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Effects of zero-valent iron on sludge and methane production in anaerobic digestion of domestic wastewater

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Case Report

Characterization and invitro toxicity assay of bio-reduced hexavalent chromium by *Acinetobacter* sp. isolated from tannery effluent

Ahmad Fatima Abdulmalik [a], Hafeez Muhammad Yakasai [a,b,*], Shehu Usman [c], Jahun Bashir Muhammad [d], Ahmad Hussaini Jagaba [e,**], Salihu Ibrahim [f], Abba Babandi [a], Mohd Yunus Shukor [g]

[a] Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University Kano, P.M.B. 3011, Kano, Nigeria
[b] Skyline University Nigeria, No. 2 Zaria Road, Kano State, Nigeria
[c] Department of Plant Science and Biotechnology, Federal University, Dutsin-Ma, Katsina, Nigeria
[d] Department of Biology, Federal Collage of Education Technical, Bichi, Kano, Nigeria
[e] Department of Civil Engineering, Abubakar Tafawa Balewa University, Bauchi, Nigeria
[f] Centre for Biotechnology Research, Bayero University Kano, P.M.B 3011, Kano State, Nigeria
[g] Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor Darul Ehsan, Malaysia

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ABSTRACT

Chromium, a transition metal element widely distributed in the earth's crust, with the valence from −2 to +6, but exists mainly in the form of trivalent and hexavalent states. Hexavalent chromium Chromium (VI) is often found in soil and ground water due to its widespread industrial use such as tannery, electroplating, and steel industries. Chromium (VI) is toxic, mutagenic, carcinogenic, and teratogenic and much more toxic to many plants, animals, and bacteria inhabiting aquatic environments because it is motile, highly toxic, soluble in water and it is a strong oxidizing agent that causes severe damage to cell membranes while the trivalent chromium Cr (III) is the most stable form of chromium and relatively immobile in the aquatic system due to its lower solubility. This study therefore, aims at identifying a bacterium that can reduce Chromium (IV) to (III) using a pour plate technique on Luria Bertani medium amended with $K_2Cr_2O_7$. Graphs and tables were used for the data analysis. The effects of incubation time, pH, temperature, inoculum size, and potassium dichromate ($K_2Cr_2O_7$) concentration were then examined to characterize the bacterium based on one factor at a time. To assess Chromium (VI) reduction, a spectrophotometric study of the 1, 5-diphenyl carbazide test (DPC) at 370 nm was utilized. By using molecular phylogenetic analysis and partial 16s ribosomal RNA analysis, the isolate was found to be *Acinetobacter* sp. strain BUK_BCH_BTE 5. The findings demonstrated that tryptic soy broth (TSB), with an optimal $K_2Cr_2O_7$ level of 200 mg/L, pH of 6.5, 35 °C temperature, inoculum size of 2%, as well as incubation time of 48 h, was the most successful approach for decreasing chromium (VI). Following Nickel (Ni), the isolate was found to withstand the highest doses of Lead (Pb), Mercury (Hg), Zink (Zn), Iron (Fe), Cadmium (Cd), Arsenic (As), and Cupper, in that order. A reduced culture's (supernatant) larvacidal bioassay showed a 30% decrease in toxicity in just 48 hours, indicating that the isolate is a promising candidate, and that the reduction method is less dangerous for decontaminating chromium-contaminated environments.

1. Introduction

An increase in population and sophisticated lifestyle increase the demand for quality industrial products at an exceptional rate. An extensive volume of wastewater originated from industries which are released into channels either untreated or inadequately treated causing water pollution. Industrialization leads to several environmental problems like water, land, and air pollution [1]. Tanning is an ancient trade that has been followed for many centuries, in the tanning process putrefiable animal hides is preserved from decomposing and is

* Corresponding author. Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University Kano, P.M.B. 3011, Kano, Nigeria.
** Corresponding author. Department of Civil Engineering, Abubakar Tafawa Balewa University, Bauchi, Nigeria.
E-mail addresses: hmyakasai.bch@buk.edu.ng (H.M. Yakasai), ahjagaba@gmail.com (A.H. Jagaba).

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converted into an enduring material, known as leather; the tanning industry has been extensively examined for its environmental impact and is branded as one of the worst anthropogenic polluters [2,3]. Tannery effluents are ranked as the highest pollutants among all industrial wastes. They are especially large contributors to chromium pollution. Long-term disposal of the tannery wastes has resulted in wide contamination of agricultural land and water sources. When tannery waste is discharged in agricultural lands or used to irrigate the lands, it affects the fertility of the soil [4]. Land pollution is common in urban areas as a result of industrial and municipal wastes which are the major pollutants in the environment and the highest quantities are generated by tannery industries where hides and skin are converted into leather. Kano has several tannery industries in Challawa, Sharada, and Bompai industrial estates, hence resolving environmental pollution from these industries has been a challenging issue because their operations could generate a large of sludge [5–9]. The quantity continually increases because of the high demand for leather materials and its status (as a second non-oil source of foreign exchange) in the nation's economic development FME, [10,11]. Naturally, chromium occurs in various oxidation states but Cr (III) and Cr (VI) are of remarkable worry biologically. Chromium is a necessary metal that is involved in the metabolism of glucose in humans and animals, but its hexavalent form is very toxic and carcinogenic and its demand is increasing day by day due to its extensive use in numerous industrial and chemical processes and these industries produce toxic waste in a large amount containing chromium with concentrations ranging from tens to hundreds of milligrams per liter and Cr (VI) is a highly toxic pollutant even at very low concentrations [12].

One of the most dangerous metal wastes is chromium and is available in a variety of oxidation states. The two most stable forms are Trivalent and hexavalent chromium, yet they have highly distinct physical, chemical, and biological properties [13]. While Chromium (III) species are more immobile and less water-soluble, Chromium (VI) species are easily dissolved in water and move around the environment. Cr (VI) is understood as a carcinogen, toxicant, and mutagen for animals such as humans, and Cr (III) is seen as major chemical needed in trace levels for amino-acid [3,14]. Glucose, lipid, and have a high nutritional metabolism. Chromium (VI) is a component of our contemporary environments because it is present in industrial wastes, the bulk of which originate from tanneries [15]. Due to the substantial demand for leather products in the nation's industrial development as of 2012 [7], numerous tanning industries located alongside Sharada, Bompai, and Challawa industrial estates in Kano State are increasingly discharging their effluents containing this toxic metal into our water bodies. It was listed as the second foreign currency source that wasn't related to oil [16].

Sludge disposal, a byproduct of wastewater treatment from these tanning companies with an extremely high chromium concentration, is another significant problem wreaking havoc on the environment today [17]. As a result, cleaning up pollution caused by this metal has been a challenging problem. However, prior to final release, these effluents must be treated [18]. Many physicochemical processes, including ozonation, electrocoagulation, reverse osmosis, and ultrafiltration, have been employed to treat tannery waste to date, but each has its limitations. Electrochemical oxidation has been proven to be inefficient when used with dye-works wastewaters because of high energy consumption and the harm that effluent does to the electrodes [19]. However, flocculation-coagulation utilizing Fe and Al salts has yielded satisfactory outcome with reference to turbidity and chromium, total suspended solids (TSS), and COD, but at the cost of increased sludge buildup. Another drawback of using membranes is a sizable fouling issue brought on by the production of cake layers, pollutant adsorption, and membrane clogging [20–26]. Treatment of industrial leachate by bio reduction is thought to be feasible [27]. According to study by Ref. [28], a lot of chromium-resistant bacteria have lately been demonstrated to be capable of changing harmful hexavalent chromium Cr (VI) to trivalent chromium Cr (III). But none of the isolates was as efficient or tolerable in reducing hexavalent chromium [29,30]. Therefore, the main objectives of this study are to identify and characterized chromium-reducing bacteria from tannery effluent been prominent contaminant that reduces the quality of the water, causes damages to the aquatic organisms, air, plants and human exposure on a large scale being the major source of water for irrigation activities in the area as a means of reducing water pollution and other health risk in Kano State, Nigeria, and also to determine the concentration of the reduced chromium and its invitro toxicity level.

2. Materials and methods

During the study, only chemicals that are of grade quality were used. Experiments that require the use of microorganisms were carried out in a class II safety cabinet [31]. Glassware was cleaned in 2 mM HNO_3, and then washed in deionized water. The study flowchart has been cited in Fig. 1.

2.1. Sample collection

Effluent samples from the tannery industry were collected in August 2019 from Challawa industrial area Kano located on latitude 11°58-11°50 N and longitude range of 8°31- 8°40 E 430 m above mean sea level on average, in sanitized bottles [32], and delivered to Bayero University Kano microbiology lab.

2.2. Identification and testing of microorganisms that reduce chromium

Bacteria were isolated from dyes-work effluent using the plating procedure [33]. 1 mL of tannery leachate was quickly assorted with 9 mL of sterilized saline (0.85%), followed by serial dilution. Hexavalent chromium containing $K_2Cr_2O_7$ was added to LB medium at dilutions ranging from 10^{-2} to 10^{-8}, and incubated for 24 hours at 37 °C. On tryptic soy broth, the most productive colonies were chosen and cultivated throughout the experiment [34–36]. Based on appearance, biochemistry, and molecular identification, the bacterial strain that could tolerate the highest concentration of Cr(VI) was isolated [37].

2.2.1. Molecular identification of the isolate

2.2.1.1. Genomic DNA Extraction, PCR amplification, and phylogenetic analysis. Genomic DNA was extracted from a pure bacterial culture that had been grown on tryptic soy broth at 35 °C by centrifuging it for 2 min at 5000 rpm. The pellet was fully dissolved in 100 L of Livak grind buffer (1.6 mL 5 M NaCl, 1.57 g Tris, 10.16 mL 0.5 M EDTA, 5.48 g sucrose, 2.5 mL 20% SDS), and then incubated for 30 minutes at 65 °C. The solution was then added, and stored at zero degrees for 30 minutes, to 14 L of 8 M K-acetate (1 M final concentration). 200 L of 100% ethanol was added to the mixture after it had been centrifuged for 20 minutes at 4 °C; the mixture was then vortexed, and the effluent was quantitatively taken to a fresh Eppendorf (1. 5 mL). The pellet was then rinsed with 100 L of ice-cold 70% ethanol, dried, and suspended in 100 L of deionized water following 10 min cultivation at 65 °C.DNA concentration was measured using a Nano-drop spectrophotometer.

KAPATaq DNA polymerase was used to conduct the PCR reaction in a 25 L total reaction volume. Approximately 0.4 M (0.85 L) of each of the forward primer Bact1442-F (AGAGTTGATCCTGGCTCAG) and reverse primer Bact1492-R (GGTTACCTTGTTACGACTT) are present in the original solution, along with 2.5 L of 10 TaqA Buffer. $MgCl_2$ 1.25 mM (1.5 L), Taq DNA polymerase in deionized distilled water and 0.25 mM (0.2 L) dNTP mixes were mixed and amplified. To see the results, ethidium bromide was used to stain the 1.5% agarose gel that was used to separate the PCR products. The 16S rRNA PCR product was extracted from the gel and sequenced using an ABI automated sequencer. The obtained sequences were compared with those obtained from the

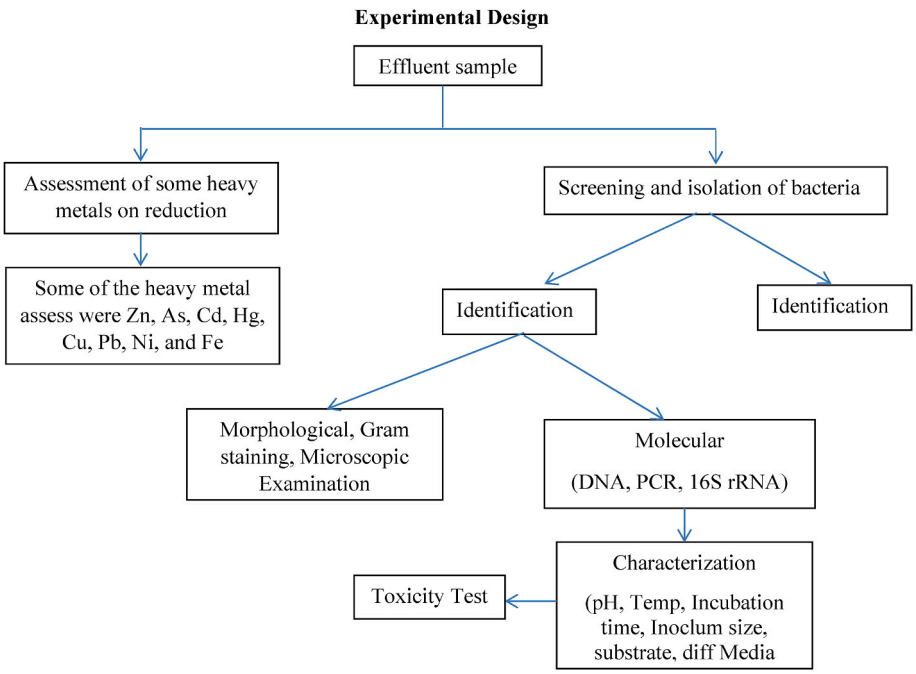

Fig. 1. Flowchart showing the design of the research work.

National Center for Biotechnology Information. To locate the currently accessible neighboring database sequences. Sequences were then submitted to GenBank and assigned an entry number.

2.3. Assessment of Cr^{6+} reduction using 1, 5-diphenyl carbazide assay

Chromium (VI) concentration in the bacterial mixture might be calculated by measuring the optical density measurement at 600 nm. About 250 mg of DPC were generated in 50 mL of acetone. The samples of the culture were centrifuged for 10 minutes at 5000 rpm. 200 L of culture supernatant, 330 L of 6 M H_2SO_4, 400 L of DPC reagent, and 10 mL of distilled water were added to the reaction mixture, which also contains 200 L of culture supernatant. After 20 minutes at room temperature, spectrophotometric measurements of the colored complex's absorbance at 370 nm were taken [12].

2.4. Characterization of hexavalent chromium-reducing bacteria

During characterization, each of the effects of temperature, pH, incubation time, chromium content, inoculum size, and heavy metals on the reduction process was tuned separately. Each experiment was repeated three times [38].

2.4.1. Effects of different media on chromium reduction

The isolate was cultivated on various media, including Luria Bertani, Nutrient Broth, Tryptic Soy Broth, and Minimal Salt Media (MSM, LB, NB, and TSB), to find the best medium for bacterial growth and chromium reduction [5].

2.4.2. Effect of incubation time on chromium reduction

The ideal incubation duration was completed in a batch culture with 100 mL of medium that had been 100 ppm $K_2Cr_2O_7$-added for a 120-h period. Using a spectrophotometer, readings were taken every 24 hours at 600nm optical density.

2.4.3. Effect of pH on chromium reduction

This was accomplished by employing 1 M HCl and 1 M NaOH to adjust the pH (5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) of the TSB media, which included 100 ppm $K_2Cr_2O_7$ as Cr(VI) compound. The 1,5-diphenylcarbazide technique was used to measure chromium reduction.

2.4.4. Effect of inoculum size on chromium absorption

Inoculum size was tested between the range of 0.5 mL and 8 mL in a 100 mL TSB media and cultivated at 37 °C for 120 h to evaluate the effect of inoculum size. Prior assessments of chromium reduction were made.

2.4.5. Effect of temperature on chromium reduction

By inoculating an overnight culture on tryptic soy broth with 100 ppm of $K_2Cr_2O_7$ and varying the incubating temperature between 25 °C and 50 °C for 120 h, the reduction in chromium was evaluated as before.

2.4.6. Effect of chromium concentration on chromium reduction

$K_2Cr_2O_7$ as Cr6+ was added to tryptic soy broth at various quantities ranging from 50 to 1000 ppm. The flasks received the strain, and they underwent an incubation period at 37 °C. As before, the decrease in chromium was assessed.

2.4.7. Impact of heavy metals on chromium reduction

On the decrease of Cr (VI), the effects of Zn, As, Cd, Hg, Cu, Pb, Ni, and Fe were investigated. These metal ions were created in the lab by combining their salts with deionized water to create aqueous solutions that ranged in concentration from 1 to 10 ppm [39]. The individual metal ions were added one at a time into a freshly made media containing $K_2Cr_2O_7$, inoculated, and cultivated at 37 °C for 120 h.

2.4.8. Effect of optimized conditions on Cr (VI) bio-reduction

To study the capacity of this isolate for the reduction, the experiment was set with the optimized conditions in this work to compare with conditions referenced in the literature [40].

2.5. Testing the toxicity of a substance bio reduced by Cr (VI) on mosquito larva

Anopheles mosquito larva was used to test the toxicity of the Cr (VI) bioreduced product (supernatant) to uphold morality. To enable bioremoval, a variety of $K_2Cr_2O_7$ samples with varying Cr (VI) concentrations (0–20 ppm) were generated and infected in various ways for 24

and 48 hours. Each culture's aliquot was centrifuged, and the supernatant was UV sterilized while the sample that wasn't inoculated served as the Cr (VI) control. Each container received about 10 Anopheles mosquito larvae, and after 24 hours the death rate was noted [41].

2.6. Assessment of residual chromate using Atomic Absorption spectroscopy (AAS)

To analyze the bio-removal efficiency of the isolate, the level of chromium in the bioremediation (cultured) samples relative to the control (uncultured) was examined using AAS [29]. To digest both samples, nitric acid was provided 5 mL and heated to 100–110 °C till it become 30 mL, nitric acid 5 mL was further added with continuous heating till it reaches 20 mL. The samples were then cooled and deionized water was supplemented to make it up to 50 mL and the chromium concentration was determined using AAS.

2.7. Statistical analysis

Graph Pad In Stat was used to examine all the data. One-way ANOVA was used to compare the groups. Statistics were judged significant at P0.05. Data are presented as mean SD of triplicates [42].

3. Results and discussions

3.1. Isolation of hexavalent chromium-reducing bacteria

Following serial dilution and a successful pour plating on LB broth and incubating at 37 °C for 48 h, appeared round milky from which a distinct colony was isolated and streaked on a fresh media to obtain a pure culture [43]. Four isolates were obtained, of which only isolate B was able to withstand extreme levels of Cr (VI) tested, therefore was chosen for the study.

3.2. Molecular identification of hexavalent chromium-reducing bacteria

Base on the findings of the morphological and biochemical characteristics as well as the 16S rRNA gene sequence of this bacterium on the Blast Server available at NCBI database, revealed that the isolate has 95.17% similarity to several Sporanaerobacter acetigenes, though, the isolate was more closely linked to *Acinetobacter* sp. than Sporanaerobacter acetigenes, according to a neighbor-joining molecular phylogenetic analysis [44,45]. Thus, tentatively identified as *Acinetobacter* sp. strain BUK_BCH_BTE 5 (Fig. 2) with accession number OM222620. In 1977, Romanenko and Koren'Kov first reported an uncharacterized anaerobic Cr (IV)-reducing *Pseudomonas* sp [46]. To date, several Cr-reducing bacteria (aerobic and anaerobic) bacteria belonging to diverse genera and environments have been published, including E*nterobacter cloacae, Ralstonia metallidurans, Desulfovibrio vulgaris, P. putida, Caulobacter crescentus, Shewanella oneidensis, Escherichia coli, Cupriavidus metallidurans, Bacillus firmus, Burkholderia cepacian*, and *Pseudomonas aeruginosa* [47], *Bacillus* [14], *Escherichia coli* [29], *Enterobacter* [48], *Pseudomonas* sp., *Shewanella* sp., *Achromobacter* sp., and others [16], *Achromobacter* sp. strain Ch1, *Sphaerotilus natans, Nesterenkonia* sp. strain MF2 [49], *Enterobacter cloacae* HO1 [15], *Arthrobacter* [6] *Escherichia* [42], and *Bacillus* sp. [50], etc signifying a very important energy-saving strategy in the bioremediation of Cr (VI) pollution [13]. The optimum incubation time of 48 h for strain BUK_BCH_BTE 5 in this study was similarly reported by Ref. [28]. Interestingly, it was shown that Cr (VI) reduction increased with longer incubation times.

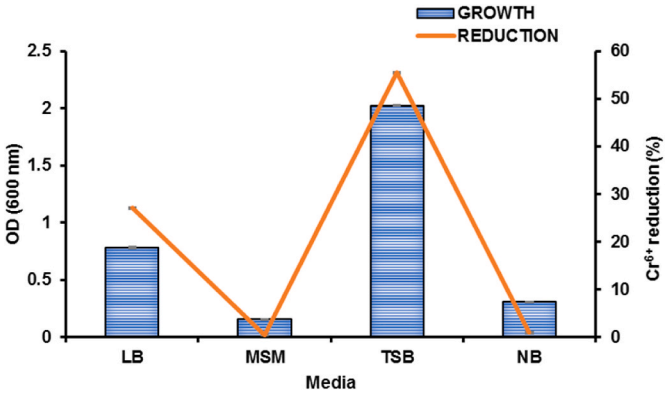

Fig. 3. Impact of different media on growth and reduction of hexavalent chromium by strain BUK_BCH_BTE 5 grown at 37 °C for 48 h.

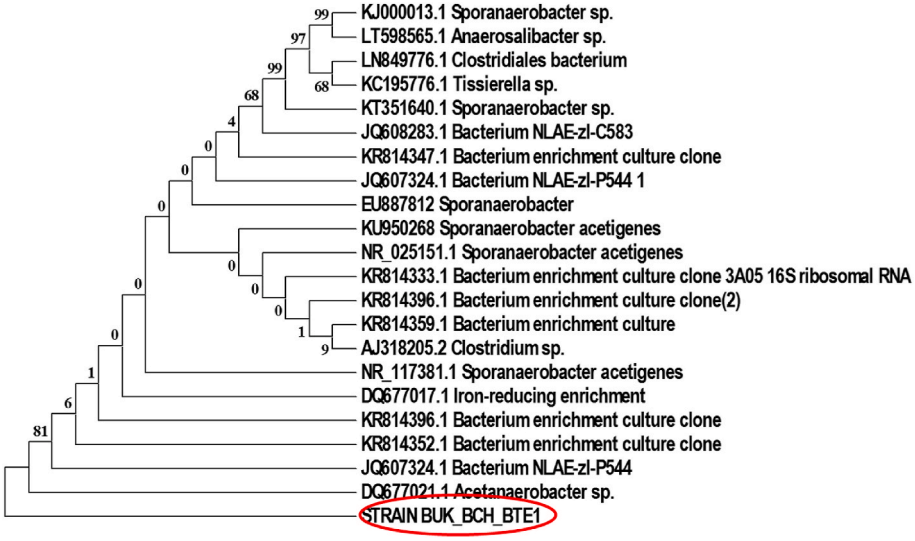

Fig. 2. Phylogram showing the evolutionary relationship between the strain BUK_BCH_BTE 5 16S rRNA gene sequence and related reference microorganisms, a program for molecular evolutionary genetic analysis. The names of the species are included beside the accession numbers.

3.3. Characterization of hexavalent chromium-reducing strain BUK_BCH_BTE 5

3.3.1. Effect of different media on chromium reduction

The impact of different media on reduction of hexavalent chromium by strain BUK_BCH_BTE 5 was presented in Fig. 3. The isolate was observed to grow best and significant ($p < 0.05$) reduced hexavalent chromium on Tryptic soy broth (TSB) when cultivated at 37 °C for 48 h.

3.3.2. Effect of incubation time on the growth of the isolate

Fig. 4 shows the impact of incubation time on the development of the hexavalent chromium-reducing strain BUK_BCH_BTE 5 cultivated on TSB. This bacterium's growth grew linearly between 24 and 48 hours, peaking at 48 hours, and then declined significantly (p 0.05) between 72 and 120 hours.

3.3.3. Impact of initial pH on the reduction of hexavalent chromium

Fig. 5 shows the impact of various initial pH levels on strain BUK_BCH_BTE 5 cultivated on TSB's ability to reduce hexavalent chromium. After 48 hours of incubation, it was found that pH 6.5 produced the highest level of chromium reduction. A significant decrease ($p < 0.05$) in the percentage reduction was observed at pH above the optimum. pH of the environment has a significant impact on the ionic state of the chromium-reducing enzyme, pH is considered to be an important factor in the Cr (VI) remediation process. As seen in Fig. 4, the isolate was cultured for 48 hours at pH values ranging from 5.5 to 8.0 to ascertain the effect of starting pH on Cr (VI) bio-reduction. The most efficient pH levels for reducing Cr (VI) are 6.5, where 55.39% of Cr(VI) were reduced, and 7.0, where 44.90% were removed. According to study by Ref. [51] pH 5.0 is where the least amount of Cr (VI) reduction occurs, demonstrating that pH is more closely related to reduction than to cell development [10]. While authors [7,28] both claimed that their maximal decrease occurred at an alkaline pH of 9.0, the findings of this investigation are different from those of those studies. The ability to remove chromium under adverse circumstances is severely impacted by pH because it affects the activity of the enzyme reductase, the binding site for metals on the cell's surface, and the shuttle rate of metal ions. The ideal pH was, however, claimed to be 7.0. According to Ref. [52], increasing pH causes a decrease in Cr (VI) elimination effectiveness. Similar to the findings by Ref. [31], it was observed that the biosorption of Cr decreased from 13% to 7% when the pH was raised from 1.0 to 6.0 due to the negatively charged oxyanion of Cr (VI) repelling the negatively charged functional groups present on the microbial cell surface. The difference in reduction efficiency brought on by pH variation is due to the release of chromate reductase, which takes place between pH 6.5 and 9.0 [53]. Similarly, at pH 6.0, there was a noticeable reduction of Cr (VI) [26]. Enzymes facilitate the reduction of Cr (VI), and changes in pH have an impact on the structure of proteins, the rate of enzyme ionization, and ultimately the activity of the enzyme. Because different cultures require different optimal pH values to achieve the greatest Cr (VI) reduction during chromate bioremediation, pH adjustment is likely required [54].

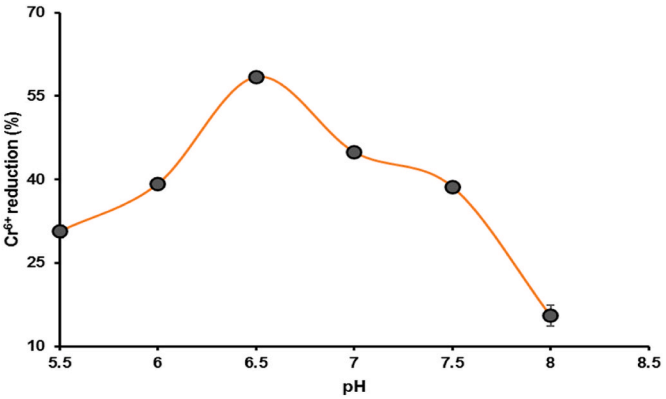

Fig. 5. Effect of initial pH on the hexavalent chromium reduction by strain BUK_BCH_BTE 5 grown in TSB at 37 °C for 48 h.

3.3.4. Effect of inoculum size on hexavalent chromium reduction

The effect of various inoculum sizes on hexavalent chromium reduction by strain BUK_BCH_BTE 5 on TSB was presented in Fig. 6. It was observed that the Cr(VI) reduction increased significantly ($p < 0.05$) as the amount of inoculum increased, reaching optimum at 2% (2 mL) inoculum following 48 h cultivation at 37 °C. A significant decrease ($p < 0.05$) in the percentage reduction was observed at an inoculum size higher than 2%. To maximize effectiveness, it is crucial to take into account the inoculum's volume Cr (VI) reduction in a liquid medium [21]. The relationship between inoculum size and Cr (VI) reduction by this isolate, as shown in Fig. 5, suggested that Cr (VI) reduction was growth connected. While only 26.6% of the Cr (VI) was reduced at the maximum inoculum of 4 mL, the highest growth is shown at a moderate inoculum of 2 mL, where 57.14% of the Cr (VI) is reduced. At 3 mL inoculum, the reported maximum Cr (VI) decrease was attained. Greater adsorption sites and reductase activity were observed to result in greater Cr (VI) reduction with increased inoculum. According to Ref. [55], Cr (VI) reduction efficacy improved up to an optimal inoculum level before remaining constant.

3.3.5. Effect of temperature on hexavalent chromium reduction

The effect of temperature on hexavalent chromium reduction by

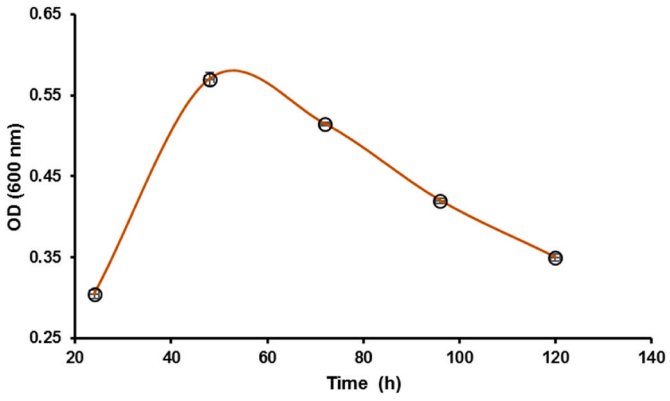

Fig. 4. Effect of incubation time on growth of hexavalent chromium-reducing strain BUK_BCH_BTE 5 grown in TSB at 37 °C.

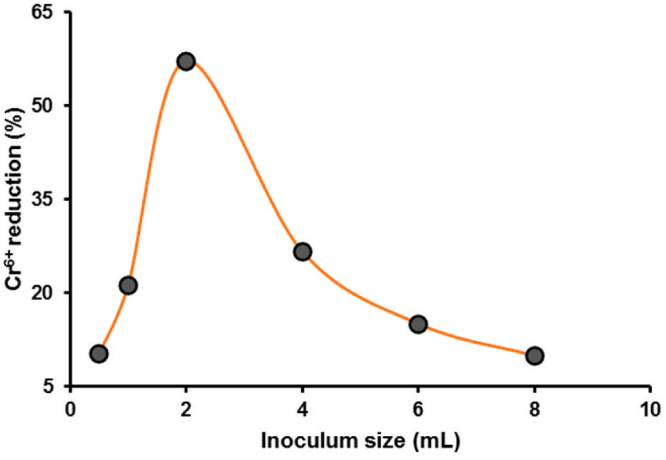

Fig. 6. Effect of various inoculum sizes on hexavalent chromium reduction by strain BUK_BCH_BTE 5 in TSB at 37 °C for 48 h.

strain BUK_BCH_BTE 5 grown in TSB was presented in Fig. 7. Hexavalent chromium reduction by this bacterium was optimum at 35 °C after 48 h incubation. A significant ($p < 0.05$) decrease in percentage reduction was observed at temperatures below and higher than 37 °C. The effect of temperature revealed that Cr (VI) reduction was linked to growth temperature for this isolate, an optimum temperature of 35 °C resulted in 70.96% of Cr (VI) being reduced, whereas, at 40 °C only 52.35% of Cr (VI) was reduced. This finding was in line with the study of [20] both of which reported an optimum Cr (IV) reduction at 35 °C. However, this deviated from the results of [9]. Generally, the optimum temperature for chromate reduction is high (around 30–40 °C), as microbes have high reducing efficiency to toxic metals at relatively high temperatures [30]. Mesophylls are more efficient in tolerating higher metal concentrations compared to thermophiles as they can make Cr (III) immobile and assemble it on the surface or inside the cell. Temperature rise brought on by the exothermic process enhances thermophile activity [10]. High temperatures can impede the growth of some bacterial strains, which can impede the bio-reduction of Cr (VI). Cr (VI) growth and reduction are negatively impacted by high temperatures. due to the fact that it stops the cell's physiological activities or makes it less viable. Extreme temperatures harm DNA and the structure of membranes in addition to denaturing proteins [56]. The fluid that makes up the cell membrane thickens at low temperatures, which causes nutrients to enter the cell very slowly. In extreme temperature conditions, the membrane's structure may even change, which would deactivate the reductase or affect the mechanism of protein synthesis [25].

3.3.6. *Influence of chromium content on the prevention and reduction of bacterial growth*

The effect of various Cr (VI) concentrations on chromium reduction by strain BUK_BCH_BTE 5 in TSB was presented in Fig. 8. Chromium reduction by this isolate was optimum at 200 mg/L following 48 h cultivation at 37 °C. As the substrate level was increased above 400 mg/L, a significant decrease ($p < 0.05$) in the percentage reduction was seen. The effect of concentrations on Cr (VI) bio-reduction by this isolate revealed that the best Cr (VI) concentration of 200 mg/L was required to achieve 65.76% reduction of the pollutant. Though, this isolate could withstand up to 1000 mg/L. These results were in line with the work done by Ref. [57], however, contradicts the works of [8,40] who reported higher optimum value. Similarly, a value lower than 200 ppm was reported [4,35]. Generally, bacterial Cr (VI) removal increases with increased cell density and decreases with increased concentration, thus, concentration decide the bio-removal efficiency of the bacteria. A higher concentration of Cr (VI) in a solution is removed by biosorption and a lower concentration is mostly bio-reduced [58]. However, as the concentration of heavy metal rises, reductase expression rises as well, leading to more reduction by the bacteria. Research has shown the bio-removal of Cr (VI) at lower concentrations [59]. According to one study, Bacillus can remove Cr (VI) from the environment to a maximum of 25 mg/L, whereas Aspergillus flavus CR500 removal percentages drop as Cr (VI) concentrations rise due to decreased biomass [60]. High Cr (VI) concentration weakens the strength of the functional group, alters the accumulation ability, and blocks the active sites responsible for the adsorption of Cr (VI) on the cell surface [61].

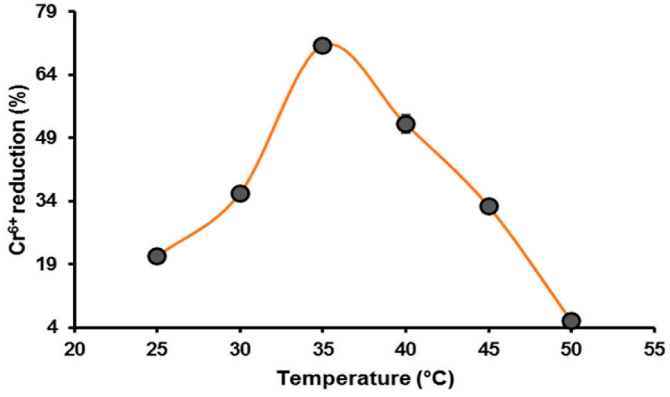

Fig. 7. Effect of temperature on hexavalent chromium reduction by strain BUK_BCH_BTE 5 grown in TSB after 48 h of incubation.

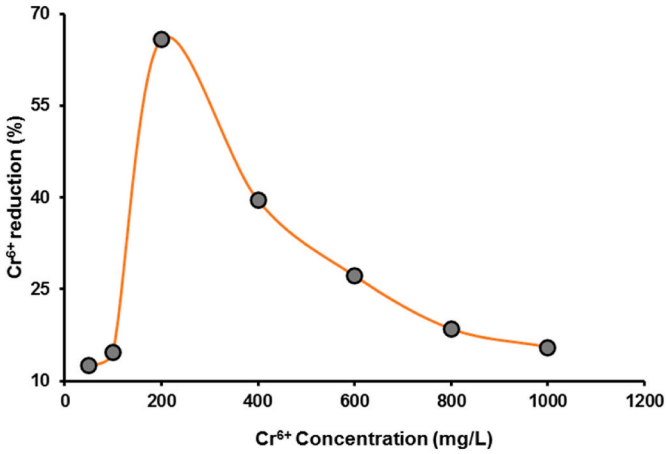

Fig. 8. Effect of chromium concentrations on Cr(VI) reduction by strain BUK_BCH_BTE 5 grown in TSB at 37 °C for 48 h.

3.3.7. *Effect of interacting heavy metals on chromium reduction*

The effect of various interacting heavy metals on hexavalent chromium reduction by strain BUK_BCH_BTE 5 was presented in Fig. 9. The optimum concentration of the heavy metals tolerated was; 1 ppm for As, Cd, and Cu, 2 ppm for Fe and Zn, 4 ppm for Hg, 6 ppm for Pb, and 8 ppm for Ni. The percentage Cr(VI) reduction in the presence of these metals was in the order Ni > Pb > control > Hg > Zn > Fe > Cd > As > Cu. Effluents containing Cr (VI) as a result of industrial processes often consist of other heavy metals, for this reason, the ability of the isolate to withstand various heavy metals was considered. The ability of microorganisms to reduce Cr (VI) and show resistance to other metallic ions is another asset for their use in bioremediation [62]. In the presence of Ni and Pb, 73.49% and 71.63% of Cr (VI) were respectively reduced, compared to Cr (VI) alone with 68.37% reduction, indicating that Ni and Pb may be promoters of Cr (VI) reduction. Ni as a promoter was also reported by Ref. [63]. In the presence of Zn, Cd, Hg, As and Fe the rate of reduction gradually decreased, Cu was found to inhibit reduction the most in which only 47.76% of Cr (VI) was reduced. This result was similar to the report of [64], suggesting their toxic and inhibitory effect.

3.3.8. *Effect of optimized conditions on bio-reduction*

The effect of optimized and un-optimized conditions on the growth and reduction of hexavalent chromium by strain BUK_BCH_BTE 5 in TSB

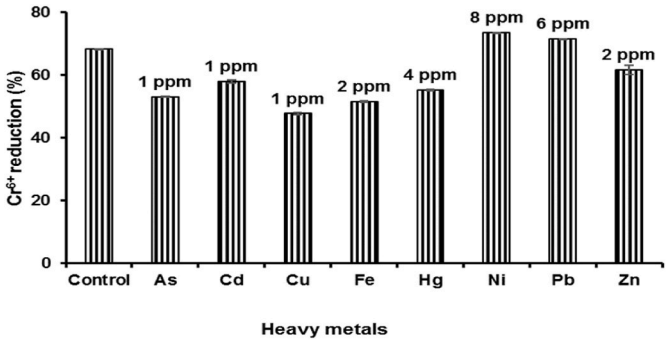

Fig. 9. Effect of various heavy metals with their optimum concentrations on the Cr(VI) reduction by strain BUK_BCH_BTE 5 in TSB at 37 °C for 48 h.

was presented in Fig. 10. Both turbidity and reduction of Cr (VI) by this strain were favored by the optimized conditions when compared with the un-optimized conditions.

3.4. *In vitro toxicity assay of Cr(VI) bioreduced product on Anopheles larva*

The toxic effect of supernatant from Cr (VI) bioreduced cultures was assessed by exposing Anopheles larva to supernatant from cultures with varying concentrations (0, 0.02, 0.2, and 20 ppm) of Cr (VI) and incubated at 0, 24, and 48 h. It was seen that as Cr (VI) level increases from 0 to 20, the percentage of mortality increases significantly ($p < 0.05$), except for concentrations between 2 and 20 which show no statistical difference ($p > 0.05$). Similarly, as the bio-removal time increases, the percentage mortality of the product (supernatant) decreases [65] i.e supernatant from the culture medium incubated for 48 h was least toxic than that incubated for 24 h and control (non-remediated). The order of toxicity was 48 h incubated<24 h incubated < control (Fig. 11). In trying to test for the toxicity of the bioreduced (bioremediated) samples relative to the non-remediated ones in vitro, an insect model was used to avoid issues due to animal ethics. The percentage mortality of Anopheles larva as influenced by different concentrations of $K_2Cr_2O_7$ were presented. The result obtained showed that there is a relationship between $K_2Cr_2O_7$ concentration and the percentage of mortality. That is, as $K_2Cr_2O_7$ concentration increases, larval per cent mortality increased. Similarly, the supernatant of both 24 and 48 h of the inoculated samples (remediated samples) showed a decreased mortality rate compared to the control (non-remediated) and thereby proving reduced toxicity of the remediated samples. Organisms striving in a metal-polluted environment are at great risk because the fitness and species diversity of such organisms is greatly reduced [66]. Insects living in the aquatic environment (aquatic insects) are abundant and are of great diversity, Due to the accumulation of metal in their systems from prolonged exposure to metal contamination, these insects frequently serve as indicators of environmental pollution and for bioassays of contaminants [33]. Additionally, because they were exposed to these heavy metals from their crucial developmental stage on, these insects are sensitive bioreporters of heavy metal pollution. Numerous studies have been conducted in the lab and in the natural environment to determine how heavy metals affect aquatic insects [67]. Heavy metal exposure to these insects causes alterations in fertility and fecundity as well as an increase in mortality rates. The percentage mortality of the larvae was shown to be concentration-dependent, meaning that as the concentration of the heavy metal increases, so does the percentage mortality of the larvae. High mortality rates as a result of heavy metal exposure were not surprising as the same results have been reported in other dipterans [68]. Generally, the detrimental effects are in direct proportion to the concentration of the metal.

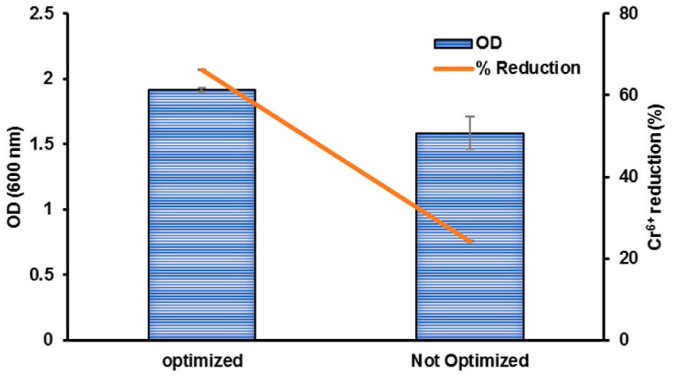

Fig. 10. Effect of optimized and un-optimized conditions on growth and reduction of Cr(VI) by strain BUK_BCH_BTE5 grown in TSB for 48 h incubation.

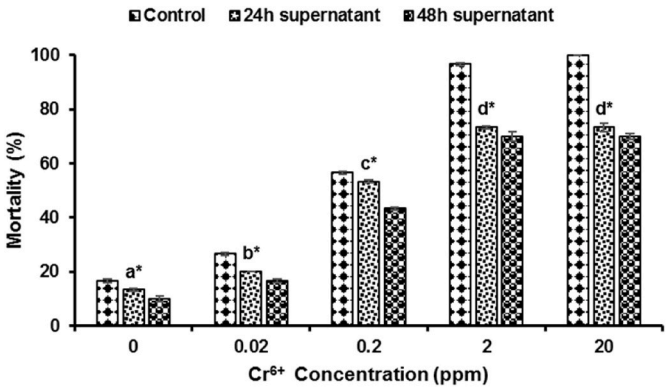

Fig. 11. Toxicity assay of the supernatant from cultured (inoculated) samples containing various chromium concentrations on Anopheles larvae at 24 h and 48 h. Groups with different letters are significantly different and (*) indicates intragroup differences ($p < 0.05$).

3.5. *Assessment of the strain BUK-BCH-BTE5 and its capacity to remove Cr (VI)*

Assessment of Cr(VI) bio-removal potential of the isolate, inoculated and non-inoculated Cr(VI) containing culture media were subjected to Atomic Absorption Spectrophotometry (AAS), following 48 h incubation at 37 °C [69]. From the result (Fig. 12) it was found about 18.32% of the Cr (VI) was taken away from the cultured sample within 48 h of incubation.

4. Conclusion

- Land pollution is common in urban areas as a result of industrial and municipal wastes which are the major pollutants that pose threat to humans, aquatic animals, plants and other organisms and the highest quantities are generated by tannery industries where hides and skin are converted into leather.
- This study therefore, aims at identifying a bacterium that can reduce Chromium (IV) to (III) using a pour plate technique on Luria Bertani medium amended with $K_2Cr_2O_7$.
- Using this media *Acinetobacter* sp. named as BUK_BCH_BTE 5 was identified from tannery effluent in the Chalawa industrial area of Kano state.
- The best conditions for the chromium reduction by the isolate were found to be pH of 6.5, temperature of 35 °C, and an inoculum concentration of 2%,.

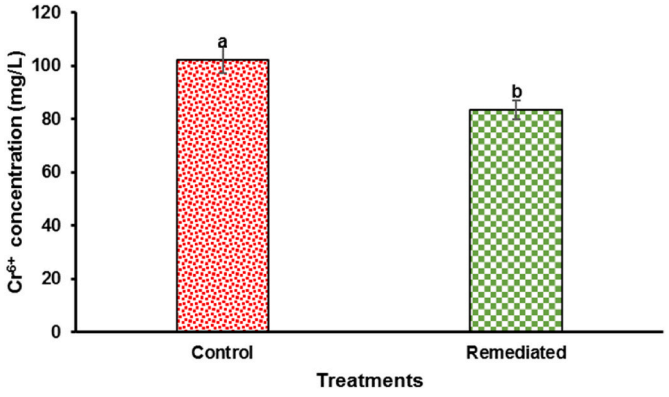

Fig. 12. Chromium concentration in inoculated (remediated) and uninoculated (control) samples incubated at 37 °C for 48 h. Different letters over the bar indicate a significant difference ($p < 0.05$).

- *Acinetobacter* sp. shows greater ability to reduce chromium up to 66.32% was reduced within 48 h.
- Wastewater treatment before their release in to the environment is a matter of serious concern. Additionally, due to the harmful effects of ternary effluent on aquatic life, plants and animals as confirmed in the toxicity test lots of pressure should be mounted on the industries along the axis to be treating their waste before been release in to the environment.
- Thus, making this isolate a candidate of choice for future chromium bioremediation. The less toxicity of the bacterial-treated supernatant suggests that it could safely be released into the environment.

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