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FULL LENGTH ARTICLE

Study of Temperature Dependent Crude Enzyme Activity and Batch Studies of Various Corn Materials for Phenol Treatment

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ABSTRACT

Industrial waste water management involves a plethora of concerns, challenges and processes. Often, the reluctance of the industries on investing huge finance for water pollution control necessitates economic ways, for treating them. Though, activated sludge process dominates the field of waste water treatment, there still lies a niche for treatment of toxic and carcinogenic compounds like phenol in the most economic and hassle free manner. Phenol samples were prepared in laboratory and the effect of crude extract of varieties of corn and its individual parts on phenol sample were determined. The temperature and time dependent studies of the crude extract showed best results for corn silk, baby corn endings and sweet corn husk, with high enzyme activity at 277K. Batch studies showed up to 85% decrease in phenol amount for 48 hours. Thus, the crude extract of corn could prove to be an efficient source for phenol treatment.

INTRODUCTION

Phenol is an aromatic organic compound found in waste water of a variety of industries like petrochemical industry, olive oil mills, textile, coal tar production industries etc. It is known to have detrimental effects on plants, animals and humans, alike. Moreover, it drastically affects the soil quality, porosity and water toxicity, co laterally affecting our entire ecosystem [3].

Industries currently employ a full-fledged activated carbon system for removal of contaminants like phenol, salts, dyes and chemicals. They even incorporate microbial degradation reactors in combination with chemical removal techniques for efficient removal of phenol.

Table 1:Different Types of Industries and Phenols in Their Waste Water [10]

Industries	Type of Phenols
Textile	Phenol, Chloro phenol, Alkyl phenols, Catechol, Chloro catechol, Nitro phenol.
Wood processing	Phenol, Chloro phenol, Alkyl phenols.
Pharmaceutical	Catechol, Chloro cetachol, Chloro phenol, Methyl phenol, Buthyl hydroxyl toluene, Buthyl hydroxyanisole.
Rubber	Aminophenol, Cetachol, Chlorocetachol, Amino phenols, Buthyl hydroxyl toluene, Buthyl hydroxyanisole
Petrochemical	Phenol,Methyl phenol.
Cosmetics	Chlorocetachols, Methyl phenol, Buthyl hydroxyl toluene, Buthyl hydroxyanisole.
Coal-tar production	Phenol, Nitrophenols, Methyl phenols.

Many agricultural sources and wastes like watermelon rinds [2], neem leaves [5], date seeds [1], rice husk [4] etc have been thoroughly worked on for efficient removal of dyes, organic compounds and phenols.

Table 2: Taxonomic Classification of Maize [8]

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Cyperales
Family	Poaceae
Genus	Zea L.
Species	Zea mays L

Zea mays (Corn) , a monocot initially domesticated by the Mexicans is cultivated largely across the world.Global corn production was 38,105 million bushels during 2015-2016 [9].
Most of the processed corn, involves discarding of the outer covering,that is, husk, silk, corn cob and endings. These maize or corn materials are known to have polyphenol oxidase [6] and peroxidase enzyme [7], that play a vital role in degradation of phenol.
The high price of activated charcoal has enhanced curiosity towards more feasible options for phenol removal. The investigation reported here examines the possibility of usage of corn crude extract for phenol degradation. A comparison of different varieites of corn and their parts is made, to gain an insight into the use of an appropriate degradation source.

MATERIALS AND METHODS

Materials

Plant Material

The corn, sweet corn and baby corn used in this experiment were obtained from the local market. The silk, husk and endings were washed and stored separately at 4°C until further experiments.

Chemicals

Phenol was purchased from NICE chemicals and Potassium Ferricyandide was procured from Merck.
4 Amino Antipyrine (4- AAP) and Boric Acid were obtained from S D Fine Chem Ltd.,
Guaiacol, Catechol, Hydrogen peroxide and Potassium Di-hydrogen was purchased from V.L Products. Phosphate, Di-potassium Hydrogen Phosphate and Calcium Chloride was obtained from Qualigens Fine Chemicals.

Methods

Standard Curve

100 mg /L of phenol stock solution was prepared.
Quantitative analysis of phenol was carried out spectrophotometrically using 4 amino antipyrine method.

Batch Studies

1gm of the sample was washed and immersed in 50 ml of phenol stock solution. Spectrophotometric analysis of phenol degradation was done after 24 hours and 48 hours.

Crude Enzyme Extraction

Three portions of the sample, 1gm each was homogenised, along with 5ml of ice cold $CaCl_2$ solution (0.5 M). It was centrifuged at 0°C for 8 minutes at 4200 rpm. The supernatant was collected and the pellet was further centrifuged with 2.5 ml $CaCl_2$ solution under same conditions. The procedure was repeated once again and the supernatant were stored at three different temperatures, that is, 298 K, 277 K and 253 K.

Peroxidase Assay

1ml of Guaiacol (substrate), along with 1ml of H_2O_2 was added to the solution of 50 µl crude enzyme and 950 µl buffer (pH 6.5). It was spun and incubated for 3 minutes. The spectrophotometric analysis was carried out at 470 nm. The control was deficient of the substrate. The value of absorbance was converted to enzyme activity.

Polyphenol Oxidase Assay

1ml of Catechol (substrate) was added to the solution containing 50µl crude enzyme and 950µl buffer (pH 5.5). It was spun and incubated for 6 minutes. The spectrophotometric analysis was carried out at 410 nm. The control was deficient of the substrate. The value of absorbance was hence converted to enzyme activity.

RESULTS AND DISCUSSION

Phenol Standard Curve

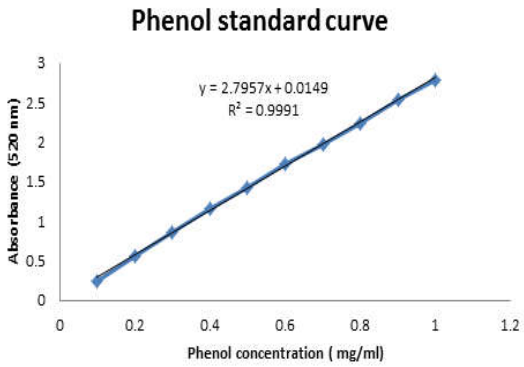

Fig 1: Phenol Standard Curve

Batch Study

Batch Study for Silk- Time dependent

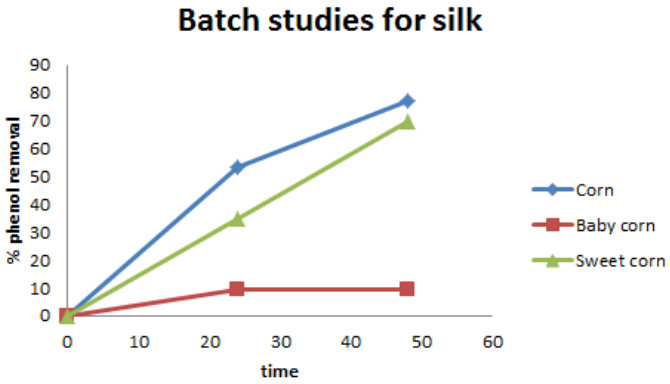

Fig 2: Graph of Time Dependent Batch Study of Silk

The time dependent batch studies of silk from corn, sweet corn and baby corn indicated that the crude extract of corn silk showed highest decrease in phenol concentration of 77% at the end of 48 hours, followed by sweet corn 69%. Baby corn crude extract showed the least phenol degradation with only 9.6% phenol being removed after 48 hours.

Batch Study For Husk- Time Dependent

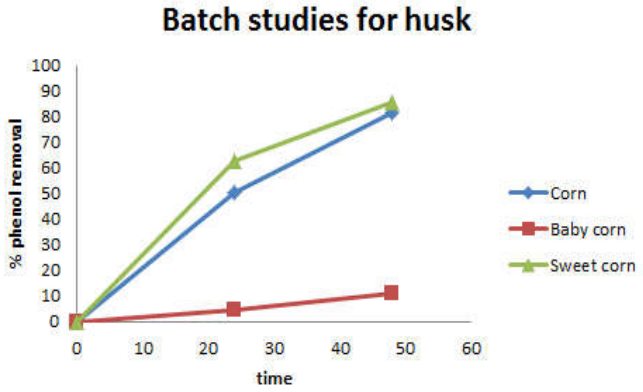

Fig 3: Graph of Time Dependent Batch Study of Husk

Sweet Corn husk crude extract had the highest phenol degradation capacity of 85.517 % at 48 hours, followed very closely by corn husk crude extract, which degrades almost 81% of phenol, at the end of 2 days. The baby corn husk crude extract has the least phenol degradation. Hence, it was concluded that the crude extract of both baby corn silk and husk showed only 9 – 11% phenol degradation ability at the end of 48 hours.

Batch Study for Endings- Time Dependent

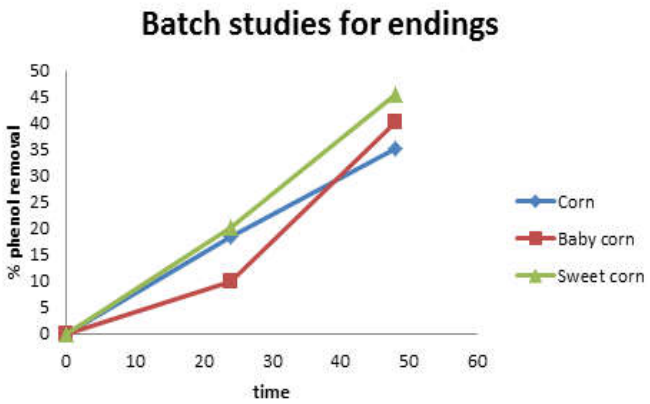

Fig 4: Graph of Time Dependent Batch Study of Endings

The crude extract of sweet corn endings showed the maximum percentage decrease in phenol of 20.28 % and 45.45 % at the end of 24 and 48 hours. Though, the percentage decrease of phenol by the crude extract of baby corn at 24 hours, had been only 10%, the process of phenol degradation seems to have accelerated during the next 24 hours, yielding 40.11% of phenol removal at 48 hours. Even the crude extract of corn exhibited almost 40% phenol decrease by 48 hours.

Peroxidase Assay

Peroxidase Assay for Silk-Temperature Dependent

At all three temperatures 298 K, 277 K and 253 K the crude extract of corn silk had maximum peroxidase activity. The highest activity of 333.3 U /min was exhibited by the crude extract of corn silk at 277K.

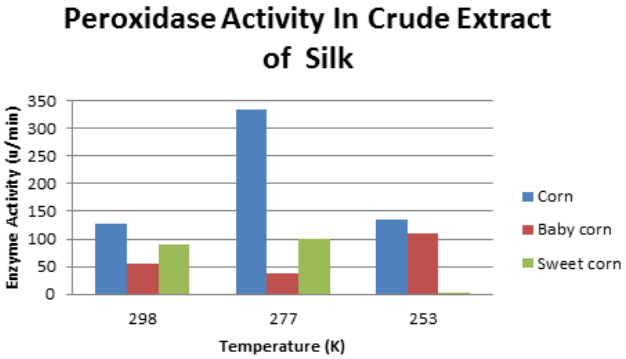

Fig 5: Graph of Enzyme Activity of Crude Silk At different Temperatures of Corn, Baby corn and Sweet Corn

At 253 K, the study showed minimal activity of the crude extract of sweet corn. The activity was enhanced at 277K for the crude extract of corn in comparison to its activity at 298 K, therefore indicating better degradation of phenol at the former temperature.

Peroxidase Assay For Husk- Temperature Dependent

At 277K, the crude extract of sweet corn husk showed maximum activity of 333.33 U/ min.

The crude extract of corn husk exhibited very similar rate of activity at all the three temperatures indicating the temperature –activity relationship and the activity of baby corn crude at 298 K and 253 K, did not have any significant difference either.

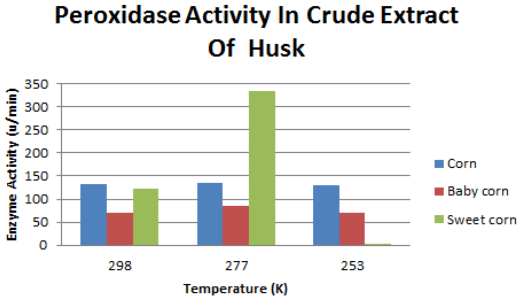

Fig 6: Graph of Enzyme Activity of Crude Husk At different Temperatures of Corn, Baby corn and Sweet Corn

Peroxidase Assay For Endings- Temperature Dependent

The crude extract of baby corn endings showed maximum activity of 101.02 U/min at 277 K. The enzyme activity in the crude extract of corn decreased from 298 K to 277 K, followed by a slight increase at 253 K.

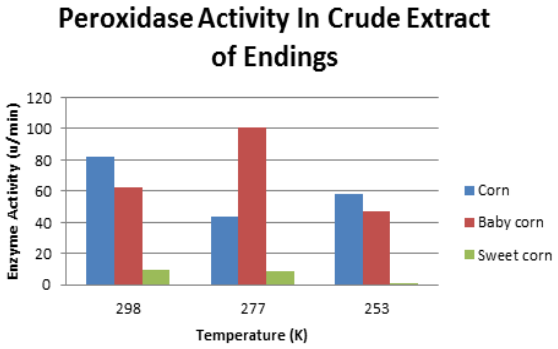

Fig 7: Graph of Enzyme Activity of Crude Endings At different Temperatures of Corn, Baby corn and Sweet Corn

Polyphenol oxidase Assay

Polyphenol oxidase Assay For Silk- Temperature Dependent

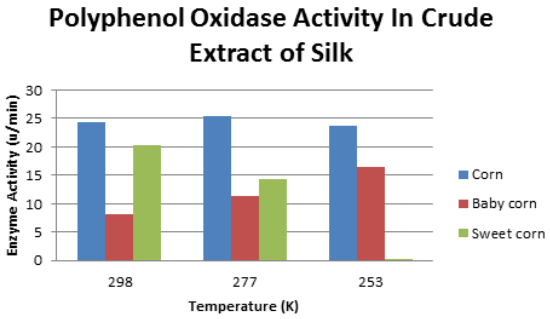

Fig 8: Graph of Polyphenol Oxidase Activity of Crude Extract of Silk At different Temperatures from Corn, Baby corn and Sweet Corn

The polyphenol oxidase enzyme activity was maximum for the crude extract of corn silk and was almost similar at all three temperatures that is around, 25 U/min. The crude silk extract of baby corn showed maximum activity at 253 K and it decreased at 277K and further decreased at 298 K. Consequently, the crude extract of sweet corn silk had 20.29 U/ min and 14.32 U/min activities at 298 K and 277 K respectively.

Polyphenol oxidase Assay For Husk- Temperature Dependent

At 298 K and 277K the crude corn husk extract showed very similar activity. Moreover, at 277K there is a consequent decrease in activity from the crude husk extract of corn to baby corn to sweet corn.

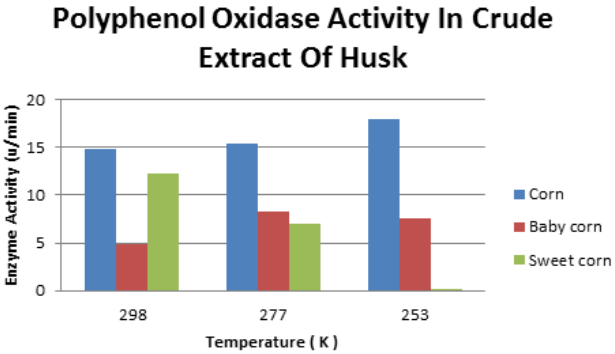

Fig 9: Graph of Polyphenol Oxidase Activity of Crude Extract of Husk At different Temperatures from Corn, Baby corn and Sweet Corn

Polyphenol oxidase Assay For Endings- Temperature Dependent

Sweet corn endings showed maximum enzyme activity at 298 K. While the enzyme activity was the most for crude extract of corn endings at 253K. The crude extract of sweet corn in general, depicts minimal enzyme activity at 253 K.

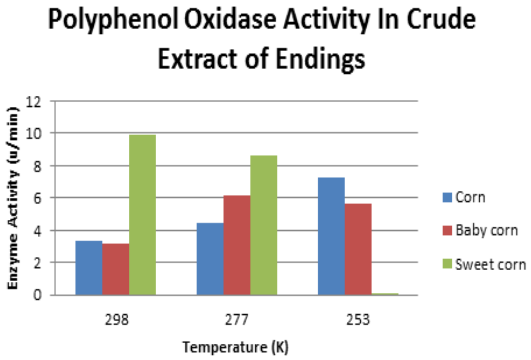

Fig 10: Graph of Polyphenol Oxidase Activity of Crude Extract of Endings At different Temperatures from Corn, Baby corn and Sweet Corn

CONCLUSION

Time dependent batch Studies indicate the possibility of usage of corn materials as one of the most economic phenol degradation agents. Further investigations, are required to optimise conditions like temperature, pH, rpm etc. for batch operations.

The enzyme activity of peroxidase and polyphenol oxidase in corn parts, are indicative of the presence of phenol degrading enzyme. Further characterisation and optimisation of appropriate enzyme is required.

Combining the data from Batch Studies and Temperature Dependent Crude Extract enzyme activity, it is possible to design a reactor with different beds of corn materials, maintained at optimum temperature and pH to achieve maximum phenol removal.

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