UNIVERSITAS INDONESIA

APPLICATION OF MICROBIOLOGY TO IMPROVE MECHANICAL PROPERTIES OF SOIL AND CONCRETE

THESIS

YUSTIAN HERI SUPRAPTO

0906644562

FACULTY OF ENGINEERING

MASTER DEGREE PROGRAM

LILLE

JULY 2011

281/FT.01/TESIS/08/2011

UNIVERSITAS INDONESIA

APPLICATION OF MICROBIOLOGY TO IMPROVE MECHANICAL PROPERTIES OF SOIL AND CONCRETE

THESIS

Proposed as one of the requirements for obtaining a Master of Engineering

YUSTIAN HERI SUPRAPTO

0906644562

FACULTY OF ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

GEOTECHNICAL ENGINEERING

LILLE

JULY 2011

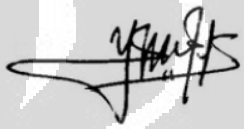
PAGE STATEMENT OF ORIGINALITY

This thesis was the work of itself, and all sources whether quoted of referenced, I have stated correctly.

Name : **Yustian Heri Suprapto**

NPM : **0906644562**

Signature :



Date : **12 July 2011**

HALAMAN PERNYATAAN ORISINALITAS

Thesis ini adalah hasil karya saya sendiri,
dan semua sumber baik yang dikutip maupun dirujuk
telah saya nyatakan dengan benar.

Nama : **Yustian Heri Suprapto**

NPM : **0906644562**

Tanda Tangan :

Tanggal : **12 Juli 2011**

APPROVAL

This Thesis is made by :

Name : Yustian Heri Suprapto

NPM : 0906644562

Program Study : Civil Engineering – Geotehcnical Engineering

Title : Application of Microbiology to Improve Mechanical Properties of Soil and Concrete

Has been successfully maintain in front of the judge and fulfil as one requirement to obtain Master of Engineering, Civil Engineering Department, Faculty of Engineering, University of Indonesia.

Judge

Supervisor : Prof. Isam SHAHROUR ( )

Supervisor : Christine LORS ( )

Supervisor : Jean-Marie LACROIX ( )

Examiner : Marwan SADEK ( )

Examiner : Ali ZAOUI ( )

Head of Department of Civil Engineering University of Indonesia

Faculty of Engineering University of Indonesia

(Prof. Dr. Ir. Irwan KATILI, DEA)

HALAMAN PENGESAHAN

Thesis ini diajukan oleh	:
Nama	: Yustian Heri Suprapto
NPM	: 0906644562
Program Studi	: Teknik Sipil
Judul Skripsi	: Aplikasi mikrobiologi dalam meningkatkan properti mekanik pada tanah dan beton

Telah berhasil dipertahankan di hadapan Dewan Penguji dan diterima sebagai bagian persyaratan yang diperlukan untuk memperoleh gelar Magister Teknik, pada Program Studi Teknik Sipil, Fakultas Teknik, Universitas Indonesia.

Dewan Penguji

Pembimbing : Prof. Isam SHAHROUR ( )

Pembimbing : Christine LORS ( )

Pembimbing : Jean-Marie LACROIX ( )

Penguji : Marwan SADEK ( )

Penguji : Ali ZAOUI ( )

Ketua Departmen Teknik Sipil University of Indonesia

Fakultas Teknik Universitas Indonesia

(Prof. Dr. Ir. Irwan KATILI, DEA)

HALAMAN PERNYATAAN PERSETUJUAN PUBLIKASI TUGAS AKHIR UNTUK KEPENTINGAN AKADEMIS

Sebagai sivitas akademik Universitas Indonesia, saya yang bertanda tangan di bawah ini :

Nama : Yustian Heri Suprapto

NPM : 0906644562

Program Study : Magister Double Degree Teknik Sipil UI dan Université Lille 1

Departemen : Teknik Sipil

Fakultas : Teknik

Jenis Karya : Thesis

Demi pengenbangan ilmu pengetahuan, menyetujui untuk memberikan kepada Universitas Indonesia Hak Bebas Royalti Noneksklusif (Non-exclusive Royalti-Free Right) atas karya ilmiah yang berjudul :

APPLICATION OF MICROBIOLOGY TO IMPROVE MECHANICAL PROPERTIES OF SOIL AND CONCRETE

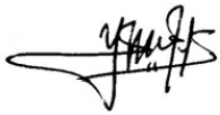
Beserta pangkat yang ada (jika diperlukan). Dengan hak Bebas Royalti Noneksklusif ini Universitas Indonesia berhak menyimpan, mengalihmedia/formatkan, mengelola dalam bentuk pangkalan data (*database*), merawat, dan memublikasikan tugas akhir saya selama tetap mencantumkan nama saya sebagai penulis/pencipta dan sebagai pemilik hak cipta.

Demikian pernyataan ini saya buat dengan sebenarnya

Dibuat di : Lille

Pada tanggal : 12 Juli 2011

Yang menyatakan



(Yustian Heri Suprapto)

ACKNOWLEDGEMENT

First, I would to thank you to my supervisor Prof. Isam SHAHROUR, who gave me an opportunity to do the thesis with the combination of biology and civil engineering. This is my first experience to study two major of science.

For My second supervisor M. Malek SIBAI, who has spent his time for me for some discuss and takes me to the laboratory of microbiology at Université Lille 1. He also

For M. Jean Marie LACROIX and the team from laboratory of microbiology Lille 1 who have assisted me to do the cultivation of *Bacillus subtilis* and to understand the characteristic of this bacteria.

Madame Christine LORS, who has spent her time from Douai to Lille to attends my presentation.

I would like to thank you to Head of Department of Civil Engineering, Faculty of Engineering, University of Indonesia Prof. Dr. Irwan KATILI, DEA who always support me and other student of Double Degree Indonesia – France. You gave me the opportunity, and I will not let you down sir.

For my family in Jakarta, Indonesia who has give me lots of support to finish my study here. My father, Sutoyo and my mother Kusmaryati, thank you so much for your support. For Putri Indah Lestari who always gave me support and had some discussion about this subject.

Abstract

Uses of microorganisms in the civil engineering domain have been developed since several years ago. From several researches, microorganisms produced calcite was able to improve the mechanical properties of the soil and concrete. In the soil, they could increase the bearing capacity by producing calcite that would fulfill the pores of soil [Ivanov, 2008]. By reducing the pores, the soil will be more compact and it can increase its capacity. On the concrete material, it could be used to fix the cracking in the concrete or it could [Achal et.al. 2010] be used in mix design which able to improve the strength of concrete. The type of the bacteria which can produce calcite is from genera Bacillus. Growth of Bacillus subtilis studied in this paper was observed in laboratory of Microbiology at Universite Lille 1.

Keyword : microorganisms, soil, concrete, Bacillus

Table of Content

Table of Figure

List of Table

CHAPTER 1

1.1 Application of Microbiology In Soil Material

Application of microbiology in the soil material has been developed by several researches. Recent researches for example bio-consolidation, bioclogging, and bio-cementation have been done in last 5 years. The principal object of the research is how to improve the mechanical properties of the soil, for example to increase the value of bearing capacity. Using the bacteria produced calcite, the calcite element are expected to reduce the pore in the soil. If the pore is reduced, the soil will has more density and the value of bearing capacity will increase.

These applications have the same type of bacteria that is *Bacillus*. This type of the bacteria is able to extract enzyme *urease* that can produce calcite. The type of Bacillus that used by the researchers are *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus sphaericus*, or *Bacillus pasteurii*. These bacteria have the ability to produce calcite more than other type of bacteria for example *Sporosarcina pasteurii*. All these bacteria are not pathogen or harmful to human, so they could be used safely. *Bacillus* is also easy to cultivate in the medium of agar-based, and the growth rate is also rapid so it can be easily used immediately after cultivated.

Type of the soil used in those researches is sand. Sand is a type of soil which has more pore than other type of soil. So, capability of bacteria produced calcite could be observed by this medium of sand. In the laboratory scale, the alteration of the sand with treated by bacteria will be clearly change and the bearing capacity will increase until five times [DeJong et al. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro-and Macro-scales*". ASCE. 2009].

For the field application, bioclogging and bio-cementation were developed by Volodymyr Ivanov and Jian Chu in 2008. Bioclogging is to reduce the hydraulic conductivity of soil and porous rocks due to microbial activity or products. It could be used to reduce drain channel erosion, from gout grout curtains to reduce the migration of heavy metals and organic pollutants, and prevent piping of earth

dams and dikes. Furthermore, bio-cementation is to enhance the strength and stiffness properties of soil and rocks though microbial activity or products. This application could be used to prevent soil avalanching, reduce the swelling potential of clayey soil, mitigate the liquefaction potential of sand, and compact soil on reclaimed land sites. For the large scale in land reclamation, utilization of microbial treatment could be one of the most cost effective methods [DeJong et al. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales*". ASCE. 2009].

1.2 Application of Bioclogging

Several experimental studies have been performed by enhanced biomass growth in soil with dextrose-nutrient solution have shown a positive correlation between attached microbial biomass and the soil hydraulic conductivity [Wu et.al. Experimental study on the reduction of soil conductivity by enhanced biomass growth. Soil sci 162:741-748. 1997].Another bioclogging experiment by Mc Conkey et al. 1990, shows that erosion was successfully diminished by reducing the soil permeability.

By injecting the microorganism into the soil which produces enzyme urease that hydrolyzes urea. The reaction is:

$(NH_2)_2CO + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH$

(Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008)

This reaction could increase the value of pH and it produces hydrocarbonate. This hydrocarbonate could initiate the precipitation of calcium carbonate which clogging the pores and binding soil particles. In 2004, a pilot scale of bioclogging using bioproduction of slime in soil was successfully carried out and after 6 weeks the hydraulic resistance of soil was enhanced by factor five [Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008].

Application of bioclogging is not only to fulfill the pore or bind the particle of the soil, but also to seal of the unforeseen leaks in the sheet piling screens around the construction wells. This application was practice in Netherlands for 10% all

construction pits dug [Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008].

There are several problems of this application of microorganism is the stability of soil properties after treatment of bioclogging. From the rapport by Ivanov, (2008) there are three general problems that exist in this treatment. First, this application would be successfully done if the soil condition is favorable for exopolysaccharide-producing microorganisms, but not favorable for exoplysaccharide-degrading microorganism. Second problem that exist is the penetration of microorganism that it could penetrate if the soil pore size limited to 0.5 – 2 μm. The last problem that could be occurs is that the growth of clogging biofilm in the soil pores affects the concentrations and mass transfer rates of nutrients and microbial metabolism.

There are several processes that could be potential to bioclogging. The formations of microorganism are that it can produce impermeable layer, slime in soil, production of undissolved sulphides metal or production of ferrous solution [Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008]. But those application have not all been tested yet in the laboratory or in the field.

Table 1. Microbial Processes that Can Lead Potentially to Bioclogging

(Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008)

Physiological group of microorganisms	***Mechanism of bioclogging***	***Essential conditions for bioclogging***	***Potential geotechnical applications***
Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
Aerobic and facultative anaerobic heterotrophic slime-producing bacteria	Production of slime in soil	Presence of oxygen and medium with ratio of C:N > 20	Provide cover for soil erosion control and slope protection.

Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepage
Nitrifying bacteria	Production of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel erosion
Sulphate-reducing bacteria	Production of undissolved sulphides of metal	Anaerobic conditions; presence of sulphate and carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO2	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Prevent piping of earth dams and dikes

In the table microbial processes that can lead potentially to bioclogging which generate by Ivanov (2008), we could see several applications that is possible to applied in the bioclogging. Different types of microorganisms could give the different impact for the soil. Hence, choice of the bacteria is one of important factor because the effect of one or other bacteria is different. Another important factor is the condition of environment of the soil that would affect the growth of bacteria or its capability to produce the chemical enzyme which can used in the bioclogging.

1.3 Application of Biocementation

Chemical cementation (chemical grouting) is to fill the sand voids with fluid chemical grouts to produce sandstone like masses to carry loads (Ivanov, 2008). This principal idea of this application is to generate the particle binding in the materials. The final result of this method is to increase the shear strength of soil. The chemicals that are used to bind soil particles include sodium silicate, calcium chloride, calcium hydroxide (lime), cement, acrylates, acrylamides, and polyutheranes (Ivanov, 2008).

For the type of material that could be treated by biocementation probably conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum, peat, lignite, sand, soil, clay, sediment, and sawdust (Ivanov, 2008). On the other hand, the type of bacteria that could be used in this application is the bacteria, which has the capability to produce urease. This enzyme is needed to synthesis urea and it will bind with the calcite in the soil. There are several bacteria from genera *Bacillus, Sporosarcina, Sporolactobacillus, Clostridium, and Desulfotomaculum* (Ivanov, 2008).

Table 2. Possible Microbial Processes that can Lead Potentially to Biocementation

(Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008**)**

Physiological group of microorganisms	Mechanism of biocementation	Essential conditions for biocementation	Potential geotechnical applications
Sulphatereducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Enhance stability for slopes and dams
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO_2	Presence of urea and dissolved metal salt	Mitigate liquefaction potential of sand Enhance stability for retaining walls, embankments, and dams; Increase bearing capacity

			of foundations
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Densify soil on reclaimed land sites and prevent soil avalanching Reduce liquefaction potential of soil

This method of microbial cementation could be applied for the following civil and environmental engineering applications (Kucharski et. al. 2005):

- Enhancing stability for retaining walls, embankments, and dams;
- Reinforcing stabilizing soil to facilitate the stability of tunnels or underground constructions;
- Increasing the bearing capacity of piled or non piled foundations;
- Reducing the liquefaction potential of soil;
- Treating pavement surface;
- Strengthening tailings dams to prevent erosion and slope failure;
- Constructing a permeable reactive barriers in mining and environmental engineering;
- Binding of the dust particles on exposed surface to reduce dust levels;
- Increasing the resistance of offshore structures to erosion of sediment within of beneath gravity foundations and pipelines;
- Stabilizing pollutants from soil by binding;
- Controlling erosion in coastal area and rivers;
- Creating water filters and bore hole filters;
- Immobilizing bacterial cells into a cemented after biofilter;

The bacteria should be injected into the soil with the certain pressure and viscosity of the suspension. There are several ways of injection in chemical grouting, which can be used also for microbial grouting (Karol, 2003). In low pressure grouting, a low-viscosity grout is injected into soil at low pressure and fills the void without the changes of soil volume and in jet grouting, grouting was injected with high pressure and flow of high velocity mixes the grout and soil (Ivanov, 2008). The depth of penetration depends on the size of the microorganism that is used in the

application. The typical size of unicellular bacteria is from 1 to 3µm, but the length could be up to 100µm (Ivanov 2008).

1.4 Disadvantages

These methods of bioclogging and biocementation have some disadvantages. According to the report by Ivanov, 2008 they have several points that used to be concerned. Table 3 is the summary of the disadvantages by using bioclogging and biocementation.

Table 3. Disadvantages Bioclogging and Biocementation

(Volodymyr Ivanov, Jian Chu. *Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ*. 2008)

Bioclogging	*Biocementation*
Transport of microbial cells into soil depends on cell size, cell surface properties, and cell physiological state	This process run slowly
Could be unstable because of their biodegradability, thermal sensitivity, and low mechanical resistance to pressure drop across the plug	Microbial activity depends on many environmental factors such as temperature, pH, concentrations and diffusion rates of nutrients and metabolites
Slow growing organism with low rate of exopolysaccharide production, this application will require long-term treatment of soil for its clogging	There isn't any test for large scale construction or land reclamation project. Neither biocementation or bioclogging

1.5 MICP Test Laboratory

MICP (Microbial Induced Calcite Precipitation) is another application which use microorganism to improve the soil's mechanical properties. This experiment was brought by De Jong et.al, 2008 where they were able to apply the microorganism to improve the strength of soil. Sand was injected by microorganism, which produce calcite so it could fill the pores in the sand. In the final result, it could increase the value of bearing capacity of the sand until five times.

The type of microorganism used in the experiment was *Sporosarcina pasteurii* which has capability to convert urea into carbonate ions due to presence of high *urease* enzyme. This bacteria injected into the sand which has the particle size 0.42mm and density relative of 35% (De Jong et.al, 2008). It is injected using aquarium bubbling tubing so they were able to penetrate into the sand. The next phase is to wait the bacteria to growth itself and produce calcite to fulfill the pores in the sand. This phase also observed by Scanning Electron Microscopy (SEM) to observe the process of production calcite (De Jong et.al, 2008).

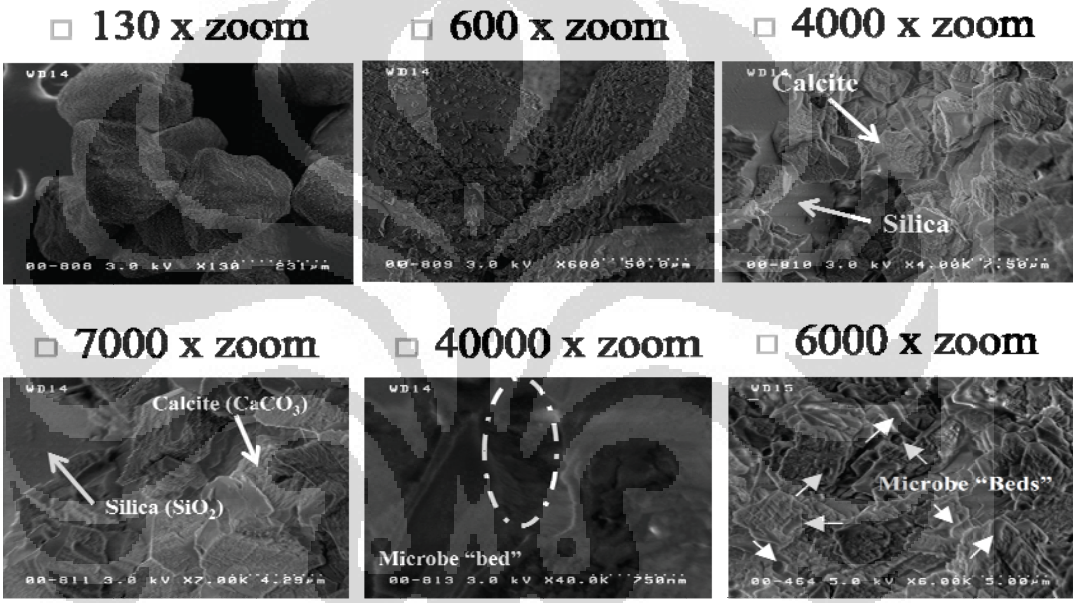


Figure 1. Observation using scanning electron microscopy (SEM)
(Brian C. Martinez, Jason T. DeJong. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales*". ASCE. 2009)

Figure 2. Application of bioconsolidation in sand
(Brian C. Martinez, Jason T. DeJong. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales*". ASCE. 2009)

Figure 3. Load testing in the sand after treatment
(Brian C. Martinez, Jason T. DeJong. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales*". ASCE. 2009)

CHAPTER 2

2.1 Application of Microbiology In Concrete Material

The requirements for high durability for structures exposed to harsh environments such as seafloor, offshore structures, tunnels, highway bridges, sewage pipes and structures for solid and liquid wastes containing toxic chemicals and radioactive elements may not be achieved using today's Ordinary Portland cement (OPC) [V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010]. In general, the durability of concrete is related to the characteristics and its pore structures and the permeability of the concrete is depending on the connectivity of the pores [Kahn. 2003]. In the other hand, deterioration of the concrete is depending on movement of aggressive gas and/or liquids from the surrounding environment into the concrete followed by physical and/or chemical reactions inside the concrete [Claise et.al. 1997]. From these theories, we can reduce the pore inside the concrete by using bacteria which produce calcite. This calcite can fill the pore inside the concrete, then the value of compressive strength will increase and its durability.

Use of bacteria in concrete technology has been revealed by several researches in last 15 years. Bacteria induce calcite is the type of bacteria that is used in most subject in this research. The idea is that bacteria have the capability to produce enzyme *urease* which catalyzes urea to produce CO2 and ammonia, resulting in an increase of pH in the surroundings where ion Ca^{2+} and CO_3^{2-} precipitate as CaCO3.

Utilization of the bacteria not only limited as admixture in concrete design, but also can be used for repairing the cracking in the concrete structure. This research was developed by K.Van Tittelboom, et al. in the paper "*Use of bacteria to repair cracks in concrete*". We usually use chemical grouting (mortar cement) to fulfill the cracking of the concrete, but now we can use the bacteria induced calcite to

fulfill the cracks of the concrete. This method has the same principal with the utilization in the concrete mixture. By producing calcite, they were injected in form paste into the crack's hole and let the bacteria growth to produce calcite and fill the crack.

2.2 Microbiology in Concrete Mixture

Recently, the application of microbiology in the concrete mixture has been discovered and developed by several researches. By adding the bacteria which can produce calcite together with the concrete mixture in the mixing process, they have been able to prove the improvement of compressive strength of the concrete about 36% [V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010]. Another experiment which is done by Ramakrishnan et al. 1998 and Ramachandran et al. 2001, by mixing the aerobic microorganism in the cement mortar, it was able to increase the compressive strength about 18%. The type of the bacteria comes from genus *Bacillus*. The species are *Bacillus sp., Bacillus megatherium, Bacillus subtilis, and Bacillus pasteurii*.

Other experiment developed by V.Achal, X. Pan, and N. Özyurt in 2010, by adding the bacteria in fly ash-amended concrete mix design. We have already known that fly ash is one of filler material used in mix design of concrete. In their experiment, they add fly ash and bacteria produced calcite into the mixture of mix design and they can increase the value of compressive strength of the concrete. The percentage of fly ash contained in the concrete surely gives the difference of increasing of compressive strength. At the fly ash concentrations of 10%, 20%, and 40% in mortars, bacterial cell enhanced mortar compressive strength by 19%, 14%, and 10% [V.Achal, X. Pan, and N. Özyurt. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010].

From these experiments could be concluded that the existence of the bacteria induce calcite in concrete mix has been able to increase the compressive strength of the concrete or mortar mix. In despite of adding the filler for example fly ash, the bacteria still able to increase the compressive strength of the concrete. The

types of bacteria that used in these experiments are *Bacillus sp* and *Bacillus megaterium*. Both of them can produce calcite by producing urease enzyme which catalyzes urea to produce CO_2 and ammonia, resulting in an increase of pH in the surroundings where ions Ca^{2+} and CO_3^{2-} precipitate as $CaCO_3$. Possible biochemical reactions in medium to precipitate $CaCO_3$ at the cell surface that provides a nucleation site can be summarized as follows.

$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+}$

$Cl^- + HCO_3\text{-} + NH_3 \rightarrow NH_4Cl + CO_3^{2-}$

$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow$

[V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010]

The bacterial degradation of urea, locally increase the pH and promotes the microbial deposition of carbon dioxide as calcium carbonate in a calcium rich environment [Warren et al., 2001]. The basic reaction of the calcocarbonic system is:

$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3$ ($Ksp = 3.8 \times 10^9$)

[V.Achal, X. Pan, and N. Özyurt. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

The value of K_{sp} is the solubility product. The driving force for precipitation of $CaCO_3$ is the supersaturation level S, defined by the ratio of the ionic product:

$S = (Ca^{2+}) \times (CO_3^{2-})/K_{sp}$

[V.Achal, X. Pan, and N. Özyurt. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

During microbial urease activity, 1mole of urea is hydrolysed intracellularly to 1mole of ammonia and 1mole of carbamate (NH_2COOH), which spontaneously

hydrolyses to form an additional 1mole of ammonia and carbonic acid [Burne and Marquis, 2000]:

$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$

$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$

[V.Achal, X. Pan, and N. Özyurt. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

These products subsequently equilibrate in water to form bicarbonate and 2moles of ammonium and hydroxide ions as derived in the following equation:

$H_2CO_3 \leftrightarrow HCO_3^- + H^+$

$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-$

[V.Achal, X. Pan, and N. Özyurt. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

These two reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions. The pH increase initially in the local micro-environment around the bacterial cell, and propagates in the bulk solution of the bacterial cell suspension.

$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$

[V.Achal, X. Pan, and N. Özyurt. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

Thus, the carbonate concentration will increase, inducing an increase in S and resulting in $CaCO_3$ precipitation around the cell, in the presence of soluble calcium ions. Those processes occur not only in the mix of the concrete, but also in the sealant to repair concrete cracking.

Recent technique for the remediation of damaged structural formations has been developed by employing a selective microbial plugging process in which microbial-metabolic activities promote precipitation of calcium carbonate in the

form of calcite [Gollapudi et al. *A New Method for Controlling Leaching Through Permeable Channels*. 1995]. As a microbial sealant, $CaCO_3$ exhibited its positive potential to selectively consolidate simulated fractures and surface fissures in granites and sand plugging [Zhong and Islam 1995; Achal et al. 2009a]. The present work deals with the compressive strength and concrete permeability using water absorption test, which are the most important parameters influencing the durability of concrete and finally its performance [V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010].

2.3 Application of Microbiology in Concrete

This chapter will discuss the experiments of utilization of bacteria in concrete mixture which has been done by several researchers. Recently, there are two experiments which done by V.Achal et al. First experiment in the paper *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010. Second in the paper *Improved Strength and Durability of Fly Ash – Amended Concrete by Microbial Calcite Precipitation*. 2010.

2.4 Materials

Materials used in this experiment for example the type of the cement and microorganisms. For the mixtures of concrete are Ordinary Portland Cement (OPC) conforming to IS 12269-1987 and locally available clean, well graded, natural river sand having fine modulus of 2.89 conforming to IS 383-1970 as fine aggregate [V.Achal et al. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010]. For the fly ash, it was obtained locally from power plant which has fineness retained on 45-μm sieve was 28.8% [V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010].

Table 4. Chemical Analysis of cement and Fly Ash (%)

[V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

	SiO_2	CaO	AL_2O_3	MgO	Fe_2O_3	K2O	Na_2O	SO_3	TiO_2
Cement	21.04	62.11	5.02	2.44	3.12	0..71	0.33	3.12	-
Fly Ash	51.47	3.82	33.72	0.67	3.16	1.56	0.65	0.51	1.32

2.5 Microorganisms

Microorganism used in this study is *Bacillus sp.* CT-5, and *Bacillus megaterium* isolated from commercially available cement. The culture was routinely maintained on Nutrient agar (pH 8.0) for *Baciullus sp.* CT-5 and pH 7.5 for *Bacillus megaterium ATCC 14581*. Nutrient broth-urea (NBU) medium (8 g nutrient broth, 2% urea and 25mM $CaCl_2$) was used to grow the isolate. Filter-sterilized urea and $CaCl_2$ was added into nutrient broth medium. Bacterial culture was grown at 37°C under shaking condition (130 rpm) [V.Achal et al. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010], [V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010].

2.6 Concrete Mixture

The composition of mixtures are cement to sand ratio was 1:3 (by weight), and the bacterial culture/water to cement ratio was 0.47. A cube mould of 70.6 mm was used, as per IS 4031-1988. Sand and cement were thoroughly mixed, adding along with grown culture of *Bacillus* sp. CT-5 correspondence to the optical density (600nm) of 1.0. Cubes were cast and compacted in a vibration machine [V.Achal et al. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010].

For the concrete with fly ash-amended, after de-molding all specimens were cured in NBU medium at room temperature until compression testing at the intervals of 3, 7 and 28 days. Control specimens were also prepared in similar way where water and NBU medium replaced bacterial culture. Compression testing was performed using automatic compression testing machine, COMPTEST 3000

[V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010].

2.7 Compressive Strength Result

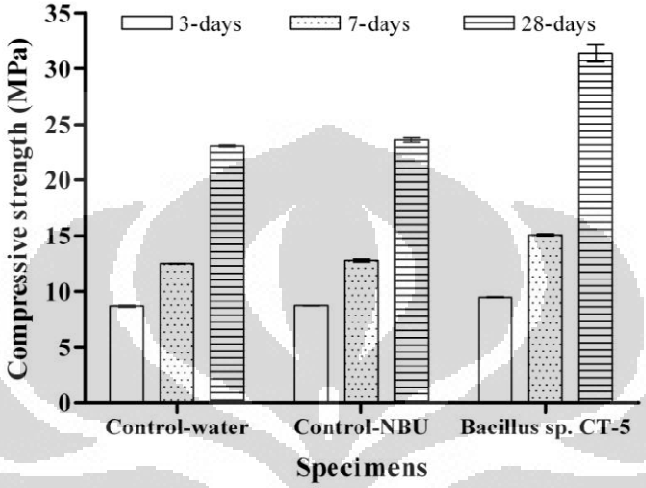


Figure 4. 3, 7 and 28 day compressive strength of different cement mortar specimens.

[V.Achal, A. Mukherjee, and M. Sudhakara Reddy. Microbial Concrete : A Way to Enhance Durability of Building Structures. 2010]

From the chart, we can infer that the compressive strength had significantly increased for the mortar cubes that contained microbial cells. The highest compressive strength was obtained with mortar cubes prepared with *Bacillus* sp. CT-5 that were incubated for 28 days (31 MPa) as compare to those prepared with water (23 MPa) and NBU medium (24 MPa). There was 36.15% improvement in the compressive strength of mortar specimens at 28 days, prepared with bacterial cells compared to control. It is noteworthy that, among the control groups without cells, the cubes cured in microbial growth medium were stronger than those cured in water although there was no significant difference. The ionic strength of medium containing Urea-$CaCl_2$ appeared to enhance the strength of the mortar cubes.

The improvement in compressive strength by *Bacillus* sp. CT-5 is probably due to deposition of $CaCO_3$ on the microorganism cell surfaces and within the pores of

cement–sand matrix, which plug the pores within the mortar [V.Achal et al. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010].

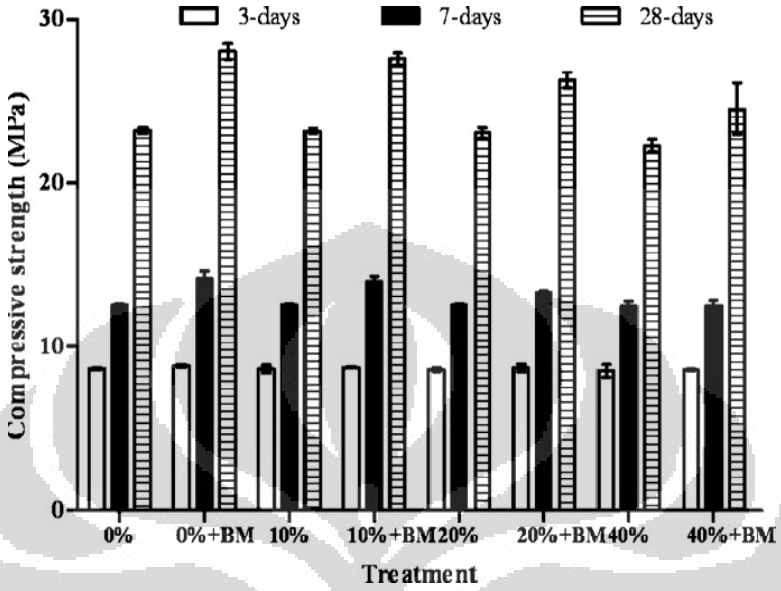


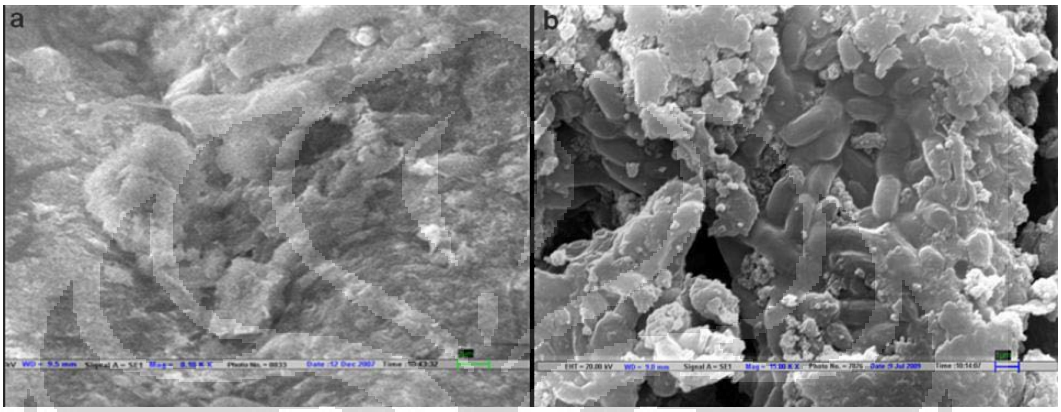
Figure 5. Effect of B. megaterium ATCC 14581 on the compressive strength of cement mortar cubes amended with different concentrations of fly ash at 3, 7 and 28 days.

Error bars show standard deviation (n = 3) (BM – Bacillus megaterium). [V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010].

The result for admixture using fly ash, and *Bacillus megaterium* ATCC 14581 also shows the improvement about 21% at 28 days. The control specimen is 23.2 MPa, and it increase until 28 MPa. Improvement in the compressive strength of mortars (containing 10% fly ash) by bacterial cells was 19% (27.6 MPa) compared to control specimen (23.1 MPa). At the 20% fly ash concentration in mortars, the bacterial cell enhanced its compressive strength by 14% compared to control specimen. There was also good improvement in the compressive strength of mortars containing 40% fly ash with bacterial cells, as it leads to 10% improvement in the strength compared to control specimen. Therefore, it can be concluded that B. megaterium ATCC 14581 significantly improved the strength of mortars amended with even higher concentrations of fly ash at 28 days. This improvement in compressive strength is probably due to deposition of CaCO3 on

the microorganism cell surfaces and within the pores of cement–sand matrix, which plug the pores within the mortar [V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010].

To determine whether the increase in compressive strength of the specimens prepared with bacteria could be attributed to the microbial calcite precipitation, the mortar samples were taken off and examined under SEM.

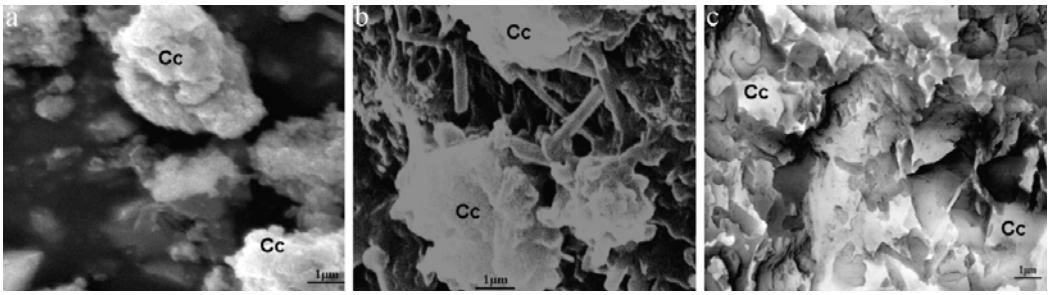


(a) **(b)**

Figure 6. Scanning Electron Micrographs of Cement Mortar Specimens.

(a) Matrix of Cement Mortar Prepared without Bacteria, and (b) Showing Dense Calcite Precipitation as Calcite Crystals with Rod Shaped Impressions Housed by *Bacillus* sp. CT-5 (V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010)

Figure 6(a) is a scanning electron micrograph of the matrix of bacteria-free cement mortar while Figure 6 (b) shows micrographs of the specimen prepared with *Bacillus* sp. CT-5 [V.Achal, et al. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010]. Sample showed calcite crystals grown all over and precipitated with rod shaped structures (typical shape of *Bacillus* species). They had distinct and sharp edges that indicating a full growth of the crystals.



(a) (b) (c)

Figure 7. Scanning electron micrographs of (a) fly ash-amended mortar, (b) close view of fly ash-amended mortar surface and (c) fly ash-amended concrete

showing microbially induced calcite precipitation (Cc – calcite crystals).
[V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]

Figure 7 shows calcite precipitation in mortar and concrete specimens by *Bacillus megaterium* ATCC 14581 which visualized by SEM analysis. A dense growth of calcite crystals embedded with bacterial cells was observed in the specimens. Bacteria were found in intimate contact with the calcite crystals. On closer observations, rod-shaped bacteria associated with calcite crystals were found. This deposition serves as a barrier to harmful substances from entering the sample, and thus improves its impermeability. The presence of crystalline calcite associated with bacteria indicates that it served as a nucleation site during the mineralization process [V.Achal, et al. *Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation*. 2010]. Based on these results it can be concluded that bio calcification by *Bacillus megaterium* plays an important role in enhancing the durability of any building materials or structures.

2.8 Permeability Result

The permeability test has been carried out to observe the impact of bacteria produce calcite on the concrete permeability. To determine the increase in resistance towards water penetration a sorptivity test was carried out. At regular time intervals (15 min, 30 min; 1h, 1.5h, 3 h, 5 h, 8 h, 24 h, 72 h, 96 h, 120 h, 144 h and 168 h) the specimens were removed from the water and weighed, after drying the surface with a wet towel. Immediately after the measurement the test

specimens were submerged again. The sorptivity coefficient, k [cm.s-1/2], was obtained by using the following expression:

$Q/A = k \sqrt{t}$

[V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010]

Where Q is the amount of water absorbed [cm^3]; A is the cross section of the specimen that was in contact with water [cm^2]; t is the time [s], Q/A was plotted against the square root of time, then k was calculated from the slope of the linear relation between the former.

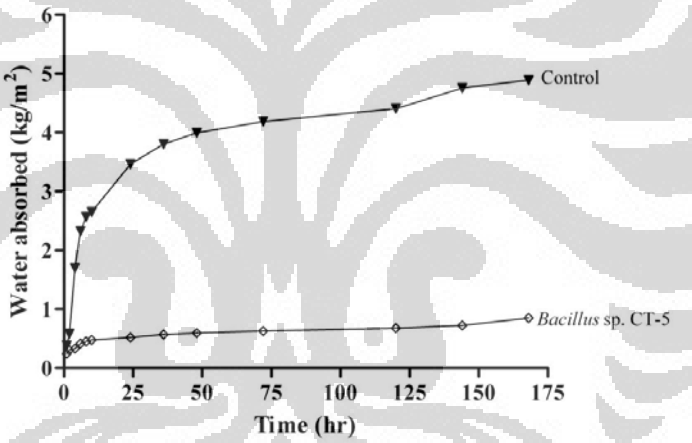


Figure 8. The Influence of the Bacterial Treatment on the Rate of Water Absorption Versus Time for Mortar Cubes.

[V.Achal, A. Mukherjee, and M. Sudhakara Reddy. Microbial Concrete : A Way to Enhance Durability of Building Structures. 2010]

Figure 8 shows the influence of the surface treatment on the water absorption rate for mortar cubes with a w/c 0.47. Over a period of 168 hours the cubes treated with *Bacillus* sp (V.Achal, A. Mukherjee, and M. Sudhakara Reddy. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010). *Bacillus* sp. CT-5 absorbed nearly six times less water than the control cubes. The presence of bacteria resulted in a significant decrease of the water uptake compared to

untreated specimens (control). The decrease in permeability of mortar specimens treated with bacteria could be seen from the water absorption experiment. The deposition of a layer of calcium carbonate crystals on the surface resulted in a decrease of the permeation properties. As a consequence, the ingress of harmful substances may be limited. Nemati and Voordouw [2003] noticed a decrease of the permeability of sandstone cores after injecting CaCO3 forming reactants. From this experiment, it is clear that the presence of a layer of carbonate crystals on the surface by bacterial isolate has the potential to improve the resistance of cementitious materials towards degradation processes [V.Achal, et al. *Microbial Concrete : A Way to Enhance Durability of Building Structures*. 2010].

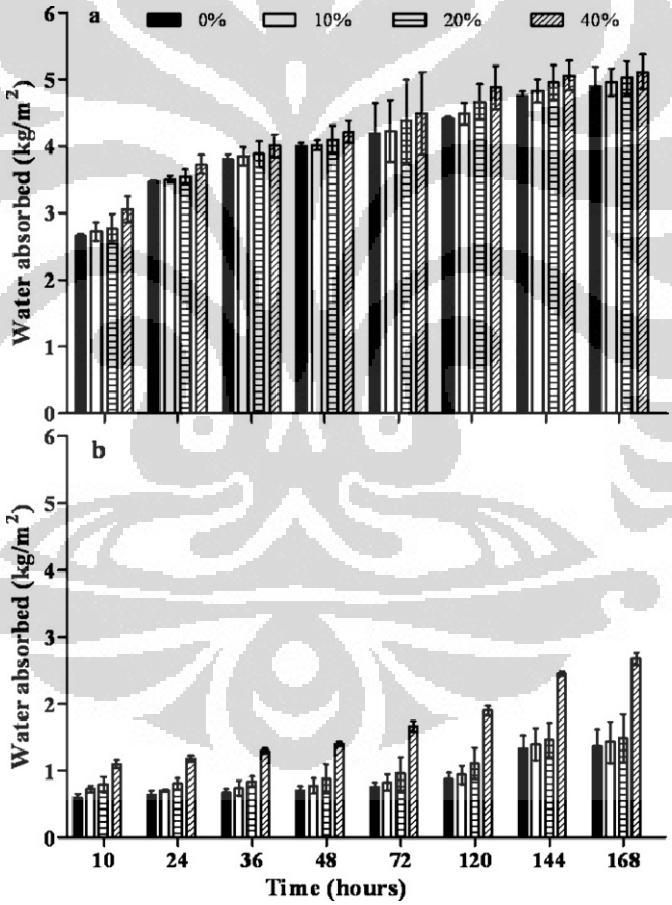


Figure 9. The influence of the (a) control and (b) microbial treatment on the rate of water absorption versus time for mortar cubes amended with different concentrations.

Error bars show standard deviation (n = 3). [V.Achal, et al. Improved Strength and Durability of Fly ash-amended Concrete by Microbial Calcite Precipitation. 2010]

Figure 9 shows the influence of the microbially induced calcite precipitation on the water absorption rate in mortar cubes. Over a period of 168 h (7 days), the cubes amended with fly ash (0%, 10% and 20%) with bacterial cells absorbed nearly 3.5 times less water (Fig. 6b) than the control cubes (Fig. 6a) while in case of cubes containing 40% fly ash, mortars absorbed two times less water compared to control. The presence of bacteria resulted in a significant decrease of the water uptake compared to control specimens. From this experiment, it is clear that the presence of a layer of carbonate crystals on the surface by bacterial cells has the potential to improve the resistance of cementitious materials towards degradation process.

2.9 Microbiology in Concrete Crack Repair

Cracking is common phenomenon due to the relatively low tensile strength. Cracks tend to expand further and eventually require costly repair [Kim Van Tittelboom, et al. "*Use of bacteria to repair cracks in concrete*". 2009]. In general, we use epoxy or grouting by cementitious material. But now, another ways to repair the cracking is revealed by utilize the bacteria which produce calcite. This method has been introduced since 1995 by Gollapudi et.al.

Uses of bacteria to repair cracking in the concrete has been realized by Ramakrishnan et al. and de Muynck et al. By injecting the paste into the crack line, we let the bacteria produced calcite to grow and produce the material which can cover the crack. This method use bacteria *Bacillus sphaericus* and *Bacillus pasteurii* [Kim Van Tittelboom, et al. "*Use of bacteria to repair cracks in concrete*". 2009]. These types of bacteria have the same characteristic with the bacteria that used in concrete mixture. They produce urease enzyme to catalyze the hydrolysis of urea into ammonium and carbonate. This technique of reparation believed to be environmentally and friendly biological process. Repair process of crack that occur equal to the process in mixing concrete.

There are several compositions to mix the bacteria and other materials to fulfill the crack. According to the research by Kim Van Tittelboom, et al. 2009, they generate the crack in the concrete by using standardized crack machine. After cracks were made, they fix it by mixing the bacteria with other chemical

compound. They combine *Bacillus subtilis* in sol-gel phase with its nutrition so they can growth well.

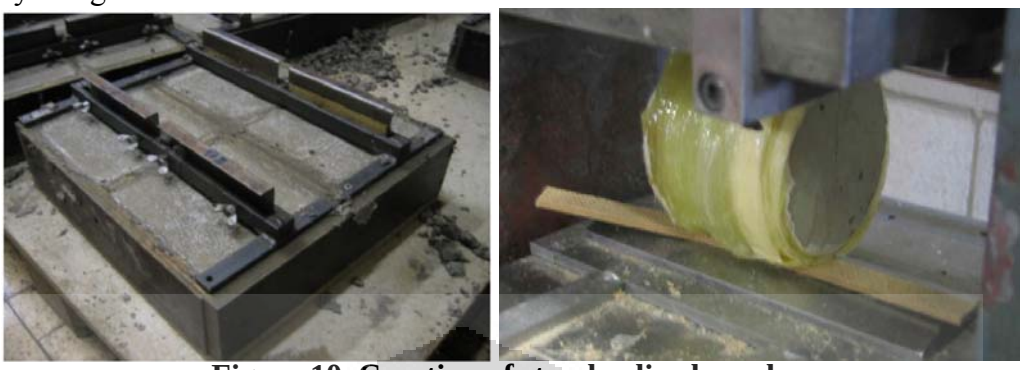


Figure 10. Creation of standardized cracks

[Kim Van Tittelboom, et al. "*Use of bacteria to repair cracks in concrete*". 2009]

Table 5. Overview of the used crack repair techniques

[Kim Van Tittelboom, et al. "*Use of bacteria to repair cracks in concrete*". 2009]

Mixture	Methods
BS + $CaCl_2$	Immersion in BS culture Immersion in $CaCl_2$ and urea solution
Sol - gel	Injection Levasil
Sol-gel+BS + $CaCl_2$	Injection Levasil Immersion in BS culture Immersion in $CaCl_2$ and urea solution
BS in Sol-gel+ $CaCl_2$	Injection Levasil and BS Immersion in $CaCl_2$ and urea solution
BS in Sol-gel+ $Ca(NO_3)_2$	Injection Levasil and BS Immersion in $Ca(NO_3)_2$ and urea solution
BS in Sol-gel+ $Ca(CH_3COO)_2$	Injection Levasil and BS Immersion in $Ca(CH_3COO)_2$ and urea solution
Autoclaved BS in sol-gel + $CaCl_2$	Autoclaving bacteria Injection Levasil and BS Immersion in $CaCl_2$ and urea solution
Autoclaved BS in sol-gel + $Ca(CH_3COO)_2$	Autoclaving bacteria Injection Levasil and BS Immersion in $Ca(CH_3COO)_2$ and urea solution

BS : Bacillus Sphaericus

After combining the bacteria and materials, they have got the following result:

Table 6. Result of composition bacteria and chemical materials

[Kim Van Tittelboom, et al. "*Use of bacteria to repair cracks in concrete*". 2009]

Mixture	**Result**
BS + $CaCl_2$	No $CaCO_3$ crystal were detected
BS in Sol-gel+ $Ca(CH_3COO)_2$	Complete filling of the crack
Grout	Only cover the surface Didn't the crack
Epoxy	Complete filling of cracks 10 mm and 20 mm deep
Sol-gel	Cracking of the gel matrix Shrinking → rise the cracking
BS in Sol-gel+ $Ca(CH_3COO)_2$	It wasn't able to fill until 20 mm deep crack

BS : Bacillus Sphaericus

From the result we can conclude that the effective composition of bacteria is *Bacillus sphaericus* in sol-gel phase with Calcium acetate '$Ca(CH_3COO)_2$'. This compound was able to fill the crack. Other compound is by injecting epoxy into the crack line.

CHAPTER 3

3.1 Laboratory Experimental

In this chapter, we will discuss about how to grow the bacteria produce calcite in laboratory of microbiology Université Lille 1, Lille. In the laboratory we grow the *Bacillus subtilis* as the selected bacteria for the research. We choose this type of bacteria because of some factors:

- Non pathogenic bacteria
- Easy to cultivate
- Simplicity of shape
- High urease enzyme
- Easy to collaborate with other type of bacteria

For the nutrition, it can be growth for example by following medium that contain one of the chemical solution for example Nitrogen (N), Sulphur (S), Phosphor (P), Iron (Fe), and Magnesium (Mg).

First of all, we have to know the basic theory of growth of the bacteria. In general, growth of bacteria is divided into three (3) phase. First phase is latency where the bacteria must adapt to the environmental condition for example temperature, pH, and humidity. In this phase, growth of bacteria will take a little long time because of the capability to adapt with the condition of environment. Second phase is augmentation of bacteria's cellule. In this phase, bacteria divide itself in exponential form.

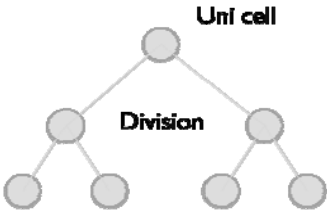


Figure 11. Growth pattern of bacteria exponential form

The final phase is constant phase. This phase is happen when the bacteria stops dividing its cells because of the availability the nutrition (source of carbon). In this phase, the rate of growth of bacteria will reduce and stop.

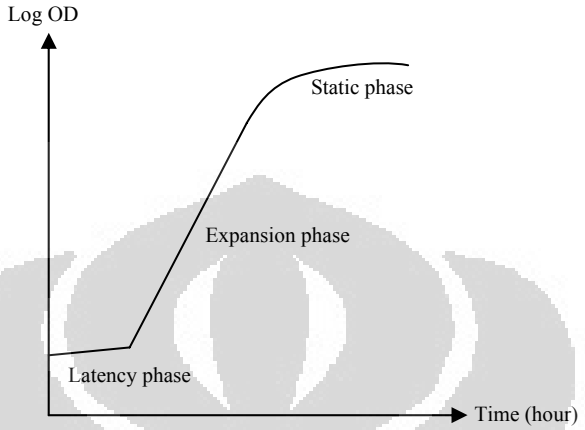


Figure 12. Growth curve of bacteria

We are able to know the growth of bacteria by spectrometer. We observe the turbidity of the solution bacteria. The value of Optical Density (OD) will increase in accordance with turbidity of solution and growth of bacteria.

3.2 Procedure Experimental

In this first experiment, we try to observe the growth of the bacteria *Bacillus subtilis* using agar based medium. We have the bacteria from the stock culture over night at laboratory of microbiology Université Lille 1. This culture were added with glycerol to protect the bacteria against low temperature and stored in temperature -20^{o}C for 1 year and -80^{o}C for 10 years in a petri dish. This storage is done in order to get the same strain of type the *Bacillus subtilis*.

For this experiment, bacteria were taken from the storage -20^{o}C and we let them for one night by adding the LB medium (Lysogeny broth) 15 g/L agar. This medium is functioned as the nutrition for the bacteria as the carbon source. In the morning, we are able to observe the growth of bacteria. First, we put 50µl of bacteria and add 5 ml of Lysogeny broth (LB). The next step is storing into the room with temperature 37^{o}C and under shaking condition 120 rpm. After storing,

we measure the value of optic density (OD) with spectrometer 20. For the first time we try to set the solution of bacteria with the value of optical density 0.1. We observe the growth of bacteria until the value of optical density is 1. When the value of optical density is one (1), we have 10^9 cell of bacteria/ml.

3.3 Measure the optical density of bacteria

For the measurement of optical density, we have noted the time of measurement to observe the growth of bacteria. First we calibrate the spectrometer until its value is zero (0).

Table 7. Value of optical density

Time	Optical Density
09:50	0.110
10:35	0.130
11:00	0.160
11:30	0.300
12:08	0.590
12:55	1.00
13:20	1.40

From the result, the normal value to observe the optical density of solution is 0.05 – 0.1. In this measurement, we get the first value is 0.110. We could see about 1 hour, the enhancement of the optical density is slow (0.050). This is the first phase where the bacteria are adapts with the condition of environment, humidity, temperature, and nutrition. But, next one hour, we can see the significant increase until 0.59 and we obtain the value of optical density equal to 1 (one) the next 40 minute. After we have obtained the result, we store it one night in the cultivation room where it has 37^{o}C of temperature and under shaking condition of 120 rpm. This condition of temperature is already set because it is the ideal condition of

environment to growth the bacteria. We can get the optimum amount of bacteria with optimum time too.

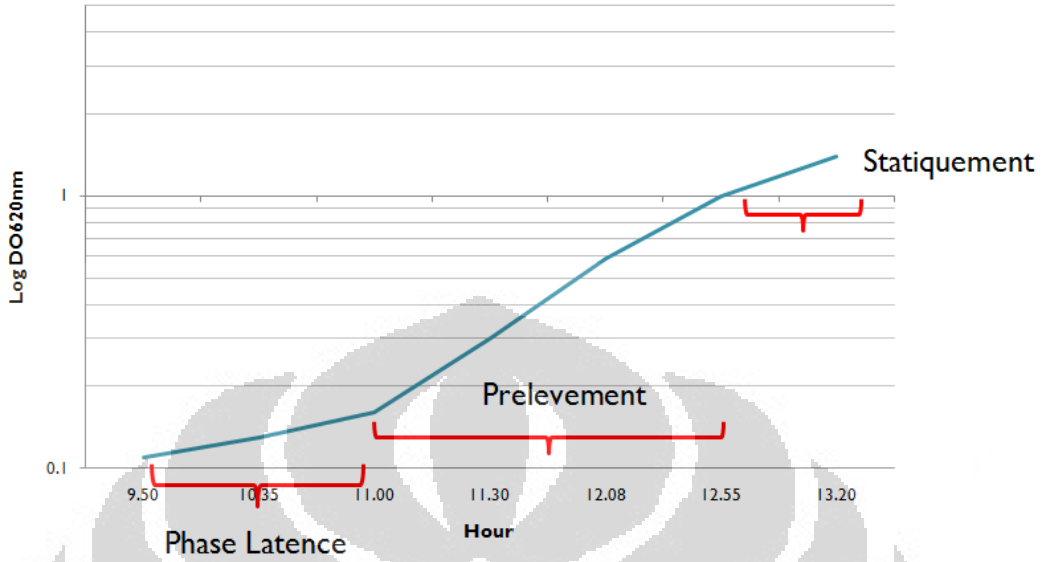


Figure 13. Result of growth of Bacillus subtilis DO620nm
Laboratory of Microbiology, Université Lille 1

The curve shows the growth of bacteria that occur rapidly after phase latency. This rapid growth of bacteria is an advantage for the research that it does not take very long time.

Figure 14. Spectrometer 20

The next step, knowing the number of colonies of the bacteria is also important. This is the step where the bacteria will form their colonies in the agar based medium.

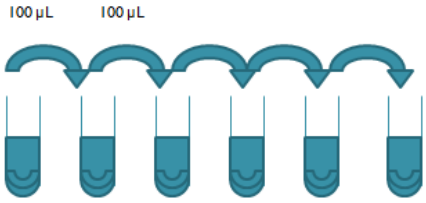


Figure 15. Observe the colonies of bacteria Bacillus Subtilis

By adding 900µL LB and 100µL solution of bacteria into the tube, we observe the strength of the solution that can influence the number of the colonies. The percentage of the solution might influence the number of the colonies of the bacteria. Weaker concentration, the numbers of the colonies will fewer than the stronger concentration.

Table 8. Number of colony of Bacillus subtilis

Solution	Colony	Colony
10^{-6}	3	4
10^{-5}	42	51
10^{-4}	312	237

This number is obtained by counting the number of the colony inside the petri dish. Weaker concentration has the lowest colonies. These colonies could be seen in the following picture.

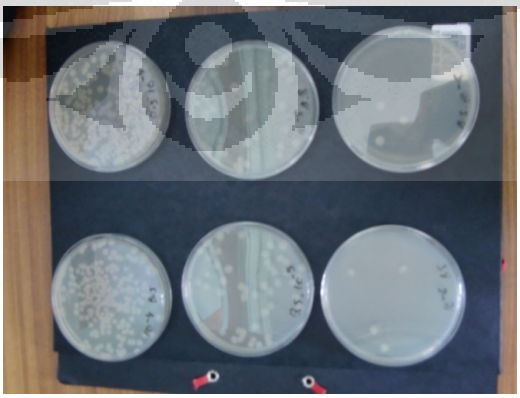


Figure 16. Colonies of the bacteria Bacillus subtilis

CONCLUSION

- Uses of microorganisms to improve the soil capacity have been reported by several researches about bioclogging and biocementation. These two methods have the same objective to fulfill the soil pores. By injecting the bacteria into the soil, it could produce calcite to fulfill the pores between it. After the treatment, it could increase the capacity of the soil until five fold.
- Application of bacteria in concrete mixing or concrete repairing has been successfully applied in several researches. This method is believed to be more economic and has more advantage to the environment. By adding the bacteria which able to produce calcite to fill the concrete's pore, it can improve the value of compressive strength. For further application, it was able to fulfill the crack of the concrete. This method is very depending into condition of environment. Factors that can influence the production of the calcite are the concentration of dissolved inorganic carbon, pH (acidity) of the soil, concentration of calcium ions, and presence of nucleation sites.
- From this experimental test in the laboratory, the method to grow the *Bacillus subtilis* is by using the medium of glucose, we can obtain the result satisfying that the bacteria able to grow rapidly. For the next experimental, it should be applied the same method to grow the bacteria in the same condition. The application of the microbiology to improve the mechanical properties of the soil is revealed and ready to be applied in the mini scale experimental. The type of the soil which will be used is the sand, because it has more pores and could be controlled if the bacteria will produce calcite.
- The disadvantage of this application is that all the experiments have to be done in sterilized condition. For the real scale, this application is more difficult because the condition reel was not sterilized. But, sterilization can be applied when injection the bacteria is launched. According to several research, application in the real condition ahs more difficulties for examples the bacteria need more time to produce calcite in the non sterile condition. In the ideal condition it takes one or two hours, but in the reel condition in the field it could takes one week and there will be competition to have the nutrition with other type of bacteria.

REFERENCE

1. Brian C. Martinez, Jason T. DeJong. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales*". ASCE. 2009
2. Kim Van Tittelboom, Nele De Belie, Willem De Muynck, Willy Verstraete. "*Use of bacteria to repair cracks in concrete*". Elsevier. 2009
3. Leon A. van Passen et al. Potential Soil Reinforcement by Biological Denitrification.
4. Marcia Aiko Shirakawa, Katia Kaori Kaminishikawahara, et.al. "*Sand Bioconsolidation Through the Precipitation of Calcium Carbonate by Two Ureolytic Bacteria"*. Mater Lett. Elsevier. 2011
5. N. De Belie. "*Microorganisms Versus Stony Materials: A Love–Hate Relationship"*. Materials and Structures (2010) 43:1191–1202. RILEM 2010
6. Varenyam Achal, Xiangliang Pan. "*Characterization of Urease and Carbonic Anhydrase Producing Bacteria and Their Role in Calcite Precipitation*". Springer Science + Business Media. LLC 2010
7. V. Achal, A. Mukhrejee, and M. S. Reddy. "*Microbial Concrete : A Way to Enhance Durability of Building Structures"*. ASCE. 2010
8. V. Acahl, X. Pan, N. Ozyurt. *"Improved Strength and Durability of Fly Ash-Amended Concrete by Microbial Calcite Precipitation"*. Elsevier. 2010.
9. Volodymyr Ivanov, Jian Chu. "*Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ"*. Springer Science+Business Media B.V. 2008

INDEX

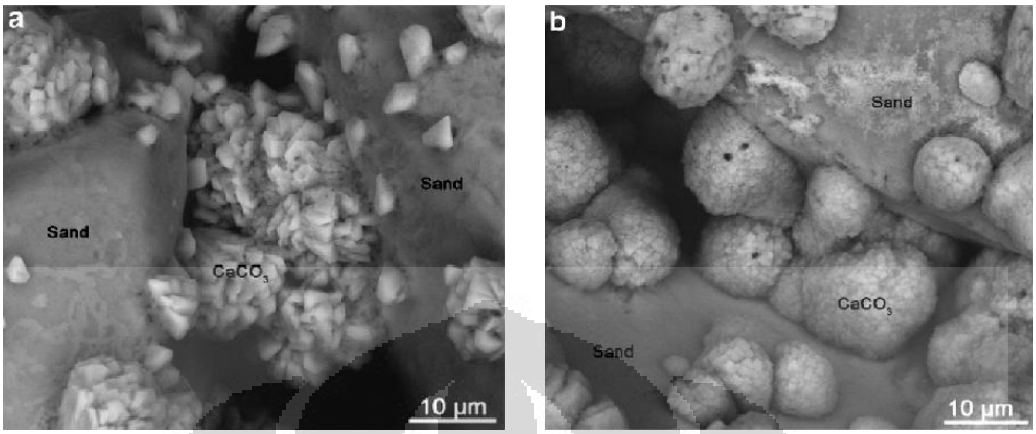


Figure 17. Scanning Electron Microscope of Bacillus sphaericus
(Marcia Aiko Shirakawa, Katia Kaori Kaminishikawahara, Vanderley Moacyr John, Henrique Kahn, Marcos Massao Futai . Mater Lett. Elsevier. 2011)

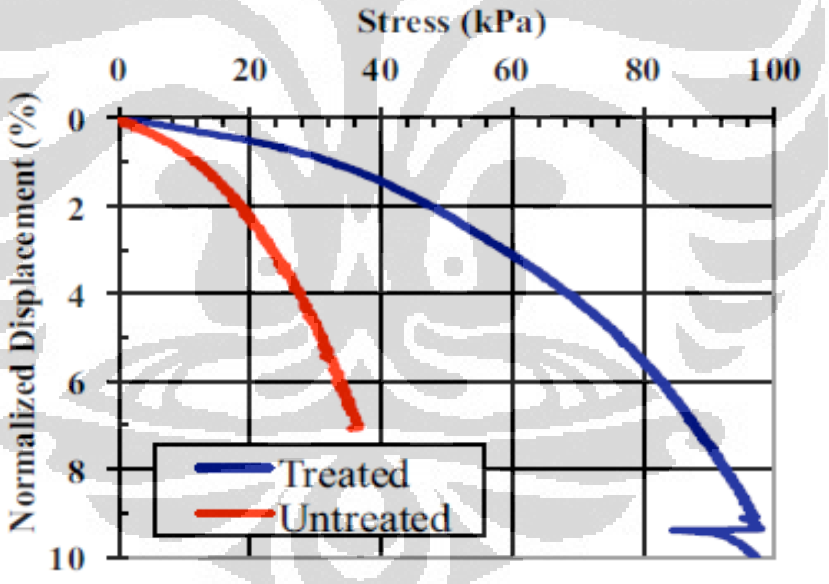


Figure 18. Quantitative results for MICP treated and untreated specimens
(Brian C. Martinez, Jason T. DeJong. "*Bio-Mediated Soil Improvement Load Transfer Mechanisms at the Micro- and Macro- scales*". ASCE. 2009.)

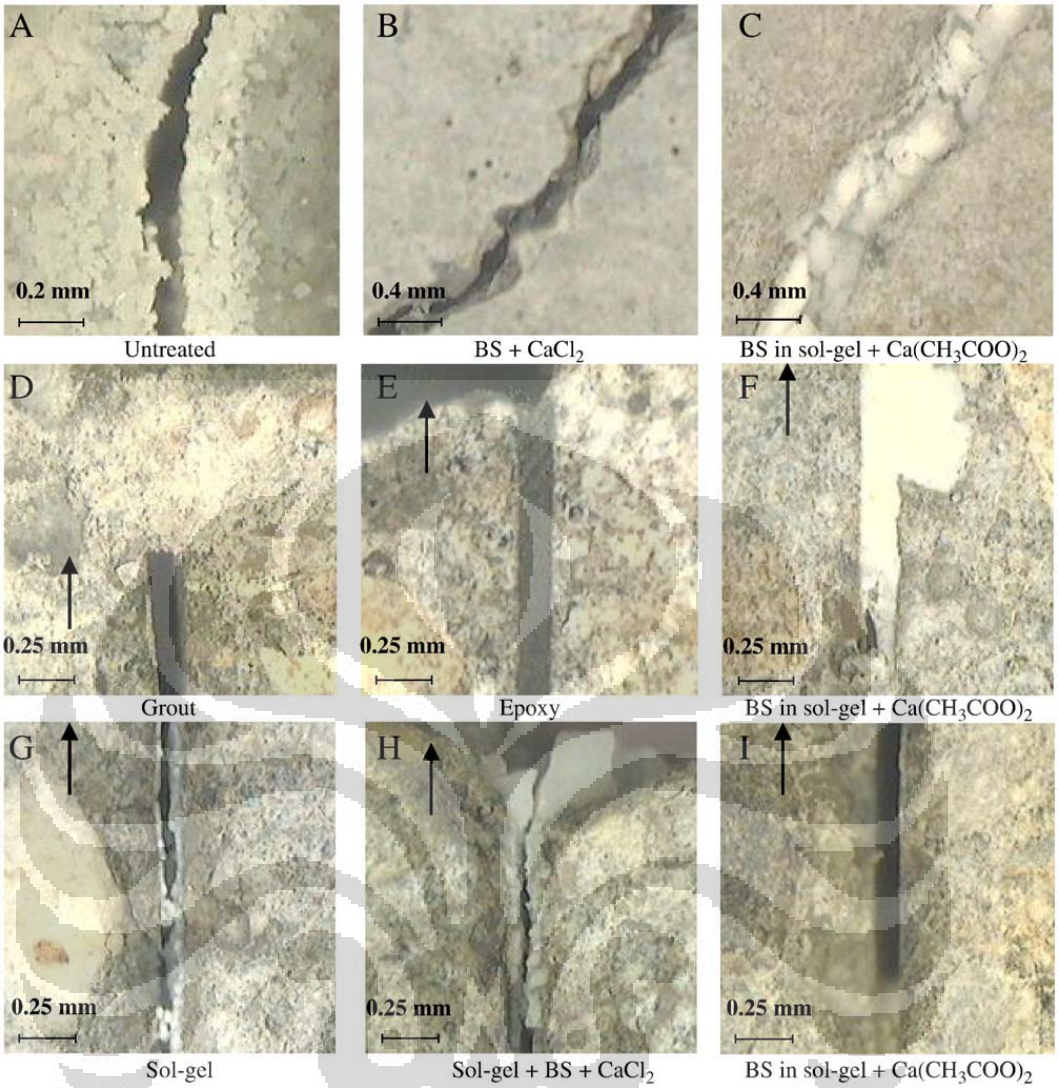


Figure 19. Mode of cracking and the treatment
(Kim Van Tittelboom, Nele De Belie, Willem De Muynck, Willy Verstraete. "*Use of bacteria to repair cracks in concrete*". Elsevier. 2009)

Figure 20. Agar medium for growth bacteria in petri dish

Figure 21. Shaker machine

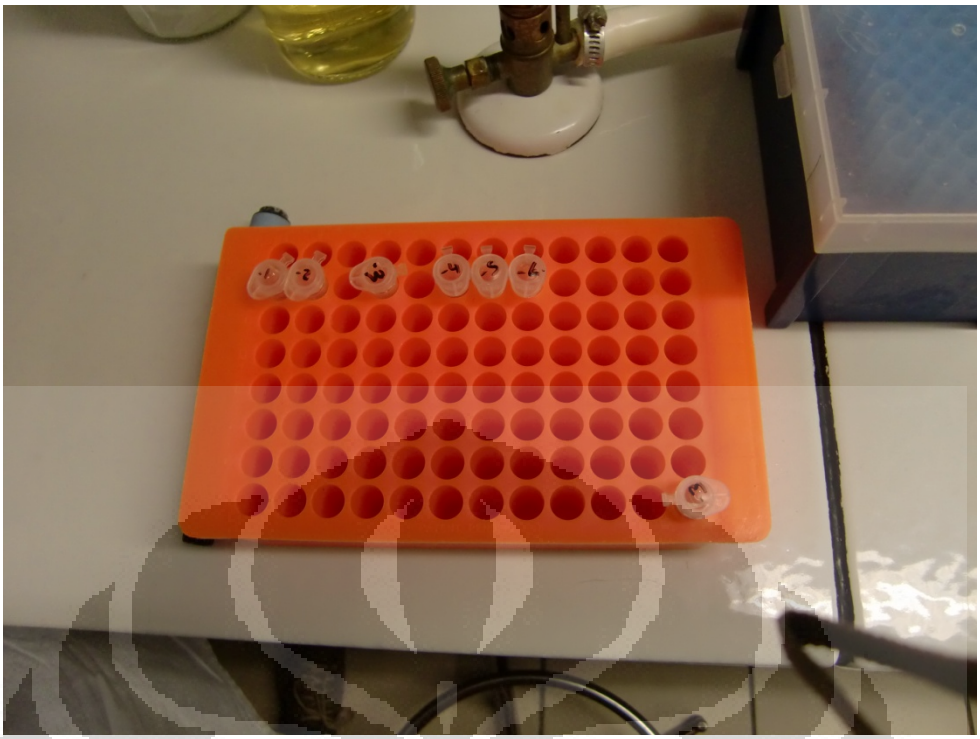


Figure 22. Culture of Bacillus subtilis in different concentration

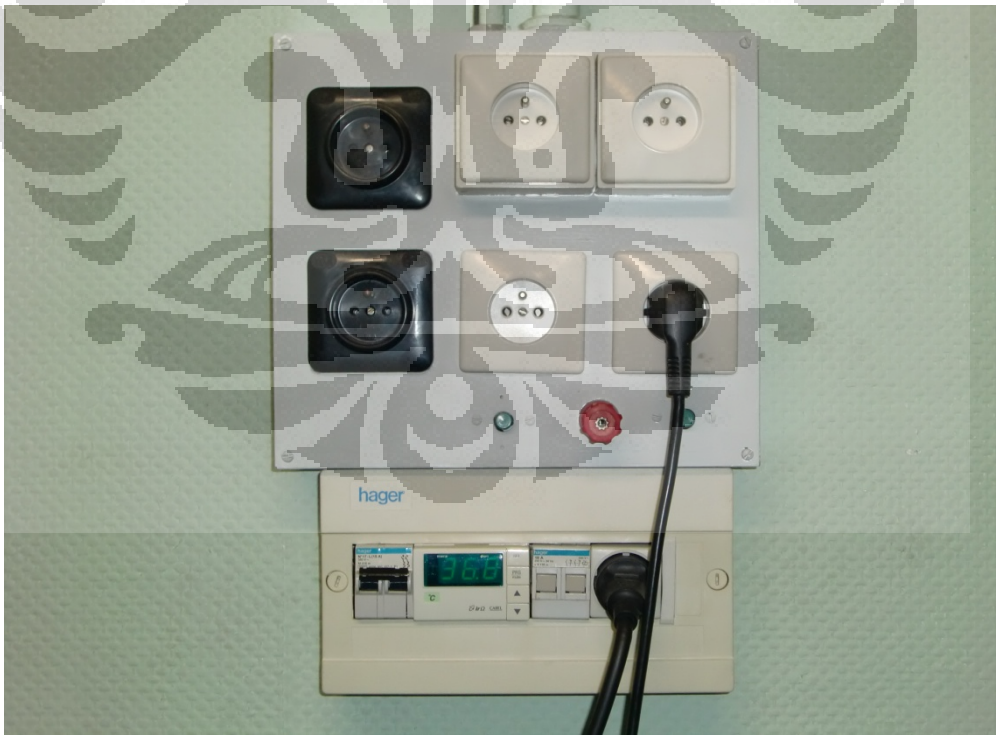


Figure 23. Temperature control

Figure 24. Drying process to avoid presence of air

Figure 25. Colonies of bacteria