Beni-Suef University Journal of Basic and Applied Sciences

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Bioreactor level optimization of chromium(VI) reduction through *Pseudomonas putida* APRRJVITS11 and sustainable remediation of pathogenic DNA in water

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Abstract

Background: Bioremediation is one of the indispensable features of *Pseudomonas putida*. The use of *Pseudomonas* has been proved to be an effective treatment of tannery released chromium (VI). The current study is the first attempt for the optimization of chromate reduction by *Pseudomonas putida* strain APRRJVITS11 in an optimized bench-scale bioreactor with successful thermo-pressure elimination of the strain thereby eliminating the health risk caused by antibiotic resistant genes (ARGs).

Results: The growth media, modified with optimized 1.0% nitrogen, 0.5% yeast extract and 0.3% sodium, showed enhanced bacterial growth for 72 h of incubation. The optimization of aeration (1.0 vvm) and agitation (150 rpm) rates enhanced the chromate reduction by about 40% at 72 h fermentation. Thermo-pressure pathogenic DNA degradation was achieved at 90 °C and 5868 Pa for 10 min.

Conclusions: Successful chromium reduction and total elimination of ARGs from effluent. A two-step treatment train was proposed for chromium reduction in the environment, which should be incorporated by the existing leather industries running on conventional treatment units.

Keywords: Chromium, Biosorption, Bioremediation, Antibiotic resistant genes, Wastewater, Bioreactor

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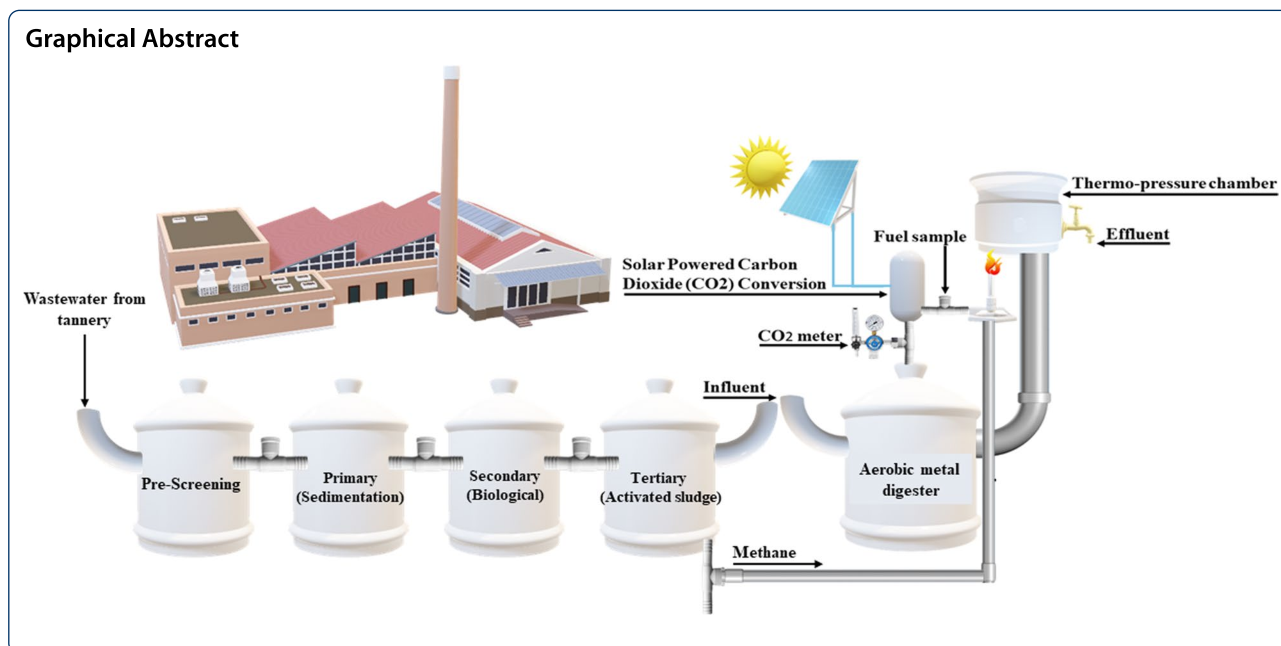
Graphical Abstract



1 Background

Global industrialization is the major contributor to environmental pollution [12]. Industrial runoffs consisting of increased concentrations of heavy metals when released without proper pre-treatment vastly contaminate the soil and freshwater bodies [15]. Among all industries, the leather industry is the most responsible for chromium emissions into the environment. Globally, an estimated 40 million litres of chromate-infused wastewater are discharged each year [23].

As Cr(VI) is persistent, bioaccumulative and toxic to human health, living resources and the ecological system, alternative treatment methods for the removal of Cr(VI) are needed. Cr(VI) causes severe skin and respiratory tract conditions and has been linked to kidney and liver failure, with the worst-case scenario being skin and lung cancer [17].

The use of bacteria for cleaning pollution sites, specifically for chromium removal has been proved with the use of different genera viz. *Geotrichum, Bacillus, Escherichia, Leucobacter, Ochrobactrum, Streptomyces, Amycolatopsis, Aeromonas, Agrobacterium, Aereobacterium* and *Xanthobacter* [4, 7, 8, 11, 27, 30]. The application of bacteria in chromate wastewater treatment systems is environmentally friendly [6]. Biofilters with simple construction and embedded with bacteria capable of reducing chromate have been studied [5].

To satisfy the rising demand for leather products while remaining cost-effective, tanneries must be remodeled with more environmentally friendly plants and processes [35]. Nitrogen inputs, growth promoters, organic compounds and metal ions for osmotic equilibrium are all-important nutritional factors influencing chromium reductase production [24]. Physical parameters such as aeration and agitation rates, pH and temperature, as well as incubation time, are also significant [38]. As a result, improving nutritional and physical parameters for optimum chromate reductase efficiency is critical for making the industrial process commercially sustainable [25].

The factors are optimized in the "one-factor-at-a-time" technique by adjusting one aspect at a time while leaving other aspects stable [20]. This method is the quickest to adopt and particularly aids in the identification of important factors influencing culture growth, thereby increasing enzyme yield [29].

An additional problem is the genes of highly resistant mutated strains used in the bioremediation process [31]. Antimicrobial resistance (AMR) has recently been linked to tolerance to heavy metals, both naturally occurring and anthropogenic, and chromium has also been confirmed to co-select such AMR [3]. The hazardous effects of a chromium-treated isolate can result in strains containing these genes (in plasmids) which commonly exist in runoff water from the tanneries and facilitate the transmission of these genes reaching higher food chains [14].

The objective of the present research work was to optimize a bench-scale bioreactor for chromate reduction by *Pseudomonas putida* strain APRRJVITS11. Followed by total incineration of the bacterial strain DNA in the water.

2 Methods

In this current study, two-unit operations (Fig. 1) were evaluated independently, which is a standard technique to determine the viability of linking and scaling them up in a concept.

2.1 Procurement and stock solution

The novel strain *Pseudomonas putida* APRRJVITS11 (Accession No. MN453582) was procured from the laboratory TT635 of Vellore Institute of Technology, Vellore, Tamil Nadu, India. In a 500 ml conical flask, 250 ml sterile modified Luria–Bertani (MLB) broth of pH 6.0 was inoculated with 2.0% (v/v) mother culture of 0.8 OD, containing 2.8×10^8 colony forming units (CFU/ml) and incubated at 37 °C in a shaker incubator (150 rpm). The broth (5.0 ml) was collected aseptically regularly, and the bacterial growth was measured by turbidity at 620 nm [32].

2.2 One-factor-at-a-time optimization of nutritional and physical parameters

The scaling-up technique was used to improve different nutritional and physical parameters by keeping all variables constant in the basal medium except the one undergoing consideration. After taking into consideration the previously optimized component(s), each following factor was evaluated [36]. Peptone in MLB broth was replaced separately by NH_4Cl and urea to test the effect of different nitrogen sources. Yeast extract was replaced by $(NH_4)_2SO_4$ and tryptone. NaCl was replaced by $MgCl_2$ and $CaCl_2$. After which the selected alternative's concentration was optimized following the optimization of $K_2Cr_2O_7$ concentration. The physical parameters of pH (4.0–11.0) and temperature (40–55 °C) were optimized keeping a shaking speed of 150 rpm. The bacterial growth was studied at an absorbance of 620 nm using a spectrophotometer [34].

2.3 Bench-scale bioreactor level optimization

Fermentation was performed in a stirred tank bioreactor (Hygene Plus Glass Bioreactor, Lark Innovation Fine Technology, India) of 3–7 L capacity (Fig. 1). The fermentor included a direct drive twin Rushton type impeller, PID temperature and agitation control, pH probes and a pH controller [34]. For chromium reduction activity, fermentation was carried out in 5 L LB broth under previously optimized nutritional (glucose 1.25%, gelatin 2%, yeast extract 0.5%, Mg^{2+} 0.01%) and physical (pH 7.0 and 37 °C) conditions. The bioreactor solution was injected with a 2.0% mother culture of *Pseudomonas putida* APRRJVITS11 (0.8 OD, A660; 2.8×10^8 CFU/ ml). Aeration of culture broth was altered at various rates (0.5,

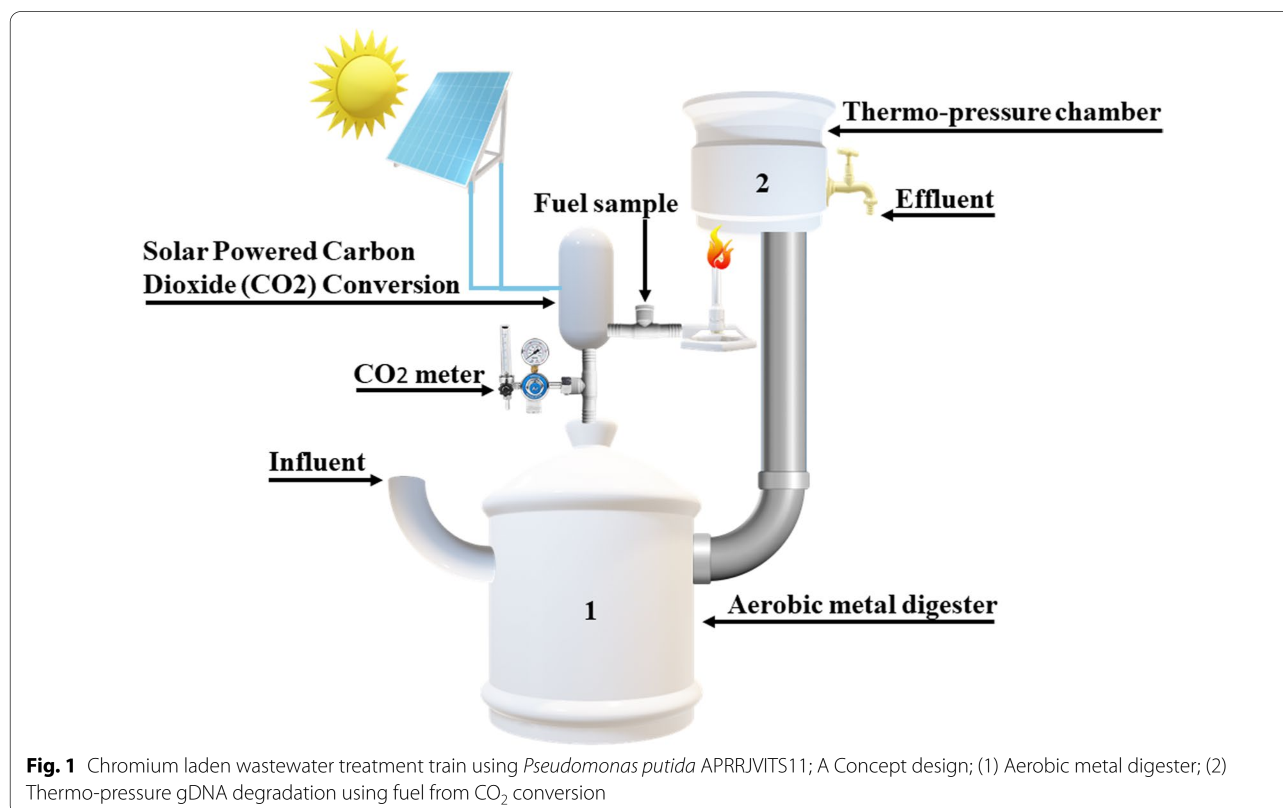


Fig. 1 Chromium laden wastewater treatment train using *Pseudomonas putida* APRRJVITS11; A Concept design; (1) Aerobic metal digester; (2) Thermo-pressure gDNA degradation using fuel from CO_2 conversion

1.0 and 1.5 vvm), and samples (5.0 ml) were taken every 24 h for three days. Turbidity at 660 nm was screened to detect bacterial proliferation. After centrifuging the samples at 12,000 rpm (4 °C) for 10 min, the cell-free supernatant was utilised to assess chromate removal efficiency using flame atomic absorption spectroscopy (AAS) and diphenylcarbazide (DPC) [13, 28].

2.4 Statistical analysis

Hexavalent chromium was assessed by its capability to react with the S-diphenylcarbazide (DPC) reagent [33]. All the statistical calculations were done on the Microsoft Excel 2019 edition software and all sampling analyses were done in triplicates and were performed with a confidence of 95% at $p < 0.05$ [9, 30].

2.5 Thermo-pressure degradation of gDNA

Reported data suggests that the thermal genomic DNA (gDNA) degradation under dry conditions happens at > 190 °C, while a complete gDNA degradation occurs somewhere between 100 and 110 °C in wet sterilization conditions [18]. *Pseudomonas putida* APRRJVIYS11 gDNA was extracted with an E.Z.N.A.® Water DNA Kit using the following protocol—The samples were filtered using microporous filter paper (0.22 µm or 0.45 µm). The filter from the adapter was removed and cut into four pieces, and placed in a clean 50 ml centrifuge tube. 3 ml of SLX-Mlus buffer and 500 mg glass beads X were added. Vortexed at for 10 min. 1 mL of DS buffer was added and incubated at 70 °C for 10 min. 1 ml of P2 buffer was added and vortexed for 30 s. Kept on ice for 5 min. Centrifuged at 4000 × *g* for 10 min at room temperature. Transferred the cleared supernatant to a new 50 ml centrifuge tube. Added 0.7 volumes of isopropanol and mixed thoroughly. Again, centrifuged at 4000 × *g* for 10 min at room temperature. The supernatant was discarded. To the DNA pellet, 400 µl of elution buffer was added and vortexed to mix thoroughly. Then incubated at 65 °C for 10 min to dissolve the DNA. The sample was transferred to a new 1.5 ml microcentrifuge tube. To that, 100 µl of cHTR Reagent was added and vortexed. Then centrifuged at 14,000 × *g* for 3 min. The supernatant was transferred to a new 1.5 ml microcentrifuge tube and added equal volume XP1 buffer. Vortexed to mix thoroughly. Transferred the entire sample to the HiBind® DNA Mini Column and centrifuged at 10,000 × *g* for 1 min at room temperature. The filtrate was discarded and to the pellet 300 µl of XP1 buffer was added. Centrifuged at 10,000 × *g* for 1 min at room temperature. Discarded the filtrate and transferred the HiBind® DNA Mini Column to a new 2 ml collection tube. Added 750 µl of DNA wash buffer. Centrifuged again at 10,000 × *g* for 1 min. Discarded the filtrate. Centrifuged the empty HiBind® DNA Mini Column for 2 min at maximum speed to dry the column matrix. Transferred the HiBind® DNA Mini Column to a clean 1.5 ml microcentrifuge tube. To that added 50 µl of sterile deionized water directly to the centre of the column membrane. Incubated at 65 °C for 5 min. Centrifuged finally at 10,000 × *g* for 1 min. Stored the DNA at − 20 °C.

Approximately 0.75 mg of gDNA was used for each thermo-pressure subjection ($n = 3$) [14]. A solar-powered CO_2 conversion device, which uses CO_2 produced from the aerobic wastewater treatment unit, can provide more sustainable temperature and pressure conditions [2].

The gDNA samples were taken in low-iron content borosilicate glass tubes containing 1.0 ml of Milli-Q water and sealed using rubber plugs. The test tubes were subjected to temperatures of 40, 60, 80, 90, 100, 120 and 150 °C, and pressure was recorded using a data logger (ALMEMO® 2690-8A). The degradation of the gDNA was observed by subjecting the DNA sample through 1 h of gel electrophoresis at 70 V and using a 1.0% agarose. An ultra-violet (UV) transilluminator was used to see the DNA bands.

3 Results

3.1 One-factor-at-a-time (OFAT) optimization of nutritional and physical parameters

3.1.1 Nitrogen source

Each organic and inorganic nitrogen source employed supported bacterial growth and chromium reductase production. However, maximum growth was observed with ammonium chloride (inorganic source) (Additional file 1: Table S1). Ammonium chloride at 1.0% (w/v) plus yeast extract (0.7%, w/v) were most suitable for maximum bacterial growth (Additional file 1: Table S1).

3.1.2 Metal ions

The use of sodium ions enhanced bacterial growth (Additional file 1: Table S1). Whereas the other metal ions (Mg^{2+} and Ca^{2+}) resulted in reduced bacterial growth. Among different levels of sodium (Na^{+}) employed, 0.3% (w/v) was the most effective [1].

3.1.3 Initial pH

The organism was able to grow in the selected pH range (7.0–11.0), but optimum bacterial growth was restricted to a pH range of 6.0 to 7.0. At pH 6.0 ± 0.1, maximum growth was observed under optimized nutritional conditions (Additional file 1: Table S2).

3.1.4 Heavy metal

Although the organism has shown a high chromate tolerance (1250 ppm) [16], the best growth results in the current bench-scale bioreactor (5 L) level study was observed at 100 ppm (Additional file 1: Table S1).

3.1.5 Temperature

Pseudomonas putida APRRJVITS11 was able to grow and produce chromium reductase in a temperature range of 10–55 °C, with maximum production at an optimum 37 °C. Temperature higher than 40 °C or lower than 37 °C, reduced the bacterial growth (Additional file 1: Table S2).

3.1.6 Incubation time

The bacterial growth and chromium reduction activity were in harmony up to 72 h under optimized nutritional and physical conditions.

3.2 Bench-scale bioreactor optimization

3.2.1 Effect of aeration

The chromium(VI) reduction was analyzed in this portion of the experiment at a fixed agitation speed of 150 rpm and varied aeration rates of 0.5, 1.0 and 1.5 vvm (Additional file 1: Table S3). The chromium(VI) reduction was a little low at an aeration rate of 0.5 vvm (51.3% at 72 h) and that of 1.5 vvm (53.1% at 72 h). The aeration rate of 1.0 vvm showed slightly better results with chromium(VI) reduction of 53.4% at 72 h (Fig. 2).

3.2.2 Effect of agitation at optimized aeration

After optimization of suitable aeration rate (1.0 vvm), agitation speed was optimized (100–200 rpm) for studying its effect on chromium(VI) reduction (Additional file 1: Table S3). The chromium(VI) reduction rate increased with an increase in agitation rate from 150 rpm, which was maximum at 200 rpm within 72 h of incubation. However, a decrease in agitation speed from 150 rpm reduced the efficiency to 53.1% at 72 h incubation (Fig. 3). At an optimized fixed aeration rate of 1.0 vvm and 200 rpm agitation, the efficiency was enhanced to 56.6% at 72 h at bench-scale bioreactor level compared to the conventional flask level optimization (51.9%) demonstrated in the previous work [16].

3.3 Thermo-pressure degradation of DNA

The genomic DNA from *Pseudomonas putida* strain APRRJVITS11 were found to be degraded at 90 °C and above (5868 Pa gauge of pressure) when exposed for 10 min (Fig. 4).

4 Discussion

Maximum growth was observed with ammonium chloride (inorganic source) (Additional file 1: Table S1). Other organic nitrogen sources showed less bacterial culture growth. Complex nitrogen sources are generally required for enzyme production; however, the requirement of a specific nitrogen source varies from organism to organism [22]. In the present study, ammonium chloride and yeast extract were most suitable for maximum bacterial growth (Additional file 1: Table S1). Yeast extract is shown to be an effective organic nitrogen source for bacterial cell growth stimulus and improved hydrogen production [10]. Singh et al. [34] found 2.0% (w/v) of gelatin (nitrogen source) with 0.5% (w/v) yeast extract most suitable for maximum protease production by *Pseudomonas putida* SKG-1.

The acidic pH optimum reveals a slightly acidophilic nature of strain *Pseudomonas putida* APRRJVITS11 [16]. Likewise, Karpouzas and Walker reported *Pseudomonas putida* (epI and epII) strains were able to grow well in the pH range 5.0–7.0 [19].

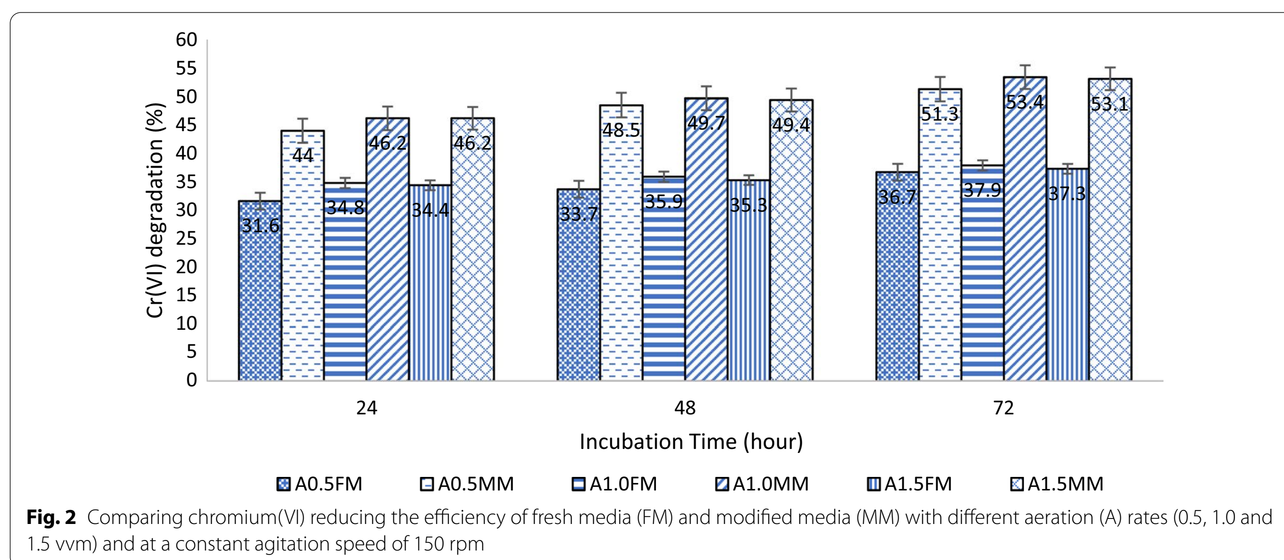


Fig. 2 Comparing chromium(VI) reducing the efficiency of fresh media (FM) and modified media (MM) with different aeration (A) rates (0.5, 1.0 and 1.5 vvm) and at a constant agitation speed of 150 rpm

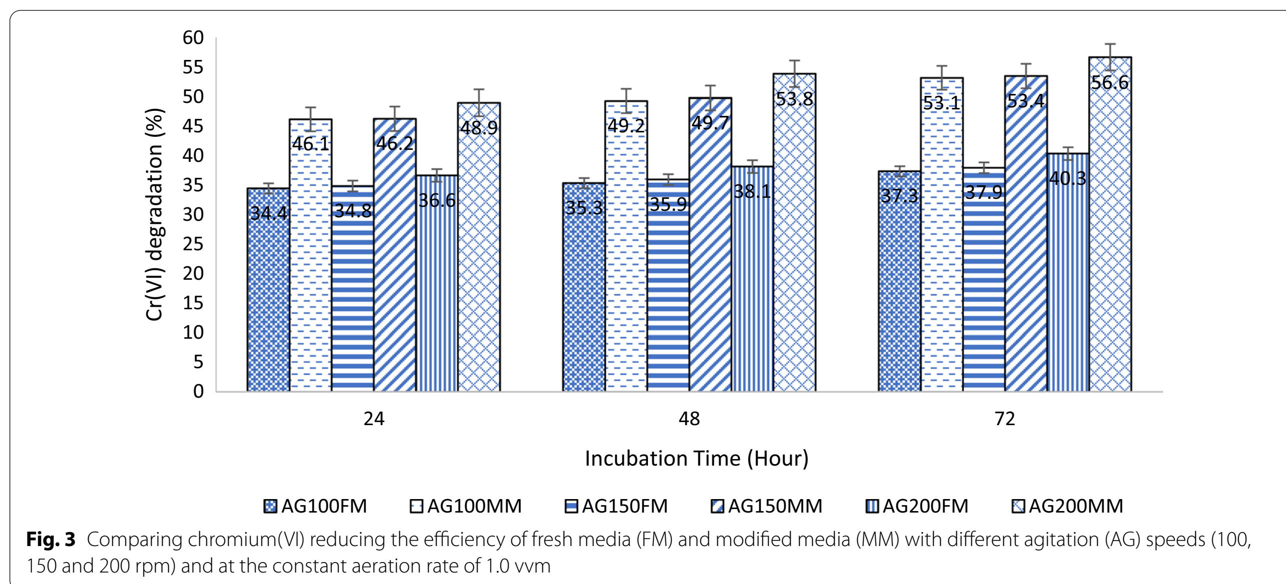


Fig. 3 Comparing chromium(VI) reducing the efficiency of fresh media (FM) and modified media (MM) with different agitation (AG) speeds (100, 150 and 200 rpm) and at the constant aeration rate of 1.0 vvm

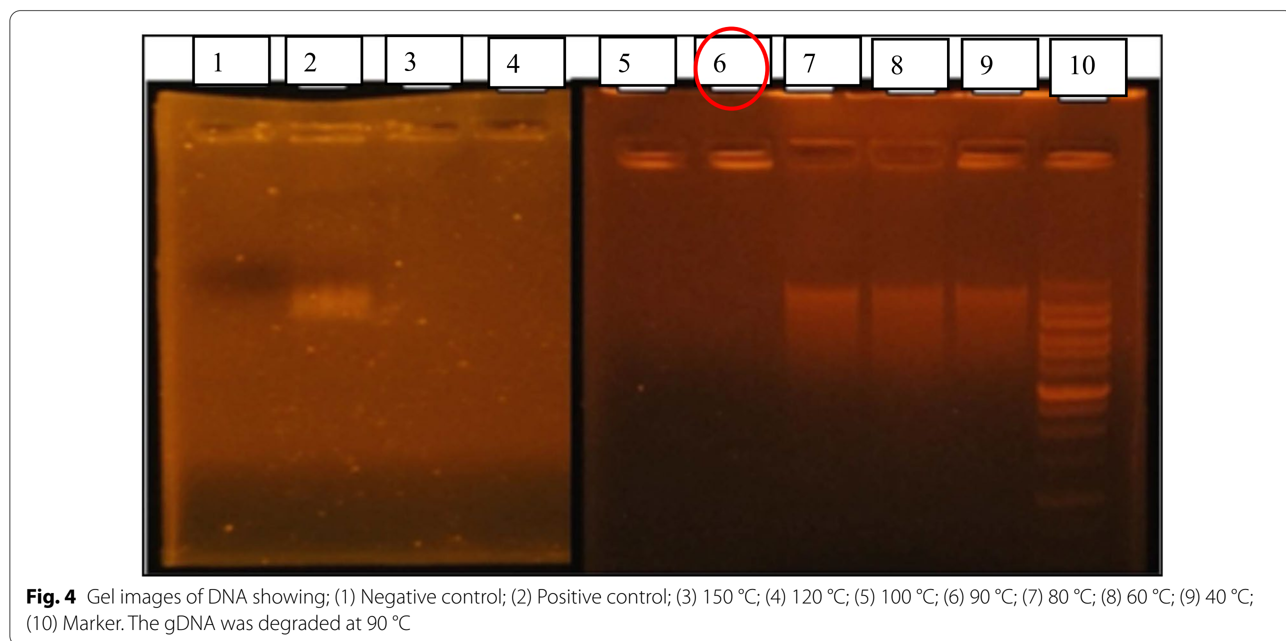


Fig. 4 Gel images of DNA showing; (1) Negative control; (2) Positive control; (3) 150 °C; (4) 120 °C; (5) 100 °C; (6) 90 °C; (7) 80 °C; (8) 60 °C; (9) 40 °C; (10) Marker. The gDNA was degraded at 90 °C

In any bioprocess, specific temperature requirement and their regulation are some of the most critical parameters. The bacterial strain was able to grow efficiently at a temperature range of 10–55 °C, with optimum activity at 37 °C, as reported by others working with the *Pseudomonas* strains [21, 26].

Bacterial growth was in the exponential phase up to 72 h. Our findings are per the results of Nowak et al. [26]. Whereas, Zeng et al. has recorded maximum growth beyond 72 h [37].

The reducing efficiency was compared with the use of fresh media (FM) and was observed that there was around a 40% increase in the efficiency when the optimized modified media (MM) was used. The findings show that optimal agitation of fermenting broth is required for efficient oxygen transport to bacterial cells. A suitable agitation/aeration ratio is required for optimum oxygen delivery to the bacterial cell, as well as to

limit the shearing impact by the impeller for optimum growth and chromium(VI) reduction efficiency of this obligate aerobic bacteria.

The genomic DNA from *Pseudomonas putida* strain APRRJVITS11 exhibited total thermo-pressure degradation of gDNA in water when exposed to 90 °C and 5868 Pa gauge of pressure for 10 min (Fig. 4). No growth was observed for the *Pseudomonas putida* APRRJVITS11 beyond this temperature and pressure in a solid or broth medium. Thereby proving as an essential condition for sustainable remediation of pathogenic DNA in water sources.

5 Conclusions

The bioreactor level optimization of chromium(VI) reduction efficiency from *Pseudomonas putida* APRRJVITS11 is being informed by a quick one-factor-at-a-time methodology. Increase in the bacterial growth was evident with optimized nutritional (nitrogen 1%, yeast extract 0.5%, Na^+ 0.3%) and physical (pH 7.0, 37 °C, 200 rpm) conditions during 72 h fermentation. Further optimization of aeration (1.0 vvm) and agitation (150 rpm) rates enhanced the chromate reduction by about 40% at 72 h fermentation (Additional file 1). Whole DNA degradation at 90 °C efficiently eliminated ARGs, limiting the harmful effect of chromium-treated isolates. The leather industries should consider adapting the train of operations (Fig. 1) described in this work, to their existing wastewater treatment units.

Furthermore, this is the first attempt at an effective reduction of chromate by *Pseudomonas putida* strain APRRJVITS11 in an optimized bench-scale bioreactor and successful thermo-pressure elimination of the strain thereby eliminating the health risk caused by ARGs.

Abbreviations

AAS: Atomic absorption spectroscopy; AMR: Antimicrobial resistance; ARGs: Antibiotic resistant genes; CFU: Colony forming unit; DNA: Deoxyribonucleic acid; DPC: Diphenylcarbazide; gDNA: Genomic DNA; LB: Luria–Bertani; MLB: Modified Luria–Bertani; OD: Optical density; OFAT: One-factor-at-a-time; PID: Proportional–integral–derivative; rpm: Revolutions per minute; UV: Ultraviolet; vvm: Vessel volumes per minute.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-021-00183-y.

Additional file 1. Supplementary Table 1. Effect of different nutritional factors on the growth of *Pseudomonas putida* APRRJVITS11 at pH 7.0, 37°C and 150 rpm during 72 h incubation. **Supplementary Table 2.** Effect of different physical factors on the growth of *Pseudomonas putida* APRRJVITS11 in nutritionally optimized media at 150 rpm during 72 h incubation. **Supplementary Table 3.** Effect of different aeration and agitation on the growth of *Pseudomonas putida* APRRJVITS11 in nutritionally optimized media at 37˚C and 7.0 pH during 72 h incubation.

Acknowledgements

The authors are thankful to the management, Vellore Institute of Technology (VIT), Vellore, for providing the essential amenities. The authors would also like to thank the Almighty God for everything.

Authors' contributions

RJ conducted methodology, software running, formal analysis, investigation, data curation, original draft preparation, and final paper's visualization. APR provided resources and conducted validation, reviewing the draft, and supervised the work. Both RJ and APR contributed to the final paper's visualization. Both the authors read and approved the manuscript.

Funding

This work was supported by the VIT SEED Grant (SEED-2576), provided by the Vellore Institute of Technology (VIT), Vellore.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All the authors, declare that they have no financial or non-financial conflict of interest.

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Received: 19 September 2021 Accepted: 11 December 2021
Published online: 18 January 2022

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