International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.03, pp 79-85, 2018

Enhancement of Water Infiltration Character of Hydrophobic Soil by Crude Cell Enzyme Mediated Bio-Alleviation

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Abstract : Hyrophopic soils are generally prevent moisture infiltration. It leads to varied water distribution over the unsaturated soil zone. Unsaturated zone is a soil-air interface, where water infiltrates into the system. Infiltration refers quantitatively the amount of water penetrating into the soil system or the quantity flows away as surface runoff causing erosion. Irregular water distribution affects the irrigation efficiency, surface runoff, and plant growth. This paper focus on the preliminary investigation on water infiltration based on crude enzyme mediated bio-alleviation of soil hydrophobicity. Based on WDPT and MED analysis, the infiltration of water can enhance upto 25 % in 19 days of incubation with repeated crude enzyme exposure.
Keywords : Hydrophobic soil, Permeability, Infiltration, Water Repellance, Crude Enzyme, Bio Alleviation.

Introduction

Hydrophobicity and hydrophobic soils are transient phenomenon, it prevent moisture penetration resulting in a varied water distribution, found to be more severe in dry soils and decreases as soil moisture content increases. Water repellent soils may be cataclysmic and impede seed germination, plant growth, reduced irrigation efficiency, water runoff and soil erosion, followed by reduced plant growth [2]. Soil repellence is accredited to accumulation of organic coatings, hydrophobic waxes, humic/fulvic acid over the soil or re-distribution or re-arrangement of accumulated organic coatings/materials [13]. Weak forces of attraction at the interface between solid and liquid molecules may/might be an attributing factor towards surface water repellence[2] [11]. Scanty literatures are available or reported on water repellence in Indian continent [6] [13], whereas soil water management are reported in countries like Australia, United States of America, Netherlands, Portugal [7] [12].

The tenacity of pollution and improper water management are strained depletion of water reserves, leading to deliberately harvest rainwater, for which the soil must be hydrophilic, as climate change models predict that soils will be much drier in summer months by 2070 leading to development of dry hotspots (Hydrophobic soils) [10]. Impending innovative cost-effective and eco-friendly methodologies are essential to

International Journal of ChemTech Research, 2018,11(03): 79-85.

DOI : http://dx.doi.org/10.20902/IJCTR.2018.110335

ameliorate water percolation/infiltration. Implication of customary methodologies such as application of wetting agents, sowing plants, extraction techniques such as introduction of wax degrading bacteria have their own advantages and practical limitations, as they sometimes fail to consider and alleviate the complexity involved [15]. Enzyme based remediation using crude extracellular enzyme fractions containing enzymes such as laccase have found to have potential applications in various fields spanning from dye decolorization, xenobiotics degradation, paper-pulp bleaching to water repellent soil management [3] [4] [14]. Crude systems are better and advantages in treating synthetic organics coatings due to its broad specificity, presence of natural mediators, high levels of titre value and low process economics (Sarnthima et al 2009, Sridhar et al 2013). A lot of studies have been carried out on enzyme mediated bioremediation using crude/purified system/live organisms, but there are vague information on enzyme mediated reduction of surface water repellency of soils. There is a gap between biotechnologist/environmentalists and soil scientists, where they are unable to bridge the gap between lacunas. The first group of scientists is aware of type of methodologies and strategies, but is not aware of mechanism and the type of soils. Similarly the second group, are not aware of production of enzymes, inter-relationship between enzymes present in the crude extra-cellular fractions and enzymatic approaches and its applications. Our proposal accentuates on bridging the gap between by solving hurdle involved in technology transfer. Many literatures have reported in enzyme mediated alleviation of soil water repellency using a crude enzyme extract [16], but we are reporting for the first time isolation of crude extracellular enzyme fractions (CEEF) by solid state fermentation by growing *Aspergillus niger* using wheat bran a solid support without addition of any external mediators/inducers and its application towards reduction of surface water repellency phenomenon in the hydrophobic soils.

Materials and Methods

Microbial source

Aspergillus niger, was gifted by Marina labs, Chennai and was maintained in Potato Dextrose Agar (PDA) slants at 4^0C and subcultured every 2 days using potato dextrose agar. After seven days, mycelia culture sample grown over potato dextrose agar is used as a source of microbial source for solid state fermentation.

Soil sampling

Four undisturbed soil samples were collected from dried lake bed during summer which was used for dumping diary effluents. The collected samples were shade dried at room temperature (25–30°C) to a constant weight and gently passed through the 2mm sieve to remove the coarse soil particles, then stored at room temperature for subsequent usage. The collected sample showed moderate water repellency based on preliminary Water Droplet Penetration Test (WDPT) analysis. The collected sample passed was designated as S1, S2, S3 and S4 to study the effect of soil size towards water percolation.

Solid state fermentation

Crude extracellular enzyme fractions (CEEF) isolation was performed according to our previous reports with minor modification (Sridhar et al 2013). The wheat bran samples were collected from chennai, Tamilnadu. The collected samples were shade dried under room temperature. The dried samples was sieved using a standard 2mm sieve and was preserved in a sealed plastic bag under room temperature till further use. Five grams of wheat bran was sterilized by autoclaving at 121°C and 20 psi for 20 minutes (minutes). Agar plug (diameter 6mm) were cut from the peripheral region of 7 day old active growing fungus (*Aspergillus niger)* culture on potato dextrose agar medium was inoculated into 250mL Erlenmeyer flask containing 5g of sterilized wheat bran as a solid-support substrate and appropriate amount of double distilled water was added to maintain 60% moisture. The culture conditions were maintained at room temperature approximately at 27±2°C under static conditions for various periods of time (days). The culture was terminated at 3, 5, 7, 9, 11 days. After appropriate days of incubation, the enzyme was harvested by the addition of 100ml double distilled water and filtered through a gauze cloth, subsequently centrifuged at 5,000 rpm for 10 min (Remi, India). The obtained crude enzyme was freeze–thawed, centrifuged at 5,000 rpm for 10 min (Remi, India), and filtered using filter paper. The supernatant obtained after centrifugation acts as a sole source of biocatalyst (crude enzyme). The clear crude enzyme was assayed for laccase and peroxidase activity using syringaldazine and guaiacol as standard substrate as described by [6].

Pretreatment of hydrophobic soils

Pretreatment of hydrophobic soils was performed according to a standard reported protocol with few modifications [15]. Twenty gram of air-dried soil sample were placed in a 90mm petri plate (Borosil), followed by addition of 20ml of crude extracellular enzyme fractions or control (double distilled water) obtained from solid state fermentation in 1:1 ratio. The Petri plate was covered with parafilm after homogeneous mixing, shaking and incubated under static condition at room temperature for 3 days with appropriate shaking. Thin needles were used to gently to stir the soils that settle down at the bottom of the Petri plate after addition of enzyme for 1min each day. The enzyme treated hydrophobic soil samples were separated by filtration under vacuum conditions and oven-dried at 40°c for 36hrs and then subjected to water-repellency analysis.

Post treatment of hydrophobic soils

Soil water repellency analysis or post treatment of hydrophobic soils was performed on enzyme treated soils using Water Drop Penetration Time (WDPT) and Molar Ethanol Droplet (MED) method. A control run was conducted in parallel, where enzyme was replaced by double distilled water in the reaction mixture in both analyses. The reactions were run in triplicate and the average of three runs was represented as percentage decrease in water repellency.

After WDPT and MED analysis on the third day, the soil samples were homogenized by thorough mixing using a glass rod, followed by manual packing in the petriplate and covered with parafilm. The process is repeated for five times on 7, 11, 15 and 19th day respectively to study repeated spiking of the enzyme over the soil to test the effect of crude enzyme towards reducing water repellency. WDPT analysis was performed with inconsequential modifications (Doerr 1998). 0.05ml of water drops were spotted on the superficial surface of enzyme treated hydrophobic soil. Water infiltration time was recorded by a timer, with the end-point being when each water drop was completely adsorbed and WDPT recorded. MED test was performed with minor modifications according to previously reported literatures (King 1981, Roy and McGill 2002). Various concentrations of aqueous ethanol solutions ranging from 0 to 6 mol L-1 were prepared in increments of 0.2 mol L–1. 90mm petriplate (Borosil) was filled with 100g of enzyme treated hydrophobic soil sample, mixed gently and pressed with a glass plate to attain a flat, smooth surface. 50µL of ethanol solutions with increasing molarity were spotted over the superficial sand using a micropipette until the drop infiltrated within 10 s [2].

Results

The primary objective of this work is application of cell free systems towards alleviation of water repellent nature of hydrophobic soil. Theoretically we try to hypothesize that repeated exposure of enzymes to the same soil may breakdown organic coatings/facilitate de-clogging of soil aggregates/increasing void space or porosity facilitating water infiltration. All statistical analysis was performed using 95% confidence limit using SPSS version 14 software.

Enzyme production

Crude enzyme production was carried out using wheat bran as solid support from *Aspergillus niger* by solid state fermentation. At regular time intervals, enzyme assays was performed to monitor the production of enzymes. The maximum enzyme (Laccase) activity was reported on 9th day of incubation (0.44 U/mL). All the experiments were conducted using ninth day crude extracellular enzyme fractions without any further dilutions.

Enzyme mediated post treatment of hydrophobic soils

WDPT analysis

Table 01 represents hydrophobic descriptive label of soil classification Water percolation/infiltration increased in soil samples after enzymatic treatment. Table 02 represents enzymatic post treatment WDPT analysis of water repellent soils. Complexity persists on water infiltration in hydrophobic soils in deciphering the mechanism of water repellency as many factors are involved. Results indicate that crude enzyme is found to have effect in breaking down organic coatings/molecules involved in the soil surface, leading to less infiltration time required. The infiltration ability of water into hydrophobic water-repellent soil was found to be 25 times

lower than similar soil made hydrophilic by invitro methods such as heating/claying (DeBano 1971). Table 03 represents MED classification of water repellency index. Table 04 MED analysis of before and after treatment.

Table 01 Hydrophobic descriptive label of soil classification (Doerr, 1998)

WDPT (s)	Classes of SWR
WDPT<5	Wettable (Hydrophilic)
05< WDPT ≤60	Slightly persistent (Hydrophobic)
60<WDPT ≤600	Moderately persistent (Hydrophobic)
600<WDPT ≤3600	Severely persistent (Hydrophobic)
WDPT >3600	Extremely persistent (Hydrophobic)

Table 02 Enzymatic post treatment WDPT analysis of water repellent soils

Soil sample	Before Treatment (Seconds)	After treatment(Seconds)				
		3rd day	7th day	11th day	15th day	19th day
S1	510±0.06	450±0.10	392±0.15	332±0.25	242±0.45	184±0.11
S2	270±0.10	214±0.40	204±0.10	184±0.20	168±0.20	60±0.10
S3	390±0.06	336±0.10	230±0.15	228±0.20	182±0.15	90±0.20
S4	432±0.10	416±0.15	286±0.25	230±1.0	196±0.25	192±0.20

Table 03 Molarity of ethanol droplet method classification

Class	MED (M)
Non repellent	0
Low-repellency	<1
Moderate-repellency	1-2.2
Severe-repellency	>2.2

Table 04 MED analysis

MED Result in molar concentration (M)						
Soil	Before treatment with enzymes	After treatment with Aspergillusfumigatus				
		3rd day	7th day	11th day	15th day	19th day
S1	6±1.0	4.53±0.02	4.07±0.07	3.55±0.04	3±0.2	2.56±0.03
S2	3.5±1.0	2.54±0.03	**2.05±0.05**	**1.5±1.0**	**1±0.15**	**0.53±0.01**
S3	4.5±0.1	3.08±0.03	2.53±0.02	**2.07±0.04**	**1.53±0.02**	**1.05±0.05**
S4	5.26±0.30	3.54±0.02	3.08±0.04	2.55±0.03	2.07±0.08	**1.56±0.02**

Discussion

Enzyme production

Solid state fermentation beneficial in achieving primary and secondary metabolites, the former are advantageous in obtain concentrated metabolites and subsequent purification procedures are not economical.Crude laccase from *Aspergillus niger* found to bio-alleviate more than 40% water repellency within 19 days of incubation at room temperature without addition of any externally added mediators. External mediators have been avoided in this experimental design because crude extracellular fractions contains an array of enzymes obtained by growing microbes over wheat bran as solid support have broader specificities.

Enzyme mediated post treatment of hydrophobic soils

One of the indices relating soil parameters such as infiltration, productivity, erodibility, capacity of the soil and soil quality is the presence of organic substances [16]. Infiltration is influenced by water flow influences, soil surface conditions, hydrophobicity, subsurface conditions, environmental factors such as frost, compaction etc. The presence of voids or pores is a critical parameter in deciding water infiltration in the soil. Smaller the pore, greater the suction force, facilitating water movement. As a thumb of rule, larger the particle size, enhanced water percolation, whereas very fine particles tend to drain poorly, but holds more water in the soil surface.

WDPT analysis

The order of decrease is hydrophobicity has been found to be S4 (44.44%)>S1 (36.07%)>S3 (23.07%)>S2 (22.22%) respectively based on WDPT analysis. The wetting front shape of water over the soil surface differentiates a soil between a hydrophilic and hydrophobic nature. As water travel through, air must either escape or be compressed below the infiltrating water, but generally, air escapes through larger pores reducing water infiltration.

MED analysis

The order of decreasing pattern based on MED test analysis indicate that S1 (42.66%)>S2 (29.66%), S3 (23.33%) and S4 (15.14%) of reduction in hydrophobicity respectively. Identification of the chemicals is a mandatory and accountable task for water repellency in the soil, as soil has an array of organic compounds has found to be occupied. MED analysis is a constructive and sensible method of evaluation of water repellency, because it is simple and can swiftly reproduce results than WDPT [8] [2].

Conclusion

- In general, a single particle of a hyrophobic soil have a negative charged ions in its surface. Hence water particle stick over the surface of each particle. It will never allow water particle to infiltrate or perculate. The permeaility character of such soil is low as water occupy the voids and bonded with particle surface.
- The crude enzyme is hyrophobic soil increase the permeability of soil by breakown the bond between soil particle and water.
- The enzyme influence increase the permeability upto 25% than untreated soil.
- The characteristic influence is high over 19 days of treatment.

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