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Study of Anti-desertification Techniques involving Microbes in Oman

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Abstract

Desertification is the development of new deserts by dry land degradation. MICP, to stabilize the soil and using organics to fertilize the soil, having immense potential to reverse the course of desertification, called as anti-desertification is being explored in this study. Microorganisms were isolated from seven different soils and tested for positive urease activity. The positive bacterial samples were then identified by biochemical tests, staining procedures and Vitek2.0 bacterial analyzer to be *Bacillus cereus*. The bacteria, cultured in Tris-YE medium, were applied to soil samples and the samples were found to have been stabilized by the solution for a test period of 6 months with over 50% more calcium carbonate compared to untreated soil. Organic wastes and seaweeds were used in different quantities and compositions to study their effect on improving soil fertility. 20ml seaweed extract per 100g soil showed great promise in being able to increase and diversify soil microbial population.

Keywords: Anti-desertification, MICP, organic wastes, seaweed

1. Introduction

Desertification is a land-degradation process in which an area increasingly becomes drier and drier, typically losing all its bodies of water as well as vegetation and wildlife, eventually turning into a desert. Desertification is a substantial scenario affecting the occupations of millions of people who depend on the aids granted by dry land ecosystems. [1] Addressing this issue is an acute and important measure for adapting to climatic changes and vindication of worldwide biodiversity losses. There are several techniques in existence for reversing or mitigating the consequences of desertification and are known as Anti-desertification techniques. We are going to study these techniques for anti-desertification which involve various micro-organisms.

1.1. MICP for Stabilizing the Soil

This sustainable alternative for the formation of sandstone from loose sand was first proposed by Professor Jason De Jong at UC Davis.[2]

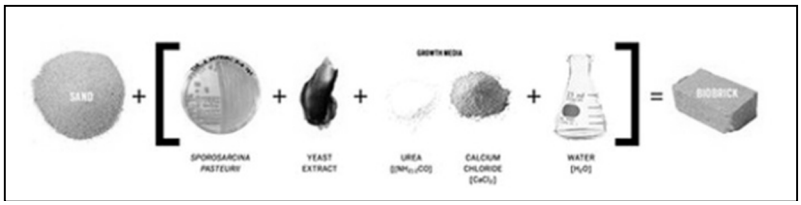


Fig.1. Formation of brick from sand through MICP
Source: http://throughthesandglass.typepad.com

This method utilizes bacteria like *Bacillus pasteurii* [3], *Bacillus cereus*[4] or *Bacillus subtilis*[5] containing a urease enzyme, which can hydrolyze the organic compound urea [CO(NH2)2] to ammonium [NH4+] [6] and while present in a Ca-rich environment (from the externally supplied Calcium chloride)[7], the precipitation of calcite occurs. Calcite is the best long-lasting polymorph of CaCO3. [8] [9] The chemical reaction which takes place is as given below.[10]

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} 2NH^{4+} + CO_3^{2-}$$
$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3$$

This calcite, acting like a bonding agent, helps in cementing the singular granules of sand collectively, thereby turning the loose sand into solid rock[11]. Calcite is that very identical natural cement which binds together manmade concrete as well as sandstone.
The advantages of this treatment include absence of toxicity problems, decreased cost because of natural material usage[12], reduced environmental impact as there is no change in basic soil structure since only the void spaces get filled in, allows water to penetrate through the sand body, including through soils with fines, improves sand characteristics, increase brick strength by decreasing absorption of water[13] and distributes and fixes bacterial activity in soil.

Fig.2. The Better Brick - 2011 Next Generation Design Competition Winner
Source: http://webecoist.momtastic.com

Reasons for choosing *Bacillus pasteurii* or *Bacillus cereus* are that it naturally occurs in the subsurface and is a common bacterium. It is an aerobic microbe especially good for making the soil water more alkaline by improving the pH value[14]. It is efficient at CO2 production along with pH rise in its neighboring environment which stimulates precipitation of CaCO3. This compels Ca and CaCO3 present within water to conglomerate, forming microscopic CaCO3 crystals – calcite. It consumes urea as the energy source and so offers dual CO2 sources – urea decomposition by urease and cellular respiration[15].

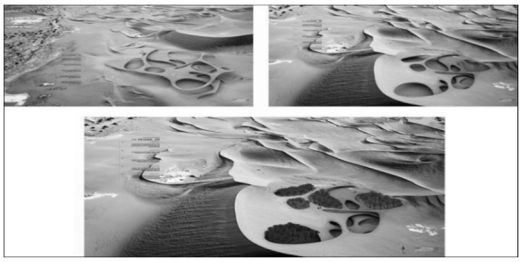


Fig.3.Shaping of dunes using *Bacillus pasteurii*
Source: http://www.theculturist.com

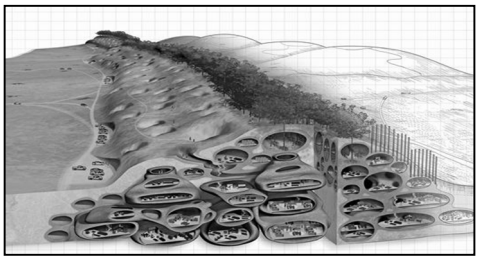


Fig.4.Magnus Larsson'sSand Dwellings
Source: http://www.popsci.com

1.2. Using Organics for Fertilizing the Soil

This technique basically involves providing the required nutrients (N, S, P) to microbes for the degradation of organic matter which contains the nutrients (C, H, O). This enables faster consumption of soil nutrients. This could be used for carbon sequestration, to convert CO2 into miniscule units of stable C – the basic foundation of rich black soil used for plant growth.

The greater lasting FF-SOM has additional N, P as well as S for every entity of C when compared to the plant substance it was derived from. The sequestration of C-rich plant remainders and organic wastes into the FF-SOM can be enhanced through addition of extra nutrients to these remains centered upon the nearly constant ratio of C:N:P:S.[16]

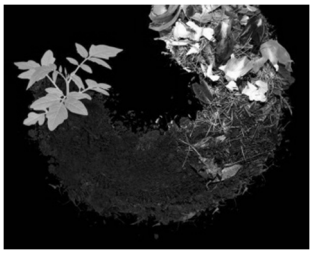


Fig.5. Organics for soil
Source: https://calorganic.org

Fig.6. Microbes for plant growth and carbon sequestration [17]

Seaweed is one of the world's most significant makers of oxygen, through photosynthesis, and on a dry-weight premise, seaweeds contain up to 1.2% N, 0.2 to 1.3% P, and 2.8 to 10% K.

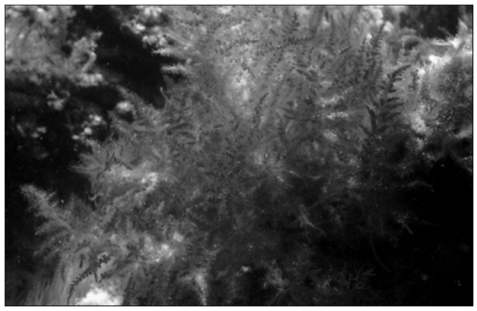


Fig.7. Seaweed *Laurencia glandulifera*
Source: http://www.algaebase.org/

Liquid seaweed fertilizer is organic, as well as originates from a feasible source and can be reaped without harming the earth.

2. Materials and Methodology

2.1. Collection of Sand Specimen

Seven types of soil samples were collected in plastic covers for the isolation of microbes. Sand specimens were collected from 7 different areas in Oman – Ruwi, Al Khuwair, Qurm heights, Bidbid, Rusayl – KOM, Wadi Kabir and Mabella.

2.2. Isolation of Microbes

Microbes were isolated using serial dilution technique. A 10^{-1} w/v mixture of soil specimen was made in distilled water [18] by taking 1g of each sand sample and it was diluted using 9ml of distilled water till a dilution of 10^{-5} was achieved.
Sterilized nutrient agar (2%) was added to the solution and the contents were mixed by rotating the petri dish and after the agar solidified, it was kept for incubation at 30°C for 24 hours.

Fig.8.(a) Fig.8. (b)
Fig.8. Nutrient agar plates of sample from Bidbid
(a) Top view (b) Side view

2.3. Selection of Microbes

Urease agar is a medium to discover the presence of the urease enzyme in microbes. The solidifying agent utilized for this procedure is agar.[19] The mixed cultures obtained after serial dilution were inoculated individually onto urease agar slants, taken in duplicates and incubated at 30°C for 48 hours. Observations were made for change in color of the slant.
The urease positive micro-organisms were observed only in the samples obtained from Bidbid.

Fig.9. Urease agar slants

2.4. Biochemical Tests and Staining

The urease positive micro-organisms were sub-cultured on nutrient agar plates by zig-zag streak plating, for performing a variety of biochemical tests and staining procedures.
Gram's staining, Voges-Proskauer test, indole test, starch hydrolysis, catalase test, mannitol fermentation, glucose fermentation, casein hydrolysis and gelatin hydrolysis were conducted.

Fig.10. Streak plated nutrient agar

2.4.1. Vitek2 Analysis

3ml sterile saline was pipetted to the test tube for Vitek2 analysis. The bacterial samples were inoculated in it till the saline matched the density of 2.0 DensiCHEK plus. The biochemical test card for *Bacillus* was inserted into the test tube. This was then put into the Vitek2, Sr. No. VK2C6879, Model No. 27630 for bacterial analysis, whose performance check and calibration was conducted with every instance of run command. The stages of the process are in the order of filling, sealing and incubation.

Fig.11.(a)

Fig. 11.(b)

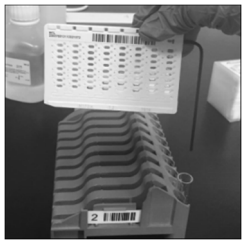


Fig. 11.(c)

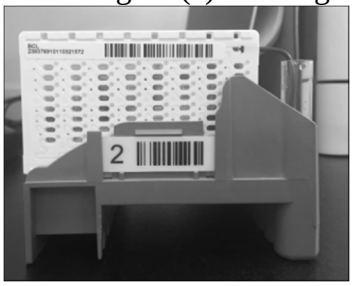


Fig. 11.(d)

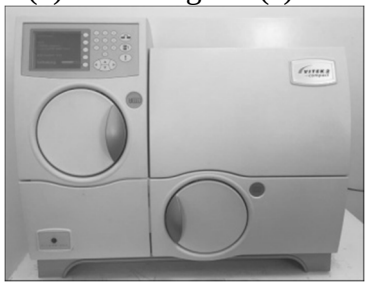


Fig. 11.(e)

Fig. 11. Vitek2 Analysis (a) 2.0 DensiCHEK plus
(b) Inoculating saline (c) Inserting biochemical card reader into saline
(d) Card reader ready for analysis (e) Vitek2 analyzer machine

2.5. Culturing of Micro-organisms

The micro-organisms were cultured in Tris-YE medium. The media were inoculated with the sample obtained from positive urease test and kept for incubation in the rotary shaker incubator at 30ºC and 100rpm for 48 hours.

2.6. Application to Soil

The soil collected from Rusayl – KOM, by using a dust pan was filtered using a 1mm pore size sieve into a 1000ml beaker to create uniformity in the experimentation setup. Two plastic cups were each filled with 100g of this filtered soil.

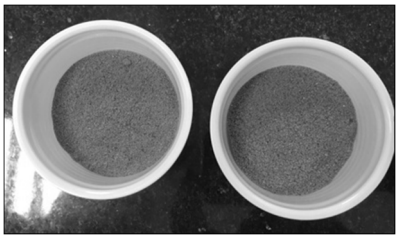


Fig.12. Soil before application of sample

20ml of the cultured sample was added to one plastic cup and 20ml of distilled water was added to the other plastic cup. Both the plastic cups were kept for incubation for 48 hours at 30ºC and the results were observed. The procedure was repeated to confirm the results.

2.7. Analysis for Calcium Carbonate

Three samples of soil were taken. The samples were of untreated soil, soil treated with control (distilled water) and soil treated with the microbial solution. These were then ground making use of a mortar and pestle. 1g of each of the soil sample was added to a conical flask. 20 ml of dilute hydrochloric acid was added to the first flask and the stopwatch was started. As soon as the reaction reached its end point and the evolution of gas stopped, the stopwatch was also stopped. The time of the reaction was noted down. This process was carried out for all the flasks.
The reaction which happens in the flask is as follows:

$$CaCO_{3\,(s)} + 2HCl_{\,(aq)} \rightarrow CaCl_{2\,(aq)} + H_2O_{\,(l)} + CO_{2\,(g)}$$

The MICP treated sample, crushed and taken in a glass petri plate, was also treated with a drop of concentrated hydrochloric acid and observed for the vigor of the reaction.

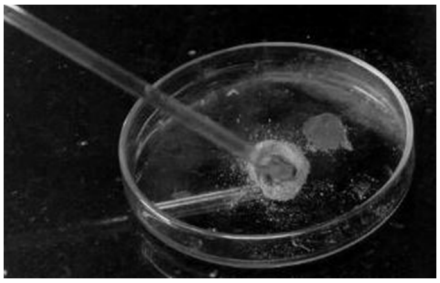


Fig.13. Reacting concentrated hydrochloric acid with MICP treated sample

2.8. Collection of Organic Specimens

Organic wastes, including dry as well as fresh components, in the form of bread, dried leaves, egg shells, dried grass, vegetable peels, spoilt fruits, used tea leaves and fresh grass clippings , were collected in small bags.
The seaweed used for the experiment was hand-picked from the algal drifts collected from the shores of Al Finz Beach, located near Bimmah Sink Hole, during high tide. The shore was lined with pebbles, stones and small rocks (lesser than 20cm diameter). Whole plants were collected and retained in plastic containers and stored in a cold place, without adding water.
We conducted four sets of experiments using different starting materials and compositions to study their effect on soil fertility.

2.9. Organic Wastes - with different Moisture Levels

Approximately 10g of the materials listed in Table 1 were crushed in a blender and taken in a china dish to determine their wet weight. They were kept in an incubator for 48 hours at 70^{o}C to find out their dry weight. From these two values, their moisture percentage was calculated using the formula in Eq. (1).

$$\text{Moisture \%} = \frac{(\text{Wet weight} - \text{Dry weight})}{(\text{Wet weight})} * 100 \quad (1)$$

Refer Table 1 for dry and wet weight values determined.

Table 1: Determination of moisture percentage of organic samples

S. No.	***Sample***	***Wet weight or W_i (g)***	***Dry weight or D_i (g)***	***Moisture % or M_i***
1.	Bread	10.01	7.63	23.776
2.	Dried leaves	10.02	7.79	22.255
3.	Egg shells	9.94	6.64	33.199
4.	Dried grass	10.03	8.92	11.066
5.	Vegetable peels	10.01	1.83	81.718
6.	Spoilt fruits	10.04	1.4	86.055

The samples were crushed in a blender with the purpose of increasing the surface area available for microbial attack and they were then taken in a 250ml beaker in the following quantities so as to achieve a N : C or green : brown or fresh : dried ratio of 2:1 (by weight) - Table 2.

Table 2: Weight of organic samples for experimentation

S. No.	***Type of Sample***	***Sample***	***Weight (g)***	***Weight of the particular type of sample (g)***	***Total weight (g)***
1.	Dried	Bread	10	40	120
2.		Dried leaves	10		
3.		Egg shells	10		
4.		Dried grass	10		
5.	Fresh	Vegetable peels	40	80	
6.		Spoilt fruits	40		

Incremental amounts of distilled water according to Table 3 were added to the beakers in the following order and the contents were mixed thoroughly with a spatula.

Table 3: Addition of water to organic samples

Beaker number	***Amount of water added (in ml)***
1	0
2	10
3	20
4	30
5	40
6	50

The beakers were covered with fling wrap and left undisturbed for 6 days. On the 7^{th} day, the same quantity of water was added and contents mixed. This was repeated on every 7^{th} day. On the 8^{th} day, this setup was kept in the incubator for 72hours at 55ºC. The total time period of this process was 9 weeks (or 63 days).

2.10. Organic Wastes – with different Nutrient Levels

Constituents with C/N ratio as in Table 4 were taken and crushed in a blender.

Table 4: C/N ratio of various constituents

S. No.	***Sample***	***C/N ratio***
1.	Bread	20:1
2.	Dried leaves	60:1
3.	Egg shells	10:1
4.	Dried grass	50:1
5.	Vegetable peels	25:1
6.	Spoilt fruits	35:1
7.	Used tea leaves	20:1
8.	Fresh grass clippings	20:1

They were added in equal parts of 20g each to 5 beakers in order to get a C/N ratio of 30:1, according to Eq. (2).

$$\text{C/N ratio} = \frac{20+60+10+50+25+35+20+20}{12}$$
$$= 30:1 \qquad (2)$$

On the 1^{st} day and thereafter on every 7^{th} day, nutrient solution of the composition as in Table 5 was added to the beakers.

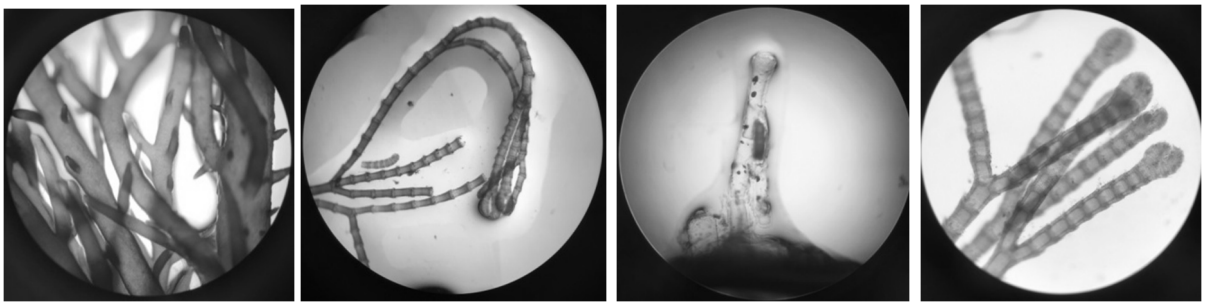
Table 5: Nutrient solution composition

S. No.	*Nutrient*	*Compound*	Quantity (g/10ml)
1.	N	Ammonium nitrate	0.64
2.	P	Potassium di-hydrogen phosphate	0.26
3.	S	Ammonium sulphate	0.17

Beaker 1 was kept as control. 1ml of the nutrient solution was added to beaker 2 and 3. 2ml of the nutrient solution was added to beaker 4 and 5. The contents in all the beakers were mixed once in every 3 days. The beakers were covered with fling wrap. This process was continued for a period of 8 weeks (or 56 days).

2.11. Fresh Seaweed

The seaweed specimen collected was identified through an analysis of its morphological structures initially under low power magnification of microbial colony counter and then under 40x, 100x and 400x magnification of compound microscope. Few drops of iodine solution were added onto one microscopic slide containing the seaweed, to observe pyrenoid presence.



14.(a) 14.(b) 14.(c) 14.(d)

Fig.14. (a), (b) Structure of seaweed under 40x magnification
(c), (d) Structure of seaweed under 100x magnification

2.12. Seaweed Extract

40g of seaweed was added to a beaker containing 200ml distilled water. This was kept undisturbed for 7 weeks. The seaweed solution was filtered using Whatman filter paper no.1 to obtain the seaweed extract and the filtrate was collected and used for further treatment.

2.13. Application to Soil

Soil collected from KOM was finely sieved using a 1mm sieve and each experimental component was added to 100g of soil in the manner according to Table 6. Plastic cup labelled 'Y' was taken as the control, to which only 100g of untreated soil was added. This soil treatment was carried out for a period of 2 weeks (or 14 days).

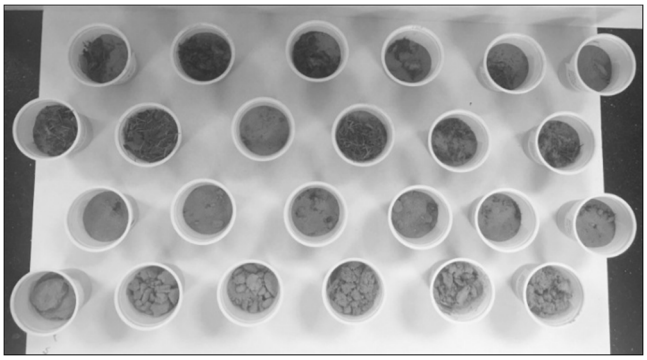


Fig.15.
Fig.15. Top view of soil treatment for fertilization

2.14. Analysis

Serial dilution of each soil treatment was performed. 1ml from each of the dilutions 10^{-4} and 10^{-5} were taken in autoclaved glass petri plates and sterilized nutrient agar medium was added. This was then allowed to solidify and kept in inverted position in incubator at 37^{o}C for 48 hours.
The individual colonies obtained were counted using a digital colony counter by standard plate count technique. Microbial population count for the soil fertilized using different organic treatments has been recorded in Table 6.

Table 7: Biochemical Test Results

Serial No.	***Biochemical Test***	***Result***
1.	Gram staining	Positive
2.	Voges-Proskauer test	Positive
3.	Indole test	Negative
4.	Starch hydrolysis	Positive
5.	Catalase test	Positive
6.	Mannitol fermentation	Negative
7.	Glucose fermentation	Negative
8.	Casein hydrolysis	Positive
9.	Gelatin hydrolysis	Positive

With reference to Table 7, the micro-organism isolated from soil has been found to be a gram positive bacillus with the appropriate urease activity. The bacillus obtained forms a fungi-like growth without distinct individual colonies. The bacillus was found to be aerobic, with a capacity to hydrolyze urea, starch, casein and gelatin. It also contains the enzymes catalase and tryptophanase. The Vitek2 analysis showed that the microbe we obtained was *Bacillus cereus.*

The primary aim was to solidify sand using microbes obtained from sand in Oman and this has been achieved. As can be clearly seen, the free space between the soil particles has been greatly reduced due to the biocementation of the soil by microbial action. The sand particles held close together were observed to remain in that stable condition for a test period of 6 months.

The soil solidified by MICP has been observed to have compacted more over a time of 2 weeks compared to the water treated soil. Also, there has been less loose sand observed in case of microbial treated soil as compared to the water treated soil.

Fig.16. Soil samples: (left) Control
(right) Solidified by MICP

The analysis for calcium carbonate in the soil samples gave the following graphical result. As can be distinctly seen from the graph, the MICP treated sample has greater than double the calcite content of the untreated soil. Also, from the extremely vigorous reaction of MICP treated soil with concentrated hydrochloric acid, we can infer that the calcite or $CaCO_3$ content of that soil is indeed very high.

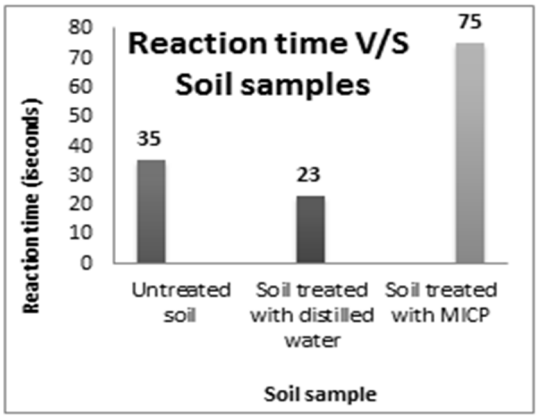


Fig.17. Graph of reaction time of dilute hydrochloric acid with soil samples

The seaweed analysis is as follows:
From fig.14.(b) and (c), we can observe that the seaweed is filamentous. The filaments are macroscopic as they are visible under the naked eye. They appear to be free living as they do not display any kind of holdfasts. The filaments are branched and multisereate (covered with many small cells) as can be seen in fig.14.(a). They are parenchymatous – made up of cell mass which has grown by cellular division in multiple planes. It does not contain pyrenoids as no black stain appeared showing the absence of starch when stained with iodine solution. The branching pattern is dichotomous (branching into a Y- like structure with 2 equal branches) as depicted in fig.14.(d). Though branching is mostly opposite, as in fig.14.(b), sometimes, small branches also arise in an alternate manner as in fig.14.(a). The seaweed has an apical cell (a noticeable cell at

the apex) which can be distinctly identified in fig.14.(d). The seaweed is corticated (which implies having an external layer of cells). The red pigmentation in fig.14.(c) is an indication of the type of algae – Rhodophyta.

4. Discussion

For obtaining the desired bacterial species having suitable urease activity, the soil obtained from Bidbid showed positive result according to Fig.9. This shows that there is a significant potential in exploring the vast diversity of soils in Oman for the isolation of bacteria for MICP. The bacteria taken from the soil can be used back in the soil to improve its structure and properties. This is an example of utilization of natural resources for nurturing nature, which is, in this case, preventing the spread of deserts. From the data collected from Table 6, it could be seen that 20ml of seaweed extract added to the soil was more potent in increasing the soil microbe population. This could be due to the fact that the seaweed extract contained all the essential nutrients leached from the seaweed which could be distributed uniformly among the soil particles, thereby nourishing the microbial world.

5. Conclusions

Isolation of microbial sample was done from soil using serial dilution and agar plating. The colonies obtained were screened for urease activity using the urease agar. Those organisms showing positive urease activity was then tested biochemically to determine their properties. The *Bacillus Cereus* obtained was cultured in Tris-YE medium and applied to soil samples to allow the solidification of individual soil particles. The solidified soil has been found to be stable for the entire duration of the testing period. The technology of MICP is environmentally friendly not only because it gives strength to sand body, but also because it does not alter the essential structure of sand. This is a sustainable technology as the bacteria can be obtained from the very sands of Oman which require stabilization.

Using seaweed extract to fertilize the soil has shown the best result in terms of increasing as well as diversifying soil microbial population. This is a very feasible technology as Oman has a long coastline, good inter-tidal zones and climatic conditions suitable for the growth of a large variety of seaweeds, which could be developed to extract their nutrients and apply it to the non-fertile soils nearby, thereby reducing transportation costs also.

Appendix

LIST OF ABBREVIATIONS USED

MICP	Microbial Induced Calcite Precipitation
S/V	Surface to volume ratio
FF-SOM	Fine Fraction pool of Soil Organic Matter
KOM	Knowledge Oasis Muscat
UC Davis	University of California at Davis

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