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Calcium ion adsorption with extracellular proteins of thermophilic bacteria isolated from geothermal sites—A feasibility study

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ABSTRACT

The scaling of geothermal wells arising from formation of calcium carbonate is one of the major problems associated with the utilization of geothermal energy. A novel eco-friendly biological-based approach for geothermal well descaling was proposed. Thermophilic bacterial strains were isolated from geothermal areas in Taiwan and were evaluated for their calcium adsorption efficiency under the extreme conditions. Among the eight strains isolated, *Tepidimonas fonticaldi* AT-A2 isolated from Antun Hot Spring, Hualien showed the highest calcium adsorption capacity. The calcium adsorbing activity of *T. fonticaldi* AT-A2 was mainly associated with the extracellular proteins and the maximum calcium adsorption capacity (1.94 g Ca/g protein) was obtained at pH 10, 150 °C and 1 atm pressure. This calcium adsorption efficiency is much higher than that of metallothioneins and other bacterial extracellular proteins. The excellent calcium adsorption efficiency of the AT-A2 proteins indicates the potential for their applications in biological geothermal well descaling.

1. Introduction

Geothermal energy is an abundant alternative renewable energy resource. Geothermal energy is the heat energy generated by the radioactive decay of long lived isotopes like potassium, uranium, radium and thorium in the earth's crust. It can be recovered as steam or hot water beneath the earth's surface, such as those in hot springs and used as a source of energy. Geothermal energy provides base-load power just like any other energy sources such as coal, oil or natural gas, and can replace fossil fuels. Geothermal power is cost effective, reliable, sustainable, environmentally friendly and virtually inexhaustible [1]. The geothermal areas or fields can be geographically classified based on their location: Volcanic or high temperature areas that lies close to active volcanoes and non-volcanic or low temperature areas that lies outside the active volcanic belts [2]. A 3 MW power plant was established in Ching-Shui non volcanic geothermal field in 1981 in Taiwan. The plant was decommissioned in 1993 due to continued decline in production. The main cause behind this operational failure was the occurrence of mineral deposits in geothermal wells, pipelines and reservoir fractures [3,4]. The mineral deposits in wells and fractures were identified as calcite ($CaCO_3$).

Calcite is a common formation mineral that often occurs as travertine deposits around neutral Na-HCO_3-SO_4 springs. In general, calcite can be generated by one of the following ways: (i) hydrolysis (involving replacement of calcium alumino-silicates), (ii) boiling of geothermal fluids (from fluids having high dissolved carbon dioxide concentrations and in the absence of mineral pH buffer) and (iii) heating of cooler peripheral geothermal fluids [5,6]. The difficulty of running a geothermal power plant in a non-volcanic area is de-trop calcite growth, when the geothermal fluid boils or degases in response to a pressure drop [7,8]. Most dissolved CO_2 is lost from the liquid phase into gaseous phase at the flash point and this causes a dramatic shift in the following equilibrium to the right [9]:

$$Ca^{2+} + 2\,HCO_3^- \rightarrow CaCO_{3\,(s)}\downarrow + CO_2\uparrow + H_2O$$

Increase in HCO_3^- content in the geothermal fluid also enhances the rate of forward reaction or formation of calcite scales ($CaCO_3$) [7,8].

The major calcite inhibition technologies applied currently include mechanical methods, chemical treatments [10], and

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biological methods [11]. Mechanical cleaning methods (such as using steam shocks and hydro blasts) were used to effectively remove scaling deposits [12]. However, the method is laborious and requires expensive equipment and manpower. Also, much technical skill is required to perform the procedure without causing any inherent damage to the infrastructure [10]. Chemical technologies have also been widely applied both in removal of calcite and inhibition of calcite formation. The chemical removal process generally used is acid wash with the use of mud acid (HCl-HF), sulfuric acid, phosphoric acid, glycine acid and barium nitrate [13]. The chemical-based calcite prevention process uses chemical inhibitors, such as organic phosphate esters, organic phosphonates [14], ethylenediamine tetraacetic acid (EDTA) [35], polyacrylic acid (PAA), polymaleic anhydrides (PMA), sodium polyacrylate (ASAP) [15]. The major disadvantage of the chemical methods is the adverse environmental impacts of the chemicals used and the high operating cost involved [16]. Moreover, both mechanical and chemical methods cannot prevent further scaling and requires periodic repetition, which may affect the productivity of the geothermal site.

On the other hand, the prevention of calcite formation can also be achieved by biological methods, in which calcium ions in aqueous solution can be removed by bacterial biomass or derived products via adsorption. Physical adsorption methods, such as using activated carbon might be feasible for this purpose because of its higher surface area and higher adsorption morphology for adsorption of various pollutants like textile dyes [17] and chromium [18]. However, so far there is no report mentioning the use of such method in the prevention of calcite formation. Biological adsorption of heavy metals has been reported in abundance in literature and heavy metal biosorption is known to be carried out by algae [19], fungi [20], bacteria [21], cyanobacteria [22] and also plant derived products [23,24]. It has been reported that cyanobacterial biomass (of the Genera *Lyngbya, Oscillatoria, Spirulina, Anabaena, Synechocystis* and *Gloeocapsa*) are capable of adsorbing calcium ions in solution [25,26]. This method was originally developed to remove traces of calcium (0.16%) from brine to improve the quality of salt, but the general theme of calcium biosorption can be extrapolated for geothermal well descaling. However, the major difference is the extreme environment involved in geothermal well descaling (such as high temperature and high pressure) compared to rather mild conditions employed in conventional biosorption based calcium removal methods. The advantages of biological method are that it is cost and energy efficient, and does not require heavy labor or expensive equipment. It is also clean and eco-friendly, as it does not generate or release any hazardous chemical at any stage during the treatment.

Based on the hypothesis that anti-scaling mechanism in geothermal wells primarily replies on the chelation of anti-scaling agents with calcium ions so that calcium ions cannot react with bicarbonate to form calcite deposition, we tried to develop eco-friendly and effective biological anti-scaling agents for the use in geothermal site. Thus, in this study, thermophilic proteins with high calcium ion binding ability were examined for their feasibility to be used as anti-scaling agents for prevention of calcite scale formation in geothermal wells. To achieve this, thermophilic bacteria were isolated from geothermal areas in Taiwan and the proteins secreted from these bacterial isolates were collected, characterized, and evaluated for their calcium adsorption capability under the geothermal site conditions. The performance of these protein-based antiscalant was justified based on their ability to bind to calcium ions under the conditions of geothermal sites (e.g., high temperature and high pressure). To the best of our knowledge, this is the first study attempting to utilize biosorption of calcium for the purpose of geothermal site anti-scaling.

2. Materials and methods

2.1. Isolation and identification of bacterial strains from geothermal sites

The bacterial strains used for the study were isolated from geothermal areas in northern and eastern Taiwan. Geothermal water was collected and 300 ml of the sample was filtered using a 0.45 μm pore size membrane. The membrane loaded with filtered bacteria was placed on Tryptic Soy Broth (TSB) agar plates and incubated at 55 °C for 5 days. The composition of the TSB medium is as follows (g/l): pancreatic digest of casein, 17; enzymatic digest of soybean meal, 3; dextrose, 2.5; sodium chloride, 5; dipotassium phosphate, 2.5. Single colonies were picked up and transferred to fresh undiluted TSB agar plates for further growth and analysis. The cycle was repeated at least three times to ensure that pure culture was obtained. Eight isolates thus obtained were used for further studies. The pure strains were cultured in 5-fold diluted TSB medium under aerobic conditions with 200 rpm agitation unless mentioned otherwise. The strains were maintained on undiluted TSB agar plates and incubated at 55 °C for 2–5 days. Cultures were preserved at −80 °C as a 20% *v/v* glycerol suspension. Identification of the strains were done based on 16S rDNA sequencing and analysis as described in our recent work [27].

2.2. Calcium adsorption with proteins produced by the isolated strains

Proteins present in the extracellular and intracellular fraction were assessed for their calcium adsorption efficiency to locate the calcium adsorption activity. The isolated strains were grown in 5 fold dilution TSB broth at 55 °C for 2–5 days, depending on the growth of individual strains. The extracellular proteins were collected by centrifugation of the culture (6000*g*, 10 min) and the supernatant obtained was concentrated by membrane filtration (10 KDa molecular cut, Amicon, Model 8200, Millipore Co., U.S.A). The concentrated protein was stored at 4 °C until further use. The remaining cell pellet was re-suspended in phosphate buffer and disrupted by sonication: 50% amplitude for 5 min, 10 s on, 5 s off in a sonicator (S-4000, MISONIX Co., New York, U.S.A). The clear cell free extract thus obtained was used as the intracellular protein for measurement of calcium adsorption. The concentrations of the intracellular and extracellular proteins were measured via Bradford method using bovine serum albumin (BSA) as the standard. Calcium adsorption assays were performed at 25 °C, pH 7 and normal atmospheric pressure for 30 min.

2.3. Measurement of calcium adsorption ability

A 25 ml of protein solution (at a protein concentration of 50 mg/L) and 25 ml of calcium chloride solution (100 mg/L $CaCl_2{\cdot}2H_2O$) were mixed by vortexing and incubated at 25 °C for 30 min. The reaction time was determined by preliminary experiments and it was performed until the adsorption reached steady state. Then, the reaction mixture was filtered through a 10 KDa membrane (Millipore) to separate the calcium bound protein and residual calcium present in the filtrate was measured by Ion Chromatography (IC 790/792, Metrohm Co., Herisau, Switzerland). The Ca^{2+} adsorption efficiency was measured as mg of Ca^{2+} adsorbed per mg of protein used.