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Biotechnological Approaches to Facilitate Gold Recovery from Double Refractory Gold Ores

Keiko Sasaki and Kojo T. Konadu

Abstract

Double refractory gold ore (DRGO) not only include ppt levels of gold grains locked in sulfide minerals but also a problematic amount of carbonaceous matter. This causes a significant recovery loss of gold during cyanidation because of the strong affinity of the $\text{Au}(\text{CN})_2^-$ with the carbonaceous matter. Combustion decreases the carbonaceous matter content, but also emits pollutant gases like CO_2 , SO_2 and As_2O_3 . Therefore, environmentally-friendly solutions have been explored by using biotechnology. Due to the very small amount of the above targets in the ore, it is challenging to show evidential changes in solid-phase before and after the biomineral processing of DRGO. This chapter introduces the mineralogical and chemical changes in the various solid residues produced during a sequential biotreatment, consisting of the liberation of gold from sulfides by an iron-oxidizer and decomposition of carbonaceous matter by lignin-degrading enzymes (lignin peroxidase, manganese peroxidase, laccase) secreted from a white rot-fungus, which successfully improved of gold recovery to over 90%. In addition, further development of biotechnology in the recovery of gold from DRGO is addressed.

Keywords: double refractory gold ore, carbonaceous matter, enzyme reaction, QEMSCAN map, biooxidation of sulfides

1. Introduction

Carbonaceous refractory gold ores are classified as double refractory gold ores (DRGO) due to containing sulfide minerals and carbonaceous matter. In DRGO, the sulfide minerals (pyrite and arsenopyrite) tend to have a significant amount of gold enclosed in it compared to other minerals in the ore and the gold confined in sulfides is difficult to recovery due to the minerals' stability during cyanidation, leading to poor recovery from sulfides. Furthermore, the gold that is successfully dissolved as $\text{Au}(\text{CN})_2^-$ in the cyanide leaching step, can be adsorbed by the organic carbon (preg-robbing), therefore, using cyanidation without DRGO pretreatment can lead to 30–70% gold recovery losses [1–9]. DRGO is produced in various parts of the world such as Ghana, Brazil, USA, Canada, Kazakhstan, Russia, Malaysia, Indonesia and China [9], and the gold production from DRGO attains to about 1/100 of the total gold production in the world. DRGO also accounts for about one-third of available gold deposits [8].

DRGO is generally subjected to flotation to recovery a sulfide concentrate that is then sent for pretreatment prior to cyanidation. However, due to the poor separation of carbonaceous matter and sulfide minerals, the carbonaceous matter also reports in the flotation concentrate [10–13]. Although, some recent studies have looked at pre-flotation as a means of improving the separation of carbonaceous matter and sulfides from DRGO [14–17]. But, for most current industrial operations, the flotation concentrate contains both the carbonaceous matter and the sulfides and therefore, it is extremely difficult to treat. It is no exaggeration to say that there is a global need for technological development to improve the gold recovery from DRGO.

The pretreatment of carbonaceous matter and sulfides in DRGO to minimize preg-robbing and liberate gold has changed with time to become more environmentally friendly (**Figure 1**). Oxidation is one of the most prominent means to pre-treat DRGO and improves gold recovery [4, 10–13, 18, 19]. In the past, thermal oxidation was used to decompose carbonaceous matter and convert sulfide minerals to iron oxides. However, the control of the roasting temperature is very important but difficult because at $\leq 500^\circ\text{C}$, there is incomplete sulfide oxidation, and also the removal of volatile matter and decarboxylation reaction in the carbonaceous matter at $100\text{--}300^\circ\text{C}$ and $400\text{--}500^\circ\text{C}$, respectively [18, 20]. In such a case, there is an incomplete liberation of gold from sulfides, and the carbonaceous matter becomes a more activate adsorbent, resulting in a higher preg-robbing ability during cyanidation. Furthermore, harmful gases such as SO_x and As_2O_3 are generated, making this process no longer as attractive, although recent advances in using gas-scrubbing techniques, the environmental concerns have been reduced [21]. In fact, some works have tried to encourage the gas-scrubbing process by proposing methods like chlorination roasting in which, the solid gold is converted to AuCl_3 gas and leaves the furnace along with other harmful gases [22, 23]. In this case, the recovery of the valuable gold necessitates the recovery of all the gases produced from the furnace and significantly decreasing the environmental impact of roasting while also increasing the cost of furnaces operation.

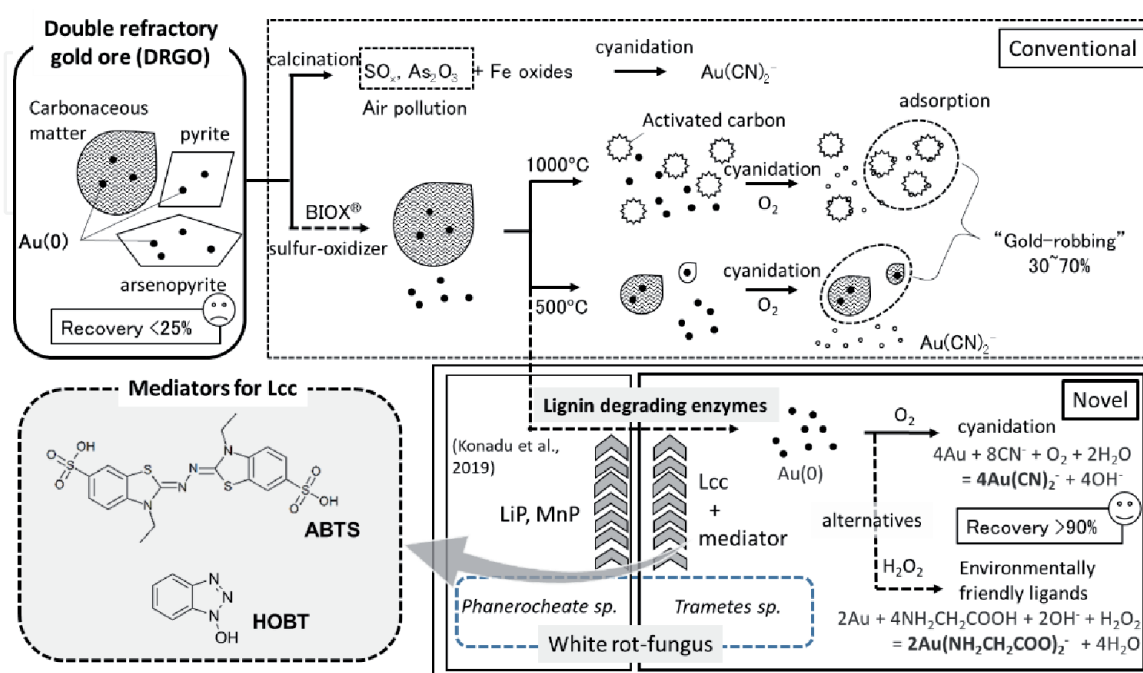


Figure 1. Conventional and novel methods in (bio) mineral processing of graphitic carbonaceous gold ores.

In place of thermal oxidation, biological (BIOX) and pressure oxidation have been applied to remove sulfides from DRGO, but these processes have a minimal effect on the carbonaceous matter [11–13, 19] (**Figure 1**). After the sulfide oxidation, the carbonaceous matter-containing residue can either undergo a treatment like roasting or blinding to minimize preg-robbing before gold recovery [24]. The roasting of the BIOX or pressure oxidation residue come with the same problems as alluded to before while blinding of the carbonaceous matter leads to the transfer of blinding reagents like kerosene and diesel oil on to the activated carbon used for $\text{Au}(\text{CN})_2^-$ recovery from the carbon-in-leach (CIL) or carbon-in-pulp (CIP) process.

A newly alternative carbonaceous matter treatment is the use of lignin-degrading enzyme released by fungi and bacteria [10–13, 25–27]. Although they are more prevalent in white-rot and brown-rot fungi, these enzymes have been isolated from some of the fungi responsible for the oxidation of lignin and a very complex polyaromatic polymer [28]. These lignin-degrading enzymes include lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lcc) and versatile peroxidase (VP) [29–31]. These enzymes accelerate the oxidative degradation of C=C and C=O bonds. These enzymes were selected for the present purpose because lignin is a precursor for the carbonaceous matter in DRGO, and it was expected that the lignin-degrading enzymes could successfully attack and oxidize this substance. The lignin-degrading enzyme treatment proceeds under very mild temperature and pH conditions and therefore has a low environmental impact [29, 30]. Additionally, the enzymes' effectiveness of these enzymes can be improved by the inclusion of mediators like veratryl alcohol and ABST [32, 33]. Although, the environmental impact assessment is subject to change once a fuller understanding of the bio-products of the process is known. Additionally, the oxidizing condition generated by these fungi also aids the dissolution of sulfides to liberate gold grains [34–36]. Several studies have shown that using lignin-degrading enzymes produced by fungi like *Phanerochaete chrysosporium* and *Trametes versicolor* can increase gold recovery by 10–20% [10–13]. Although these enzymes are produced in some quantities from white-rot fungi and are beginning to be used for decomposing harmful polyaromatic compounds such as dioxins and for producing biofuels, there is no application research to the mining industry [37].

So that the present study reviews DRGO treatment, with particular focus on the carbonaceous matter treatment by lignin-degrading enzymes. It covers the application of these enzymes to surrogates for the carbonaceous matter to understand the enzyme-substrate interactions and finally move on to using these enzymes on the DRGO to improve gold recovery.

2. Utilization of lignin-degrading enzymes on a surrogate for carbonaceous matter in DRGO

White-rot fungus and brown-rot fungi are a class of microbes known for producing very valuable enzymes. In wood chemistry, a lot of research has focused on determining the properties, reactions, and enzymes at the molecular level [28–33]. A very popular white-rot fungus is *P. chrysosporium*, which grows at a flexible pH range, relatively moderate temperature and produces a variety of lignin-degrading enzymes with low substrate specificity. These include Lip, MnP and Lcc, which have been used been to oxidize substrates from several industries including pulp, agricultural waste, dye treatment but not in the mining industry [37]. There are very few studies that have looked at the performance of these enzymes against substrates with high crystallinity as can be found in DRGO.

Invariably, authors considering the application of the lignin-degrading enzymes to carbonaceous matter in DRGO have started by working with surrogates in a simplified setup to get a better understanding of the oxidation mechanism and bioproduct. Some authors have used activated carbon [9], coal [38] and elemental carbon extracted from DRGO [39] as a model of the graphitic carbon in DRGO. The interaction between the lignin-degrading enzymes and the carbonaceous matter surrogate were facilitated by either growing the fungi in the presence of the carbonaceous matter or harvesting the spent culture liquid and using it for the treatment. It has generally been observed that the lignin-degrading enzymes attack the aromatic C=C bonds in the graphitic carbon and converted it to aliphatic C-C and oxygen-containing functional groups like carbonyl C=O and alcohol C-O. This was confirmed by FTIR and solid-state ^{13}C -NMR spectra, showing the relative intensity of aromatic carbon C=C decreasing after the application of the lignin-degrading enzymes and the relative increase in aromatic carbon C-H and aliphatic carbon C-H (Figure 2).

Some consequences of the oxidation of the aromatic C=C, which serve as the backbone for these surrogate materials, is an increase in the surface roughness and a reduction in the specific surface area as shown in Figure 3. Several works have shown a reduction in the specific surface area by 76% for anthracite, 34.5% for carbonaceous matter extracted from DRGO and 38% for activated carbon [9, 38, 39]. All of these chemical and physical changes in the graphitic carbon resulting from the interactions with the lignin-degrading enzymes lead to a significant reduction in the $\text{Au}(\text{CN})_2^-$ uptake ability.

There is still some amount of work required to improve our understanding of the impact of the lignin-degrading enzymes on the carbonaceous matter. These include establishing a relationship between the amount of enzyme consumed to decompose the carbonaceous matter and a better characterization of the bioproducts of the treatment.

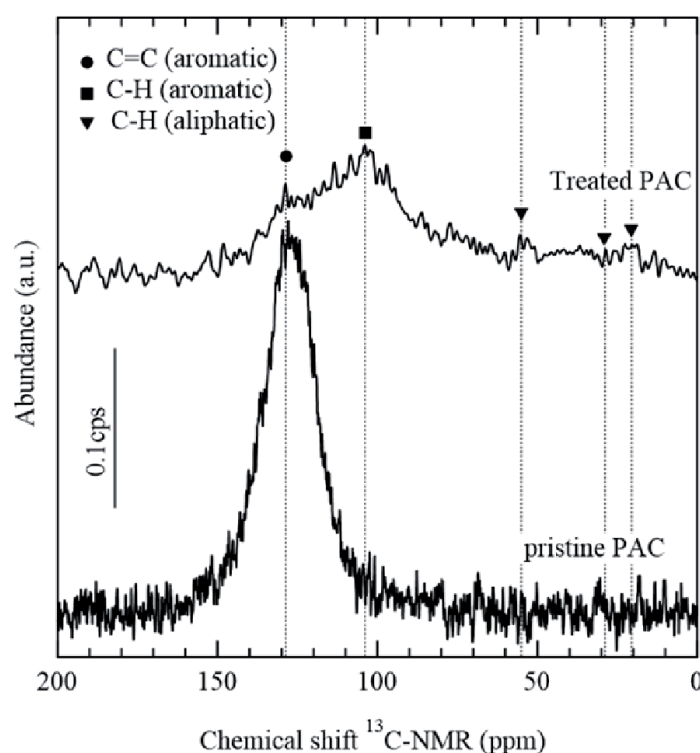


Figure 2.

^{13}C -NMR spectra for powdered activated carbon (PAC) before and after treatment by spent medium of *P. chrysosporium* (modified [9]).

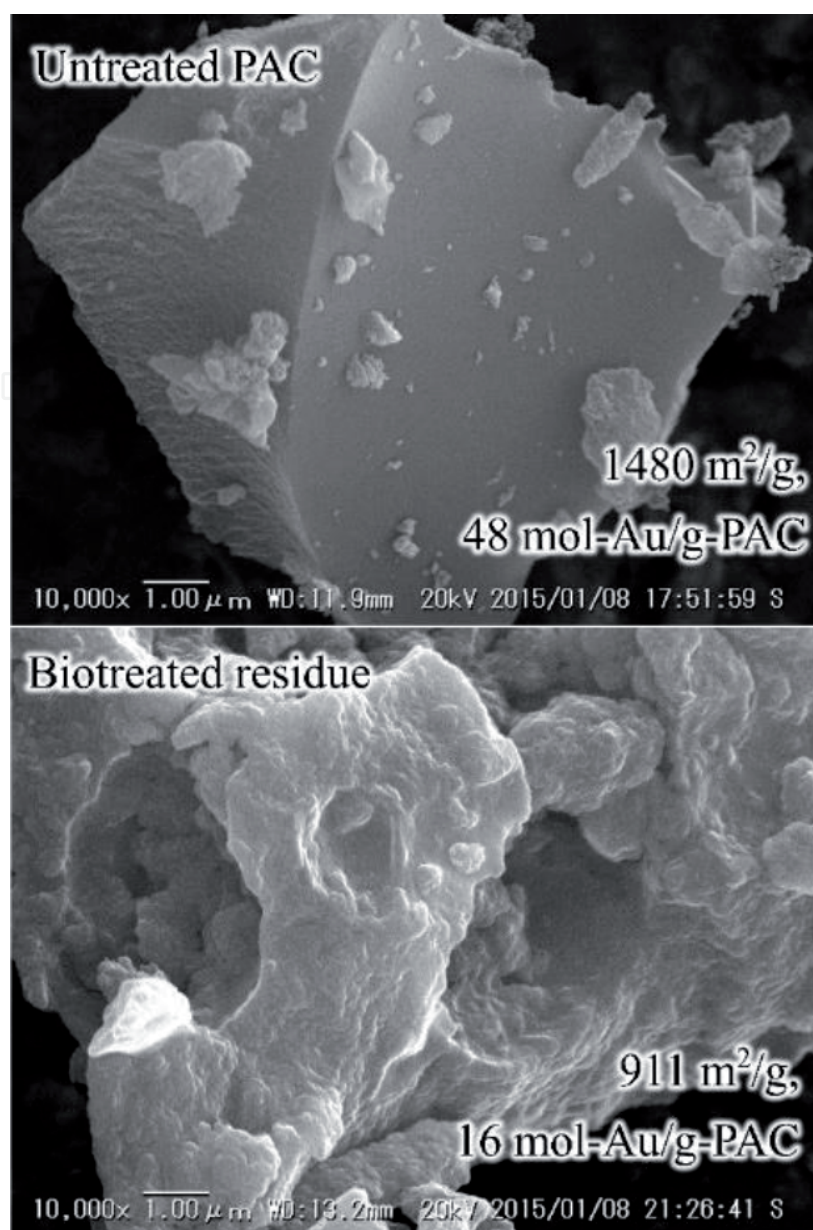


Figure 3.
 SEM images of powdery activated carbon before (top) and after (bottom) treatment by spent medium of *P. chrysosporium*. Horizontal bars indicate 1.00 μm .

3. Utilization of lignin-degrading enzymes on DRGO

3.1 Decomposition of sulfide minerals

In DRGO, gold ($\text{Au}(0)$) is mainly confined in sulfide minerals, especially arsenopyrite (FeAsS) and pyrite (FeS_2), which must be decomposed to liberate gold. Sulfide minerals in DRGO typically make up 10 ~ 20% after flotation [10–13]. Most of the other major mineral components in are quartz and clay minerals, which do not participate in the oxidative decomposition of sulfide minerals. The standard BIOX treatment of sulfides utilizes chemoautotrophic bacteria *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, heterotrophic bacteria *Sulfolobus acidocaldarius*, *Sulfolobus* sp., *Sulfobacillus* sp., etc., to oxidize and dissolve, or decompose, sulfides by using Fe^{3+} ions as oxidizing agents by oxidizing Fe^{2+} ions in strongly acidic solutions to Fe^{3+} ions [11, 40].

Some white-rot fungi have been shown to improve sulfide oxidation. *P. chrysosporium* produced a mixture of enzymes like LiP and MnP, which needed to be activated

by H_2O_2 [18]. Some studies have shown that the H_2O_2 produced by the fungi might be involved in a Fenton reaction with pyrite and arsenopyrite in the DRGO [34–36]. The oxidizing conditions lead to the generation of Fe^{3+} ions as an additional oxidizing agent. However, the competition for H_2O_2 between the lignin-degrading enzymes and the Fenton reaction leads to the inadequate oxidation of both the carbonaceous matter and sulfides [11]. Also, the pH 4–5 used in this treatment results in precipitation of the Fe and reduces the efficacy of Fe^{3+} as an oxidizing agent. Therefore, most studies that have achieved very high gold recoveries have used a sequential treatment.

3.2 Sequential decomposition sulfides minerals and carbonaceous matter

DRGO often contains less than 7% of carbon content, and it is considered to have a more non-uniform structure compared to activated carbon, making it relatively easier to decompose [8]. However, the susceptibility to carbonaceous matter to decomposition by lignin-degrading enzymes should vary depending on the ore. Raman spectroscopy was used to generate **Figure 4**, which shows the difference in graphiticity of carbonaceous matter from various gold ores. The C=C peak for continuous graphitic carbon near 1580 cm^{-1} (G-band) and the C=C peak for vibration adjacent to the graphite defect

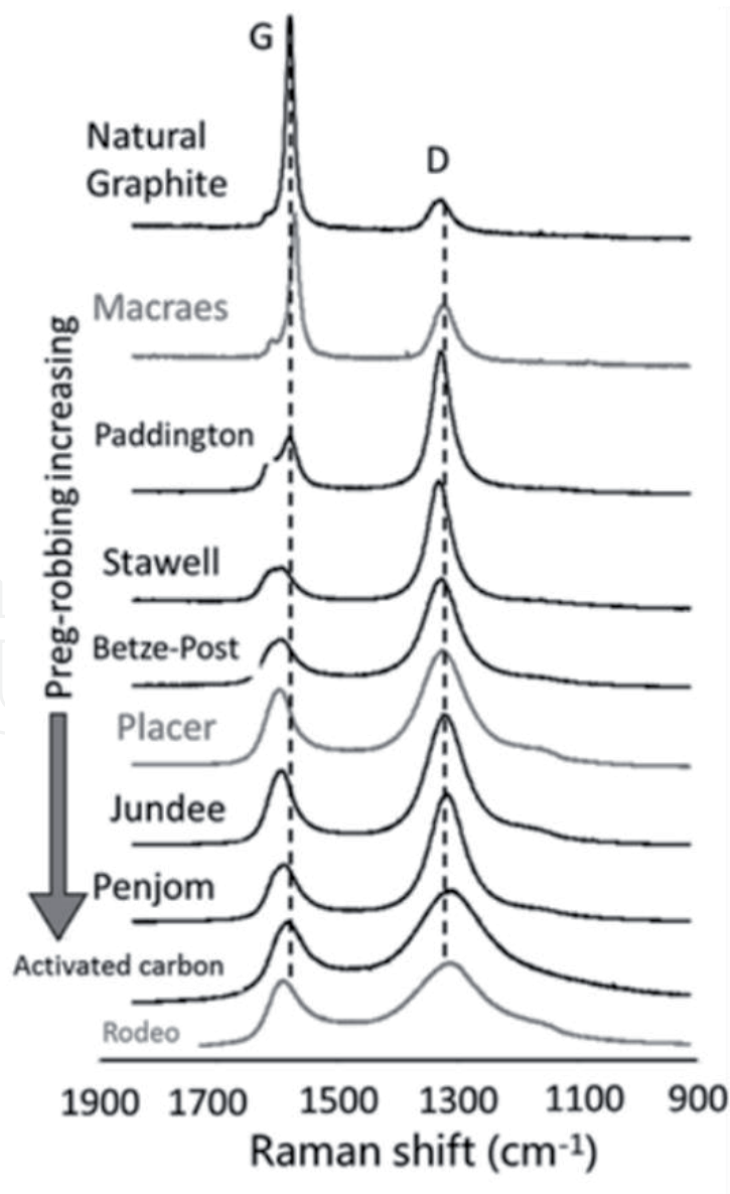


Figure 4.
Raman spectra for DRGOs from different mines (modified [41]).

near 1320 cm⁻¹ (D-band) was distinguished, and the relative intensity I_D/I_G and can be used as an index of the degree of the defect [42]. Relative intensity I_D/I_G varies with ore and is an indicator of the preg-robbing ability of the carbonaceous matter. The gold ore from the Paddington mine in Australia has a very high defect compared to carbonaceous matter from the Macraes mine in New Zealand, which has a very aromatic structure. This indicates that the carbonaceous matter from Paddington mine might be more susceptible to lignin-degrading enzymes than the ore from Macraes mine.

The sequential treatment of DRGO has shown significant results, as seen in **Table 1**. Using lignin-degrading enzymes to treat DRGO began with work by Yen et al., [10] who applied the fungi *T. versicolor*, but information about this work is limited because it is a patent. This was followed by Ofori-Sarpong et al. [11] who built open their previous work using *P. chrysosporium* to decompose coals of various ranks [38]. They found that applying the lignin-degrading enzymes directly to the DRGO to decompose both the sulfides and carbonaceous matter improved the gold recovery from 41% - 78% which was slightly less than 81% recovery obtained when the sample was only subjected to sulfide oxidation by chemoautotrophic bacteria. This indicates that the fungal treatment was less effective at oxidizing both refractory materials. Therefore, sequential treatment was used, with the sulfide oxidation by bacteria preceding the carbonaceous matter treatment by *P. chrysosporium*, and they reported a final gold recovery of 94%.

They attempted to further understand the effect of the *P. chrysosporium* on the DRGO by using only the spent medium to treat the DRGO [12]. This was done to check the ability of the lignin-degrading enzymes to oxidize the carbonaceous matter while also avoiding complications like the fungal biomass. This work did not yield as high a gold recovery compared to when the *P. chrysosporium* was cultured with the DRGO, but it did show that it could be viable for DRGO treatment.

Authors	Gold grade (g/t)	Carbon content (%)	Fungus	Treatment conditions	Au recovery (%)	
					Before treatment	After treatment
Yen et al. [10]	—	—	<i>Trametes versicolor</i>	Flotation concentrate	54.1–64.5	95.2
Ofori-Sarpong et al. [11]	30.2	3.6	<i>Phanerochaete chrysosporium</i>	Flotation concentrate, 30% pulp density, 21 days, pH 4	41 (78) ^{*1}	94
Ofori-Sarpong et al. [12]	30.2	3.6	<i>Phanerochaete chrysosporium</i> (spent medium)	Flotation concentrate, 30% pulp density, 3 days, pH 4	38	66
Liu et al. [36]	2.18	0.2	<i>Phanerochaete chrysosporium</i>	Artificial DRGO	44	62
Konadu et al. [13]	40.4	5.8	<i>Phanerochaete chrysosporium</i> (spent medium)	Flotation concentrate, 5% pulp density, 15 days, pH 4	24 (77) ^{*1}	92

^{*1}Numbers in brackets mean the gold recovery after 1st step of microbiological oxidation and before 2nd step of enzyme treatment.

Table 1.
Summary of the previous works on bio-treatment of carbonaceous matter in DRGO by lignin-degrading enzymes.

To this end, Konadu et al. [13] used the spent medium of *P. chrysosporium* to treat a DRGO, which had a Raman spectrum similar to the Rodeo gold ore in **Figure 4**. It was observed that starting with an iron oxidizer, followed by the spent medium of *P. chrysosporium*, help to improve the gold recovery from 24–92%. Although, an alkaline washing step had to be incorporated after the spent medium treatment to remove some of the byproducts of the carbonaceous matter decomposition to allow for the final 15% recovery to attain overall the 92% recovery. On the other hand, using the spent medium before the iron oxidizer appeared to inhibit sulfide oxidation and lead to a final gold recovery of 45% for that sequence.

The changes in the mineral phases were observed by Quantitative Evaluation of Minerals using Scanning Electron Microscopy (QEMSCAN) [3, 43]. The sulfides (yellow color) were observed to be relatively liberated while the carbonaceous matter was associated with illite as seen in by the dark green color (**Figure 5a**). After the best sequential treatment condition (sulfide oxidation followed by carbonaceous matter treatment by spent medium, DAC) was applied, most of the sulfide minerals were decomposed and disappeared (**Figure 5b**). Additionally, a new mineral phase (C-Si-Al) containing currently unknown amounts of carbon, aluminum and silicon was observed. A backscatter image of two organic carbon-containing particles is

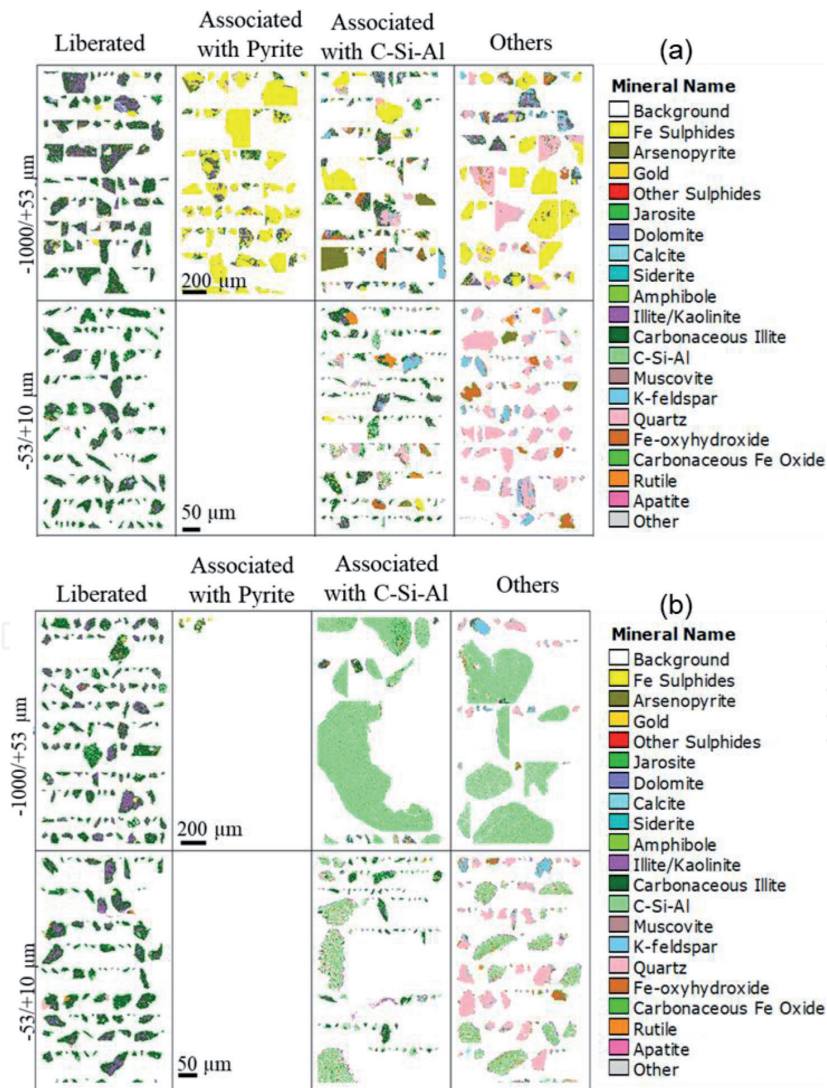


Figure 5. QEMSCAN maps for DRGO (a) and DAC (b): Particles are classified into liberated carbonaceous illite, associated with illite, associated with quartz, and others according to particle sizes. Notable changes from DRGO to DCA are the disappearance of pyrite and formation of larger particles than 100 µm which are categorized to C-Si-Al, and formation of larger particles than 100 µm which are rich in C, Si and Al (modified [43]).

shown in **Figure 6**. **Figure 6a** shows a particle in which the carbonaceous matter is associated with the illite in the original DRGO while **Figure 6b** shows a residue produced after the sequential treatment was applied (DAC). The sediment-like morphology of particle in **Figure 6b** indicated that it was most likely a product of the carbonaceous matter decomposition.

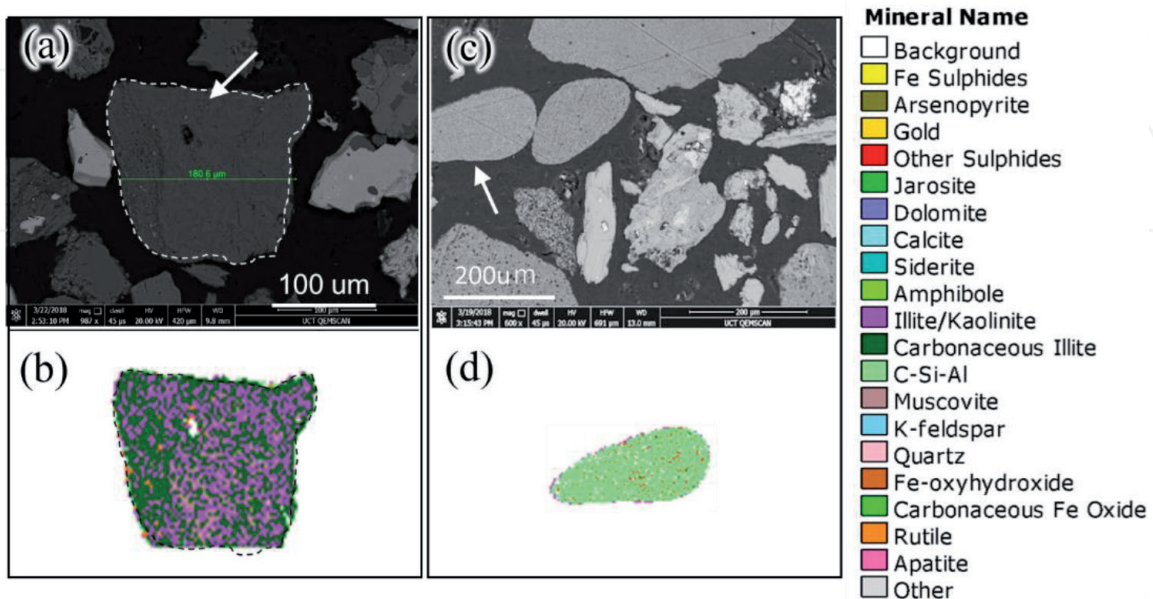


Figure 6. Backscattering images of (a) DRGO and (c) residue after sequential biotreatment with an iron-oxidizer and spent medium of *P. chrysosporium* (DAC). Images (b) and (d) are false color renditions of the arrowed particles in (a) and (c), respectively.

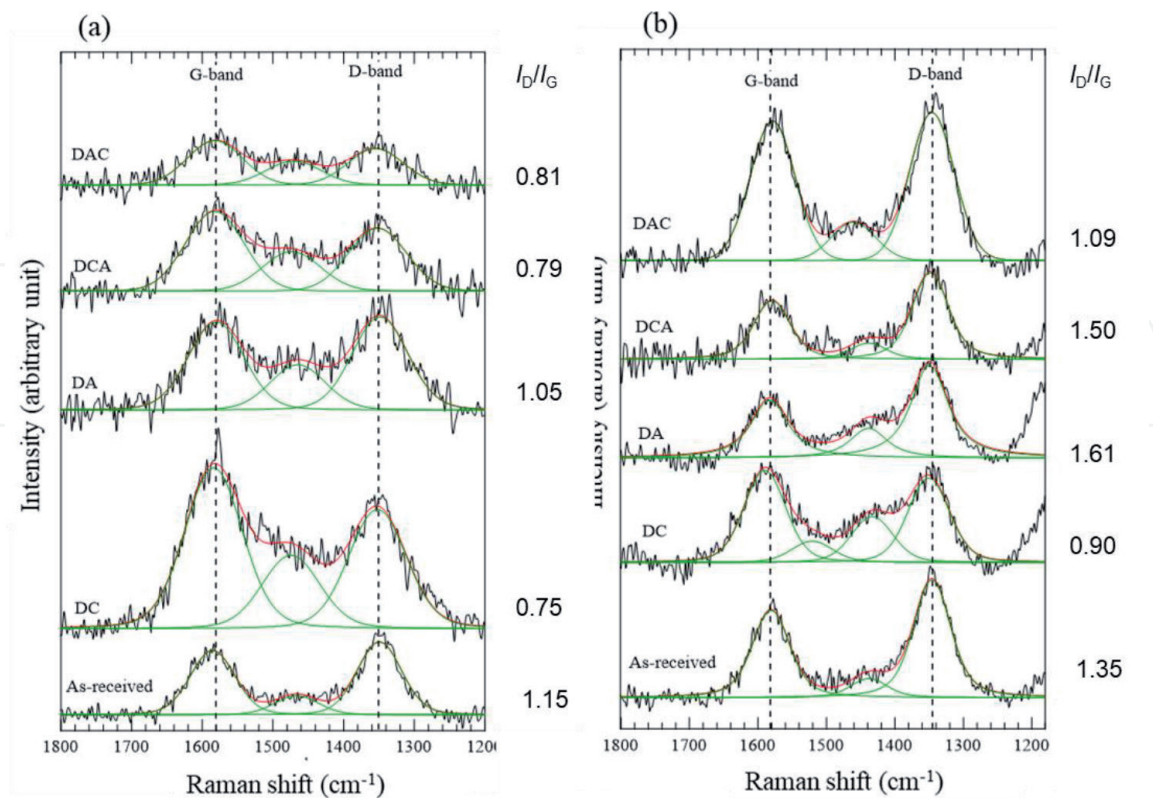


Figure 7. Raman spectra (a) before and (b) after 1 M NaOH washing of the as-received ore, and the solid residues after treated by CFSM (DC), *A. brierleyi* (DA), CFSM followed by *A. brierleyi* (DCA), and *A. brierleyi* followed by CFSM. Numbers beside figures indicate the intensity ratio (I_D/I_G) for the relative quantity of the defect in all samples with graphitic structures (modified [43]).

Raman spectra of the samples before and after each treatment showed a relative decrease in the D-band compared to the G-band (**Figure 7a**). This change was most significant after the spent medium treatment indicating that the lignin-degrading enzymes might have preferentially oxidized aromatic C=C carbon with some type of physical or chemical defects [43].

After the sequential treatment, the gold recovered before and after the spent medium treatment was unchanged at 77%, indicating that some of the by-products of this treatment were interfering with the recovery (DAC, **Figure 8**). Therefore, 1 M NaOH washing was incorporated into the sequential treatment to remove what

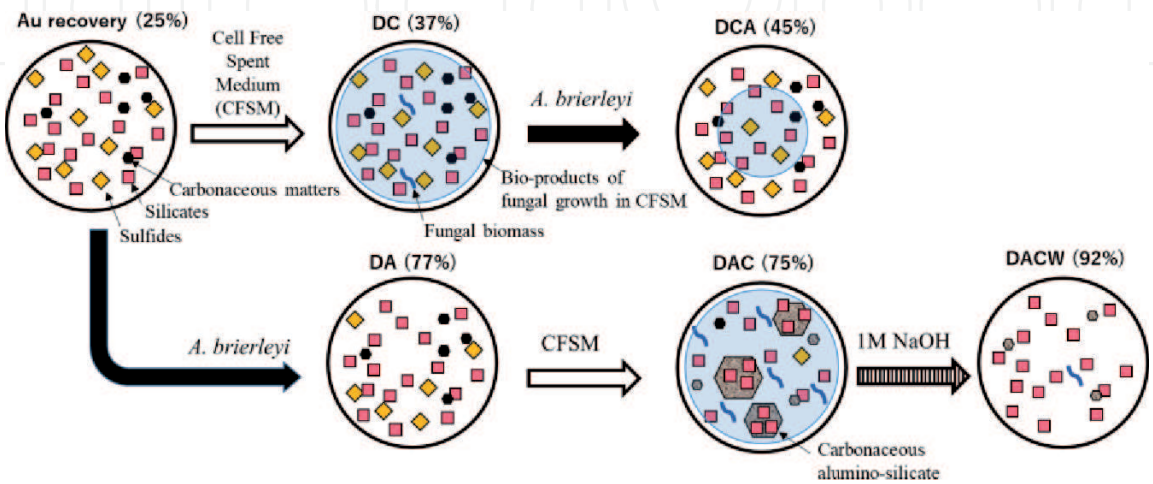


Figure 8. Gold recovery in each step of sequential biotreatment of DRGO (modified [13]).

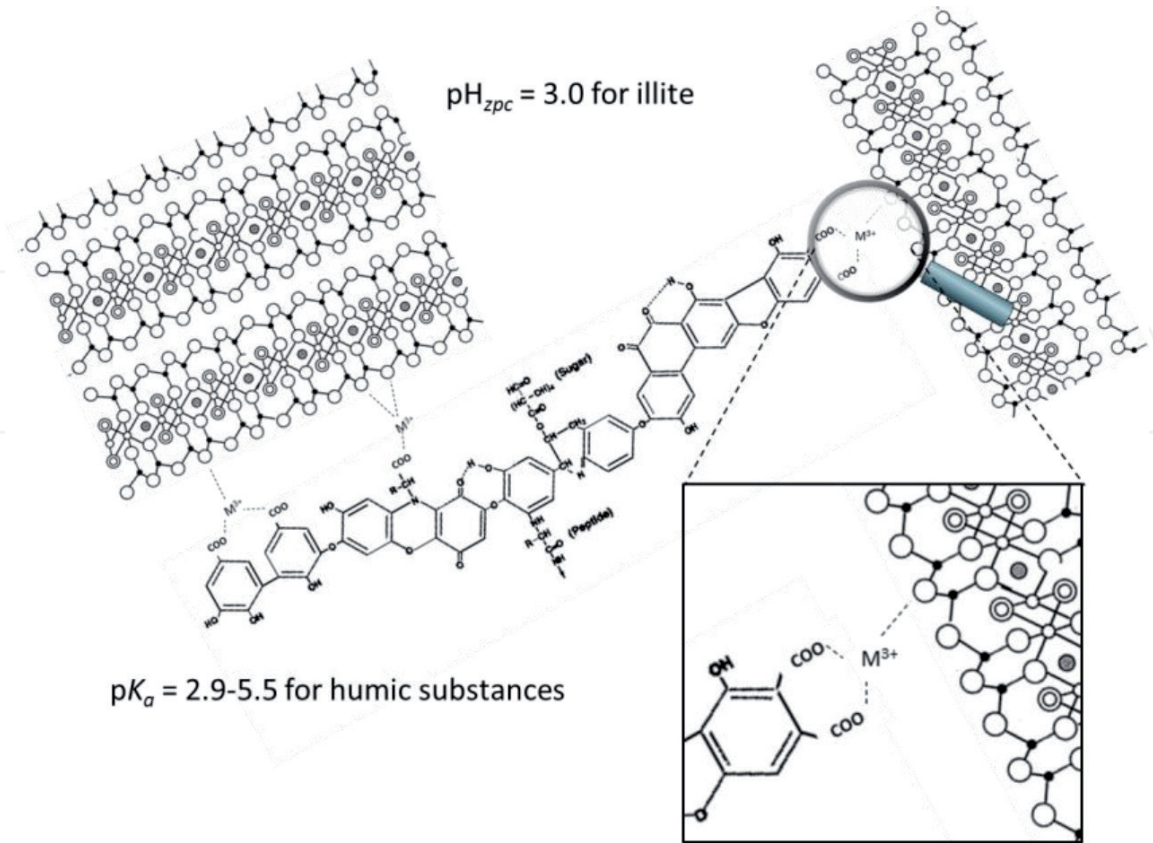


Figure 9. Schematic illustration of agglomerated particles in DAC after enzyme treatment, which are rich in C, Al and Si. After enzyme treatment, humic-like substances are formed to interact with clay minerals through metallic ions like Fe^{3+} .

was identified by 3D fluorescence spectroscopy as humic-like substances interfering during the gold recovery process, which lead to the observed changes in the Raman spectra and increase in gold recovery to 92% (**Figure 7b**) [43]. Thus, it was proposed that sediment-like C-Si-Al phase could have been produced by an electrostatic aggregation of clay minerals like illite and the humic-like substances. Under the condition of pH 4.0, where the enzyme treatment is performed, the surface of illite is negatively charged (isoelectric point 3.0) (**Figure 9**). While the humic-like substances have a large, irregular structure, and thus, the acid dissociation constant does not have a uniform value and has a width of 2.9–5.5. At pH 4.0, if the humic-like substances have a positive charge, then it might direct form an agglomerate with the negatively charged illite. However, if charged negative, then some of the cations in the solution like Fe^{3+} might be involved in the agglomeration of C-Si-Al.

4. Conclusions

Based on the above, a sequence for DRGO bio-processing was proposed in **Figure 10**. After crushing and flotation, the biooxidation of the sulfide mineral should be performed under strongly acidic conditions to liberate the gold. Afterwards, the spent medium collected from a separate culturing bioreactor for *P. chrysosporium* will be applied to the residue from the sulfide biooxidation tank. This is to decompose the carbonaceous matter in the sample. The residue will then be sent for alkaline washing to remove the humic-like substances and break apart the C-Si-Al agglomerates before cyanidation to recover gold.

These steps need to be reviewed according to the content and localization of gold in the gold ore, the aromaticity of carbon, and the concentration of soluble iron components in the system. From the Raman spectroscopy analysis of the solid

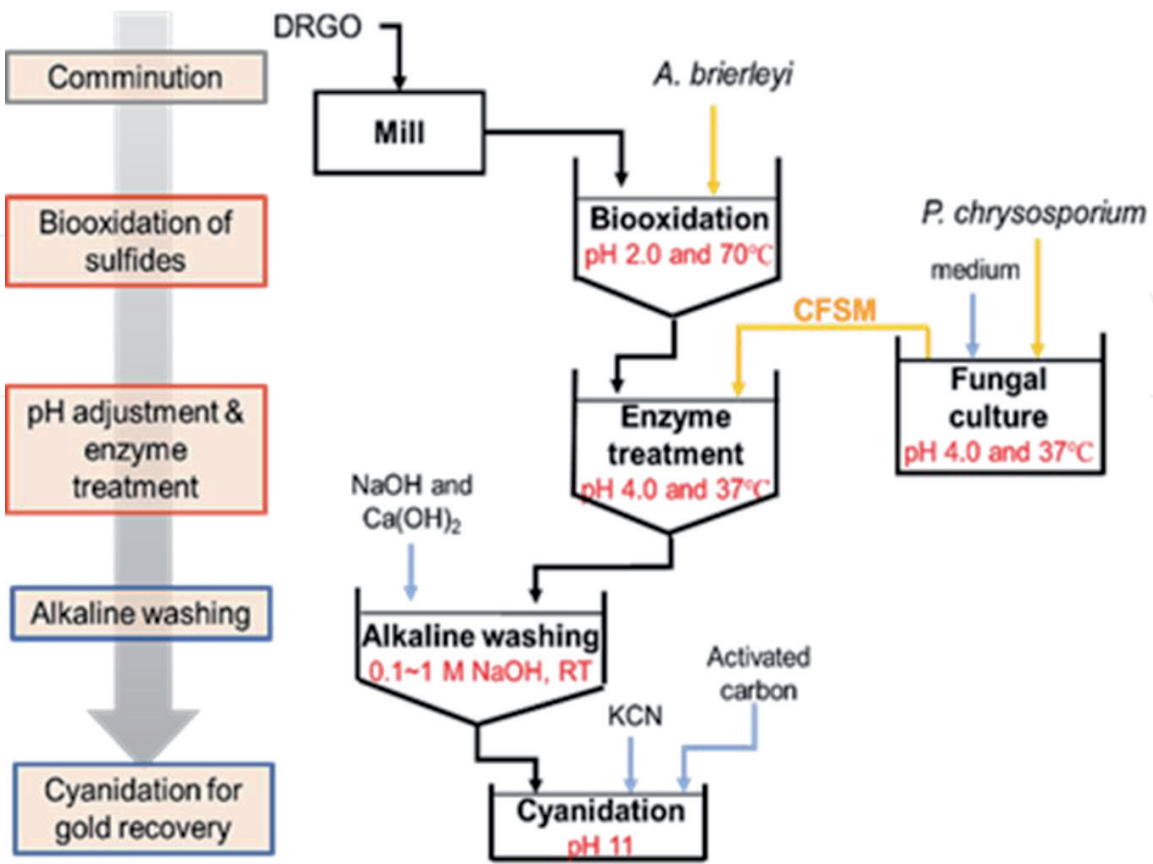


Figure 10.
Proposal of sequential biotreatment process of DRGO.

residue, after the spent medium treatment and alkaline washing, the lignin-degrading enzymes are more effective at decomposing defective graphite, and alkaline washing is more effective in converting graphitic carbon to defective form. Now it is known that it is advantageous for DRGO, which has a high aromatic attribute, to be first alkali-treated before biotreatment.

In the future, the treatment process for various types of carbonaceous gold ore will be organized and targeted for development by utilizing the characteristics of bio-treatment and chemical treatment and will contribute to the production of gold. Additional investigations will be conducted into utilizing less harmful gold lixiviants like amino acids to compared their efficacy against the cyanide system for DRGO processing (**Figure 1**) [44, 45]. Such research, in addition to using lignin-degrading enzymes for biooxidation, will greatly contribute to decreasing the environmental impact of processing DRGO.

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