CHAPTER 5

HYDROGEN PEROXIDE AS A NON-INVASIVE AGENT FOR THE REMOVAL OF MANGANESE OXIDE COATINGS FROM FOSSIL SPECIMENS

5.1 Pilot study

A pilot study was undertaken in order to determine the feasibility of using hydrogen peroxide for the removal of manganese oxides from fossil bones and teeth. The main objective was to validate the use of hydrogen peroxide as a non-invasive chemical method for the removal of manganese iron bone coatings. This was achieved by first determining an optimized lowest hydrogen peroxide concentration that sufficiently removes the bone and teeth coatings, and then using Scanning electron microscopy and gravimetric analysis as validation of the technique.

Procedure

The cleaning method depends on the use of hydrogen peroxide as a reducing agent to convert Mn^{4+} into soluble Mn^{2+} under optimised conditions. The products of the reaction (Evangelou, 1998) are manganese(II) and oxygen:

$$MnO_2(s) + H_2O_2\ (aq) + 2H^+ \rightarrow Mn^{2+}\ (aq) + O_2\ (g) + 2H_2O\ (l) \qquad 5.1$$

Fe^{3+} is similarly reduced by hydrogen peroxide:

$$Fe_2O_3\ (s) + H_2O_2\ (aq) + 4H^+ \rightarrow 2Fe^{2+}\ (aq) + O_2\ (g) + 3H_2O\ (l) \qquad 5.2$$

These reactions (5.1 and 5.2) seem to require acidic conditions to drive the reaction. As a result of hydrogen peroxide being naturally acidic (pH = 2 for a 30% solution) and that this low pH value can cause the dissolution of carbonates which bone predominantly comprises, the hydrogen peroxide was diluted.

Iron and manganese can also form a redox couple. The Fe^{2+} is oxidized resulting in the reduction of Mn^{4+} according to the following reaction (5.3) (Evangelou, 1998):

$$MnO_2 + 4H^+ + 2Fe^{2+} \leftrightarrow Mn^{2+} + 2H_2O + 2Fe^{3+} \qquad 5.3$$

Iron present in the oxide coatings could thus indirectly be a major factor in the reduction of manganese oxide fossil bone coatings (5.3). This reaction could also explain why some manganese oxide coated fossil bones exhibit different degrees of cleaning, when placed into a hydrogen peroxide solution under optimal conditions. A specimen with an oxide coating containing a higher concentration of iron would be expected to be more favourably reduced. This is because, once the Fe^{3+} is reduced into solution by the hydrogen peroxide, it can itself act as a reducing agent in the presence of the manganese oxide fossil bone coating to form Mn^{2+} as shown in 5.3.

5.1.1 Optimization of the hydrogen peroxide concentration

All optimization was performed on a qualitative basis using a Sony Cyber Shot T7 digital camera. Fossil bones of similar size coated with the manganese oxide layers, were placed into a range of hydrogen peroxide concentrations and left for a given time interval. The degree of manganese dioxide removal was used as a direct indicator as to which was the optimum hydrogen peroxide concentration for that range.

The concentration of the hydrogen peroxide, reaction time and various other conditions were studied in order for optimisation and validation of the procedure. pH measurements and Mn^{2+} and Ca^{2+} concentrations with time were used to monitor the progress of the reaction.

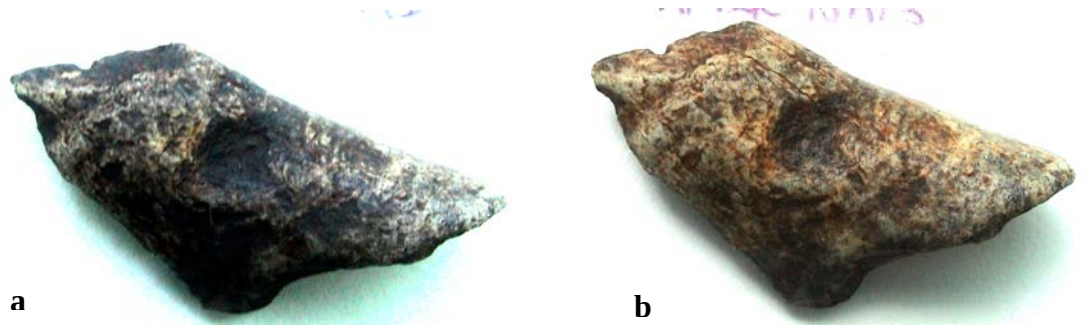

Figure 5.1 Manganese oxide coated fossil bone fragment **a,** before treatment; **b**, after treatment in 0.2 mol L^{-1} hydrogen peroxide.

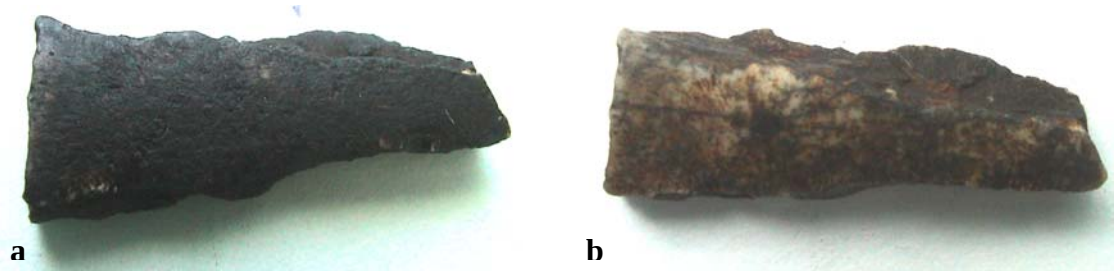

Figure 5.2 Manganese oxide coated fossil bone fragment **a,** before treatment; **b**, after treatment in 0.5 mol L^{-1} hydrogen peroxide

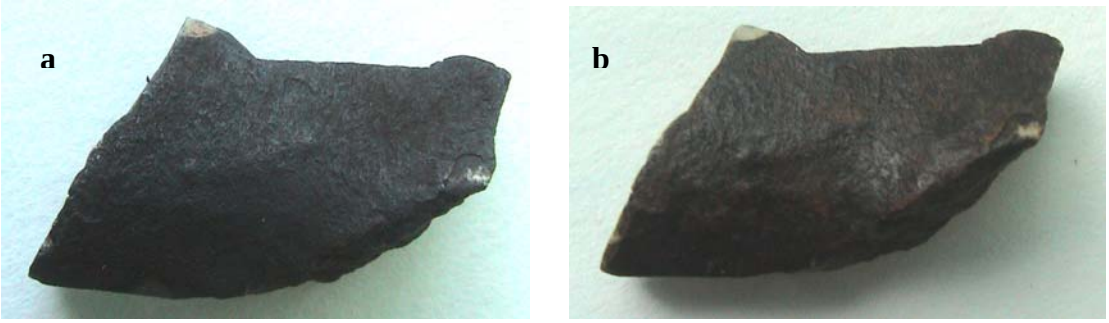

Figure 5.3 Manganese oxide coated fossil bone fragment **a,** before treatment; **b,** after treatment in 1.0 mol L^{-1} hydrogen peroxide.

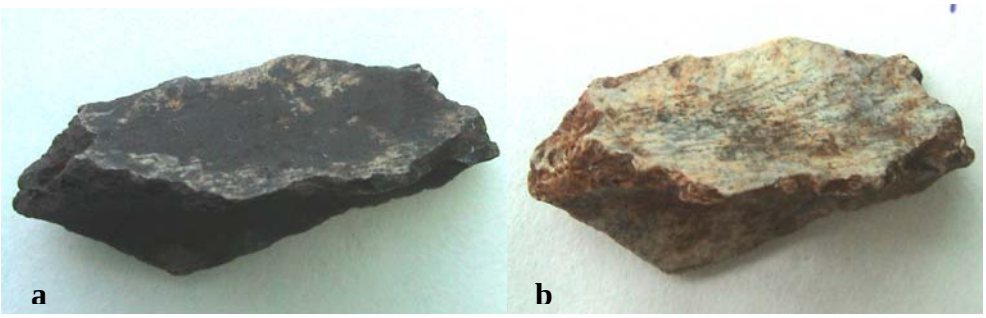

Figure 5.4 Manganese oxide coated fossil bone fragment **a,** before treatment; **b,** after treatment in 1.7 mol L^{-1} hydrogen peroxide.

5.1.2 Temporal profile of pH for the hydrogen peroxide reaction system

All the pH measurements of the hydrogen peroxide solutions for the concentrations used in the pilot study were their natural pH values. Thus the initial pH for each solution did vary slightly. The pH and time relationship was investigated and it was determined that the pH of the solution increases at a rapid initial rate (Fig. 5.5) thereafter it remains constant, suggesting that equilibrium has been established. Even once this equilibrium is reached, evolution of oxygen gas continues on the surface of the bone, because MnO_2 also catalytically decomposes hydrogen peroxide (Brown *et al*., 1999; Ni *et al*., 2000).

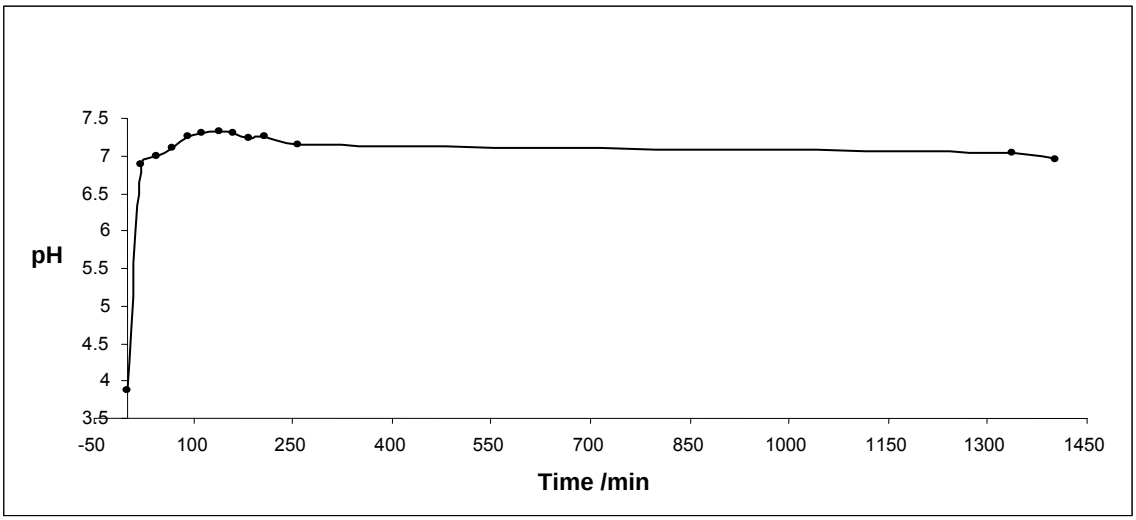

Figure 5.5 Evolution of pH with time for a manganese coated fossil bone placed in 1.6 mol L^{-1} hydrogen peroxide.

5.1.3 Manganese evolution

The quantification of the manganese dissolved during the bone cleaning process was determined by measuring the concentration of Mn^{2+} in the solution as a function of time. It was found that the concentration increases rapidly during the first hundred minutes, thereafter the rate decreases, and eventually levels off after approximately 3 hours. This indicates that equilibrium is reached after 3 hours. It is thus important for the reaction solution to be replaced regularly in order to maintain the forward reaction. Because of this equilibrium, optimal removal of the coating requires repeated immersion of the specimen in a fresh hydrogen peroxide solution on an hourly basis.

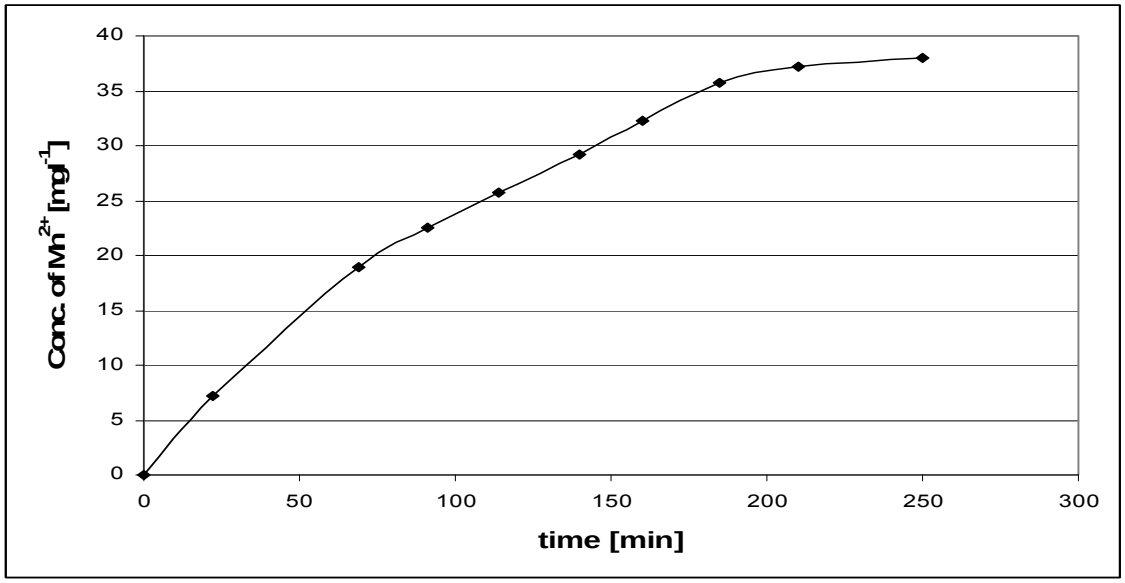

Figure 5.6 Manganese evolution as a function of time for a coated fossil specimen in a 1.6 mol L^{-1} hydrogen peroxide solution.

The rate of manganese evolution during the reaction between the oxide coating increases almost in a linear relationship until equilibrium is reached where the pH is above 5. Equilibrium is reached after 200 minutes for the above reaction. After equilibrium the reaction solution has to be replaced to maintain the pH (proton gradient) which is one of the main factors in the driving force of the forward reaction. After 200 minutes, at equilibrium stage, the concentration of protons in the solution is depleted and Mn(II) can be re oxidized back to Mn(III) and in some cases Mn(IV). This would result in competition for surface sites on the fossil bone, thus also slowing down the reduction of manganese oxide.

5.1.4 Calcium evolution

In order to determine whether this method of cleaning does not destroy the underlying bone, the concentration of calcium in the reaction solution was measured as a function of time. This experiment was performed for coated and uncoated fossil bones. It was determined that for the coated bone the calcium concentration increase was greater than that for the uncoated bone. It is thought that this may be as a result of the presence of calcium (finely divided $CaCO_3$) on or near the surface of the bone coating. As the reaction proceeds and the coating is removed, the rate of calcium evolution decreases. After 20 minutes the rate of calcium evolution equals the rate of calcium evolution for the uncoated bone. For both coated and uncoated bones the calcium concentration appears to level off, indicating that equilibrium conditions are reached.

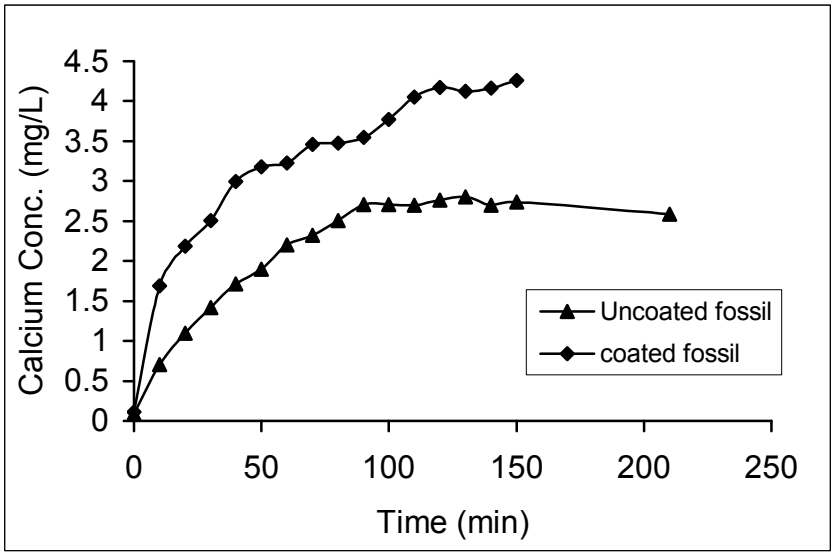

Figure 5.7 Calcium evolution with time for a coated and uncoated fossil bone treated in a 1.6 mol L^{-1} hydrogen peroxide solution.

From characterisation of the fossil bone coatings it was determined that the coating is mainly of a manganese/iron oxide in the form of birnessite. Birnessite is composed of manganese and oxygen as well as sodium and calcium. When the coated fossil bones are placed into a hydrogen peroxide solution and dissolution of the coating occurs, the calcium concentration would thus be expected to increase with time, which is shown to occur (fig. 5.7). This is validated by the fact that the rate of the calcium that evolves for the clean fossil bone is much lower, thus attributing the source of calcium being evolved to the coating and not the bone matrix. The calcium evolved when the clean fossil bone is placed into the hydrogen peroxide originate from loose soil debris and dust particles inside the porous matrix of the bone, which is also detached from the evolution of molecular oxygen produced during the main reaction. Once this material is removed and dissolved the pH value of the solution shifts closer to alkalinity, and thus the calcium concentration reaches equilibrium.

5.1.5. Optical and SEM validation

Sample surfaces were examined using optical and SEM methods to determine whether the method caused any visible surface etching or other damage to, the fossil. Images showed no visible signs of any damage or alteration to the bone surface.

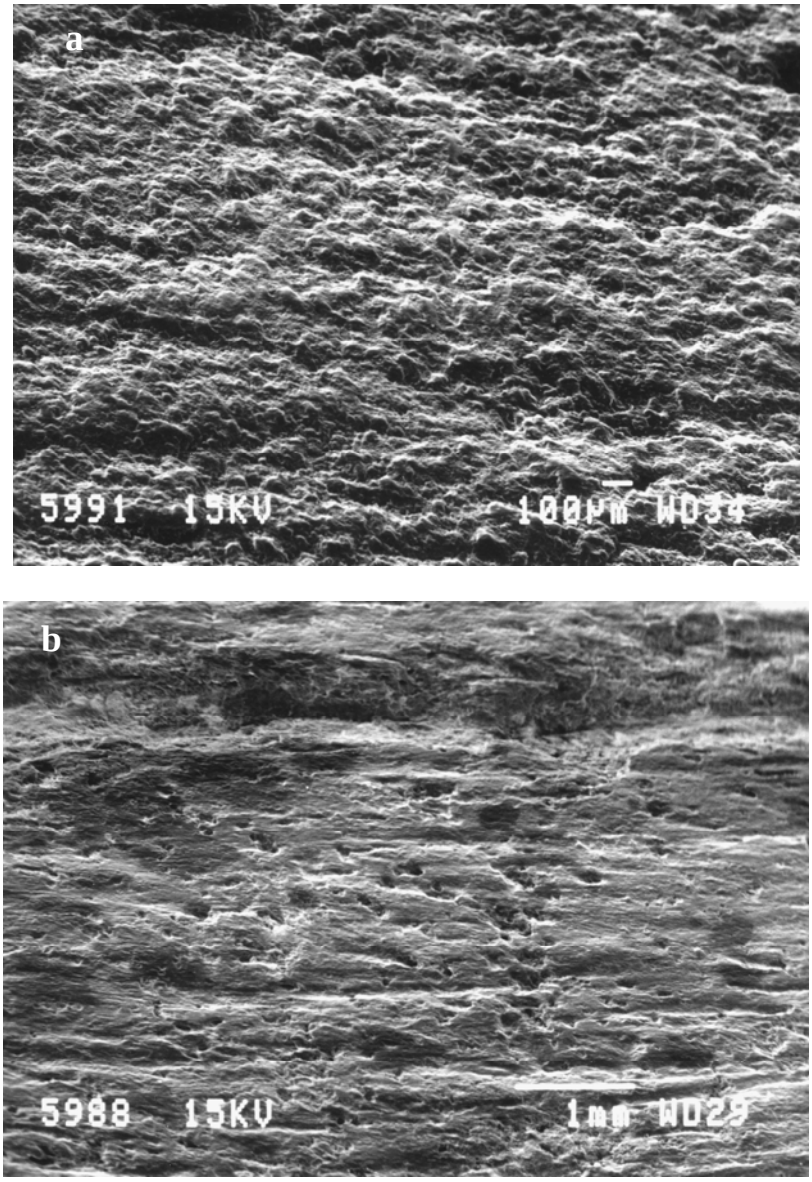

Figure 5.8 Scanning electron microscope image (16X magnification) for a manganese oxide coated bone surface **a**, before cleaning **b**, after cleaning in 1.6 mol L^{-1} hydrogen peroxide solution.

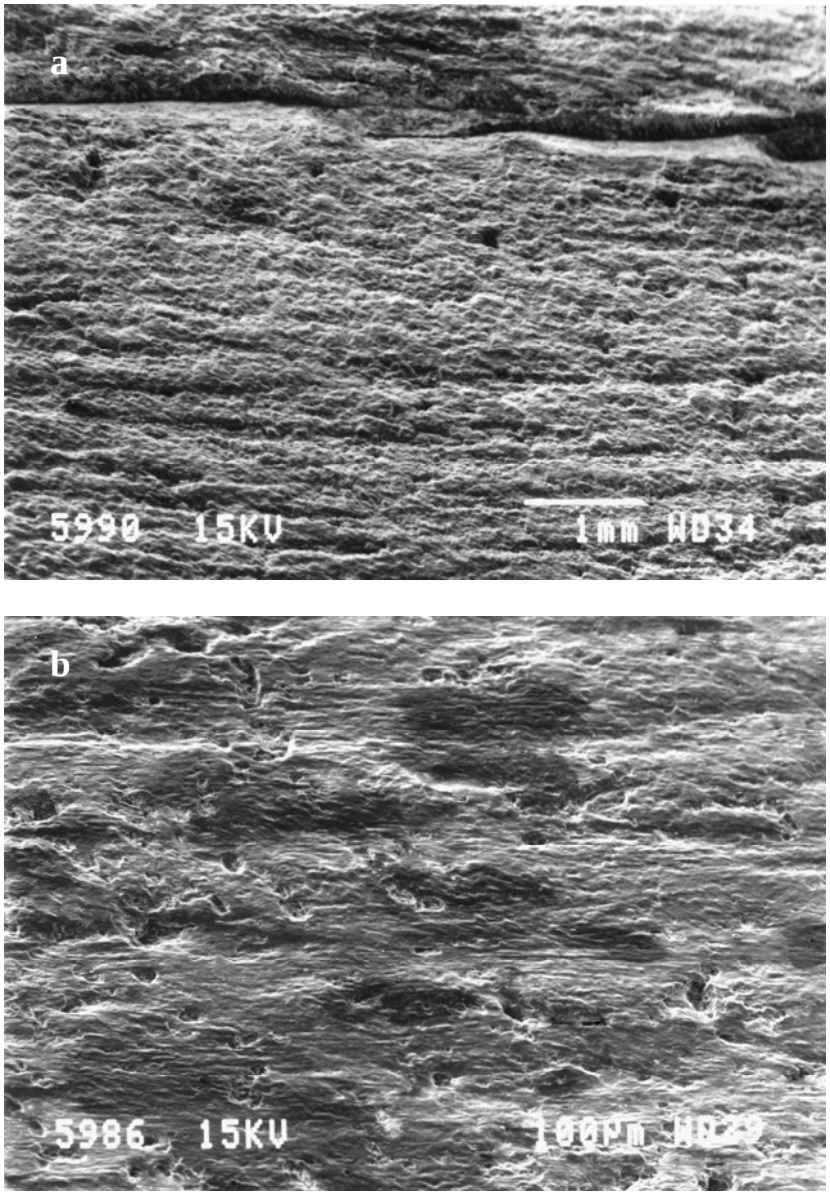

Figure 5.9 Scanning electron microscope image (40X magnification) for a manganese oxide coated bone surface **a**, before cleaning; **b**, after cleaning after cleaning in 1.6 mol L^{-1} hydrogen peroxide solution.

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Figs. 5.9a and b show the same bone surface, before and after removal of manganese coating using hydrogen peroxide. Before the coating is removed, the surface of the bone takes on a bubble wave like surface structure. Once this coating is removed as shown in

fig. 5.9b the exposed flouro hydroxy apatite matrix has a well defined appearance typical of bone matrix.

5.2 Removal of hematite from rocks and fossil bones using hydrogen peroxide

A piece of hematite rock, which had been mechanically removed from a fossil dinosaur bone using an air scribe, was placed into a 1.6 mol L^{-1} hydrogen peroxide solution. The hematite coating was removed by replacing the hydrogen peroxide solution after approximately every 2 hours.

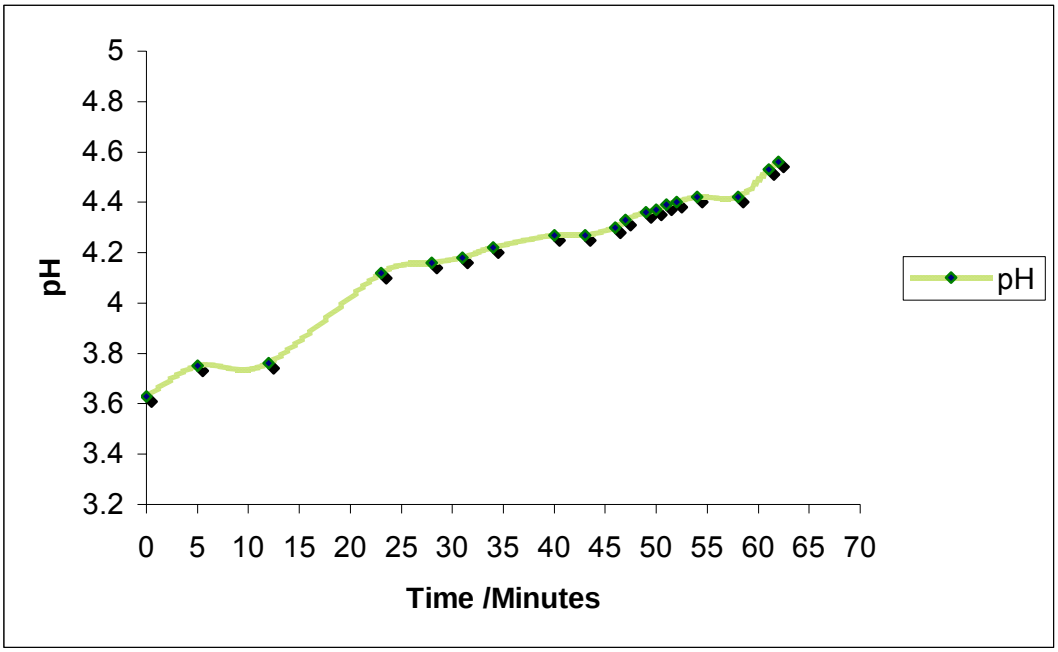


Figure 5.10 Evolution of pH with time for the reaction of 1.7 mol L^{-1} hydrogen peroxide on the surface of a hematite rock.

As the reaction between hydrogen peroxide and the hematite coating proceeds, the pH increases. However the rate of increase appears to be slower than that of the manganese oxide /peroxide reaction. This is opposite to what would be expected, as the redox potential of iron is much lower than that of manganese oxides, thus it would be more thermodynamically favourable for the iron to be reduced before manganese. A possible reason for the above trend in fig. 5.10 is the hematite samples appear to contain more silicious material and are heavier for their size. This could result in the hematite coating being composed of many other more resistant minerals that would increase the redox potential. These minerals would have combined with the coating during diagenesis thus making the coating and bone matrix more resistant to acid attack.

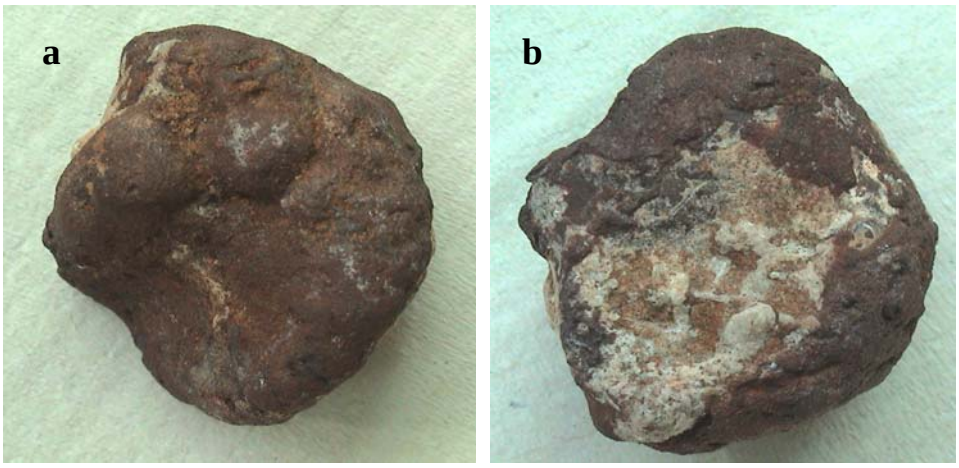


Figure 5.11 Hematite coated fossil bone specimen **a,** before treatment **b,** after treatment with 1.7 mol L^{-1} hydrogen peroxide showing partial removal of the top section of the coating.

The hydrogen peroxide removes some of the hematite coating from the crystallized fossil bone. As indicated by the Eh and pe diagrams for manganese and iron, in fig.5.34 the iron has a lower reduction potential so it would be expected to be removed from the above sample much easier than for fossil specimens coated with a manganese oxide layer. However as can be observed in fig.5.34 the opposite occurs. There are two possible reasons for this:

1) The hematite sample is remarkably different from all the manganese coated fossil bones in that the underlying material on which the hematite is deposited consists more of silica quartz materials, as opposed to the fossils coated with manganese that sits on a flouro apatite matrix. This flouro-apatite is mainly composed of calcium carbonate (Mann, 1996; Skinner, 2004) which could possibly have a partial catalytic effect by lowering the surface activation energy of many different redox reactions (Mann, 1996; White, 1997).
2) The samples are of different ages, thus the mineral diagenetic processes that occurred would have resulted in different minerals existing in the two samples. These different minerals play a major role in the reaction of hydrogen peroxide with bone coatings and the overall resistance of the bone specimen to certain chemical reactions.

5.2.1 Gravimetric analysis

The hematite rock was weighed before and after treatment with hydrogen peroxide. From the masses given below it was found that the percentage of hematite removed from the rock was 6% (w/w) of the total specimen mass.

Mass before treatment: 9.622 g

Mass after treatment: 9.000 g

5.3 Further optimisation of the hydrogen peroxide concentration used for the removal of manganese oxide fossil bone coatings

In order to determine the maximum rather than optimum concentration at which the removal of manganese coating can be achieved in a safe and efficient manner, the entire concentration range of hydrogen peroxide was tested. It is also important to note that not all the bones were of the exact same size and texture. This is important as these physical differences and features can play a major role in the surface chemistry and reaction kinetics of the manganese oxide-hydrogen peroxide reaction.

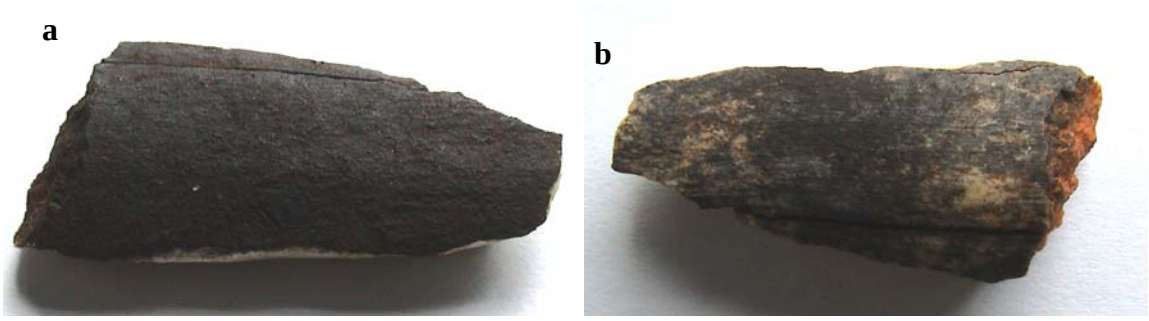


Figure 5.12 Manganese oxide coated fossil bone fragment **a,** before treatment with hydrogen peroxide solution; **b**, after treatment using 2.5 mol L^{-1} hydrogen peroxide.

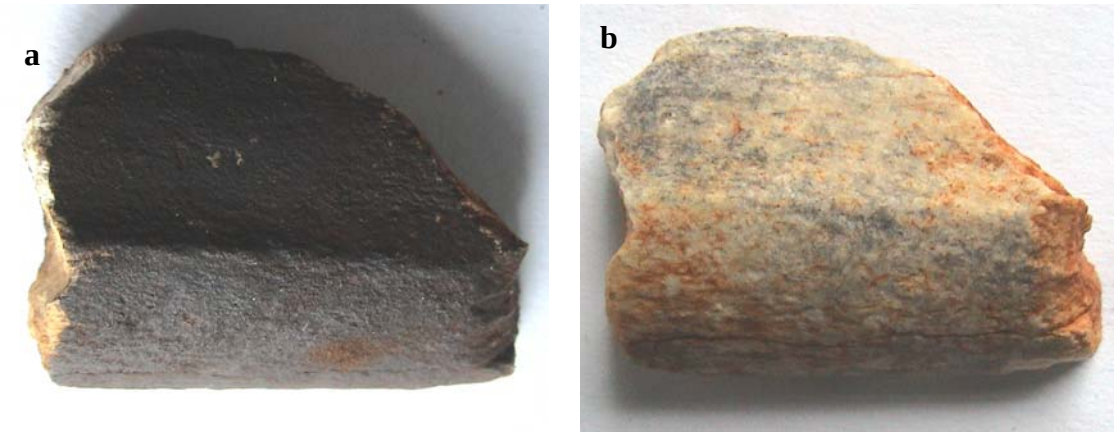


Figure 5.13 Manganese oxide coated fossil bone fragment **a,** before treatment with hydrogen peroxide solution; **b**, after treatment using 5.5 mol L^{-1} hydrogen peroxide.

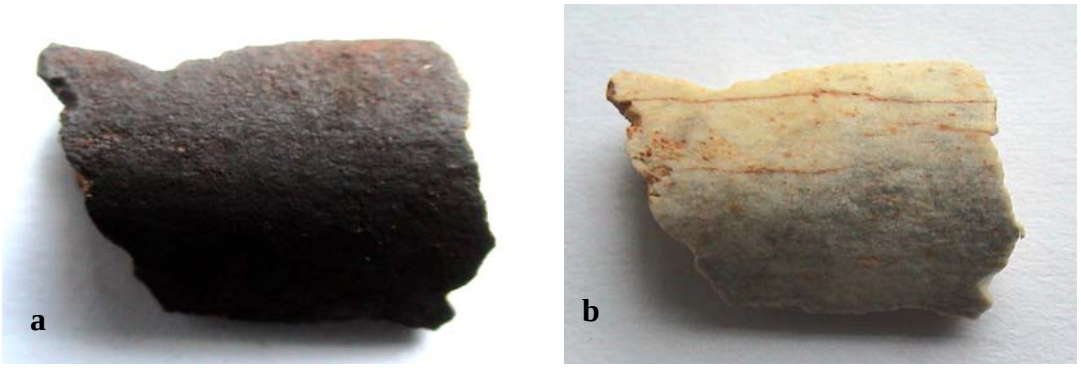


Figure 5.14 Manganese oxide coated fossil bone fragment **a,** before treatment with hydrogen peroxide solution; **b**, after treatment using 7.5 mol L^{-1} hydrogen peroxide.

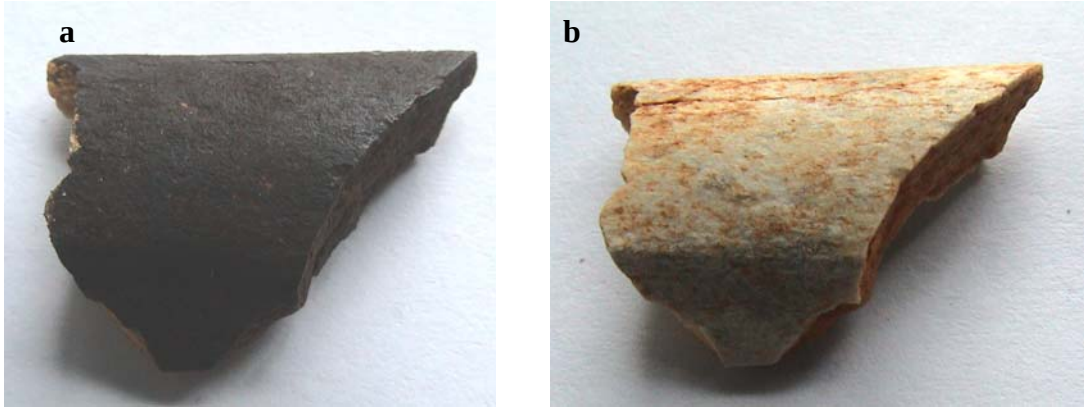


Figure 5.15 Manganese oxide coated fossil bone fragment **a,** before treatment with hydrogen peroxide solution; **b**, after treatment using 9.7 mol L^{-1} hydrogen peroxide.

From the above qualitative optimization, it can be observed that an increasing trend with regards to hydrogen peroxide concentration occurs. The 7.5 mol L^{-1}, 8.5 mol L^{-1} and concentrated hydrogen peroxide (9.7 mol L^{-1}) all appear to remove the manganese oxide coatings as efficiently, with no obvious damage (powdering and flaking-off) or surface etching of the fossil bones. Based on this the lower 7.5 mol L^{-1} hydrogen peroxide concentration can be classified as the optimum concentration of the cleaning solution to be used.

5.3.1 Kinetics features of the reaction

Kinetic experiments were performed to determine some of the basic kinetic parameters and factors of the reaction.

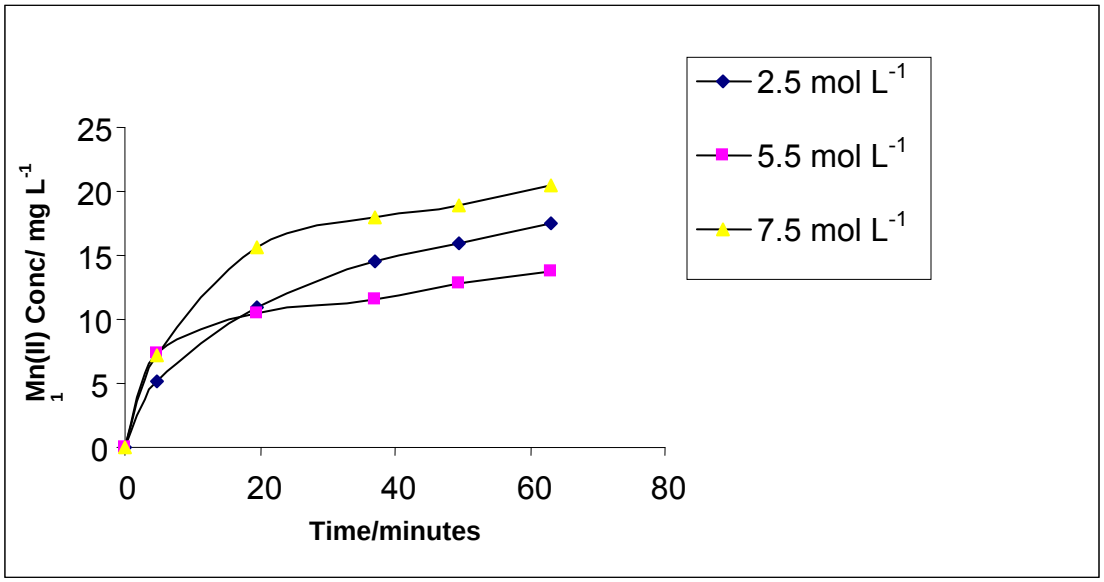


Figure 5.16 Rate of manganese(II) evolution trend for three different hydrogen peroxide concentrations.

The trend in the above figure is expected. For the 2.5 mol L^{-1} concentration of hydrogen peroxide coated fossil bone the rate is larger than that of the 5.5 mol L^{-1} solution. This is because the bone fragment used in the 2.5 mol L^{-1} experiment had a larger surface area that of the 5.5 mol L^{-1} bone. All fragments were derived from the same original coated fossil bone. As the concentration of the hydrogen peroxide is increased, the pH value of the solution is initially more acidic, thus the proton driving force for the reaction is greater, so equilibrium is reached later.

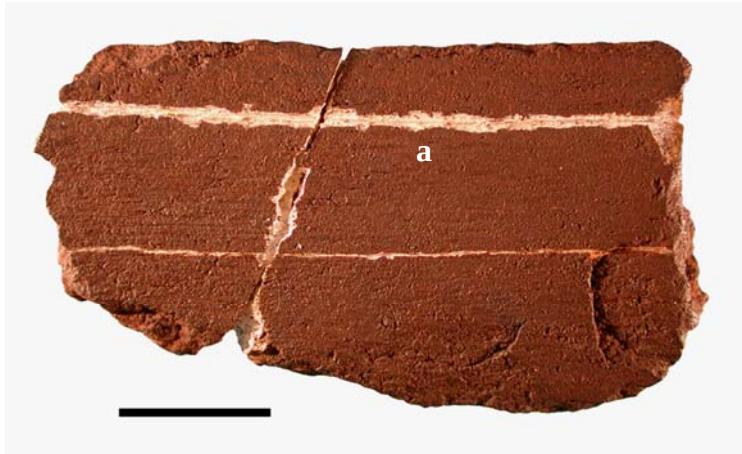
5.3.2 Validation

There are two main objectives in the validation process; firstly the hydrogen peroxide method was investigated to determine whether the underlying bone is damaged during the chemical cleaning process. This is of great importance to the success of the overall cleaning method as a significant amount of bone matrix dissolution will also alter the surface modification features that are important taphonomic indicators of each fossil excavated. Secondly, more intensive experiments were carried out in order to determine the chemical nature of these reactions occurring and to try and determine the basic mechanistic and kinetic features of the hydrogen peroxide reaction.

It has been observed that when bone dissolution occurs, the surface can either become more etched or smooth and powdered depending on the age, texture and type of bone-chemical interaction.

a) Artificially incised coated fossil bone

A manganese coated bone was broken into two equal fragments after two parallel incisions were made along one side. After treatment of the one fragment, the texture and diameter of the incisions were compared to determine whether the hydrogen peroxide method caused dissolution along the newly exposed bone.



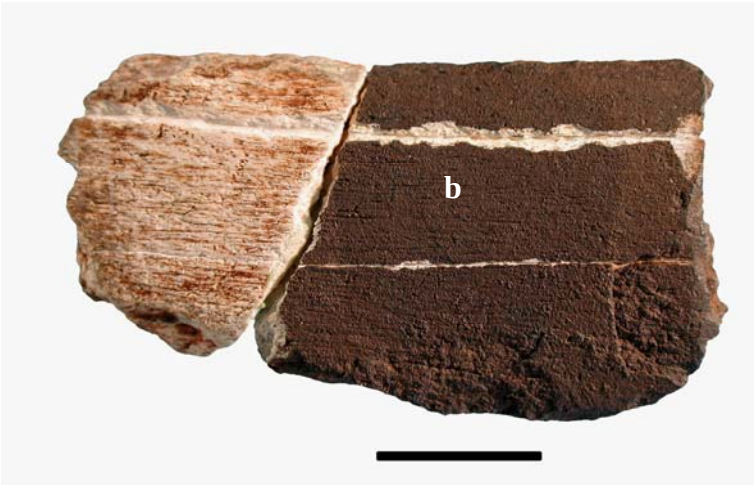


Figure 5.17 Optical image of manganese oxide coated fossil bone broken into two fragments **a,** both untreated; **b**, treated fragment (left) placed alongside the untreated fragment (right). The edges align with the untreated fragment and artificial grooves are still aligned after the smaller fragment is placed into a 1.6 mol L^{-1} solution of hydrogen peroxide and the manganese oxide coating is removed from the surface fragments placed together fit and artificial grooves are still aligned.

It can be observed and concluded from fig. 5.17a and b that there is no visible dissolution along the exposed terrace as both grooves align when placed back together. This is a very

important result in terms of validation of this method as it would be expected for dissolution to occur more readily along new un-weathered or non siliceous mineral replaced bone. The outer bone matrix is far more resistant to weathering or mineral dissolution due to diagenetic processes having already taken place in that region of the bone.

b) Preservation of taphonomic features

It has been shown that bone fragments coated with manganese oxide with visible taphonomic features to undergo even greater enhancement of those features once the specimen is treated with the hydrogen peroxide solution as shown in fig. 5.18 below.

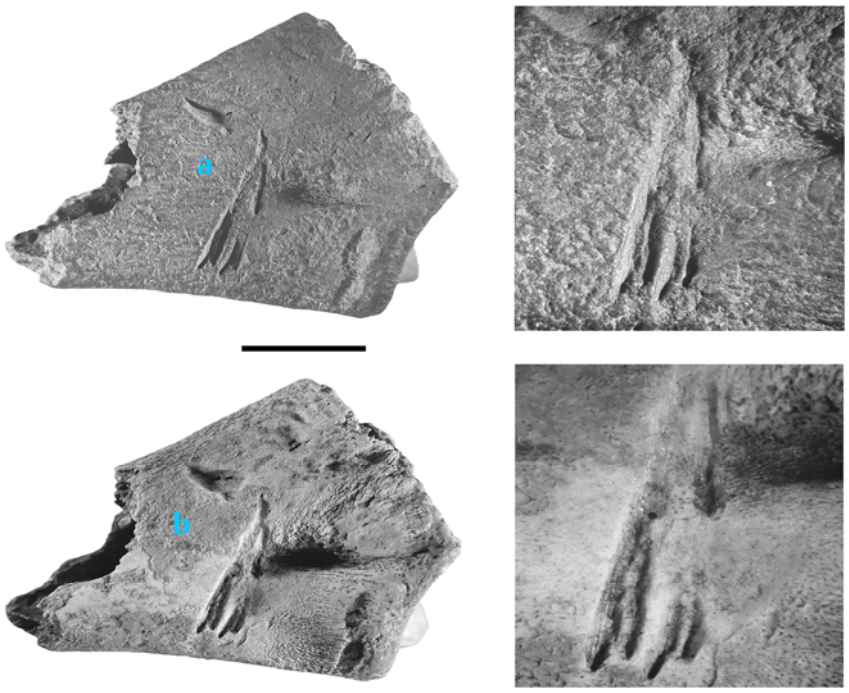


Figure 5.18 Fossil bone fragment with taphonomic features coated with manganese dioxide **a,** before treatment with hydrogen peroxide; **b,** after treatment with 1.6 mol L^{-1}

hydrogen peroxide showing surface modifications on the bone after the manganese dioxide is removed.

From the fig. 5.18 above it is observed that this cleaning method dramatically enhances the surface modifications on the bone surface while not altering the natural topographical bone structure. This is of great significance as previously vague and obscured, unidentifiable modifications can now be enhanced and studied using this cleaning method with no damage to the bone matrix.

Figure 5.19 Manganese dioxide coated fossil bone fragment broken into two pieces.

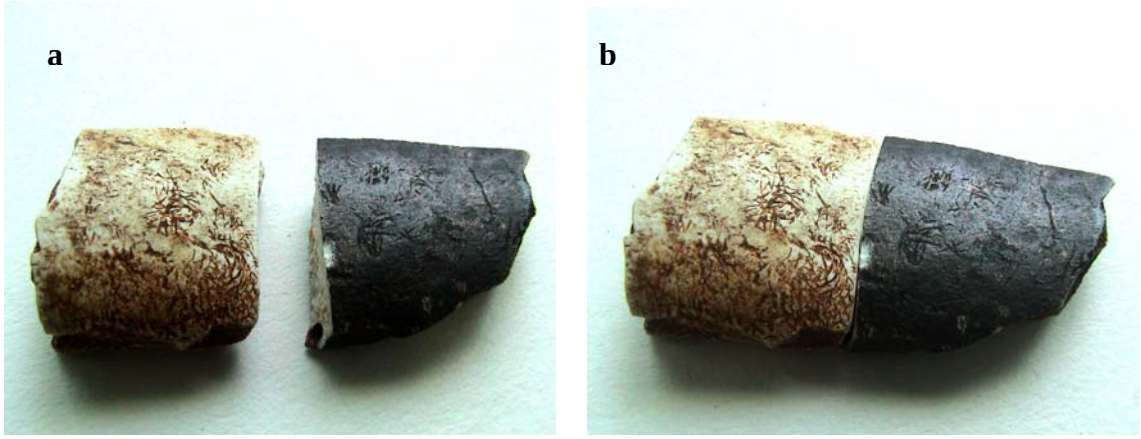


Figure 5.20 Fossil bone fragment broken into two fragments, showing the difference in surface features **a,** after treatment of one of the fragments (left) in a hydrogen peroxide solution; **b**, the same fossil bone fragment rejoined to the untreated bone fragment.

The above illustration and validation of the hydrogen peroxide cleaning method is a good illustration, as the surface modifications on the surface is slightly visible on the coated bone and then enhanced for the cleaned bone fragment thus validating that no overall change or alteration of the taphonomy or bone matrix occurs when the fossil bone is placed into the cleaning solution.

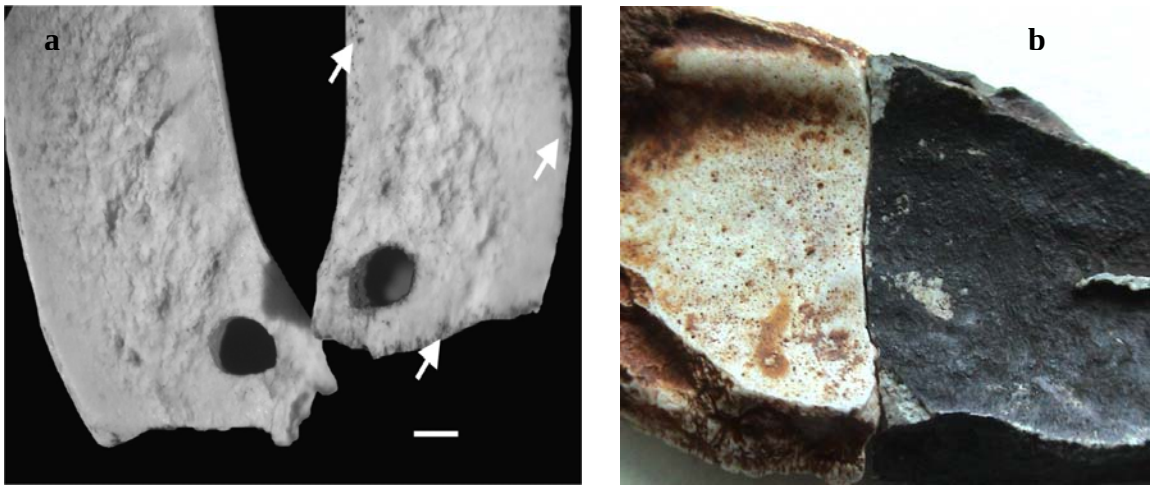


Figure 5.21 Fossil bone fragment broken into two fragments **a,** Cross section view of the treated (left) and untreated (right) fragments showing impregnation of the manganese oxide into the sub surface layer for the untreated fragment; **b,** underside view of the hydrogen peroxide treated (left) and untreated (right) fossil bone fragments.

The diameters of the two holes in each fragment in figure a where identical, thus proving that bone matrix dissolution does not occur.

c) Hollow bone validation

A maximum concentration of 9.7 mol L^{-1} hydrogen peroxide was selected so as to expose a fossil bone to the most severe optimized conditions that could be applied. The fossil hollow bone was carefully broken into two pieces. One of the fragments was placed in the 9.7 mol L^{-1} hydrogen peroxide solution. Using a vernier calliper the treated bone fragment's diameter and thickness from hollow to exterior surface was measured. These measurements were then repeated once the bone was removed from the treatment solution.

Figure 5.22 A hollow cylindrical manganese oxide coated fossil bone fragment broken into two pieces and rejoined before treatment in 9.7 mol L^{-1} hydrogen peroxide solution.

Figure 5.23 A hollow cylindrical manganese oxide coated fossil bone fragment after treatment in a 9.7 mol L^{-1} hydrogen peroxide solution rejoined to the untreated fragment.

After treatment using a 9.7 mol L^{-1} hydrogen peroxide solution both bone fragments fit back into their original position identically. This is a very important feature when determining whether bone dissolution is occurring, as the softer, less resistant middle matrix and sides would show or be prone to dissolution first.

Figure 5.24 Cross sectional view and comparison of the fossil bone fragment after treatment (left) with the untreated fragment (right).

Other features and measurements (diameters and thickness)

Before 30% hydrogen peroxide treatment

Inner vertical hollow cross sectional diameter: 4.95 mm

Inner horizontal hollow cross sectional diameter: 6.85 mm

After 30% hydrogen peroxide treatment

Inner vertical hollow cross sectional diameter: 4.94 mm

Inner horizontal hollow cross sectional diameter: 6.84 mm

5.4 Validation of three optimized hydrogen peroxide concentrations using long flat bones

A long flat fossil bone was broken into three fairly equal fragments and each fragment was placed in a different hydrogen peroxide concentration. The concentrations used were 7.5 mol L^{-1}, 8.5 mol L^{-1} and 9.7 mol L^{-1}.

Figure 5.25 Single long flat manganese oxide coated fossil bone broken into three approximately equal fragments and rejoined to fit before treatment in the hydrogen peroxide solutions.

Figure 5.26 Enlarged view of a joint between two of the coated fossil fragments illustrating the fitting pattern.

Figure 5.27 View of two adjacent fragments showing perfect realignment before hydrogen peroxide treatment.

Figure 5.28 All three individual fragments before and after treatment using three different optimized concentrations of hydrogen peroxide.

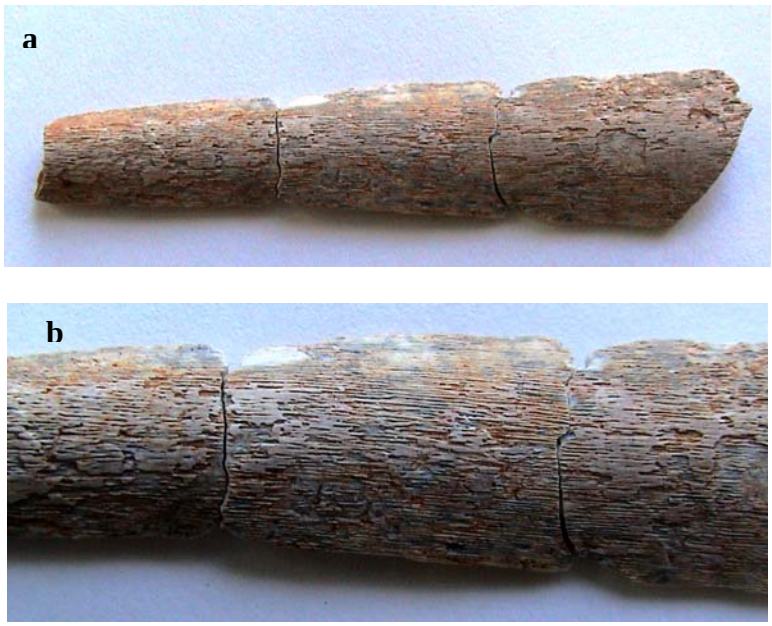


Figure 5.29 Surface features for all three fragments **a,** showing perfect re-fitment and realignment after treatment with the three different optimized concentrations of hydrogen peroxide; **b,** enlarged image of the three cleaned bone fragments rejoined.

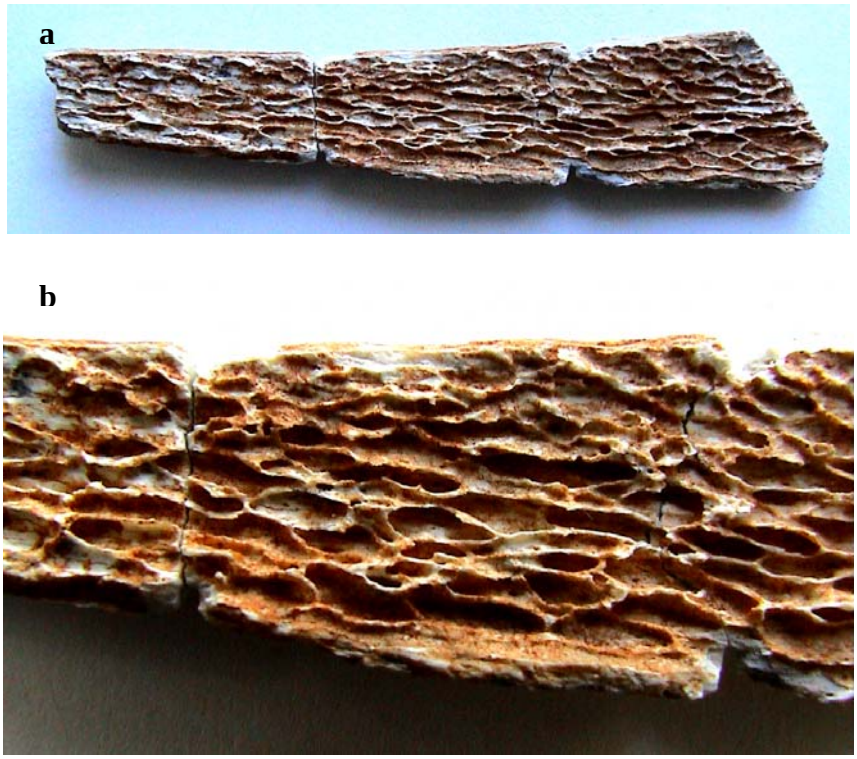


Figure 5.30 Underside view of the break zones for a fossil bone broken into three fragments treated in three different optimized hydrogen peroxide concentrations **a,** three bone fragments rejoined; **b,** enlarged image of the underside break zones showing perfect re fitment and re-alignment after the three coated fossil bone fragments were treated with three different optimized hydrogen peroxide concentrations.

Figure 5.31 Enlarged image of the underside of two rejoined sections of the treated fossil bone fragment.

It can be observed in figs. 5.30 and 5.31 that after treatment of the manganese oxide coated fossil specimen, which was fragmented into three pieces, the manganese coatings are efficiently removed and complete realignment of the bone is achieved when placed back together. The most consolidating evidence for validating that bone matrix dissolution is not occurring, comes from the fact that the ripple like underside surface of the bone realigned with each groove perfectly preserved with sharp edges. If mineral dissolution of the bone was occurring, these sharp edges would be smoothed considerably and the edges would not join edge to edge.

Most fossil bone specimens that are covered with manganese oxide coatings do not exhibit this web-like feature, and it is only visible once the coating and organic material from soil is removed.

5.4.1 Scanning electron microscopy validation

In order to validate the developed and optimized hydrogen peroxide method on a microscopic level, SEM was used to observe the surface of coated bone fragments before and after treatment using the optimized hydrogen peroxide solution.

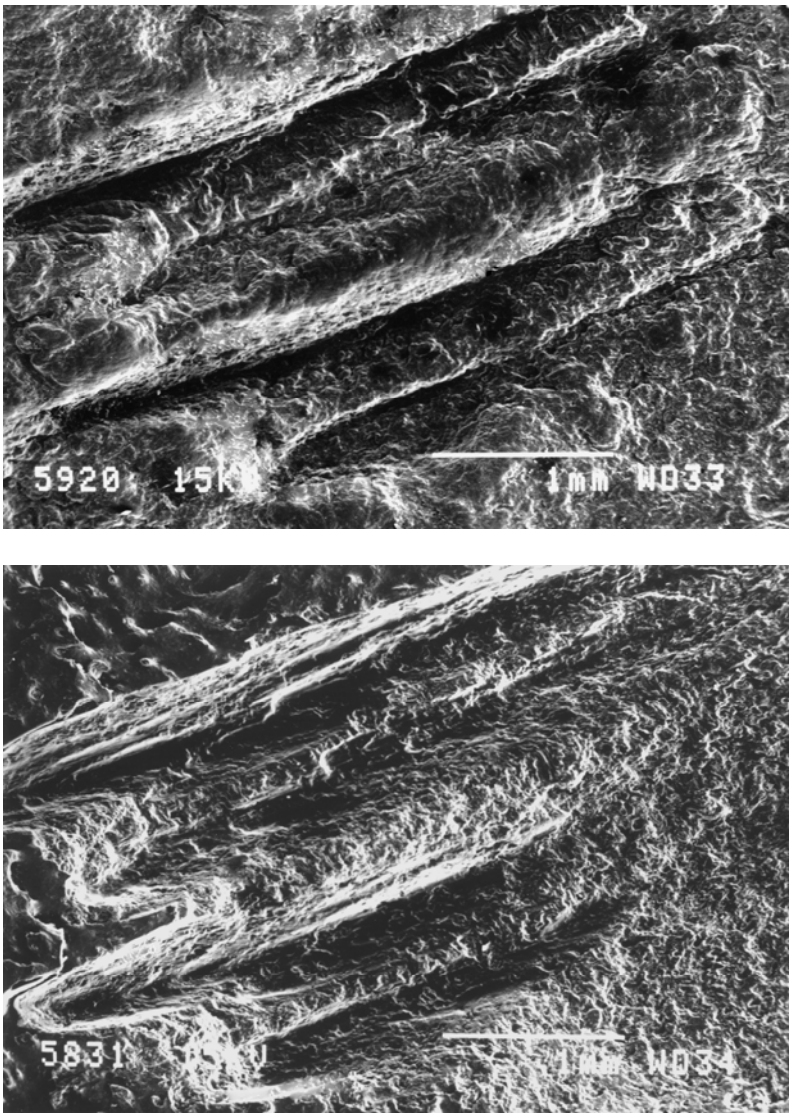
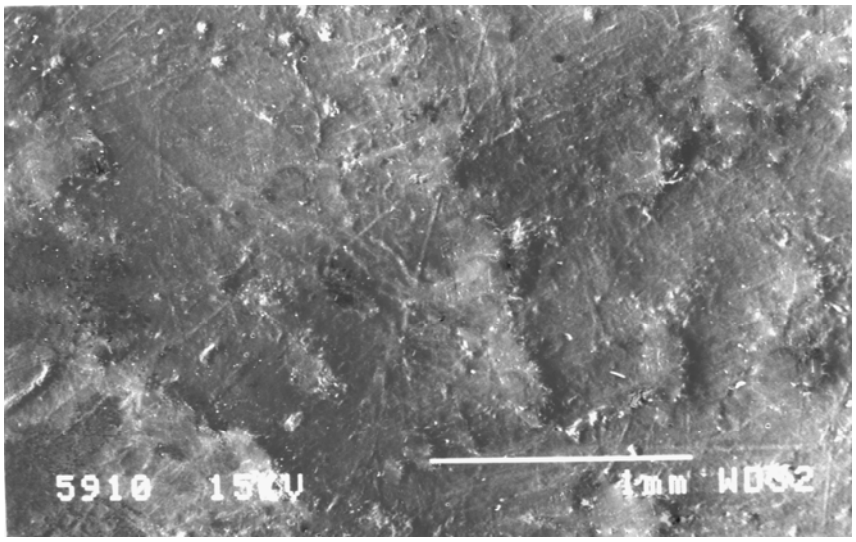


Figure 5.32 Scanning electron microscope image of surface modifications on bone covered and obscured by a manganese coating; **a,** before treatment in the hydrogen peroxide solution **b,** the same modifications after treatment with hydrogen peroxide, identified as stone tool cut–marks based on the presence of fine internal striations.

Figure 5.32a shows an SEM image of a resin replica of manganese-coated bone surface containing modifications. In figure 5.32b the identical surface is shown after cleaning. The definition on the grooves in the cleaned image is greatly enhanced, revealing micro-striations within the grooves that are characteristic of cut-marks. This is evidence of early human behaviour that was not visible before the fossil specimen was cleaned using hydrogen peroxide.

5.4.2 Validation using an uncoated fossil bone

Validation using an uncoated fossil bone fragment was performed to determine the direct effect the treatment solution (hydrogen peroxide) has on the bone surface.



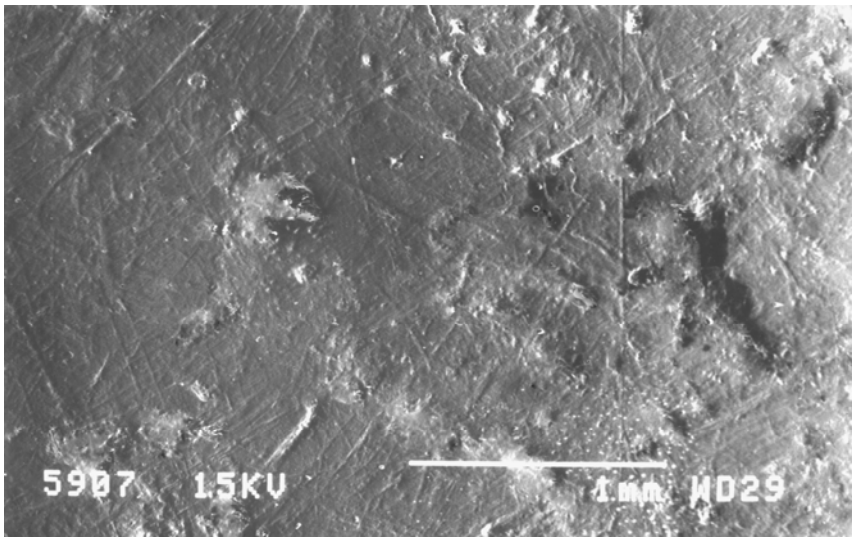


Figure 5.33 Scanning electron microscope image of the surface of; **a,** an uncoated bone flake with virtually no manganese coating; **b,** the same surface after treatment with hydrogen peroxide, with no evidence of surface alteration having occurred.

Fig. 5.33a shows a resin replica of a control surface on a bone that was almost entirely free of manganese oxide coating before treatment; Fig. 33b is of a replica of the same surface after exposure to a hydrogen peroxide cleaning solution. The surface shows no evidence of etching or other alteration by the peroxide solution.

5.4.3 Oxygen isotopes

Oxygen isotope readings were checked to determine whether the cleaning treatment solution had any effect on the oxygen isotopic signatures of fossil remains. Oxygen isotopes are used to determine the dietary behaviour of mammals and have environmental implications. No evidence of oxygen isotope changes were recorded.

5.5 Reactivity of manganese oxide, oxygen evolution and synthetic manganese iron oxides

Manganese oxides can act as catalysts in the decomposition of hydrogen peroxide (Hasan *et al*., 1999; Deraz *et al*., 2002) as well as be reduced by hydrogen peroxide under acidic conditions (Banerjee *et al*., 1997; Evangelou, 1998). This dual capability of hydrogen peroxide is important when investigating the true nature and mechanism of the reaction. The reduction of manganese and iron oxides has been shown to require the use of protons; however manganese reduction differs from that of iron as it can often occur at high pe in the presence of oxygen (Bartlett, 1981).

In order to differentiate between the reactions observed that occurs on the bone surface and a generalized manganese oxide/hydrogen peroxide reaction, synthetic manganese/iron oxide was precipitated in the presence of calcium carbonate. When the synthetic derivative was placed into the identical hydrogen peroxide solution under the same conditions as those used in the fossil bone oxide removal process, dissolution of the solid manganese and iron oxides did not occur. Only disproportionation of the hydrogen peroxide occurred. This proves that certain properties of the fossil bones, i.e. carbonate surface and stratigraphy could be a major factor in catalyzing the dissolution reaction.

Another important factor in the cleaning mechanism of the fossil bones and teeth is the evolution of oxygen at high concentrations. As oxygen is evolved, it moves along the bone surface and also escapes from within the porous structure of various bones to eventually diffuse out of the solution. This collective physical action of oxygen is also thought to weaken and in some cases dislodge small pieces of manganese and iron oxide from the bone surface.

5.6 Surface potential

The redox potential of hydrogen peroxide and manganese oxides are not in the correct orientation, as shown on the Eh diagram in fig. 5.34, to facilitate the direct reduction process. There appears to be a lowering of the surface potential that allows the redox potentials to switch, so as to cause the reduction of manganese dioxide by hydrogen peroxide.

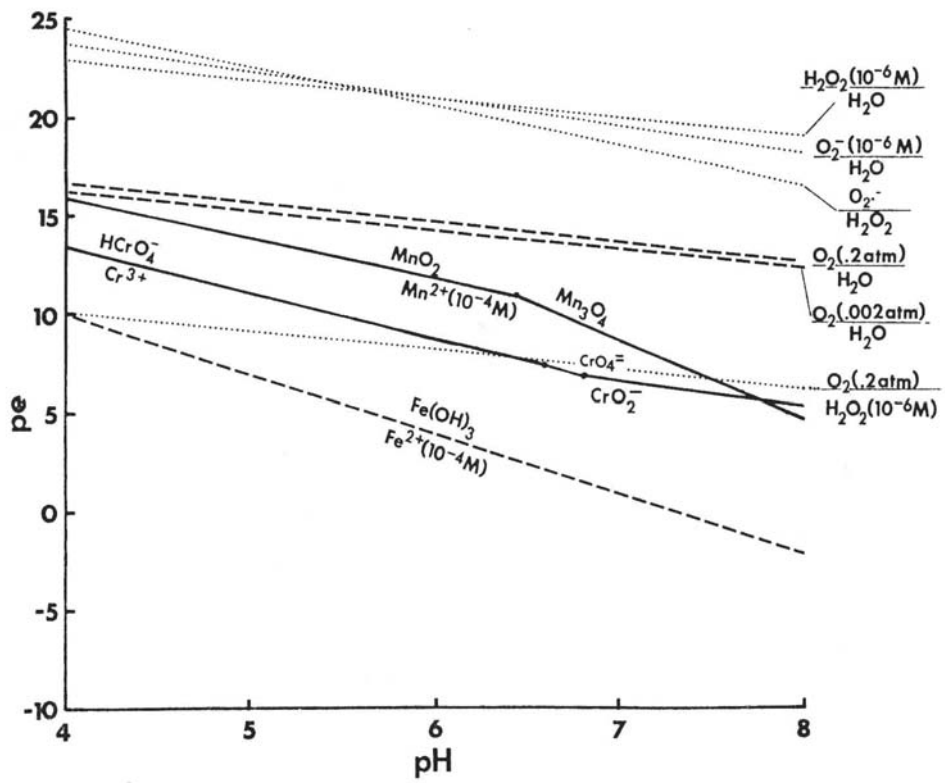


Figure 5.34 Stability lines between oxidized and reduced species for several redox couples. Solid phases of manganese and iron are at unit activity, and activities of other species are designated if not equal within a couple (Bartlett, 1981).

From fig. 5.34 it is clear that there had to be a change in the pe of the MnO_2 in order for the hydrogen peroxide to be oxidized, and in turn reduction of the manganese oxide to occur. Over and above the proton gradient, which is a key factor in the driving force of this reduction reaction, another major factor could be a lowering of the surface potential on the surface of the manganese oxide coated fossil bones.

5.7 Influence of specimen pre-treatment

Certain sample pre-treatment methods and procedures were investigated to determine the effect they have on the overall reaction with respect to cleaning time and efficiency.

5.7.1 Rinsed versus un-rinsed fossil bones

Using the previously optimized hydrogen peroxide conditions, two bones were prepared from the same bone fragment. One bone was lightly rinsed with deionised water so as to remove only loose soil and any organic debris. The other bone was left untreated. Both specimens were then placed into two hydrogen peroxide solutions each with the same concentrations and pH values.

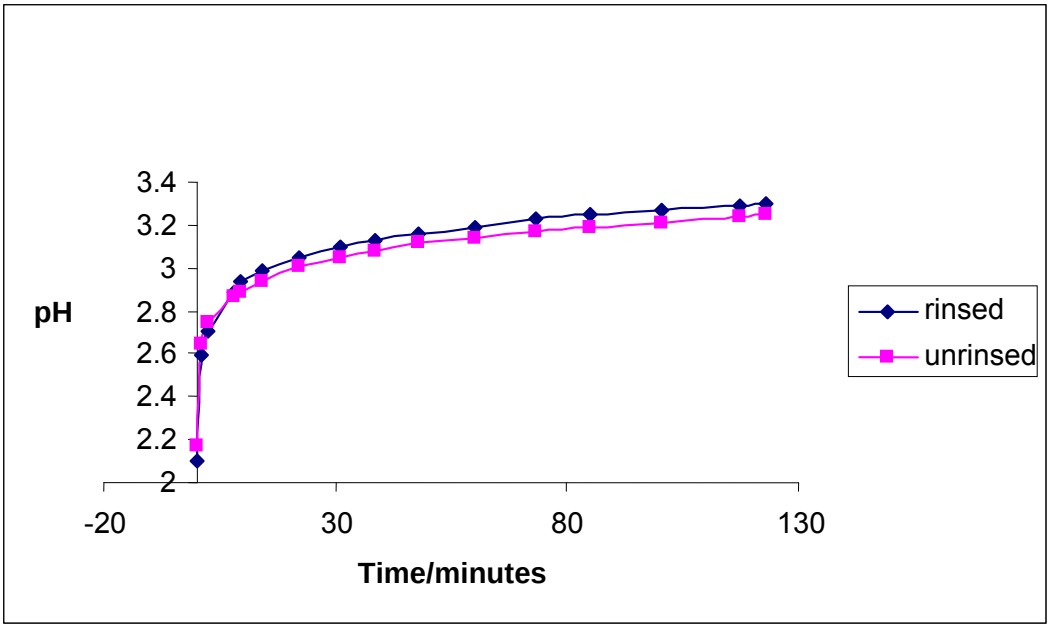


Figure 5.35 pH and time relationship for a manganese oxide coated fossil bone lightly rinsed with deionised water and the other fragment placed in 7.5 mol L^{-1} hydrogen peroxide solution unrinsed.

There is a similar relationship between pH and time for both rinsed and unrinsed; however the rinsed bone has a slightly higher rate of pH increase. This slightly elevated rate for the rinsed bone could be due to less competing oxidation of organics on the surface of the specimen and the more rapid penetration of the peroxide solution into the surface sites of the manganese oxide coating, resulting in faster reduction of the mixed manganese iron oxide layer (Hem, 1978; Stumm and Wollast, 1990).

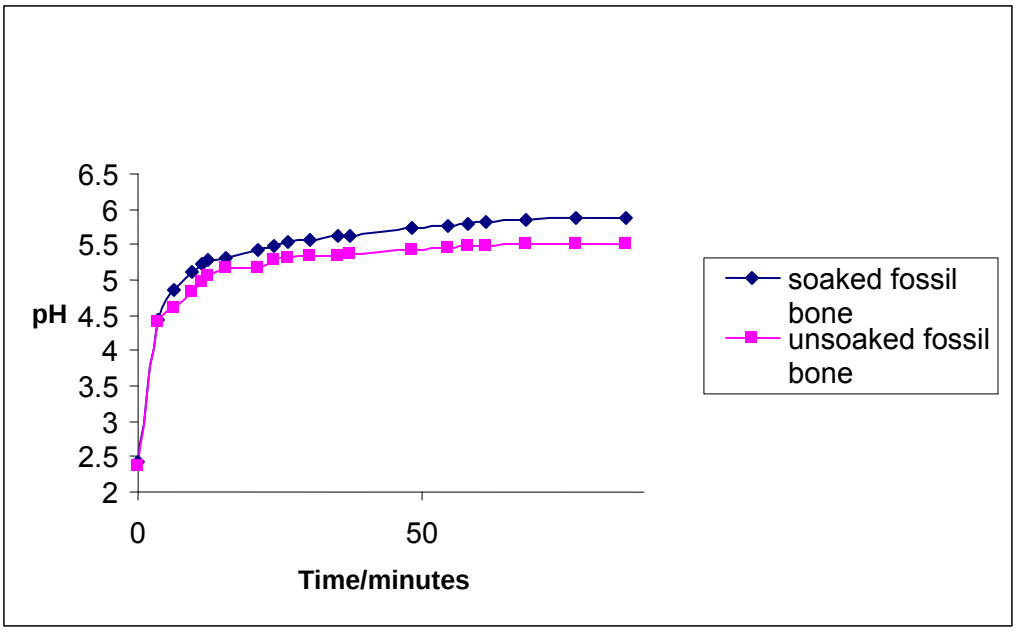


Figure 5.36 pH and time relationship for a manganese oxide coated fossil bone soaked in deionised water and the other fragment placed in 7.5 mol L^{-1} hydrogen peroxide solution without soaking.

Bone soaking also appears to have a similar, but slightly greater effect on the rate at which equilibrium is reached. There are two main considerations that need to be taken into account when trying to explain the above trends.

1) By soaking the fossil specimen, the water penetrates deep into the porous structure of the bone matrix and dilutes the hydrogen peroxide entering the bone. This effect could thus result in the equilibrium of the reaction taking slightly longer to be reached.
2) As water is also known to have an effect on the redox chemistry of manganese oxides in soils, it is assumed the same effect could take place when water is absorbed into the matrix containing the oxide coating. This would result in pre-

reduction/oxidation reactions taking place (Evangelou, 1998), thus lowering the surface potential and Eh even before the main reductant, namely hydrogen peroxide is added. This can be partially observed as the pH for the soaked bone rises more rapidly with respect to time.

3) Another possible reason for the soaked and rinsed bones having a sharper increase in the pH with time could be due to the fact that all or most surface organics are minimized or removed, thus preventing the peroxide from being reduced and lowering its reducing capability on the manganese oxides. Thus when the organics and siliceous materials are removed the manganese can also have a more direct effect in the decomposition of the peroxide, and also be reduced directly by the peroxide with fewer side reactions, thus explaining the faster kinetics towards alkaline pH value for the soaked or rinsed samples.

5.8 Multiple specimen cleaning experiment

5.8.1 Experimental set up

A larger scale cleaning operation was undertaken in order to test and implement the optimized hydrogen peroxide method for large scale use. The outcome of the cleaning operation would be to study the previously covered surfaces of approximately 7000 bone pieces all excavated from various deposits from Coopers cave in the Cradle of Humankind.

5.8.2 Flow through system

The main idea of the flow through system was to ensure the optimization of the cleaning process and to reduce the labour intensive aspect of the cleaning procedure. By removing the reacted hydrogen peroxide solution containing Mn(II), and replacing it with fresh hydrogen peroxide solution, the equilibrium of the system would be driven to favour the forward reaction.

Furthermore, the reverse reaction for the oxidation of Mn(II) to Mn(IV) in the presence of hydrogen peroxide is also a possible competing reaction (5.4) (Evangelou, 1998).

$$Mn^{2+} + H_2O_2 \leftrightarrow MnO_2 + 2H^+ \qquad 5.4$$

The reaction solution has to be replaced to maintain the proton gradient that is an important driving forces for the manganese oxide reduction reaction, and to remove the Mn(II) produced in order to:

a) Drive the forward reduction reaction (5.1).
b) Prevent the reverse reaction (5.4) whereby the Mn(II) is re-oxidised to manganese oxide.

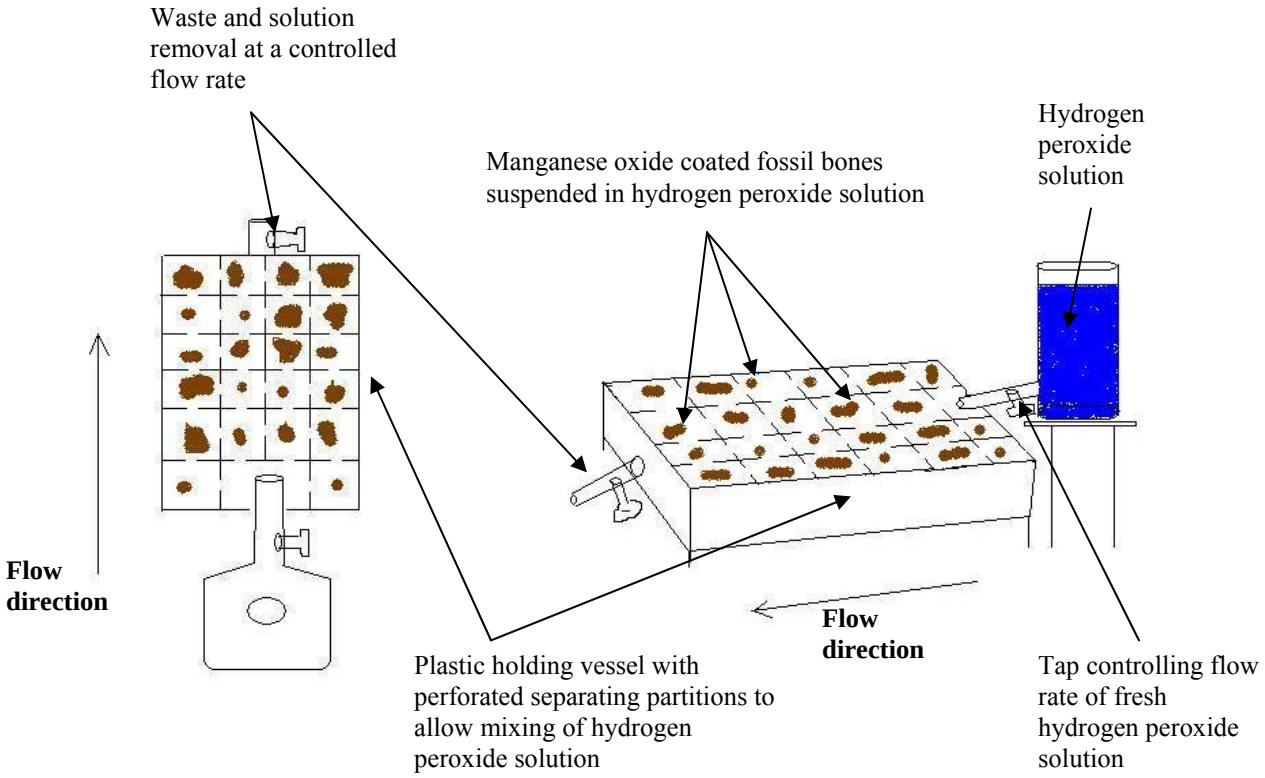


Figure 5.37 Hydrogen peroxide flow through system used for the cleaning of approximately 7000 manganese dioxide coated fossil bone fragments.

The above system was set up at a 15 degree angle so as to facilitate the removal of any loose debris removed, but not dissolved from the bone coatings. The debris would collect at the bottom and be removed on a daily basis. Each fossil specimen was suspended into the hydrogen peroxide solution to ensure equal cleaning on all surfaces of the sample. The specimens were also separated from touching one another by a plastic perforated partition placed inside the holding tray.

Typically, the initial pH of the hydrogen peroxide solution in the holding tray is approximately 2.9. As the manganese oxide coatings on each fragment react in the solution, the solution pH initially increases fairly rapidly, thereafter slowing down. In

order to maintain the efficiency of the reaction the flow through system is designed with a waste tap located at the bottom of the tray. The solution that has already reacted with each manganese oxide coated bone fragment suspended in the hydrogen peroxide solution is then released out at a constant flow rate so as to not remove all the fresh solution entering the system from the top container. This flow through system thus achieves two main objectives:

1) By removing the waste products of the reaction it is ensured that the forward reaction is favoured, thus removing more manganese oxide from the fossil bone fragments.

2) Sediment that would normally accumulate at the bottom of the container is also removed, which ensures that the hydrogen peroxide does not react with any material surfaces other than the manganese oxide coating on the bone fragments.

5.9 Catechol/hydrogen peroxide combined cleaning method investigation

The role of catechol for the aided removal of more resistant manganese oxide coatings was also investigated. Catechol is an organic ligand that is known to reduce manganese oxides in soils (Matocha, *et al*., 2001) and is part of the hydroquinone family which has been extensively studied with regard to their reduction capabilities of various metal oxides in soil environments.

The experiments performed using catechol where carried out on a pilot study level.

Figure 5.38 Fossil bone fragment before treatment with a combination of first catechol and followed by hydrogen peroxide solution.

Figure 5.39 Manganese oxide-coated fossil bone fragment after catechol treatment showing the thick dark polymeric layer from the oxidation of the catechol by the manganese oxide at the bone surface.

Figure 5.40 After hydrogen peroxide treatment the thick dark polymeric catechol surface coating is removed.

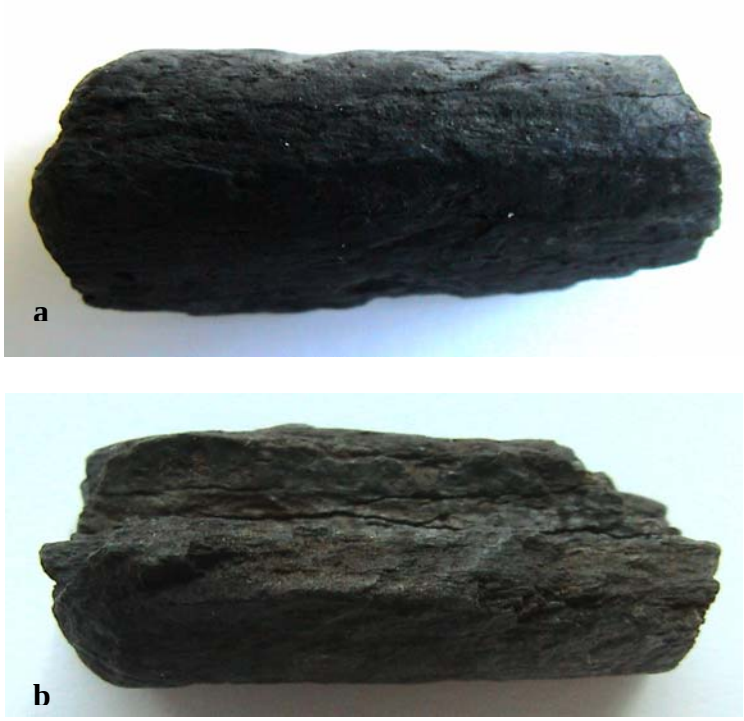


Figure 5.41 Top **(a)** and underside **(b)** view of a fossil bone fragment after treatment in a 0.1 mol L^{-1} catechol solution.

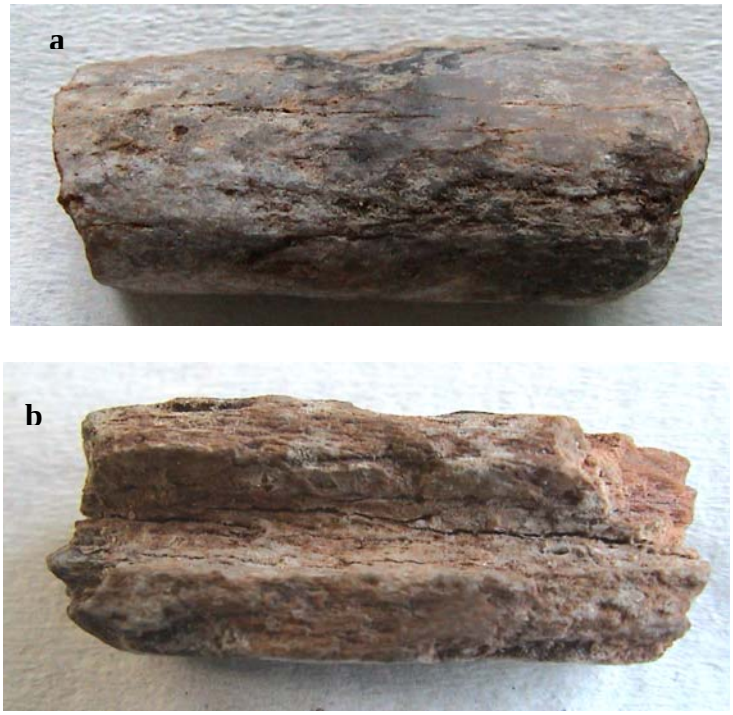


Figure 5.42 Top **(a)** and underside view **(b)** of a 0.1 mol L^{-1} catechol treated fossil bone fragment after treatment in a 1.6 mol L^{-1}hydrogen peroxide solution.

5.10 Manganese oxide-coated fossil teeth

Fossil teeth were found to clean much faster and more efficiently than bone specimens. This could be due to the nature of the surface of the teeth as opposed to the bone surface, which is far more irregular, with a more porous structure. The concentration optimized and used for cleaning teeth was far lower as a result of the ease at which the manganese oxide coating was removed. A concentration of 1.6 mol L^{-1} hydrogen peroxide was used for all tooth cleaning procedures. Specimens were found to possess manganese coatings varying in thickness from-1 nanometer to a few millimeters. The cleaning of fossil tooth specimens is important as teeth can show the animal's eating habits and immediate

environment. Texture and composition differ from bone (apatite and flouro hydroxy apatites) depending upon the age of the bone.

5.10.1 pH dependence for hydrogen peroxide reaction with manganese oxide coated fossil teeth

Manganese oxide coated fossil teeth were rinsed with water to remove loose debris and the specimens were placed in 1.6 mol L^{-1} hydrogen peroxide for approximately 2 hours.

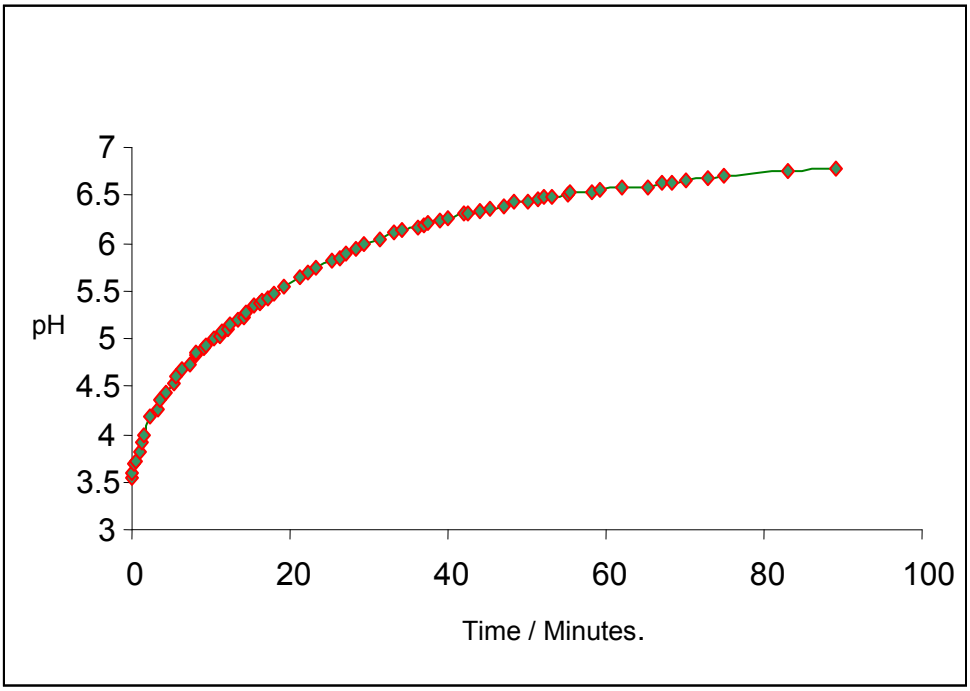


Figure 5.43 pH and time relationship for the reaction of hydrogen peroxide (1.6 mol L^{-1}) with a manganese oxide coated fossil tooth specimen.

The relationship between pH and time shows a similar trend for fossil teeth specimens as observed for fossil bones for the removal of manganese oxide. One difference however is the initial increase in pH for the fossil teeth. This increase is less rapid for the reaction of hydrogen peroxide with teeth than for fossil bone specimens. This could be due to the different surface textures and topographies for bone versus tooth samples. The evolution of oxygen for teeth would appear to be more regular due to the non-porosity of the sample. A porous sample, as in the case of most bones, would produce fluxional changes in the amount of oxygen evolved from the solution and sample. This is because the porous structure can trap already produced gas in the bone cavities, resulting in flux evolution of oxygen. This could explain the more gradual increase in pH versus time for the tooth specimen. According to the overall reaction an increase in the oxygen evolution would drive the forward reaction and thus increase the pH of the solution at a greater rate. Equilibrium appears to be reached faster for the fossil bones than teeth. This could be attributed to the porosity of the bones which increase the overall surface area of the reaction surface, thus increasing the rate of manganese reduction and similarly the rate of hydrogen peroxide decomposition.

Figure 5.44 Fossil tooth specimen heavily coated in manganese oxide obscuring all surface detail; **a,** before treatment in a 1.6 mol L^{-1} hydrogen peroxide solution; **b,** Anterior view of a fossil tooth after treatment in a 1.6 mol L^{-1} hydrogen peroxide solution; **c,** posterior view of the cleaned fossil tooth.

From the figures above, it can be observed that the fossil tooth is efficiently cleaned after the treatment using hydrogen peroxide. It is important to note that the hydrogen peroxide also has a bleaching effect on the tooth enamel. Hydrogen peroxide is used in the dental field as a tooth whitening agent, thus the above observation is expected for this sample type. The time required to achieve total removal of the thick manganese dioxide coating was also shorter (2 hours with no replacement of the cleaning solution required). This increased efficiency and rate of removal of the coating could be due to the difference in surface structure and composition between fossil bone and tooth material. Fossil teeth have a markedly flatter, smoother and less porous texture. This would mean that the tooth has less sites of attachment for the manganese to bind to the surface, making it weaker.

The porosity of the bone allows the manganese to impregnate deeper into the matrix, thus making it more difficult to remove via physical and chemical processes.

5.11 Case study (Pancake)

Pancake is the name given to (COB 707) a hominid cranial specimen that has been compressed over millions of years. The deformation was due to fragmentation in its burial site over thousands of years followed by compaction into a distorted fossil specimen.

The specimen was rinsed with deionised water to remove any loose unwanted debris and organics which could cause heat generation from the oxidation reaction with hydrogen peroxide. Any excessive heat could cause weakening and possible specimen damage. The specimen was then placed in the orientation as shown in fig. 5.46b in a 1.6 mol L^{-1} hydrogen peroxide solution. The solution was replaced every 24 hours. Some of the darker more resistant coated areas were also treated by using a soft sponge.

Figure 5.45 Close up view of a dark manganese oxide coated area which is one of many located on the fossil specimen.

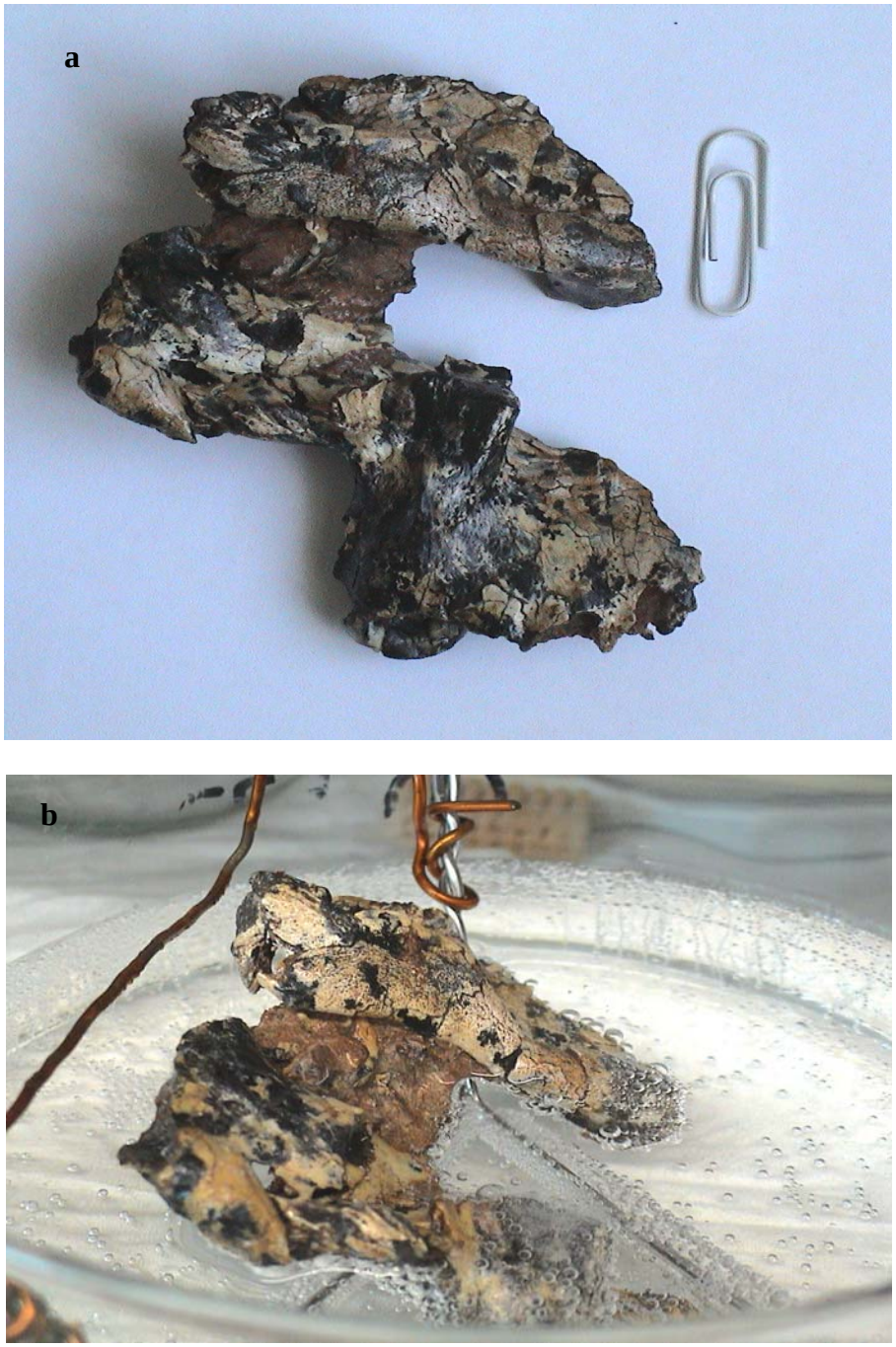


Figure 5.46 Top view of 'Pancake' **a,** before treatment using a hydrogen peroxide solution; **b,** reaction of 1.6 mol L^{-1} hydrogen peroxide with extensive oxygen evolution at the manganese coating on the fossil bone surface.

After the cleaning process was complete, the specimen was re-evaluated by palaeontologists, and the interpretation of the specimen was modified due to the fact that more detail could be seen after the removal of the manganese oxide coatings.

5.12 Method overview

The optimized fossil bone preparation method using hydrogen peroxide to remove manganese oxide fossil bone coatings is an efficient non-invasive chemical method whereby implementation is fairly straight forward. Furthermore this method is environmentally friendly. An important aspect of the hydrogen peroxide as a reducing agent in this reaction is the evolution of high concentrations of oxygen. Although the oxygen does aid the fossil specimen cleaning process, monitoring of specimens is recommended so as to prevent any mechanical damage from excessive oxygen gas evolution.