African Journal of Biotechnology Vol. 7 (8), pp. 1087-1095, 17 April, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

*Full Length Research Paper*

# Enzymatic recovery of platinum (IV) from industrial wastewater using a biosulphidogenic hydrogenase

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Accepted 7 March, 2008

**It has been established that dissolved heavy metals escaping into the environment pose a serious health hazard. As a result, there is an urgent need for controlling metal emissions into the environment. The aims of this study were to purify and biochemically characterise hydrogenase(s) from sulphate reducing consortium (SRB) and investigate the potential of the purified enzyme(s) in the recovery of platinum from wastewaters. A hydrogenase from sulphate reducing consortium was purified by a combination of PEG 20000 concentration, ion exchange (Toyopearl-Super Q 650 S) and size exclusion (Sephacryl S-200) chromatographies. SDS-PAGE analysis revealed a distinct protein band with a molecular mass of 58 kDa. The investigation of enzymatic platinum (IV) reduction *in vitro*, showed highest hydrogen-dependent platinum (IV) reducing activity in the presence of hydrogenase and its physiological electron carrier, cytochrome $c_3$. When the purified hydrogenase enzyme (with and without cytochrome $c_3$) was used with the industrial effluent, containing 7.9 mg.l$^{-1}$ platinum, only 10 – 15% recovery was noted pointing to a suppression of enzyme activity due to the low pH (0.38) of the effluent. Bioremediation studies on industrial effluent using resting SRB cells showed a 34% platinum removal from the effluent while growing SRB cells, within a sulphidogenic reactor, gave a platinum removal of 78%, with the pH of the system fluctuating at around 5.6. Evidence of sulphate reduction and sulphide generation were not observed during this treatment process suggesting that platinum sulphide was not formed and supporting the argument that the increased amount (78%) of platinum removal from the industrial wastewater by the growing SRB cells was due to more hydrogenase/cytochrome $c_3$ enzyme complex being available.**

**Key words:** Biosulphidogenic reactor, industrial effluent, hydrogenase enzyme.

## INTRODUCTION

Though the enzymatic reduction of metals by sulphate reducing bacteria (SRB) (Macaskie et al., 1994, 2001) have been examined in the context of environmental biotechnology (Whiteley and Lee, 2006), the enzymes and mechanisms responsible for these reductions have not yet been completely characterized. Several studies have revealed the role of cytochrome $c_3$ and hydrogenase enzymes to couple the oxidation of hydrogen, or simple organic acids such as lactic acid to metal reduction in lieu of sulphate as a final electron acceptor, e.g. Rh(III) (Ngwenya and Whiteley, 2006), Pt(IV) (Rashamuse and Whiteley, 2007), Fe(III), U(VI) (Lovely et al.,1993; Woolfolk and Whiteley, 1962), Cr(VI) (Lovely and Philips, 1994)), Tc(VII) (Lloyd et al., 1998a), Pd(II) (Lloyd et al., 1998b) and Mo(VI) (Tucker et al., 1998).

The abundance of platinum in the earth's crust is about 5 µg.kg$^{-1}$ (Ashwertn et al., 1999) and so there is an urgent requirement to reclaim platinum from industrial wastewaters and other secondary sources. We have previously demonstrated that cells from a sulphate reducing consortium obtained from a biosulphidogenic reactor can

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**Abbreviations:** DCIP, 2,6-dichloroindo-phenol; $DdH_2O$, duble distilled water; $H_2S$, hydrogen sulphide; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel eletrophoresis; and SRB, sulphate reducing bacteria.

reduce Rh(III) (Ngwenya and Whiteley, 2006) and Pt(IV) (Rashamuse and Whiteley, 2007) to their metallic form under a hydrogen atmosphere. During the present study a mixed SRB culture was used as it offers an advantage over a pure culture in environmental biotechnology context, in that it is less liable to contamination from other organisms (Gadd and White, 1996a, 1996b). Models for the SRB enzymatic reduction of metals can be used to develop and design the bioremediation of wastewater treatment systems and there are a number of features that make the SRB attractive for this purpose. 1). Although, the enzymes from the sulphidogenic bioreactors are produced under anaerobic conditions, they are perfectly able to work within an aerobic environment. 2). Using enzymes in a sulphidogenic environment supports the fact that the sulphide produced would be strongly inhibitory to the survival and proliferation of pathogens. 3). Biological (enzymatic) processes have an added advantage over traditional chemical/physical methods as they are regarded as "clean and green". 4). The high cost of industrial enzymes is prohibitively expensive and so the use of these enzymes, from a biosulphidogenic reactor is a cheap alternative and a tool for bioremediation.

The present work reports on the use of SRB cells from the resting and growth phases as well as a purified hydrogenase enzyme, with and without its physiological electron carrier, cytochrome $c_3$, and a soluble cell-free extract on the recovery of platinum from industrial effluents.

## MATERIALS AND METHODS

### MATERIALS

Unless stated otherwise all reagents were purchased from Sigma-Aldrich and Fluka-chemicals (Germany). Sephacryl-S-200 and Dialysis tubing were obtained from GE Healthcare (Sweden) while Toyopearl-Super Q 650S was obtained from Tosoh (Japan). Gases were purchased from Afrox (South Africa).

### Reactor design and operation

An experimental sulphidogenic bioreactor was set up, with slight modification, and tested as previously described (Rashamuse and Whiteley, 2007).

### Analytical procedures

All analyses were carried out in triplicate and the values reported as the means with standard deviations. Hydrogenase activity was assayed using 1 mM of methyl viologen as an electron acceptor and hydrogen gas as an electron donor essentially as described by De Lacey et al. (2000). Protein concentration was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard. The molecular mass of each of the pooled fractions containing purified hydrogenase was estimated using the SDS-PAGE (Laemmli, 1970).

### Hydrogenase isolation and purification

During mid-stationary phase SRB cells were harvested by centrifugation (7000 x g, 15 min, 4°C), washed in double distilled water ($ddH_2O$) then re-suspended anaerobically in Tris-HCl buffer (20 mM, pH 7.6, 50 ml) under oxygen-free nitrogen at a biomass density of 5 g (wet weight).$l^{-1}$. These cells were termed resting phase. Cell disruption by sonication based on a surface response model was carried out.

A sonicated extract (50 ml) was completely covered with a dry matrix of PEG 20000. The sample was left at 4°C for 2 h until the desired sample volume (5 ml) was attained and the protein content and hydrogenase activity determined. A sample (5 ml) of the concentrated extract was applied to a Toyopearl-Super Q 650S ion exchanger column (2.5 x 25 cm) equilibrated with Tris-HCl buffer (20 mM, pH 7.6). The unbound protein was washed from the column with buffer until the absorbance at $A_{280nm}$ of eluate had reached base line. The bound proteins were eluted by a stepwise (0-1M) NaCl-in Tris-HCl buffer (20 mM, pH 7.6), at a flow rate of 1 ml.$min^{-1}$. Fractions (5 ml) were collected, monitored for protein and hydrogenase activity and the active fractions collected and analysed by SDS-PAGE. The pooled active fractions were freeze dried, redissolved in Tris-HCl buffer (20 mM, pH 7.6, 3 ml) and loaded on to a sephacryl S-200 column (1.5 x 20 cm) equilibrated with the same buffer. Active hydrogenase fractions were eluted from the column at a flow rate of 1 ml.$min^{-1}$, collected, dialysed and analysed by SDS-PAGE.

### Electron acceptor specificity

Enzyme activities were determined using the standard assay containing the following electron acceptors: cytochrome $c_3$ (5 µM, 1.0 ml) ($\varepsilon_{550nm}$ = 7.18 $mM^{-1}cm^{-1}$), potassium ferricyanide (100 µM, 1.0 ml) ($\varepsilon_{420nm}$ = 1.04 $mM^{-1}cm^{-1}$), 2,6-dichloroindo-phenol (DCIP) (100 µM, 1.0 ml) ($\varepsilon_{600nm}$ = 20.6 $mM^{-1}cm^{-1}$) and methyl viologen (1.0 mM, 3.0 ml ) ($\varepsilon_{604nm}$ = 13.9$mM^{-1}cm^{-1}$) and hydrogen gas as the electron donor.

### Platinum recovery from industrial effluent

Industrial effluent, obtained from AngloPlatinum (South Africa) was analysed and contained 7.9 mg.$l^{-1}$ platinum, 169 mg.$l^{-1}$ sulphate and < 1.0 mg.$l^{-1}$ sulphide and had a pH of 0.38.

## RESULTS AND DISCUSSION

### Extraction of hydrogenase

There was little evidence of hydrogenase activity in any of the supernatant liquors prior to cell disruption reflecting that the enzyme was predominantly intercellular and/or membrane bound. Two different extraction methods – sonication and detergent (sodium cholate) were employed to ensure maximum release of the enzyme. Since the loss in enzyme activity during sonication is a common occurrence (Scopes, 1982), a surface response model was used to determine optimum conditions that afforded the highest activity.

A 2-dimensional cross-section contour map was constructed with varying sonication power amplitude and time (minutes) as variables (Figure 1). The best conditions for maximum extraction of hydrogenase activity

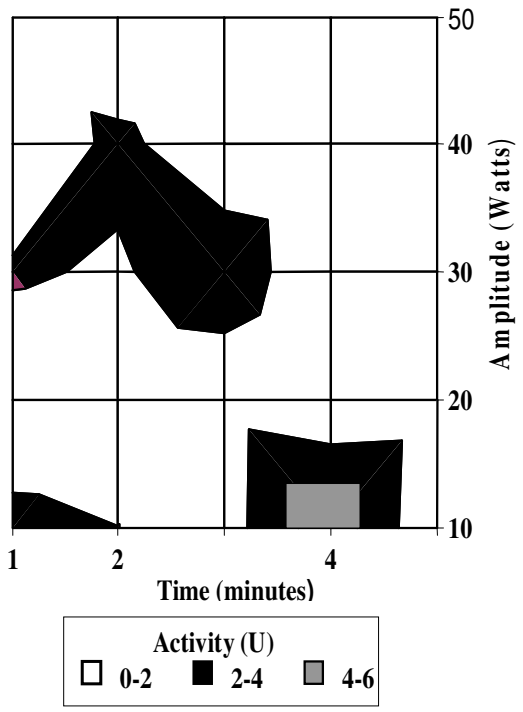


**Figure 1.** Contour map for sonication conditions that yield the highest hydrogenase activity.

were 10 W (power amplitude) for 4 min. It was observed that the release of hydrogenase activity from SRB pellet into the aqueous medium (supernatant) increased with an increase in the sonication time. However, no increase in hydrogenase activity was observed after 4 min of sonication, which implied that all of the cytoplasm associated hydrogenase activity was released. Despite an overall 16% loss in enzyme activity with the sonication method the temperature during the process was kept at 4°C in order to keep this loss to a minimum. Sonication alone was not sufficient for the release of the entire hydrogenase activity entrapped within the SRB cells, as it resulted in the liberation of only 56% into the medium, whilst about 27.5% activity still remained inside the SRB cells. The use of sodium cholate solution (3%) for the extraction of membrane-bound hydrogenase enzyme, however, was satisfactory as a further 23% of the enzyme was released to give a total yield of 79% (5% remained within the SRB cells and could not be extracted).

## Purification of hydrogenase

In view of the fact that the hydrogenases are extremely oxygen sensitive and require pre-activation before assay (Henry et al., 1980) a fast purification procedure comprising minimal steps, while not compromising the purity of the enzyme, was selected. A summary of the hydrogenase purification is shown (Table 1).

## Concentration of enzyme extract

Extensive loss in enzyme activity was experienced when either freeze-drying or an ammonium sulphate fractionation protocol was employed during the purification process. A soluble extract (50 ml) from the sonication protocol, initially containing 5.11 mg protein, was concentrated with PEG 20000 to 5.0 ml and the protein to 4.7 mg with a complete recovery of the enzyme (99.3%) and a purification fold of 1.08 (Table 1).

## Toyopearl Super Q - Sephacryl S-200

The concentrated extract (5.0 ml) was loaded onto a Toyopearl Super Q 650S ion exchange column with a step-wise addition of 0 – 1 M NaCl in Tris-HCl buffer (20 mM, pH 7.6). Hydrogenase-active fractions were eluted at high salt concentration of 750 mM indicating that the enzyme is relatively strongly bound to the resin. The pooled hydrogenase-containing fractions showed a specific activity of 6374 $U.mg^{-1}$ with 69.1% recovered activity and a purification factor of 1.67 (Table 1). These active fractions were concentrated by freeze drying, then redissolved in 3 ml of buffer before being loaded onto a Sephacryl S-100 column to give a single hydrogenase active fraction with a specific activity of 35341.7 $U.mg^{-1}$, 51.8% recovery and 9.26 fold purification. This fraction was subjected to SDS-PAGE analysis (Figure 2). The summary of the purification is shown in Table 1.

## Characterization of hydrogenase

### *pH*

In this study, the pH profile of the hydrogenase was determined over a pH range of 4 - 11, and it was found to be most active between pH 7.5 and pH 8 (data not shown). This was in accordance with other studies done, where hydrogenase enzymes were found to be active in the same pH range (Ngwenya and Whiteley, 2006; Zehender, 1990; Bianco et al., 2001). The enzyme displayed a high degree of pH sensitivity, and though considerable loss in activity was observed on either side of the pH optima the hydrogenase enzyme functioned optimally in a slightly alkaline environment. Enzymes have been known to generally operate within the growth pH optima of the host organisms; as a result the hydrogenase purified in this study had an optimal pH that corresponded with the conditions under which the SRB are cultured.

## Temperature

The effect of temperature on the activity of the hydrogenase enzyme was studied over a temperature range 0

**Table 1.** Purification of hydrogenase from sulphate reducing bacterial consortium.

| Step | Volume (ml) | Total protein (mg) | Total activity (U) | Specific activity ($U.mg^{-1}$) | Yield (%) | Purification fold |
|---|---|---|---|---|---|---|
| Crude sonicate | 50 | 5.11 | 19503 | 3816.6 | 100 | 1.00 |
| PEG 20000 | 5 | 4.7 | 19373.4 | 4122 | 99.3 | 1.08 |
| Toyopearl Super Q | 50 | 2.11 | 13476.6 | 6374 | 69.1 | 1.67 |
| Sephacryl S-100 | 3 | 0.286 | 10102.6 | 35341.7 | 51.8 | 9.26 |

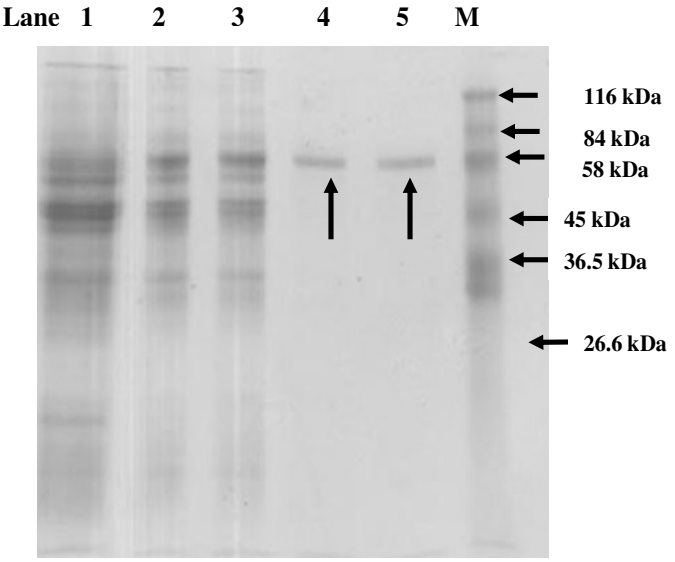


**Figure 2.** SDS-PAGE (12%) analysis of purified hydrogenase. Lane 1: Crude sonicate; lane 2: PEG concentration; lane 3: Toyopearl –S Q 650S; lane 4 and: Sephacryl S-200; lane M: Sigma prestained molecular weight markers.

- 70°C and it was found that the enzyme had its highest activity of 80 – 100% at a temperature of between 35°C and 40°C (data not shown), which is within the growth temperature range of mesophilic SRB. The enzyme was completely denatured at temperatures exceeding 55°C, and was gradually inactivated at temperatures below 35°C.

### *Electron acceptor specificity*

The specific activity of almost all hydrogenases largely depends on the nature of electron donor and acceptor used (De Lacey et al., 2000). *In vitro* hydrogenases have been shown to catalyze the reactions of hydrogen uptake with a number of electron acceptors such as cytochrome $c_3$, potassium ferricyanide, dichloroindolephenol (DCIP) and methyl viologen (Peck and LeGall, 1994). In this study methyl viologen (redox potential = -446 mV) was found to be the most suitable electron acceptor with hydrogen gas as the electron donor (Figure 3) (Ngwenya and Whiteley, 2006). The relative activity of each fraction in the presence of other electron acceptors was found to be 48% with cytochrome $c_3$; 34% with potassium ferricyanide and 30% with dichloroindophenol, DCIP. It was not unusual to find considerable hydrogenase activity (48%) when cytochrome $c_3$ was used as the electron acceptor given the proposed role of cytochrome $c_3$ *in situ* where it acts as an energy transducing device by capturing both protons and electrons produced by the hydrogenases in SRB (Dolla et al., 2000; Okura et al., 2001). Furthermore cytochrome $c_3$ is a naturally occurring electron carrier of hydrogenases and it is found among the periplasmic proteins in a ratio of 4.5 cytochrome molecules per hydrogenase molecule (Odom and Peck, 1984).

### Thermal stability

The thermal stability of the hydrogenase was investigated at its optimum pH and temperature. Minimal activity loss was observed during the first 10 to 20 min and thereafter, activity steadily decreased as incubation time increased (data not shown). After 80 min of incubation, the enzyme had lost 75 - 80% of all of its activity. The purified hydrogenase fraction had a half life of 35 minutes ($t_{1/2}$ = 35).

### *Metal ions*

Metal ions play an integral role in the life processes of micro-organisms. Essentially, they function as catalysts for biochemical reactions; they stabilize proteins and bacterial cell walls, and serve in maintaining osmotic balance (Hughes and Poole, 1997). Only 21% hydrogenase activity remained in the presence of 1 mM zinc (Figure 4) while complete hydrogenase inhibition was seen in the presence of chromium. Only 3.5 and 5% activity remained in the presence of silver and copper, respectively. Not surprisingly the presence of nickel and iron, which play an indispensable role during the hydrogenase redox processes, resulted in a 1.6 and 1.8 fold increase in hydrogenase activity. Though the mechanism of hydrogenase inhibition by Zn(II) and Cu(II) is not fully understood it is possible that these metals bind irreversibly into the nickel and/or iron binding sites in the

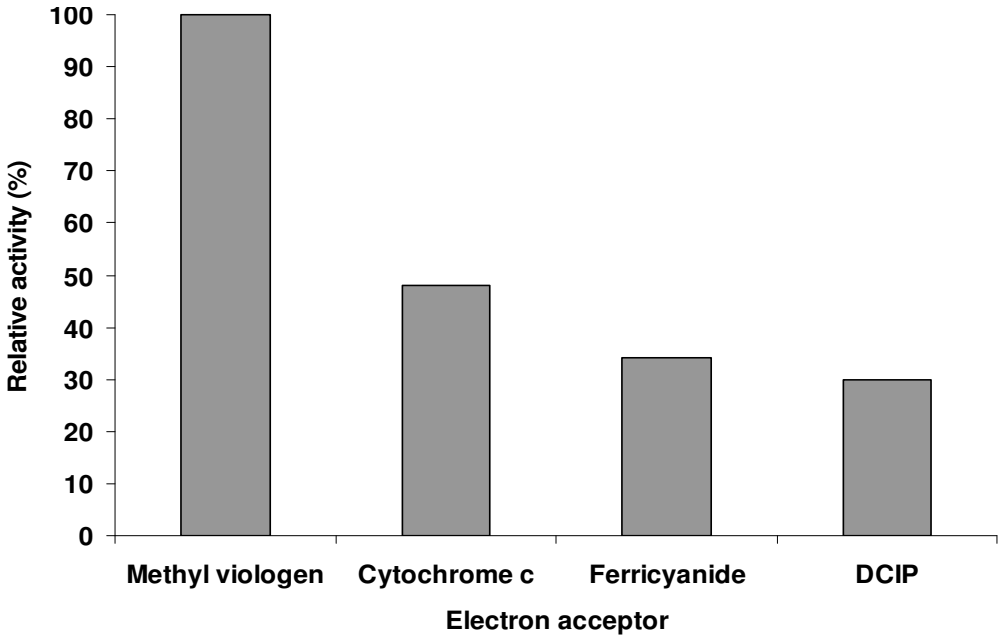


**Figure 3.** Effect of different electron acceptors on hydrogenase activity. Hydrogen is the electron donor. 100% activity = 35341.7 U.mg$^{-1}$.

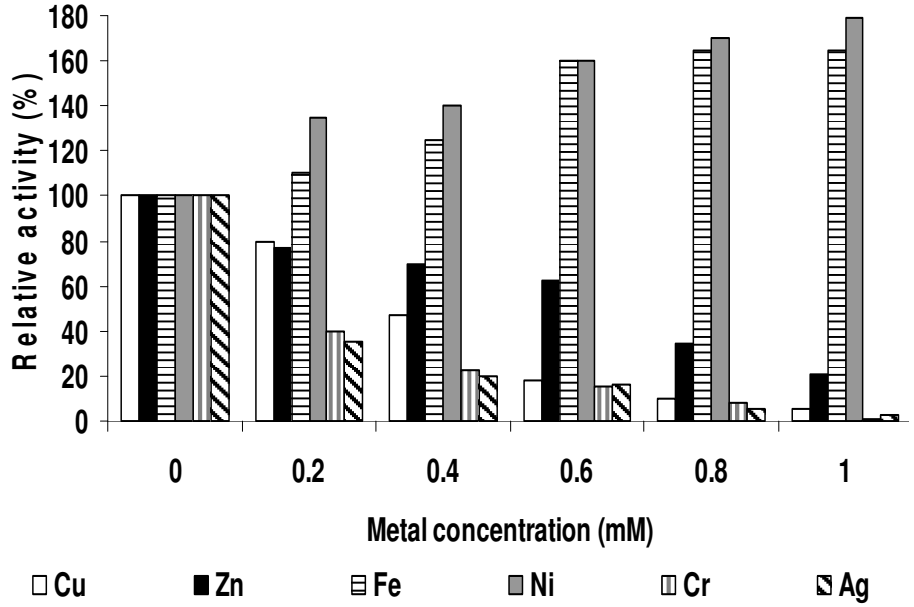


**Figure 4.** Effect of heavy metal ions on hydrogenase activity. Hydrogen is the electron donor. 100% activity = 35341.7 U.mg$^{-1}$.

hydrogenase active site, resulting in precursors containing Zn(II) or Cu(II) which cannot readily form a stable substrate-enzyme complex (Bock et al., 2001). In addition, complete inhibition of activity with Cu(II), a specific inhibitor of the periplasmic hydrogenase *in vivo*, is in accordance with other previous reports (Fernandez et al., 1989) and, consequently, establishes unequivocally that the enzyme being characterized is ofthe hydrogenase family.

### Kinetic parameters ($V_{max}$ and $K_m$)

The dependence of each of the hydrogenase fractions on

the concentration of methyl viologen as a substrate electron acceptor was also investigated. The maximum hydrogenase activity ($V_{max}$) and Michaelis constant ($K_M$) were 1312 U.mg$^{-1}$ and 5.2 µM respectively suggesting that the enzyme follows Michaelis Menten kinetics with an enzyme efficiency ($K_{cat}$) (turnover number) of 3.6 s$^{-1}$ (data not shown).

**Molecular weight analysis**

Molecular mass markers, crude sonicated extract and active fractions from the chromatographic separation were loaded onto a 12% SDS gel. From the SDS-PAGE (Figure 2) the hydrogenase appeared to be monomeric since a single distinctive protein band corresponding to molecular weight of 58 kDa (Figure 2) was detected, respectively. The molecular mass was in the same order of magnitude (48 – 89 kDa) as that of a Fe-only hydrogenase found in *Desulfovibrio desulfuricans* or the Ni-Fe hydrogenase, localized within the cell membrane (Grahame, 1988; Nicolet et al., 2000). Periplasmic localized nickel containing hydrogenases from *D. desulfuricans* (NRC 49001) and *D. desulfuricans* (Norway 4), for example, are monomeric with molecular weights of 52 and 58 kDa, respectively (Reider et al., 1984; Teixeria et al., 1987; Lissolo et al., 1986; Cammack et al., 1986). Some nickel-containing hydrogenases are heterodimeric, such as those purified from *D. gigas* and *D. baculatus* which have molecular weights of 89.5 kDa (63 + 26.5 kDa) and 85 kDa (56 + 29 kDa), respectively (Winter et al., 2005).

It has been postulated that these monomeric nickel-containing hydrogenases, might be an active large subunit of the heterodimeric nickel-containing hydrogennases (Hausinger, 1987). It appears that molecular weights of the hydrogenases are species dependent, varying from one organism to another. Since the hydrogenase in the present study was purified from a mixed culture and because hydrogenases are uniformly distributed in SRB it is impossible to speculate which sulphate reducing species produced which enzyme and whether they are metal-free, Fe-only or Ni-Fe hydrogennases. In addition many studies conducted so far on the bioreduction of metals by hydrogenase have not been concerned with the class of hydrogenase involved in the process. Nevertheless the objective of identifying, purifying and characterising the hydrogenase involved has been achieved.

**Platinum recovery from industrial effluent**

Recently we reported that hydrogenase enzymes isolated from a biosulphidogenic reactor were capable of bioremediating both rhodium (Ngwenya and Whiteley, 2006) and Pt(IV)and platinum (Rashamuse and Whiteley, 2007) from aqueous solutions. Investigation of enzymatic platinum (IV) reduction *in vitro*, showed highest hydrogen-dependent platinum (IV) reducing activity in the presence of hydrogenase and its physiological electron carrier, cytochrome $c_3$.

In order to study the enzymatic reduction of platinum (IV) by the sulphate reducing consortium and to provide evidence of the involvement of a hydrogenase in the platinum (IV) reduction process different extracts of SRB cells were tested with industrial effluent for platinum removal. The reduction of platinum by the purified hydrogenase enzyme alone yielded about 10% removal after 3 h incubation while after the addition of cytochrome $c_3$ there was a slight increase to 15% (Figure 5). The low overall capacity for platinum reduction by 'pure' enzyme with and without cytochrome $c_3$ may be attributed to the suppression of enzyme activity due to the extreme acid pH of 0.38 of the effluent.

Platinum (IV) reduction from industrial effluent by the soluble cell-free fraction gave a 31% reduction after 3 h of incubation (Figure 5) while with resting cells a 34% removal of platinum, was observed after the same time period. These results indicate that, regardless of a low pH level of the effluent, resting SRB cells still offer great potential for removal of platinum from industrial effluent. Since resting cells were used the possibility of platinum removal by precipitation as platinum sulphide is ruled out. It is therefore postulated that under growing SRB conditions platinum removal should increase significantly as a result of sulphide precipitation. Indeed a platinum removal of over 50% (Figure 5) was observed by the growth phase cells within the biosulphidogenic reactor, during the first hour after the introduction of industrial waste effluent. This increased to 78% after 3 h and since 34% was effectively removed by a resting consortium it is believed that the remainder (44%) was due to precipitation of platinum as its platinum sulphide.

In order to investigate whether sulphate reduction was occurring in the presence of growing cells, sulphate and sulphide content were monitored throughout the process. During the first hour after industrial effluent had entered the biosulphidogenic reactor the sulphide concentration within the reactor decreased from 300 mg to between 150 and 200 mg.l$^{-1}$ mainly due to the continuous flow of hydrogen gas, resulting in some of the sulphide escaping as gaseous hydrogen sulphide. After industrial waste, containing 169 mg.l$^{-1}$ sulphate and < 1.0 mg.l$^{-1}$ sulphide, entered the reactor the sulphate concentration in the reactor increased from an initial value of about 100 to around 280 mg.l$^{-1}$ during the first 3 h. A slight decrease in sulphate concentration to around 180 mg.l$^{-1}$ was noted after 3 h, suggesting that sulphate reduction was taking place, in the presence of hydrogen as an electron donor. A seriously limiting factor, however, was that the concentration of sulphate contained in the reactor is too low (169 mg.l$^{-1}$) compared to the sulphate concentration required for effective sulphate reduction (ca. 1500 mg.l$^{-1}$) (Rashamuse and Whiteley, 2007).

The fact that the sulphate levels remained constant for

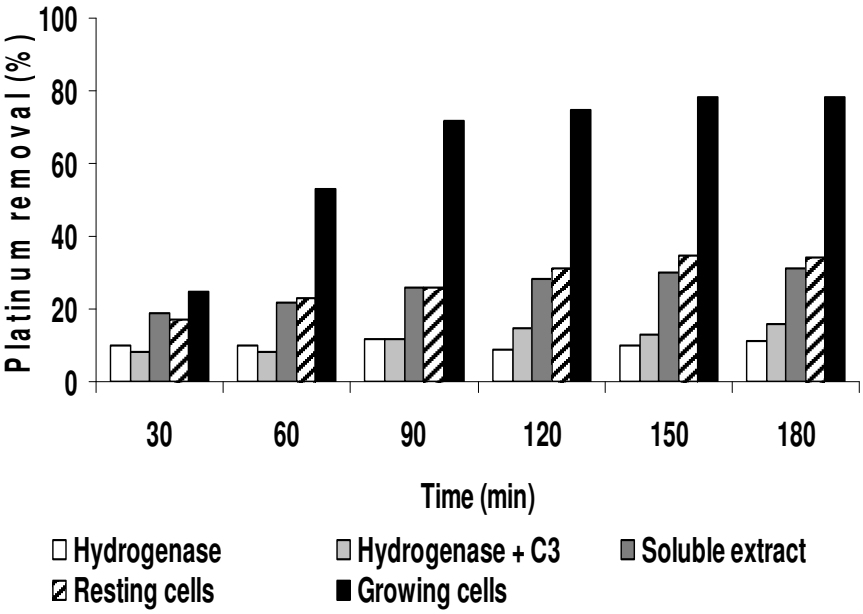


**Figure 5.** Removal of platinum from industrial effluent by hydrogenase (specific activity = 35341.7 $U.mg^{-1}$) and with cytochrome $c_3$; soluble cell-free extract and resting and growing SRB cells.

the first 3 h without any further production of sulphide must illustrate that the 44% increase in platinum removal was not due to precipitation as platinum sulphide. Consequently it is in our opinion that this increase by the SRB cells from the growing phase between one and three hours can only be due to further production and reaction of the hydrogenase-cytochrome $c_3$ enzyme system.

Previous investigations of metal reducing activity by various cell extracts revealed similar results. Investigation of the enzymatic-dependent Cr(VI) reduction by *Desulfovibrio vulgaris* (Lovely and Philips, 1994), showed that the cell-free extract accounted for all major Cr (VI) reducing activity (86%). Earlier observations of enzymatic reduction of U (VI) by the same sulphate reducing bacterium also showed major U(VI) reducing activity (95%) in the soluble fractions (Lovely et al., 1994). In both studies, metal reducing activities were shown to be hydrogen dependent, suggesting direct involvement of a hydrogenase enzyme, in the soluble extracts, in removing the electrons for metal reduction from the hydrogen.

Conventionally, metal ions have been removed from effluent streams by increasing the pH of the solution by addition of chemical ligands like lime or sulphides. In contrast enzymatic processes can generate metal-desolubilising ligands on a continuous basis with extracellular or intracellular deposition of metals as a metal-ligand precipitate. Metal reduction usually results in the precipitation of low valence, reduced forms of metals and a mechanism for the involvement of hydrogenases is proposed (Figure 6). Under strict anaerobic conditions

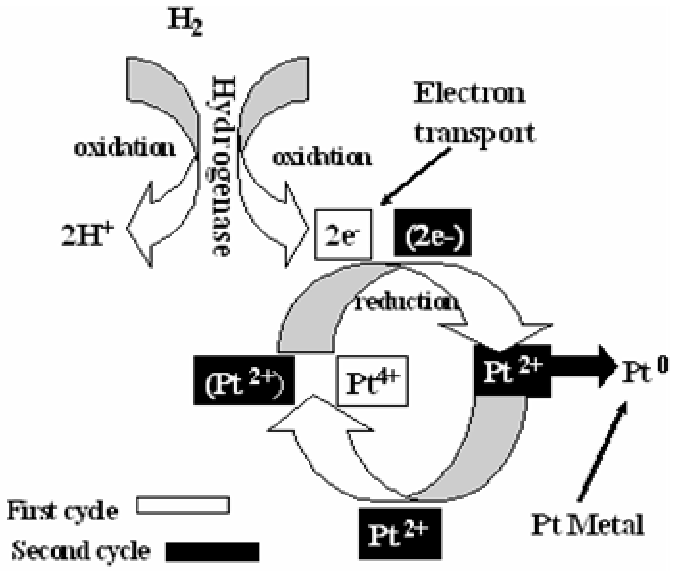


**Figure 6.** Mechanism for a two-cycle reduction of platinum by hydrogenase enzyme.

the SRB can strip a metallic compound back to a base metal via hydrogenase activities. Protons generated from the oxidation of molecular hydrogen by hydrogenases are used for metabolic activities of the cell while electrons released are channeled to the electron acceptor through the electron transport chain. Ordinarily this electron acceptor is the sulphate ion, but if platinum ions are pre-

sent the bacteria can use these as the electron sink leading, via two cycles (Figure 6), to the reduction of the base metal which is then precipitated in the periplasmic space.

The initial pH of the industrial effluent was 0.38 while the pH of the biosulphidogenic reactor was 7.6 making the pH within the reactor, after 60 min reaction time, at 7.3. After the same period of time the pH of the effluent increased from 0.38 to 5.6.

This study, therefore, has shown that platinum (IV) recovery from an industrial effluent through a biosulphidogenic SRB consortium is via a hydrogenase-cytochrome $c_3$ enzyme system that removes electrons from hydrogen to the platinum metal that acts as a final electron acceptor. If this is true, other PGMs should be reduced by the same mechanism (Ngwenya and Whitely, 2006). The use of this enzyme complex system in the treatment of platinum effluent offers several advantages over biogenic sulfide precipitation including the following: (i) there is no need to supplement low-sulphide wastewater streams with added sulphate; (ii) non-growing cells can be used, leading to the generation of a low-biomass waste for disposal; (iii) potentially hazardous toxic $H_2S$ is not generated as a by- product of the process, and indeed it has been noted (Rashamuse and Whiteley, 2007) earlier about technical difficulties in using resting SRB cells to reduce sulphate to $H_2S$; (iv) the process is potentially environmentally benign if hydrogen is used as a feedstock because there is no additional carbon substrate added to the wastewater; and (v) the reduced platinum (IV) is held within the outer compartments of the cell, potentially spatially separated from oxidizing and chelating agents that may be present in the effluent.

We are currently undertaking the N-terminal amino acid sequencing of the purified hydrogenase with the aim of designing degenerate oligonucleotides in order to determine hydrogenase encoding sequence. This will help with the design of the recombinant hydrogenase over-expression system which would allow the process optimization for the enzymatic recovery of platinum from the wastewater streams.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from AngloPlatinum Management Services (Pty) Ltd, South Africa and Deutscher Akademischer Austausch-dienst (DAAD) for MSc scholarship to K. J. Rashamuse.

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