

Isolation of Urease Producing Bacteria to Produce Biocement via MICP Process

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(Received 16 April, 2023; Accepted 19 June, 2023)

ABSTRACT

In the present study alkaliphilic bacteria, isolated from paddy field i.e. rich in urea was used for production of biocement. Urea hydrolysis is carried out by enzyme urease and is the most effective way of the generating calcium carbonate via producing CO_3^{2-} and NH_3 . Ammonia increases the pH in the environment causing Ca^{2+} and Co^{3+} to precipitate in the form of calcium carbonate, which can be used as biocement. It is one of the mechanisms of microbially induced calcium carbonate precipitation (MICP). Among the 5 isolates, potent urease producer which can tolerate urea up to 3.5% was used for the study. Tap and Borewell water having hardness of 22 mg/ml and 17 mg/ml was used for the biocement production. Urease activity was calculated using electrical conductivity method and it was in the range of 1.602-1.901 mS/min. *In vitro* biocement was produced by isolate (B) by incubating it for 7 days and the biomass precipitated was filtered, air dried and analysed by SEM and FTIR. The present ecofriendly approach can be potentially useful for removal of water hardness along with production of biocement.

Key words: Biocement, Urease, Hardwater, SEM, FTIR etc.

Introduction

The continuous development in the field of civil engineering and in the developing industrial activities has created progressing demand of good quality material for constructing industry. Material used for the construction of the building is porous in nature. This type of material (porosity) comes in contact with different environmental conditions like humidity or other harmful chemicals like acid chloride and sulphates that affects the quality of the material and decrease the capacity of the holding object and their life span. The organism producing urease enzyme is a solution for the key problem in the field of civil engineering which can overcome this problem in an

eco-friendly way. Urease (urea aminohydrolase: EC3.5.1.5) is an enzyme that hydrolyzes urea into one mole of carbon dioxide and two moles of ammonia per mole of urea, resulting in an increase of the pH and carbonate concentration in the bacterial environment, which induces the formation of calcium carbonate. This enzyme is widespread in nature.

The microbial induction mechanism for the precipitation of calcium carbonate is called Microbial Induced Calcite Precipitation (MICP). Biologically controlled or biologically induced is some mechanism by which CaCO_3 is precipitated (Lowenstan and Weiner, 1988).

Hard water is major problem that affects quality

of water worldwide. Out of 97% of sea water only 0.5% is fit to drink. Hard water interferes with many processes such as laundry, bathing etc. and can create other issues. Hard water contains calcium and magnesium ions. It can be determined by the presence or measuring, of calcium and magnesium ion present in water sample by titration method (EDTA).

In recent study attempts are made to isolate bacterial strain with high urease activity from paddy field that can be used to remove hardness of water along with production of biocement through precipitation of calcium carbonate.

Materials and Methods

Sample collection

Soil sample was aseptically collected from paddy soil, i.e. rich in urea and kept in a sterile zipper bag. The sample was transported to laboratory and stored at 4 °C till use.

Enrichment and isolation

Sample were enriched by inoculating in 50 ml sterile nutrient broth of pH- 9 containing 2 % urea and incubated at 37 °C. Isolation and enumeration was done by serial dilution method.

Qualitative urease test

This test is used for detecting capability of microbe to produce urease enzyme. For this test Christensen's urea agar medium (Himedia) was used and inoculated with isolates and incubated at 37 °C and were examined continuously to record pink colour development.

Quantitative urease assay by electric conductivity

To check urease activity, electric conductivity method was used in absence of calcium ion (AL-Thawadi, 2008; Whiffin, 2004). For enzyme assay, 1ml of 24 hrs old bacterial broth was added to 9 ml of 1.11 M urea solution. Conductivity reading was taken till 5 min at 20 °C by electric conductivity meter (EQUIP-TRONICS NO. EQ – 660A). Urease activity is presented by the rate of conductivity increase as mS/min.

Urea tolerance test

Isolates were tested for urea tolerance and were added to 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 M urea con-

centration and incubated at 37 °C for 48 hrs. Density of cultures was determined at O.D at 600nm calorimetrically (Anitha *et al.*, 2018).

Determination of water hardness by EDTA method: Tap water and Borewell water samples was collected from college campus and hardness was determined using EDTA method using Erichrome black indicator (Varalakshmi *et al.*, 2014).

Production of Biocement

To 500 ml water sample, 100 ml (3M) concentration of sterile urea and 100 ml of cell inoculum were added and incubated for 1 week at 37 °C without agitation (Anitha *et al.*, 2018). After incubation deposited CaCO₃ was filtrated and filter paper containing deposits were dried in hot air oven at 60 °C for 5 hrs.

The yield of Biocement was determined using formula: -

$$W_c = W_{fc} - W_f$$

Where W_{fc} = weight of filter paper containing precipitate

W_f = Empty weight of filter paper.

Characterization of Biocement

Biocement produced was characterized using light microscope, Scanning electron Microscopy (SEM), Fourier Transmission Infrared Spectrophotometer (FTIR).

Results and Discussion

Isolation and Screening of microorganism

Total 8 isolates were isolated on nutrient agar having pH -9. They were further screened for production of urease enzyme using Christensen's urea agar base. The change of colour in the media from yellow to pink was observed and was considered as posi-

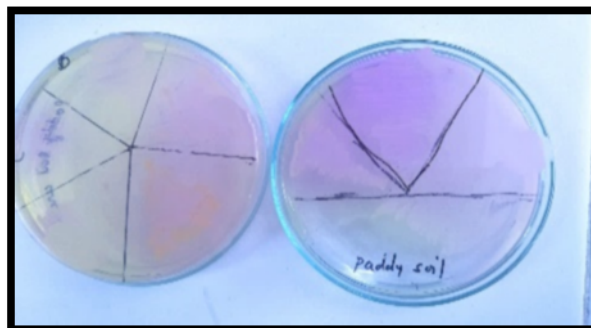


Fig. 1. Screening of urease positive isolates.

tive test. Out of 8, 5 isolates showed positive test and were used for further studies.

Quantitative urease assay

Conductometric method was used in which conductivity was recorded at 0 time and at 1, 2, 3, 4, 5 min as showed in Table 1, conductivity increase with time. The rates of enzyme activity were in the range of 1.602-1.901 mS/min.

Table 1. Electric conductivity (mS/min) of urease assay at different time intervals.

Isolate	Electric Conductivity (mS/min)	
	0 min	5 min
A	0.023	0.029
B	1.602	1.901
C	1.512	1.66
D	1.007	1.012
E	0.007	0.009

Urea tolerance test

Isolate 'B' showed highest tolerance of urea about 3M and was used for production of Biocement.

Determination of water hardness by EDTA method

Hardness of borewell water was about 17mg/ml And Hardness of tap water was 22 mg/ml The change of colour from wine red to blue by reducing EDTA was observed.

Production of Biocement

Biocement production was more in tap water than borewell. 500 ml of bore water produced 2.3 g of biomass and Tap Water produced 4.2 g of biomass which showed tap water has more water hardness than bore water.



Fig. 2. Biocement precipitate

Characterization of Biocement

The precipitate of Biocement was observed under light microscope and SEM analysis was done. Under

10X magnification of microscope (a) and (b) SEM image of Biocement precipitated showed different size structures (Fig. 3a & b). The EDS mapping is shown in Fig. 4 which indicates the elemental composition of biocement. FTIR spectrum of Biocement production by isolate B is shown in (Fig. 4) and spectra were matched with reference spectrum of calcite from RRUFF Database (<http://rruff.info/calcite/R050128>). The FT-IR analysis revealed the presence of carbonate and calcium in the precipitated sample.

Determination of water after Biocement Production: After biocement production, again water hardness was determined. Water hardness was reduced by 14 mg/ml in tap water and 10mg/ml in bore water. In this way this method can be used to remove hardness of water along with biocement Production.

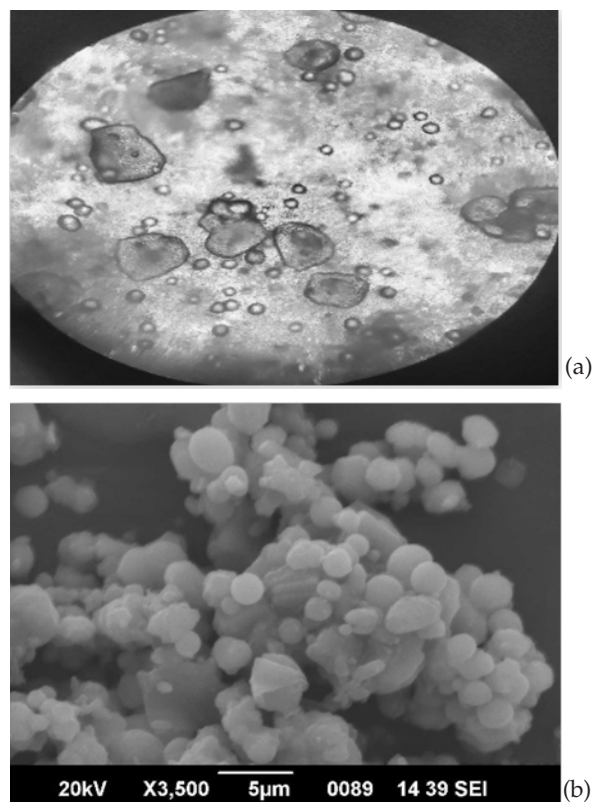


Fig. 3. Microscopic image of crystals of different types and shapes of CaCO_3 precipitated by isolate 3a and 'B' SEM image of bio cement.

Conclusion

The purpose of this study was to produce biocement from urease positive isolates screened from paddy

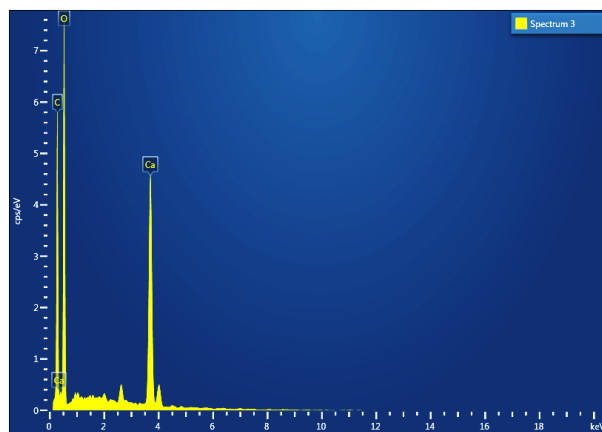


Fig. 4. EDS mapping of biocement produced by isolate 'B'.

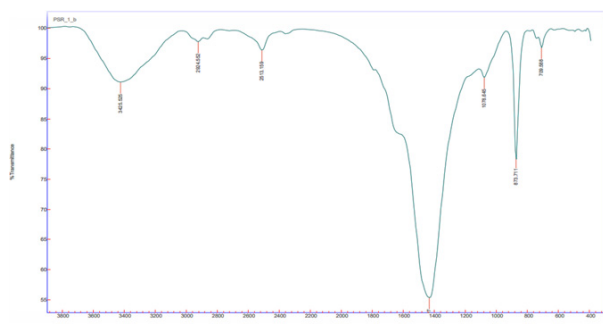


Fig. 5. FT-IR analysis of bio cement produced by isolate B.

soil along with removal of hardness from the tap and borewell water. The biocement produced was characterized using light microscopy, FT-IR, SEM with EDS. In future optimization process will be carried out for different parameters used for improved biocement production which can be used in construction industry in cost effective way.

Acknowledgement

The authors acknowledge, Principal, MVP's, S.V.K.T College, Deolali Camp and Principal, K.T.H.M College, Nashik (Research Centre) for their support.

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