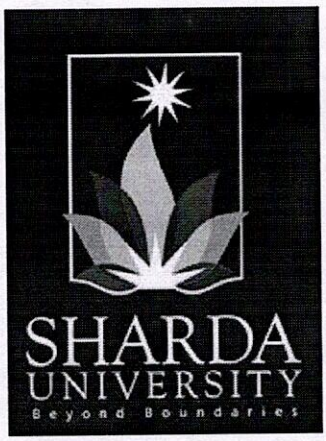
Use of *Bacillus subtilis* for the formation of self-healing concrete



By

Pragati Mahur- BTech Biotechnology (2016-2020)- 2016011680

Supervisor: Prof Shahana Majumder

CERTIFICATE

This is to certify that this project work entitled " Use of *Bacillus subtilis* for the formation of self-healing concrete" submitted to the Department of Biotechnology, School of Engineering & Technology, Sharda University, Greater Noida, is a record of research work carried out by-Ms Pragati Mahur under my guidance and supervision.

Place: Sharda University

Supervisor: S. Majumder

Date: April 29, 2019

INTRODUCTION

Bacillus subtilis is a gram-positive bacterium. It is also known as hay *bacillus*, grass *bacillus* or *Bacillus globigii*. It was firstly discovered by Christian Gottfried Ehrenberg in 1835, who named it *Vibrio subtilis*. Later it was renamed as *Bacillus subtilis* by Ferdinand Cohnin 1872. It got its name due to its Rod shape i.e. *Bacillus* and *subtilis* means slim, slender. They are most widely studied gram positive bacterium and considered as model organism in the study of Bacterial Chromosome replication and Cell differentiation.

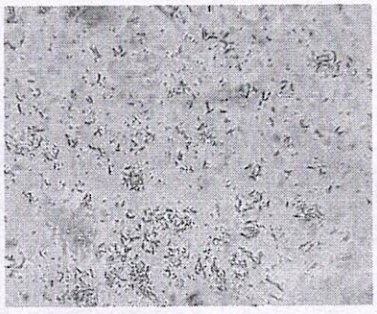


Figure 1. Shape of *Bacillus subtilis*

The figure shows the shape and structure of *Bacillus subtilis* under compound microscope (40X), stained with methylene blue.

Apart from being available in nature, *Bacillus subtilis* is one of the important organisms in gut microflora. The United State Food and Drug Administration (FDA) has classified *Bacillus subtilis* as a GRAS, i.e., Generally Recognized as Safe organism. That means this organism is safe to work with, and can be used in S1 laboratory.

Bacillus subtilis grows in irregular colonies, their colony size is large in size and have undulate margin. They have white and dull color and a dry texture.

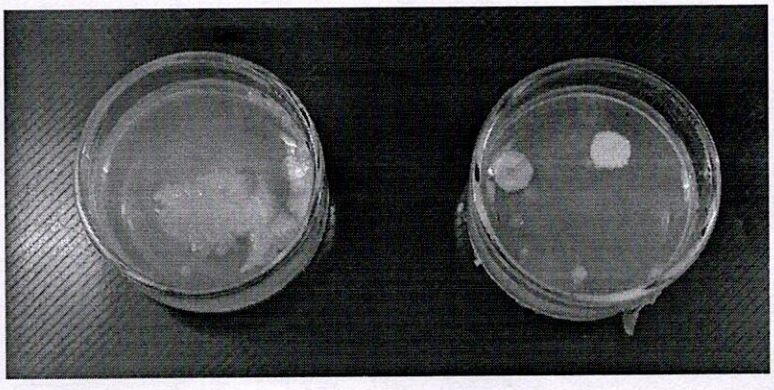


Figure 2. Colonies of *Bacillus subtilis*

Colonies of *Bacillus subtilis* grows in irregular shape and their boundary is wavy.

Bacillus subtilis can be found in variety of environment like terrestrial and aquatic. It may appear to be ubiquitous and widely adapted to grow in diverse condition within biosphere. However, it is observed that *B. subtilis* can form a highly resistant dormant spore in condition of lack of nutrient and other environmental stresses. The spores are easily dispersed by air. *B. subtilis* are most often in its vegetative form when they are in saprophytic nutrient but when nutrient

depletes, they once again get sporulated. After germination the cells forms bundled chain which moves on the surface in flagella-independent manner.

It is observed that *B. subtilis* can grow in close association with plant root surfaces. Rhizosphere of a variety of plant contain more *B. subtilis* than any other spore forming bacteria. Through these associations *B. subtilis* may promote plant growth.

Possible reasons for plant growth are:

A) It out competes other microbes that would have adverse effect on plant growth.
B) It activates host defense system so that plant is resistant to other pathogens.
C) It makes nutrients like phosphorus and nitrogen more readily available to plants.

During sporulation the rod-shaped *Bacillus subtilis* cell divides asymmetrically, forming two genetically similar daughter cells that later undergo different cell fates. *Bacillus subtilis* is naturally competent and often genes for spore formation are non-essential for growth and maintenance.

For the formation of reproducible and homogeneous batches, precise regulation of growth and sporulation parameters are of great importance. A single chemically defined media allows both the rapid growth and complete *B. subtilis* sporulation. This media contains only known quantities of chemically defined ingredients. Defined media do not contain any complex ingredient such as proteins, hydrolysates, animal-derived ingredient or constituent of unknown composition, all components of chemically defined media have a fixed and known structure, which helps in homogeneous culture formation of microorganism.

Self-healing concrete can pe produced by using metabolic activity of some bacteria, such as *Bacillus subtilis,* that converts organic compounds in presence of oxygen forming Calcium Carbonate or Limestone. Bacteria when added top concrete mix as healing agent can lead to limestone production using metabolic activities. This process also known as biomineralization, and can seal cracks of up to 0.8mm width which helps in waterproofing and increased frost damage resistance.

Biomineralization in nature happens in alkaline environment where the product of calcium and carbonate ion concentration exceeds the solubility product of calcium carbonate. Although the metabolic activity of these bacteria does not affect calcium ion concentration but it does affect carbonate ion concentration due to production of carbon dioxide by degradation of organic compounds. Carbon dioxide molecules are rapidly converted into carbonate ions in alkaline media in presence of calcium ion subsequently forming Calcium Carbonate mineral as precipitate. Bacterial driven production of substantial amount of calcium carbonate requires availability of suitable organic carbon source. When Calcium Lactate is present in concrete

matrix, it acts as carbon source, the biochemical reaction resulting in production of limestone is as follows:

$$Ca(C_3H_5O_3)_2 + 6CO_2 \longrightarrow CaCO_3 + 5CO_2 + 5H_2O$$

In the initial reaction bacteria mediate the conversion of one molecule of Calcium lactate into one molecule of calcium carbonate with the help of 6 molecules of oxygen, additionally 5 molecules of carbon dioxide and 5 molecules of water is also produced. When this reaction occurs within the concrete, the produced 5 carbon dioxide, reacts with present calcium hydroxide to produce additional 5 molecules of calcium carbonate:

$$5CO_2 + 5\ Ca(OH)_2 \longrightarrow 5CaCO_3 + 5H_2O$$

The sequential reactions thus yield 6 molecules of calcium lactate from one molecule of calcium lactate.

METHODOLOGY

1 SPORE PRODUCTION

1.1 Media Formulation

For the production of spore three concentration of Nutrient Broth was taken. 100%, 50% and 25% was made with following concentration:

	100%	50%	25%
Beef Extract	1g	0.5g	0.25g
Peptone	1g	0.5g	0.25g
NaCl	0.5g	0.25g	0.125g

*All the concentration is for 100 ml of solution.

Media was sterilized by steam sterilization method using autoclave. Repetitive autoclaving was used to ensure proper sterilization.

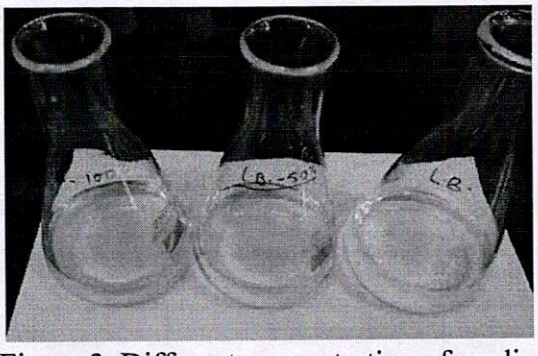


Figure 3. Different concentration of media

1.2 Inoculation

All the three flasks were inoculated with 200µl of *Bacillus subtilis* culture.

1.3 Incubation

After inoculation the flask was kept in rotatory shaker for three days at 37°C. After every 24 hours a small amount of broth was taken out and optical density was measured.

A small amount of broth was used for the slide preparation for the visualization of growth.

2 SPORE VIABILITY

2.1 Agar Preparation

100 ml of nutrient agar was prepared by following composition:

Constituents	Amount
Peptone	0.5g
Beef Extract	0.3g
Agar	1.5g
NaCl	0.5g

Above mentioned constituents were mixed in 100ml of water. The mixture was heated while stirring to fully dissolve all components. Autoclaving was done at 121° C for 15 minutes for sterilization. After sterilization the agar was allowed to cool and was poured into plates. The plates were left for solidification.

2.2 Inoculation and Incubation

After solidification spores from the growing media was streaked on the Agar plates. The lids were replaced and the plates were sealed.

The plates were kept for incubation at 37°C in BOD Incubator for 24 hours.

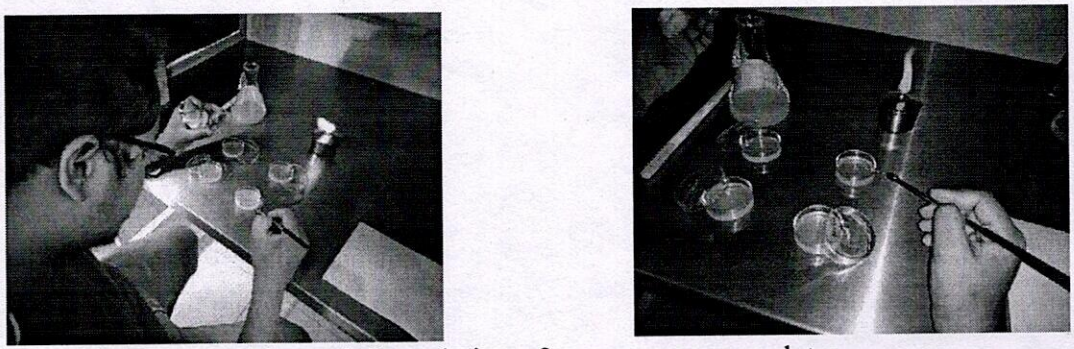


Figure 4. Inoculation of spores over agar plates

3 CALCIUM LACTATE CONVERSION

3.1 Media Preparation

100 ml of media was prepared with following composition:

Constituents	Amount
Beef Extract	1g
Peptone	1g
NaCl	0.5g

3.2 Calcium Lactate Mixing

Media was divided into 2 sets of 4 test-tubes. One of each was used as control, and in other Calcium Lactate was added in concentration of 2%, 5%, 10% respectively.

Other set of same concentration of Calcium Lactate was prepared with Water.

Autoclaving was done at 121°C for 15 minutes for sterilization.

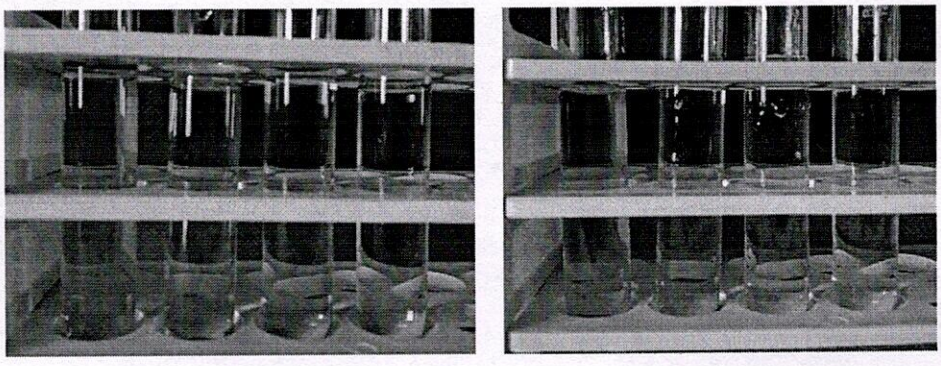


Figure 5. Calcium Lactate solution with Nutrient Broth and Water

3.3 Inoculation

After cooling the media 200µl of culture was inoculate in 4 tubes containing nutrient media and water. The mixture was mixed thoroughly.

Other set of media and water was inoculated with 50µg of spores and the mixture was mixed thoroughly.

3.4 Incubation

The test-tubes were incubated at 37°C for 3 days in rotatory shaker.

4 Calcium Carbonate Presence Test

After 3 days, the mixtures were centrifuged at 1000 rpm for 5 min. precipitate was collected in test tubes and 500µl of water was added.

1N HCl was added to the solution for testing the presence of Calcium Carbonate in the mixture.

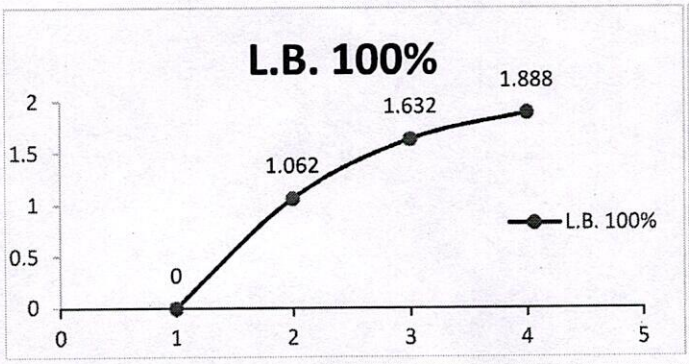
RESULTS

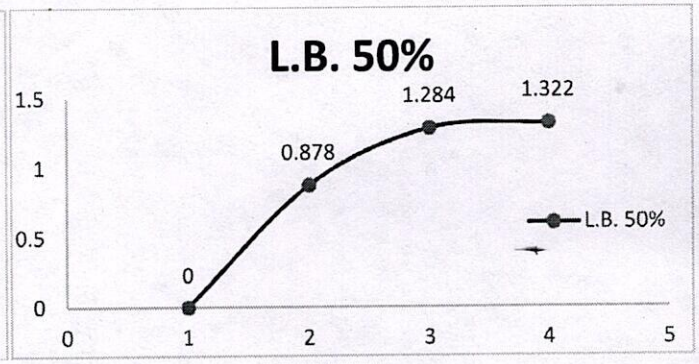
1 Spore Production

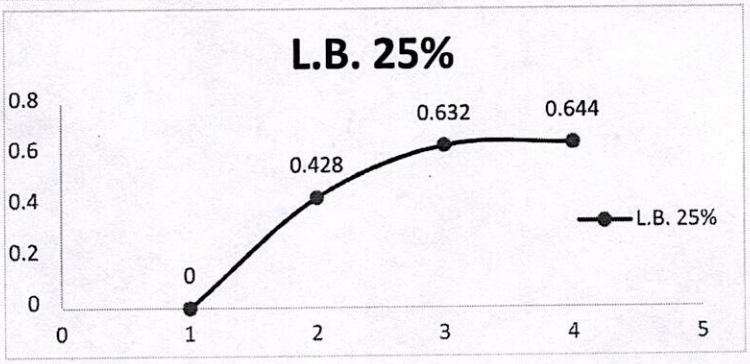
Followings are the findings of the Optical Density measurement:

	DAY 1	DAY 2	DAY 3	DAY 4
L.B. 100%	0	1.062	1.632	1.888
L.B. 50%	0	0.878	1.284	1.322
L.B. 25%	0	0.428	0.632	0.644

From the findings it was concluded that, the growth was fastest and maximum in the media with 100% concentration, while the spore formation was fastest in the media with 25% concentration. The spore concentration was observed maximum in 100% on the end of third day.







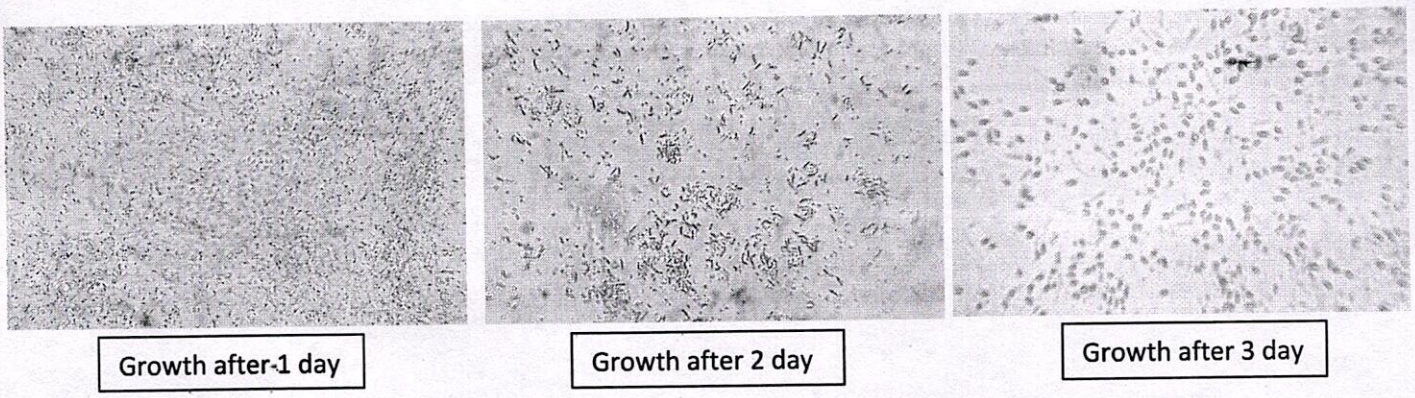


Figure 6. Growth of Culture in three days

2 Spore Viability Test

After 24 hours of incubation it was observed that the spores were able to regenerate and form colony over the agar plate.

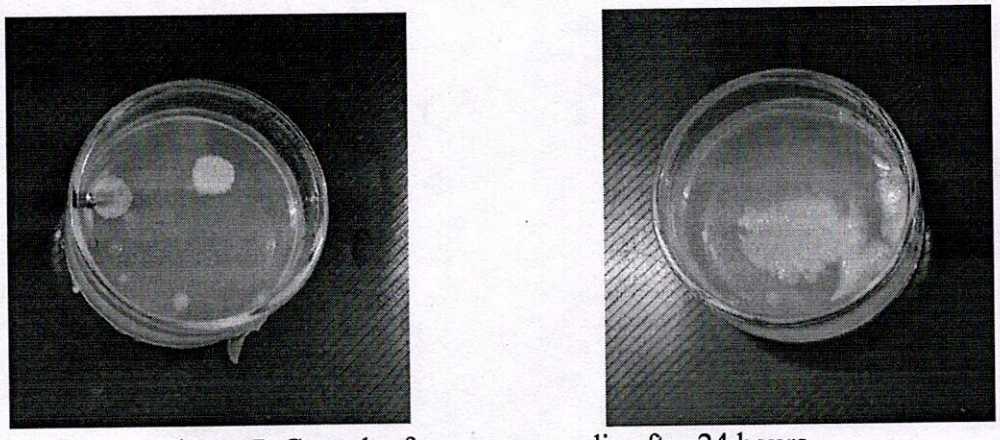


Figure 7. Growth of spores on media after 24 hours

The growth of *Bacillus subtilis* spores on the agar plates were showing undulated margin. They formed white colonies over the media.

3 Calcium Carbonate Formation

After 3 days of incubation it was observed that there was a small amount of Calcium Carbonate was precipitated at the bottom of the test tubes, a ring of solid Calcium Carbonate was also observed on the top of the broth.

Same observation was found in the test tubes containing water, this proved that the spores are capable of conversion of calcium lactate to calcium carbonate in the presence of water.

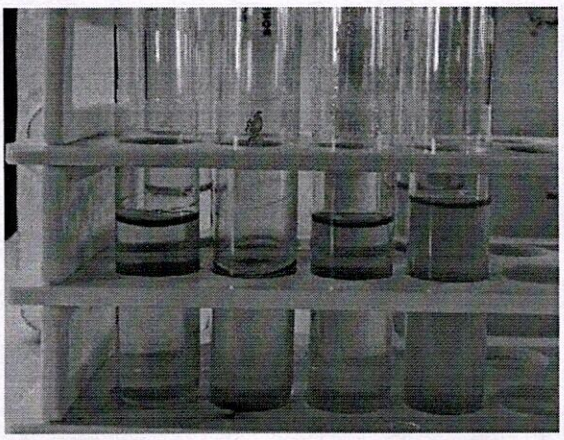


Figure 8. Calcium Carbonate ring on broth

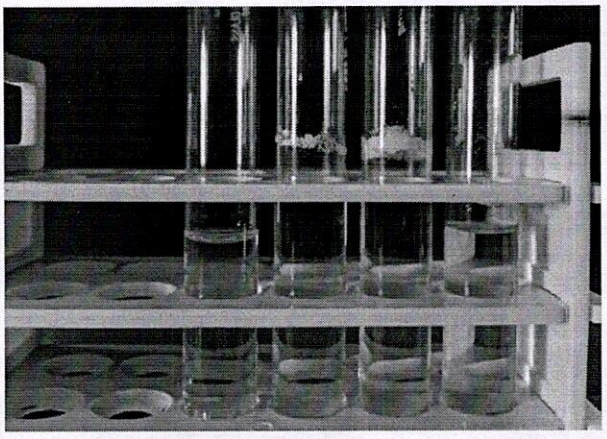


Figure 9. Calcium Carbonate ring on water

4 Test for the presence of Calcium Carbonate

When 1N HCl was added to the solution of precipitate formed CO_2 was evolved with small fizz. Confirming the presence of $CaCO_3$.

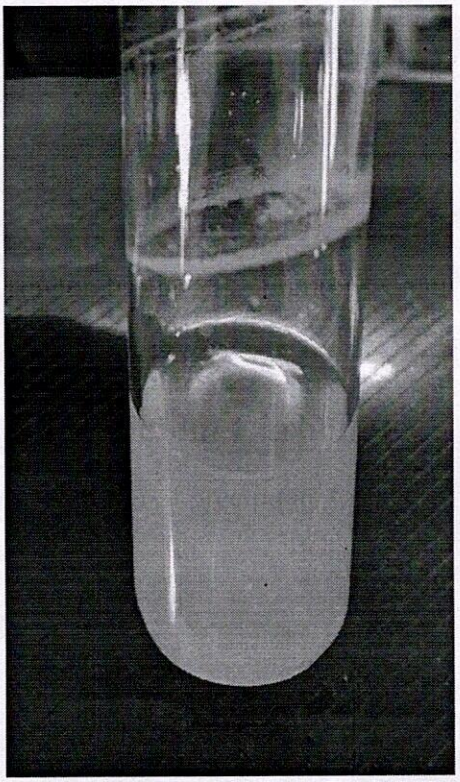


Figure 10. Fizz with evolution of CO_2 confirming the presence of $CaCO_3$.

CONCLUSION

The effect of calcium lactate on biomineralization was tested in this study. The bacteria were added to the nutrient broth and water both in spore form and culture form. The results verified that addition of bacteria in both the forms yields in the precipitation of $CaCO_3$. The results show that calcium lactate should be added in a lower concentration to the concrete so as to improve the compressive strength.

REFERENCES

Enhanced Spore Production of *Bacillus subtilis* Grown in a Chemically Defined Medium by S. M. S. Monteiro, J. J. Clemente, M. J. T. Carrondo, A. E. Cunha

Effect of calcium lactate on compressive strength and self-healing of cracks in microbial concrete by Kunamineni VIJAY, Meena MURMU

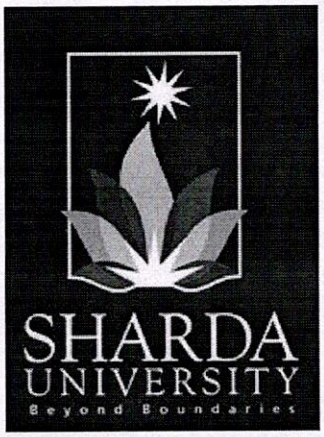
Spore formation in Bacillus subtilis by Irene S. Tan, and Kumaran S. Ramamurthy

Earl, A. M., Losick, R. & Kolter, R. Ecology and genomics of Bacillus subtilis. Trends Microbiol. 16, 269–275 (2008).

Driks, A. (1999). Bacillus subtilis spore coat. Microbiol. Mol. Biol. Rev. 63, 1 – 20.

McKenney PT, Driks A, Eichenberger P. The Bacillus subtilis endospore: assembly and functions of the multilayered coat. Nat Rev Microbiol. 2013; 11:33–44.

Bioremediation of textile dyes by *Aspergillus niger*



By

Abhishek Sharma, B-Tech (2016-2020), System Id - 2016007326

Supervisor: Prof Shahana Majumder

CERTIFICATE

This is to certify that this project work entitled "Bioremediation of textile dyes by *Aspergillus niger*" submitted to the Department of Biotechnology, School of Engineering & Technology, Sharda University, Greater Noida, is a record of research work carried out by- Abhishek Sharma under my guidance and supervision.

Supervisor: S. Majumder

Place: Sharda University

Date: April 29, 2019

1. Introduction

Colors have always been part of human life and culture. Today natural pigments have been substituted by synthetic dyes that usually end up threatening nature itself by polluting the environment. To what extent can we use chemistry and biology to minimize the environmental impact of the textile industry? During the dyeing process a certain amount of dye, 2 to 50 percent, does not bind to the fabric and is washed away. Companies are working on improving this process. Meanwhile something needs to be done to avoid the dye effluents ending up untreated in the natural environment. Discharge of untreated textile effluents in the natural environment is a widespread problem where the production of textiles is concentrated, for example in the south of Asia. This is caused by lack of care and awareness, and defective regulations. Much research has been performed to reduce the environmental impact of the dyeing process units and appropriate technologies are now available. However, most units are very small, sometimes just the size of a family company, and they do not have money for treating their effluent. Why are colors threatening the environment? Aquatic plants and algae are indispensable for aquatic ecosystems. They use carbon dioxide and light to produce oxygen and food, which are consumed by higher organisms like fish. The presence of colors in water tends to stop the penetration of sunlight, hence the gradual decrease in living organisms in rivers. Dyes can be harmful for humans, acting as irritants for eyes and skin. Moreover, the problem does not disappear with the color! In fact, in time the dye molecules will eventually break down and become colorless. The resulting compounds are even more harmful than the original dyes and in some cases carcinogenic. Researchers all around the world are looking for an optimal treatment for textile effluents.

Common solutions for wastewater treatment include aerobic biological processes, adsorption and coagulation. Can these be used for textile effluents as well? Microorganisms are the main actors in aerobic biological processes because they remove nutrients like organic carbon, nitrogen and phosphorous from water without the addition of chemicals. The downside is that a lot of oxygen is needed for this process and excess sludge that may contain pollutants is generated and needs to be handled in an appropriate way. In addition, most dyes used in textile industry are not fully degradable and the treated water could contain dangerous amines. Adsorption and coagulation remove dyes and other pollutants by transferring them from a liquid phase, the wastewater, to a solid

phase, the adsorbent or coagulant. This is however only a temporary solution. In fact, the resulting sludge produced contains dyes and needs to be properly disposed of, with additional costs. Dyestuff and other chemicals found in textile effluents are no exception. However, industries are often not ready to put money into waste handling, something that will not make them richer. Therefore, the future of wastewater should be centered on the concept of water reuse, which is profitable for industries as well as the environment.

Biological processes are often the preferred choice for treatment of wastewater. They are considered to have low environmental impact and costs in comparison with other types of treatments, because they require only slight or no addition of chemicals and reasonable amounts of energy. They are based on the ability of microorganisms to transform the contaminants and use them as sources of energy, carbon and other minerals which are essential for their growth.

2. Review of Literature

The textile industry is a water intensive industry (Brik et al., 2006; Badani et al., 2005; Chakraborty et al., 2003; Barclay & Buckley, 2002) producing wastewater containing varying concentrations of both organic and inorganic compounds (Libra & Sosath, 2003). Treating industrial textile wastewater is complicated due to the high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS) and nonbiodegradable nature of the organic dyes present in the wastewater (Badani et al., 2005; Kim et al., 2002). When treating textile wastewater for re-use by the industry colour removal, as well as total suspended solids (TSS), BOD and COD reduction must be addressed in the primary treatment stage. The most commonly used treatment methods for textile wastewater are the following : 1) physico-chemical treatment using lime and ferrous/alum that generates large quantities of hazardous sludge and is ineffective in the removal of colour, TSS, BOD and COD; 2) biological treatment with aeration in order to reduce BOD and COD levels; 3) chlorination that successfully removes colour and reduces BOD and COD, though this method produces chloro-organic compounds that are potentially carcinogenic and therefore not eco-friendly; and 4) ozonation with or without ultraviolet irradiation (You & Teng, 2009; González-Zafrilla et al., 2008; Badani et al., 2005; Chakraborty et al., 2003; Kural et al.,2001; Gupta et al., 2000; Slokar & Le Marechal, 1998). The key factors affecting the reclamation of treated wastewater are suspended solids (SS), turbidity, colour and non-biodegradable substances.

Therefore, advanced treatment processes are required to improve the quality of the treated wastewater up to re-use criteria (Feng et al., 2010).

2.1. Textile Dyes

Dyes are classified by their chemical type (azo, anthraquinone, indigo,…) and by their method of application (acid, direct, reactive,…). Azo dyes are the most widespread dyes and also the ones that raise the biggest concern in terms of mutagenicity and carcinogenicity (O'Neill et al. 1999).

Synthetic dyes may be toxic to humans by causing allergies and skin- or lung sensitization. In addition, some dyes and their derivatives are mutagenic and have therefore been indexed (European Parliament 2002). However, some carcinogenic dyes may still be in use and the products dyed with them may be sold also where the compounds are banned. The main concern is for the workers, but as a consequence of inadequate disposal of wastewater and waste material there can also be consequences for living organisms that come into contact with such wastes.

Dye molecules are comprised of two key components: the chromophores, responsible for producing the color, and the auxochromes, which can not only supplement the chromophore but also render the molecule soluble in water and add an enhanced affinity toward the fiber (Christie, 2001).

Azo dyes are the most widespread dyes and also the ones that raise the biggest concern in terms of mutagenicity and carcinogenicity (O'Neill et al. 1999). Their main feature is the presence of one or more azo bonds (-N=N-) linking aromatic structures. The azo bond can be cleaved chemically or biologically to release aromatic amines, which are often more toxic than the mother compound and mutagenic. Azo dyes have a more intense color than anthraquinone dyes and this advantage, together with the fact that they are relatively cheap to produce, has resulted in their dominance of the market (Koh 2011).

Weber and Wolfe (1987) found that azo-and nitrocompounds are reduced in sediments and in the intestinal environment, resulting in the regeneration of the parent toxic amines, also Chang and Lin (2001) stated that the release of residual azo dyes into industrial effluents deteriorates the water quality, and may cause a significant impact on human health due to mutagenic or carcinogenic effects of some azo dyes or their metabolites. Thus, particularly in the case of azo dyes, effluent treatment becomes a serious issue because of their negative impact on water

ecosystems and human health, especially that thousands of azo dyes have been developed for use on every type of fiber (Reife and Othmer, 1993 and Yesiladali et al., 2006).

2.2. Effect of textile wastewater discharge on the environment

There are main three stages involved; they are spinning, knitting or weaving and wet processing the later involves many steps like sizing, desizing, scouring, bleaching, mercerizing, dyeing, printing and finishing. Each of these operations generates huge amounts of wastewater and pollution from wet processing steps desizing is one of the largest sources of wastewater pollutants and often contributes up to 50% of the Biological Oxygen Demand (BOD) load in wastewater. The scouring process also has a high BOD and also uses the highest volumes of water in the preparatory stages. The major pollution issues in the bleaching process are chemical handling, water conservation and high pH values. Also, using pentachlorophenol (PCP) during scouring, bleaching, dyeing and printing which is removed from the fabric and discharged into the wastewater. It is toxic due to its relative stability against natural degradation processes and it is also bioaccumulative (EEAA, 2003).

But the majority of wastewater containing residual dyes is generated after dyeing and printing. Colored wastes reportedly contribute about 10-30% of the total BOD and in many cases reach 90%. Dyes also contribute about 2-5% of the Chemical Oxygen Demand (COD), while dye bath chemicals contribute about 25-35%. In addition to the high BOD and 10 Literature Review COD values of dyes, toxicity to aquatic organisms and fish toxicity have also been reported.

Heavy metals present in textile wastewater may typically include copper, cadmium, chromium, nickel and zinc. Toxic effects of heavy metals to animal and aquatic life are dependent on the physico-chemical interaction. Dyes and pigment from printing and dyeing operation are the principal sources of colors in textile effluent. Finishing processes typically generate wastewater containing natural and synthetic polymers and a range of other potentially toxic substances (EEAA, 2003).

It is estimated that 280.000 tons of textile dyes are discharged every year in such industrial effluents worldwide (Maas and Chaudhari, 2005). Direct discharge of these effluents causes formation of toxic aromatic amines under anaerobic conditions in receiving media. In addition to their visual effect and their adverse impact in terms of COD, many synthetic dyes are toxic, mutagenic and carcinogenic

(Jin et al., 2007), therefore, water pollution control is currently one of the major areas of scientific activity.

Effluents from the textile industries containing dyes are highly colored and are therefore visually identifiable (Kilic et al., 2007). The complex aromatic structure of the dyes is resistant to light, biological activity, ozone and other degradative environmental conditions, thus conventional 12 Literature Review wastewater treatment remains ineffective (Kaushik and Malik, 2009). Also, anionic and non-ionic azo dyes release toxic amines due to the reactive cleavage of azo groups (Joshi et al., 2004). Presence of heavy metals like chromium, cobalt and copper in wastewater is also an environmental concern (Freeman et al., 1996). Up till now scientists have been trying to develop a single and economical method for the treatment of dyes in textile wastewater but still it remains a big challenge (Dos Santos et al., 2007 and Kaushik and Malik, 2009).

2.3. Biological treatment of waste water

Since the removal of dyes from effluents by physicochemical means are often very costly, though efficient accumulation of concentrated sludge creates a disposal problem. Thus, there is a need to find alternative treatments that are effective in removing dyes from large volumes of effluents, low in cost and technically attractive (Dias et al., 2003). Biological methods being cheap and simple to use are resorted to as the proposed solution. The ability of microorganisms to carry out dye decolorization has received much attention and is seen as a cost-effective method for removing these pollutants from the environment. Lately, fundamental work has revealed the existence of a wide variety of microorganisms capable of decolorizing wide range of dyes (Robinson et al., 2001).

Microbial decolorization involving suitable bacteria, algae and fungi has attracted increasing interest (McMullan et al., 2001), these microorganisms are able to biodegrade and/or bioabsorb dyes in wastewater (Fu and Viraraghavan, 2001). Efforts to isolate bacterial cultures capable of degrading azo dyes started in the 1970's with report of a Bacillus subtilis (Horitsu et al., 1977), followed by numerous bacteria: Aeromonas hydrophila (Idaka and Ogawa, 1978), Bacillus cereus (Wuhrmann et al.,1980), Pseudomonas strains (Kulla, 1981), Proteus mirabilis (Chen et al., 1999) and Mycobacterium avium (Jones and Falkinham, 2003).

Algae have been suggested to remove color from textile wastewater (Tarlan et al., 2002). The potential use of commonly available green algae belonging to species Spirogyra was investigated as viable biomaterials for biological treatment of simulated synthetic azo dye (reactive yellow 22) effluents, their ability to remove color was dependent both on the dye concentration and algal biomass (Mohan et al., 2002).

A number of simple azo dye was degraded in liquid aerated batch cultures by a strain of yeast Candida zeylanoides (Martins et al., 1999). Only limited studies about yeast decolorization were reported, the ability of Kluyveromycse marxianus IMB3 to decolorize remazol black b dye was investigated (Meehan et al., 2000).

2.4. Role of Fungi in treatment of textile waste water

The role of fungi in the treatment of wastewater has been extensively investigated (Azmi et al., 1998; Coulibaly et al., 2003 and Brar et al., 2006). Fungi have proved to be a suitable organism for the treatment of textile effluents and in dye removal (Banat et al., 1996). The fungal mycelia have an additive advantage over single cell organisms by solubilising the insoluble substrates by producing extracellular enzymes, due to an increased cell-to-surface ratio; fungi have a greater physical and enzymatic contact with the environment. The extracellular nature of the fungal enzymes is also advantageous in tolerating high concentrations of the toxicants (Kaushik and Malik, 2009).

Many genera of fungi have been employed for the dye decolorization either in living or dead form. Based on the mechanism involved through biodegradation, biosorption and/ or bioaccumulation. Biodegradation is an energy dependent process and involves the breakdown of dye into various byproducts through the action of various enzymes. Biosorption is defined as binding of solutes to the biomass by processes which do not involve metabolic energy or transport, although such processes may occur simultaneously where live biomass is used. Therefore, it can occur in either living or dead biomass (Tobin et al., 1994). Bioaccumulation is the accumulation of pollutants by actively growing cells by metabolism (Aksu and Donmez, 2005).

Several fungal cultures capable of decolorization have been isolated by Ohmomo et al. (1985) and were identified as Coriolus versicolor, Mycelia sterilia and *Aspergillus fumigatus*. Several other brown rot fungi capable of decolorizing a

wide range of structurally different dyes were also isolated and were found to be more effective than *Phanerochaete chrysosporium* (Knapp et al., 1995).

Several fungal cultures capable of decolorization have been isolated by Ohmomo et al. (1985) and were identified as Coriolus versicolor, Mycelia sterilia and *Aspergillus fumigatus*. Several other brown rot fungi capable of decolorizing a wide range of structurally different dyes were also isolated and were found to be more effective than *Phanerochaete chrysosporium* (Knapp et al., 1995).

Also, there are various fungi such as *Aspergillus niger* (Fu and Viraraghavan, 1999, 2000), *Rhizopus arrhizus* (Zhou and Banks, 1991, 1993), *Rhizopus oryzae* (Gallagher et al., 1997) which can also decolorize and/or biosorb diverse dyes. Aspergillus species are a ubiquitious group of filamentous fungi which are commonly isolated from soil, plant debris and indoor air environments, *Aspergillus niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil (Bennett, 1979).

Aspergillus niger grows rapidly on a variety of artificial substrates producing colonies which consist of a compact white or yellow basal felt covered by a dense layer of dark brown to black conidial heads, their mycelial hyphae are septate and hyaline(Frisvad et al., 1990). Many Aspergillus enzymes are used in brewing and textile industries. Several researches suggest that Aspergillus fungi could be used to remove toxic and radioactive metals from the environment.

Aspergillus niger fermentation is generally regarded as safe (GRAS) by the Food and Drug Administration (FDA), also *Aspergillus niger* is used to test the efficacy of preservative treatments (Jong and Gantt, 1987). In addition, *Aspergillus niger* has been shown to be exquisitely sensitive to micronutrient deficiencies prompting the use of *Aspergillus niger* strains for soil testing (Raper and Fennell, 1965).

3. OBJECTIVE

3.1 Bioremediation of textile dyes by *Aspergillus niger*.

3.2 To develop an efficient inoculation method for industrial use.

4. MATERIALS AND METHOD

4.1.1) Preparation of Potato Dextrose Agar and Potato Dextrose Broth.

i) Take 250 gram potatoes, cut in small pieces.

ii) Boil them in water and filter the extract.

iii) Add and dissolve 20grm dextrose to it.

iv) Make its volume up to 1 liter by adding distilled water.

v) Put few volume of this solution to the 6 conical flasks.

vi) Take 200ml of solution and add 4grm agar in it, heat this mixture till agar dissolved

vii) Pour it in 20 test tubes.

viii) Autoclave the test tubes and conical flasks.

ix) Make the slants by placing the test tubes in a slant position immediately after autoclave.

4.2) Revival of culture

Revive the culture that has taken from ITCC by sub culturing in Potato dextrose agar.

4.3) Addition of Dye (Alizarin Yellow/ Mordant orange 1)

Add 0.06 grams of dye in each conical flask containing 100 ml PDB and mix it.

4.4) Autoclave the luffa and inoculate it with *Aspergillus niger*.

Fig1: Autoclaved luffa

4.5) Introduction of luffa containing *Aspergillus niger* into the media in each conical flask.

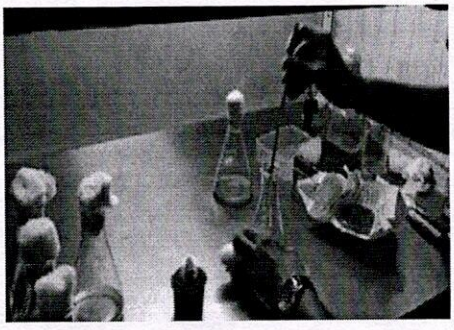


Fig2: Introduction of luffa into PDB

4.6) Place 3 conical flasks in static incubator and other 3 in shaker incubator for 3 days at 33^{0}C.

4.7) Filter the culture of all conical flasks after taking out the luffa from both types of conical flasks with the help of filter paper.

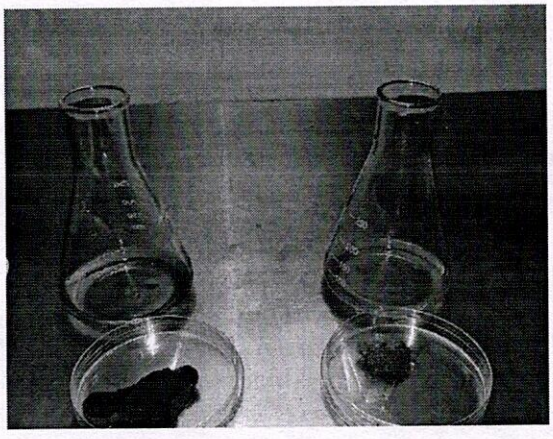


Fig 3: luffa from both the samples

4.8) Study the effect of fungal growth and estimate the dye absorption by taking O.D at 450 nm using spectrophotometer.

RESULT

Fig: 4