



# Carbon-in-Leach Gold Recovery from Fungi-treated Carbonaceous Ore: Effect of Entrained Biomass on Activity of Activated Carbon

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**Abstract:** The ability of the fungus, *Phanerochaete chrysosporium* to reduce preg-robbing of carbonaceous matter (CM) in gold ores has been confirmed by many researchers, and studies are ongoing to minimize the effect of entrained biomass on subsequent gold leaching and adsorption processes. This paper presents a study on gold extraction from surrogate carbonaceous gold ore (CGO), and fungal-treated CGO to ascertain the influence of entrained biomass on the downstream carbon-in-leach (CIL) process. The surrogate CGO was prepared by adding 3% anthracite-grade CM to free-milling gold ore (FGO). The main minerals in the FGO were quartz, feldspar and sericite, with 8.4 g/t gold, 0.18% sulphide sulphur and 0.06% organic carbon. Aside biotreatment of the CGO with cell-free liquor of *P. chrysosporium*, anthracite and activated carbon (AC) were also contacted with *P. chrysosporium* to confirm the direct effect of biomass on the CMs' ability to preg-rob aurocyanide. Preg-robbing effect of the as-received anthracite and AC were 95% and 80% respectively, and these reduced to 81% and 13% respectively. Water-washing, acid-washing and alkaline-washing of the treated CMs returned respective preg-robbing effects of 88%, 92% and 85% for AC and 32%, 38% and 28% for anthracite. Scanning electron microscopy and Raman spectroscopy of anthracite revealed a decrease in the degree of orderliness in the structure required for gold adsorption. The FGO and the prepared CGO gave cyanidation gold recoveries of 94.5% and 54.8% respectively. Following cell-free treatment of the CGO, direct cyanidation recorded 88.3% solution recovery, which was increased to 91.3% in CIL. The overall recovery onto activated carbon in CIL was 81%, which improved to 82% and 85% respectively after washing the fungal-treated CGO with water and NaOH. The results here affirm that thorough washing of fungal-treated CGO assists in removing some entrained biomass. However, the results also call for additional studies on purification of the cell-free liquor to further minimize the biomass effect, and sustainability of the enzyme activity during pretreatment to enhance the overall gold recovery.

**Keywords:** Carbon-in-Leach, Fungal Biomass, Activated Carbon, Anthracite, Carbonaceous Gold Ore, Free-milling Ore

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## 1. Introduction

Extraction of gold from refractory ores poses serious challenges to mining companies, and raises scientific and engineering concerns regarding innovations in processing

techniques. In a typical gold processing plant treating refractory ores that contain sulphides, the main biotic pretreatment option is the biooxidation process where iron- and sulphur- oxidising bacteria are used to liberate gold from the sulphides [1, 2]. The pretreated material undergoes washing to get rid of excess acid, toxic materials and microbial matter, after

which it is conditioned appropriately and fed into the Carbon-In-Leach (CIL) circuit, where leaching of gold and adsorption of gold ions onto activated carbon (AC) are done simultaneously. The biooxidation process has been commercially available for about thirty years with great success [3-5]. However, there are challenges with recovery when the ore contains carbonaceous matter (CM), which being organic, is generally not degraded by the bacteria which are autolithotrophs [6-8]. The CM thus enters the CIL circuit and pre-robbs dissolved gold, leading to decreased recovery and high tailings value [6, 9-14].

Research efforts have resulted in microbial methods that can biotransform the CM and attenuate its gold adsorption ability [15-18]. One of such probable methods is the use of the fungus *Phanerochaete chrysosporium*, and there are reports on drastic reduction in pre-robbling [19-22] and increase in overall gold recovery [10, 23-25]. However, the fungus grows into thick biomass that clings to the substrate, and one challenge encountered in its usage has to do with washing and removal of the biomass from the treated material prior to the CIL process [10]. This challenge led to the investigations into cell-free biotransformation by Ofori-Sarpong *et al.* [26], thus reducing drastically the clinging effect of cells on the treated material. The use of crude enzymes and liquid cultures to biotransform different types of carbon surrogates for CM; including carbon black, AC, anthracite coal and elemental carbon have been investigated by several researchers who have reported reduction in gold adsorption activity of these carbons [22, 27-28]. Studies by Bonnah *et al.* [27] and Azzigbli *et al.* [29] employing AC as surrogate CM recommended sufficient water washing as a requirement to reduce the effect of entrained biomass on carbon.

The adhesion and entrainment of biomass on treated ore could pose post-treatment challenges in downstream cyanidation, and spores in the cell-free liquor could germinate into cells when the operation is prolonged [31]. Commercial cyanidation employs the CIL process where AC is added purposely to adsorb gold, and such carbons have to be very active. If the CIL process follows fungal pretreatment, the entrained biomass could passivate the surface of AC and reduce its ability to adsorb gold in the CIL circuit. Some researchers have used water and other solvents to wash AC after its treatment with cell-free liquor, and in all cases, the washing led to increase in the ability of the AC to adsorb gold relative to the unwashed material [22, 27, 29]. After cell-free treatment of carbon black by Konadu *et al.* [22], the authors observed higher adsorption after washing the treated carbon with propanol and sodium hydroxide. Work done by Adzigbli *et al.* [29] on contacting activated carbon with cell-free liquor also reported that higher volumes of water and longer washing hours lead to increased ability of treated AC to adsorb gold.

With this knowledge, it is important to assess how carbonaceous gold ore (CGO), which has been treated with *P. chrysosporium* will respond to gold recovery in CIL circuit, and the effect of possible microbial biomass on the AC introduced. The present investigation therefore considers a series of studies which mimic the CIL process as a post-fungal treatment gold recovery process. The CGO was

prepared by introducing anthracite-grade CM into free-milling ore. After treatment with cell-free liquor of *P. chrysosporium*, the ore was leached by cyanidation in the presence and absence of AC to assess the impact of entrained biomass on AC, which was used in recovering gold during the subsequent CIL process. The treated materials were also subjected to a series of washing protocols, and the varying effects on pre-robbling are reported in this paper.

## 2. Experimental Investigations

### 2.1. Materials Used

The gold ore utilized in the study was a geological sample obtained from the free-milling Tarkwaian system of Ghana. Anthracite coal which served as carbonaceous matter in the free-milling ore, was obtained from the Coal Bank of Penn State University, USA. Reagent grades of dextrose powder, ammonium tartrate, succinic acid, thiamin, sodium tartrate, and standard gold solution were supplied by VWR, while manganese sulfate, hydrogen peroxide, hydrochloric acid, sodium hydroxide and sodium cyanide were obtained from the Minerals Laboratory of University of Mines and Technology, Tarkwa, Ghana. Anthracite was washed with dilute HCl, rinsed with water, and dried at 37°C before it was added to the free-milling material. Fungal spores of *Phanerochaete chrysosporium* ME446, were obtained from Prof Ming Tien (Penn State University).

### 2.2. Method

#### 2.2.1. Characterization of Oxide Gold Ore and Carbonaceous Matter

The geological samples used in this paper were analysed megascopically and also by thin and polish sections. Thin and polish sections were observed using Leica 2700 MP Petrological Microscope, and the volume percentages of the minerals observed were obtained using a point counting device. The tests were carried out along guidelines in ASTM C295-18a and ASTM C856-18a. Sulphur and carbon speciation were determined using the combustion volumetric technique. Gold in particulate samples were determined by conventional fire assaying and that in solution samples by Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES). Determination of selected metals was done using aqua-regia digestion and ICP-AES.

The anthracite used in this study, before and after fungal treatment, were characterized with Raman spectroscopy and scanning electron microscopy (SEM). For Raman spectroscopy, samples were diluted into 1 wt% with spectroscopy grade KBr and compacted into tablets. The samples were then mounted on adhesive sample holder, and the spectra were collected via the Confocal WITec XY Raman spectrometer, with a polarized laser light of 488 nm wavelength using a 40X objective lens. The spectra were collected at 5 min interval with 1 s integration time. SEM analysis was carried out using Camscan Model CS44FE scanning electron microscope at an acceleration voltage of 20 kV and 30 kV.

### 2.2.2. Preparation of Medium, Incubation of Fungus, and Extraction of Cell-free Components

Glucose broth (liquid) medium was prepared with dextrose powder (carbon source) and ammonium tartrate (nitrogen source), and the other components as described by [19, 22-23, 30]. Succinic acid was used to buffer the solution, and the pH adjusted to 4.0 with hydrochloric acid or sodium hydroxide. The medium without thiamin (protein source) was autoclaved at 121°C for 30 minutes and cooled down before addition of thiamin, which is heat-sensitive.

The medium was inoculated with 1 vial of spore suspension of *P. chrysosporium* per 1000 mL of medium as detailed in [19]. The inoculated medium was distributed as 200-mL solution into 1000-mL Erlenmeyer flask, and allowed to incubate for 7 days under stationary culturing at 37 °C. The flasks were covered by porous plugs to allow for aeration as described by [30].

At the end of the incubation period, the cell-free liquid (supernatant) was extracted from the culture by sieving through sterilized quadruple-layered cheesecloth and 0.22 µm micropore filter unit. The cell-free extract (crude enzymes) was buffered with sodium tartrate to pH of 4 and used immediately.

### 2.2.3. Treatment of Anthracite and Gold Ores with Cell-free Liquor and Harvesting of Treated Material

To ascertain the influence of fungal treatment on the gold leaching and adsorption properties, triplicate samples weighing 10 g each of activated carbon and anthracite were contacted with cell-free liquor at 20% pulp density, for 24 hours at 37°C. Also, five batches of triplicate samples of the prepared carbonaceous gold ore, weighing 100 g each, were pulped with the cell-free liquor to 20% pulp density in 1 L Erlenmeyer Flask. The flasks were covered with foam and incubated for 24 hours at 37°C and 150 rpm on a New Brunswick Series 25 incubator shaker. Control experiments were also set up under similar conditions as described above, except that there was no addition of cell-free liquor. At the end of the residence time, the samples were washed under different washing regimes (no washing, water-washing, acid-washing and alkaline-washing). The supernatant was decanted and the residue filtered and dried at 37°C for 3 days.

### 2.2.4. Gold Recovery by Carbon-in-Leach

The possibility of cell-free liquor carrying entrained biomass to partially passivate the activated carbon (AC) in CIL was assessed by conducting cyanidation with and without AC for the treated materials after the washing regimes. These were compared with cyanidation of the untreated gold ores. Cyanidation was conducted at 25% pulp density and 350 mg/L cyanide concentration. The pH was maintained between 11 and 11.5 by the addition of sodium hydroxide. The samples were agitated at 250 rpm for 24 hours. At the end of the leaching period, the pulp was filtered, and the solutions obtained was analyzed for gold by ICP-AES whereas the residual solids (tailings) were assayed for residual gold by fire assaying. Equation 1 was used in calculating the percentage gold recovery (PGR), where W is the weight of feed material, V is the volume of water used in the cyanidation experiment,

C is the concentration of gold in the leachate after cyanidation as determined by ICP-AES, whereas the CHG is the calculated head grade of the gold-containing material [10].

$$\text{PGR}(\text{wt}\%) = \frac{\frac{1}{W}(\text{g}^{-1}) \times V(\text{mL}) \times C(\mu\text{g}/\text{mL})}{\text{CHG}(\text{g}/\text{t})} \times 100\% \quad (1)$$

### 2.2.5. Effect of Washing on Preg-robbing Activities of Treated Carbonaceous Materials

To evaluate the extent of surface deactivation of CM in the CGO, preg-robbing test was used to compare the adsorption ability of the AC and anthracite; as-received, after contact with the cell-free liquor, and after washing of the treated CM with water and with 1 M NaOH. The CM, weighing 1 g each was contacted with 25 mL of gold solution obtained from the cyanidation of the oxide ore, at 8 mg/L gold concentration. Triplicate samples of the CM were agitated for 24 hr at 150 rpm on an incubator shaker. At the end of the contact time, the residual solution was filtered, and gold in the filtrate determined using ICP-AES. The difference between the concentration of gold in solution before (Initial conc) and after (Final conc) the adsorption test was computed and expressed in percentage according to Equation 2. The extent of preg-robbing was estimated by comparing the gold adsorbed on the as-received with the CMs that have been in contact with the fungal extracts.

$$\% \text{ Adsorption} = \frac{\text{Initial conc.} - \text{Final Conc.}}{\text{Initial conc.}} \times 100\% \quad (2)$$

## 3. Results and Discussion

This study hypothesized that trace quantities of biomass can entrain fungal-treated refractory ore and become available in the downstream carbon-in-leach process, where it can interact with, and reduce the gold adsorption activity of activated carbon (AC) introduced purposefully to adsorb dissolved gold. To ascertain this effect, the study set out to simulate carbonaceous gold ore (CGO) by dosing 3% anthracite-grade carbonaceous matter (CM) into the free-milling gold ore (FGO). The FGO and CGO were treated with cell-free fungal extract, subjected to different washing regimes, and leached with and without activated carbon. The ensuing sections presents and discusses results on characterization of the FGO, and as-received and treated anthracite, and preg-robbing effect of anthracite and AC after contact with cell-free liquor and under different washing regimes. Cyanidation of treated CGO with and without activated carbon is also presented.

### 3.1. Characterization of Free-Milling Gold Ores and Carbonaceous Matter

#### 3.1.1. Chemical and Mineralogical Analysis of Free-Milling Ore

The petrographical analysis showing the rock and ore forming minerals are presented in Table 1, with photomicrographs depicted in Figure 1, while partial chemical analysis of the as-received FGO is presented in Table 2.

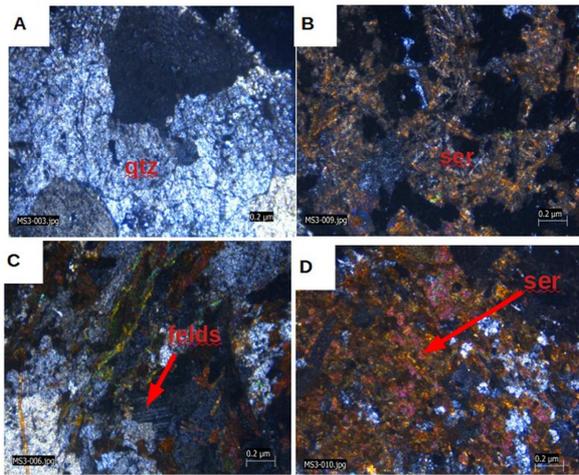
**Table 1.** Modal analysis of minerals in the as-received free-milling gold ore.

Rock and ore forming minerals, %			
Quartz	Feldspar	Sericite	Iron hydroxides
35	25	25	15

**Table 2.** Partial chemical analysis of the as-received free-milling gold ore.

Assay, g/t					
Au	Ag	Cu	As	Sb	Hg
8.43	0.85	42.	<2	<3	<1

Sulphur and carbon speciation, %				
S <sub>total</sub>	S <sup>2-</sup>	C <sub>total</sub>	C <sub>organic</sub>	C <sub>inorganic</sub>
0.52	0.18	0.06	0.06	<0.01

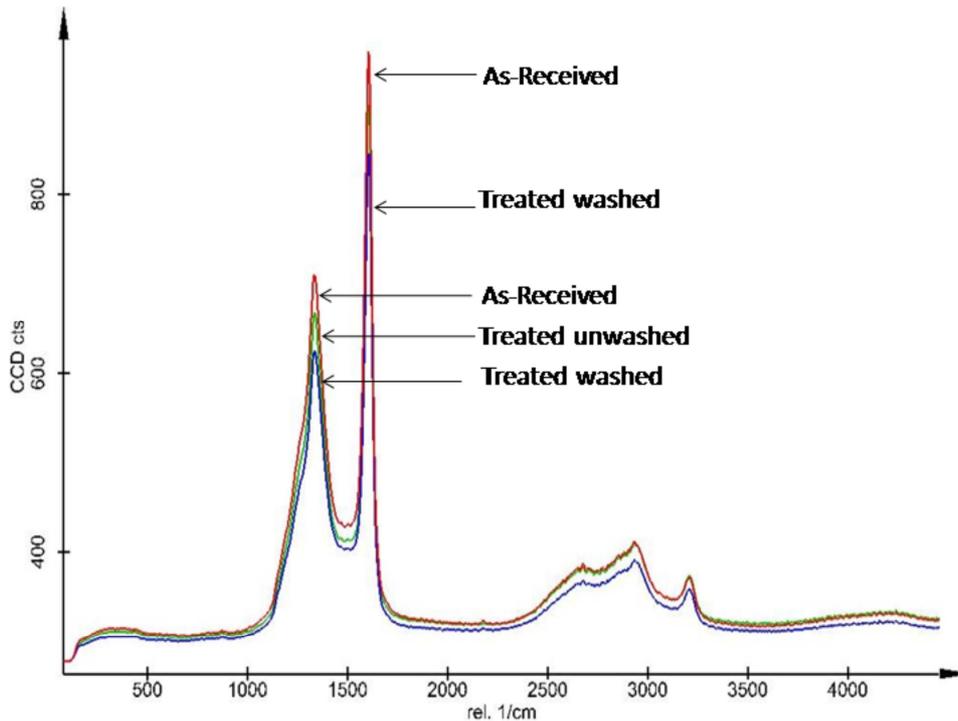


**Figure 1.** Petrographic evaluation of free-milling gold ore showing quartz (qtz), sericite (ser) and feldspar (felds).

The major minerals were quartz, feldspar and sericite. The presence of iron hydroxides and sericite indicates some amount of weathering. The grade of gold was high at 8.43 g/t. The total sulphur content was 0.52% while the sulphide sulphur was 0.18%, confirming the weathering observed from the mineralogical study. With sulphide sulphur content of 0.18%, it can be confirmed that the ore is non-sulphidic. The organic carbon content was also very low at 0.06%, and so the ore is not carbonaceous.

**3.1.2. Raman Spectroscopy Studies**

The changes in degree of graphitization of the as-received and treated anthracite were studied using Raman spectroscopy, and the spectrograms are presented in Figure 2. Though there seems to be some controversy regarding the assignment of various peaks to the graphitic and disordered structures in CM by different researchers, there are some consensus regarding the two most distinct peaks around wavelengths of 1580 cm<sup>-1</sup> known as the graphite band (G), and 1370 cm<sup>-1</sup>, the defect band (D) [32, 33]. All the spectra showed three distinct bands at 1597 cm<sup>-1</sup> and 1346 cm<sup>-1</sup>, and 2500-3200 cm<sup>-1</sup> for the aliphatic C-H functional groups. The G-band gives an indication of the degree of graphitization of carbon, which is known to favour gold adsorption, while the D-line portrays disorderliness in the structure due to edge defects or lattice disorder. It can be seen from the figure that the drops in the two major peaks were not distinct, probably due to exhaustion of the cell-free liquor within the 24 hours of treatment [26, 31]. The slight reduction in the G line may, however, lead to lower carbon-to-oxygen ratio, and this is known to be unfavorable for gold adsorption [19, 20-21, 31, 34-35].



**Figure 2.** Raman spectroscopy of anthracite.

### 3.1.3. Scanning Electron Microscopy Studies

Changes in surface morphology of the as-received and biotreated anthracite were pictured using SEM as presented in Figure 3. Under SEM, the as-received anthracite showed an undisturbed surface, however, following microbial action, the surface was seriously etched and disturbed. The change in surface properties led to amorphization of the graphitic structure as suggested in Figure 2, and this can cause a reduction in gold adsorption capabilities of the microbial-treated anthracite.

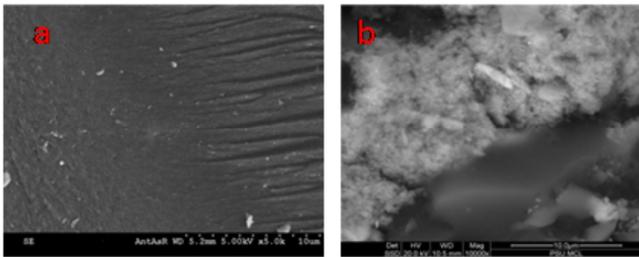


Figure 3. As-received anthracite (a), anthracite surface after biotreatment (b).

## 3.2. Recovery of Gold by Carbon-in-Leach

### 3.2.1. Cyanidation of Free-milling Gold Ores and Prepared Carbonaceous Gold Ores

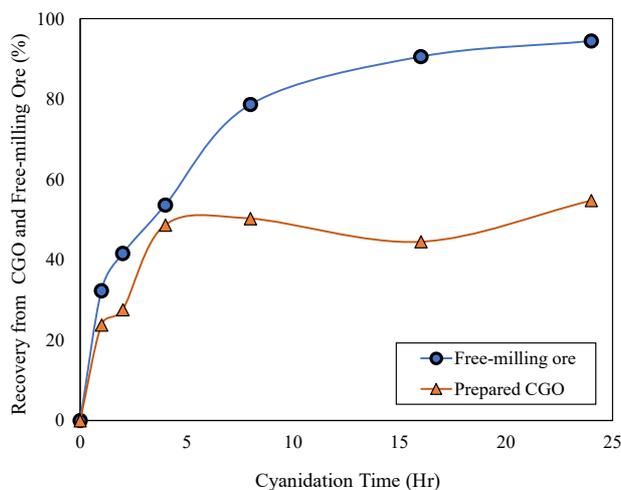


Figure 4. Cyanidation recovery from free-milling gold ore and prepared carbonaceous fold ore.

Refractory carbonaceous ores pose serious challenges during cyanidation of gold due to the ability of the carbonaceous matter (CM) to preg-rob dissolved gold that should be available for recovery. This effect is depicted in Figure 4, which shows cyanidation recovery from the free-milling gold ore (FGO) and the simulated carbonaceous gold ore (CGO). The FGO gave a recovery of 94.5% at the end of 24 hours of cyanidation, as is expected [2]. When the FGO was dosed with 3% anthracite-grade CM to prepare CGO, gold recovery dropped to 54.8%, showcasing the strong ability of the CM to preg-rob aurocyanide in line with results reported by other researchers [6, 9-13, 22-23]. The trend in the graph indicates that all the gold that

came into solution after 8 hours of leaching was sorbed back into the solid residue by the CM, accounting for 40% of what should have been available in solution for recovery. To reduce the loss in recovery, CGOs have to undergo pretreatment to deactivate the active sites of CM for gold adsorption.

### 3.2.2. Cyanidation of Prepared and Biotreated Carbonaceous Gold Ore

To reduce the preg-robbing effect, the CGO was subjected to treatment using cell-free liquor of *P. chrysosporium*, and the deactivation effect is portrayed by comparing gold recoveries from the surrogate CGO and treated CGOs over 24-hr leaching in Figure 5. As is expected, gold recovery from the treated CGO increased from 1 hr of leaching consistently to the 8<sup>th</sup> hour, after which the rate reduced and almost plateaued. Such an increase in gold recovery following fungal treatment has been recorded by other researchers [9-13, 18, 22-23, 25, 36]. The cell-free treatment increased recovery from 54.75% to 88.25% in 24 hours, implying 11.75% of Au was left in the solid residue. This is equivalent to about 1 g/t out of the initial head grade of 8.43 g/t.

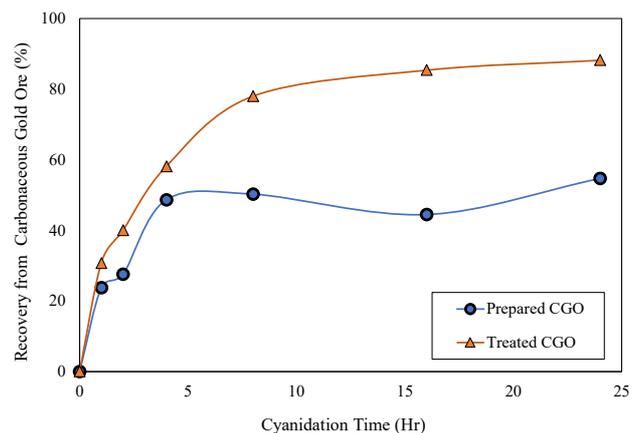
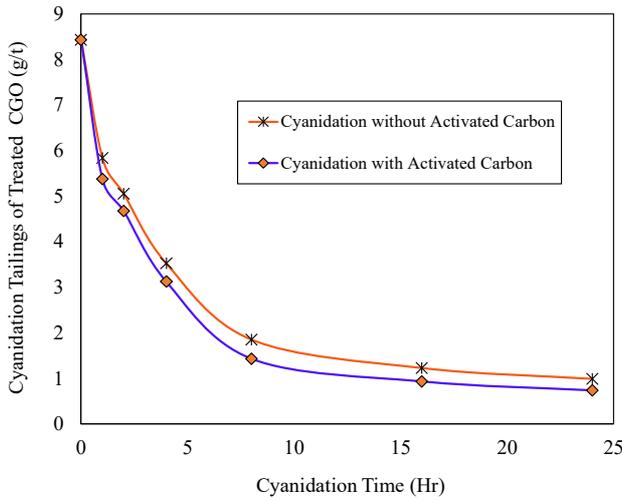


Figure 5. Cyanidation recovery from the prepared and treated CGOs.

### 3.2.3. Comparison Between Carbon-in-Leach and Conventional Cyanidation of Treated CGO

The 1 g/t tailings grade recorded after 24-hr conventional cyanidation of the treated CGO gives an indication of a portion of the CM being still active after the cell-free fungal treatment. In the presence of CM, gold recovery is more effective when activated carbon is added as it is done in carbon-in-leach (CIL) to outcompete naturally-occurring carbons [2]. The treated CGO was thus leached by CIL, and the gold in residual solids compared as presented in Figure 6. The figure gives a value of 0.73 g/t at the end of cyanidation with activated carbon (AC), lower by 0.25-0.46 g/t as compared with cyanidation without AC. This translated into a recovery difference of 5.5% in favour of the CIL process at 1 hour of cyanidation, and 5.0% and 3% respectively at the end of 8 and 24 hours. Nevertheless, Residual tailings grade of 0.73 g/t for the CIL still appears to be high, and this could be attributed to possible interaction of the AC with entrained fungal biomass, which is reported to reduce the activity of AC

[21-22, 27, 29].



**Figure 6.** Residual gold in solid tailings after leaching treated CGO with and without activated carbon.

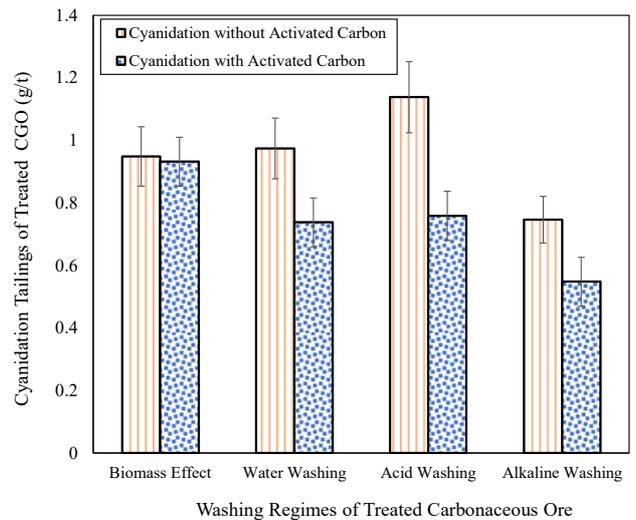
**3.3. Effect of Entrained Biomass on Gold Recovery by Activated Carbon (AC) in CIL Process**

Fungal-treatment may leave entrained biomass that may negatively influence AC’s ability to adsorb aurocyanide ion. A number of researchers subjected AC to various treatment using *P. chrysosporium*, and reported a decrease in the adsorption activity of AC [21-22, 27, 29, 31]. Bonnah *et al.* [27] theorized that sufficient water washing after contact of the fungus with AC could increase the ability of the AC to adsorb gold. This was tested by Adzigbli *et al.* [29] who subjected treated AC to different degrees of water washing before contact with standard gold solution. The authors concluded that sufficient water washing was necessary to increase the ability of the treated AC to adsorb gold from about 70% without washing to about 90% after washing 25 g of processed material with 1000 mL of water.

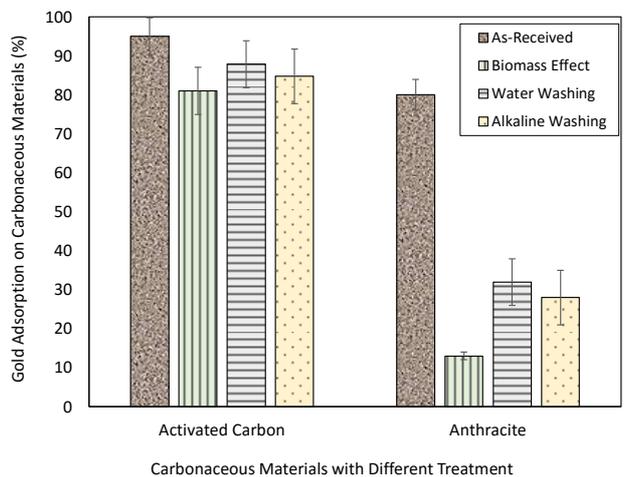
In this paper, the AC was introduced during cyanidation of the treated CGO as it occurs in commercial CIL operations to assess whether the ability of AC to adsorb the dissolved gold could be reduced due to entrained biomass on the treated CGO. To measure this, the treated CGOs were subjected to series of post-treatment washing prior to the CIL cyanidation process. Figure 7 shows results for gold recovery after no washing (biomass effect), washing in water, acid solution and alkaline solution. The figure gives an indication that washing in alkaline improves on dissolution of gold after fungal treatment, which is opposite to acid washing that can reactivate the surface of CM to preg-rob and increase the gold in residue [25, 37]. The acid washing exposes the surface of some of the treated CM in the ore, which increases preg-robbing and reduces the recovery of gold into solution; and in this case, the AC is also activated to adsorb more. Water-washing also increased the residue in leaching without activated carbon. The biomass appears to have a negative impact on the AC, as it returns the highest tailings value of 0.93 g/t for the CIL. The

fungal treatment leads to formation of organic products which passivate the surface of the treated carbon, reducing its ability to interact with aurocyanide ions, and this product can be washed off with NaOH [36]. In the CIL, however, all the tailings values were relatively lower.

To confirm directly the relative effect of the different washing regimes on AC and the anthracite, these carbons were contacted with gold solution after the treatments, and the effects are demonstrated in Figure 8. It is clear from this figure that the fungal interaction has effect on both carbonaceous materials but to different degrees; the highest being treatment without washing (biomass effect), followed by alkaline-washing before water-washing.



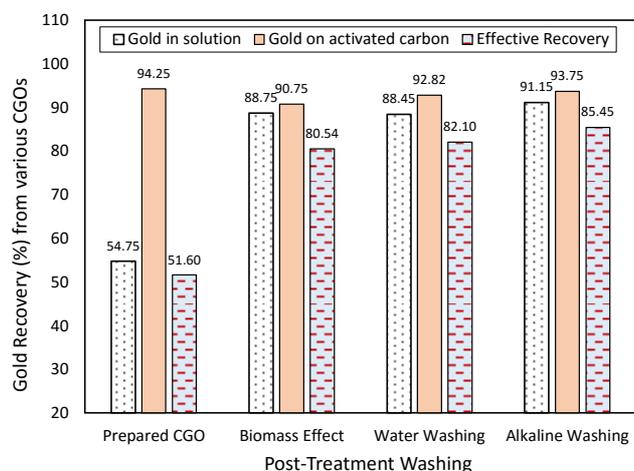
**Figure 7.** Effect of different washing regimes of treated CGO on CIL process.



**Figure 8.** Effect of different washing regimes of treated AC and anthracite on preg-robbing activities.

Figure 9 summarizes the overall effect by showing the recovery of gold from the ore into solution, the adsorption of gold from the leachate onto activated carbon, and the effective (overall) recovery which expresses the gold adsorbed as a percentage of what was dissolved from the ore. The highest overall recovery of 85.45% was achieved after washing the

treated CGO with sodium hydroxide. The NaOH-washing removes some surface coatings on the treated CGO and enhances dissolution of gold from the treated CGO [25, 36], and then, the AC is able to adsorb the dissolved gold. This was followed by recovery from the water-washed material (82.10%) and the material affected by biomass (80.54%). These two recorded higher recoveries than the untreated CGO, which had overall recovery of 51.60%. The unwashed treated CGO has entrained biomass, which partially blinds the surface of the AC when it is introduced, and reduces AC's ability to adsorb gold from the leachate, thus causing the highest solution tails of 18.45%. Altogether, washing with NaOH appears to be a good idea since it also conditions the slurry to the required pH for the downstream cyanidation process.



**Figure 9.** Overall CIL recovery of gold from untreated and fungal-treated CGOs.

## 4. Conclusions

The ore utilized in this study was free-milling gold ore with gold assay, sulphide sulphur, and organic carbon contents being 8.43 g/t, 0.18% and 0.06% respectively. The main minerals were quartz, feldspar and sericite. This ore was used in preparing carbonaceous ore by adding 3% anthracite-grade coal. Both the prepared carbonaceous gold ore and anthracite were acted upon by the fungus *Phanerochaete chrysosporium*. Fungal action led to changes in anthracite, and SEM showed a disturbed surface while Raman spectra indicated an increase in amorphous nature. As-received anthracite and activated carbon preg-robbed 95% and 80% of aurocyanide respectively, and these decreased to 81% and 13% after fungal treatment. When the treated CMs were washed with water, acid and alkaline preg-robbing effects recorded were 88%, 92% and 85% for activated carbon and 32%, 38% and 28% for anthracite respectively. Changes in carbonaceous ore before and after fungal action were observed through gold recovery by direct cyanidation and also by CIL. Gold extraction from the free-milling ore was 94.5% but reduced to 54.8% after addition of the anthracite. Biotreatment of the carbonaceous ore followed by direct cyanidation improved solution recovery to 88.3% while CIL recovery was higher at 91.3%.

The effective recovery of gold on activated carbon was 81% after the fungal treatment. This was improved to 82% and 85% respectively after washing the fungal-treated CGO with water and NaOH. The washing assisted in removing some entrained biomass. However, there is a need to extend the study to cover purification of the cell-free liquor and sustainability of the enzyme activity at the pretreatment stage to improve upon the overall recovery.

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