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Enhancing the Structure of Sandy Soil by Biological Molecules: An Innovative Approach to Water Conservation in Newly Reclaimed Desert-Agricultural Land

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ABSTRACT

The main aim of this study was to enhance the water holding capacity of the reclaimed desert sandy soil using an environmentally friendly biological technique that would minimize the loss of irrigation water from plant root zone. by downward seepage. Two environmentally friendly bacteria (*Azotobacter chroococcum* and *Lactobacillus fermentum)* capable of producing insoluble polysaccharides as a soil pores plugging agent were selected. The polysaccharides production efficiencies of these bacteria were evaluated. The effectiveness of the polysaccharides in enhancing the water holding capacity of the soil was evaluated. The result showed that the sandy soil used in this study had a particle density of 2.58 g/cm^3, a bulk density of 1.6 g/cm^{3}and a porosity of 37.98 %. The particle size varied from 0.150 to 2.000 mm with most of the soil particles having a diameter within the range of 0.425-0.850 mm indicating that the soil was free of silt and clay. This soil had a loose texture, high infiltration rate and low water holding capacity. *Azotobacter chroococcum* and *Lactobacillus fermentum* were capable of producing levan from sucrose. The levan yield was 0.248 glevan/g sucrose (62.78% of theoretical yield) and 0.371 g levan/g sucrose (93.92% of theoretical yield) for *Azotobacter chroococcum* and *Lactobacillus fermentum,* respectively. The cell yield was 0.074 g cell/g sucrose (47.69 % of theoretical yield) and 0.062 g cell/g sucrose (56.92 % of theoretical yield) for *Azotobacter chroococcum* and *Lactobacillus fermentum,* respectively. The polymer was effective as a plugging agent to plug the pores of the high permeability sandy soil. The results showed that increasing the concentration of bacteria had no significant effect on the amount of leachates collect from the soils treated with both bacteria. However, the leachates collected from the soils treated with *Azotobacter chroococcum* were much larger than those collected from the soils treated with *Lactobacillus fermentum.* Also, the leachates collected from the control (soils received no bacterial treatment) were much larger than soils treated with both bacteria. These microorganisms were suitable for production of levan from sucrose, fixing nitrogen in soil and producing growth hormones, thereby improving the water holding capacity of the soil and enhancing its nutrient content and stimulating plant growth.

Keywords: Sandy soil, Particle density, Bulk density, Porosity, Water holding capacity, Infiltration, Seepage, Bio-cementation, Bio-logical sealing

INTRODUCTION

Egypt is a transcontinental country situated mostly in north-eastern Africa, with the Sinai Peninsula in Western Asia. Egypt has a coastline at the Mediterranean Sea in north, and the Gulf of Suez and the Red Sea in east. The country lies in the dry arid region except for the northern part which enjoys a Mediterranean climate during winter (December-March) which is cool, windy and humid, with occasional rains. Summer in Egypt (June-September) is very dry with extremely hot temperatures into the 32-38°C, sometimes breaking into 48°C. Shoulder seasons (April-May and

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October-November) are particularly pleasant months but with no rain. Egypt receives 20-200 mm of annual precipitation along the narrow Mediterranean Coast and nearly 0 mm in the central and the southern parts of the country [1, 2].

The total land area of Egypt is 1,000,450 km^2, of which 32,425 km^2(3.24%) is the Delta which is made of silt deposits carried by the River Nile and in which is most agricultural land and live most of the population. The Western Desert of Egypt (681,000 km^2 or 68.07%) is an area of the Sahara which lies west of the River Nile up to the Libyan border, and from the Mediterranean Sea to the border with Sudan. The Eastern Desert (223,000 km^2 or 22.29%) extends east from the Nile to the Red Sea, and from the Mediterranean Sea to the border with Sudan. The area of Sinai is 61,000 km^2 (6.10%) and Northern Lakes cover an area of 3025 km^2 (0.30%) and is main source of aquaculture in Egypt. Generally, deserts are barren areas of landscape **(Figure 1)** where little or no precipitation occurs and, consequently, living conditions are hostile for plant and animal life. With 96% of Egypt's land is uninhabitable desert (never receives any rain) in both sides of the Nile, the population is concentrated around the narrow Nile Valley and Nile Delta, with smaller numbers along the Mediterranean and Red Sea coasts [3].

Egypt's worrying population boom poses very real dangers to the economic development of the country and is considered as a major challenge to the government. In 2000, the United Nations estimated that Egypt's population would hit 96 million in 2026. However, in 2017, there were some 104.5 million Egyptian, of which 9.5 million lived outside the country. With current population growth rate (2.6 million babies born in 2016)), Egypt's population is expected to grow to 128 million by 2030 [4]. According to Egypt's Statistical Agency, the population growth rate must be one-third that of economic growth to prevent living standards from deteriorating [5]. Once the breadbasket of the Roman Empire, Egypt began to import large quantities of wheat in the 1980s and is now importing 50% of its food [6].

The quest to bring desert land under cultivation has been a cornerstone of Egyptian Government Agricultural Policy since the 1952. The total area reclaimed reached 1.92 million *feddans* (*feddan*=0.42 hectare) in 1987. By 2002, the total reclaimable land was estimated at 2.8 million *feddans* [7]. However, the increase in agricultural land has not kept pace with the population increase in Egypt since 1950's. As a result, the country is facing unprecedented challenges as the agricultural lands are increasingly strained due to urban expansion and depletion of scarce water resources as the Nile faces upstream challenges with Ethiopia building Africa's largest dam [8]. Land reclamation in the Egyptian context means converting desert areas into agricultural land by extending water canals into the desert, enhancing soil fertility, and providing infrastructure for new village construction. If the unlimited desert sandy soil can be improved and provided with water, it can grow a lot of food for the growing population.

Therefore, in 2013, the Egyptian Government began an effort to reclaim approximately 1.5 million *feddan* of desert lands for agricultural use as a first stage of a major project aiming at the reclamation of 4 million *feddan*. Due to Nile water shortage, ground water will be used to irrigate 1,322,000 *feddan* (88.5%) and surface water will be used to irrigate 172,000 *feddan* (11.5%). The hope is that, with new wells, desalination plants and better infrastructure (new towns), farmers will be able to grow more wheat [2]. Example of this new development is the land reclamation project that began in 2015 in the Farâfra Depression (980 km^2) in Western Desert. The white desert of Farâfra **(Figure 2)** has been converted into agricultural land capable of producing wheat, potato, radish and other produces **(Figure 3)**. By adding the new farmland to Egypt's current 8.4 million *Fedde*n, it is hoped to free the population from the narrow confines of the Nile Valley and have the capacity to meet food production needs [9].

Figure 1. Nature of Eastern and Western deserts of Egypt.

Egypt desert soils originated by mechanical disintegration and wind deposit. They are mostly loamy sand (of 95-97% sand and 3-5% clay). These soils are coarse, porous and well-drained and have a red to brown color. They contain salts and are high in potassium, phosphorus and nitrates. These soils have very low moisture, very low organic matter and a basic pH (7.5-8.0). Generally, the sandy soils of Sahara are one of the poorest types of soil for growing plants because of their very low nutrients and very poor water holding capacity [10-14]. In addition, surface irrigation in this dry climate can cause the water to evaporate very quickly leaving salts behind on the soil surface causing salinization. Furthermore, water uses (agricultural, industrial, municipal, transportation and electricity generation) and management in Egypt are very complex and there is a great deficit between the demand and supply [15]. Therefore, it is important to consider (a) water conservation through use of new irrigation technology, (b) new water sources such as desalination and municipal wastewater treatment and reuse and (c) improving the quality of the soil and its water holding capacity [4,7,16].The farmer are already adopting new irrigation technology to conserve water [7] and the government has imparked on major desalination, and wastewater treatment and use projects as well as drilling wells for underground water [17]. However, building an adequate soil structure in the newly claimed land is still a major challenge.

In order to improve the soil properties, farmers are planting crops that fixes nitrogen such as alfalfa, but this process is unduly time-consuming for many farmers [4]. There are, however, several other techniques for land improvement including: (a) addition of biochar which significantly and permanently increase soil cation exchange capacity (the soil's ability to hold nutrients), creates habitats for beneficial microbes and increases water retention [18-22], (b) addition of organic matter such as well-rotted manure or finished compost which decomposes quickly (since microbial activity is so fast in hot climate) and improves the physical properties of the soil [23,27], (c) application of chemical grouting to stabilize soil structure and modify the pore geometry of the soil by chemical reactions or ionic exchange resulting in a reduced fluid movement and improved water holding capacity and reduced water and nutrient seepage [28-32] and (d) application of microorganisms to alter the oil structure in order to reduce porosity and enhance water and nutrient retention [33-48].

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There are many microbiological activities that can be used to alter soil structure and improve the properties of soils which include bio-cementation (or bio-mineralization), gleization and bio-sealing. Bio-cementation is the process where microorganisms produce elemental compounds such as calcium carbonate as a basis for bio-grout that can improve the mechanical properties of the soil and decrease its porosity [33-37]. Gleization is a process in which breakdown of soil structure takes place by strong oxidizing or reducing gelatinous agents which are the products of microbial metabolisms [38-41]. Soil bio-sealing is a process in which microbially induced compounds are utilized to plug the soil pores and reduce soil porosity, leading to increased water holding capacity and reduced loss of water and nutrient through seepage [42-48].

Natural bio-seal (biological soil crust) can develop from the intimate association between soil particles and microorganisms that live within soil such as cyanobacteria, green algae, fungi, bacteria, lichens and bryophytes [44]. They are typical of arid and semi-arid regions but can occur in most ecosystems [45-46]. Some strains of bacteria produce water insoluble polysaccharides which appear to be promising selective plugging agents that can be used to create bio-seal in the sandy soil of Egypt [49,50]. Microbial polysaccharides which have potential in the sealing mechanisms include dextran, xanthan, curdlan, indicant, pullulan, heteroglycan and zenflox-polysaccharides. This study proposes to investigate the possibility of applying biological sealing into the sandy soil of reclaimed Egyptian deserts and evaluate its effectiveness in improving water retention.

OBJECTIVES

The main aim of this study was to enhance the water holding capacity of the reclaimed desert sandy soil using environmentally friendly biological technique that will minimize the loss of irrigation water by downward seepage out of the plant root zone. The specific objectives were: (a) to select environmentally friendly bacteria capable of producing insoluble polysaccharides as a plugging agent in order to minimize soil porosity, (b) evaluate the polysaccharides production efficiency of these bacteria and establish the optimum concentrations of the bacterial

Figure 2. White desert of Farâfra before reclamation.

Figure 3. Fields of crops in newly reclaimed agricultural land of Farafra.

cultures and (c) evaluate the effectiveness of the polysaccharides in enhancing the water holding capacity of the sandy soil.

MATERIALS AND METHODS

Selection of polysaccharide

The polysaccharide levan was selected for this study. Levan is a polymer made up of fructose (a monosaccharide sugar) connected in 2, 6 beta glycosidic linkages as shown in **Figure 4** [51, 52]. Levan can be in both branched and linear structures **(Figure 5)** of relatively low molecular weight [52]. In the branched version, levan forms a very small, sphere-like structure. This structure has basal chains of 9 units long which contain 2, 1 branching, allowing for the methyl ethers to form and create a spherical shape. The ends tend to contain a glucosyl residue. The branched structure of levan tends to be more stable than the linear structure. However, the amount of branching and length of polymerization tends to vary among different species. The shortest levan is 6-kestose, essentially a chain of two fructose molecules and a terminal glucose molecule [52-53]. Levan contains a diverse set of properties **(Table 1)**. The beta 2, 6 linkages of levan allow for it to be insoluble in water, oil and many organic solvents (methanol, ethanol, and isopropanol. The branching of levan also allow for it to have a large amount of tensile and cohesive strength, while the hydroxyl groups contribute to adhesion with other molecules [52-55].

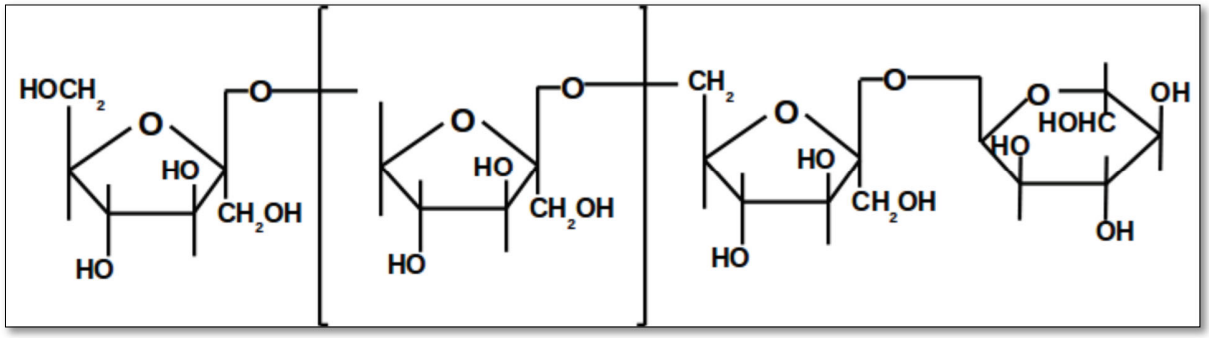

Figure 4. Structural Formula of Levan [51,52].

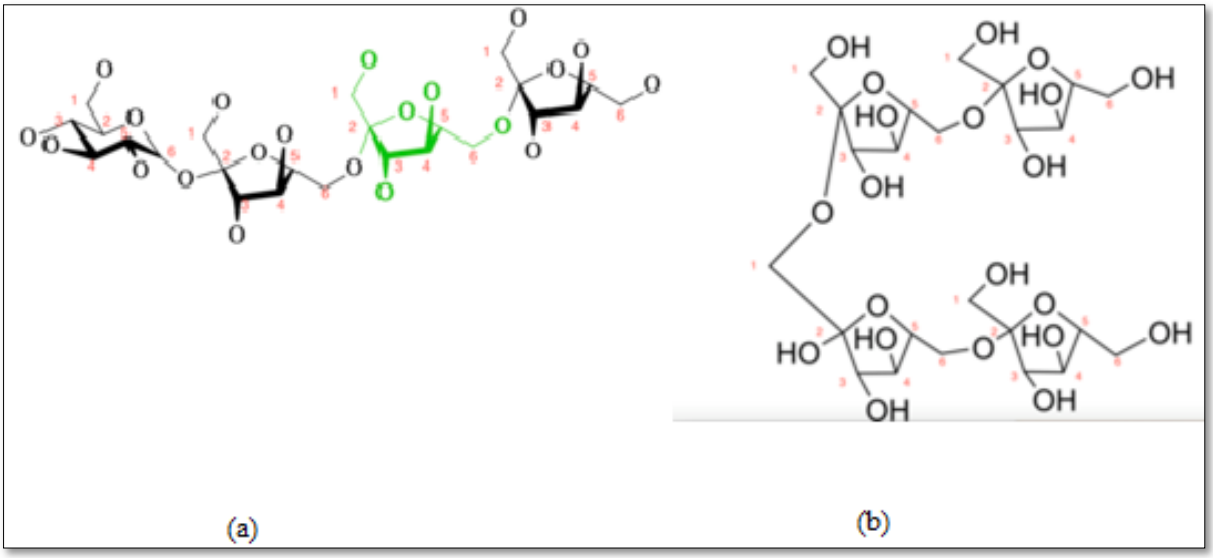

Figure 5. Levan forms [52]. (a) Linear form with beta 2,6 glycosidic linkages. (b) Branched from with beta 2,1 glycosidic linkages.

Levan is diversity distributed in plants and microorganisms. It is usually found in the stems and leaf tissues of Levan is also produced as exopolysaccharides usually from sucrose (a disaccharide sugar containing glucose and fructose) based substrates by a variety of microorganisms *Agropyroncristatum, Dactylisglomerata, Pea secunda, Ttriticumaestivum* and *Pachysadra terminalis* [56, 57]. including bacteria, fungi and algae. However, there are some reports indicating that microbial levan can be produced from fructose, glucose and raffinose substrates [56, 58, 59]. The

main reaction in levan biosynthesis is the transfructosylation by the extracellular enzyme levansucrase. The enzyme forms the 2, 1 linkages in the linear basal chains of levan to allow for branching points to occur. This production of levan is sensitive to temperature, oxygen concentration, pH and other factors [56, 60-62].

Table 1. The main properties of levan [51-70].

Property	Description
Molecular Formula	$C_{18}H_{32}O_{16}$
Molecular Weight	504.4 g/mol
Solubility	Water and oil insoluble due toβ-(2→ 6) linkage
Viscosity	Low viscosity (0.07-0.18 dL/g for molecular weight 16-24 million Da)
Particle Size	Nanoparticle in water = 224.3 nm Nanoparticles in ethanol = 251.8 nm
Stability	High stability to heat, acid and alkali media Melting point = 225 ºC Glass transition temperature = 141 ºC Boiling point = 900 ºC
Enthalpy of Vaporization	150 kJ/mol
Ionic Bonding	Non-ionic
Assembling	Self-assembled in aqueous solution
Tensile Strength	Up to 10.3 MPa (1500 psi)
Polarity	Amphiphilic (poses water and fat loving properties)
Shape or Form	Amorphous (lacking clear structure)
Chemical Compatibility	Compatible with salts and surfactants
Biomedical Benefits	Non-toxic Antioxidant Anti-inflammatory Anticarcinogenic Antihyperlipidemic Antidiabetic Ameliorate stress Hyperglycaemic Prebiotic and immuno-nutrient Not hydrolyzed by human digestive enzymes Nanocarrier system of peptides, proteins and drugs

Selection of microorganism

Selection of microorganisms used in this study was based on the criteria shown in **Table 2**. As the land will be used for agriculture production, contaminated soil with pathogens could spread diseases to crops and vegetables or to healthy animals and human. Thus, the selected microorganisms must be non- pathogenic. Microbial cells smaller than the average pore sizes of the soil are desirable. Insoluble polysaccharide is required to plug the soil pores and form stable sealing. *Arthrobacter* and *Bacillus* are the most common bacterial genera found in soils and any microbes introduced into to the soil for the purpose of clogging the soil pores (bio-sealing) must compete with these indigenous bacteria for substrate [42,49,50].

Table 3 shows some of the levan producing bacteria. The bacterial species *Azotobacter chroococcum* and *Lactobacillus fermentum* were selected for this study. *Azotobacter chroococcum*are capable of producing levan and have a full range of enzymes needed to perform nitrogen fixation (ferredoxin, hydrogenase, and an important enzyme nitrogenase). Owing to their ability to fix molecular nitrogen and produce growth hormones, and therefore increase the soil fertility and stimulate plant growth, *Azotobacter* species are widely used in agriculture as a source of nitrogen biofertilizer [71]. *Lactobacillus fermentum* bacteria are a levan producing bacteria. The use of these two microorganisms would be suitable for production of levan from sucrose, fixing nitrogen in soil and producing growth hormones, thereby improving the water holding capacity of the soil and enhancing its nutrient content and stimulating plant growth. The scientific classifications of *Azotobacter chroococcum* and *Lactobacillus fermentum are* shown in **Table 3** and their biological and biochemical characteristics are shown in **Table 4**.

Table 2. Selection criteria of microorganisms used in the study.

Criteria	Descriptions
Pathogenicity	As the treated soil will be used for agricultural production, contaminated crops and vegetables can spread the diseases to healthy animals and human. Thus, the selected microorganism must be non-pathogenic
Size	Cells size must be smaller than the average pore diameter of the soil (the average pore diameter of agricultural soils is 7 - 15 µm and for sandy soil that drains freely by gravity is >150 µm)
Type of Polymer Produced	Non-soluble polysaccharide required to form stable sealing
Competition with Soil Microorganisms	*Arthrobacter* and *Bacillus* species are the most dominant soil microorganisms and the selected microorganisms must be able to compete with these as well as other microorganisms in the soil such as yeast and fungi.

Soil collection and preparation

The soil was collected from the Teaching and Experimental Farm of the Faculty of Agriculture, Cairo University. About 100 kg of soil were collected in plastic bags and transported to the Bioengineering Laboratory. The visible organic matter was removed from the soil and soil clumps were crushed.

A soil sample of 500g was used to determine the particle size distribution using a mechanical sieving apparatus (Vibratory Sieve Shaker, Series AS200, Retsch GMBH, Haan, Germany. The pan was first placed onto the sieving apparatus. The sieves with the smallest mesh were stacked on the top of the pan and successively larger meshes were placed above. The sample was placed into top sieve and the lid was placed on top of the stack. The shaker was turned on for 30 min. The soil collected from each sieve was weighed and the percentage of each soil fraction from the original soil weight was calculated.

Soil samples of 50 g each were used to measure the soil particle density, bulk density and porosity. A soil sample of 50 g was placed in a 100 ml graduated cylinder and the actual volume of the soil sample was determined. Another soil sample of 50 g was placed into a graduated cylinder containing 100 ml of water. The volume of water that resulted from the addition of soil is considered the volume of the soil particles. The particle density is defined as the weight of the soil particles divided by their volume. The bulk density is defined as actual weight of the soil divided by its apparent volume. The soil porosity is defined as these were calculated as follows:

$$\rho_p = W/V_p \quad (1)$$

$$\rho_b = W/V_b \quad (2)$$

$$P = (\rho_b - \rho_p)/\rho_b \quad (3)$$

where:

P = Porosity (%)

V_b= Volume of the soil(cm^3)
V_p= Volume of the particles (cm^3)
W = Weight of the soil (g)
ρ_b= Soil bulk density (g/cm^3)
V_p=Particles density (g/cm^3)

The rest of the soil was placed in 10 plastic bags, each containing 1 kg of soil. The bags placed in an autoclave (Tabletop Autoclave, Tuttnauer 2340M, Alpha Scientific, Vancouver, British Columbia, Canada) for sterilization at a temperature of 121°C and a pressure of 103 KPa for 20 min. This process was carried out to kill any soil microorganisms. The sterilized soil was used later to test the effectiveness of bio-cementation and bio-sealing (clogging of the soil pores).

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Preparation of the growth medium and microbial cultures

Samples of *Azotobacter chroococcum* and *Lactobacillus fermentum* **(Figure 6)** were obtained from the Department of Microbiology, Faculty of Agriculture, Ein Shams University and the Department of Microbiology, Faculty of Agriculture, Cairo University, respectively. Liquid growth medium was prepared using Bacto® Nutrient Broth, which was obtained from Difco Laboratories, Detroit, Michigan, USA.

The Nutrient broth is composed of a simple peptone and a beef extract. The peptone contributes organic nitrogen in the form of amino acids and long-chained fatty acids while the

Table 3. Some levan producing bacteria.

Microorganism	Reference
Acetobacter aceti^{SG-}	Loewenberg and Reese [72], Moonmangmee et al. [73], Tomulescu et al. [74]
Acetobacter diazotrophicus^{SNG-}	Tomulescu et al. [74], Hemandez et al. [75], Arrieta et al. [76], Tambera et al. [77], Batista et al. [78]
Acetobacter Pasteurianus^{SNG-}	Loewenberg and Reese [72], Tomulescu et al. [74], Perumpuli et al. [79], Minakami et al. [80], Tayama et al. [81]
Acetobacter xylinum^{SG-}	Srikanth et al. [56], Tomulescu et al. [74], Tayamaetal [81], Jasson et al. [82]. Wong et al. [83]
Acinetobacter nectaris^{PG-}	Gozalez-Garcinuno [84], Bansal et al. [85], Tabernero et al. [86]
Actinomyces viscosus^{HAG+}	Pabst [87], Warner and Miller [88], Miller and Somers [89], Igarashi et al. [90]
Achromobacterxylosoxidans^{HG-}	Han [91], Yamasato et al. [92]
Aerobacteraerogenes^{HG-}	Han [91], Srinivasan and Quastel [93], Wilkinson et al. [94]
Aerobacter levanicum^{SG-}	Evans and Hibbert [95], Takeshita et al. [96], Feingold and Gehatia [97]
ArthrobacterUreafaciens^{SG+}	Tomulescu et al. [74], Han [91], Song et al. [98], Tanaka et al. [99], Tanaka et al. [100]
Azotobacterchroococum^{SG}	Tomulescu et al. [74], Hestrin and Goldblum [101], De La Vega et al. [102], Han [103]
Bacillus arrophilus^{HG+}	Tomulescu et al. [74], Bansal et al. [85], Abou-Taleb et al. [104]
Bacillus atrophaeus^{SG+}	Tomulescu et al. [74], Bansal et al. [85], Hestrin and Goldblum [101]
Bacillus amyloliquefaciens^{SG+}	Tomulescu et al. [74], Han [91], Tian et al. [105]
Bacillus lentus^{SG-}	Tomulescu et al. [74], Bansal et al. [85], Abou-Taleb et al. [104]
Bacillus licheniformis^{SG+*}	Ghaly [42], Ghaly et al [49], Ramsay et al. [54],Tomulescu et al. [74], Bansal et al. [85], Xavier et al. [106], Kekez et al. [107], Mamay [108], Larpin et al. [109], van Dyke et al. [110]
Bacillusmegaterium^{SG+}	Tomulescu et al. [74], Evans and Hibbert [95], Strube et al. [111]
Bacillus mesentericus^{SG+}	Tomulescu et al. [74], Han [91], Tanaka et al. [112]
Bacillus methylotrophicus^{G+}	Tomulescu et al. [74], Zhang et al. [113], Li et al. [114], Jadan et al. [115]
Bacillus polymyxa^{SG+}	Han and Clark [51], Tomulescu et al. [74], Bansal et al. [85], Han and Watson [116], Han [117], Liu et al. [118]
Bacillus subtilis^{SG+}	Shih et al. [60], Bansal et al. [85], Jensen et al. [119], Ing-Lung et al. [120], Abdel-Fattah et al. [121], Benigaret al. [122], Ahmed [123], Esawy et al. [124], Vaidya and Prasad [125], Goncalves et al. [126], Abdul Razack [127], Abdel-Fattah et al. [128]
Bradyrhizobiumdenitrificans^{SNG-}	Bansal et al. [85], Sutherland [129]
Bradyrhizobiumelkanii^{SNG-}	Bansal et al. [85], Sutherland [129], Sucawara et al. [130]

Bradyrhizobiumembiopense^{SNG-}	Bansal et al. [85], Sutherland [129]
Bradyrhizobium japonicum^{SNG-}	Bansal et al. [85], Sutherland [129], Sudtachat et al. [1317], Dake [132]
Bradyrhizobiumoligotrophicum^{SNG-}	Bansal et al. [85], Sutherland [129], Resenberg et al. [133]
Bradyrhizobiumyuanmingense^{SNG-}	Bansal et al. [85], Sutherland [129]
Brenneriagoodwinii^{SPG-}	Tomulescu et al. [74], Liu et al. [134], Xu et al. [135]
Clostridium acetobutylicum^{SG+}	Gao et al. [136], Dahech et al. [137]
Corynebacterium laevaniformans^{SG+}	Han [91], Dias and Bhat [138]
Corynebacterium beticola^{SHG+}	Tomulescu et al. [74], Han [87], Chen et al [139]
Erwinia amylovora^{SPG+}	Tomulescu et al. [74], Wuerges et al. [140], Gross et al. [141]
Erwinia herbicola^{SPG-}	Tomulescu et al. [74], Benigar et al. [122], Keith et al. [142], Keith et al. [143]
Geobacillus stearothermophilus^{SG+}	Inthanovong et al. [144], Li et al. [145]
Gluconobacteraquatilis^{SPG-}	Tomulescu et al. [74], Ua-AraK et al. [146], De Muynck et al. [147]
Gluconobactercerinus^{SPG-}	Tomulescu et al. [74], De Muynck et al. [147], Jakob et al. [148]
Gluconobacteroxydans^{SG-}	Velazquez-Hernandez et al. [149], Park et al. [150]
Gluconoacetobacterdiaztrophicus^{SNG-}	Han [91], Serrato et al. [151], Banguela et al. [152]
Gluconacetobacterxylinus^{SG-}	Jakob et al. [148], Kommann et al. [153],
Halomonassmyrnensis^{SG-}	Sarilmiser et al. [59], Kazak et al. [154], Poli et al. [155]
Kozakiabaliensis^{SWG-}	Ua-AraK et al. [146], Brandt et al. [156]
Lactobacillus fermentum^{SWG+}	Dutta et al. [157], Badel et al. [158], Heinemann et al. [159], Galle and Avendt [160]
Lactobacillus gasseri^{SWG+}	Anwar et al. [161], Diez-Municio et al. [162]
Lactobacillus reuteri^{SWG+}	Tomulescu et al. [74], Sims et al. [163], van Hijum et al. [164], Kaditzky and Vogel [165], Ni et al. [166]
Lactobacillus sanfranciscensis^{SWG+}	Bansal et al. [85], Tieking et al. [167],
Leuconostoccitreum^{SWG+}	Tomulescu et al. [74], Han et al. [168], Ortiz-Soto et al. [169], Bounaix et al. [170]
Leuconostocmesenteroides^{SWG+}	Han [81], Xu et al. [171]
Mesorhizobiumalhagi^{SNG-}	Bansal et al. [85], Liu et al. [172]
Mesorhizobiumamorphae^{SNG-}	Bansal et al. [85], Priest and Goodfellow [173], Kimbrel [174]
Mesorhizobiumaustralicum^{SNG-}	Bansal et al. [85], Priest and Goodfellow [173], Reeve et al. [175]
Mesorhizobiumciceri^{SNG-}	Bansal et al. [85], Priest and Goodfellow [173], Das et al. [176]
Mesorhizobiumhuakuii^{SNG-}	Bansal et al. [85], Priest and Goodfellow [173], Chen et al. [177]
Mesorhizobiumjapanicum^{SNG-}	Bansal et al. [85], Priest and Goodfellow [173]
Mesorhizobium loti $^{SNG-}$	Bansal et al. [85], Priest and Goodfellow [173], Kawaharada et al. [178], Kelly et al. [179]

Mesorhizobiummediterraneum $^{SNG-}$	Bansal et al. [85], Priest and Goodfellow [173], Ray [180]
Mesorhizobiumplurifarium $^{SNG-}$	Bansal et al. [85], Priest and Goodfellow [173]
Micbacteriumlaevaniformans $^{SG+}$	Bansal et al. [85], Han [91], Bae et al. [181]
Odontomycesviscosus $^{SWHAG+}$	Han [91], Krichevsky et al. [182]
Paenibacillusbovis $^{SG+}$	Gozalez-Garcinuno [84], Xu et al. [183], Hang et al. [184].
Pediococcusacidilactici $^{SHG+}$	Youssef et al [62], Petrov and Petrova. [185]
Phytomonaspruni $^{SPG+}$	Han [91], Haworth and Stacey [186], Lyne et al. [187]
Pseudomonas aureofaciens $^{SPG-}$	Tomulescu et al. [74], Fuchs [188], Alamäe et al. [189]
Pseudomonas brassicacearum $^{SPG+}$	Tomulescu et al. [74], Alamäe et al. [190], Al Qysi [191]
Pseudonomaschlororaphis $^{SG-}$	Tomulescu et al. [74], Fuchs [188], Alamäe et al. [189]
Pseudonomas fluorescens $^{SG-}$	Jathore et al. [58], Tomulescu et al. [74], Bansal et al [85], Fuchs [188], Alamäe et al. [189]
Pseudomonas syringae $^{SPG-}$	Tomulescu et al. [74], Alamäe et al. [189],Kasapis et al. [191], Laue et al. [192]
Rahnellaaquatilis $^{SWHG-}$	Yoo et al. [193], Kim et al. [194], Kim et al. [195]
Rhizobium leguminosarum $^{SNG-}$	Bansal et al. [85], Priest and Goodfellow [173]. Karunaratne [196], Tikhonovich et al. [197]
RhisobiummelilotiS $^{NG-}$	Bansal et al. [85], Priest and Goodfellow [173], Tikhonovich et al. [197]
Rhisobiumraiobacter $^{SNG-}$	Bansal et al. [85], Priest and Goodfellow [173], Tikhonovich et al . [197]
Rothiadentocariosa $^{SWHG+}$	Tomulescu et al. [74], Han [91], Lesher and Gerencser [198], Willner et al. [199], Hill [200]
Saccharomyces cerevisiae $^{SG-}$	Tomulescu et al. [74], Bansal et al. [85], Franken et al. [201], Elorza et al. [202]
Streptococcus mutans $^{SHG+}$	Han [91], Yoo et al. [203], Ebisu et al. [203]
Streptocuccussalivarius $^{WHG+}$	Tomulescu et al. [74], Fuchs [188], Yoo et al. [193], Ebisu et al. [204], Newbrun et al. [204]
Xanthomonas axonopodis $^{SPG-}$	Tomulescu et al. [74], Han [91], Yoo et al. [193], Moosavi-Nasab et al. [205]
Zymomonasmobilis $^{SG-}$	Silbir et al. [57], Tomulescu et al. [74], Bansal et al. [88], Benigar et al. [122], Abdul Razack [127], Yoo et al. [193], Vigants et al. [206], Calazans et al. [207], Bekers et al. [208], Melo et al. [209], Ananthalakshmy and Gunasekaran [210], Shaheen et al. [211], Santos et al [212] , De Oliveira et al. [213]

A = Animal Pathogen
G+ = Gram Positive
G- = Gram Negative
H = Human Pathogen
N = Nitrogen Fixing
P = Plant Pathogen
S = Soil microorganism
W = Water microorganism

beef extract provides vitamins, carbohydrates, salts and other organic nitrogen compounds. An amount of 6.5 g of the nutrient broth was added to two Erlenmeyer flasks, each containing 500 ml distilled deionized water. Each flask was capped and thoroughly mixed. The flasks were placed in an autoclave (Tabletop Autoclave, Tuttnauer 2340M, Alpha Scientific, Vancouver, British Columbia, Canada) at a temperature of 121°C and a pressure of 103 KPa for 20 min

to sterilize the media. The flasks were left to cool down. One flask was inoculated aseptically with *Azotobacter chroococum* while the other flask was inoculated aseptically with *Lactobacillus fermentum.* The two cultures were grown on a controlled environment laboratory shaker (MaxQ™ 4000 Benchtop Orbital Shaker, Thermo Fisher Scientific, Montreal, Quebec, Canada) at room temperature (21°C) for 24 h.

Table 4. Scientific classification of *Azotobacter chroococum*and *Lactobacillus fermentum* [214-216].

Taxonomy	*Azotobacter chroococum*	*Lactobacillus fermentum*
Kingdom	Bacteria	Bacteria
Phylum	Proteobacteria	Firmicutes
Class	Gammaproteobacteria	Baccilli
Order	Pseudomonadales	Lactobacillales
Family	Pseudomonadaceae	Lactobacillaceae
Genus	Azotobacter	Lactobacillus
Species	Azotobacterchroococum	Lactobacillus fermentum

The cell number was determined according to the procedure described by Ghaly and Mahmoud [217].

Culture propagation and polymer production in laboratory

The two cultures were then grown on liquid growth medium containing sucrose. The liquid growth medium consisted of 50.0 g sucrose, 2.5 g tryptone, 2.5 g K_2 HPO_4 and 5.9 g yeast extract per liter of distilled deionized water. The media were transferred to several 1 L Erlenmeyer flasks and sterilized in an autoclave (Tabletop Autoclave, Tuttnauer 2340M, Alpha Scientific, Vancouver, British Columbia, Canada) at a temperature of 121°C and a pressure of 103 KPa for 20 min. Each microbe was transferred aseptically from the nutrient broth to ten 750 mL Erlenmeyer flasks, each having 500 ml of sterilized liquid media. Each flask was inoculated with 10% (v/v) of the homogeneous mixture of the nutrient broth culture. The cultures were grown in a controlled environment laboratory shaker ((MaxQ™ 4000 Benchtop Orbital Shaker, Thermo Fisher Scientific, Montreal, Quebec, Canada) at room temperature (21°C) for 5 days. Samples were drawn from the flasks for biomass, sucrose and polysaccharide determination. Sampling was done every 4 h during the first 24 h every 6 h during the period of 24-72 h and then every 12 h until the end of the 5 days. The cell biomass was determined according to the procedure described by Ghaly and Mahmoud [218]. The polysaccharide concentration analysis was determined according to the procedure described by Ramsay [219]. The sucrose concentration was determined according to the procedure described by Borji et al. [220].

Polymer production in soil (bio-cementing and bio-sealing)

The setup for testing sandy soil bio-cementation and bio-sealing is shown in **Figure 7**. It consisted of 5 infiltration soil columns, each was constructed a PVC cylinder of 7.5 cm diameter and 40 cm height, a plastic filtration funnel of 7.5 cm diameter and a 1 L flask. The funnel was placed on the top of the flask and a filter bad was placed inside the funnel. This filter bad has a pore size smaller the that of the smallest sand particles (does not allow the soil particles to pass through). The cylinder was connected to the funnel and sealed together. One kg of the sterilized soil was placed in the cylinder and packed to achieve field density. This was done to simulate the soil root zone.

The application of microbial culture and water was carried out as shown in **Table 6**. 400 ml of microbial culture of *Azotobacte rchroococcum* were added on day 1 to each column. On Day 3, 400 ml of the diluted microbial culture (each soil column received different concentration of the microbial) were added to the columns.

On day 5, the moisture content and pH were measured. The moisture content was measured using a portable soil moisture measurement meter (TOR 150 Soil Moisture Meter, Edaphic Scientific, Moorabbin, Victoria, Australia). The pH was measured using a portable pH meter (Hanna H199121 Digital pH Meter, ITM Instruments Inc, Sainte Anne de Bellevue, Quebec, Canada). Then, 400 ml of water were added to each column and the leachate collected in the flasks were measured after 12, 24 and 48 h from addition of water. Finally, the water holding capacity was determined.

After completing the experiment with Azotobacter *chroococcum,* the component of each column were dismantled and washed thoroughly with water and disinfected with alcohol. They were the sterilized in an autoclave (Tabletop Autoclave, Tuttnauer 2340M, Alpha Scientific, Vancouver, British Columbia, Canada) at a temperature of 121°C and a pressure of 103 KPa for 20 min. The 5 columns were reassembled again. The same experimental procedure was followed with *Lactobacillus fermentum.*

RESULTS AND DISCUSION

Soil characteristics

The result showed that the sandy soil used in this study had a particle density of 2.58 g/cm^3, a bulk density of 1.6 g/cm^{3}and a porosity of 37.98 %.

Table 5. The biological and biochemical characteristics of *Azotobacter chroococum* and *Lactobacillus fermentum* [74,101-103,157-160].

Parameter	*Azotobacter chroococum*	*Lactobacillus fermentum*
Habitats	Neutral to alkaline soils, aquatic environments and on some plants.	Fermented milk products, sourdough, fermenting plant materials, faeces and ewage
Motility	Free-living microbes	Non motile
Staining	Gram negative	Gram positive
Bacteria Shape	Oval or spherical	Rod-shaped
Bacteria Size	0.6 - 0.9 µm by 1.5 - 3.0 µm	0.5 - 0.8 µm by 2 - 9 µm
Spore Shape	A closed sac containing a cluster of cells	Non spore forming
Oxygen	Obligate aerobes	Facultative anaerobes
Temperature	Optimum growth at 20 - 33oC	Optimum growth at 20 - 30oC
pH	4.8 - 8.5 with optimum at 7.0-7.5	Strong pH tolerance (pH 3)
Tolerance to Salt	Good growth up to 6% NaCl	Ferment sugars up to 6-8% NaCl
Growth on Agar	Large spreading flat, slimy colonies with a diameter of 5-10 mm and a dark brown to green color	*Colonies* on agar media are usually 2-5 micrometers, convex, entire, opaque, and without pigment
Substrate	Wide variety of carbohydrates and organic metallic salts with mannitol as a source of energy	Ferment ribose, galactose, D-glucose, D-fructose, D-mannose, maltose, lactose, melibiose, saccharose, D-raffinose, D-tagatose and gluconate.
Applications	Fixing nitrogen (bio-fertilizer) Production of growth hormones Production of polysaccharides	Potential probiotic Production of lactic acid Production of polysaccharides

The fraction of the soil collected from each sieve as a percentage of the original soil weight is shown in **Table 7**. The particle size varied from 0.150 to 2.000 mm. Most of the soil particles had a diameter in the range of 0.425-0.850 mm **(Figure 8)**. All sand particles have a diameter within the range 0.05 mm and 2.00, all silt particles have a diameter within the range 0.002 mm and 0.05 mm while all clay particles are less than 0.002 mm in diameter as shown in **Figure 9** [221].

The results indicated that the soil used in this study is typical sandy soil with a rough texture and free of silt and clay. This soil has a loose texture resulting in wind erosion, low organic matter, low nutrient content, high infiltration rate, low water holding capacity, high temperature resulting in faster plant growth, high aeration rate resulting in faster decomposition of organic matter [222]. For a sustainable agriculture, it is important to consider applying biotechnological techniques for building an adequate soil structure in these types of soils as well as water conservation by adopting new irrigation technology.

Figure 6. Samples of *Azotobacter chroococum* and *Lactobacillus fermentum.*

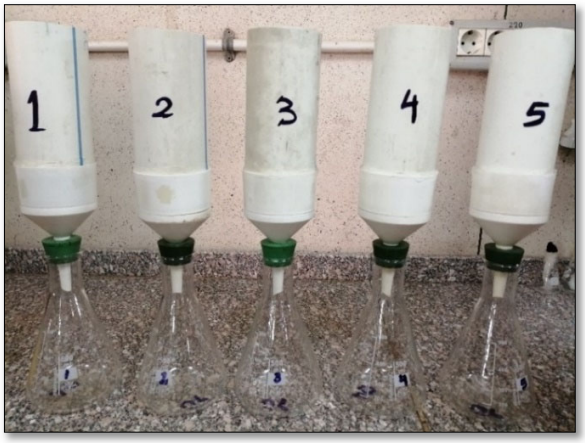

Figure 7. Bio-cementation and bio-sealing testing

Bacterial growth and levan production in bioreactor

The bacteria *Azotobacter chroococcum* and *Lactobacillus fermentum* were first grown on a sucrose in shake flasks to produce levan. The plate count test performed on the media obtained from the shake flasks containing nutrient broth revealed that there was a count of approximately 7.29 x 10^8 microbial cells/mL for *Azotobacter chroococcum* and 8.23 x 10^8 microbial cells/mL for *Lactobacillus fermentum*. The result of batch culture propagation of *Azotobacter chroococcum* and *Lactobacillus fermentum in the* shake flasksare presented in **Table 8**. The maximum biomass concentrationwas3.6 g/L and 3.0 g/L after 22 h and 26 h for *Azotobacter chroococcum* and

Table 6. Microbial culture and water applications.

Time	Application
Day 1	Units 1-5 400 ml microbial culture
Day 3	Unit 1 (control): 400 ml water+0 ml microbial culture
	Unit 2 (treatment): 300 ml water+100 ml microbial culture
	Unit 3 (treatment): 200 ml water+200 ml microbial culture
	Unit 4 (treatment): 100 ml water+300 ml microbial culture
	Unit 5 (treatment): 0 ml water+400 ml microbial culture
Day 5 (Low moisture content)	Units 1-5 400 ml water

Lactobacillus fermentum, respectively. The concentration of sucrose decreased reaching 1.6 g/L and 1.5 g/L after 56 h and 61 for *Azotobacter chroococcum*and *Lactobacillus fermentum,* respectively. With the depletion of sucrose, the bacterial cell mass decreased reaching 0.16 g/L and 0.13 g/ L after 68 h and 72 h for *Azotobacter chroococcum*and *Lactobacillus fermentum,* respectively. The bacteria produced the enzyme levansucrase which converts the soluble sucrose into the polysaccharide ß-D fructoside (levan) and glucose. The production of levan reached a maximum of 14 g/L and 17 g/L for *Azotobacter chroococcum*and *Lactobacillus fermentum,* respectively.

During the fermentation process, the bacteria utilize sucrose for production of levan and for cell maintenance and growth. The following equations describe product formation, respiration and energy production and growth and reproduction.

(a) Respiration and energy production

$$\underset{\text{sucrose}}{(C_{12}H_{22}O_{11})} + 12O_2 \xrightarrow{\text{Cells}} 12CO_2 + 11H_2O + \Delta \quad (4)$$

Table 7. Soil Particle size distribution.

Sieve	Diameter	Retained	Soil	Passing
Number	(mm)	(Kg)	(%)	(%)
10	2	48	9.6	90.4
20	0.85	105	21	69.4
40	0.425	199.5	39.6	29.8
60	0.25	137	27.4	2.4
100	0.15	12	2.4	0
Pan	0.075			

Soil Sample = 500 g

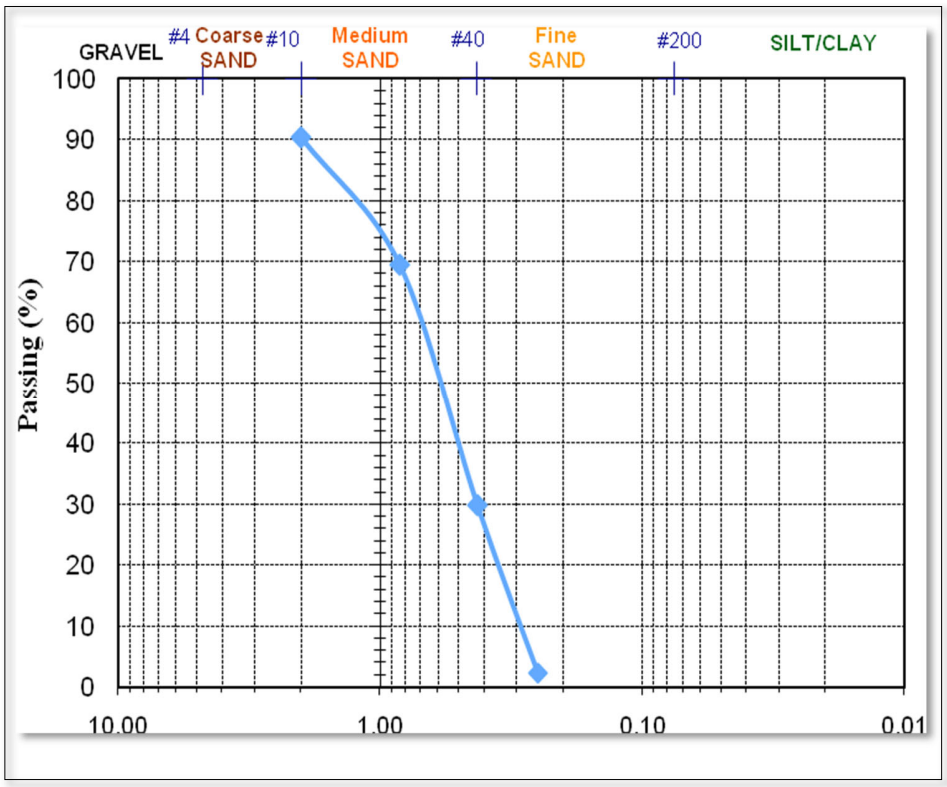

Figure 8. Sand particle sizes and percentage of particles passing through sieves.

(b) Growth and reproduction

$$\underset{\text{sucrose}}{5\,(C_{12}H_{22}O_{11})}+12\,NH_4^{+}\xrightarrow{\text{Cells}}\underset{\text{cells}}{12\,C_5H_7O_2N}+31H_2O+12H^{+} \quad (5)$$

(c) Product formation

$$\underset{\text{sucrose}}{30(C_{12}H_{22}O_{11})}\xrightarrow{\text{levansucrase}}\underset{\text{levan}}{(C_{66}H_{10}O_5)}+\underset{\text{glucose}}{30(C_6H_{12}O_6)} \quad (6)$$

Equation (4), (5) and (6) can be combined to yield the following equation:

$$36(C_{12}H_{22}O_{11})+12O_2+12\,NH_4^{+}\rightarrow(C_6H_{10}O_5)_{30}+30(C_6H_{12}O_6)+12C_5H_7O_2N+42\,H_2O+12CO_2+12\,H^{+}+\Delta \quad (7)$$

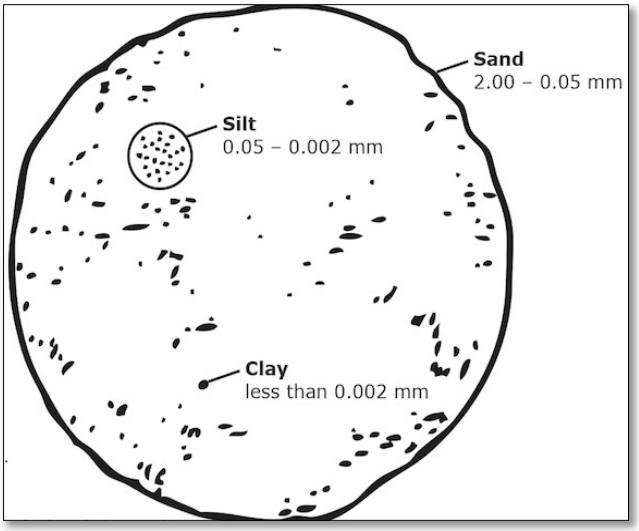

Figure 9. Sand, silt and clay particle sizes [217].

From equation (7), it appears that the theoretical levan yield is 0.395 g levan/g sucrose and the theoretical cell yield is 0.130 g cells/gsucrose. In this study, the levan yield was 0.248 glevan/g sucrose (62.78% of theoretical yield) and 0.371 g levan/g sucrose (93.92% of theoretical yield) for *Azotobacter chroococcum* and *Lactobacillus fermentum,* respectively. The cell yield was 0.074 g cell/g sucrose (47.69 % of theoretical yield) and 0.062 gcell/g sucrose (56.92 % of theoretical yield). The results showed that *Lactobacillus fermentum* was mor efficient in converting sucrose to levan than *Azotobacter chroococcum.* However, *Azotobacter chroococcum* produced more bacterial biomass (g cells/g sucrose) than *Lactobacillus fermentum.* This may be due to the fact that*Azotobacte rchroococcum* is a nitrogen fixing microorganism, a process that require organic matter. Thus, some of the sucrose may have been utilized in nitrogen fixation.

The results showed that it is feasible to use growing cultures of *Azotobacter chroococcum* and *Lactobacillus fermentum.* From the biological and biochemical characteristics of the *Azotobacter chroococcum* and *Lactobacillus fermentum,* it appears that the organisms can produce levan from sucrose under most field and soil conditions and they should be able to compete with most common soil microbial species.

The polysaccharide (levan) produced in this study was non-viscous and water insoluble. The viscosity of the culture broth was the same as that of water. The polymer was a non-transparent suspension and was found to deflect visible light. The polymer can be used as a plugging agent to plug the pores of high permeability soils. Microbial levan contains up to 3 million residues compared to plant levan which contains about 100 residues [51]. The polysaccharide levan $(C_6H_{10}O_5)_n$ consists of fructose monomers linked mainly by β(2→6) linkages [91].

Bio-cementation and bi-sealing

The moisture content and pH measurements taken on day 5 before the application of 400 ml water to each of the soil bio-cementation and bio-clogging columns are presented in Table 9. The results indicated that the moisture content of the soils receiving the bacterial culture of *Azotobacter chroococcum* (22.3%) was lower than that of the soils receiving the bacterial culture of *Lactobacillus fermentum* (25.3%). The soils designated as control (received no bacterial treatment) had lower moisture content than the soils treated with both bacterial cultures. The moisture content of the soils receiving the bacterial culture of *Azotobacter chroococcum* was higher than that of the control by13.78% while the moisture content of the soil receiving the bacterial culture *Lactobacillus fermentum* was higher than that of the control by 29.59%. However, increasing the concentration of the bacteria cultures that were added on day 3 did not have any significant effect on the moisture content. There was also no change in the soil pH as a result of addition of bacterial cultures or varying the concentration of bacterial culture added on day 3.

The volumes of leachates collected from the soil bio-cementation and bio-clogging experiment after the addition of 400 ml water for *Azotobacter chroococcum* and *Lactobacillus fermentum* are shown in **Table 10**.

The results showed that increasing the concentration of bacteria from 25 to 100% in the bacterial culture added on day 3 did not have any significant effect on the amount of leachate collect for both bacteria. However, the leachates collected from the soils receiving Azotobacter chroococcum (205 ml) were much larger than those collected from the soils receiving Lactobacillus fermentum (105 ml). Also, the leachates collected from the control (received no bacterial treatment) were much larger (310 ml) than both soils treated with both bacterial cultures. In other words, 90 ml (22.5%), 190 ml (47.5%) and 295 ml (73.75%) of the added water on day 5 were retained by the control, the soil receiving Azotobacter chroococcum and the soil receiving Lactobacillus fermentum, respectively. This amount to a water conservation of 100 ml (25%) and 205 ml (51.25%) for the soil receiving Azotobacter chroococcum and the soil receiving Lactobacillus fermentum, respectively.

The results obtained from the study showed that it is feasible to use growing cultures of Azotobacter chroococcum and Lactobacillus fermentum to produce a water insoluble levan. The polymer can be used as a plugging agent to plug the pores of the high permeability sandy soils. Upon production of levan, pore spaces would be reduced and, hence, the hydraulic conductivity would be substantially reduced. In addition to producing levan, these bacteria also produce gelatinous agents and elemental compounds that cause soil bio-cementation as shown in Figure 12.

Table 8. Biomass andlevan and conversion efficiencies.

Parameter	***Azotobacter chroococcum***	***Lactobacillus fermentum***
Cell Count (Cells/mL)	8.23×10^8	7.29×10^8
Maximum Biomass (g /L)	3.6	3
Time to reach Maximum Biomass (h)	22	26
Minimum Biomass (g /L)	0.16	0.13
Time to reach Minimum Biomass (h)	68	72
Starting Sucrose (g/L)	50	50
Final Sucrose (g/L)	1.6	1.5
Time to Reach Final Sucrose (h)	56	61
Maximum Levan (g/L)	14	17
Time to Reach Maximum Levan	24	28
Theoretical Levan Yield (g levan/g sucrose)	0.395	0.395
Experimental Levan Yield (g levan/g sucrose)	0.248	0.371
Levan Production Efficiency (%)	62.78	93.92
Theoretical Biomass Yield (g cells/g sucrose)	0.13	0.13
Experimental BiomassYield (g cells/g sucrose	0.074	0.062
Biomass Production Efficiency (%)	47.69	56.92

Table 9. Soil moisture content and pH before the addition of water on day 5.

Parameter	**Bacteria**	**Value**				
		Control	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Moisture Content (%)	*Azotobacterchroococcum*	19.6	22.4	22.3	22.2	22.3
	Lactobacillus fermentum	19.7	25.4	25.4	25.3	25.4
pH	*Azotobacterchroococcum*	8.1	8.3	8.2	8.3	8.2
	Lactobacillus fermentum	8.1	8.2	8,3	8.3	8.2

The bacteria could be grown in the laboratory either in the non-polysaccharide producing mode or in the polysaccharide producing mode. The first would permit distribution of the bacteria to the lower soil layers but would delay the production of the polysaccharide due to the extension of the lag period required to produce the enzyme (levansucrase).

Improving soil properties using biological techniques such as gleization, bio-grouting or bio-cementation and bio-plugging or bio-sealing has been reported by many authors. Kumariad and Xiang [33] stated that bio-grout is an excellent technique for reducing the permeability of porous soils and improving their mechanical properties. Mujab et al. [34] reported that bio-cementation binds soil particles together leading to increased soil strength and stiffness against wind erosion. Ivanove and Chu [36] evaluated the application of bio-cementation and bi-clogging techniques for reducing the porosity and hydraulic conductivity of soils and found facultative and microaerophilic bacteria to be the most suitable organisms for these techniques. McConkey et al. [40] applied an enhanced gleization technique into irrigation canal and reduced water seepage by 30%. Ghaly [42] developed an enhanced bio-sealing mechanism for earthen manure storage using levan producing microorganism and reported that the infiltration rate was affected by the soil type and was correlated to the percentage of sand in the soil. Knapenetal [47] studied the effect of microbiotic crust on soil erodibility by wind and reported a

37% reduction in soil detachment. Ghaly et al. [49] studied the plugging effect of levan produced by *C* in earthen manure storage and found the bacteria converted sucrose into levan under field condition and the exopolysaccharide plugged the pores of a highly porous soil. Stewart and Folger [50] used polymer producing bacteria to modify soil profiles for enhanced oil recovery and reported that the bacteria utilized sucrose to produce exopolymer which created plugged regions of the porous media leading to enhanced oil recovery. Ramsay et al. [54] used *Bacillus licheniformis* to produce water insoluble levan that was used as a selective plugging agent in microbial enhanced oil recovery under a temperature of 55°C, a pH between 6 and 9, a pressure less than sooata and a salt concentration of 4%.

CONCLUSION

The result showed that the sandy soil used in this study had a particle density of 2.58 g/cm^3, a bulk density of 1.6 g/cm^{3}and a porosity of 37.98 %. The particle size varied from 0.150 to 2.000 mm with most of the soil particles having a diameter in the range of 0.425-0.850 mm indicating that the soil was free of silt and clay. This soil has a loose texture, low organic matter, low nutrient content, high infiltration rate, low water holding capacity.

Table 10. Leachates collected after the addition of 400ml water on day 5.

Time (h)	Bacteria	Leachate (ml)				
		Control	Treatment 2	Treatment 3	Treatment 4	Treatment 5
12	*Azotobacterchroococcum*	309	205	205	205	205
	Lactobacillus fermentum	310	105	104	105	104
24	*Azotobacterchroococcum*	310	206	205	205	205
	Lactobacillus fermentum	311	105	105	105	104
48	*Azotobacterchroococcum*	310	206	205	205	204
	Lactobacillus fermentum	311	105	108	105	105

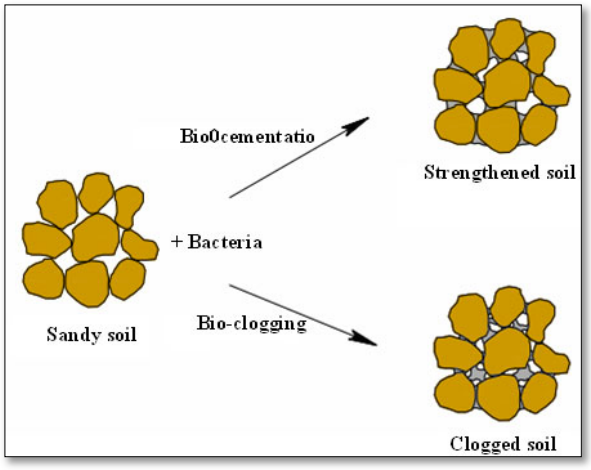

Figure 10. Bio-cementation and clogging of soil.

Azotobacter chroococcum are capable of producing levan from sucrose and have ability to fix molecular nitrogen and produce growth hormones, and therefore increase the soil fertility and stimulate plant growth, *Lactobacillus fermentum* bacteria are a levan producing bacteria. The viscosity of the culture broth was the same as that of water. The polymer can be used as a plugging agent to plug the pores of high permeability soils. The levan yield was 0.248 glevan/g sucrose (62.78% of theoretical yield) and 0.371 glevan/g sucrose (93.92% of theoretical yield) for *Azotobacter chroococcum* and *Lactobacillus fermentum,* respectively. The cell yield was 0.074 g cell/g sucrose (47.69 % of

theoretical yield) and 0.062 g cell/g sucrose (56.92 % of theoretical yield).

The results showed that increasing the concentration of bacteria had not significant effect on the amount of leachate collect for both bacteria. However, the leachates collected from the soils receiving *Azotobacter chroococcum* were much larger than those collected from the soils receiving *Lactobacillus fermentum.* Also, the leachates collected from the control (received no bacterial treatment) were much larger than soils treated with both bacterial cultures. These microorganisms can be used together for production of levan from sucrose, fixing nitrogen in soil and producing growth hormones, thereby improving the water holding capacity of the soil and enhancing its nutrient content and stimulating plant growth.

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COMPETING INTRESTS

The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors contributed equally in various roles including formulation research goals, development of methodology, performing the experiments and analysing data and writing the initial draft. The corresponding author coordinated the research activity as agreed by all authors. All authors read and approved the final manuscript.

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