Suppression of Preg-Robbing Activity in Carbonaceous Gold Ores by the Fungus *Trametes Versicolor*

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Abstract

Carbonaceous matter in gold ores adsorbs dissolved gold during leaching and reduces extraction; a phenomenon known as preg-robbing. Microbial suppression of preg-robbing activity in a carbonaceous sulphidic gold ore was investigated using a white rot fungus, *Trametes versicolor* (ATTC 20869) with the aim of enhancing gold extraction. A study was conducted to monitor the effect of microbial-ore interactions on preg-robbing and the process variables were pulp density, temperature, pH and processing time. In addition, both biotic and abiotic investigations were performed to ascertain the effects of fungal culture and culture medium alone on reduction of preg-robbing. Analysis of ore carbon content after microbial contact indicated that *Trametes versicolor* reduced preg-robbing activity by passivation of carbonaceous matter and not degradation; as preg-robbing activity significantly decreased from 18.1% to below 1.0 % with insignificant changes in carbon content. Three (3) days of microbial-ore interaction at pH 4.5 and 30^{o}C temperature were the established optimum conditions for passivation. Various types of coal samples were utilized as surrogates and the study showed that the characteristics of the carbon material influenced the ability of *Trametes versicolor* to reduce preg-robbing. This novel application of *Trametes versicolor* to process carbonaceous gold ore resulted in significant suppression of preg-robbing activity.

Key Words

Preg-robbing; Passivation; Carbonaceous Matter; Fungus

1 Introduction

Carbonaceous gold ores are those containing carbonaceous matter which adsorb dissolved gold (or preg-rob) during cyanidation, resulting in reduced gold extraction. The most important classes of carbonaceous matter are organic carbon (hydrocarbons, humic acids and other organic substances) and graphitic or amorphous elemental carbon (Radtke and Scheiner, 1970; Osseo-Asare et al., 1984; Hausen and Bucknam, 1985; Stenebraten et al. 1999, 2000; Rees and Van Deventer, 2000).

Several investigations have indicated that all the components of carbonaceous matter may not be present in a given carbonaceous ore and that there may be wide variations in carbonaceous matter content form one deposit to another as well as composition and preg-robbing behavior from sample to sample within a deposit. Variations in carbon activity are also considerable as may be expected from a surface phenomenon (Osseo-Asare et al., 1984; Hausen and Bucknam, 1985; Stenebraten et al. 1999, 2000).

Based on their constituents, many researchers are of the view that the formation of carbonaceous matter is similar to that of coals. For example many of the organic compounds in carbonaceous matter associated with gold ores such as poly aromatic hydrocarbons and humic acids are present in lignite while the more matured graphitic carbon is also present in bituminous coal and anthracite. Carbonaceous matter and coal also have similar carbon to hydrogen ratio (Zumberge et al., 1978, Hallbauer, 1986, Sibrell et al., 1990, Stenebreten et al., 1999) and coal is also referred to as carbonaceous rock (Hippo et al., 1989, Faison, 1992). However, graphitic carbon is the main carbonaceous matter in carbonaceous gold ores and poses serious recovery concerns during leaching.

During processing of carbonaceous ores, preg-robbers are either degraded/passivated during pre-treatment stages or passivated during gold dissolution (Henley, 1975; Osseo-Asare et al., 1984; Demopoulos and Pangangelakis, 1987; Afenya, 1991; Linge, 1991) most pre-treatment steps do not oxidize or passivate carbonaceous matter significantly and thus continue to pose serious recovery challenges during the leaching stage. Oxidation or passivation of preg-robbers is the focus of most research in bio-mining in recent times (Portier, 1991; Brierley and Kulpa, 1992;

Kulpa and Brierley, 1993; Amankwah et al., 2005; Amankwah and Yen, 2006; Yen et al., 2008; Afidenyo et al, 2008; Ofori-Sarpong et al, 2010).

Portier (1991) used other heterotrophic bacteria and some fungi to degrade carbonaceous matter and reported increases in gold recovery due to microbial action. Brierley and Kulpa (1992, 1993) utilised a mixed regime of heterotrophic bacteria, many of which are from the *Pseudomonas* family and naturally associated with gold ores, to deactivate the active sites on carbonaceous components leading to increase in gold extraction during cyanidation. Investigations in biotransformation of carbonaceous matter by *Streptomyces setonii*, using two gold ore samples gave different responses to degradation and alteration of preg-robbing behaviour. This was attributed to differences in the characteristics of carbonaceous matter (Amankwah et al., 2005). In a further study, *Streptomyces setonii*, was used to study the effects of carbonaceous characteristics on microbial degradation and preg-robbing behaviour (Amankwah and Yen, 2006). Ofori-Sarpong et al. (2010) utilized the fungus *Pharnerochaete chrysosporium* to reduce preg-robbing activity of anthracite grade coal used as surrogate for coal by over 90%.

In a previous study by Afidenyo et al. (2008), refractory gold ores was subjected to biotransformation using the fungus *Trametes versicolor* leading to increases in gold recovery. This present study is an extension of the initial investigation. In this research, suppression of preg-robbing activity in carbonaceous gold ores and various coal samples was investigated by employing the versatile white-rot fungus *T. versicolor*. The preg-robbing behavior of the coal samples following biotransformation was compared with that of the carbonaceous gold ore. Fungal biotransformation of carbonaceous refractory gold ores presents a new platform in the application of fungi in mineral processing.

2 Experimental Investigation

2.1 Materials

Three coal types, lignite, bituminous coal and anthracite which represent virtually all the types of carbonaceous matter present in gold ores were used as models in studying preg-robbing suppression potential of the fungus. The samples were obtained from Ward's Natural Minerals Company while the carbonaceous gold ore was sourced from a plant which treats double refractory gold ore. The samples were crushed and pulverized to 95% passing 75 µm. Carbon content in the samples was determined by the combustion and volumetric method using a Leco titrator, SC-444DR. The carbon content of the as-received samples is indicated in Table1.

Table 1. Carbon content of samples used

Sample	Carbon, %
Lignite	62.8
Bituminous coal	85.5
Anthracite	99.44
Ore	0.63

2.2 Microorganisms and inoculum preparation

The fungus, *Tramestes versicolor* (ATTC 20869) was maintained on plates containing Kirk's medium, 1.5% agar and 3% malt extract. Agar plugs of the actively growing fungus were used to inoculate 500 ml of Kirk's medium in 1000 ml Erlenmeyer flasks. The medium contained 10 g/l glucose, 1.2 g/l ammonium tartrate, 0.05 g/l MnSO4 .7H2O, 0.01 g/l CaCl2 .$2H_2O$, 1 mg/l thiamine and 1 ml/l (Kirk et al. 1978). The medium was also supplemented 2.92 g/l 2, 2-dimethylsuccinate. The trace mineral solution contained 30 g/l $MnSO_4$. $7H_2O$, 10 g/l NaCl, 5g/l $MnSO_4$.H_2O, 1 g/l $CoSO_4$, 1 g/l FeSO4 .7H2O, 1 g/l $ZnSO_4$.$7H_2O$, 0.82 g/l $CaCl_2$, 0.1g/l $CuSO_4$.$5H_2O$, 0.1 g/l NaMoO4. $2H_2O$, 0.1 g/l H_3BO_3 and 1 g/l EDTA. Shake-flask cultures were grown at 30 ^oC with continuous agitation at 180 rpm for 10 – 14 days.

2.3 Microbial-Carbonaceous Matter Interactions

The ore and coal samples were contacted with the pregrown *Trametes versicolor* culture to investigate the biotransforming action of the fungus on carbonaceous matter and preg-robbing activity. Each sample of mass 10 g was contacted with 90 mls of freshly grown fungal culture in Erlenmeyer flask and agitated at 180 rpm. Process variables monitored were processing time, pH and temperature. Temperature was maintained at 28 – 30 ^oC and the pulp density at 10% solids. The test was conducted at pH between 3 and 10.5 and the natural (unadjusted) pH of the sample-fungal culture (4.5). Processing time was between 3 and 28 days. Sodium hydroxide (10 N) and hydrochloric acid (6 M) obtained from Alfa Aesar were used as pH modifiers. The microbial pre-treatment products were thoroughly washed with water and dried at 60 - 70^{o}C. Abiotic test (control) which involved only the culture medium was conducted to investigate the effect of the medium. Duplicate experiments were run and the differences in the values were within 1-3%.

2.4 Evaluation of Fungal Action on Carbonaceous Matter

Techniques employed to evaluate microbial actions included analysis of carbon content of fungal contacted samples, determination of mass changes of samples, infrared analysis, gold sorption before and after microbial contact and visual observations.

Pre-treated samples were digested with sodium hypochlorite to eliminate the interference of fungal biomass on the pre-treated sample weight and carbon content. During digestion, a sample of the product was contacted with 5-6% hypochlorite for 1 hour as described by Berger et al (1989) and Ramsay et al (1990) and thereafter thoroughly rinsed with water. Percentage reduction in carbonaceous matter was determined quantitatively using the Leco volumetric combustion technique.

Gold sorption tests were used to evaluate the preg-robbing properties of products obtained after the microbial contact. All pre-treated samples were washed with water and dried before evaluation. The tests were conducted on both pre-treated and untreated samples. Each sample of mass 3 g was placed in 27 ml solution of potassium gold cyanide containing 5.0 mg Au/l in 50-ml flasks. The pH was kept at 10.5 and free cyanide concentration of 0.5 g/l. Gold dissolution was not observed in the course of the preg-robbing tests. The samples were agitated at 75 rpm for 24 hours. The ore was then separated from the solution by filtration and the final solution gold determined using an atomic absorption spectrophotometer. The difference in gold concentration before and after the solution-sample contact is the measure of the preg-robbing activity.

3 Results and Discussion

3.1 Effect of Fungal Biotransformation on Carbon Content

Results of carbon analysis after fungal contact showed that carbonaceous matter in both the ore and coal samples was not degraded. There was rather an increase in the carbon content which was due to adhering fungal biomass. However, upon digestion of the fungal biomass with sodium hypochlorite, the carbon assayed for the pre-treated samples were almost at par with the as-received. This means *Trametes versicolor* did not degrade carbonaceous matter irrespective of the level of maturity of the coal sample.

3.2 Preg-robbing Studies on Fungal Treated Samples

When pre-treated coal and ore samples were subjected to gold sorption studies, the results indicated a decrease in gold sorption for bituminous and anthracite coals and an increase in sorption activity of lignite as shown in Table 2. Gold adsorption ability of bituminous coal dropped from 40.3% for the untreated sample to between 8 and 13% with the sample pretreated at near neutral having the lowest value of 8.3%. Gold adsorption by anthracite dropped from 99.7% for the as-received to 27.7% after pretreatment under alkaline conditions and 30.7 under acidic conditions. Neutral and unadjusted pH of 4.5 did not result in appreciable decrease in preg-robbing behavior as shown in Table 2. For all pH values, there was significant reduction in the preg-robbing behavior of the ore from an as-received value of 18.1% to values ranging from 0.4% for neutral pH to 2.3% for alkaline pH.

Generally, pre-treated samples recorded dips in gold sorption but for lignite as indicated in Table 5.1. In spite of the stable values of carbon content after pre-treatment, gold sorption values dropped significantly indicating passivation of the carbonaceous matter after microbial contact.

Table 2. Gold Sorption Data for Ore and Coal Samples after 14 days contact

Sample	**Gold Sorption after fungal treatment at various pH values, %**				
	As-received	**Acidic (3.0)**	**Neutral (7.0)**	**Alkaline (10.5)**	**Unadjusted (4.5)**
Lignite	33.5	59.8	35.8	40.1	56.9
Bituminous Coal	40.3	9.4	8.3	13.1	11.1

Anthracite	99.5	30.7	79.1	27.7	85.9
Ore Sample A	18.1	1.0	0.4	2.3	0.27

3.3 Effects of Process Variables on Preg-robbing Behaviour of the Ore Sample

Effect of Pulp Density

The effects of pretreatment pulp density was studied at various pulp densities between 5% and 50% solids for the biotic process at fixed alkaline pH of 9.5 -10.5 and temperature of 30^{o}C for 2 weeks. It was observed that pretreatment reduced preg-robbing activity and at 5% solids, preg-robbing dropped to a low of 0.7% from an as-received value of 18.1%. It was equally low for all pulp densities investigated and even at 50% solids preg-robbing was 6.5%. Increase in pulp density leads to a consequent increase in the rate of oxygen consumption. Since the microbes are aerobes, the resulting oxygen limitation at higher pulp densities gives rise to lower conversions. In addition, increase in pulp solids might result in high attrition rate. In a study by Bailey and Hansford (1993) it was shown that higher solids densities cause mechanical damage of microbes and this may be partly responsible for the trend observed.

Effect of pH

The fungus *Trametes versicolor* is a versatile fungus known to thrive in a wide range of pH. Thus the effect of pH was investigated for 3.5, unadjusted (4.25), 4.5 and 10.0 for both abiotic (control) and biotic process for 14 days studied at temperature of 30^{o}C. Carbon degradation was not observed but generally carbon values were higher than the as-received due to introduction of organic carbon by the fungal biomass and culture medium. However, preg-robbing trends were clear. Generally reduction in preg-robbing for the biotic was better than the abiotic as shown in Fig 1. This indicates that there is a microbial involvement in the carbon passivation process.

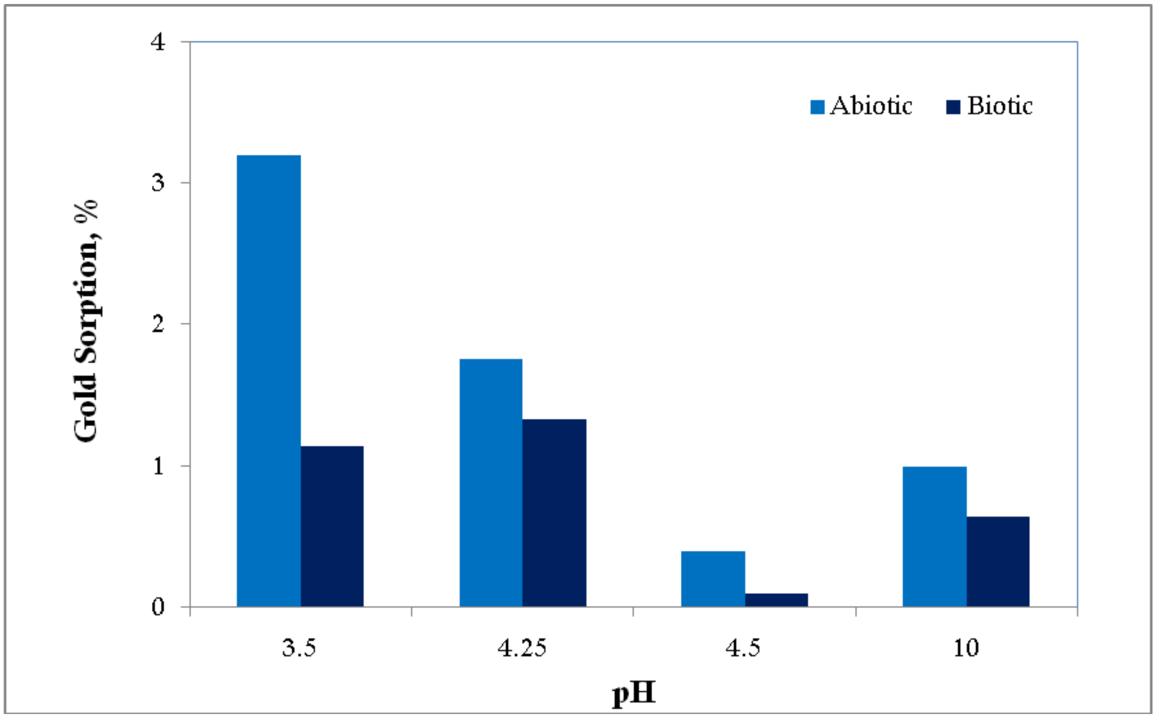

Fig 1. Gold sorption by ore sample after microbial contact at various pH values

Effect of Contact Time

Carbonaceous matter passivation was optimum at shorter contact times. Thus as the contact time was increased, passivation decreased and the ability of the bio-treated material to preg-rob increased. Since passivation is a surface phenomenon, it is possible that the continuous stirring and subsequent increase in attrition led to a reduction in passivating action of the fungal metabolites with time.

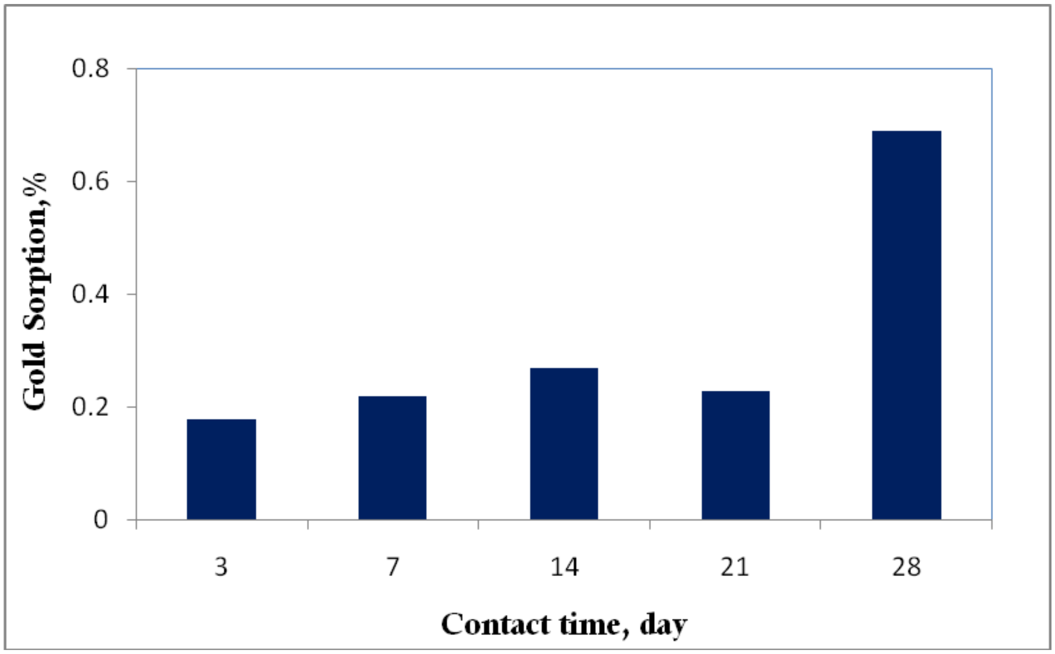

Fig 2. Gold sorption by ore sample after microbial pre-treatment for various contact time.

Analysis of Infra red Patterns after Fungal Contact

As-received and pre-treated ore sample were subjected to infra-red analysis and their spectra compared. The peak assignments, on the as-received and the pre-treated samples were deduced by comparing with earlier works by Rao (1963), Silverstein et al (1981) and Amankwah and Yen (2006).

Infrared analyses on various coal samples by Amankwah and Yen (2006) showed that lignite has more oxygen containing groups such as the -C=O and -COOH groups than bituminous coal and anthracite. Significant differences in their surface functional groups were also observed. Summarized in Table 3 are the characteristic peaks of the various components present in untreated lignite, bituminous coal and anthracite. Peaks of various carbon components present in the ore sample are also shown in Table 4.

The OH peak is generally attributed to the presence of water while CH_3 are the aliphatic hydrogen groups. Amankwah (2005) reported that oxidation or biodegradation of carbonaceous matter resulted in the formation of more oxygen containing groups (C=O) with the disappearance of aliphatic hydrogen groups (CH_2 and CH_3).

Table 3. Peaks of carbon components in as - received coal samples (Amankwah, 2004)

Coal Sample	**Peaks Wave number, cm^{-1}**				
	C=O	**CH_2**	**CH_3**	**OH**	**C=C**
Lignite	1650	-	2850 – 2980	3000 – 3800	-
Bituminous Coal	1620	1460	2980	-	-
Anthracite	-	1460	-	-	1580

Table 4. Peaks of carbon components present in ore sample

Pre-treatment	**Peaks Wave number, cm^{-1}**				
	C=O	**CH_2**	**CH_3**	**OH**	**COOH**
Untreated	1618-1658(5)	-	-	3426(1)	1092(2)
Abiotic(pH 10.5)	1638-1655(2)	-	-	3439(1)	1165-1091(2)
Biotic(Natural)	-	-	2895(1)	3253-3439(2)	1096(1)
Biotic(pH 10.5)	1636(1)	-	2544(1)	-	1089-1170(2)

Numbers of peaks are indicated in bracket

Comparing the as-is sample's peak components with the coal samples it could be deduced that the level of carbonaceous matter maturity in the sample is between lignite and bituminous coal as none of the peaks in anthracite was detected. Comparing the microbial treated samples with the as-received, the product of the abiotic test as shown in Fig. 4 showed similar peaks as the as-received in Fig. 3 except that there was reduction in the C=O peaks from 5 to 2 and the formation of another COOH group. This indicated that not much change has taken place in the carbonaceous matter components. For the biotic pre-treatment at unadjusted pH conditions, no C=O peak was detected but rather the formation of an aliphatic group and a reduction in the

COOH group as indicated in Fig 5. For the biotic process under alkaline conditions, only one C=O group was detected while an aliphatic group formed.

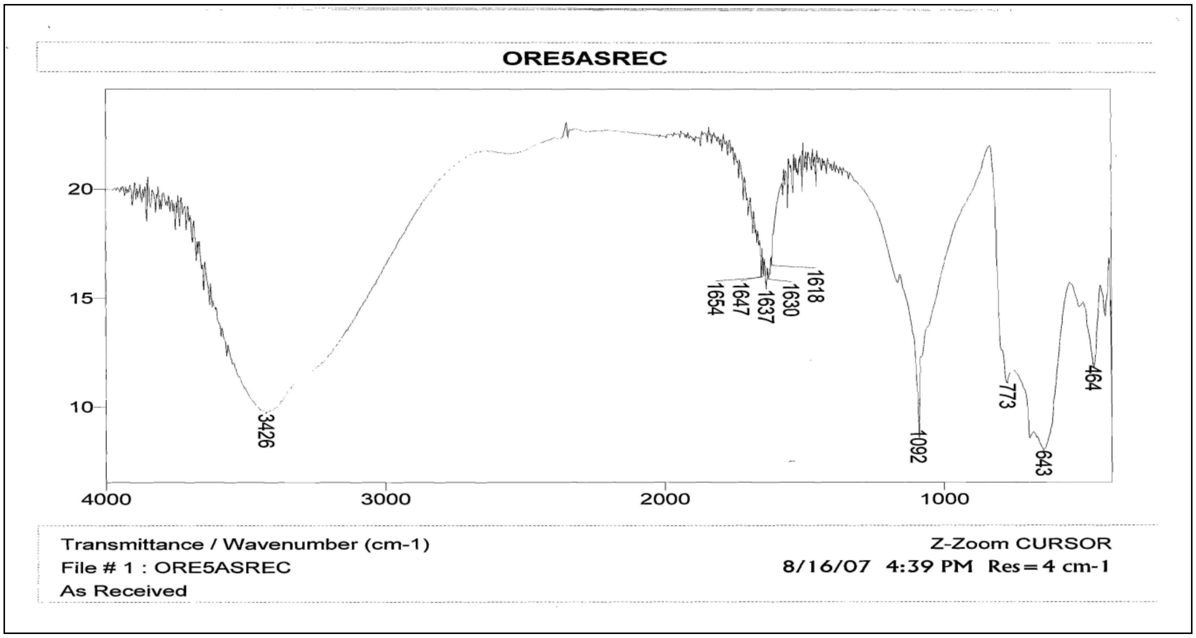

Fig. 3. As-received ore sample infrared diffraction pattern.

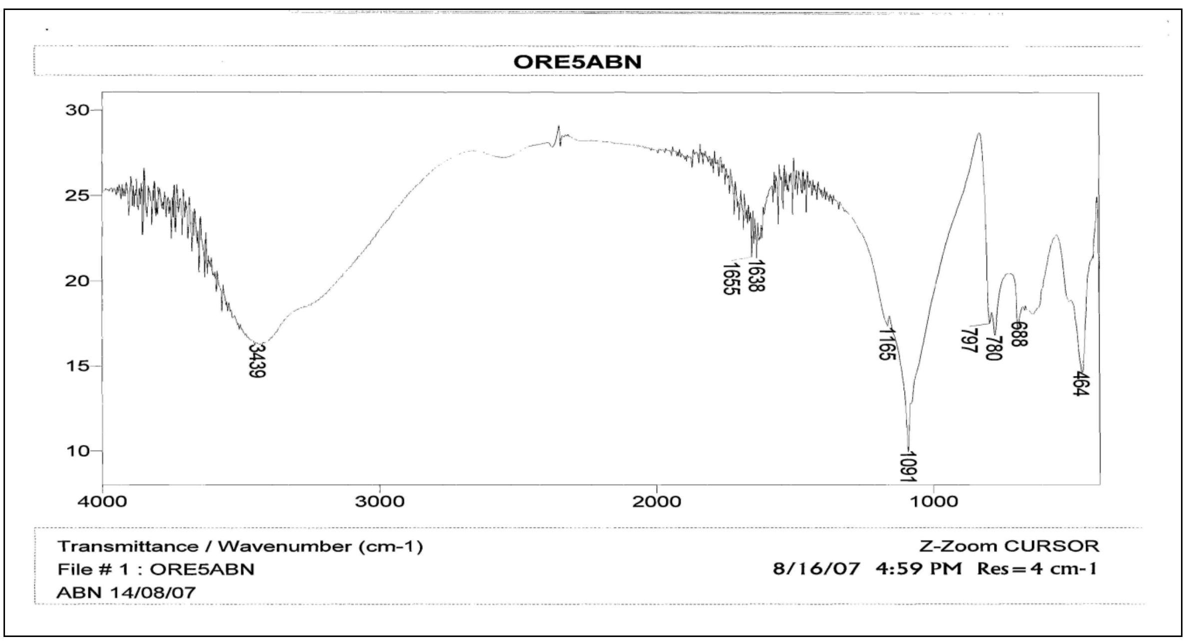

Fig 4. Ore sample infrared diffraction pattern after *Trametes versicolor* contact under abiotic alkaline condition.

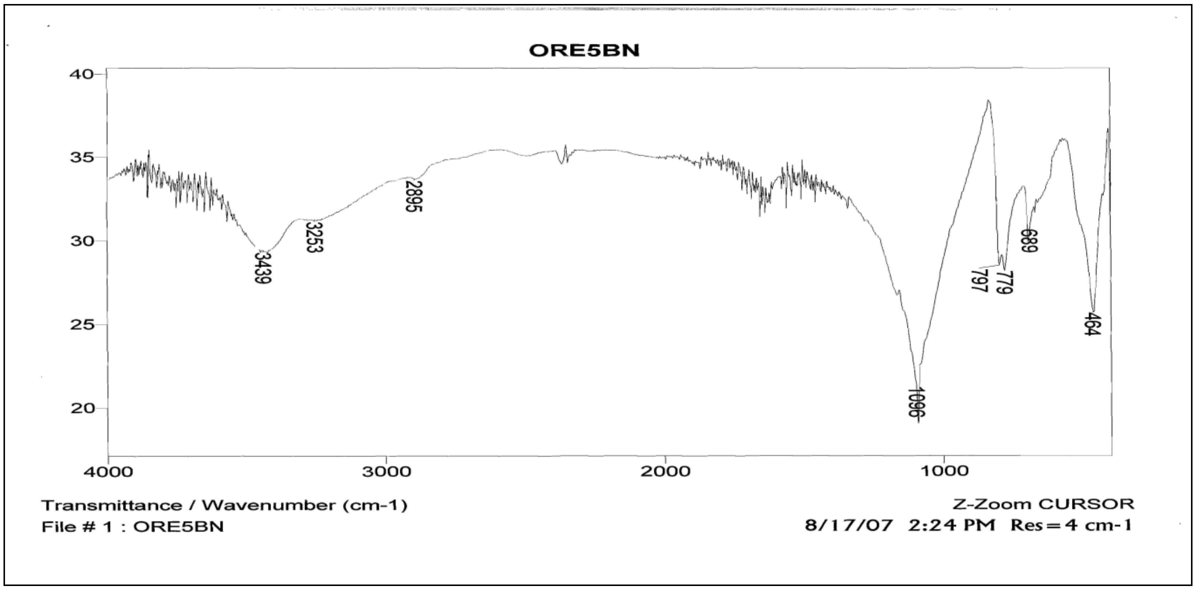

Fig 5. Ore sample infrared diffraction pattern after *Trametes versicolor* contact at optimum pH (4.5) condition.

Analysing the data, biodegradation of the carbonaceous matter was not observed as no new oxygen groups were formed after the *Trametes versicolor* contact. However, the preg-robbing activity of the pre-treated samples decreased significantly; thus confirming some surface passivating phenomenon rather than degradation. Carbon values after microbial contact indicated no significant changes as well. Both the fungal culture and the medium have carbonaceous matter passivating property.

Conclusion

No significant change in carbonaceous matter content after fungal contact with ore and coal samples was observed during the investigation. However, preg-robbing activity reduced drastically by 98.3% for the ore sample after 3 days contact at pH 4.5 indicating that carbonaceous matter was passivated. Passivation effect in the presence of *Tramestes versicolor* may be attributed to enzymatic secretions by the fungus.

This novel process is a potential for pre-treating double refractory gold ores. Fungal passivation of carbonaceous matter can be used to complement the traditional processes like pressure oxidation and bacterial leaching where the carbonaceous matter is not oxidized significantly.

The application of this process to passivate carbonaceous may not be universal and limits will need to be set. As an initial step, the carbonaceous gold ores may be investigated to find out whether the carbonaceous matter present is amenable to passivation by *Tramestes versicolor*.

Lignite did not respond well to passivation by *Tramestes versicolor* metabolites and as such carbonaceous matter of this rank may not respond to passivation. Double refractory gold ore with carbonaceous matter maturity of bituminous coal may not be passivated extensively but gold sorption could be reduced considerably.

Anthracite did not respond well to *Tramestes versicolor* passivation but better than lignite. The process is therefore recommended for ores in which the carbonaceous matter maturity is similar to bituminous coal and to a lesser extent anthracite. Gold ores containing lignite grade of carbonaceous matter may not be suitable for this process.

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Table 2: Percentage changes in samples' carbon content

	% Increase in Carbon Content			
Sample	**Acidic**	**Neutral**	**Alkaline**	**Control**
Lignite	14.25	9.47	8.68	16.84
Bituminous Coal	3.51	2.75	-0.18	-5.51
Anthracite	-8.47	-6.40	0.88	-2.63
Ore	24.92	15.40	-5.56	96.83