The Influence of Microbiology on Soil Aggregation Stability

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Abstract. In the present study, this work investigated the effects of bacteria (Bacillus sp.) decay and biological aggregating factors (organic matter, microbial calcium carbonate precipitation) at various steps on soil aggregate stability. Experiments carried out at different cultivation time 2, 5, and 8 weeks. The soil samples were compared to the control (pure soil) versus soil treated with bacteria. The results showed that bacterial activity played an essential function in the macro-aggregate foundation. Nevertheless, the influence of organic matter (OM) on soil aggregate stability was important, which might be related to the microbial activity of bacteria to decay the organic material to an organic mineral that is connected with soil particles to form stable micro-aggregates. The CaCO₃ is one of the most stable bonding agencies in nature as it is called natural cement. The soil aggregates stability increased as calcium carbonate increased. No change of soil pH levels observed due to microbial reactions with soil. Hence, the appropriate pH was 7.5. In addition, the higher pH decreases enzymatic activity and carbonate tends to dissolve at pH levels are very low. This study affords the helpful of soil stabilization in the presence of natural cementation of bacteria in contrast with the previous studies.

Keywords: Bacteria, Organic matter, Microbial calcium carbonate precipitation, Aggregation stability

1. Introduction
Microorganisms are the most important causes of aggregate stability. Bacteria participate in the stabilization of soil aggregates by precipitation of extracellular polysaccharides (EPS) and the creation of hemic materials that form polyvalent metal-organic matter complexes. Soil aggregation, a key component of soil structure, fertility, and soil loss are one of the explanations for soil erodibility [1]; [2]; [3]; [4]. Applying ecological technique would be a workable solution for doing sustainable rebuilding of degenerate soils. Soil aggregation is a complex associate among biological, chemical, and physical processes in the soil [5]; [6]; [7]. Factors affecting aggregate stability include carbon storage, organic matter (OM) stabilization, soil microorganisms, water holding capacity and resistance to erosion [8]. The enhancement of soil stability is relevant for agroecosystems sustainability, avoidance of erosion. Numerous studies reported that extracellular polysaccharides (EPS), calcium carbonate, contents of organic matter, and pH are the soil characteristics could be stabilized soil and increased their aggregates. Gasperi-Mago and Troeh [9] studied the EPS to produce the best consideration for soil aggregation
consist of strains (Pseudomonas, Bacillus, and Paenibacillus), genera simply grownup in laboratory conditions, creating high quantities of EPS. Moreover, strains of (Streptomyces and Penicillium) presented an important positive influence on soil loss and erodibility, after rainfall simulation. Haynes and Swift [10] and Guerra [11] investigated the soil aggregate stability that is positively related to organic carbon (OC). A reduction in organic carbon (OC) with the following decrease in aggregate stability in cultured soils usually leads to soil degeneration problems like runoff and erosion. Ekwue [12] and Loch [13] reported the measure of organic carbon (OC) and aggregate stability assist us to evaluate the danger of structural degradation. In addition to runoff and erosion measurements commonly, increase as organic carbon content declines. Amézkaeta et al. [14] evaluated the influence on soil stability of the polysaccharides using synthetic soil treated with bacteria formed in situ. The creation of polysaccharides was fifty percent of the aggregates due to injected by bacteria and the other part was conserved without inoculation (pure soil). Water stable aggregates (WSA) of the aggregates incubated with bacteria were higher than WSA of the aggregates pure. The polysaccharides created by the bacteria and the bacteria themselves were answerable for the improved aggregate stability. The organic carbon was reduced in the soil aggregate stability as reported by Le Bissonnais and Arrouays [15]. They found that the infiltration rate was observed for a set of soils of identical physical and chemical properties. The outcomes found that soil building stability dropped as organic carbon reduced and there was an important decline in infiltration rate. Miller and Jastrow [16] studied the ability of bacteria to recover soil assembly by enhancing the creation of soil combinations and pores inside. The results showed that bacteria released exopolysaccharides that form the organic mineral compound, which acts as a binding material to soil aggregates. Some studies have been investigated the role of organic matter and clay content on soil aggregation stability. Seybold and Herrick [17] tested the use of organic matter and clay act as adhesive and cementation materials in the soil and revealed that increasing in clay fraction noticeably enhanced aggregates stability and suggested an aggregate model. A fine network of microorganism and roots in soils with greater percent of organic matter holds aggregates together. Extracellular polysaccharides at most answerable for the foundation of aggregates are valuable for enhancing absorbency and ventilation in the soil. Meanwhile, numerous studies have been globally showed on the effect of pH on calcium precipitation and microbial activity. Annamalai et al. [18] studied the effect of bacteria (Bacillus pasteurii) on calcium precipitation at a different value of pH and determined pH conditions for the culture used to produce calcium carbonate crystals, and they concluded that the suitable pH was 7.5. Nevertheless, soil pH of 8.0 has also been described as acceptable for bacteria (Sporocarcina pasteurii and Bacillus pasteurii), greater pH reductions enzymatic activity [19]. Furthermore, the carbonate was dissolved when soil pH stages was reduced. Different types of bacteria were found to be capable to produce different quantities of urease and calcium carbonate precipitation. Bang et al. [20] and Dhami et al. [21] reported the types of Bacillus pasteurii and Sporosarcina pasteurii are the most favoured bacteria for the microbial induced carbon precipitation (MICP) process because of their capability to form a great numbers of precipitates within a short time. Gupta and Germida [22] have been experimentally proved that the mineral components of the soil do not only influence soil structure but also by the existence of microorganisms in pores. Walkley and Black [23] suggested the mechanisms of bacterial inoculated with organic fertilizer could be a very valued means for enhancing soil fertility and aggregation, which may additionally help in returning the enrichment of degenerate soil. Miller and Jastrow [16] studied the effect of inoculation of bacteria and fungi of degraded land. The results showed that bacteria and fungi increased the nutrient availability and improving soil aggregation through nitrogen fixation and mobilization of key nutrients (phosphorus, potassium, and iron). On other hand, using organic nourishment with the mixing of bacterial strains could have a possible for the repair of decadent soils. The organic matter with bacteria and fungi improve soil aggregate foundation and stabilization. The aim of recent scientific work is to determine the effect of microbiological (bacteria) isolated from agricultural soils on soil characteristics like (organic matter, pH, aggregate stability, calcium carbonate) related to variable periods of soil inoculated by bacteria.
2. Experimental Work and Methodology

2.1. Sample Collection and Analysis of Control Soil (without inoculation)
Soil samples were achieved from agricultural soil located at Yusufiya region, Baghdad, Iraq. Soil samples were transported to Soil Laboratory, Civil Engineering Department, Mustansiriyah University as shown in Figure (1). Air-dried soil samples as shown in Figure (2). The soils were crushed and passed via a two millimetres sieve. The digital pH meter was utilized to measure the soil pH levels by mixing 1:1 of soil/water ratio. The conductivity meter was utilized to measure salinity levels with 1:1 of soil-water ratio solution. Total calcium carbonate content was determined according to Jackson [24]. Sub-samples passed through a 0.25-mm sieve were used in the analysis of SOC. The SOC was determined using the dichromate oxidation method [24]. Sieve analysis and hydrometer tests were conducted according to ASTM Standards [25]. Aggregate stability was measured according to Mutter [26]. The soil samples were analysed to determine the physio-chemical properties before treatment are summarized in Table 1.

![Figure 1. Soil samples before air drying.](image)

![Figure 2. Soil samples after air drying.](image)

| Physical properties | Values |
|---------------------|--------|
| Particle size distribution |        |
### Chemical composition

| Component        | Value          |
|------------------|----------------|
| CaCO₃ (%)        | 26.43          |
| CaSO₄ (%)        | 5.30           |
| Ca (meq/l)       | 31             |
| Mg (meq/l)       | 19.3           |
| Na (meq/l)       | 6.83           |
| SAR              | 0.57           |
| CEC (meq/l)      | 16.04 meq/100 g soil |
| OM               | 0.62           |
| OC               | 0.34           |

### 2.2. Bacteria Isolation

Soil samples (4 grams per sample) were collected from agricultural soils at Baghdad according to Berkeley and Logan [27] in isolating the bacteria (*Bacillus sp.*). The soil sample was diluted by added 1gm of soil in 9 ml distilled water of 1:10, 1:100, and 1:1000 as shown in Figure 3.

![Isolated bacteria from agricultural soils.](image)

Bacteria were prepared in the laboratory by propagated in a growth media (nutrient agar medium) modified under 29 ± 1°C in Incubator. Spread 0.1 ml of the soil diluted (1:1000) over the nutrient medium as shown in Figure (4) and incubate the dishes for 24 hours at 30 °C. Then, the considerable bacteria growth was collected and put in nutrient broth as shown in Figure (5) and return it in an incubator. After two days, after drying and burn soil sample in 400 °C to remove all organisms in the soil, the considerable bacteria growth was mixed with soil and put in the Incubator to homogenize the sample. Two weeks later, the growth of the bacteria covered the surface of the soil-forming thick mat, and then (after different incubation periods, 5, 8 weeks) taken the treated samples for physio-chemical analysis to determine the effect of treatments by bacteria on the soil properties.
3. Results and Discussions
The type of bacteria (Bacillus sp.) were tested and compared with the control soil (without inoculation) to compare the influence of bacteria (Bacillus sp.) versus control soils related to measuring of organic matter, aggregate stability, calcium carbonate, and pH at 2, 5 and 8 weeks of inoculated soil with bacteria.

The effect of organic matter on the various periods of inoculated soil with bacteria is shown in Figure (6). Increased organic matter measurements were observed in soils for all inoculated periods. Note that the organic matter was 0.62 % at initial (before soil inoculated) and reached up to 2.1% (after soil inoculated) eight weeks later due to Bacteria decomposed organic material to form organic mineral products that are related with soil particles to building micro-aggregates (diameter of 2 to 20 mm). These
small micro-aggregates are bound by bacterial products to build greater aggregates (diameter of 20 to 250 mm). These aggregates adhere together to build macro-aggregates (>250 mm) and thus increase inter-particle cohesion, which is in agreement with the findings of [16].

To assess the effect of bacteria on soil aggregation, Figure (7) represented the relationship between incubation period and soil aggregates stability. It was observed that aggregates stability tends to increase for the soil aggregates were inoculated with bacteria compared with the control soil (without inoculation). It is clearly indicated that this increase was due to the polysaccharides produced by the bacteria were accountable for the increased aggregate stability. Amézketa et al. [14] reported similar observation.

![Figure 7. The relation between incubation period and aggregates stability.](image)

Organic matter is the prime factors in combined establishment and stabilization. In order to test the effects of organic matter (OM) on soil aggregates, Figure (8) reports the correlation between aggregation stability versus OM for soil samples at different inoculated times. Ours observed of improvement of stable aggregates over time due to the increase in organic matter. Furthermore, through bacterial growing, extracellular polymeric materials are produced as form of slime polymers into the soil solution. These polysaccharides and amino acids with sticky property enable of bonding clay particles to building aggregates. Tang et al. [28] reported similar observation.

Calcium Carbonate (CaCO$_3$) is another important factor often affecting soil aggregate stability. Figure (9) shows the significant increase in aggregates stability due to increased precipitation the CaCO$_3$ to soils surface. As bacteria results in the formation of a biofilm on the soil surface that enables the precipitation of CaCO$_3$ crystals, which obviously increased the soil aggregates stability. As reported by [20] the type of Bacillus pasteurii is the most preferred bacteria for the precipitate calcite due to their capability to products a great quantity of occasions within a small duration.

The influence of microbial activity (bacteria) on pH is complex because they affect different processes including urease activity and the CaCO$_3$ solubility. No general behaviour was observed of soil pH of bacteria (Bacillus sp.) versus control soil related to different incubation periods. The soil pH values from incubation time of 2 to 8 weeks ranged from 6.5 to 6.54 and 7.26 to 7.65 for control soil (without inoculation), incubation soil, respectively. Slightly increased of soil pH of incubation soil versus control soil was detected due to precipitation CaCO$_3$. Dupraz et al. [29] reported the calcium precipitation is carried out at a pH between 7.0 and 9.5. Stocks-Fischer et al. [30] Concluded the enzymatically action was much great at pH 7.7 and this range favoured to calcium carbonate precipitation.
Figure 8. The correlation between aggregate stability and organic matter (OM).

Figure 9. The Relationship between aggregate stability and Calcium Carbonate (CaCO3).

4. Conclusions
This study has shown that the effectiveness of bacteria (Bacillus sp.) as a bonding material in soil aggregation. The effect of different factors was studied such as (organic matter, microbial calcium carbonate precipitation, pH) on soil aggregate stability. The actions by bacteria use increased the soil aggregate stability via increasing the organic matter content and the formation of a biofilm of the surface of soil macroaggregates, which proven the main role of bacteria (Bacillus sp.) on soil aggregate stability. The precipitation of CaCO3 crystals also increased the foundation of aggregates stability, indicating the important effect of bacteria (Bacillus) due to their capability to produce a great number of precipitates (CaCO3) within a short period because of their high urease action. No general pattern of soil pH was detected. The enzymatic activity was considerably high at pH 7.7, where calcite precipitation was preferable in this extent similar to reported in the previous studies.

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