

Lecture 39

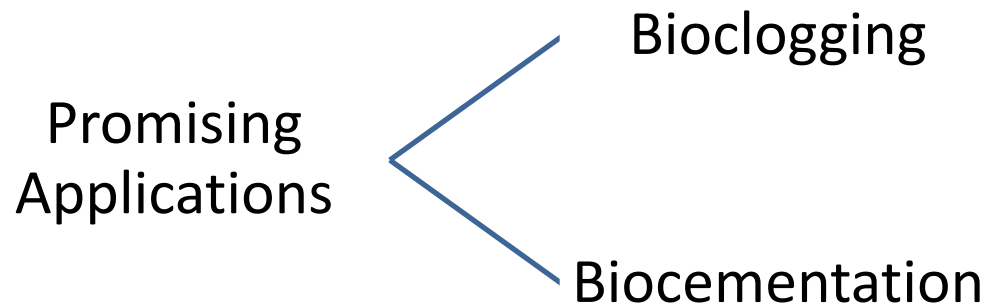
Emerging aspects in Microbial Geotechnology and Ground Improvement



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INTRODUCTION

- Microbial Geotechnology – Emerging branch of Geotechnical engineering



- Bioclogging – Production of pore-filling materials
- Biocementation – Production of particle binding materials

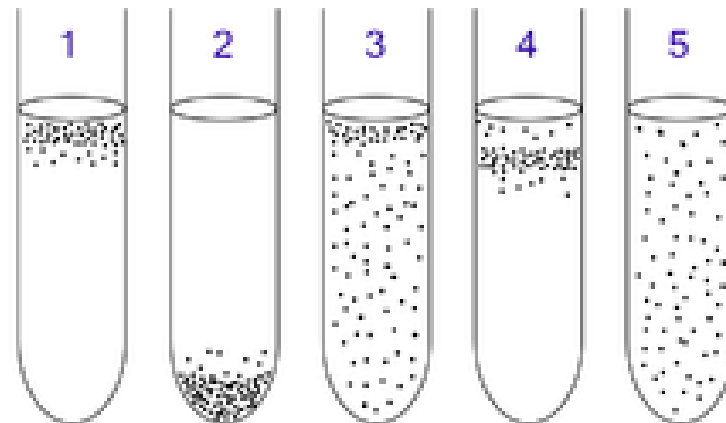
Ref: Ivanov V. and Chu J. (2008) Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. *Reviews in Environmental Science and Biotechnology*, , 7: 139-153

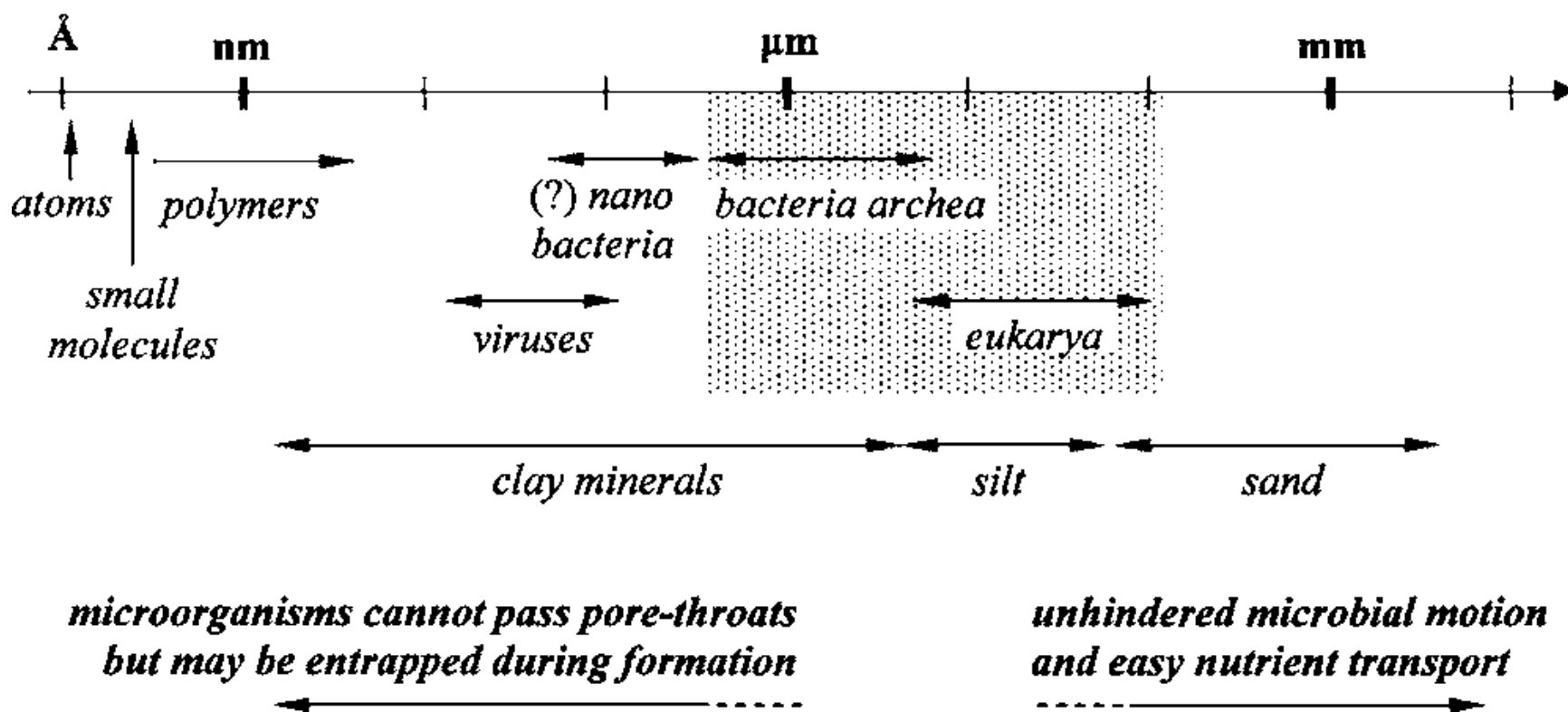
Mitchell J.K. and Santamarina J.C. (2005) Biological Considerations in Geotechnical Engineering. *J. Geotech. Geoenviron. Eng.* 131: 1222-1233

- Suitable microorganisms :
 - Facultative anaerobic bacteria
 - Micro-aerophilic bacteria
 - Anaerobic fermenting bacteria
 - Anaerobic respiring bacteria
 - Obligate aerobic bacteria

Aerobic and anaerobic bacteria can be identified by growing them in liquid culture:

Obligate aerobe
Obligate anaerobe
Facultative anaerobic organism
Microaerophile
Aerotolerant





- Factors affecting applications of microorganisms:
 - Screening and identification of suitable microorganisms
 - Optimization of microbial activity in situ
 - Biosafety of the application
 - **Cost effectiveness** : Most important for large-scale application
 - Stability of soil properties after biomodification

BIOCLOGGING



- **AIM:** To reduce porosity and hydraulic conductivity of soil and porous rocks due to microbial activity or products
- Potential of use as sealing material for leaking construction pit, landfill or dike
- Use as grouting material for soil erosion control, mitigating soil liquefaction and enclosing of bioremediation zone
- **Examples of Bioclogging processes:**
 -  Microbial production of water-insoluble polysaccharides in situ
High cost involved
 -  Production of bacterial exopolymers in situ
Adopted for soil bioremediation

Table 1 : Microbial Processes that can lead to bioclogging

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	Avoid 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 metals	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 CO ₂	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

MICROORGANISMS USED FOR BIOLOGGING

Oligotrophic bacteria

Caulobacter

Aerobic Gram-negative
bacteria

*Acinetobacter, Agrobacterium,
Alcaligenes, Cytophaga, Flavobacterium,
Pseudomonas, Rhizobium*

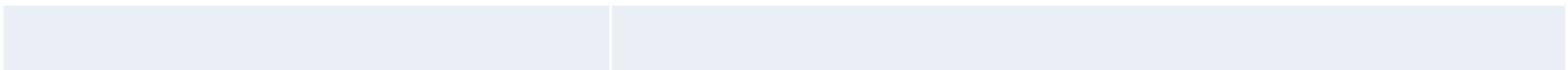
Cellulose degrading
bacteria

Cellulomonas flavigena

Gram –positive bacteria

Leuconostoc mesenteroides

Nitrifying bacteria

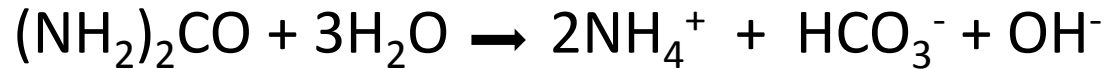


APPLICATIONS

- Exopolysaccharide producing bacteria may be used to :
 - Reduce permeability of soil
 - Selective zonal bioremediation
 - Harbour and dam control
 - Earthquake liquefaction mitigation
- Cost of soil clogging can be reduced by using organic wastes for producing exopolysaccharide
- To reduce drain channel erosion
- To form grout curtains to diminish migration of heavy metals and organic pollutants
- To prevent piping of dams and dikes

APPLICATIONS (contd.)

- Enzymatic reaction by *Bacillus pasteurii* to form soil plugs:



- pH increases
 - Initiation of precipitation of calcium carbonate
 - Leads to clogging of pores and binding of soil particles
- Bioclogging can be used to:
 - Enhance recovery of oil from reservoirs
 - Repair cracks in concrete
 - Seal unforeseen leaks in the sheet piling screens around construction wells
- Estimated tens of millions of Euros of savings in cost

LIMITATIONS AND POTENTIAL PROBLEMS

- Stability of soil after biomodification will be achieved under favorable conditions for growth of exopolysaccharide-producing microorganisms
- Slow growth of nitrifying and oligotrophic bacteria implies long-term treatment of soil for clogging
- Penetration of microbial cells in soil depth is limited by minimum soil pore size (0.5 to 2 μm)
- Clogging biofilm in soil pores affects the concentrations and mass transfer rates of nutrients and microbial metabolites between biofilm and flow through the pores

BIOCEMENTATION-INTRODUCTION

- Microbial cementation (Biocementation)- formation of soil particle-binding material with aid of microbes and additives.
- It is different from biobinding! (biobinding utilizes fungi, it is unstable and is easily degradable hence not suitable for large scale operations.

CHEMICAL CEMENTATION

- Occurs due to precipitation of material in spaces between soil particles and binds them to form a hard rock like mass.
- Ex- drying of soil sample containing iron hydroxide (needs high pH or redox potential).
- Similarly precipitation of silica dioxide(also known as natural soil calcification).
- It fills pores and glues soil particles together.

BIOCEMENTATION PROCESS

- Only processes that can be mediated by microbial activity such as
 - Oxidation
 - Reduction
 - Dissolution
 - Precipitation

of inorganic substances in soil are **STRONG** and **STABLE**.

POSSIBLE MICROBIAL PROCESSES

- Binding of soil particles with sulphides of metals produced by sulphate - reducing bacteria.
- Binding of soil particles with carbonates of metals produced due to hydrolysis of urea.
- Binding of particles with ferrous and ferric salts and hydroxides, produced due to activity of iron-reducing bacteria.

MICROORGANISMS ASSOCIATION

- Calcium
- Magnesium
- Iron
- Manganese
- Aluminium which are crystallized as
 - Carbonates
 - Silicates
 - Phosphates
 - Sulphides
 - Hydroxides (especially iron hydroxides)

HIGH STRENGTH CEMENT

- Material + urease-producing microorganisms + urea + soluble calcium=Cement (Kucharski et al. (2005))
- Materials- Conglomerate, Breccia, Sandstone, Siltstone, Shale, Limestone, Gypsum, Peat, Lignite, **Sand, Clay**, Sediments, Sawdust.
- Urease producing microorganisms-*Bacillus*, *Sporosarcina*, *Sporolactobacillus*, *Clostridium* and *Deslfotomaculum*.

BIOCEMENTATION OF SAND

- Sand with enrichment culture of iron-reducing bacteria, fine particles of iron ore and cellulose as electron donor demonstrated that Fe^{2+} formed can produce cementation effects.
- Fe^{2+} is oxidized by air.
- Unconfined shear strength increases from 0 to 140 kPa.

COMPARISON WITH MECHANICAL COMPACTION

- Mechanical compaction
 - Shallow (rolling or vibrating)
 - Deep (vibro or dynamic compaction)
- These are economically viable up to **10 m**.
- Dynamic compaction cannot be used for clayey soil and municipal landfills.

COMPARISON WITH CHEMICAL GROUTING

- Chemical grouting- Inject chemical grout into pores to enhance physical properties.
- Grout can be dissolved or suspended.
- They diminish permeability and increase mechanical strength.
- Usually cement, bentonite, silicates, lignosulfonates, pozzolanic-based materials, thermoplastic polymers, organic polymers etc. are used.

GROUT INJECTION

- Pressure injection- most effective in sandy soils and cracked rocks.
- Grout must have proper hardening time
 - Less- sets before reaching
 - More-spreads too thinly.
- Low pressure- Injection of low-viscosity grout at low pressure, no change in soil volume.
- High pressure- Jet grouting, high flow velocity mixes soil and grout and sets.
- Stage up or Stage down.

BIOCEMENTATION REQUIREMENTS

- Depth of penetration depends on size of microorganism used.
- For filamentous microorganisms typical size 1-3 μm , length up to 100 μm .
- It requires optimal pH, salinity, oxidation-reduction potential, concentration of nutrients and water content.

ADVANTAGES OVER CHEMICAL GROUTING

- Non-toxic (Lignosulfonates, acrylamides and polyurethane are toxic-chemical grouts).
- Low cost of reagents.

COST COMPARISON

Material	Price (\$/kg)	Amount of additives required (kg/m ³)	Cost of additives (\$/m ³)
CHEMICAL			
Lignosulphites-Lignosulphonates	0.1–0.3	20–60	2–18
Sodium silicate formulations	0.6–1.8	10–40	6–72
Phenoplasts	0.5–1.5	5–10	2.5–15
Acrylates	1.0–3.0	5–10	5–30
Acrylamides	1.0–3.0	5–10	5–30
Polyurethanes	5.0–10.0	1–5	5–50

Materials	Price (\$/kg)	Amount of additives required (kg/m ³)	Cost of additives (m ³ of soil)
MICROBIAL			
Molasses + microorganisms	0.1–0.2	5–20	0.5–4.0
Homogenized food-processing wastes + microorganisms	0.05–0.1	10–20	0.5–2.0
Iron ore + organic wastes + microorganisms	0.1–0.2	10–20	1.0–4.0
Organic wastes (agricultural, horticultural, food-processing wastes)	0.05–0.1	10–20	0.5–2.0
Calcium chloride + urea + microorganisms	0.2–0.3	20–30	4.0–9.0

APPLICATIONS

- Enhancing stability for retaining walls, embankments and dams.
- Reinforcing or 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.

APPLICATIONS

- Binding of the dust particles on exposed surfaces to reduce dust levels.
- Increasing the resistance to petroleum borehole degradation during drilling and extraction.
- Increasing the resistance of offshore structures to erosion of sediment within or beneath gravity foundations and pipelines.
- Stabilising pollutants from soil by the binding.
- Controlling erosion in coastal area and rivers.
- Creating water filters and bore hole filters.
- Immobilising bacterial cells into a cemented active biofilter.

LIMITATIONS

- Microbial process is slow.
- It is complex (depends on multiple factors like pH, concentration of donor and acceptors of electrons, temperature, concentrations and diffusion rates of nutrients and metabolites).
- Apart from soil conditions and grouting medium content, microbiological, ecological and geotechnical aspects must also be considered.

LIMITATIONS

- Bioclogging and biocementation requires data of
 - Growth
 - Biosynthesis
 - Biodegradation
 - Bioreduction
 - Biooxidation
 - Specific enzymatic activities
 - Precipitation
 - Crystallization
 - Adhesion.
- No large scale testing done yet!

ASSUMPTION

- Assumption derived from general consideration of physiological activity of prokaryotes:
 - Bacteria with Gram-positive type of cell wall are most suitable for soil bioclogging and biocementation.
 - These bacteria are most resistant to the changes of osmotic pressure which is typical condition for soil on construction reclamation sites.

SCREENING OF MICROORGANISMS

- Group of chemotrophic prokaryotes (cyanobacteria) have wide physiological diversity.
- Grows only on soil surface and produces rigid crust which diminishes soil infiltration and improves slope stability.
- Lower layers become anaerobic hence less efficient.

SCREENING OF MICROORGANISMS

- Anaerobic fermenting bacteria- used when calcium, magnesium or ferrous ions.
- Cementation is due to increase in pH caused by ammonification and carbon dioxide production in soil added with urea or waste protein.
- If carbohydrates are added the bacteria diminishes the pH and causes precipitation of silicates.

SCREENING OF MICROORGANISMS

- Anaerobic fermenting bacteria- They cannot clog soil pores by synthesis of extracellular polymers because they cannot produce large quantity of slime.
- Organic acids, hydrogen and alcohols can be used as donors of electrons.
- It can be used in treatment of waste water and groundwater.

SCREENING OF MICROORGANISMS

- Sulphate reducing bacteria- They produce dihydrogen sulphide using organic acids, hydrogen or alcohols as electron donors and sulphate as electron acceptor.
- Sulphide reacts with iron and other metal to form insoluble sulphides of metals
- These clogs the soil pores and binds the soil particle.
- Soil compaction created is unstable because they can be oxidized to sulphuric acid or sulphates.

SCREENING OF MICROORGANISMS

- Most suitable bioagents- they produce big quantity of exopolysaccharides, which usually promote formation of cell aggregates.
- It can work both in aerobic and anaerobic conditions.
- Both microzones of aerobic and anaerobic co-exist.
- Ex- *Alcaligenes*, *Enterobacter*, *Staphylococcus*, *Streptococcus*, *Rhodococcus*, corynebacteria (*Gordonia*, *Nocardioides*), gliding bacteria (*Myxococcus*, *Flexibacter*, *Cytophaga*) and oligotrophic bacteria (*Caulobacter*)

SCREENING OF MICROORGANISMS

- Aerobic bacteria- they produce big quantity of slime, form chains and filaments, increase pH and oxidize different organic and inorganic substances.
- *Actinomyces* are commonly used.
- pH increases due to ammonification and then insoluble carbonates are precipitated forming particles that clog the soil pores.
- Formation of anaerobic zones below makes the bacteria inactive.

Table 2: Periodic table- Screening of microorganisms

Ecology of origin	Relation to oxygen and type of energy generation			
	Anaerobic fermenting prokaryotes	Anaerobic respiring prokaryotes	Facultative anaerobic and microaerophilic prokaryotes	Aerobic respiring prokaryotes
Prokaryotes of aquatic origin	<i>Bacteroides</i>	<i>Desulfobacter</i>	<i>Escherichia</i>	<i>Pseudomonas</i>
	<i>Prevotella</i>	<i>Geobacter</i>	<i>Shewanella</i>	<i>Acinetobacter</i>
	<i>Ruminobacter</i>	<i>Wolinella</i>	<i>Beggiatoa</i>	<i>Nitrosomonas</i>
Prokaryotes of terrestrial origin	<i>Clostridium</i>	<i>Desulfotomaculum</i>	<i>Microthrix</i>	<i>Bacillus</i>
	<i>Peptococcus</i>	<i>Desulfitobacterium</i>	<i>Nocardia</i>	<i>Arthrobacter</i>
	<i>Eubacterium</i>	<i>Bacillus infernus</i>	<i>Streptococcus</i>	<i>Streptomyces</i>
Prokaryotes originating from extreme environments (Archaea)	<i>Desulfurococcus</i>	<i>Methanobacterium</i>	<i>Metallosphaera</i>	<i>Picrophilus</i>
	<i>Thermosphaera</i>	<i>Thermococcus</i>	<i>Acidianus</i>	<i>Ferroplasma</i>
	<i>Pyrodictium</i>	<i>Haloarcula</i>	<i>Haloferax</i>	<i>Sulfolobus</i>

BIOSAFETY

- The most suitable microorganism for biocementation and bioclogging must be safe too.
- It can be solved by selecting safe bacterial strain.
- The biomass of this safe strain can be produced in bioreactor and used as starter culture for bioclogging or biocementation.

BIOSAFETY

- To diminish the risk of pathogenic accumulation the following conditions are used-
- An application of carbon sources, which are used in nature by saprophytic microorganisms, such as cellulose, cellulose-containing agricultural waste, vegetable-processing waste, molasses.
- The conditions that are suitable for the growth of autolithotrophic bacteria. Carbon dioxide is used as a carbon source and inorganic substances (NH_4^+ , Fe_2^+ , S) are used as electron donor.
- The conditions that are suitable for application of bacteria able for anaerobic respiration with SO_4^{2-} or Fe^{3+} as electron acceptors.
- An application of solution with low concentration of carbon source for preferable growth of oligotrophic microorganisms in soil.

CONCLUSIONS

- Bioclogging and Biocementation processes could be used to improve mechanical properties of soil in situ. They can replace energy demanding, expensive and environmentally unfriendly methods with significant reduction in cost.
- Most suitable microorganisms for large-scale construction and environmental problems are facultative anaerobic and microaerophilic bacteria.
- Industrial-scale applications of microorganisms in geotechnical engineering is yet to be determined.