

Biological detoxification of Cr(VI) using wood-husk immobilized *Acinetobacter haemolyticus*

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Abstract

Acinetobacter haemolyticus, a Gram-negative aerobic locally isolated bacterium, immobilized on wood-husk showed the ability to detoxify Cr(VI) to Cr(III). Wood-husk, a natural cellulose-based support material, packed in an upward-flow column was used as support material for bacterial attachment. Around 97% of the Cr(VI) in wastewater containing 15 mg L⁻¹ of Cr(VI) was reduced at a flow rate of 8.0 mL min⁻¹. The wastewater containing Cr(VI) was added with liquid pineapple wastewater as nutrient source for the bacteria. Electron microscopic examinations of the wood-husk after 42 days of column operation showed gradual colonization of the wood-husk by bacterial biofilm. The use of 0.1% (v/v) formaldehyde as a disinfecting agent inhibited growth of bacteria present in the final wastewater discharge. This finding is important in view of the ethical code regarding possible introduction of exogenous bacterial species into the environment.

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1. Introduction

Chemical reduction followed by precipitation is the most common technique used in industry for the removal of Cr(VI) [1]. However, this technique has its own serious disadvantages such as the possibility of chemical spillage and the high cost of treatment chemicals, while the large generation of sludge leads to disposal problems. This prompts the need to look into safer and cheaper alternatives to carry out the Cr(VI) reduction process such as biological processes. Numerous reports have demonstrated the feasibility of using bacterial processes for the treatment of Cr(VI)-containing industrial wastewaters by use of either a pure culture or a bacterial consortium [2–5]. Bacteria of various genera have been used including *Achromobacter*, *Aeromonas*, *Agrobacterium*, *Bacillus*, *Desulfovibrio*, *Enterobacter* and *Pseudomonas* [6]. These bacteria showed different Cr(VI) reducing capacity depending on factors such as availability of organic compounds as electron donor, dissolved oxygen, Cr(VI) concentration, pH, redox potential, temperature, presence of other electron acceptors and inhibition effects by

metallic or phenolic compounds [6,7]. In this study, wood-husk was chosen as the column support material for bacterial attachment because it is a natural source for cellulose that is known for its bacterial attachment property, inexpensive and stable. Another point to note is that the glucose-containing pineapple waste [8] is readily consumed by *Acinetobacter haemolyticus*, and so is an effective substitute for expensive growth medium such as nutrient broth (NB). One example of a bioremediation process that was short lived due to its requirement for a high cost nutrient was the bacterial-based metal removal system developed by Advanced Mineral Technologies, Inc., in Colorado that used *Bacillus* sp. as the biosorbent [9].

The objective of this study is to determine the ability of a Cr(VI)-resistant bacterium, *A. haemolyticus*, to reduce Cr(VI) from raw electroplating wastewater in a bench-scale laboratory Cr(VI) column reduction system.

2. Materials and methods

2.1. Bacteria

In this work, *A. haemolyticus* was isolated from the Cr(VI)-containing wastewater from a batek (textile-related)

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manufacturing premise in Kota Bharu, Kelantan, Malaysia. *A. haemolyticus* was cultivated in NB (8 g L^{-1} , Merck) at 200 rpm and 30°C (Certomat, B. Braun). It was identified via the 16S rRNA gene sequencing analysis carried out by First BASE Laboratories Sdn. Bhd., Malaysia, where a 99.5% similarity with *A. haemolyticus* (AY586400 and X81662) was obtained from the nucleotide sequence of 597 bp. The nucleotide sequence was deposited to GenBank where it was given the accession number EF369508.

2.2. Electroplating wastewater

The electroplating wastewater was obtained from the rinse-bath tank of a local electroplating company in Masai, Johor. The wastewater was characterised by pH, microbiological count, sulphate concentration, heavy metals and Cr(VI) concentration.

2.3. Liquid pineapple wastewater

In this study, liquid pineapple wastewater was used as the energy source for bacteria. It was obtained from a pineapple-processing premise in Tampoi, Johor. The following parameters were determined: pH, microbiological count, heavy metals, together with sulphate, Cr(VI), glucose, fructose and sucrose concentrations.

2.4. Laboratory scale bioreactor

A glass column with inner diameter (i.d.) 8.0 cm, outer diameter (o.d.) 8.70 cm and height 100 cm was used. Inlet and outlet points were set at 2 cm from the bottom and top of column, respectively. Teflon tubing with i.d. of 2.0 mm and o.d. of 4.0 mm was fitted to the inlet and outlet points, respectively. The teflon tubes were sterilized by soaking in 100% ethanol before use. Inert stones were packed to 75 cm^3 at the bottom of the column to ensure good flow distribution inside the column and to retain the column content. Following this, wood-husk was packed into the column to a volume of 825 cm^3 . This volume is considered as the working volume of the column. Inert stone was then

packed on top of the working volume for 50 cm^3 . A headspace of around 30 cm^3 was allowed in the column. The total volume of the column is therefore 1000 cm^3 .

2.5. Immobilization of *A. haemolyticus* onto wood-husk

A modified procedure from Von Canstein et al. [10] was used during the immobilization of *A. haemolyticus* cells onto wood-husk packed in the column. The wood-husk was obtained from a local sawmill factory. It is brown and cut to irregular lengths of between 0.35 and 0.21 cm. Using a peristaltic pump (Eyela MP-1000 D), the column was first rinsed with deionised water to prevent clogging by large particulate substances on the support material and to allow the wood-husk surface material to acquire necessary charge for bacterial attachment [9]. Then, 1 L of the *A. haemolyticus* culture (grown for 24 h in NB) was pumped using the same flow rate as a rinsing step. The wastewater collected was recycled back into the column and continuously pumped for 6 h to allow bacterial attachment. A mixture of 20% (v/v) NB in 1 L pineapple waste (final pH of mixture 7.00) was pumped into the column using the same flow rate as *A. haemolyticus* cells for 24 h to ensure initial formation of biofilm by the attached bacteria.

2.6. Cr(VI) reduction system

The experimental setup for the Cr(VI) reduction system consists of a holding tank, peristaltic pump, column (bioreactor), precipitation tank and disinfection tank and is shown in Fig. 1.

Solution from the holding tank consisting of 15 mg L^{-1} Cr(VI) from electroplating and pineapple wastewaters was first adjusted to pH 7.0 using 15% (v/v) NaOH solution. The solution was then pumped into the *A. haemolyticus*-immobilized column at 3.0 mL min^{-1} until the Cr(VI) concentration in the effluent fraction was more than 0.5 mg L^{-1} . At this point, a decrease in the population of the Cr(VI)-reducing *A. haemolyticus* was expected due to Cr(VI) toxicity. The column was then regenerated using NB followed by pineapple waste. Then, 200 mL of fresh *A. haemolyticus* culture grown for 24 h in NB was

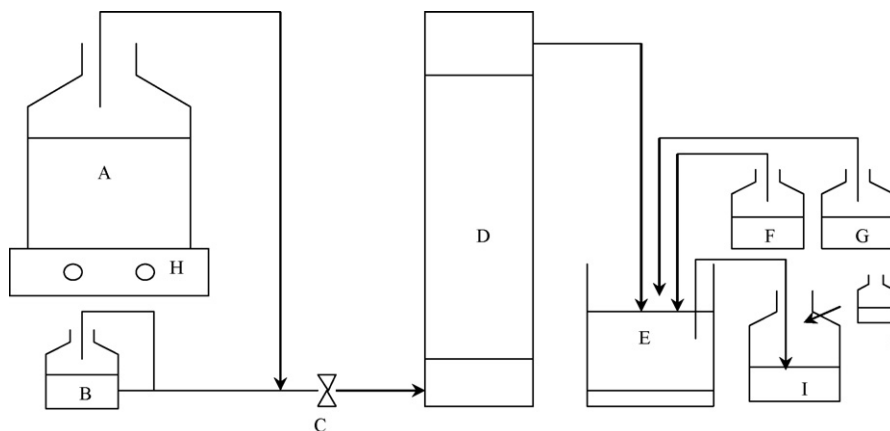


Fig. 1. Schematic representation of the experimental setup for Cr(VI) reduction system: A, holding tank; B, culture of *A. haemolyticus*; C, peristaltic pump; D, column (bioreactor); E, precipitation tank; F, NaOH solution; G, coagulant solution; H, magnetic stirrer; I, disinfection tank; J, disinfectant.

introduced before the column was left idle for 3 days to allow biofilm formation [12]. Then, Cr(VI) reduction was resumed for 30 days when the influent flow rate was varied between 3.0 and 8.0 mL min⁻¹. The following parameters were periodically measured: Cr(VI) and total Cr concentrations, heavy metals, pH, microbiological count and dissolved oxygen (DO).

2.7. Electron microscopy analysis on the development of biofilm on the wood-husk in column

The field emission scanning electron microscope (FESEM) and the scanning electron microscope (SEM) were used to determine the development of biofilm on wood-husk in the column at the end of column operation, *i.e.* 42 days. Sample preparation is as follows: cellulose cubes was first cut into small pieces using a flame-sterilized knife before being immersed in 2.5% glutaraldehyde for 1–2 h. It was then washed with deionised water before immersed in 2% osmium tetroxide (OsO₄) in 0.1 M phosphate buffer saline (PBS) was carried out for about 1 h and the sample was washed with deionised water. The small cellulose pieces were then dehydrated using increasing concentrations of absolute ethanol before drying at 70–80 °C overnight in an oven. The dried cellulose pieces were then mounted on a sample holder before viewing under the electron microscope.

2.8. Neutralization/precipitation of treated electroplating wastewater

Treated wastewater containing Cr(III) from the column was collected in a 2 L Schott Duran bottle. A modified procedure from Von Canstein et al. [12] was used where the Cr(III) was precipitated using 15% (v/v) NaOH until a pH of 7.00 ± 0.10 was achieved [1]. Coagulant A, a commercial coagulant obtained from S.J. Coating Sdn. Bhd., was added at 0.75% (v/v) during the precipitation process to accelerate the Cr(III) precipitation process. Supernatant obtained was pooled into a 15 L plastic container while the sludge formed was discarded. At regular time intervals, the bulk supernatant was analyzed for heavy metals and bacterial count. Both solid and liquid wastes generated in this study were sent for disposal *via* Unit Pengawasan dan Keselamatan Makmal, Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM) Skudai.

2.9. Disinfection of bacteria from the bulk supernatant prior to disposal

The experiment was carried out to ensure that no bacteria would be present in the bulk supernatant prior to disposal as a safety measure. Modified procedure as used in the S.J. Coating Sdn. Bhd. (S.J. Coating Sdn. Bhd., personal communication) was employed where the treated Cr(VI) wastewater was disinfected through 20 min contact with UV followed by 45 min contact with H₂O₂.

In this study, the bulk supernatant was disinfected using 20% (v/v) ethanol (99.9%, v/v), 10% (v/v) hydrogen peroxide (35%, v/v) and formaldehyde (40%, w/v) in a series of 100 mL Schott Duran bottles before shaking in an orbital shaker at 100 rpm

for 3 h at room temperature (25 °C). At the end of experiment, the mixture was determined for bacterial count using the serial dilution technique. The disinfecting agent showing the highest ability to decrease the bacterial content of the wastewater was further optimized for concentration between 0.1 and 10% (v/v).

2.10. Analytical method

The Cr(VI) concentration was determined colorimetrically at 540 nm using the diphenylcarbazide (DPC) method with a detection limit of 5 µg L⁻¹ [13]. The method is as follows: in a 10 mL volumetric flask, 1 mL of sample was mixed with 9 mL of 0.2 M H₂SO₄. Then, 0.2 mL of freshly prepared 0.25% (w/v) DPC in acetone was added to the volumetric flask. The mixture was then vortexed (Maxi Mix-II Thermolyne) for about 15–30 s and let to stand between 10 and 15 min for full colour development. The red-violet to purple colour formed was then measured at OD₅₄₀ using distilled water as reference. The instrument used was calibrated using 0.4–2.0 mg L⁻¹ Cr(VI) prepared from Cr(VI) stock solution (1000 mg L⁻¹).

pH was determined using a pH meter (WTW, Germany) and microbiological count *via* the spread-plate technique. Sulphate concentration was determined against a sulphate standard solution, 70 mg L⁻¹ (DR400 Spectrophotometer, HACH) while heavy metals were determined using the inductively coupled plasma mass spectrometer (ICP-MS, Toshiba UP6100). Total Cr concentration was determined using atomic absorption spectrophotometry (AAS, Perkin-Elmer AAnalyst 400), and dissolved oxygen using a DO meter (Cimanec-2 Thermolyne). ICP-MS analysis was carried out at the Chemical Engineering Pilot Plant, UTM. Biofilm development were monitored using an FESEM instrument (Hitachi S-4500 FESEM) at Material Science Laboratory, UTM while the pure culture of *A. haemolyticus* was analyzed using SEM (SEM, Philips XL-40, Netherlands) at the Electron Microscopy Unit, Institute for Medical Research, Kuala Lumpur. Glucose, fructose and sucrose concentrations were determined using high performance liquid chromatography, HPLC (Waters 410) with a Refractive Index detector and Autosampler (Waters 717 Plus, Waters Associates Milford, USA).

3. Results

3.1. Characteristics of electroplating wastewater

Results from the characterization of the wastewater are as follows: colour, yellow to dark orange; turbidity, clear; pH, between 2.30 and 2.70 ± 0.05; temperature, 30.7 ± 3.39 °C; sulphate, 4.45 ± 3.32 mg L⁻¹. Microbiological count did not yield any colonies. Profile for the heavy metals content of the raw electroplating wastewater is shown in Table 1.

3.2. Characteristics of liquid pineapple wastewater

The liquid pineapple wastewater has the following characteristics: colour, yellowish green; turbidity, slightly turbid; pH, 3.19–4.17 ± 0.28; temperature, 33.9 ± 1.61 °C; sulphate,

Table 1
Profile of metal concentration in pre- and post-treated electroplating wastewater

Element	Pre-treated electroplating wastewater	Post-treated electroplating wastewater (mg L ⁻¹)	Standard B (mg L ⁻¹)
Pb	1.97 ± 0.43	1.34 ± 0.22	0.50
As	0.29 ± 0.13	0.29 ± 0.07	0.10
Hg	0.02 ± 0.01	0.03 ± 0.02	0.05
Cu	0.63 ± 0.11	0.66 ± 0.07	1.00
Fe	1.49 ± 0.18	1.48 ± 0.07	5.00
Ni	4.76 ± 1.67	4.62 ± 0.72	1.00
Cd	0.08 ± 0.01	0.06 ± 0.02	0.02
Cr	28.77 ± 2.38	0.18 ± 0.49	0.05

Values shown are means of triplicate sample; Standard B, permissible discharge limit for industrial wastewater outside the catchment area in Malaysia [14].

58.2 ± 1.41 mg L⁻¹; microbiological count, 4 colonies isolated. From the ICP-MS analysis (Table 2), concentrations of Pb, As, Hg, Cu and Cr exceeds the permitted level. Sugar content was as follows: glucose 4.95 g L⁻¹ and fructose 4.49 g L⁻¹. Sucrose content was relatively low in comparison with the glucose and fructose, and hence was not readily detected.

3.3. Optimizing reduction of Cr(VI)

During phase I, initial influent Cr(VI) concentration was 8.37 mg L⁻¹ with Cr(VI) reduction around 55.33%, determined using the DPC method. Lowering of influent Cr(VI) concentration did not result in increased Cr(VI) reduction. More than 95% of Cr(VI) reduction was achieved after 3 days of column operation. However, the inability to achieve effluent Cr(VI) concentration below the discharge limit (Std. B, 0.05 mg L⁻¹) prompted the column operation to be stopped to allow a regeneration step (phase II). After regeneration, the effect of influent flow rate and column retention time on Cr(VI) reduction was investigated, *i.e.* phase III. Cr(VI) reduction was not affected up to influent flow rate of 8.0 mL min⁻¹ at Cr(VI) concentration of 7.37–8.44 mg L⁻¹ when more than 99% Cr(VI) was reduced. Variation of influent flow rate (3.0–8.0 mL min⁻¹) corresponds to R_t of 1.72–4.58 h (volume of column, 825 cm³). During phase IV, a flow rate of 8.0 mL min⁻¹ and Cr(VI) concentration less than 15 mg L⁻¹ was used throughout the study. Around 93.50–96.93% of influent Cr(VI) was converted to Cr(III). AAS analysis of the influent and effluent fractions shows that a high

Table 2
Profile of metal concentration in liquid pineapple wastewater

Element	Pineapple waste (mg L ⁻¹)	Standard B (mg L ⁻¹)
Pb	1.24 ± 0.18	0.50
As	4.04 ± 1.41	0.10
Hg	0.11 ± 0.00	0.05
Cu	9.06 ± 10.3	1.00
Fe	22.21 ± 2.62	5.00
Ni	0.63 ± 0.25	1.00
Cd	0.05 ± 0.00	0.02
Cr	1.28 ± 0.08	0.05

Values shown are means of triplicate sample, Standard B, permissible discharge limit for industrial wastewater outside the catchment area in Malaysia [14].

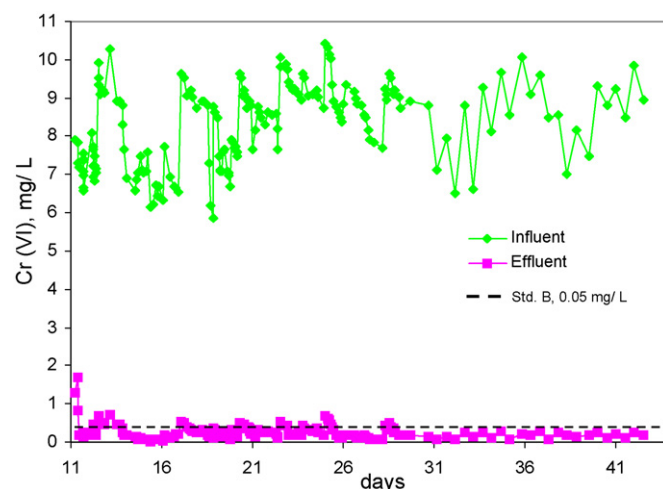


Fig. 2. Profile for bioremediation of Cr(VI) from electroplating wastewater by wood-husk immobilized *A. haemolyticus* in a column system.

percentage of the total Cr was recovered in the effluent portion with average value of 83.77 ± 5.48% (Fig. 2).

3.4. Electron microscopy on the development of biofilm on wood-husk

Fig. 3 shows the development of bacterial biofilm on wood-husk in the column. On day 0, the surface of the wood-husk support material appeared void of bacteria (Fig. 3C). However, after 30 days of column operation, biofilm-containing microorganisms in various shapes and sizes had grown massively and filled the substratum cavity (Fig. 3A and B). At this stage, the biofilm has developed into a stable microbial unit. It is advantageous to have a uniform colonization of the column with the bacteria to minimize clogging from the effect of dead-zones and to have a uniform flow over the entire cross-sectional area of the column [15].

In this study, the type of cells present in the biofilm was not identified. This is because the use of simple isolation method such as nutrient enrichment to isolate bacterial species from biofilm might not be useful due to the subjective colony morphology-based isolation procedure and the possibility of uncultivable bacteria being present in the biofilm [15,16]. Wagner-Dobler et al. [17] suggested the use of thermogradiant gel electrophoresis (TGGE) technique combined with 16S rDNA sequencing to identify invading microorganisms presence in biofilm of *P. putida*. However, the use of such technique or approach was not within the scope of this study, and was not pursued.

Possible sources for biofilm contamination in the column was investigated *via* culture enrichment using NB on individual components present in the column influent. Results are summarized in Table 3.

No Cr(VI) resistant bacteria could be isolated either from pre-treated electroplating wastewater (PTEW) or pH 7.00-adjusted PTEW. This was due to the high Cr(VI) concentration of 28.77 ± 2.38 mg L⁻¹ (Table 1). Possible contamination of the electroplating wastewater by air-borne bacteria was discounted

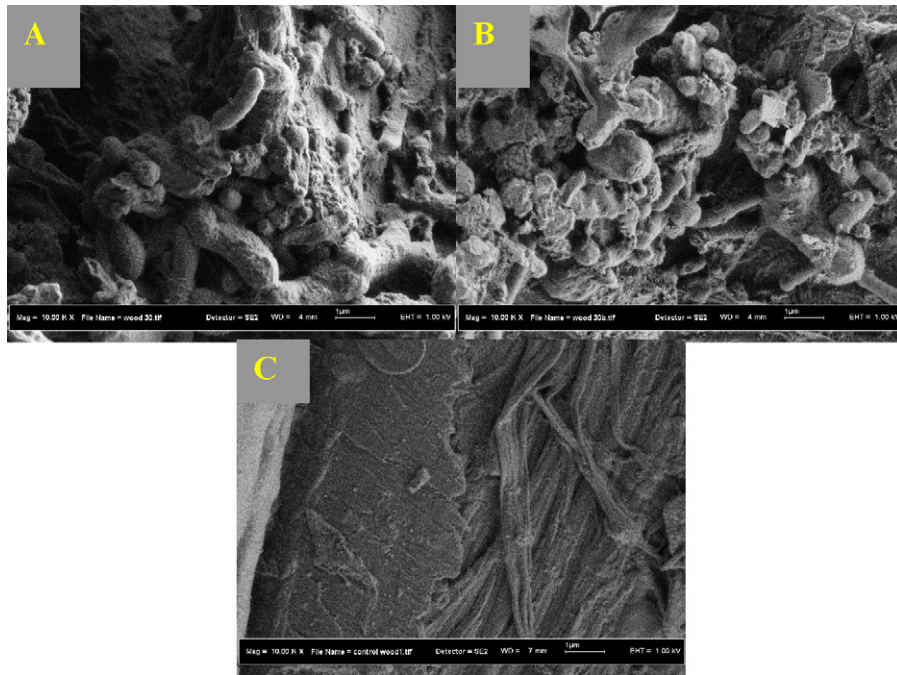


Fig. 3. Development of biofilm on wood-husk support material: (A and B) microorganisms in various shapes and sizes on wood-husk after 30 days of column operation compared to at day 0 (C).

from unsuccessful isolation of bacterial colony after exposing the electroplating waste to room condition overnight. Based on these findings, it can be stated that the electroplating wastewater (at pH 7.00) in the influent did not contribute to column colonization by exogenous bacteria. Three bacterial colonies were isolated from the pH 3.00–3.50 raw pineapple waste. When pre-treated liquid pineapple wastewater (PTLPW) was mixed with PTEW in sterile deionised water and the pH adjusted to 7.00, only two colonies were isolated. Since, no bacteria were isolated from either the electroplating wastewater or the sterilized deionised water, the two colonies isolated should originate from the PTLPW. The role of bacteria from the tap water (unfiltered and unsterilized) in column colonization may also be substantial

as three distinct colonies were isolated at 10^6 CFU mL⁻¹. However, upon subculturing into NB medium with added 10, 50 and 100 mg L⁻¹ Cr(VI), none of the colonies isolated from the tap water survived after 24 h of incubation at 30 °C. The two bacterial colonies isolated from PTLPW showed good survival ability with bacterial counts of $18 \pm 0.3 \times 10^6$ CFU mL⁻¹ at 10 mg L⁻¹ Cr(VI), $30 \pm 1.2 \times 10^6$ CFU mL⁻¹ at 50 mg L⁻¹ Cr(VI) and $25 \pm 0.8 \times 10^5$ CFU mL⁻¹ at 100 mg L⁻¹ Cr(VI). These results indicate the substantial role of bacteria from PTLPW in column colonization.

3.5. Neutralization/precipitation of treated electroplating wastewater

Two distinct layers of hydroxide sludge and Cr-deficient wastewater were obtained after 30 min of contact with 15% (v/v) NaOH and 0.75% (v/v) commercial coagulant.

3.6. Disinfection of Cr-deficient wastewater from the neutralization/precipitation process

In view of the possible detachment of bacteria from the biofilm, disinfection of wastewater was carried out as a preventive measure for bacterial contamination of the final wastewater. Both H₂O₂ and HCHO (10%, v/v) were found to be good inhibitors for bacterial growth based on the absence of bacterial colonies formed on NA plates (Table 4). However, ethanol (20%, v/v) was not an effective disinfecting agent as significant bacterial population remained, *i.e.* $5 \pm 1.53 \times 10^3$ CFU mL⁻¹. No formation of bacterial colonies was observed on NA plates supplemented with up to 0.1% (v/v) HCHO.

Table 3
Isolation of culture from individual components of column influent

Individual component	pH	Results
Pre-treated electroplating wastewater (PTEW)	2.30–2.70 ± 0.41 ^a	No colony isolated
PTEW (pH adjusted)	7.00	No colony isolated
PTEW exposed to air overnight (pH adjusted)	7.00	No colony isolated
Pre-treated liquid pineapple wastewater (PTLPW)	3.00–3.50 ± 0.55 ^a	Three colonies isolated
PTLPW + PTEW + sterilized deionised water	7.00	Two colonies isolated at 10 ⁵
Raw tap water	7.24 ± 0.13 ^a	Three colonies isolated at 10 ⁵
PTLPW + PTEW + tap water	7.00	Three colonies isolated at 10 ⁶

^a Values are mean from five readings; culture enrichment in NB using serial dilution technique.

Table 4
Effect of disinfecting agent on bacterial population in Cr-deficient wastewater after neutralization

Disinfecting agent	Initial CFU (mL ⁻¹)	Final CFU (mL ⁻¹)	%Cell kill
10% (v/v) H ₂ O ₂		0	100
10% (v/v) HCHO		0	100
20% (v/v) ethanol	4 ± 0.58 × 10 ⁴	5 ± 1.53 × 10 ³	87.5
Control		3 ± 0.58 × 10 ⁴	–

Control, not treated with disinfecting agent; CFU mL⁻¹ based on number of colonies formed on nutrient agar plates (triplicates) after 24 h incubation at 30 °C.

4. Discussion

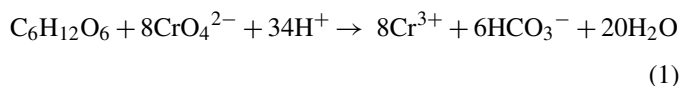
Parameters characterized for the electroplating wastewater were based on their potential influence on bacterial Cr(VI) reduction [18]. For example, low concentrations of sulphate ions may not compete with Cr(VI) uptake by *A. haemolyticus*. For the liquid pineapple wastewater, the presence of four bacterial colonies indicates possibility of competition to *A. haemolyticus* for nutrients and dissolved oxygen. The presence of heavy metals in the pineapple wastewater are assumed to originate from various sources such as processing steps involving metallic materials (canning and fruit-cutting), run-off water from cleaning of equipments, or from fruit-washing where trace concentrations of metal may be leached out from soil particles attached to pineapple parts such as root and leaves.

Wood-husk was chosen as the support material for *A. haemolyticus* in the column because it is a natural source for cellulose material. Cellulose contains the glucose group that has the easily substituted hydroxyl groups that provide a weakly basic and acidic ion exchange conditions that enhance bacterial attachment [19]. Cellulose is also porous, hence potentially high surface area is available for bacterial attachment. Both these features have been suggested by Macaskie and Dean [20] as prerequisite for bacterial attachment. Cellulose is also cheap and stable at different operating conditions such as temperature and pH [19].

Most studies on biological reduction of Cr(VI) have been conducted in batch reactors using pure cultures [18,21,22]. It is only recently that continuous-flow and fixed-film bioreactors were used for biological reduction of Cr(VI) [23–25]. This study consists of four main phases, *i.e.* optimization of influent Cr(VI) concentration, column regeneration, variation in influent flow rate and sustainability of Cr(VI) reduction in continuous operation. A regeneration step was necessary to counter the expected decrease of bacterial population in column from Cr(VI) toxicity during optimization of influent Cr(VI) concentration.

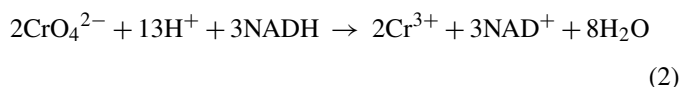
The ability of *A. haemolyticus* to use pineapple waste as carbon source has been reported [8]. *A. haemolyticus* showed good growth in 15% (v/v) pineapple waste, with glucose identified as the main sugar component utilized by the bacteria compared to sucrose and fructose. Around 80% of 2.97 g glucose L⁻¹ was consumed by *A. haemolyticus*. The effective role of glucose as carbon source in Cr(VI) reduction can be described

by Eq. (1) [26]:



From Eq. (1), complete breakdown of 1 mol glucose would yield sufficient energy to reduce 8 mol Cr(VI). For example, complete breakdown of 5 g L⁻¹ glucose (28 mM) is expected to yield sufficient energy to reduce 500 mg L⁻¹ Cr(VI) (192 mM). Based on the reaction stoichiometry between glucose and Cr(VI) of 1:8, breakdown of 28 mM glucose would yield enough energy to reduce approximately eight times the amount of glucose, *i.e.* 204 mM Cr(VI), which is higher than 192 mM Cr(VI) targeted to be reduced [23]. Raw glucose concentration was determined at 4.95 g L⁻¹. However, column influent mixture contains 15% (v/v) of pineapple that correspond to 0.74 g L⁻¹ or 4.16 mM of glucose. Influent Cr(VI) concentrations ranged from 10 to 15 mg L⁻¹ or 3.84 to 5.76 mM. From the molar ratio, complete breakdown of 4.16 mM glucose would produce enough energy to reduce 33.26 mM Cr(VI). Taking into consideration the range of influent Cr(VI) concentrations of 3.84–5.76 mM, it can be said that excess carbon source was supplied for the Cr(VI) reduction system throughout the study.

The most common pathway for the breakdown of hexose sugars such as glucose (catabolism to the final products, CO₂ and H₂O) is *via* the formation of pyruvate through glycolysis in citric acid cycle with at least two molecules of NADH released for each glucose molecule oxidized. Bacterial species such as *Bacillus* sp. may produce a soluble reductase that complexes Cr(VI) in an enzymatic reaction in which Cr(VI) receives electrons to form Cr(III). NADH is required in the reduction of Cr(VI) to achieve maximum activity as shown in Eq. (2) [7,27]:



$\Delta G^{\circ} = -87$ kJ electrons⁻¹ transferred (calculated from reported standard electrode potential, E°).

The energy required for the reduction of Cr(VI) is lower than the energy released in the complete oxidation of glucose (yielding CO₂ and H₂O), *i.e.* $\Delta G^{\circ} = -121$ kJ electrons⁻¹ transferred. The enzyme-mediated reduction of Cr(VI) involves overall the deprotonation of NADH and the release of two electrons per mole which are transferred to Cr(VI) [7].

Since the column operation was carried out under non-sterile conditions, *A. haemolyticus* biofilm formed may be subjected to colonization by ubiquitous Cr(VI) resistant bacteria. At this time, it can be stated that the invading microorganisms were beginning to have a role in the biofilm as evident from its good survival ability. The invading bacteria colonized the biofilm during the operation of the column that has been washed thoroughly with 90% ethanol prior to packing with wood-husk material. This could explain the high Cr(VI) reduction capacity of cells. During biofilm development, portions of biofilm peel away from the surface and are entrained in the fluid flow. Detachment is a process of continuous biofilm removal and is highly dependent on hydrodynamic conditions such as

flow rate and toxicity. The presence of toxic substances or the limitations on by-product diffusion from the biofilm further aggravate the situation and would lead to death or lysis of the bacteria [11].

Although the main objective of this study was establishing an alternative treatment method for Cr(VI) reduction, large volumes of the treated-electroplating wastewater generated prompted a neutralization and precipitation step to be carried out for disposal purposes where NaOH was used. Precipitated sludge obtained in this study was found to be soft and have the tendency to dissolve back into solution with gentle shaking. This was expected as floc or sludge from NaOH precipitation is known to be light, fluffy, *i.e.* compacted poorly and easily disturbed [1]. Since, it is not possible to remove Cr(VI) directly from solution *via* precipitation, all of the 97% of Cr removed from solution can be said to be that of Cr(III), *i.e.* the product of reduction of Cr(VI). The remaining 3% of Cr in final wastewater can then be recovered using ion exchange or activated carbon filter, two of the most widely used polishing unit installed at Cr(VI) wastewater treatment plant (S.J. Coating, personal communication). Normally, treatment using either one of these techniques would result in final wastewater containing Cr at concentration less than 0.05 mg L⁻¹, *i.e.* Standard B [14]. However, this step was not carried out in this study.

The use of ethanol at 20% (v/v) was not successful in inhibiting bacterial growth because of the low concentration used. Ethanol was used because of its ability to denature protein and disrupt lipid structure at cell membranes [28]. This could be due to possible utilization of ethanol as carbon source by bacteria especially for *A. haemolyticus* [29]. A point to note is that industrial application of ethanol as disinfecting agent for bacteria might be limited because of its high pricing. Therefore, ethanol was not studied further. The same consideration goes for the selection of HCHO over H₂O₂. Even though, both disinfecting agents showed good bacterial inhibitory capacity, the higher industrial cost of H₂O₂ prompted the selection of HCHO. The capacity of HCHO to be a good bacterial growth inhibitor was due to its enzyme-disrupting ability *via* oxidation of important functional groups on the enzyme [18].

5. Conclusion

The bioreactor packed with wood-husk for the attachment of *A. haemolyticus* effectively reduced Cr(VI) without the need to resupply fresh cells. Wood-husk looks promising as support material for biofilm development while minimizing operating cost due to its abundance. The use of indigenous microbes such as *A. haemolyticus*, which was isolated from a Cr(VI) containing wastewater, provides a certain advantage and ensures durability under various operating conditions. Reduction of Cr(VI) by *A. haemolyticus* was significantly influenced by influent Cr(VI) concentration. The ability of *A. haemolyticus* to utilize liquid pineapple wastewater as a nutrient is an excellent example of the substitution of a cheap and readily available industrial waste in place of expensive growth medium and could be a significant factor in the commercial use of a process such this. The high percentage conversion of Cr(VI) to Cr(III) by the system suggests

this may be an efficient and economical method for removing Cr(VI) from industrial wastewater.

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