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Redox Potential and Proton Demand in an Anaerobic Palladium (II) Reducing Culture of Desulfovibrio Desulfuricans Seroval

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Microbial recovery of Pd is emerging as a clean alternative bioremediation processes as compared to the traditional physical and chemical recovery processes, and Sulphate-reducing bacteria have drawn a great deal of attention because they have proven to have excellent metal reaction properties for Pd. However, to effectively reduce Pd (II) to its elemental Form a clear understanding of its particle physics is needed as well as the limitations posed by its occurrence in chelated states on the adsorption and uptake by living organisms. Thus, the pH of the solution has a significant role in the interaction and uptake Pd (II) ions leading to its reduction. Therefore, the aim of the study was to investigate the use of sulphate-reducing bacteria isolated from sludge from a wastewater treatment plant, and a pure isolate of Desulfovibrio desulfuricans DSM642 in the reduction of 2mM of Pd (II) from pH 1 – 10 at the expanse of formate as an electron donor, using HCl and NaOH to adjust the pH. After 12 h of incubation the results revealed a maximum of 90 % and 83 % of palladium reduction at pH 4 by sulphate-reducing bacteria and Desulfovibrio desulfuricans respectively and a low reduction percentage was observed at pH values lower than 3. This was attributed to chloride ion interference at low pH values. Nevertheless sulphate-reducing bacteria proved to be the better choice as a potential organism to bioremediate Pd contaminated environments.

1. Introduction

Palladium [Pd] a precious metal belonging to the platinum group metals (PGMs) is highly valuable because it is resistant to corrosion and oxidation. In addition to having good electrical conductivity, it has excellent catalytic activity and disinfection properties. Palladium's high catalytic activity for a range of substrates has resulted in its use in many industrial synthetic processes ranging from reforming reactions in the petroleum refining industry to hydrogenation and dehydrogenation reactions in the pharmaceutical industry (Bernadis et al., 2005). In addition, Pd is used in automotive catalytic converters to reduce gaseous emissions in vehicle exhausts to decrease the carbon emissions footprint (Yong et al., 2002). However, due to the widespread usage of Pd its demand has exceeded its supply, thus, it has to be recovered and recycled. Chemical treatments such as pyrometallurgical and hydrometallurgical processes are widely used however these methods are expansive, time consuming and generate large amounts of waste into the environment (Das, 2010). In addition, Pd is highly mobile in the environment because of its solubility in water, thus it has potential ecosystem alterations. Therefore, economic, environmental and efficient methods need to be developed to recover Pd.

The microbial reduction of metals has attracted recent interest because it is regarded as a clean alternative to the traditional chemical processes. Microbes offer an advantage in that they play a crucial role in the cycling of organic and inorganic species in the environment and if harnessed they may offer a wide range of innovative biotechnological processes (Lloyd, 2003). In addition, they are sensitive enough to recover metal concentrations at ppm concentrations which are below the economic threshold of traditional recovery methods (Zhang and Hu, 2007). Recent studies have demonstrated the ability of microorganisms to reduce metals through metal resistant mechanisms that incorporate changes in the oxidation state of the toxic metals; Capentier et al. (2003) was able

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to reduce V (V) to V (IV) using Shawanella oneidensis, Bansal et al. (2019) showed the reduction of Cr (VI) to Cr (III) by using chromium reducing organisms in the presence of Fe (II) where Fe (II) acted as a catalyst in the reduction processes. Lloyd et al. (1998) noted the reduction of Pd (II) to Pd (0) by Desulfovibrio desulfuricans. The application of microbial metal reduction is endless. However, to effectively reduce Pd to its elemental form the is a need to better understand the following: i) the fundamental basis of biogeochemical cycles of Pd reducing microbes so they can be harnessed for a range of biotechnological applications, ii) the Pd particle physics and limitations posed by its occurrence in chelated states on the adsorption and uptake by living organisms (Lloyd, 2003), and the pH of the environment which plays an important role in the interaction and uptake of Pd (II). Considering the background above, this study aimed at investigating the usage of Sulphate-reducing bacteria isolated from sludge from a wastewater treatment plant and a pure isolate of Desulfovibrio desulfuricans DSM642 in the reduction of Pd (II) at different pH ranges. The work presented in this paper suggest the biorecovery processes studied here has potential application for recovery and remediation of Pd contaminated environments. However, to understand the reduction capability, the pH of the medium should be considered.

2. Methods and materials

2.1 Bacterial preparation

Desulfovibrio desulfuricans DSM620 was cultured using modified Postgate medium C (0.5 g K_2HPO_4, 1.0 g NH_4Cl, 1.0 g Na_2SO_4, 0.1 g $CaCl_2$ x 2 H_2O, 2.0 g $MgSO_4$ x 7 H_2O, 2.0 g Na-DL-lactate, 1.0 g Yeast extract, 0.5 ml Na-resazurin solution (0.1% w/v), 0.5 g $FeSO_4$ x 7 H_2O, 0.1 g Na-thioglycolate, 0.1 g Ascorbic acid in 1 L distilled water) in butyl-rubber sealed 100mL serum bottles at 30 ºC under 120 rpm (Ngwenya and Chirwa, 2015). Mid-logarithmic phase cultures were prepared by anaerobic withdrawal of 10 mL of an actively growing culture into 100 mL of Postgate's medium C under oxygen free nitrogen and grown at 30 °C for 48 h. The cells were harvested by centrifugation, kept on ice before and after centrifugation and washed with 20 mM MOPS-NaOH buffer (pH 7.0) three times. Then resuspended in 20 mM MOPS-NaOH buffer to provide the stock suspension for the preparation of bio-Pd (0), then stored at 4 °C until use within 24 h (Mabbett et al., 2006). 0.2 g of sludge from the Brits wastewater treatment plant was placed in a butyl-rubber sealed 100 mL serum bottle, filled up to brim with fresh medium C so that no air was trapped in the bottle. Incubated at 30 ºC shaken at 120 rpm for 5 d (Molokwane and Chirwa, 2009). The presence of Sulphate-reducing bacteria was indicated by the blackening of the medium which was the production of FeS (Postgate, 1979) as shown in fig 1. Mid-logarithmic cultures were prepared as above.

2.2 Reduction of Pd (II) metal ions

The concentrated cell suspension of 2 mL with an OD_{600} of 0.920 and 0.899 for Desulfovibrio desulfuricans and the consortium respectively was diluted in a 5 mL buffer containing 2 mM of $Pd(NH_3)_4Cl_2$ from Sigma-Aldrich and 25 mM of formate, at a pH ranging from 1 - 10 adjusted using NaOH and HCl, sparged with nitrogen for 6 min in a 100 mL serum bottles to form the headspace gas, incubated at 30 ºC, 120 rpm for 12 h. Then sparged with air immediately to stop the reduction, centrifuged at 6000 rpm for 5 min then analyzed (Yong et al., 2002).

2.3 Assay of metal ions

Pd (II) levels in the supernatants were determined by Atomic Absorption Spectrometry, Spectrometer model AAnalyst 400, S/N 201S8070301 Autosampler Model 510. Using air-acetylene flame, Parkin-Elmer Lumina Pd hallow cathode lamp at a slit size of 1.8/1.35, lamp current of 30 and wavelength of 244.79 nm at an energy of 79.

3. Results and discussion

3.1 Reduction through biosorption Mechanism

Biosorption is a very complex metabolism-independent process which includes physical or chemical sorption on the cell wall which includes physio-chemical mechanisms such as ionic interactions, complexation, coordination, and chelation between metal ions and ligands which depend on the specific properties of the biomass, or biosorption can be related to cell metabolism which includes metal precipitation as sulfides or phosphates, sequestration by metal binding proteins, peptides or siderophores, transport and internal compartmentalization (Vargas et al., 2004). In this study no growth occurred during the reduction instead aggregates of bio-Pd formed, shown as darkening of the buffer in Fig 3b, and palladium aggregates being visible from pH of 3 as shown in Fig 3a. Biosorption was via a metabolism-independent process, where the microbes used chelation mechanisms between the metal ion and ligands to reduce Pd.

3.2 Influence of medium pH on reduction

According to Yong et al. (2002) sulphate-reducing bacteria are very sensitive to moderate low pH values, however the opposite was observed in this study. Even though the organisms thrive and grow in neutral pH values, in this study they were seen to be active at very low pH values where 48 % and 58 % of Pd (II) was reduced at pH 1 and 2 by sulphate-reducing bacteria and 38 % and 49 % reduction for pH 1 and 2 was archived by Desulfovibrio desulfuricans as shown in Fig 2. With an increase in pH there was an increase in reduction until a pH of 4, where reduction of Pd reached a maximum of 90% for sulphate-reducing bacteria and an optimum of 83 % for Desulfovibrio desulfuricans. The was a slight decrease in reduction from pH 8 – 10, 68 % and 64 % of Pd (II) was reduced by sulphate-reducing bacteria and Desulfovibrio desulfuricans respectively as shown in Fig 2. The results suggest that pH dependent Pd (II) reduction could be due to various functional groups on the bacterial cell walls as well as the chemistry of Pd. According to Rashmause and whiteley, (2007) the functional groups capable of metal sorption are usually basic, for example carboxyl, phosphate and amine groups, which are deprotonated at high pH values. As the pH increases more functional groups dissociate and become available for ion reduction due to less competition from protons.

However, to explain the differences between the metals in solution matrices at a given pH factors that determine possible sorption mechanisms need to be considered. The main factor that influences the biosorption process is the property of the metal solutions, for example the pH, metal concentration and metal ion chemistry. According to their chemical characteristics, the metal ionic species exhibit different preferences for ligand binding sites of the biomass. Palladium speciation is strongly related to pH and chloride concentration conditions (de Vargas et al., 2004). According to Ruiz et al. (2000) when chloride concentrations are high above 10 mmol/dm^3 approximately 75% of anionic species such as $PdCl^-$ and $PdCl^{2-}$ are predominant at low pH values and metal hydroxylation becomes significant at pH values higher than 3.5. When chloride concentrations are lower than 0.5 mmol/dm^3 approximately 90% of cationic species such as $PdCl_2$, $PdCl^+$ and Pd^{2+} are predominant at low pH values and hydroxyl complexes such as $Pd(OH)^+$, $Pd(OH)_2$, and $Pd(OH)_4^{2-}$ appear at a pH above 2.5. This phenomenon explains the high reduction observed at pH 4 instead of 2, chloride concentrations where above 10 mmol/dm^3 at low pH values because of chloride anions from the HCl used to adjust the pH in this study. However, it is likely that cationic and hydroxy complex species predominant at a higher pH values are more favorable for palladium biosorption (de Vargas et al., 2004).

Comparing these organisms there was no significant difference in the reduction efficiencies, However the consortium of sulphate-reducing bacteria had a higher reduction percentage then the isolate. Suggesting the consortium is more flexible it can quickly adapt to minor environmental changes. This ability offers an advantage over pure cultures in environmental biotechnology because it is less liable to contamination from other organisms and it has varying optima for culture variables such as nutrient concentration, temperature, redox potential and pH (Gadd and White, 1996).

Figure 1: Blackening of the medium due to bacterial growth

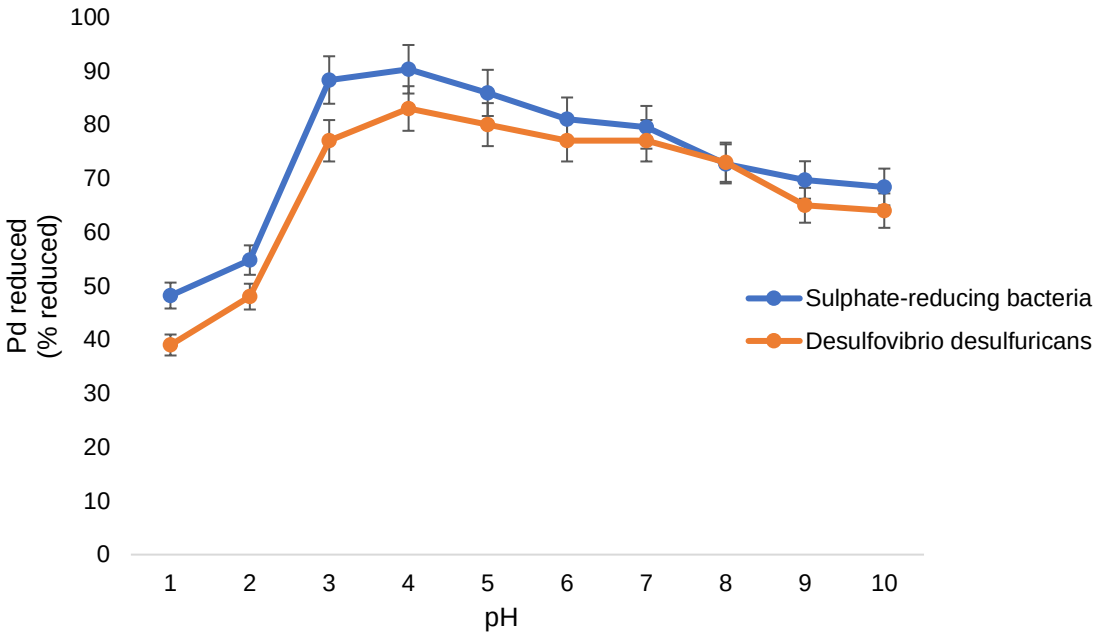

Figure 2: The effects of pH on Pd (II) reduction by Sulphate-reducing bacteria in comparison with a pure isolate of Desulfovibrio desulfuricans after 12 h of incubation.

3.3 Influence of pH on microbial biomass

Microbial biomass provides polymers as ligand groups on to which metal species can bind, polymers such as proteins, nucleic acids, and polysaccharides which would give the biomass a charge on its surface in the form of insoluble functional groups (Niu and Volesky, 1999). Surface charges depend on the types of compounds in the cell wall. In Gram-negative bacteria such as Desulfovibrio, about 5 % – 20 % of the wall is peptidoglycan which mainly provides carboxyl and amine groups because it comprises a polymer of two sugar derivatives; *N*-acetylglucosamine and *N*-acetylmuramic acid. In addition, gram-negative bacteria have an outer membrane that contains lipids, lipopolysaccharides, proteins, and extracellular polymeric substances (EPS) which contain sugar residues. The pKa of carboxyl groups in the cell wall is 4.8 while the amine groups have a pKa of approximately 7 – 10 (Fen et al.,1997). As the pH decreases, the negatively charged carboxyl groups and the neutral weak base amine groups become protonated, offering positive binding sites. For example, at low pH values due to HCl, the surface presents protonated groups which attract chloride anions electrostatically and positions them as counter anions that are exchanged with anionic palladium chloride species. Previous studies confirm there is a competition between anionic palladium complexes and chloride anions for adsorption sites, this was shown by low palladium biosorption when chloride anions were added to the solution (de Vargas et al., 2004), and This was in accordance with the study conducted by Yong et al. (2002), the reduction of palladium was studied across pH 2 - 7 at the expanse of formate or hydrogen. The results showed no reduction at a pH of 2 for formate however 50% of the reactivity was retained with hydrogen and a maximum rate was seen at pH values 3 - 7, which was similar to the rate with format at neutral pH values. The rate was affected by chloride and nitrate ions.

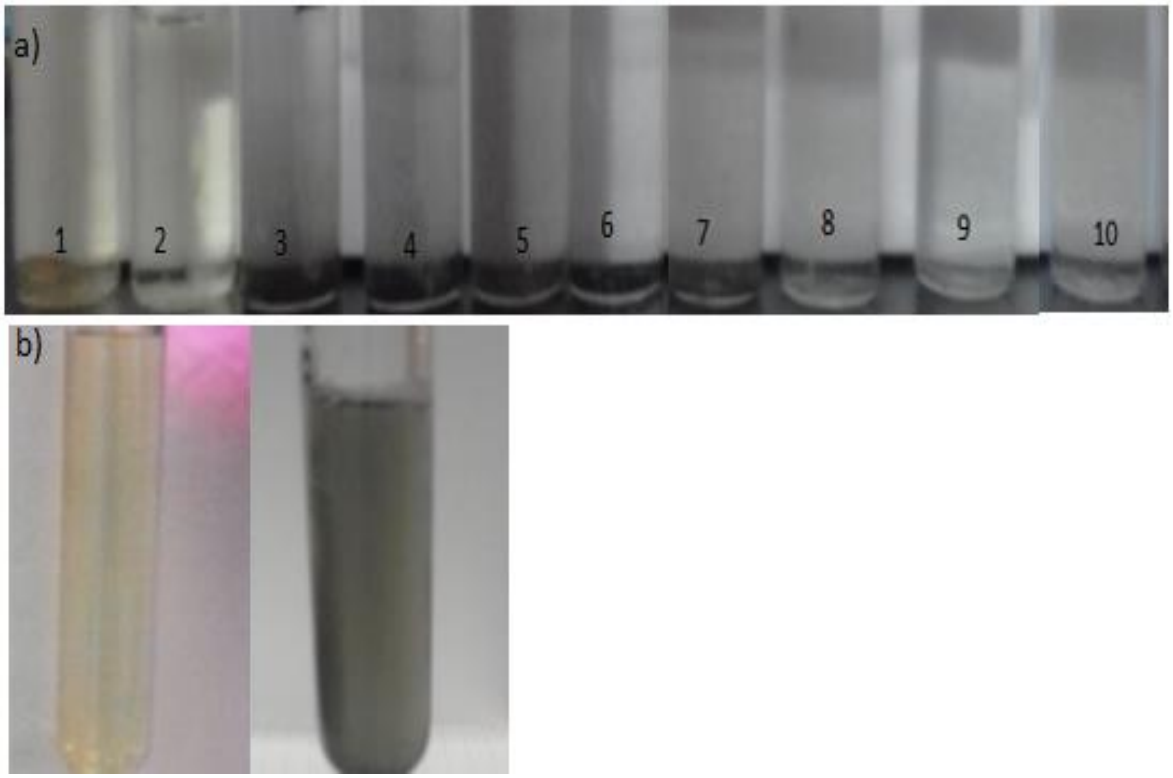

Figure 3: Reduction of 2mM of Pd(II) after 12 h of incubation at the expanse of fornate: a) Reduction from pH 1 - 10, there is a clear indication of reduction from pH 3 - 7 as the buffer is darkened by black precipitates. b) Darkening of the buffer due to Pd(0) nanoparticle formation by Sulphate-reducing bacteria

4. Conclusions

The reduction of Pd (II) by sulphate-reducing bacteria and Desulfovibrio desulfuricans at different pH values ranging from 1 - 10 revealed significant activity of the bacteria from pH 3, with 4 being the optimum pH for reduction where 90 % and 83 % of Pd (II) was reduced by sulphate-reducing bacteria and Desulfovibrio desulfuricans. However, a competitive effect of chloride ions was discovered at low pH levels, resulting in 54 % and 48 % of the palladium being reduced by sulphate-reducing bacteria and Desulfovibrio desulfuricans. This study demonstrated that the pH of an environment collates strongly with microbial communities across a wide range of biogeochemical conditions, it shapes microbial metabolism by affecting environmental conditions that are needed for microbial growth and survival and It defines the chemical activity of protons which are a key player in redox reactions, mineral dissolution and precipitation. Future work is required to fully understand biosorption kinetics of the biomass for future applications in pallidum bioremediation and recovery. as well as catalytic efficiencies of the bio-Pd in the remediation of other contaminants.

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