting hydrolytic enzymes in their culture media. *Neurospora crassa* is a filamentous fungus, capable of producing high level of secreted active cellulases when induced with cellobiose as compared to enzyme levels observed during growth on crystalline cellulose. *N. crassa* has a robust cellulolytic response to growth on plant cell walls and crystalline cellulose, including induction and secretion of a large number of cellulases and hemicellulases. Production of hydrolytic enzymes is induced to high levels only in presence of biopolymers or their derivatives. Ample of literatures are available on different species of the genus *Neurospora*. Cellulase and β-glucosidase were produced by *N. sitophila* cultured in media containing corn stover or sugarcane bagase as carbon sources by Oguntimein, Gbekeloluwa et al., (1992). Moo-Young Murray et al. (1992) used N. sitophila to convert solid cellulosic substrates, such as, sugarcane bagase, corn stover, wood cellulose etc. to protein rich materials for food and fodder. However, the production of cellulase as well as aryl-β-D-glucosidase has been reported (Rao et al., 1983) by selecting two promising strains of *N. crassa*. Also, the fungus is responsible for the production of an extracellular cellulase complex and has got the ability to ferment D-glucose, D-xylose and treated cellulosic substrates directly to ethanol in a single step process.

II. EXPERIMENTAL

A. Materials & Method:

The fungus *Neurospora crassa* was obtained from Chandra Shekhar Azad Agricultural and Technological University, Kanpur. The culture was grown on potato dextrose agar slants containing filter paper strips as cellulosic substrate at a temperature of 30 ± 2° C for a period of ten days and maintained at 4°C by subculturing every month.

From ten days old culture, spores' suspension was prepared by adding sterile distilled water to the culture tubes under aseptic conditions. The suspension was filtered through sterile muslin cloth, adjusted to pH under aseptic conditions and incubated at 30 ± 2° C on rotatory shaker for ten days.

Various growth media were tried for maximum growth of organisms and maximum elaboration of enzymes from the cultures.

B. Result and Discussion:

Neurospora crassa was grown on Malt-yeast extract medium containing different nitrogenous compounds, vitamins and growth promoting substances. The flasks were incubated at 28 ± 1° C for 7 days under stationary conditions. Control flasks, lacking any nutrient, were also incubated.

After 7 days of incubation the growth of the fungal cultures was observed and calculated in terms of dry mycelial weight. While, the activities of the elaborated enzymes were determined as the amount of reducing sugars formed at the end of the reaction. The results are recorded in the Table.

Table

Effect of growth factors and nutrients on growth and elaboration of enzymes

Growth Factors/ Nutrients	N. crassa	
	Cell mass (mg/100ml)	Enzyme activity (ug/ml)
Control	60	500
Biotin	87	580
Calcium pentothenate	85	542
Riboflavin	82	538
Thiamine-HCl	80	530
Asparagine	20	230
Peptone	60	525
Ammonium chloride	61	520
Ammonium sulphate	59	490
Ammonium phosphate	58	522
Sodium nitrate	20	240
Urea	59	515

Number of determinations were three in each case.

Tian C, et al. (2009) carried out the identification by mass spectrometry in the supernatant of a *N. crassa* culture grown on Avicel and Miscanthus. *N. crassa* was identified in the cell wall fraction of conidia by Maddi A, et al (2009) and its enzymatic activity was verified by Bohlin C, et al. (2010). Trivedi and Rao (1979) reported maximum production of all cellulase components of *Aspergillus fumigatus* on 12th day of growth in basal medium containing cellulose as sole carbon source and a combination of ammonium sulphate and ammonium di hydrogen phosphate as nitrogen sources. Park and Ryu (1983) studied the favorable effect of yeast extract on cell growth of *Clostridium thermocellum* in glucose medium, due to either organic nitrogen sources or growth factors contained in yeast extract. They found that biotin had the most favorable effect on glucose utilization and cell growth, followed by thiamine-HCl, pyridoxal-HCl and pentothenic acid.

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