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# Potential of sodium dichromate and sodium silicate to control in vitro growth of *Bacillus cereus*, a metal corrosion-causing bacterium

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**Abstract.** *Ciawi Y, Inabuy FS, Teriyani NM, Ramona Y. 2023. Potential of sodium dichromate and sodium silicate to control in vitro growth of* Bacillus cereus, *a metal corrosion-causing bacterium. Biodiversitas 24: 1530-1537.* The maintenance cost of metal-based objects in industrial and construction sectors has been found to significantly increase due to corrosion. Most corrosion is caused by metal oxidation, and about 2% of this corrosion is induced by microbial activity (MIC). The main objectives of this research were to isolate, and identify corrosion-causing bacteria, and to find out the optimum concentration of sodium dichromate and sodium silicate to control their growth in vitro. These compounds have been used to protect the metal surface from corrosion caused by non-microbial-induced corrosion. Three different bacterial isolates were obtained in this study and the black colony (the predominant isolate) was further investigated in the determination of their optimum inhibitory concentrations. Application of sodium dichromate and sodium silicate at the rates of 0.1% w/v and 2% w/v, respectively were found to be optimum to inhibit the in vitro growth of this black bacterial isolate in our study. The predominant isolate found in our study was identified as *Bacillus cereus*, following the alignment of its 16s rDNA sequence with those deposited at the GenBank (NCBI). Additionally, *Enterobacter asburiae* was also found on the surface of corroded water tanks.

**Keywords:** Biodeterioration, chemical control, microbe-induced corrosion, microbial activity

## INTRODUCTION

Corrosion is a natural phenomenon involving a series of complex chemical reactions in which construction materials (metal-based tools used in human life) are returned to their origin (returned to nature) (Kip and van Veen 2015; Dong et al. 2021). Based on the process, corrosions have been classified into several types, namely uniform corrosion, pitting corrosion (Li et al. 2020; Tran et al. 2021), crevice corrosion, inter-granular corrosion, galvanic corrosion, selective corrosion, stress corrosion cracking, erosion corrosion, cavitations, fatigue corrosion, hydrogen embrittlement, and microbial-induced corrosions (Cai et al. 2021). The latter accounts for 2% of all corrosion and have caused a high cost of maintenance. Microbial-induced corrosion (MIC) can be noticed by the blackening corrosion products and has a distinct $H_2S$-like smell. MIC may occur in tools and utilities of chemical-related industries, biotechnology and pharmacy-related industries, wastewater treatment plants, cooling water installation with open circulation where seawater is used as cooling agent, part of construction materials as well as part of building foundations (Omar et al. 2021)

In Indonesia, where the air humidity is very high, metal corrosion induced by bacteria frequently occurs (Salgar-Chaparro et al. 2020). Some corrosion-causing bacteria, such as those that reduce sulphate (Dong et al. 2021), sulphur bacteria, iron bacteria, thiosulphate bacteria, hydrogen bacteria, and nitrogen bacteria, have been extensively reported. The sulfate-reducing bacteria could increase its surrounding environmental pH which leads corrosion to occur (Cui et al. 2021). These scientists further reported that corrosions promoted by sulphate-reducing bacteria are 3.5 folds higher than those caused by natural metal oxidation. Acidic compounds (Wang et al. 2020) produced by bacteria or bacteria-facilitated extracellular electron transfer (Kato 2016) have been well-documented as the main cause of metal corrosion. Kato (2016) further elaborated on three mechanisms by which microbial extracellular electron transfer (EET) occurs, and these are summarized in Figure 1. These EETs may occur either directly [through membrane redox proteins (Figure 1.A) or solid conductive matrix (Figure 1.B)] or indirectly which is facilitated by an electron mediator compound (Figure 1.C). According to Suarez et al. (2022), *Enterobacter roggenkampii* is one of many bacterial species causing corrosion. This bacterial species has been widely reported to have the capability to oxidize iron or reduce nitrate which leads to damage to the metal protective layers.

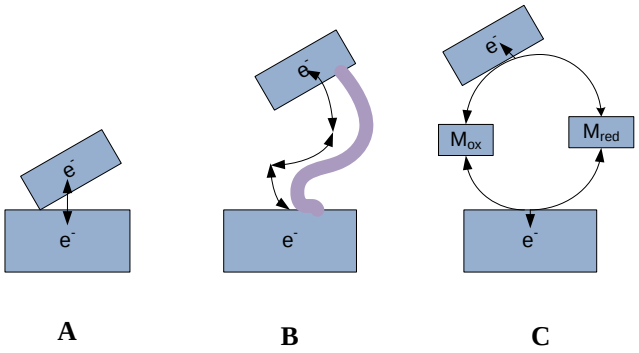


**Figure 1.** The schematic images of three microbial extracellular electron transfer (EET) mechanisms as proposed by Kato (2016). A. Direct EET via membrane redox proteins, B. Direct EET via the solid conductive matrix, such as conductive pili, C. Indirect EET via electron mediator compounds ($M_{red}/M_{ox}$)

The loss caused by corrosion may reach approximately 3-4% of a country's GNP (Procópio 2019; Little et al. 2020). An alternative method to prevent corrosion is the use of inhibitors. Some potential chemicals that have been applied to inhibit MIC include 1,8-dimethyl-1,3,6,8,10,13-hexaazacyclotetradecane (Nwankwo et al. 2016), chitosan/lignosulfonate nanospheres (Rasheed et al. 2020), *Andrographis paniculata* or the kalmegh leaves extract (KLE) (Kamaruzzaman et al. 2021), and tetrazolium violet. The latest has been applied as a corrosion inhibitor on copper-based materials exposed to sulfate-rich environments. The such compound acts through physical and chemical absorption (Tao et al. 2020). The development of MIC mitigation technology including the use of environmentally friendly biocides, such as antibacterial agents, paint coatings, and biocontrol approach to replace conventional biocides, has also been proposed in the last two decades (Omar et al. 2021; Royani et al. 2022).

In recent years, sodium dichromate and sodium silicate, which acts by the formation of a veil on metal surfaces (so that it is protected/prevented from direct contact with its surrounding environment), have been widely proposed and applied to prevent corrosions, particularly those induced by non-microbial reactions. The use of such compounds to prevent microbe-induced corrosion is rarely reported/documented until recently. Based on the above rationale, the main objectives of our research were to investigate the effectiveness of sodium dichromate and sodium silicate to prevent the in vitro growth of a corrosion-causing bacterium (isolated in our study) and to investigate their effectiveness to inhibit such bacteria in vitro, to develop a novel method for prevention of microbe-induced corrosions.

## MATERIALS AND METHODS

### Sample sources

Samples were collected from corroded water tanks (by scraping the inside wall of the tanks) in houses around the Batu Bulan Village, Gianyar District, Bali, Indonesia.

### Media

Nutrient agar, nutrient broth, and TSIA (triple sugar iron agar) products of Oxoid used in this project were purchased from suppliers in Jakarta. Pure distilled water was purchased from a chemical store in Bandung. Inhibitor media with sodium dichromate and sodium silicate in nutrient broth solution prepared for this study were 0%, 0.01%, 0.1%, 0.5%, and 1% w/v.

### Sampling procedure

Samples were collected by scraping rusts (corroded inner wall of water tanks) using a sterile spatula, soaked in 100 mL sterile saline solution, and stored at 4ºC until required. For bacterial isolation, the samples were diluted with the same solution, before being transferred into a nutrient broth for acclimation prior to bacterial isolation.

### Bacterial isolation

Prior to isolation, 10 mL of samples were enriched in 250 mL of nutrient broth medium and shaken overnight at ambient temperature with a rotation rate of 200 rpm. Dilution and spread method on nutrient agar was applied in the isolation of corrosion-causing bacteria. The previously enriched samples were diluted in saline solution to achieve dilution factors of $10^{-2}$, $10^{-3}$, $10^{-4}$, and $10^{-5}$, and 1 mL from each diluted sample was dispensed into petri dishes, added with melted nutrient agar medium (app. 40ºC), let it settled for 20 minutes, and incubated for 72 hours at 37$^0$C until bacterial colonies appeared on it. Colonies with different characteristics were streaked for a single colony on a TSIA agar medium and incubated at 37$^0$C for 24 hours to obtain bacterial pure cultures. Black bacterial colonies on the TSIA medium were the targeted bacteria and were further purified on the new TSIA medium. When white colonies appeared, they were further incubated for 7x24 hours and terminated if they did not change into black colonies in this medium.

### Effectiveness of sodium dichromate and sodium silicate to inhibit corrosion-induced bacteria

The effect of sodium dichromate and sodium silicate to inhibit the growth of isolated corrosion-induced bacteria (black bacterial colony) was investigated in a nutrient broth medium supplemented with these compounds. The concentrations of these compounds in the medium were adjusted to 0.01%, 0.1%, 0.5%, and 1% w/v. Nutrient broth medium without supplementation of those compounds served as control. The experiment was conducted in 250 mL Erlenmeyer flasks (mini fermenter) with a working capacity of 100 mL, in triplicate experiments. All flasks were inoculated with a loop full of tested bacterial pure culture, incubated at ambient temperature with a rotation rate of 200 rpm, and regularly sampled (5 hours intervals) for optical density determination ($OD_{660nm}$ reading) at the wavelength of 660 nm. The cultures were terminated 48 hours after inoculation.

**Molecular identification of the suspected corrosion-causing bacterium**

*Extraction of 16s rDNA*

Kit of Isoplant II (Isoplant code No. 310-04151, Nippon Gene, Toyama Japan) was used in the extraction of LAB's DNA. The procedures followed the instruction of the KIT. A volume of 1 mL bacterial suspension with a cell density of approximately $10^8$ cells/mL was centrifuged at 5000 rpm in Eppendorf tubes for 7 minutes followed by decanting of supernatant to obtain cell mass in the form of pellets. The pellets were then washed twice with sterile distilled water, added with 300 µL solution I, vortexed for 5 seconds, added with 150 µL solution II, and vortexed again for 5 seconds. The mix was then added with solutions IIIA and IIIB (each 75 µL), vortexed for 2 seconds, cooled on ice for 15 minutes and centrifuged at 4ºC and 15,000 rpm for 30 minutes to obtain DNA pellets. The DNA pellet was subsequently added with 400 µL of absolute ethanol, centrifuged at 4ºC and 15,000 rpm for 30 minutes, decanted to obtain pellet, added with 100 µL of 70% ethanol, centrifuged as above, evaporated, and added with 25 µL TE pH 7.5, and vortexed for 5 seconds.

*Amplification of 16s rDNA by PCR*

Amplification of 16s rDNA was conducted according to the method specified in Sintyadewi et al. (2015) with minor modifications, using primers of 27F and 1492R. The total volume of the PCR reaction mix was 50 µL, consisting of 46 µL PCR *master mix* and 4 µL isolated DNA. The PCR master mix contained 25 µL 2X My Taq HS Red Mix (Bioline), 2 µL of 10 pmol primer 27F, 2 µL of 10 pmol primer 1492R, and 17 µL Nuclease Free Water (Qiagen). Amplification was done in an *Infinigen Thermocycler* TC-25/H, with pre-denature at 95ºC for 1 minute, followed by 25 cycles of PCR. Each cycle of this PCR process consisted of DNA denature at 95ºC for 30 seconds, annealing at 58ºC for 30 seconds, and elongation at 72°C for1minute. The final cycle was followed by final elongation at 72ºC for 10 minutes. This PCR product was then stored at -20ºC until required.

*Electrophoresis*

Electrophoresis was conducted on 1% agarose gel in a chamber filled with TAE buffer. DNA *ladder* of 10.000 bp (Promega) was loaded into a well beside the well where the 3 µL of PCR products was loaded on this agarose gel. The electrophoresis was run for 30 minutes at 100 Volt and the bands of DNA fragments were visualized with a computer-connected UV-transilluminator where a DNA amplicon with the size of 1.500 base pair was the target band of this electrophoresis.

*Sequencing of the PCR product*

Sequencing of the 16s rDNA of our isolate was conducted at the 1st base, Malaysia via the PT. Genetika Science Indonesia. The PCR product or the isolate was first purified and sequenced by application of the BigDye terminator sequencing method in an automated capillary sequencer. The 16s rDNA sequence obtained was then aligned with those deposited in the GenBank (NCBI) to determine the molecular identity of our isolate and followed by the construction of a phylogenetic tree using Custal W2 software combined with Njplot.

**Data analysis**

Data obtained in this research were analyzed descriptively.

## RESULTS AND DISCUSSION

Bacterial colonies appeared following dilution and the spread of samples on the TSIA medium and nutrient agar medium is shown in Figure 2. The characteristics of bacterial isolates collected from corroding materials are presented in Table 1. Bacterial colonies with black color on the TSIA medium were being the target to be controlled in further studies as they were suspected to produce $H_2S$ (indicated by black deposits on this medium). According to Kiani Khouzani et al. (2019), bacterial colonies on TSIA agar with black deposits around them have the capability to form biofilm on metal surfaces and induce corrosion processes. Bacteria, sulfate-reducing bacteria (SRB) in particular, can utilize iron elements as an alternative source of energy as they can donate its electron in anodic reactions (Jia et al. 2019). These SRBs subsequently utilize sulphate as an electron acceptor terminal in the cathode reactions, following the reaction of $SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$ which is induced by microbes (El Hajj et al. 2013).

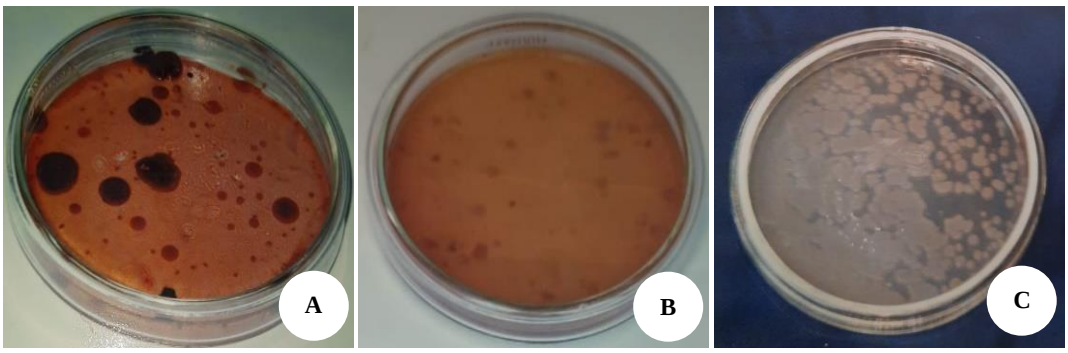


**Figure 2.** Various colony morphologies of suspected corrosion-causing bacteria isolated from the inner wall of corroded metal-made tanks. A, B, and C are colonies from sample 2 on TSIA, sample 1 on TSIA, and sample 4 on NA, respectively

**Table 1.** Characteristics of bacterial colonies on TSIA and NA media following 24 - 48 hours of incubation

| Medium | Sample codes | Medium color after incubation | Shape of colonies | Color of colonies | Incubation time |
|---|---|---|---|---|---|
| Tsia | 1 | Brownies Red | Round | White | 48 Hours |
| Tsia | 2 | Dark Orange | Round | Black | 48 Hours |
| Tsia | 3 | Red | Round | White | 48 Hours |
| Tsia | 4 | Yellow | Round | White | 48 Hours |
| Tsia | Blank | Dark Brown | - | - | 48 Hours |
| Na | All samples | White | Round | White | 24 Hours |

The appearance of the black colony in such a medium indicated $H_2S$ formation which is deposited in the TSIA medium by this isolate (Braccia et al. 2021; Thakur and Kumar 2021; Braccia et al. 2021; Thakur et al. 2021). Although $H_2S$ is a weak acid, dissociation of this compound will produce hydrogen ions and decrease the surrounding pH (Murros 2022), which leads corrosion to occurring on iron-based objects.

TSIA medium contains glucose, lactose, and sucrose in addition to iron in the form of ferric citrate. This medium has been used to initially indicate the bacterial ability to produce gas and acidic compound, such as $H_2S$. This is a colorless gas with the capability to react with metal salt incorporated and subsequently form a visible insoluble black ferrous sulfide precipitate (Thakur et al. 2021). As shown in Figure 2, colonies with black color appeared in the isolation process of this research, indicating that the corroded metal tanks have been deposited by these corrosion-causing bacteria. Some bacterial species belonging to the genera *Desulfovibrio* and *Bilophila* have been indicated by Braccia et al. (2021) and Murros (2022) to produce $H_2S$ in the gut of a human. *Proteus* sp., *Escherichia coli* and *Klebsiella oxytoca* have also been reported to produce $H_2S$ from the assimilation of sulfur-containing compounds (Saimin et al. 2020).

All those bacteria may contaminate water bodies and often become part of biofilm on the surface of any objects, including metal-based materials (such as water tanks) (Kahraman and Karaderi 2022). Microbes in the biofilm play important role in the corrosion of metal-made materials (Procópio 2019). This phenomenon was also observed on the inner wall of the water tanks, where we obtained our samples for this research. The scraps of the samples also contained bacterial colonies with the capability to produce $H_2S$, which are indicated by the formation of a black deposit on the TSIA medium (Table 1A and Figure 2). The results of our study confirmed that these black bacterial colonies were the agents causing corrosion on the inner wall of the water tanks from which our samples were obtained.

The mechanism by which corrosion of iron-based materials happens was comprehensively reviewed by Lewandowski and Beyenal (2009). This review mentioned that corrosion may occur both under anaerobic-aerobic conditions. The following equations explain the 3 stages of the corrosion process that occurs under anaerobic conditions as reviewed by Lewandowski and Beyenal (2009) and Imo et al. (2016):

$$Fe \rightarrow Fe2^{+} + 2e^{-} \quad \text{(anodic)}$$
$$2e^{-} + 2H_2O \rightarrow H_2 + 2OH^{-} \quad \text{(cathodic)}$$
$$2H_2O + Fe \rightarrow Fe(OH)_2 + H_2 \quad \text{(net)}$$

Similar reactions of corrosions under aerobic conditions have also been proposed by these scientists. Under such conditions, the product of iron dissolution that occurred in stage 1 of the reaction is further hydrolyzed and oxidized in the presence of oxygen. The following stages summarize the corrosion process that occurred under the aerobic condition, and this was reviewed by Lewandowski and Beyenal (2009), Lewandowski et al. (2009), and Imo et al. (2016). Kiani Khouzani et al. (2019) indicated in their study that reaction (3) was one of the main products of microbe-induced corrosion.

$$4OH^{-} + 4Fe(OH)_2 \rightarrow 4Fe(OH)_3 + 4e^{-} \quad \text{(anodic)}$$
$$O_2 + 2H_2O + 4e^{-} \rightarrow 4OH^{-} \quad \text{(cathodic)}$$
$$4Fe(OH)_2 + O_2 + 2H_2O \rightarrow 4Fe(OH)_3 \quad \text{(net)}$$

It was further elucidated and summarized by Kiani Khouzani et al. (2019) that the sequential processes of microbe-induced corrosions are as follows:

$$SO_4^{2-} + 9H^{+} + 8e^{-} \rightarrow HS^{-} + 4H_2O$$
$$Fe^{2+} + HS^{-} \rightarrow FeS + H^{+}$$
$$Fe_2 + + 2OH^{-} \rightarrow Fe(OH)_2$$
$$Fe(OH)_2 \rightarrow Fe_3O_4 + H_2 + 2H_2O$$

Modifications of the above reactions may occur within the biofilm, where the causative agents of corrosion reside. Biofilm has been reported to have a significant role in the corrosion of metals in several ways, such as oxygen consumption (Sierra and Gomez 2007), increasing the mass transport of corrosion reactants and products (Varseev and Alekseev 2015), generating corrosive substances (Procópio 2019), and generating substances that function as auxiliary cathode reactants (Ye et al. 2021).

It was noticed in our experiment that bacterial colonies appeared on the nutrient agar medium following 24 hours of incubation, while at least 48 hours of incubation was needed on the TSIA medium (Table 1). This indicates that the carbon source of the nutrient agar medium is more digestible for more bacterial species when compared to those contained in the TSIA. Therefore, nutrient agar medium can be considered a universal medium for bacterial cultivation (Uthayasooriyan et al. 2016).

Pure culture of the black colony (suspected to be a cause of corrosion-causing bacterium) that appeared on the TSIA medium was tested for its survival rate in the nutrient broth medium supplemented with sodium chromate or sodium silicate at various concentrations. The results of these treatments are shown in figures 3 and 4. Both compounds effectively reduced or inhibited the growth of the suspected corrosion-causing bacterium, isolated in this study. The growth of the tested bacterium was significantly lower than that of the control (bacterial cells grown in NB only), and at concentrations of higher than 1% and 0.1% for $Na_2SiO_3$ and $Na_2Cr_2O_7$, respectively, these compounds almost totally inhibited the growth of this bacterium (Figures 3 and 4). It can also be seen in these figures that the bacterial growth reduction is proportional to the concentrations of sodium dichromate and sodium silicate applied in the experiments (Figures 3 and 4). Application of sodium dichromate and sodium silicate at the rates of 0.1% w/v and 2% w/v, respectively were found to be optimum to inhibit the in vitro growth of this corrosion-causing bacterium (Figures 3 and 4).

In addition, to prevent microbe-induced corrosion, sodium dichromate has also been effective for use to inhibit non-microbe-induced corrosion by forming a thin protective layer over the metal surface (Khan and Hadromi 2020). In the prevention of microbe-induced corrosion, chromate inhibits metabolic pathways in bacterial cells as well as disturbing the function of proteins and DNA (Nickens et al. 2010; Zhu and Costa 2020). Due to its high toxicity to prevent metals from microbe-induced corrosion, chromium has been used to protect alloys (such as stainless steel) as well as cookware, automobile parts, and sink faucets. The high toxicity level of this compound, due to its heavy metal content should also be considered (Chen et al. 2019), because it will have bad side effects on human health following chromate exposure (Kozłowska et al. 2022; Santonen et al. 2022; Tavares et al. 2022). This compound has also recently been reported to have carcinogenic effects (Chen et al. 2019; Zhu and Costa 2020).

In addition to sodium dichromate, sodium silicate has also been well-known as an anticorrosive agent. This compound protects iron-based materials by forming a silicon-rich coating so that these protected materials are prevented from microbe-induced corrosion (Li et al. 2021). Commercially, sodium silicate has been applied at a concentration of 2% and is very effective to control various types of corrosion (Mainier et al. 2016). This concentration was also effective to control the in vitro growth of corrosion-causing bacterium isolated in our study (Figure 3). In food industrial sectors, therefore, sodium silicate has also been widely used as a stabilizer due to its antibacterial and antifungal properties (Zhang et al. 2021).

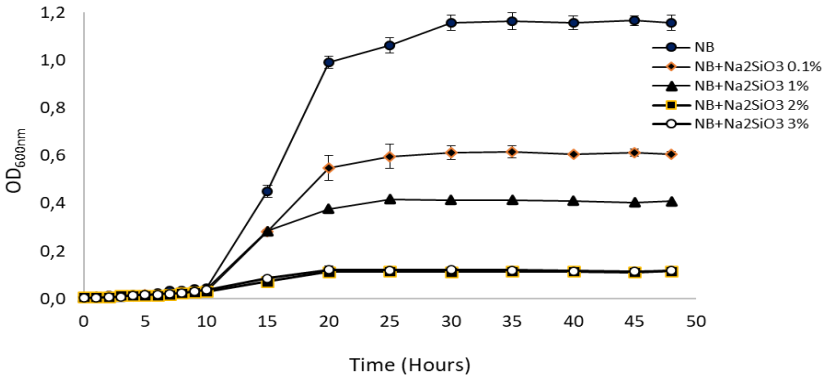


**Figure 3.** Growth inhibition of the suspected corrosion-causing bacterium following application of $Na_2SiO_3$ at various concentrations in nutrient broth medium. Values in this figure ± standard deviation bars are averages of five replicate measurements

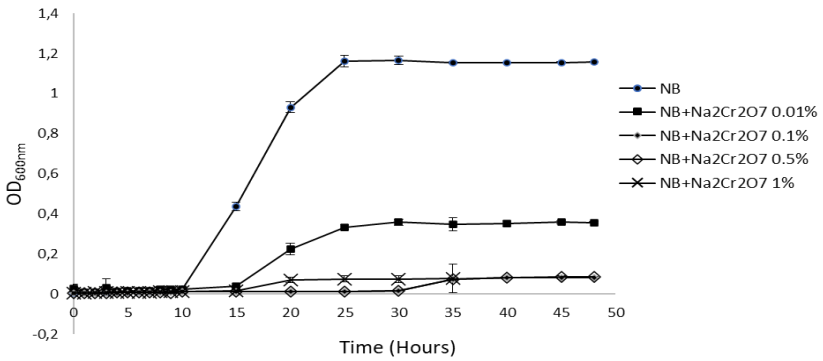


**Figure 4.** Growth inhibition of the suspected corrosion-causing bacterium following application of $Na_2Cr_2O_7$ at various concentrations in nutrient broth medium. Values in this figure ± standard deviation bars are averages of five replicates

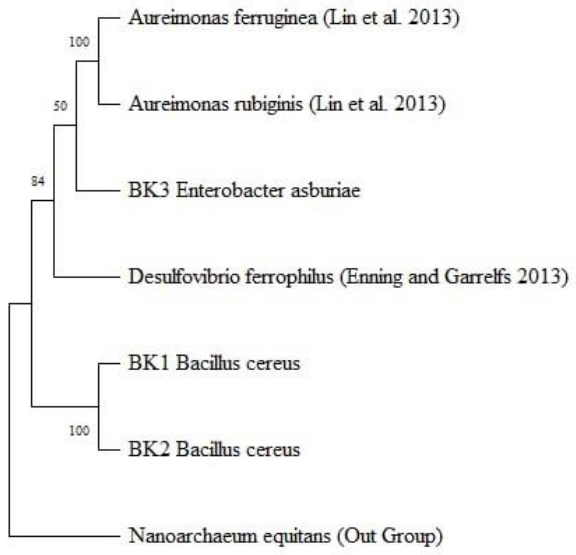


**Figure 5.** Phylogenetic tree of the black isolates (BK1 and BK2) on TSIA medium, relative to other bacterial species causing metal corrosion

Being chemical-based compounds, both sodium dichromate and sodium silicate are hazardous to human health. As it was mentioned previously, sodium dichromate in particular has been claimed to have a carcinogenic effect due to its heavy metal content in it. Some efforts to reduce the use of chemical-based compounds to prevent metal corrosion have been carried out globally, to avoid further exposure to these hazardous compounds. These include the application of more environmentally friendly organic biocides (Royani et al. 2022). According to Faiq and Mokhtar (2022), organic biocides were very effective to decrease the rate of the pitting corrosion process. CTA-4OHcinn is an excellent example of an organic biocide with biocidal properties that have been found to significantly inhibit the growth of biofilm formation (Tuck et al. 2022).

The biocontrol approach (application of Methanobacteriales and Syntrophobacterales) has also been researched to avoid applications of chemical-based compounds, especially in the prevention of microbe-induced corrosions (in ‘t Zandt et al. 2019). However, the application of such microbial competitors to prevent microbe-induced corrosion has some drawbacks. Some methanogenic Archaea, for example, are among biocontrol agents with the capacity to induce corrosion, besides their capacity to compete with other corrosion-causing microbes (Hirano et al. 2020, 2022).

The black colonies (two colonies encoded with BK1 and BK2) purified on the TSIA medium in our study were identified as *B. cereus*, following the alignment of their 16s rDNA with those deposited at the GenBank (NCBI). The position of this species in the phylogenetic tree, relative to other species, causing metal corrosion is shown in Figure 5.

*Bacillus cereus* has been reported by many researchers to induce corrosion on metal-based materials. Parthipan et al. (2017) and Wan et al. (2018) for example reported that this bacterial species accelerated the corrosion of steel. In a more recent study, Moreira-Filho et al. (2022) also found *B. cereus* as the main cause of corrosion on stainless steel materials. They concluded that such species accelerated the corrosion process by speeding up anodic dissolution reaction on such steel-made materials. These studies indicated that *B. cereus* plays a significant role in microbe-induced corrosion, as we found in our current study. In addition to *B. cereus*, *Enterobacter asburiae* (coded as BK 3) was also identified in our study (Figure 5). Bacterial species, such as *Aureimonas ferruginea* and *A. rubiginis* (Lin et al. 2013) as well as *Desulfovibrio ferrophilus* (Enning and Garrelfs 2013) were also reported to cause corrosions on metal-based materials.

In conclusion, various types of bacterial species can accelerate metal corrosion by increasing anodic dissolution reaction. Two species were identified in our study as *B. cereus* and *E. asburiae*, following the alignment of its 16s rDNA sequence with those deposited at the GenBank (NCBI). Sodium dichromate and sodium silicate effectively controlled the in vitro growth of *B. cereus*, the predominant metal corrosion-causing bacterium found in our study. The optimum concentration of these compounds to control the growth of this bacterium appeared to be at the rates of 0.1% w/v and 2% w/v, respectively.

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