Genetically Modification of Amylolytic Bacterial Consortium with Physical Mutagen (UV) for Bioremediation

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ABSTRACT

A genetically modified organisms (GMOs) or genetically engineered organisms (GEOs) are an organism whose genetic material has been altered using genetic engineering techniques. The use of genetic engineering to create organisms specifically designed for bioremediation has great potential. The goal in bioremediation is to stimulate microorganism with nutrients and other chemicals that will enable them to destroy the contaminants. The bioremediation systems in operation today rely on microorganisms' native to the contaminated sites, encouraging them to work by supplying them with the optimum levels of nutrients and other chemicals essential for metabolism. However, researchers are currently investigating ways to augment contaminated sites with nonnative microbes---including genetically engineered microorganisms, especially suited to degrading the contaminants of concern at particular sites. Bioremediation uses naturally occurring microorganisms to degrade various types of wastes.

Like all living creatures, microbes need nutrients, carbon, and energy to survive and multiply. Such organisms are capable of breaking down chemicals to obtain food and energy, typically degrading them into harmless substances such as carbon dioxide, water, salts and other innocuous products. Microbial bioremediation has proven to be an important remediation technology because it (a) destroys or immobilizes contaminants rather than transfers them from one environmental media to another (b) conserves limited financial resources due to shortened cleanup times and/or lower capital expenditures related to many other remediation technologies. Starch or amylum is a carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds, and the important sources of energy for microorganisms, plants and animals. The rapid growth of various types of chemical industries including the starch industries have resulted in the increased discharge of the effluent in water bodies which contain toxic chemicals and hazardous compounds. Amylolytic enzymes form a large group of enzymes

are produced by a great variety of living systems. Properties of enzymes hydrolyzing starch and related saccharides vary and more or less linked to the environmental niche occupied by the producing organisms, which is especially true for microbial amylolytic enzymes. The physical mutagens are fixed radiations of fixed specific wavelength and energy. Ultra violet light is a kind of radiation that can cause genetic mutation. Sometimes the mutant strains of bacteria show better enzymatic activity. So in present study researcher tried to generate mutant bacterial consortium for better amylolytic activity through UV radiation.

Keywords: Amylolytic Bacterial Consortium, Bioremediation, Genetically Modification, Physical Mutagen, UV-Rays, etc.

1. INTRODUCTION

Starch and its derivatives and hydrolysis products have important industrial applications.[1][2] Utilization of starch as a renewable raw material has increased considerable over the last decade.[3] To hydrolyze starch, organisms must produce amylase, an enzyme capable of cleaving α (1→4) glycosidic linkages between α-D-glucopyranosyl residues in the starch molecule. **Amylolytic** microorganisms commonly found in soil, freshwater reservoirs, and seawater hydrolyze starch into mono- and disaccharide units.

Mutation is changes in the DNA sequence of a cell's genome and are also caused by radiation. The radiation, a physical agent that changes the genetic materials usually DNA that increases the frequency rate of mutations in the organisms. The radiations have a fixed specific wavelength and energy. They include, heat, x-rays, UV-rays, ionizing radiation, gamma rays etc. Ultraviolet means beyond the violet in the electromagnetic spectrum, corresponding to light having wavelengths shorter than 4000Å (or 400 nm), and the radiation energies are from 3 eV to 124 eV.

It is so named because the spectrums consist of electromagnetic waves with frequencies higher than those that humans identify as the color violet. Ultraviolet light in wavelength of approximately 200 to 300 nm destroy microorganisms by damaging their DNA. It induces pyrmidine dimmers in the DNA that results in the distortion of the DNA molecule and which interferes in the replication and transcription of DNA molecule during protein synthesis and cause **mutation.** If mutation takes place in the critical genes death of the cell results unless the damage is repaired.[4] Thus the mutants are genetically modified organisms (GMOs). Sometimes these organisms produce more enzymes due to modification of their genome. These GMOs are also used for biodegradation of pollutants, and the approach is known as bioremediation.

In present study the amylolytic bacterial consortium which are previously isolated from waste water of Phagwara are exposed with UV-rays in different durations and the most efficient mutants are identified and separated for better enzymatic activity.

2. REVIEW OF LITERATURE

Sharifi-Yazdi and Darghahi (2006) worked on inactivation of pathogenic bacteria using pulsed uv-light and its application in water disinfection and quality control. The lethality of pulsed ultra-violet (UV) rich light for the inactivation of pathogenic bacteria has been investigated. A low pressure xenon filled flash lamps that produced UV intensities have been used. The pulsed operation of the system enable the release of electrical energy stored in the capacitor into the flash lamp within a short time and produces the high current and high peak power required for emitting the intense UV flash. The flash frequency was adjusted to one pulse per second. Several types of bacteria were investigated for their susceptibility to pulsed UV illumination. The treated bacterial populations were reduced and determined by direct viable counts. Among the tested bacteria *Pseudomonas aeruginosa* was the most susceptible to the pulsed UV- light with a 8 log10 cfu/ml reduction after 11 pulses, while the spores of *Bacillus megaterium* was the most resistant and only 4 log10 cfu/ml reduction achieved after 50 pulses of illumination. The results of this study demonstrated that pulsed UV- light technology could be used as an effective method for the inactivation, of pathogenic bacteria in different environments such as drinking water.[5]

Nahrstedt *et al.* (2005) studied the mutant strains of bacillus by introducing defined deletions in *recA* and an essential sporulation gene (*spoIV*), stable mutant strains of *Bacillus licheniformis* were obtained which are totally asporogenous and severely affected in DNA repair and thus being UV-hypersensitive. Studies on growth in various liquid media as well as on amylase production revealed no differences of the mutants when compared to the wild type. Hence, such genes appear to be suitable disruption targets for achieving passive biological containment in this industrially exploited species.[6]

Bayliss & Waites (1980) worked on the effect of hydrogen peroxide & ultraviolet irradiation on non-sporing bacteria. A kill of 99.99% was obtained in cell suspensions of *Escherichia coli* and *Streptococcus faecalis* by incubation with hydrogen peroxide 1.0% (w/v) for 75 and 180 min respectively. The same kill was produced by 30 s irradiation with ultraviolet (u.v.) light in the presence of hydrogen peroxide 1.0% (w/v). This simultaneous treatment with U.V. and hydrogen peroxide produced a synergistic kill at least 30-fold greater than that produced by irradiation of cell suspensions of *E. coli* with or without subsequent incubation with hydrogen peroxide.[7]

3. PLACE AND IMPORTANCE OF THE STUDY

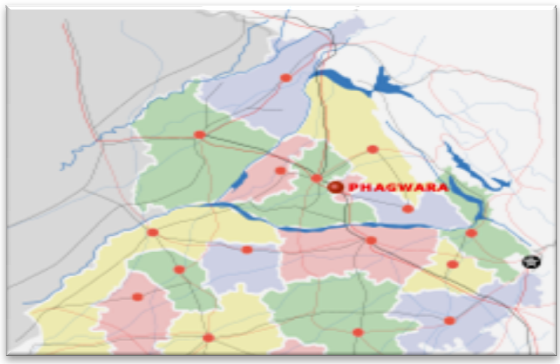

Fig.1 : Phagwara in Punjab. **Fig.2 : Sukhjit Starch & Chemical Ltd..**

Phagwara (31°13'11"N 75°45'36"E), is a city as well as a Municipal Council in District Kapurthala in one of the North Indian State named as Punjab. It was founded by Samrat Shah Jahan during the Mughal times. Phagwara is located on the Delhi – Amritsar National Highway and also on the main railway link between Amritsar – Delhi and Delhi – Jammu. It is located between two big cities Ludhiana (38 kms) and Jalandhar (21 kms). The main industries of Phagwara are Jagjit Cotton Textiles, Wahid Sugar Mill, Guru Nanak Autos, Dairy Industry, etc. They generate lot of industrial effluent which creates major problems in the city and surrounding areas. In presents study researcher tries to generate some mutant amylolytic bacterial consortium to degrade the pollution creating starch particles in waste water of Phagwara.

4. OBJECTIVES

Ob1. To **prepare** the Selective Starch Agar Media.

Ob2. To **inoculate** Amylolytic Bacterial Consortium in Petri Plates.

Ob3. To **give** UV-radiation Treatment for Various Durations of that Inoculated Bacterial Consortium in Petri Plates.

Ob4. To **identify** the Genetically Modified Amylolytic Bacterial Consortium for better Enzymatic Activity.

Ob5. To **separate** those Modified GMOs for further study.

5. EXPERIMENTAL MATERIALS AND METHODS

5.1 Preparation of Selective Starch Agar Media

Starch agar medium was prepared by dissolving peptone (5g), beef extract (3g), soluble starch (2g) and agar (15g) in 1000 ml of distilled water. Heat the medium till it melts and autoclave it at

121^0C for ten minutes. After 10 minutes pour the medium into the sterilized Petri plates (10-15ml) and allow it to solidify.

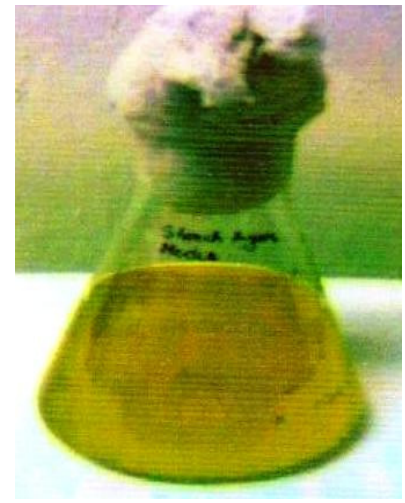

Fig.3: Preparation of Starch Broth Media. **Fig.4: Preparation of Starch Agar Media**

5.2 Flow chart for isolation of Amylolytic bacteria through selective media

Fig. 5: Inoculated Starch Broth Conical Flask were kept inside Shaker for overnight

Preparation of selective broth and divide into 20 ml in 10 conical flasks → Inoculate it with different strains → Incubate for 24 hours at 37°C → Prepare selective media plates → Inoculate them with broth of conical flask to corresponding plates.

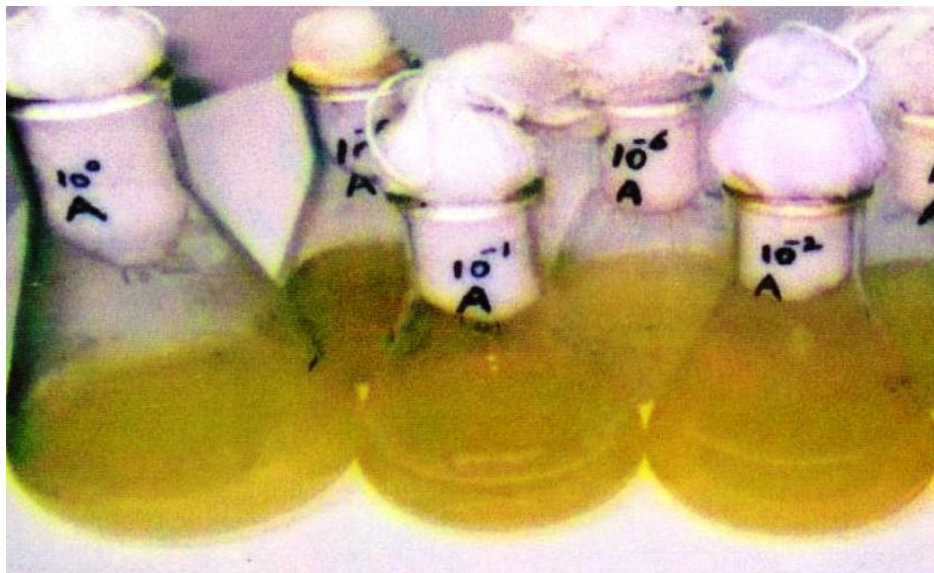

Fig.6: Conical Flasks Containing 24 hrs old culture in Starch Broth

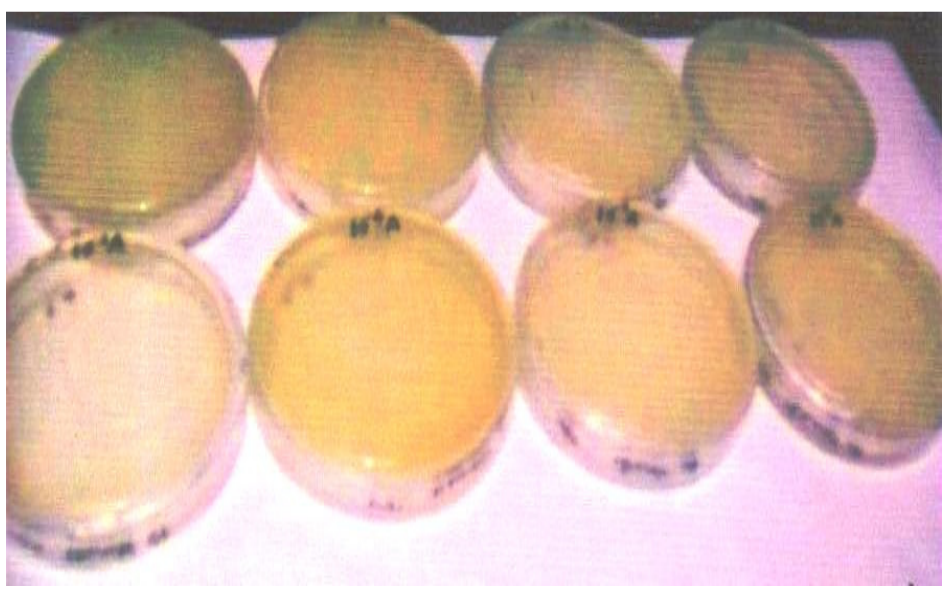

Fig.7: Inoculated Starch Agar Plates

5.3 To give UV-radiation Treatment for Various Durations of that Inoculated Bacterial Consortium in Petri Plates.

For the modification of bacteria, the physical mutagen was used that is U.V. radiation in inoculation chamber for 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 minutes with glass lids → After exposure those Petri Plates were kept in incubator for at least 24 hours → Repeated these steps for all consortium → Then on the basis of hydrolysis of media by the bacterial consortium the results were observed.

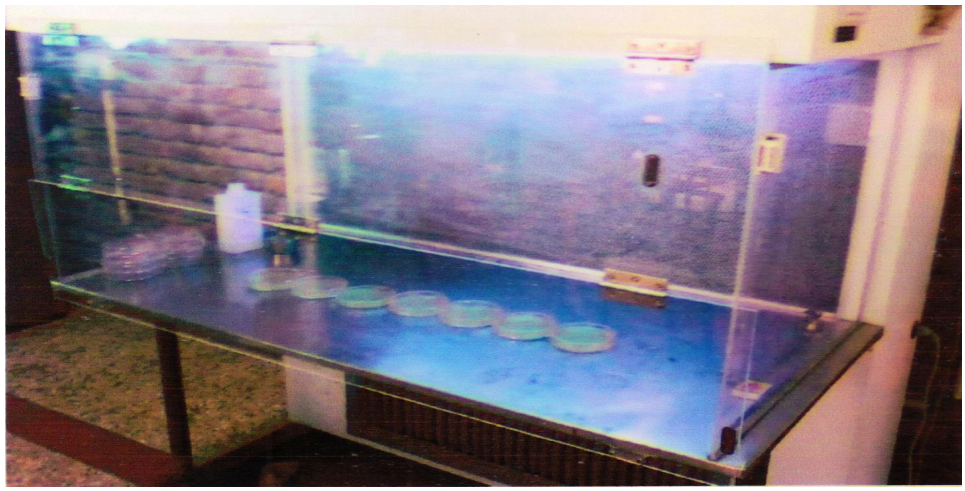

Fig.8: UV Radiation treatment of Inoculated Plates at different durations

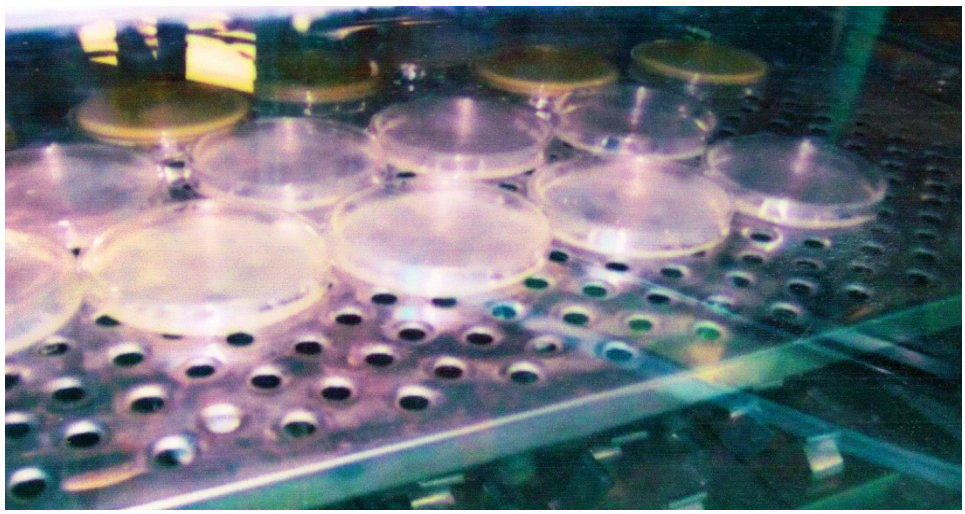

Fig.9: Plates were kept in Incubator for 24 hrs at 37°C