SCREENING MICROORGANISMS FOR REMEDIATION OF WELLS VIA CARBONATE PRECIPITATION

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Abstract

Borehole infrastructure in near end-of-life oil and gas reservoirs can potentially be reused for CCS operations when repurposing fields for use as CO_2 storage reservoirs. The integrity of such wells may not be sufficient for immediate applications; however remediation could make them suitable for reuse. Microbial induced carbonate precipitation (MICP) is ubiquitous in nature and had been proposed as a suitable technological solution for reducing permeability in several geotechnical scenarios. However, the relatively high temperatures, and high pH of down well environments provide challenges to microorganisms. We present a suite of screening techniques and simplified flow apparatus to enable screening of MICP microorganisms and optimization of parameters to better understand the application of novel microorganisms for remediation in deep subsurface conditions.

Keywords: *MICP, bioprecipitation, borehole remediation, CCS, microbiology*

1. Introduction

A large number of oil and gas reservoirs are coming to end-of-life and are of interest as potential sites for large scale CCS. REX-CO_2 is a multidisciplinary project addressing the potential for reuse of existing oil and gas wells for CCS. Assessing well integrity and exploring potential remediation techniques form part of this work.

Microbial induced carbonate precipitation (MICP) is a biochemical process that is widespread in nature, both in terms of environments and organisms involved [1]. Several different biochemical pathways can result in carbonate precipitation; primarily from the carbon, nitrogen and sulfur cycles. These all differ in the details of the pathway. However, MICP generally relies on the microbial production of carbonate or bicarbonate compounds and microbial induction of local increases in pH. Then, if there are suitable cations in the environment, precipitation occurs chemically.

MICP is utilised in biotechnology applications e.g. Biocalcis® for soil stabilization (Soletanche Bachy, France) and is a rapid area of development for subsurface geotechnical applications such as borehole sealing [2], and self-healing cement [3]. Field scale borehole sealing at 340m depth was demonstrated using a wireline dump bailer, delivering fluids at depth. It was achieved by introducing alternating solutions of microbial growth media containing either *Sporosarcina pasteurii* or Ca ions and achieved flow rate decrease from 1.9L min^{-1} to 0.47L min^{-1} after 4 days treatment [2].

The bacterium *S. pasteurii* induces MICP via urea hydrolysis, and is commonly used as a focus of biotechnology development as precipitation proceeds by a well-defined pathway, and can lead to high carbonate yields [1]. Modifying factors such as microbiological media composition or flow rate can affect precipitation and therefore permeability [2]. However, MICP via the ureolytic pathway is not suitable in all applications due to environmental and other constraints [4].

We explored non-ureolytic nitrogen microbial metabolic pathways for down well remediation, it was essential that microbial isolates were capable of carbonate precipitation in fluids at the moderate temperatures (due to depth) and higher pH (due to cement) that may be encountered when remediating oil and gas wells. Several suitable environments were targeted for bacterial isolation and simple screening techniques were applied to identify essential and desirable properties of the microorganisms. Additional simple flow experiments were developed to enable the optimization of parameters, such as media concentration, prior to application in a laboratory scale remediation test with aged cement and suitable reservoir or caprock.

2. Materials and Methods

2.1 Field sites and isolation of novel bacteria

MICP microorganisms are commonly found in carbonaceous soils, so a lime-rich field site was selected as source of microorganisms. As growth at elevated temperatures was also of interest two thermal springs were sampled, both of which passed through carbonate rocks. Lime-rich soil samples were taken from a stream draining a lime waste site near Buxton, Derbyshire, UK (soil identification from landis.org). Initially two samples were selected to test a range of precipitation media, and modified B4 microbiological growth agar was selected for further isolations. Modified B4 agar (per L): yeast extract (4g), glucose (1g), calcium acetate (2.5g), Tris HCl (4.42g), Tris base (7.72g), phenol red (12mg) and agar (15g). Final pH 8.5. Glucose and calcium acetate were added as 0.2 µm filter-sterile fluids after autoclave sterilization of the remining media components. An additional ten samples were collected along a transect of the stream. Spring water was collected from Buxton St Anne's Well (~27°C) and Thermae Bath Spa (~40°C), 500mL to 4 L was filtered onto sterile 0.22 µm membranes, split in half then pressed onto modified B4 agar. Plates from all sites were incubated at 30 and 50°C.

Table 1: Selected screening results from unique isolates

Isolate	Provisional identification	Metabolic pathways		Carbon source						pH		**Anaerobic**	Liquid	**50 °C**
		Urease	Am.	G+Ac	YE+G	YE+Ac	G	YE	Ac	9.5	10.0			
BH01	*Bacillus flexus*	Y	Y	W	Y	Y	W	Y	W	W	W	**N**	Y*	**N**
BH03	*B. paralicheniformis*	N	Y	Y	Y	Y	Y	Y	W	Y	Y	**Y**	Y*	**Y**
HH11	*Microbacterium maritypicum*	Y	Y	W	W	W	N	W	W	W	W	**Y**	xls	**W**
HH18	*Arthrobacter gandavensis*	N	Y	Y	Y	xls	xls	Y	xls	Xls	xls	**N**	xls	**Y**
HH19	*Brevibacterium frigoritolerans*	Y	Y	Y	Y	xls	Y	Y	xls	Xls	W	**Y**	xls	**W**
HH21	*M. oxydans*	Y	N	xls	Y	xls	xls	W	W	W	W	**W**	xls	**N**
HH26	*B. licheniformis*	N	Y	Y	Y	xls	W	Y	xls	Y	Y	**Y**	xls	**W**

Am. Ammonification, YE yeast extract, G – glucose, Ac – Ca acetate. Y – growth, N – no growth, W - weak growth, xls - growth with visible crystals observed.
* no crystal could be seen but thick biofilms formed, potentially containing crystals

2.2 Screening techniques

Visually unique isolates with evident crystal growth were selected and re-streaked on modified B4 agar. Bacterial isolates were identified by partial DNA sequencing of the 16s rRNA gene, and potential pathogens discarded. Further adjustments were made to modified B4 agar to test for the following properties of each of the bacterial isolates:

- Growth with Na acetate replacing Ca acetate
- Growth with single or dual carbon sources (yeast extract, glucose and Ca acetate)
- Crystal formation in liquid media (these were static to preserve local pH changes around cells)
- Growth at different temperatures: 25, 35 and 50°C
- High pH tolerance: 9.0, 9.5 and 10.0

Isolates were tested by resuspending a single colony in sterile saline and spotting three volumes of 10µL onto appropriate agar or 10µL into 20mL fluid.

Selective media for urease production and ammonification were also used to test the bacterial isolates. Urease agar (per L): peptone (1g), NaCl (5g), glucose (1g), urea (20g), phenol red (12mg), agar (15g). Final pH 6.5. Urea and glucose were added as filter sterile solutions. Ammonification agar (per L): peptone (5g), yeast extract (3g), NaCl (5g), phenol red (12mg), agar (15g). Final pH 7.0. For both tests, positive identification is associated with a colour change in agar surrounding the isolate. A pink colour is seen as the pH increases.

2.3 Development of low-cost flow experiments

Simple flow experiments were developed to enable test for MICP of bacterial isolates in the presence of unconsolidated materials. *S. pasteurii* (DSM33, purchased from DSMZ, Germany) was tested alongside the seven isolates identified from the initial screening stage of this study. Figure 1 shows an example set-up. All isolates and were grown in liquid broth for three days at 35°C. Suspension was then centrifuged at 4000 x g to concentrate microbial cells and 0.5 mL of concentrated isolate was added to top of syringe barrel, and topped up with 2.5mL unbuffered liquid media (i.e. modified B4 without Tris buffer components or agar). Experiments were left for 4 hours to allow for attachment of cells (three-way valve closed). Then, unbuffered B4 media was supplied to the top of the syringes at nominal flow rate of 0.5 mL h^{-1}. The three-way valve was opened, allowing for excess fluid to flow out via tubing under gravity.

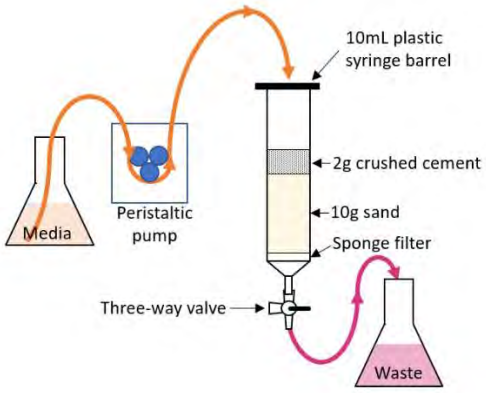

Figure 1: Example set up of low-cost flow apparatus. The syringe barrels are housed in incubator.

At the end of experiment DI water was flowed through system for 24 hours, at 0.5 mL h^{-1}. If out flow under gravity was not possible, excess fluid was removed using syringe at time intervals between 4 to 8 hours. Syringes barrels and contents were then dried at 35°C. The end of the syringe barrels were cut off with craft knife and contents ejected using syringe plunger. The samples were imaged using Olympus TG-6 in macro mode.

3. Results

3.1 Colony Screening

Thirty-one isolates (showing crystal growth) were picked for further screening (Figure 2), of which seven unique

isolates were identified by colony morphology and subsequent DNA sequencing (Table 1). Five were isolated from sediments (HH), and the remaining two from Thermae Bath Spa water (BH). All isolates were capable of growth with Na acetate replacing Ca acetate and at 35°C (data not shown), with five capable of growth at 50°C. All isolates were capable of growth at all pHs tested, and could grow with just yeast extract, however it was observed that spots were generally slightly larger with the addition of glucose.

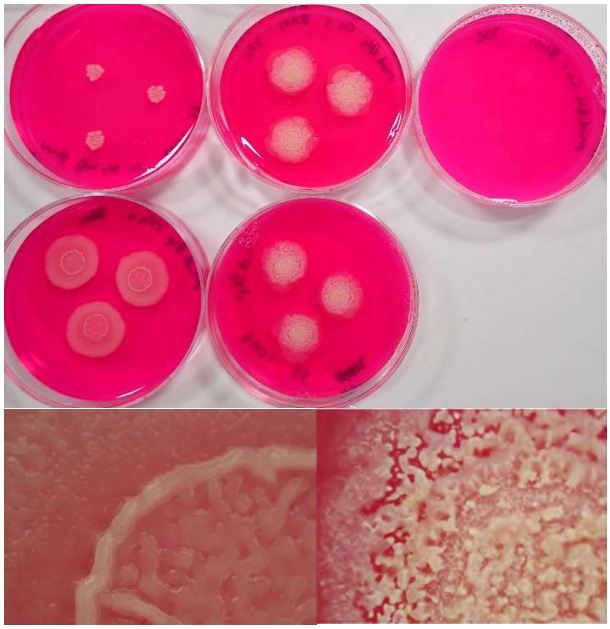

Figure 2: example of isolate screening with isolate BH01. Petri dishes are 55 mm diameter. Top row left to right, growth at 25°C, 35°C and 50°C. Middle row, growth at 35°C left with Na acetate, and centre 50% Ca acetate. Bottom row, close up images showing differences between growth in the absence (left) and presence (right) of Ca ions. Image width ~3 mm.

All seven isolates, along with *S. pasteurii*, were tested in the first round of flow experiments as all were capable of growth at 35°C, pH 10.0 and likely to produce crystals in liquid media.

3.2 Flow experiments

Phenol red is a pH sensitive dye and was added to the microbiological media as carbonate precipitation is pH dependent and is favoured at higher pH. The unbuffered B4 media starts orange (~ 7.0), and will turn yellow at pH < 6.2 and pink at pH > 8.0. During microbial growth in unbuffered B4 the growth media initially turns yellow, likely through organic acid production during glucose consumption. After this the solution turns pink e.g. through ammonification. In the flow experiments the media turns pink once added to the crushed cement. After 24 hours of flow through the cements and sand the colour, hence pH, varied among the isolates from orange to pink (lowest to highest) in the following order: HH26, HH10, HH19, *S. pasteurii*, BH03, BH01, HH18 and HH21. The higher pH indicates a more favourable environment for carbonate precipitation.

Waste flow under gravity was no longer possible in BH01 and BH03 after 7 days, and all flow experiments were stopped after 8 days. Blockages were likely to be a combination of crystal and biomass growth. After drying, crystals and finer powdered material were seen on the surfaces of the crushed cement. Some consolidation of the crushed cement was observed with all isolates, this was not quantified however was noticeably weaker with HH26 (Figure 3).

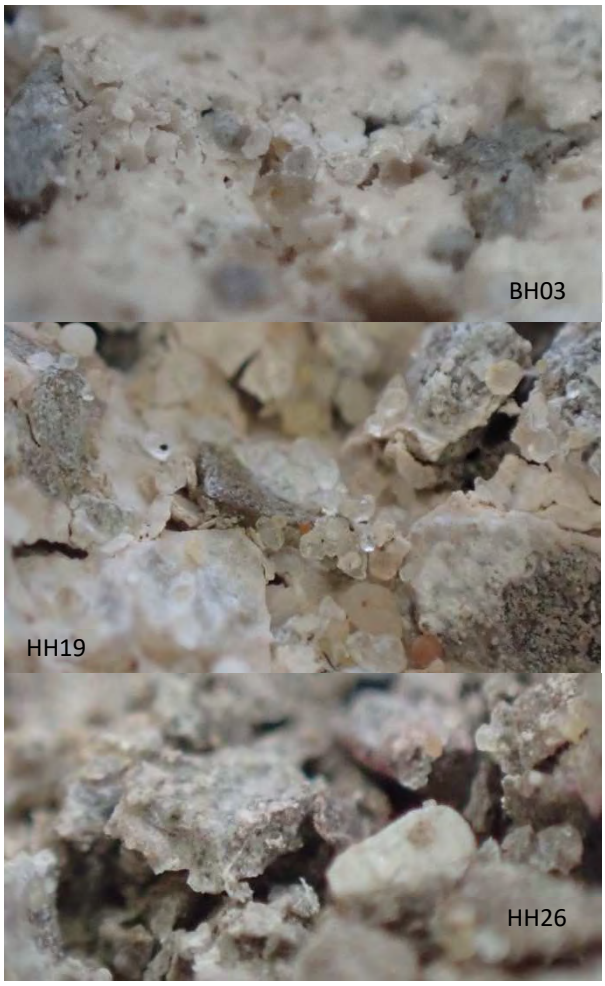

Figure 3: Crystal growth on upper surface of crushed cement. Image width ~2.5 mm.

Given that all isolates met the essential characteristics and were capable of growth with just yeast extract, the desirable characteristics and performance in flow tests were used for further screening. Only BH03 has strong growth in the remaining two desirable characteristics (50°C and anaerobic growth). HH11, HH18 and HH19 are also of interest as they show strong growth in one of the desirable characteristics and either weak growth in the other, or promise in flow experiments.

4. Discussion and Conclusion

Screening was divided into two different categories, those that are essential for bioremediation at depth and those which are desirable as they support practicalities and reduce cost implications of bioremediation. Due to selection of appropriate field samples and initial isolation criteria (modified B4 agar and minimum 30°C growth) it is not unsurprising that all seven isolates satisfied the essential characteristics (growth at moderate temperature, 35°C, and precipitation of minerals at moderate temperatures at high pH and in liquid media). The desirable characteristics were higher growth

temperature (50°C), reduced nutrient requirements and anaerobic growth. For biotechnology applications fast growth would also be desirable, and could be estimated by recording size of spot after a given time, this was not recorded systematically during screening, but could be estimated from images. The provisional identifications of the good (*B. paralicheniformis*), and potential (*M. maritypicum*, *A. gandavensis* and *Br. Frigoritolerans*) candidates come from a range of genera but limited to two classes of soil associated bacteria.

Due to the high cost of Tris buffer this was excluded for the flow experiments, as would drastically increase the cost of industrial applications. Phenol red, a pH sensitive dye, was included during these experiments as it allows for easy trouble shooting, but would be unnecessary during upscaling. Screening has demonstrated that only yeast extract was required for growth, and the low-cost flow experiments developed here are perfectly suited to test effect of media composition on carbonate precipitation, leading to increased efficiency and cost reduction.

Successful consolidation of the crushed cement by six of the seven isolates (and *S. pasteurii*) demonstrates that simple flow experiments can be used as a basis for microbial screening and testing precipitation parameters. The flexibility of this system can be easily adapted to different flow regimes, *i.e.* bottom fed, and along with precise measurements of mass change can be used to quantify the effect of these changes on precipitation. This system will also be used to refine parameters such as media composition, starting cell density or pulsed flow, before being used in more complex laboratory test systems [5,6].

Laboratory test systems for bioprecipitation, including those planned as part of REX-CO2 project, generally involve complex, or large-scale systems and can include high pressure pumps, pressure vessels, cored material, or sand tanks along with instrumentation to monitor changes in pressure and flow. Although these systems provide essential insight into how these systems behaving in near real-world scenarios [5, 6], their nature means that only a limited number of parameters can be measured.

Down hole MICP technologies have a huge potential in geotechnical engineering including the remediation of oil and gas wells for CCS. Similar technologies have already been demonstrated to promote borehole sealing at 340 m depth in oils and gas wells through urea hydrolysis by *S. pasteurii* [2]. This presented here has identified one good (provisional identification *B. paralicheniformis*), and three potential candidates (provisional identifications *M. maritypicum*, *A. gandavensis* and *Br. Frigoritolerans*) for bioremediation of oil and gas wells. As urea was not used in these experiments an additional pathway for MICP must be involved, potentially via the ammonification of the nitrogen sources in the yeast extract. As urease hydrolysis is not appropriate in all environments, microorganisms capable of MICP via alternative metabolic pathways are required to fulfil the full potential of MICP as a geotechnical tool.

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