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Mycohydrometallurgy Review - Part 1: Preg-Robbing Reduction in Carbonaceous Materials in Double Refractory Gold Ores Using *Phanerochaete chrysosporium*

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Abstract: To contribute to research efforts in pretreatment processes, "mycohydrometallugy" was introduced into the literature of hydrometallurgy in 2010 by employing the fungus, *Phanerochaete chrysosporium* in a "one-pot" process, to pre-treat carbonaceous and sulphidic materials usually encountered in refractory gold ores, with the ultimate aim of enhancing gold extraction. Several mycohydrometallurgical studies have been conducted over the past ten years, and this paper presents a review of the findings reported on carbonaceous materials, with recommendations for future research directions. Qualitative assessment of fifteen direct articles and twenty auxiliary ones revealed an agreement in the findings that biotransformation by *P. chrysosporium* results in preg-robbing reduction in all the carbonaceous materials (CM) used as surrogates. Statistical analysis of the secondary data revealed a mean preg-robbing reduction of 33% to 79% with a standard deviation of 4 to 26, depending on the CM type. Techniques such as XRD, Raman, NMR, FTIR and XANES showed reduced aromaticity and increased oxygen content, while TEM and SEM exhibited increased amorphous nature of CM. Porosity measurements also demonstrated a general decrease in surface area and micropore volume, but increase in pore diameter. The findings largely agree that the oxidative enzymes of *P. chrysosporium* break graphitic bonds in CM, destroy micropores and introduce oxygenated-functional groups, while fungal metabolites passivate the surface of CM. The resultant effect is a decrease in the affinity of CM for aurocyanide ions. With this milestone achieved, future research should focus on increasing the efficiency of enzyme extraction and kinetics of the pre-treatment processes.

Keywords: Review; *Phanerochaete chrysosporium*; Carbon-containing materials; Characterisation; Preg-robbing reduction

1. Introduction

Refractory gold ores contain recalcitrant components like sulphides, carbonaceous matter, tellurides and cyanicides that compete against the smooth extraction of gold by conventional methods. These components require pretreatment to enhance their responses to conventional gold extraction. Abiotic pretreatment processes have been explored with attendant difficulties; including environmental pollution, operational safety challenges and high cost, among others. These difficulties generated interest in the use of biotic processes, leading to the commissioning of the first commercial biooxidation (BIOX®) plant at Fairview in 1986.[1-3] The success of this biotic process led to the switch of pretreatment processes from roasting to biooxidation in many countries.

The BIOX® technology makes use of chemolithoautotrophic bacteria, which are organisms that obtain energy from the oxidation of inorganic compounds, and use carbon dioxide as their carbon source. Being lithotrophic iron- and sulphur-oxidising bacteria, the consortium in the biooxidation circuit uses ferrous ion and elemental sulphur from the sulphide minerals as electron donors, and ferric ion and molecular oxygen as electron acceptors, to generate energy for their biosynthetic processes. This results in the oxidation of sulphides with consequent liberation of gold for subsequent extraction.[2,4-8] As autotrophs, they make use of only carbon dioxide as their source of carbon to synthesise complex organic carbon for their cell growth. Accordingly, any organic carbonaceous material (CM) naturally present in the ore does not get oxidised by the bacteria, and thus, enters into the downstream gold dissolution circuit and adsorbs the dissolved gold. Since the natural CMs generally have fine particle sizes (2-50 µm), they cannot be separated from the rest of the ore slurry after gold adsorption, and this occurrence leads to reduction in recovery, a phenomenon termed preg-robbing.[2,9-15] Gold mining companies employing the BIOX® process on refractory ores and concentrates that contain CM are therefore confronted with preg-robbing issues.[6,8]

This research gap in biooxidation of refractory gold ores with chemolithoautotrophic bacteria have prompted researchers the world over to put in efforts into finding alternative and/or complementary processes such as roasting, blanking with organic reagents, and other biotic regimens.[5,16-18] One such biotic treatment

efforts led to the introduction of the term "Mycohydrometallugy" into the literature in 2010 to describe the application of mycology in hydrometallurgy.[19] The authors employed the fungus *Phanerochaete chrysosporium* to biotransform the major refractory components in refractory gold ores; thus, surrogate carbonaceous materials on one hand, and surrogate sulphidic materials on the other hand; with the aim of developing a 'one-pot' process that pretreats refractory ores to enhance overall gold recovery. This idea has been advanced by several researchers who have used wide-ranging growth media to culture the fungus and different substrates for the fungal biotransformation under varied incubation conditions.

The substrates include carbon-containing materials,[15,20-24] sulphidic materials,[21,25-28] and refractory gold ores.[21,25,29,30] The growth media include glucose broth, glucose agar, millet, wheat bran, corn bran and corn cob.[19,22,23,31-33] These authors have used temperatures (25 ^{o}C – 37 ^{o}C), pH (2 -10.5), medium (whole cells in liquid and solid, and cell-free), residence time (1 – 24 days) and agitation (0 – 250 rpm) in the treatment processes. All the authors have reported promising results with some degree of variability, and some shortcomings which require future attention.

This paper, comes up as the first of three in series, which reviews the studies made so far on carbon-containing materials with the aim of reducing their ability to preg-rob gold, and the proposals put forth to elucidate the underlying theories. This present review paper evaluates the findings reported, summarises the trends reported, highlights the strives made and genuine inconsistencies, and presents the future research direction of mycohydrometallurgy of carbonaceous materials.

2. Resource and Analysis

2.1. Resource

The materials used for this review comprise journal and conference publications from 2010 to 2020, focusing on the use of *Phanerochaete chrysosporium* in the biotransformation of carbonaceous materials (CM) encountered in refractory gold ore treatment. In all, fifteen articles published by the following authors[15,19-26,31-35] were used as direct raw data. About twenty additional articles were used to supplement the discussions.

2.2. Analysis

Google search was used to obtain the references of papers on carbon-containing materials (generally encountered in the treatment of refractory gold ores), which have been used as the substrates in biotransformation by *P. chrysosporium* with the aim of reducing their preg-robbing ability. Sampled papers were categorised into summary of culturing and incubation conditions, gold adsorption test and results on reduction in preg-robbing, characterisation facilities used and results reported, and mechanisms proposed. In some cases, the results presented were normalised to allow for quantitative comparison, and the general trends, presented. The review combines the *narrative* and *systematic methods*, and thus combines qualitative presentations of patterns from the published papers and the use of statistical methods to process some of the data in the published papers as secondary data to highlight the general inclinations.

3. Results and Discussions

This paper presents a review of findings published over the past ten years on using *Phanerochaete chrysosporium* to biotransform carbonaceous materials (CM) with the aim of deactivating the active surface of CM for gold adsorption. The published papers were evaluated, and the findings processed using qualitative and quantitative techniques. The results obtained are presented in the ensuing sections.

3.1. Culturing of Phanerochaete chrysosporium and incubation conditions

This section presents summaries and evaluations of growth media used in culturing the fungus, carbonaceous materials (CM) used as substrates for the fungus, environmental conditions like pH and temperature of the media, and the duration for incubating the CMs with the fungus. The section also highlights on the use of whole cell medium and cell-free extracts, and the incubation of CMs under stationary and agitated modes at various levels of agitation.[22,23,32,34]

3.1.1. Various media used in culturing P. chrysosporium

Culturing of *P. chrysosporium* has been done in several growth media including glucose broth, glucose agar, millet, wheat bran, corn cob and corn bran at various solid to liquid ratios, and in some cases, a combination of two media have been used. Fig. 1 presents in percentages, the CM biotransformation studies that used the media mentioned above to culture the fungus. The figure shows that glucose broth (solution) has been used the most (26%), and this is because glucose has been the conventional media used in studying the behavior of the fungus.[36]

In a study by Ofori-Sarpong et al.,[19] millet was adjudged the best after comparison with wheat bran and glucose. Millet forms a non-continuous phase that allows for inflow of air, thus, creating the needed environment for the aerobic fungus, which requires oxygen for its growth and reproduction.[37] The use of millet, however, has ethical implications since it is a food source for humans. Wheat bran, corn bran and corn cob alone or in combination with millet have also been used to effectively culture the fungus.[19,31,32] These are waste products, and so, their usage makes economic and environmental sense. However, they have low density, and thus require huge volumes in their usage. They also have the capacity to create a

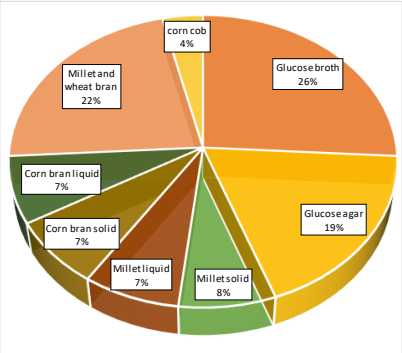


Fig. 1. Percentages of media usage by authors in culturing *P. chrysosporium* for CM biotransformation studies.

viscous medium after heat-sterilisation, which complicates separation between the medium and treated materials. Mixing these low-cost materials with millet may therefore be a better option.

3.1.2. Various carbonaceous materials treated with P. chrysosporium

The different CMs that have been used in the biotransformation studies by the various authors employing *P. chrysosporium* for reduction in preg-robbing are presented in Fig. 2, which gives activated carbon and anthracite as the CMs used the most in the researches under review. These two CMs are of interest because activated carbon is used in commercial recovery of dissolved gold,[2,35] while anthracite coal has similar maturity to more than half of the natural CMs found in carbonaceous gold ores.[11,15,19,38] Natural CM forms an integral part of refractory carbonaceous gold ores, and is composed mainly of hydrocarbons, humic acids, and graphitic/amorphous elemental carbon. The elemental carbon component behaves like activated carbon and, accordingly, adsorbs gold.[38-40]

In commercial gold extraction, there is competitive gold adsorption by these two CMs, and so, researchers have interest in studying their behaviour.[10,14] Flotation concentrate of refractory gold ores may contain natural carbonaceous materials which remain after biooxidation (BIOX® concentrate),[5,8,19,21] and these have the capacity to preg-rob gold in subsequent cyanidation circuits. The other CMs; lignite and bituminous coals are all used as surrogates to study the natural CMs found in gold ores due to the complexity of the ores.[11,19] All the CMs presented in Fig. 2 have exhibited various degrees of preg-robbing effects.[22,24,26,31]

3.1.3. Environmental conditions used in P. chrysosporium biotransformation of CM

Percentages of studies that utilised various pH and temperature values in the culturing/incubation of *P. chrysosporium* with CM are presented in Figs. 3a and 3b respectively. From the figures, most of the authors culture the fungus and/or incubate the CMs at 37 ℃ (61%) and at pH 4 (33%), as these are the conditions for optimum growth of the fungus. The use of lower temperatures has been explored with the aim of cutting down heating cost. The use of pH 2 is to increase the stability of the enzymes in the cell-free liquor, pH 6-7 ensures incubation at near-neutral pH and cuts down on acids and alkaline used in conditioning the media, while pH 10.5 is strategically selected as the required minimum pH in post-treatment cyanidation.

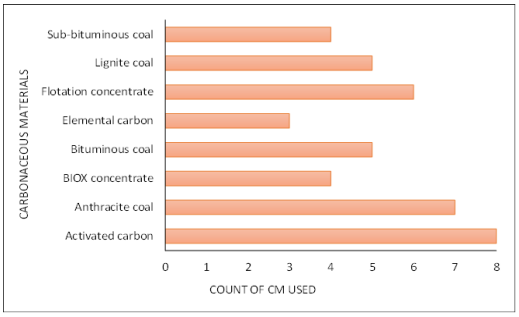


Fig. 2. Use of carbonaceous materials by authors in biotransformation by *P. chrysosporium*.

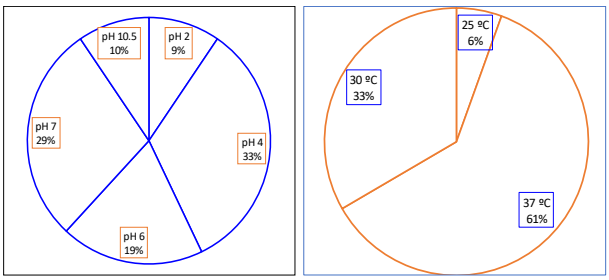
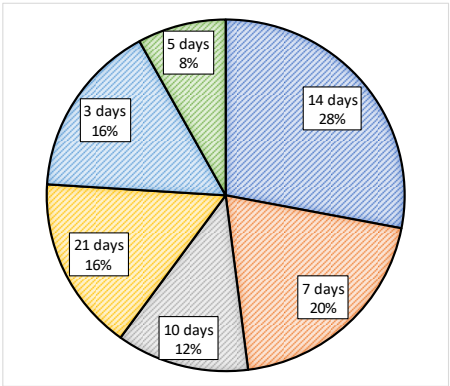


Fig. 3. Percentages of pH (a) and temperature (b) by authors used in culturing/incubation of *P. chrysosporium* with CM.

The fungus has adapted well to all these conditions, and performed well due to its non-specific nature.[2,22,23,33-34] The use of lower temperatures allows for reduction in heating cost.

Fig. 4a shows the percentages of residence time by authors used in culturing/incubation of P. chrysosporium with CM, while Fig. 4b gives a pictorial view of the use of whole cell and cell-free biotransformation of CM by authors. The processing times have ranged from 7 to 21 days mainly for either culturing the fungus for extraction of the crude enzymes[23,24,31,32,35] or for in vivo (whole cell) studies with the CMs.[19-22,33,34] The residence times for optimum biotransformation with whole cells have ranged between 5-7 days. Cell-free studies have been done with residence times of up to 7 days, and there are reports indicating lower biotransformation rates beyond 24 hours.[23-25] Whole cell treatment allows for continuous interaction of the cells and the enzymes secreted with the substrates under treatment, but poses a challenge with post-treatment separation of the treated



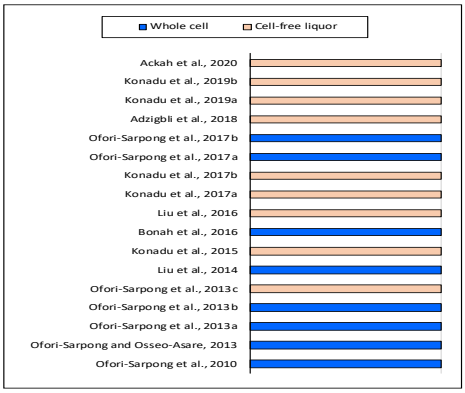


Fig. 4. Percentages of residence time used in culturing/incubation of *P. chrysosporium* with CM (a) and the use of whole cell and cell-free liquor by authors (b).

materials, and this leads to substantial losses.[21] This challenge is overcome by pre-growing the fungus, and extracting the cell-free liquor for use in the treatment. The use of cell-free liquor slightly outweighs that of whole cells, and this trend is expected to increase but that will require an increase in the efficiency of the enzyme extraction process to boost the activity of the enzyme.

3.2. Preg-robbing reduction by various carbonaceous materials after fungal treatment

The experimental conditions used by the various authors in testing the preg-robbing ability of the various CMs before and after fungal treatment, and the results reported have been summarised in Table 1. This valuation of the data presented by the various authors was necessary to normalise the highly varied results that have been reported, and create a good foundation for comparison. The experimental conditions and adsorption results were normalised to the same units.

The summary indicates that lower masses (1-12.5 mg) are used for the stronger, more mature and purer forms of carbon (with > 80% fixed carbon), which have the ability to exhibit higher adsorptivity, while with those having relatively lower amount of mature CMs, higher masses (50-100 mg) are used for the gold adsorption/preg-robbing test. The volume of gold solution used for the adsorption test ranges from 2 mL to 100 mL, while the concentration ranges from 5 µg/mL to 50 µg/mL in all the work done by the various authors. The gold adsorbed on CM was estimated from the pool of secondary raw data by the various authors with the aid of Equation 1, modified after.[19] PEC is the preg-robbing effect of carbon showing specific adsorption values in µg of gold per gram of CM, IC and FC respectively are the initial and final concentrations of gold in solution, while V is the volume (mL) of gold solution used, and WC is the mass (mg) of carbon material used in the adsorption test.

$$\text{PEC}\left(\text{in }\frac{\mu\text{g of gold}}{g\text{ of carbon}}\right) = V, \text{mL}\ x\ \left(\frac{10(\text{IC}-\text{FC}),\ ^{\mu g}/_{mL}}{\text{WC, mg}}\right) \quad (1)$$

The calculated PEC before and after fungal treatment were used to estimate the reduction in preg-robbing, and all these values were summarised into ranges as shown in Table 1. It is clear from Table 1 that activated carbon, elemental carbon and anthracite coal used so far could adsorb very high amounts up to 1850, 1570, and 1050 µg Au per gram of the CM respectively, and these adsorption values decreased correspondingly to 1479, 1115 and 250 µg per gram of CM after fungal treatment. The lower rank coals could adsorb a maximum of 38-85 µg/g CM which decreased to 6-50 µg/g. The preg-robbing reduction by various CM after fungal treatment was estimated from the secondary data of the various authors with the help of Equation 2, and the results on mean reduction in preg-robbing with the standard deviations are presented in Fig. 5.

$$Pregrobbing\ reduction\ (\%) = \frac{PEC\ by\ as-received\ CM - PEC\ by\ treated\ CM}{PEC\ by\ as-received\ CM} \quad (2)$$

Since the means and standard deviations vary significantly, it was necessary to estimate the coefficient of variability (CV) for comparison of the degree of variation presented. It is clear from Fig. 5. that the data series of the coal materials and elemental carbon showed relatively very low variability (<15%), while the CV of flotation concentrate was a little higher at 24%. The CV for preg-robbing reduction in the case of activated carbon was 66%; relatively far higher than the rest. This is so because about 70% of the raw secondary data showed preg-robbing reduction of less than 40% while 30% of the authors reported 70-80% reduction in preg-robbing.[22-24,31,32,35] This wide variation will definitely result in high CV. The variability suggests that there are inconsistencies based on the experimental protocols, and this calls for robust research to streamline the varied methodologies explored.

3.3. Characterisation of CM to evaluate the extent of biotransformation

Several characterisation analysis have been used by the various researchers to expound the mechanisms by which *P. chrysosporium* modifies the surface of carbonaceous materials (CM) to reduce their ability to interact with and adsorb gold cyanide from solution. The objectives of choosing particular characterisation equipment is therefore contingent on the mechanisms by which aurocyanide adsorbs on activated carbon and the elemental carbon associated with the CM. The mechanism of gold adsorption depends, among other factors, on the graphitic structure, porosity and surface functional groups of carbon.[10,13,41-47] In order to assess the extent to which these factors have been affected by the fungal treatment, the characterisations performed by the various authors are grouped into four and discussed in the succeeding sections.

Table 1. Experimental conditions for gold adsorption on carbonaceous materials and results reported by various authors.

Carbonaceous materials (CM)	Weight of CM (mg)	Vol of Au soln (mL)	Conc of Au soln (µg/mL)	Au Adsorbed on CM (µg/g)		Reductio in preg-robbing (%
				Control CM	*Treated CM*	
Anthracite coal	1-10	25-50	5-10	125-1050	6.25-250	65-95
Flotation conc	50-100	25-100	5-10	25-85	10-50	33-60
Bituminous coal	50-100	25-50	5-10	12.5-38.5	1.5-6.4	69-88
Lignite coal	50-100	25-50	5-10	15-45	3.75-12.5	62-75
Activated carbon	1-12.5	2-100	5-50	200-1850	158-1479	13-80
Elemental carbon	5-10	50-100	10-20	1500-1570	500-1115	28-39

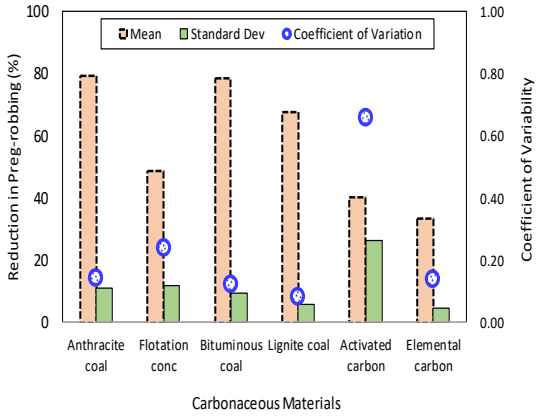


Fig. 5. Preg-robbing reduction by various carbonaceous materials after fungal treatment.

3.3.1. Studies on changes in surface morphologies of CM by TEM and SEM

Ofori-Sarpong et al.[20] studied the changes in surface morphologies of anthracite using transmission electron microscopy (TEM), and the results shown in Fig. 6 indicates a more amorphous surface (b) after fungal-biotransformation as compared with a well-ordered structure of as-received anthracite (a). The authors ascribed the amorphous nature to surface oxidation and surface coatings with fungal metabolites. Coating of the graphitic structure leads to blinding of adsorption sites with subsequent reduction in gold cyanide adsorption.[17] For effective gold adsorption, a well-structured carbon is required to increase interaction of the gold central ion with pi electrons of the graphitic planes.[43,45-47]

Scanning electron microscopy (SEM) has also been used by some authors to study changes in surface morphology of CM following fungal treatment. A representative figure is presented here as Fig. 7 from work done by Liu et al.,[22] who made similar observations to those on the TEM. The SEM analysis showed extensive micropores (Fig. 7a) on untreated activated carbon (20–40 µm), but the pores faded off (Fig. 7b) after the fungal treatment. The observation was attributed to possible mycelium and metabolites covering the surface and changing the physicochemical properties, which leads to reduction in preg-robbing capacity.[20,22,35] In a similar study, Liu et al[33] observed that the untreated elemental carbon was generally massive with a smooth surface which had some pores with diameter of 50-3000 nm. After treatment with *P. chrysosporium*, the surface morphology was damaged significantly, and most of the pores were no more visible.

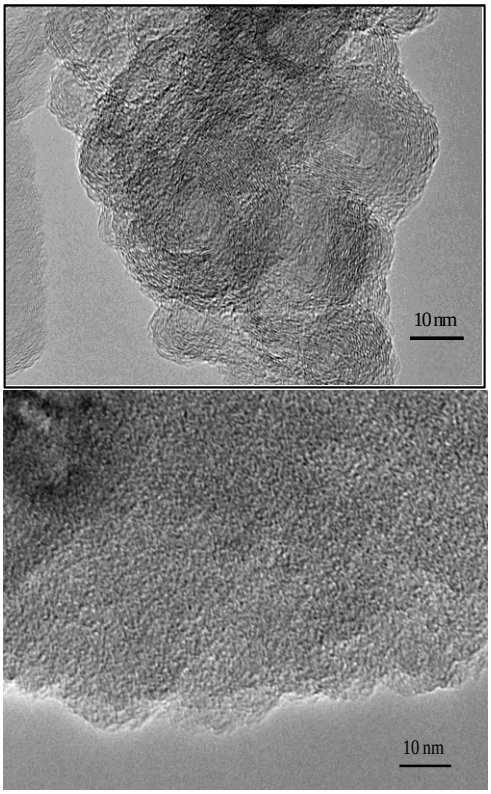


Fig. 6. TEM analysis of as-received anthracite (a) showing structured surface and treated anthracite (b) showing a disturbed surface.[20]

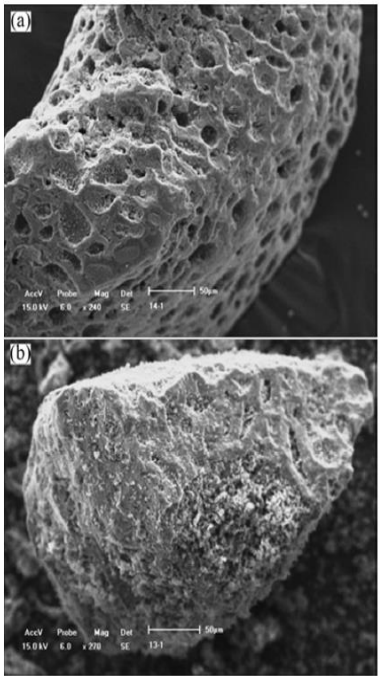


Fig. 7. SEM analysis of as-received anthracite (a) showing structured surface with open pore spaces and treated anthracite (b) showing a surface with pores covered.[22]

3.3.2. Studies on changes in degree of graphitisation of CM using XRD, Raman and NMR

The degree of graphitisation of the various CMs have been studied by various authors using x-ray diffractometer (XRD), Raman spectrogram and nuclear magnetic resonance (NMR) spectroscopy.[20,22,23,26,35] XRD analysis of as-received and treated activated carbon by Liu et al[22] revealed distortion of microcrystalline structure of activated carbon, which decreased the number of aromatic rings and aromatic layers, the stacking height and interlayer distance of microcrystal layers, and the condensation degree of aromatic nuclei. Similar observations by XRD on destruction of microstructure and macromolecules were made when elemental carbon was treated with *P. chrysosporium.*[33] A decrease in the graphite peak at 27 deg on the 2-theta axis after fungal treatment of anthracite was also observed by Ofori-Sarpong et al[26] as shown in Fig. 8. The peak is characteristic of structured graphite, and its decrease suggests an increase in the degree of disorderliness/amorphousity.

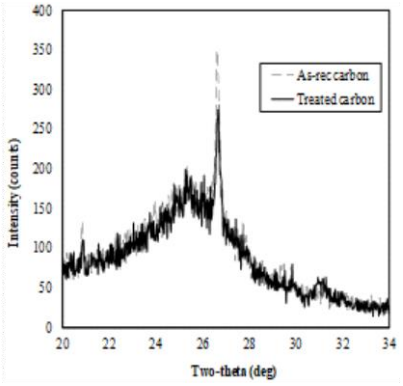


Fig. 8. XRD of as-received and treated anthracite coal.[26]

Raman spectroscopy of CM can generally be used to explain the differences in the relative abundance of graphitic or ordered structure designated by the peak around wavelength of 1580 cm^{-1} known as the G line, and disordered structure assigned to 1370 cm^{-1}, the D line.[48,49] Fig. 9 presents the Raman spectrograms of as-received and treated anthracite in a study undertaken by Ofori-Sarpong et al.[20] The figure shows a general decrease in both peaks, depicting reduction in carbon content and thus a lower carbon-to-oxygen ratio, which is known to be unfavourable for gold adsorption.[19,26,45] A study by Konadu et al.,[23] showed a shift in the G line from 1597 cm^{-1} to 1600 cm^{-1} in all the bio-treated residues, which they ascribed to a decrease in the amount of aromatic carbon layers.

Konadu et al[23] employed ^{13}C NMR on activated carbon, and the analysis showed degradation of aromatic C-C bond into a mixture of aromatic and aliphatic C-H bonds after fungal treatment of activated carbon as shown in Fig. 10.

3.3.3. Studies on changes in modification of surface functional group on CM using FTIR and XANES

The relative intensities of Fourier-transform infrared spectroscopy (FTIR) peaks can be used to quantify the number of functional groups of different CMs. FTIR analysis of untreated and fungal-treated elemental carbon by Liu et al[33] showed a decrease in the metamorphic grade of the CM and the condensation degree of aromatic rings, and this is presented in Fig. 11. The authors attributed the decrease to destruction of some aromatic rings by the fungus with introduction of aliphatic groups, surface exfoliation, and destruction of microcrystalline and pore structure of elemental carbon. A decrease in the content of aromatic structure, an increase in the number of aliphatic structure and the number of oxygen-containing functional groups following treatment of activated carbon have also been reported.[22]

In FTIR studies of anthracite, Ofori-Sarpong et al[20] also reported changes in the peaks at 2929 cm^{-1} and 2856 cm^{-1} which are due to the presence of aliphatic hydrocarbons and the peak at 1715 cm^{-1} ascribed to carbonyl (C=O) groups.[50] The C=O group in the treated anthracite sample implied more oxygen-containing groups on the surface of anthracite.

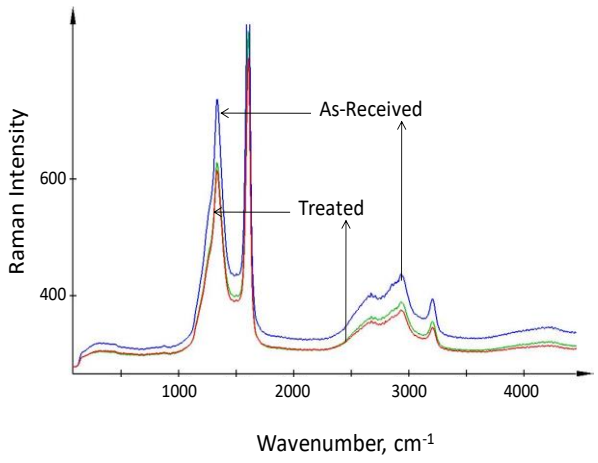


Fig. 9. Raman spectra of as-received and treated anthracite.[20]

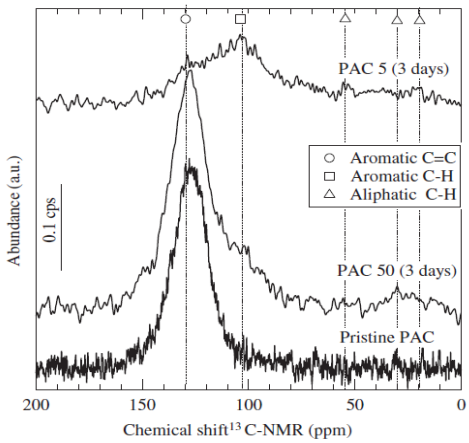


Fig. 10. ^{13}C NMR spectra of pristine powdered activated carbon (PAC), PAC 50 after 3 days and PAC 5 after 3 days of bio-treatment.[23]

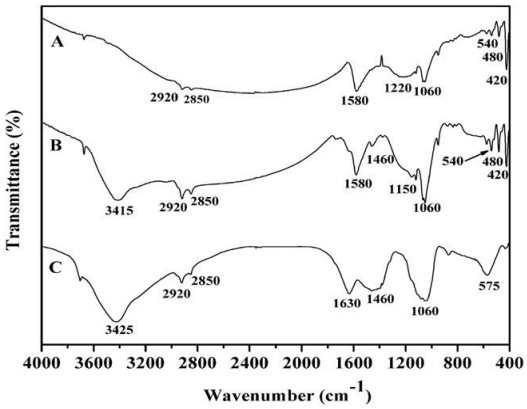


Fig. 11. FTIR spectra for elemental carbon (A), the degradation residue (B) and the water-soluble alkaline precipitate (C).[33]

With the increase in the aliphatic groups and the carbonyl groups, all the authors inferred that the degree of aromaticity was reduced due to fungal treatment. These changes will result in reduced aurocyanide adsorption on CM as the adsorption requires large continuous areas of graphitic plates, not interrupted by edges terminating with C-H or C-O groups.[41,45-47] Observation of increase in oxygen-containing groups is buttressed with the use of x-ray absorption near edge structure (XANES) spectroscopy on anthracite by Ofori-Sarpong et al[20] as shown in Fig. 12. The zenith of this technique was the increase in the peak between 288 and 289 eV which signifies the presence of carboxylic acids[51] after fungal treatment. Increase in carboxylic acids on the surface of CM does not favour aurocyanide adsorption.

3.3.4. Studies on changes in surface area and pore characterisation of CM using porosity measurements

The surface area and porosity of CM are very important factors in gold adsorption, and most of the authors have compared these values before and after treatment. BET surface area of the as-received anthracite was found to be 4.08 m^2/g by Ofori-Sarpong et al,[20] similar to that of anthracite samples studied by Sibrell and Miller[52] who obtained 4.0 m^2/g. After fungal treatment by Ofori-Sarpong et al,[20] the BET surface area reduced by 76 % to 0.98 m^2/g.

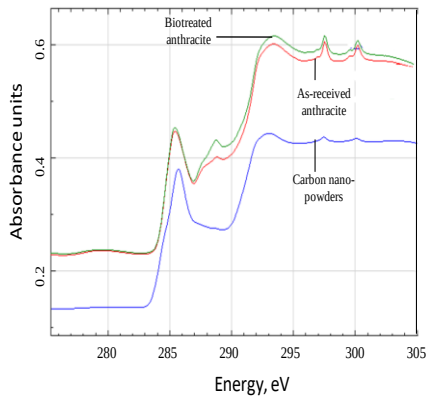


Fig. 12. A comparison of the as-received and 14-day biotreated anthracite spectra from XANES.[20]

In addition, the micro pore volume and average pore diameter of the as-received sample were 15.09 x 10^{-4} cm^3/g and 5.7 nm respectively, and after treatment, the micro pore volume reduced by 80% while the average pore diameter increased by 65%. The BET surface area and the micropore area of elemental carbon studied by Liu et al[33] were 21.62 m^2/g and 14.14 m^2/g, respectively in the as-received, and these decreased by about 38% and 92% after fungal treatment. The total pore volume and the micropore volume after treatment reduced by about 77% and 98% respectively. Similar results have been reported by other authors.[23,26] All these infer that *P. chrysosporium* destroys micropores, which are the most important pores for gold adsorption.

3.4. Proposed mechanism responsible for reduction in gold adsorption after biotransformation of CM by P. chrysosporium

Aurocyanide adsorption on carbon is influenced positively by a high degree of graphitic nature of CM, low oxygen-containing groups on CM and high surface area with high amount of micropore volume.

This is because gold dicyano complex adsorbs on the surface of graphitic planes of carbon via donation of delocalised pi electrons from the graphitic planes to the empty 6s shell of gold ion. Though the surface of carbon is negatively charged due to sp^2 hybridisation, this interaction outweighs the electrostatic repulsion between the negatively charged surface of carbon and the $Au(CN)_2^-$ complex.[20,41,43,45-47] According to Klauber[43] and Poinen and Thurgate,[46] the continuous nature of the graphene layers is required for the adsorption of aurocyanide complex from alkaline solutions, since gold cyanide ion, $Au(CN)_2^-$, lies approximately on 3 adjacent graphitic planes. Any treatment that leads to destruction of graphitic planes, complete and/or partial oxidation of carbon leading to increase in oxygen-to-carbon ratios will therefore decrease the gold adsorption capacity of carbon.[5,19,23,33,34,45]

The mechanism by which *P. chrysosporium* degrade CM in refractory gold ores have been explained extensively by Ofori-Sarpong et al.[20,26] A simplified pictorial version developed by Liu et al[22] is presented in Fig. 13. The fungus secretes oxidative enzymes (($P(Fe^{3+})$), which become two-electrons oxidised into Compound I (($P{=}Fe^{4+}\cdot$)) by interacting with hydrogen peroxide, also produced by the fungus. Compound I undergoes reduction in two steps to regenerate the native enzymes by oxidizing an aromatic molecule into an aromatic radical in each step. The radical in turn reacts with other molecules creating chain reactions, which may lead to complete conversion of carbon to carbon dioxide or introduction of oxygen-containing functional groups on the carbon as shown in Fig. 14.[20]

The analyses by the various authors using XRD, Raman, NMR, FTIR and XANES spectra on various CM all agree that biotransformation of CM by *P. chrysosporium* lead to interruptions in the graphitic layers and subsequent introduction of oxygen/hydrogen groups, which can partly explain the reduction in gold adsorption following fungal biotransformation. The analyses using TEM and SEM also attest to preferential attachment of organic compounds secreted by the fungus to the hydrophobic graphitic planes.[20,22,33]

Microbial transformation of solids begins with the formation of a slime layer around the area of attack. This layer is composed of polysaccharides[37] and it is possible that these organic substances penetrated and coated more of the smaller pores. The findings reported by the various authors on surface area and pore size measurements also indicate a drastic reduction in surface area and micropore volume with increase in average pore diameter following fungal-treatment. These will reduce drastically, the active surface for gold adsorption which is reported to occur mostly in the micropores.[2] All the mechanisms by which *P. chrysosporium* biotransforms CM to reduce its ability to preg-rob gold, as detailed by the various authors considered in this paper, can be summarised as follows:

(a) surface oxidation, which disrupts the continuous graphitic structure, thus decreasing the active sites necessary for adsorption;
(b) reduction in surface area via plugging of pores possibly by fungal biomass and/or slimy substances formed through fungal interaction with the growth medium, and hence decreasing accessibility of gold to the adsorption sites; and
(c) Cleavage of bonds leading to pore enlargement, thus rendering the pores too large for adsorption of the aurocyanide ion which is known to adsorb into micropores.

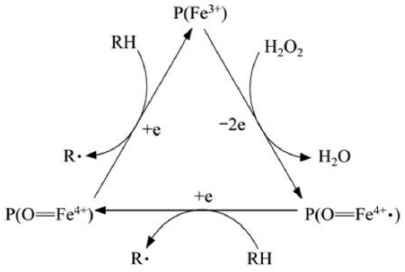


Fig. 13. Simplified proposed mechanism of biotransformation of carbonaceous matter by *P. chrysosporium*.[22]

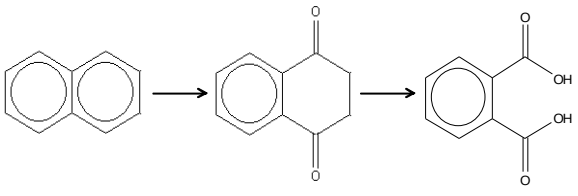


Fig. 14. Pictorial illustration of the disruption of graphitic planes and subsequent introduction of oxygen/hydrogen groups.[20]

4. Conclusions

This paper comes as the first in a series of three papers dedicated to conducting reviews of published work on the use of *Phanerochaete chrysosporium* to biotransform refractory carbonaceous and sulphidic materials to improve on gold extraction. Qualitative analyses of fifteen direct articles and twenty auxiliary ones published in the past ten years on carbon-containing materials associated with refractory gold ores revealed a general consensus that *P. chrysosporium* has the ability to transform a wide range of carbonaceous materials (CM) and reduce their ability to preg-rob dissolved gold from alkaline cyanide solution. Contact of the CM with gold solution gave a mean preg-robbing reduction of 33% to 79% with a standard deviation of 4 to 26, and a coefficient of variability of 0.12-0.66 depending on the CM type. Destruction in the degree of graphitisation, with introduction of more oxygenated-functional groups has been expounded with techniques such as XRD, NMR, Raman, FTIR and XANES. Studies on surface morphology using techniques like TEM and SEM show increase in amorphous nature, while porosity measurements prove a general decrease in surface area and micropore volume, and an increase in pore diameter after fungal interactions.

The findings generally agree that the oxidative enzymes of the fungus are responsible for breaking the graphitic bonds, allowing for introduction of oxygenated-functional groups and destruction of micropores, while the fungal metabolites also passivate the surface of the CM. All these factors reduce the affinity of CM to adsorb aurocyanide ions. Both whole cells and cell-free liquor have been explored with varied advantages and disadvantages. While whole cells provide continuous supply of enzymes, difficulty in post-treatment separation leads to losses in treated material and carry-over of biomass to downstream processes. Cell-free process eliminates the repercussions of entrained biomass, but suffers limited enzyme activity which leads to abrupt shutdown of the pre-treatment step. The future research direction in mycohydrometallurgy of CM should focus on increasing the efficiency and effectiveness of enzyme extraction processes, and exploring enzyme production in a continuous mode. This will guarantee continuous supply of enzyme in an adjacent CM pre-treatment vessel which will improve on the kinetics and yield.

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Conflicts of Interest

The authors declare no conflict of interest.

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