Linnaeus University
School of Natural Sciences

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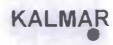
International Conference on Natural Sciences and Technologies for Waste and Wastewater Treatment, Remediation, Emissions Related to Climate, Environmental and Economic Effects



PROCEEDINGS

Linnaeus
ECO-TECH ´10
22-24 November, 2010
KALMAR, SWEDEN

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LINNAEUS ECO-TECH' 10

INTERNATIONAL CONFERENCE
ON
NATURAL SCIENCES AND TECHNOLOGIES
FOR

WASTE AND WASTEWATER TREATMENT
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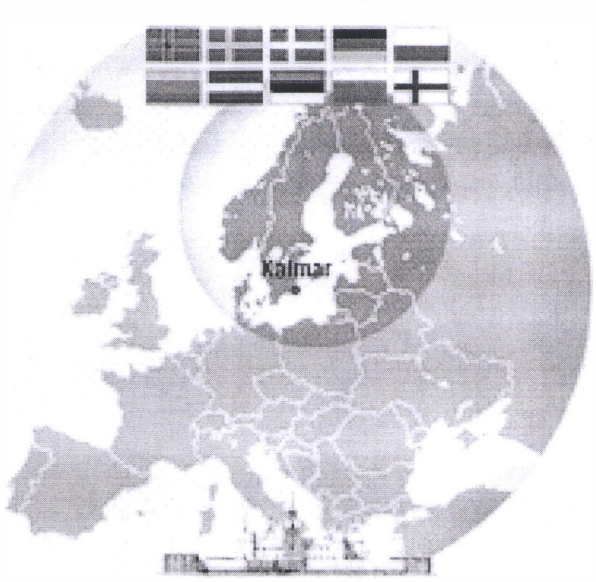
The Seventh International Conference on the Establishment of Cooperation between Companies and Institutions in the Nordic Countries, the Baltic Sea Region, and the World



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EFFICIENCY OF RYE BIOMASS ON OIL HYDROCARBON BIODEGRADATION

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ABSTRACT

The majority of the plants secrete their enzymes in the soil, where they can decompose various organic compounds. Extra cellular plant enzymes can be effective against a number of contaminants. To estimate the plants, their enzymes and micro-organism effect on hexadecane content in the soil, the sterile soil samples was amended with 0.5 % hexadecane and sowed with accordingly prepared rye seeds. Till increasing the quantity of micro-organisms and the formation of rye seedlings, hexadecane content remained practically unchanging in all samples. After 21 days hexadecane content in the soil with rye seedlings was about 5 % of the contributed content. The intrinsic soil processes through 21 days to shrank the hexadecane content in the soil without rye seedlings up to 17 %. Oxidases, catalases and peroxidases were detected in rye biomass during all test period. As shown by the results, polyphenoloxydases were detected only in root biomass, the early germs of germination and roots formation stages.

KEYWORDS

Phytoremediation, enzyme, micro-organisms

1 INTRODUCTION

Phytoremediation is a quite novel technique to clean polluted soils using plants. Plants affect the water balance of a site; they change redox potential and pH, and stimulate microbial activity of the soil. Currently, phytoremediation is used for treating many classes of contaminants including oil hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and other. There are numerous mechanisms by which plants may remediate contaminated sites. Many plant enzymes appear to play an important role in xenobiotic degradation, including dehydrogenases, peroxidases, nitroreductases, dehalogenases and others mono- and dioxygenases [4, 11, 15]. The majority of these plants secrete their enzymes in the soil, where they can decompose various organic compounds. The enzymes are involved in the xenobiotics digestion, after the plants to soak up a simple diffusion path. Pollutant intake leads to close contact with detoxicated plant enzymes, which are located in cell citozol. Plant organs are the further

transformation of these compounds, conjugation and the transfer of the various plant tissues. Later, they may even be evaporated [1]. Some of these enzymes appear to be naturally released into the soil, where they are capable of degrading organic pollutants ranging from solvents to explosives [17].

Innovative, new techniques are required for cleaning up hydrocarbon-contaminated environments. Our interest focuses on the interaction of oil hydrocarbons with plant biomass enzymes in soil. The processes that favour phytoremedation may be optimized by the choice of plant species. The evidence has been mainly based on higher plant species, in particular crop plant species. The aim was to assess the impact of the plant enzyme degradation of oil hydrocarbons.

2 MATERIALS AND METHODS

The laboratory tests were performed in plastic boxes. For control rye seeds were twice washing with 70% ethanol and overspreading in plastic boxes with sterile soil (25 % w/w). Rye seeds were germinated at room temperature (20±2°C). Plants were harvested 20 days after seedling emergence. The part of the rye shoots from control plastic boxes were cut and twice washed with sterile distilled water and mashed up. The 2.5 % rye mash prepared from rye shoots was added to mineral salt medium M9 without carbon source [12]. The medium was supplemented with 0.5 % hexadecane. Control was prepared with mineral M9 salt medium supplemented with 2.5 % preheated rye mash and with 0.5 % hexadecane. Biomass was heated for 48 hours at 60°C. The flasks were incubated at room temperature for 21 days. All experiments were performed in triplicate. To estimate the plant and micro-organism effect on hexadecane content in the soil, the soil samples were sieved (2 mm), steriled at 121°C for 1.5 hours. Before application, one part of the soil samples was amended with 0.5 % hexadecane and sowed with 70% ethanol washed rye seeds. Every 7 day three replicate boxes and flasks were collected for enzymes, micro-organisms and hexadecane content assays. Hexadecane extraction and gravimetric measurements were used to determine the concentration in experiments [6].

For qualitative enzyme assays the rye 20-30 cm shoots and the roots from every box and flask were cut and mashed up. The filter paper discs soaked with test reagent, N, N, N`, N`-tetra-ethyl-p-phenylenediamine dihydrochloride were used to oxidase detection. The oxidase test is a test used to determine cytochrome c oxidases. The reagent is a dark blue to maroon colour when oxidized and colourless when reduced [2]. Catalase - peroxidase test was done as advocated by Bogen (1957). 2.0 ml of a freshly prepared mixture of equal volumes of 0.2 % catechol and 1 % hydrogen peroxide solutions in distilled water was poured on the rye mash. Evolution of bubbles within 3 to 5 minutes was taken as an indication of the presence of catalase. If the colonies turned brown or black within 45 to 60 minutes they were termed as peroxidase positive. The filter paper strips soaked with 0.5M catechol solution were used for polyphenol oxidase detection [9]. Oxygen-dependent formation of o-quinones leads to the formation of brown and black pigmented polymers.

The micro-organism cultures were obtained by standard serially diluting method. Serially diluted samples were plated on plate count agar medium (Oxoid) and nutrient broth agar (BIOKAR) for bacteria. Mitosporic fungi were isolated on Czapek agar (Merck).

3 RESULTS AND DISCUSSION

Plants perform phytoremediation by multiple processes including metabolism, volatilization, and sequestration followed by harvesting. Additionally, plants exude compounds and enzymes that facilitate the breakdown of pollutants [8, 10, 13], and stimulate microbial biodegradation in the rhizosphere. The main plant enzymes involved in degradation and detoxification of organic pollutants, including petroleum products, belong to oxidoreductase class. It is established that a large proportion of these enzymes found in plant root exudates distinction along with the other metabolites [14]. Extra cellular plant enzymes including laccases, dehalogenases, nitroreductases, peroxidases and hydrolases can be effective against a number of contaminants.

Before starting to investigate the plant influence on the oil hydrocarbon degradation by selected compounds we tested their volatility. The high volatility and low boiling point of organic compounds have a negative impact on the reliability of research results. Therefore, the tests were to choose less volatile oil hydrocarbons, choicely decane, hexadecane, xylene, naphthalene. The first test was checked at the beginning of hydrocarbon evaporation intensity. After 7 days the control flasks found that xylene decreased by an average of 96.6 %, decane -70.5 %, naphthalene - 26.4 %. Hexadecane average remained in flasks to 95 %. Therefore, for further research has been selected hexadecane of.

As shown the studies the number of micro-organisms began to rise for the second day in the sterile soil sown with ethanol washed rye seeds (Fig. 1.)