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Bioflocculation of high-ash Indian coals using *Paenibacillus polymyxa*

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Abstract

Most Indian coals have high ash content of the order of 25–35%. High ash in the coal not only reduces the thermal value of coal but also leads to production of fly ash, which is a major environmental problem. Cleaning with gravity concentration techniques is ineffective and more efficient techniques need to be developed. In recent times, bioflocculation as an alternative preparation method has been reported for a number of mineral systems including high-sulfur coals. In this paper, bioflocculation of high-ash Indian coals has been studied using *Paenibacillus polymyxa* for two coal samples. A quartz sample was used for comparison purposes. Zeta-potential measurements showed that coal samples and the bacterium were negatively charged over most of the pH range with a point-of-zero-charge (PZC) around pH 2–3. Surface free energy, determined through contact angle measurements, showed that the coal samples were hydrophobic while the bacterium was hydrophilic. Among the coal samples, the coal with the lower ash content exhibited greater hydrophobicity. Adhesion tests revealed that adhesion took place in about 25 min and that maximum adhesion occurred around pH 2. Similarly, flocculation tests showed that the bacterium flocculated coal effectively and efficiently with the best results around pH 2. More than 90% of the coal flocculated in about a minute in the presence of the bacterium while compared to about 20–30% in the absence of the bacterium. Flocculation of quartz was retarded under the same conditions, indicating that it is dispersed. Ash analysis of the flocculated portion showed a decrease in ash by 60% thereby suggesting that selective flocculation of coal is possible.

Keywords: coal; ash; adhesion; surface free energy; *Paenibacillus polymyxa*

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1. Introduction

Separation of unwanted mineral matter from coal to produce clean coal of high BTU value is a problem faced worldwide. Generally, gravity concentration techniques are used in most preparation plants to remove mineral matter from coal. In case the mineral matter is finely disseminated in coal, then gravity concentration techniques are not efficient. In such cases, methods such as flotation (Aplan, 1976; Klimpel, 1988; Misra and Anazia, 1987), flocculation (Attia, 1985; Palmes and Laskowski, 1993), agglomeration (Drzymala and Wheelock, 1993) and coagulation (Honaker et al., 1991) have been shown to be effective in removing mineral matter to low levels.

In the past decade, bioprocessing of coal as an alternative to conventional processes listed above has been shown to have a great potential for cleaning coal. Attia et al. (1993) showed that enhanced pyrite separation from coal by flotation is possible with biosurface modification using *Thiobacillus ferrooxidans*. Similarly, desulphurization of coal using various strains of *T. ferrooxidans* by column flotation has been reported (Ohmura and Saiki, 1994). Selective flocculation of fine coal using the bacterium *Mycobacterium phlei* has been reported and shown to be very effective in reducing both ash and pyritic sulphur content (Misra et al., 1993; Raichur et al., 1996). The selectively is based on the differential adhesion of bacteria to coal and its associated minerals (Raichur et al, 1995). It has also been shown that flocculants of biological origin are more effective than synthetic ones (Raichur et al., 1997).

In the above studies, the difference in surface properties of coal and its associated minerals has been the primary reason for selective adhesion of the bacteria, thus leading to selective flocculation or flotation. The surface properties of coal also vary depending on the composition and rank of coal. Flocculation with synthetic flocculants is very much dependent of the type of coal, which in turn affects the separation of mineral matter from coal (Palmes and Laskowski, 1993). So far, no studies have been reported on how the type or source of coal affects the adhesion of bacteria to its surface and hence bioflocculation.

The microorganism *Paenibacillus polymyxa* has been used for flocculation and flotation of various minerals including hematite (Deo and Natarajan, 1998), pyrite and chalcopyrite (Sharma et al., 2001). In this paper, an attempt has been made to study the adhesion of the bacteria *P. polymyxa* on to two types of coal and its consequent effect on flocculation and deashing. The effect of time, pH and surface properties, i.e. zeta potential and surface free energy on adhesion and flocculation, have been investigated in detail. A pure quartz sample has also been used for comparison purposes.

2. Materials and methods

2.1. Culture of microorganism

A pure culture of the microorganism *P. polymyxa* was used in this study. The organism was cultured using Bromfield medium. The microorganism was harvested by centrifugation and the pellet was washed several times before the bacterium was

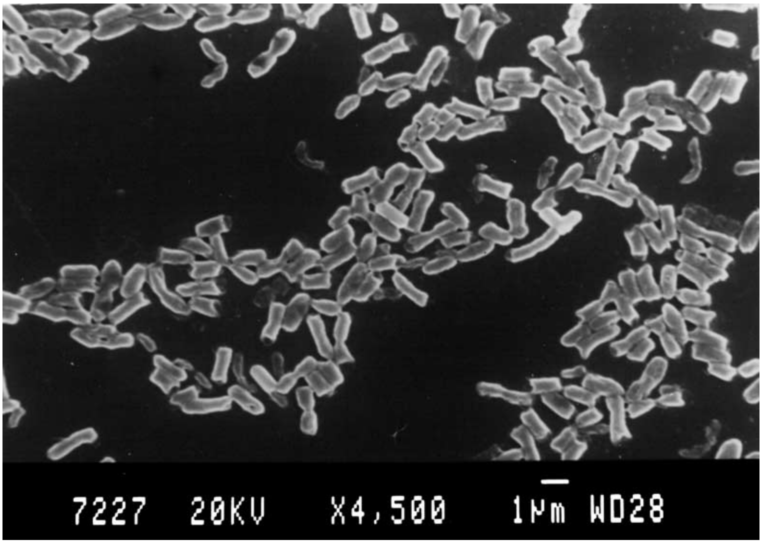

Fig. 1. Scanning electron micrograph of *P. polymyxa*.

resuspended in distilled water. This suspension was used in further studies. The cell count was monitored as a function of time by counting the cells using a Petroff–Hausser counter. The growth characteristics of *P. polymyxa* have been reported elsewhere (Shashikala and Raichur, 2001). The microorganism is about 1.5 μm in length and about 0.6 μm in diameter and a scanning electron micrograph is shown in Fig. 1.

2.2. Coal samples

Two coal samples were obtained for this study and the source and analysis of the coals are shown in Table 1. As can be seen, both the coals are high in ash and volatile matter. The samples were ground in a dry mill and then packed and stored. The ground samples were used for adhesion tests and zeta-potential measurements. Some large lumps were broken down to pieces of approximately 2×2×1 in. size, which were used for contact angle measurements. X-ray diffraction studies showed the presence of

Table 1
Proximate analysis of coal samples

Composition	N-coal (%)	M-coal (%)
Moisture	1.08	0.66
Ash	23.5	33.33
Volatiles	27.39	20.89
Fixed carbon	48.03	45.12

various minerals such as SiO_2, Al_2O_3, and oxides of iron, which contribute towards the ash formation.

2.3. Zeta-potential measurements

Zeta potential of the coal samples, quartz and the bacterium was measured using a Zeta-Meter 3.0 system. About 1 g of the mineral sample was added to 50 ml of 1×10^{-2} M KNO_3 solution. The suspension was conditioned using magnetic stirrer until the pH stabilized. Then, the pH was adjusted to the required value using dilute HNO_3 or NaOH and further conditioned until the pH stabilized. The suspension was allowed to stand for a couple of minutes before the supernatant was transferred to the electrophoresis cell for measurements. About 10 separate readings were taken and the average value is reported here. In case of the bacterium, a 50 ml suspension was made in distilled water to which another 50 ml of KNO_3 solution was added so that the required electrolyte concentration was obtained. Then the conditioning was done as described above.

2.4. Contact angle measurements

Contact angle measurements were performed using a contact angle goniometer (Ramé-Hart, NJ, USA). The coal samples were first molded in epoxy resin with one flat surface being exposed for contact angle measurements. The surface of each sample was polished using 1000 and 4000 grit SiC paper. Final polishing was carried out with 0.05 μm alumina suspension. The samples were thoroughly washed with distilled water before making the measurements. In case of the bacterium, the suspension containing the microorganism was filtered using a 0.2 μm Millipore filter paper as described by van der Mei et al. (1991). Enough bacteria were filtered so that a uniform layer is formed on the filter paper. This bacterial lawn was used for contact angle measurements.

Contact angles were measured using the sessile drop technique. A 2 μl drop was placed onto the surface and measurements were made within 2–3 min of placing the drop. Angles on both sides of the droplet were measured. The average of about eight readings is reported here. The contact angles could be reproduced with an accuracy of $\pm 2°$.

2.5. Adhesion tests

Adhesion tests were performed in shake flasks and the change in cell number was monitored by counting using a Petroff–Hausser counter. About 1 g of coal sample was conditioned in 50 ml of distilled water at the required pH. Similarly, the bacterial suspension was conditioned in 50 ml of distilled water of the same pH as that of the mineral suspension. After a few minutes, the two suspensions were mixed and the time from that point was taken into account. The effect of conditioning time and pH on adhesion were studied.

2.6. Flocculation tests

Flocculation tests were carried out in 100 ml graduated cylinder. The coal and bacterium were mixed in a beaker for about 25 min and the required pH and then

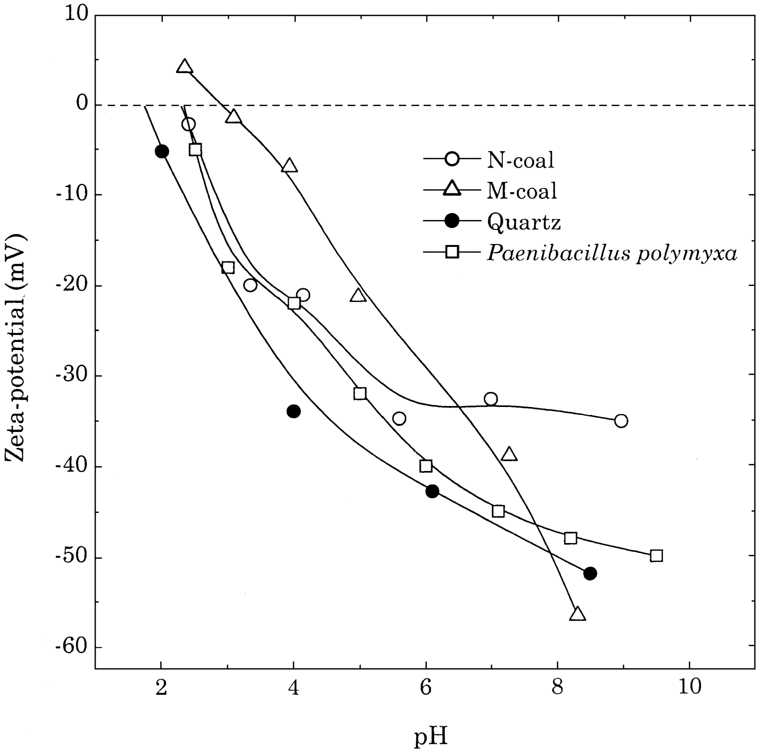

Fig. 2. Zeta potential of minerals and the bacterium in 1×10^{-2} M KNO_3 solution.

transferred to the graduated cylinder. The amount of coal settled after 1 min was determined at different pH values. The flocculated coal was analyzed for ash. Ash was determined as per the ASTM method D-3174.

3. Results and discussion

3.1. Zeta-potential studies

The surface charge of all the surfaces was determined by measuring the zeta potential as a function of pH at 1×10^{-2} M KNO_3 and the results are presented in

Table 2
Measured values of the contact angle on all surfaces with different diagnostic liquids

	θ_{water}	$\theta_{formamide}$	$\theta_{diiodomethane}$
N-coal	79	49	57
M-coal	70	55	58
Quartz	39	28	42
P. polymyxa	42	54.5	66

Table 3
The surface free energy components of different surfaces (mJ/m^2)

	γ_s^{LW}	γ_s^{+}	γ_s^{-}	γ_s^{AB}	γ_s
N-coal	31.45	0.94	5.86	4.70	36.15
M-coal	34.83	0.06	15.87	1.87	36.7
Quartz	38.59	1.135	36.0	12.78	51.37
P. polymyxa	25.13	0.286	54.70	7.91	33.04

Fig. 2. It can be seen that all the coal samples exhibited similar change in zeta potential with increase in pH and had a point-of-zero-charge (PZC) in the pH range of 2–3. Generally, coal samples have a PZC around pH 5–6, while in this case, it is much lower. This may be due to the presence of high amounts of ash forming minerals, which probably change the PZC to lower values. The zeta-potential curve of the quartz sample supports this reasoning.

The surface charge of *P. polymyxa* was also determined and is plotted in Fig. 2. The bacterium had a PZC around pH 2 and the zeta potential became more negative with increase in pH. It is interesting to note that the mineral samples and the bacterium exhibit a

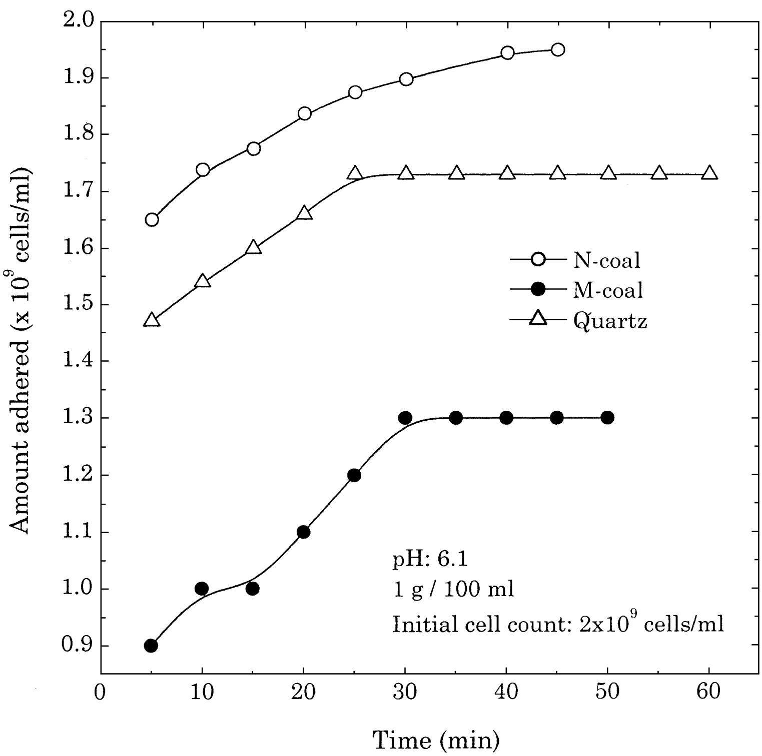

Fig. 3. Adhesion kinetics of *P. polymyxa* onto coal and quartz.

very similar change in zeta potential with change in pH and are negatively charged over most of the pH range.

3.2. Surface free energy calculations

The contact angle measurements were carried out to determine the surface free energy parameters for the mineral samples as well as the bacterium by the van Oss (1994) technique. Three diagnostic liquids, viz. water, diiodomethane and formamide, were used in this study and the measured contact angles are shown in Table 2. It can be seen that the contact angle of water is high for the coal with lower ash content (N-coal) followed by M-coal. Quartz and the bacterium had the lowest contact angle. However, the contact angle of water alone does not yield complete information about the surface. Using the van Oss technique, the surface free energy parameters were calculated and are tabulated in Table 3. The Lifshitz–van der Waals component was found to be independent of the type of coal. This is consistent with the observations of Parekh and Aplan (1978) where it was reported that critical surface tension of wetting of coal is independent of rank of coal. It should be noted that the critical surface tension of wetting is a very close estimate of the Lifshitz–van der Waals component of surface free energy. The electron acceptor component of the

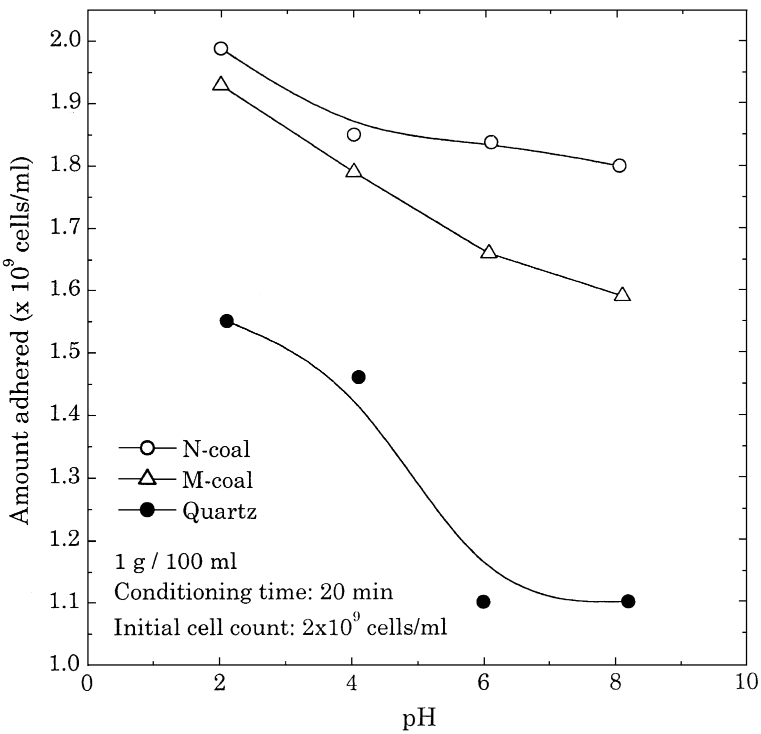

Fig. 4. Adhesion of *P. polymyxa* onto coal and quartz as a function of pH.

solid surface free energy (γ_s^+) did not vary significantly for both the coal samples. But the electron donor component (γ_s^-) changes with coal type. The electron donor component is high for the M-coal, which has about 33% ash. This means that the bulk composition of coal has a direct role in determining the surface free energy components. Even though the value of γ_s^- increases, it is still below the value of 28.8 mJ/m^2, indicating that the coal samples are hydrophobic (van Oss, 1994). On the other hand, quartz and the bacterium were found to be hydrophilic with γ_s^- values greater than 28.8 mJ/m^2.

3.3. Adhesion measurements

Adhesion of the bacterium on to the solid surfaces was carried out as a function of time and pH. The kinetics of adhesion (Fig. 3) revealed that most of the adhesion took place in about 25 min. The interesting thing to note is the amount of bacterium adhering to the different surfaces. Maximum adhesion took place onto the N-coal followed by M-coal while the lowest amount of bacteria adhered to quartz surface. The effect of pH on adhesion is shown in Fig. 4. All the surfaces exhibited similar behavior, i.e. maximum adhesion occurred around pH 2 and then decreased with increase in pH. As can be seen, maximum adhesion occurs near the PZC of the mineral and the bacterium. As pH is increased, both the surfaces become more and more negatively charged, thus decreasing the amount of bacterium adhering to the surface. The most important thing to be noted is that adhesion does occur in spite of both surfaces being negatively charged. This means that the surface hydrophobicity may be more dominant in determining the adhesion of bacterium to coal and its associated impurities. Coal is hydrophobic while quartz is hydrophilic. The difference between coal and quartz in adhesion is quite obvious.

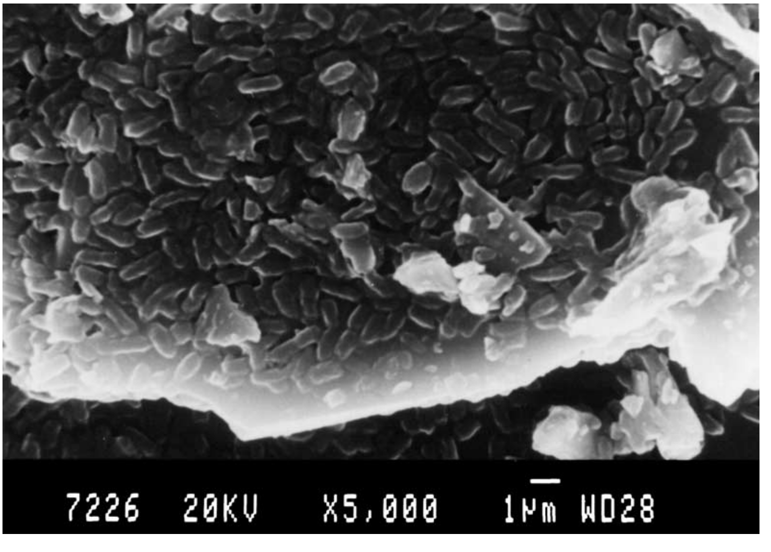

Fig. 5. Scanning electron micrograph of *P. polymyxa* adhering to coal surface.

Moreover, the difference in adhesion between the two coal samples can be attributed to the difference in hydrophobicities indicated by the difference in the γ_s^- values as described in the previous section.

After adhesion, the samples were washed with water to determine the nature of adhesion. It was observed that bacterium adhered to coal tenaciously and no cells were removed. The micrograph of *P. polymyxa* adhering to coal surface is shown in Fig. 5. A uniform layer of the bacterium is formed on the surface of the coal. In case of quartz, some cells were removed with each washing, indicating that the bonding was not as strong as with coal.

3.4. Flocculation tests

Flocculation tests were conducted in the absence and presence of the bacterium and the amount of material settled was determined. The results of these tests are presented in Figs. 6 and 7 in the absence and presence of *P. polymyxa*, respectively. In the absence of bacterium, quartz settles much quicker compared to coal, which is due to the effect of density. Also, coal is hydrophobic and it takes time to be wet by water. In the presence of the bacterium, coal flocculated and settled at a very high rate as compared to quartz.

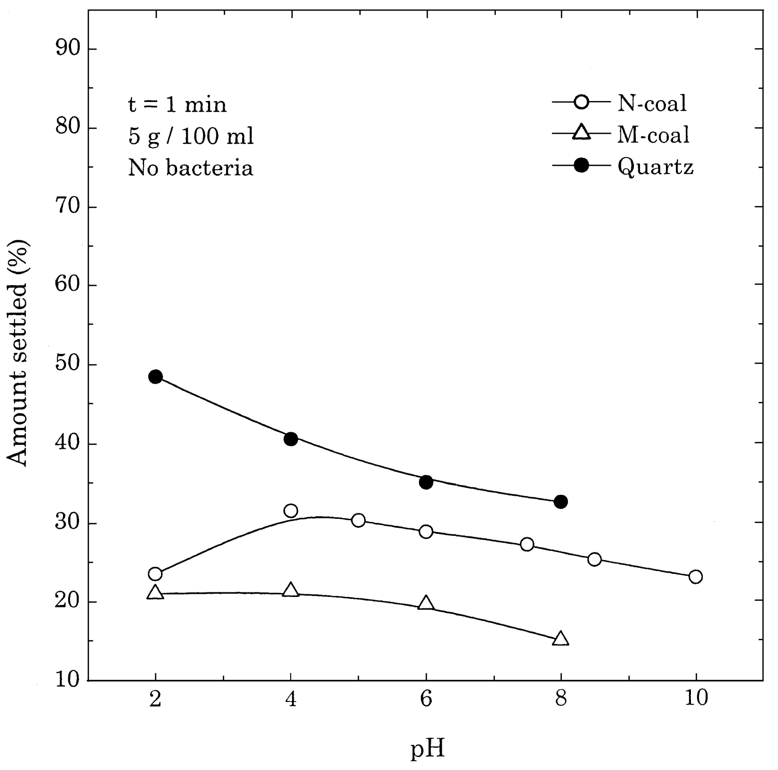

Fig. 6. Flocculation of coal and quartz as a function of pH in the absence of the bacterium.

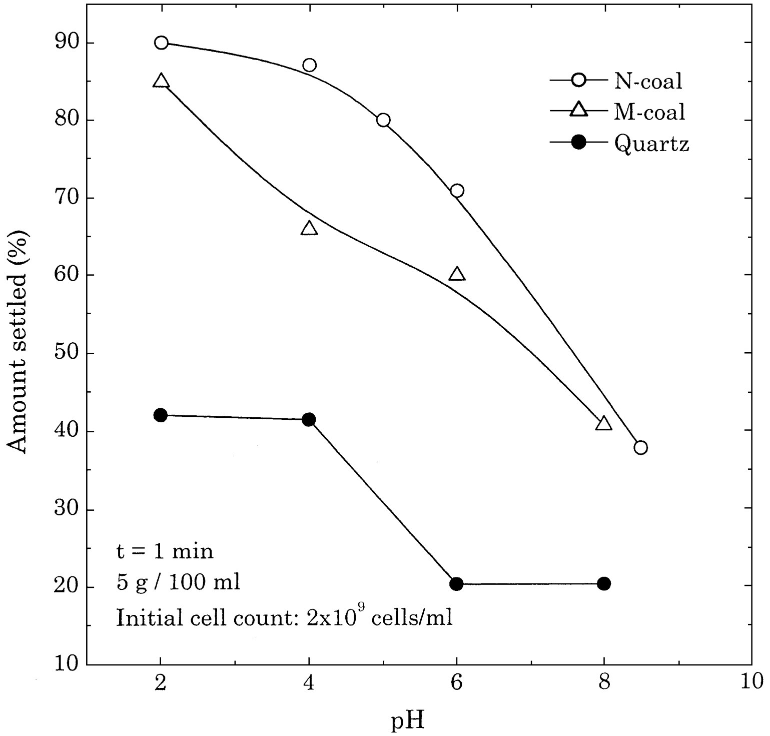

Fig. 7. Flocculation of coal and quartz as a function of pH in the presence of the bacterium.

Most of the material had settled in the first minute itself and maximum flocculation occurred in the acidic pH range of 2–4. This corresponds well with the adhesion tests where maximum adhesion of the bacterium was observed in the same pH range. As the pH was increased, the amount settled decreased steadily with both the coal samples and quartz. Again, the difference in settling behavior between the two coal samples can be attributed to the surface hydrophobicities. The most significant result is that the settling rate of the quartz in the presence of the bacterium was less when compared to in the absence of the bacterium. This means that quartz particles are getting stabilized in the suspension and settle at slower rates, which means that coal and quartz can be selectively separated by bioflocculation.

The flocculated and unflocculated coal samples were separated and dried and analyzed for ash and the results are tabulated in Table 4. With both the coal samples, the maximum

Table 4
Percentage ash rejected from flocculated coal (%)

	pH			
	2.1	4.08	6.15	8.2
N-coal	61.70	57.44	50.0	25.10
M-coal	54.50	45.0	38.2	23.19

amount of ash rejection was at pH 2 and decreased with increase in pH. For N-coal, the maximum ash rejected was about 62% while for M-coal, it was around 55%. This clearly demonstrates that bioflocculation can be used for cleaning coal from high-ash coals. The amount of ash rejected can be increased with some more optimization studies that are underway.

4. Conclusions

The following conclusions can be arrived at from this study:

(1) Zeta-potential studies showed that both the coal samples exhibited a PZC in the pH range of 2–3. The negative charge on the coal samples over most of the pH range can be attributed to the high ash content of the coal samples and is supported by the zeta-potential curve of quartz. The bacterium *P. polymyxa* also had a very similar zeta-potential profile.

(2) Surface free energy calculations showed that as the ash content of coal increased the electron donor component of surface free energy increased. This also means that coal becomes less hydrophobic with increase in ash content. Quartz and the bacterium were found to be hydrophilic.

(3) Kinetics of adhesion of *P. polymyxa* on to coal and quartz showed that maximum adhesion is achieved in about 25–30 min.

(4) Studies on effect of pH showed that maximum adhesion occurs at pH 2, which is close to the PZC of the coal samples and that of the bacterium. Hydrophobicity of the solid surface appears to be the primary criteria for determining adhesion of bacterium to mineral surfaces.

(5) At any given time and pH, the bacterium adhered more on to the coal with the least ash content and highest hydrophobicity. The amount adhering decreased with increase in ash content and a decrease in hydrophobicity.

(6) Flocculation studies demonstrated that the bacterium flocculates coal effectively while the settling of quartz is retarded, thus indicating that selective flocculation is possible. Nearly 55–60% of the ash could be removed in a single-stage flocculation experiment with both the coal samples, thus demonstrating the potential for bioflocculation in deashing of high-ash Indian coals.

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References

Aplan, F.F., 1976. Coal flotation. In: Fuerstenau, M.C. (Ed.), Flotation—A.M. Gaudin Memorial Volume. SME, Littleton, CO, USA, pp. 1235–1264.

Attia, Y.A., 1985. Cleaning and desulfurization of coal suspensions by selective flocculation. In: Attia, Y.A. (Ed.), Processing and Utilization of High Sulfur Coals. Elsevier, Amsterdam, pp. 267–285.

Attia, Y.A., Elseky, M., Ismail, M., 1993. Enhanced separation of pyrite from oxidized coal by froth flotation using biosurface modification. International Journal of Mineral Processing 37, 61–71.

Deo, N., Natarajan, K.A., 1998. Studies on interaction of *Paenibacillus polymyxa* with iron ore minerals in relation to beneficiation. International Journal of Mineral Processing 55, 41–60.

Drzymala, J., Wheelock, T.D., 1993. Organic thiols as pyrite depressants in oil agglomeration of coal. Coal Preparation 13, 53–62.

Honaker, R.Q., Luttrell, G.H., Yoon, R.-H., 1991. The application of hydrophobic coagulation for upgrading ultrafine coal. SME Preprint No. 91–149. SME, Littleton, CO.

Klimpel, R.R., 1988. The industrial coal flotation system. In: Klimpel, R.R., Luckie, P.T. (Eds.), Industrial Practice of Fine Coal Processing. SME, Littleton, CO, USA, pp. 113–127.

Misra, M., Anazia, I., 1987. Ultrafine coal flotation by gas phase transport of atomized reagents. Minerals & Metallurgical Processing 4, 233–236.

Misra, M., Smith, R.W., Dubel, J., Chen, S., 1993. Selective flocculation of coal using hydrophobic *Mycobacterium phlei*. Minerals & Metallurgical Processing 10, 20–23.

Ohmura, N., Saiki, H., 1994. Desulfurization of coal by microbial column flotation. Biotechnology and Bioengineering 44, 125–131.

Palmes, J.R., Laskowski, J.S., 1993. Effect of the properties of coal surface and flocculant type on the flocculation of fine coal. Minerals & Metallurgical Processing 10, 218–221.

Parekh, B.K., Aplan, F.F., 1978. Chap. 9: The critical surface tension of wetting of coal. In: Li, N.N., Long, R.B., Stern, S.A., Somasundaran, P. (Eds.), Recent Developments in Separation Science. CRC Press, Cleveland, OH, pp. 107–113.

Raichur, A.M., Misra, M., Smith, R.W., 1995. Differential adhesion of a hydrophobic bacterium onto coal and its associated impurities. Coal Preparation 16, 51–63.

Raichur, A.M., Misra, M., Smith, R.W., 1996. Flocculation and flotation of fine coal by adhesion of hydrophobic *Mycobacterium phlei*. Colloids and Surfaces, B: Biointerfaces 8, 13–24.

Raichur, A.M., Misra, M., Davis, S.A., Smith, R.W., 1997. Flocculation of fine coal using synthetic and biologically derived flocculants. Minerals & Metallurgical Processing 14, 22–26.

Sharma, P.K., Hanumantha Rao, K., Forssberg, K.S.E., Natarajan, K.A., 2001. Surface chemical characterization of *Paenibacillus polymyxa* before and after adaptation to sulfide minerals. International Journal of Mineral Processing 62, 3–25.

Shashikala, A.R., Raichur, A.M., 2001. Role of interfacial phenomena in determining adsorption of *Bacillus polymyxa* on to hematite and quartz. Colloids and Surfaces, B: Biointerfaces 24, 11–20.

van der Mei, H.C., Rosenberg, M., Busscher, H.J., 1991. Chap. 10: Assessment of microbial cell surface hydrophobicity. In: Mozes, N., Handley, P.S., Busscher, H.J., Rouxhet, P.G. (Eds.), Microbial Cell Surface Analysis: Structural and Physicochemical Methods. VCH Publishers, New York, pp. 263–287.

van Oss, C.J., 1994. Interfacial Forces in Aqueous Media. Marcel-Dekker, New York.