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Isolation of Coal-Solubilizing Microorganisms and Utilization of the Solubilized Product

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ABSTRACT

Twenty-eight strains of microorganisms able to solubilize pretreated subbituminous coal were isolated. One fungal strain was selected as the most effective isolate, because it produced black liquid rapidly from coal. The black liquid contained about 2% (w/v) of a coal-derived material that had a high molecular weight and gave precipitate in an acidic condition. Characteristics of the precipitate that were investigated and compared with the original coals were elemental composition and IR spectrum. Coal-water mixture containing 64% (w/v) of bituminous coal and 0.7% (w/v) of the material had a viscosity of 0.31 Pa·s. It might be possible to use the microbial product as an additive for coal-water mixture.

Index Entries: Coal; low-rank coal; solubilization; microbial solubilization; alkaline extract; surfactant; coal-water mixture; additive.

INTRODUCTION

Low-rank coal is an abundant resource, perhaps two hundred billion tons existing worldwide. This enormous resource has hardly been used as a fuel because of the problems of low heating value and possibility of spontaneous combustion.

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Microbial solubilization of coal was first introduced in 1982 as the phenomenon of solid coal being converted to black liquid by wood-rotting fungi (*1*). Since then, a large number of researchers have been attracted by this interesting phenomenon. Ward (*2*) and Scott et al. (*3*) isolated fungi and a yeast from lignite-associated samples, which could solubilize lignite. Fakoussa (*4*) and Maka et al. (*5*) isolated coal-solubilizing bacteria by an enrichment procedure. Strandberg and Lewis (*6*) and Gupta et al. (*7*) found coal-solubilizing strains among ligninolytic actinomycetes. Pretreatment of coal was found to be effective for enhancement of the microorganisms' ability to solubilize coal (*8*). Characteristics of the microbial product were also examined in detail (*3,7,9,10*). Some mechanisms were proposed for coal solubilization. In the mechanisms, various proposed substances were suggested to be concerned with solubilization: metal-chelator (*11,12*), alkaline substance (*5,6,13*), biosurfactant (*4*), or enzyme (*14*).

The microbial solubilization of low-rank coal had been expected to provide a new method of utilizing this inconvenient resource, but until now, there were only a few reports that actually examined the possibility of its utilization. This article describes the isolation of coal-solubilizing microorgamisms, the characterization of the coal-solubilized product from a subbituminous coal, and the possibility of utilization as an additive for coal–water mixtures.

METHODS

Media for Microorganism Isolation

Sabouraud dextrose agar (SDA) (containing glucose, 40 g; polypeptone, 10 g; and agar, 15 g/L [pH 7.0]) was used for isolation of fungi. Nutrient agar (containing beef extract, 5 g; polypeptone, 10 g; NaCl, 5 g, and agar, 15 g/L [pH 7.0]) was used mainly for isolation of bacteria. The coal medium (containing KH_2PO_4, 1.2 g; $Na_2HPO_4 \cdot 12H_2O$, 10.8 g; $MgSO_4 \cdot 7H_2O$, 0.04 g; $FeSO_4 \cdot 7H_2O$, 0.02 g; $MnCl_2 \cdot 4H_2O$, 0.02 g; NH_4Cl, 3 g; and Joban coal, 50 g/L [pH 5.0]), the lignin medium (containing $[NH_4]_2SO_4$, 1.0 g; KH_2PO_4, 0.2 g; K_2HPO_4, 1.6 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 0.01 g); and dialyzed lignin, 10 g/L [pH 7.0]) were used for enrichment cultures. All media were prepared with deionized distilled water and then autoclaved at 121°C for 15 min.

Isolation of Microorganisms from Coals

For isolation of microorganisms, a subbituminous coal derived from Joban seam in Ibaraki, Japan was tested as the source of microorganisms. This coal had been piled on the ground for dozens of years. Small pieces of coal (approx 1 cm in diameter) were placed on surfaces of SDA plates.

After 3–7 d of incubation at 25°C, some fungus hypha had grown on the plates from the coal. The fungus hypha points were taken for isolation.

For isolation of bacterium and yeast, small pieces of the coal were suspended in sterile distilled water. The resulting liquid was used directly as a source for isolation.

Enrichment cultures with coal were also used for isolation. The nonsterile pieces of Joban coal were inoculated to flasks containing 100 mL of the autoclaved coal medium. Then the flasks were reciprocally shaken at 120 rpm at 30°C. Microorganisms were isolated from these cultures after 1 mo of cultivation.

Enrichment cultures with lignin were also carried out. To 10 mL of the sterile lignin medium in test tubes, bituminous coal or the mixed soils from the coal storage site of Takehara coal power plant in Japan were added as microbial sources. The cultures were incubated at 30°C, and 1 mL of each culture was transferred to fresh media after 2 wk of cultivation. Microorganisms were isolated following several culture transfers. All isolates were purified by being streaked a series of several times on SDA plate.

Detection of Coal-Solubilizing Activity

All isolates were inoculated onto the whole surfaces of SDA plates. Plates were incubated at 30°C until the isolates grew sufficiently (3–10 d). Pretreated coal (*see below*) was then placed on the cultures on the plates to detect their coal-solubilizing abilities. Visual observations were kept during 2 wks of incubation. Coal-solubilizing ability was defined as being able to stain the medium to black under the coal particles or as producing black droplets on the particles.

Pretreatment of Coal

Joban coal was crushed into particles. The particles were sieved to adjust size between 0.5 and 0.7 mm in diameter. The coal particles (100 g) were added into 1 L of 10% (w/v) hydrogen peroxide. The slurry was periodically stirred for 24 h. Coal particles were filtered and washed with distilled water prior to being air-dried for 1–2 d.

Preparation of Solubilized Product

One isolate, strain F-10, was selected as the most effective isolate of all for rapid droplets formation. It was cultured for 3 d at 30°C in 500-mL Erlenmeyer flasks containing 100 mL of Saboulaud dextrose liquid medium with rotary shaking at 120 rpm. The fungus mycelia grown in the liquid culture was aseptically accumulated, and then disrupted using a mortar and pestle. The suspension of hypha was mixed with liquid SDA medium, and 5 mL of the mixture were then transferred onto SDA plates. These plates were incubated at 30°C for 10 d to make fungal mats, and then 0.5 g of the pretreated coal particles was dispersed on the mats. The black

droplets were produced on the coal particles in 3 d. The droplets were harvested twice by a pipet after 10 and 20 d of incubation. Approximately 1 mL of the black liquid could be obtained from a plate. The product was filtered through a 0.22-μm membrane filter and stored at 4°C until use.

Molecular-Weight Fractionation

The filter units of ultrafiltration (Ultrafree-C3, with a mol-wt limit of 10,000, 30,000, 100,000, Nihon Millipore Kogyo Co., Yonezawa) were used to estimate molecular-weight distribution. The amount of each fraction was decided by measuring total organic carbon with TOC-500 (Shimadzu Co., Kyoto).

Alkaline Extraction

The pretreated coal was dissolved into 10 vol of 0.1*N* NaOH. The solid impurity was then removed from the solution by centrifugation at 15,000 $\times g$ for 10 min and by filtration through a 0.22-μm membrane filter. The filtrate was acidified with 12*N* HCl to give a black precipitate. The precipitate was then washed twice with 0.1*N* HCl and freeze-dried.

Preparation of Coal–Water Mixture (CWM)

Workworth bituminous coal from Australia was pulverized and ground to obtain 80% passing a 200 mesh sieve (pore-size 74 μm). Coal powder (186 g) was mixed with 100 mL of an aqueous solution that contained 2% additive, and then stirred mechanically at 720 rpm for 10 min. Approximately 200 mL of CWM, which contained 64% of coal powder and 0.7% of additive, were made up with the microbial product, alkaline extract, sodium ligninosulfonate (anion surfactant), or polyoxyethylene laurly ether (nonion surfactant) as additives.

Measurement of Characteristics of CWM

The viscosity of a CWM was measured by a rotational viscometer (Tokyo Keiki Co., Tokyo) at 20°C. For the evaluation of stability, CWM was poured into a plastic bottle (60 mm in diameter, 250-mL vol) and left for 1 d. The formation of sediment at the bottom of the bottle was visually observed. The degree of the sediment formation was used as a stability indicator.

Analytical Procedures

The infrared absorption of coals and acid precipitate of solubilized product were measured by KBr method with an infrared spectrophotometer 260-10 (Hitachi Co., Tokyo). Composition analysis of the coals was done for C, H, O, N, and S. The elemental composition, ash content, and heating value were analyzed by JIS method (*15,16*). The elemental con-

tents of the solubilized product and the alkaline extract were obtained with CHN coder MT-3 (Yanagimoto Seisakusho Co., Kyoto) for C, H, N, and ash, CHN-O RAPID (HERAEUS Co., Hanau), for O, and TSX-10 (Mitsubishi Kasei Co., Tokyo) for S. The surface tension was measured by the Wilhelmy method using a KYOWA CBVP Surface Tensiometer A3 (Kyowa Interface Science Co., Tokyo) with a glass plate.

RESULTS

Isolation of Coal-Solubilizing Microorganisms

Various microorganisms were isolated from coals and coal-associated samples (Table 1). Among 22 isolates from Joban coal, 14 strains were active coal solubilizers. They consisted of eight strains of fungi, five strains of bacteria, and one strain of yeast. Among 15 isolates from coal-enrichment culture, eight strains were active, consisting of seven strains of bacteria and one strain of actinomycetes. Among 14 isolates from lignin-enrichment culture, six strains were active, consisting of four strains of fungi and two strains of bacteria. Controls consisting of the microorganisms on the plates in the absence of coal showed no media staining, indicating that agar staining was from a coal derivative.

Of the 12 coal-solubilizing fungal isolates, nine were able to produce black droplets on the coal particles. The F-10 fungal strain produced droplets the most rapidly on the nine and was therefore selected for the following study. The strain F-10 had the characteristics of the genus *Penicillium* in both conidial structure and other morphological features.

Characteristics of Microbial Product and Alkaline Extract

The black liquid produced by strain F-10 was an aqueous solution that contained 2.1–2.3% (w/v) of a coal derivative. The solution gave black precipitate in an acidic condition. The weight of the precipitate corresponded to more than 90% of freeze-dried weight. In the molecular-weight fractionation experiment, 95% of total organic carbon of the black liquid was retained on a 10,000-mol-wt membrane, and 40% was retained on a 100,000-mol-wt membrane (data not shown). This indicates that if the substance consisted of globular molecules, 95% of the organic matter had a mol wt of more than 10,000, and 40% had more than 100,000.

The elemental compositions of coals and the derivatives are shown in Tables 2 and 3. The hydrogen-peroxide-treated coal apparently increased in ash content and decreased in carbon content, when compared to the original coal (Table 2). The microbial product and alkaline extract increased in oxygen content, and remarkably decreased in ash content rather than

Table 1
Isolates of Coal-Solubilizing Microorganisms

Isolation method	Isolation source	Isolates	Microbial type[a]	Solubilizing ability[e]	
				Staining[b]	Droplet[c]
Direct	Joban coal	B-1	B	+	−
		B-3	B	+	−
		B-8	B	+	−
		B-11	B	+	−
		B-12	B	+	−
		F-1	F	+	+
		F-4	F	+	+
		F-5	F	+	−
		F-6	F	+	+
		F-8	F	+	−
		F-10	F	+	+ +
		F-13	F	+	−
		F-14	F	+	+
		Y-1	Y	+	−
Coal-enrichment culture	Joban coal	E-1	B	+	−
		E-2	B	+[d]	−
		E-3	B	+	−
		E-5	B	+[d]	−
		EC-3	B	+	−
		EC-4	A	+[d]	+
		EC-5	B	+[d]	−
		EC-6	B	+	−
Lignin-enrichment culture	Bituminous coal and mixed soil	L-1	F	+	+
		L-3	F	+	+
		L-5	F	+	+
		L-6	F	+	+
		L-13	B	+	−
		L-14	B	+	−

[a] B, F, Y, and A indicate bacterium, fungus, yeast, and actinomycetes, respectively.
[b] Black staining of an agar medium.
[c] Black droplet producing on the surface of the hyphal mat.
[d] Staining immediately after dispersion of coal.
[e] Indicated on a relative basis, from + + (strongly positive) to − (negative).

the untreated and treated coal. Alkaline extract had a slightly higher carbon content and lower hydrogen content than microbial product. Heating values of the both acid precipitates were apparently higher than coals. When comparing the values on an ash-free basis (Table 3), however, both precipitates decreased in heating value rather than untreated coal.

Untreated and treated coal, and microbial product were analyzed by IR spectroscopy. The adsorption spectra are shown in Fig. 1. The broad

Table 2
Composition of Elements and Ash in Coals and Derivatives

Coals and derivatives	Composition wt%[a]						Heating value, J
	C	H	O	N	S	Ash	
Untreated coal	45.3	3.7	16.0	0.6	0.2	34.3	17,960
Treated coal[b]	38.3	3.2	16.7	0.5	0.2	41.2	14,820
Solubilized product[c]	47.8	3.8	39.4	4.0	0.1	4.5	-
Solubilized product acid-precipitated[d]	55.8	4.1	32.0	1.9	0.6	0.7	22,820
Alkaline extract acid-precipitated[e]	60.1	2.7	29.7	0.8	0.4	0.3	23,150

[a] All values are indicated on dry basis.
[b] Treated with 10% hydrogen peroxide solution for 1 d.
[c] Product from treated coal by strain F-10 was direct-dried.
[d] Acid-precipitated product was freeze-dried.
[e] Acid-precipitated alkaline extract was freeze-dried.

Table 3
Elemental Composition of Coals and Derivatives

Coals and derivatives	Composition wt%[a]					Heating value, J
	C	H	O	N	S	
Untreated coal	68.9	5.6	24.4	0.9	0.3	27,340
Treated coal[b]	65.1	5.4	28.4	0.9	0.3	25,210
Solubilized product[c]	50.0	4.0	41.3	4.2	0.2	-
Solubilized product acid-precipitated[d]	56.2	4.1	32.2	1.9	0.6	22,980
Solubilized product acid-precipitated[e]	60.3	2.7	29.9	0.8	0.4	23,220

[a] All values are indicated on ash-free dry basis.
[b,c,d,e] *See* Table 2.

absorption around 3400 cm^{-1}, which indicates hydrogen-bonded OH, was observed in all samples. The bands of 2920 and 2850 cm^{-1}, which indicate aliphatic C—H stretch, were observed in both of untreated and treated coal; these bands were faint in microbial product. The absorption around at 1720 cm^{-1}, which indicates carbonyl stretch, was observed in both of microbial product and pretreated coal, whereas it appeared on the shoulder in untreated coal. The absorption around 1600 cm^{-1}, which is regarded as typical for coal, was observed in all samples. The two bands along the broad hydrogen-bonded OH stretch at 3600 and 3800 cm^{-1} in untreated and treated coal were assigned as nonhydrogen-bonded OH in clay structure included in ash component. The absorption at 1100 cm^{-1} related to the Si-O band was also shown on untreated and treated coal.

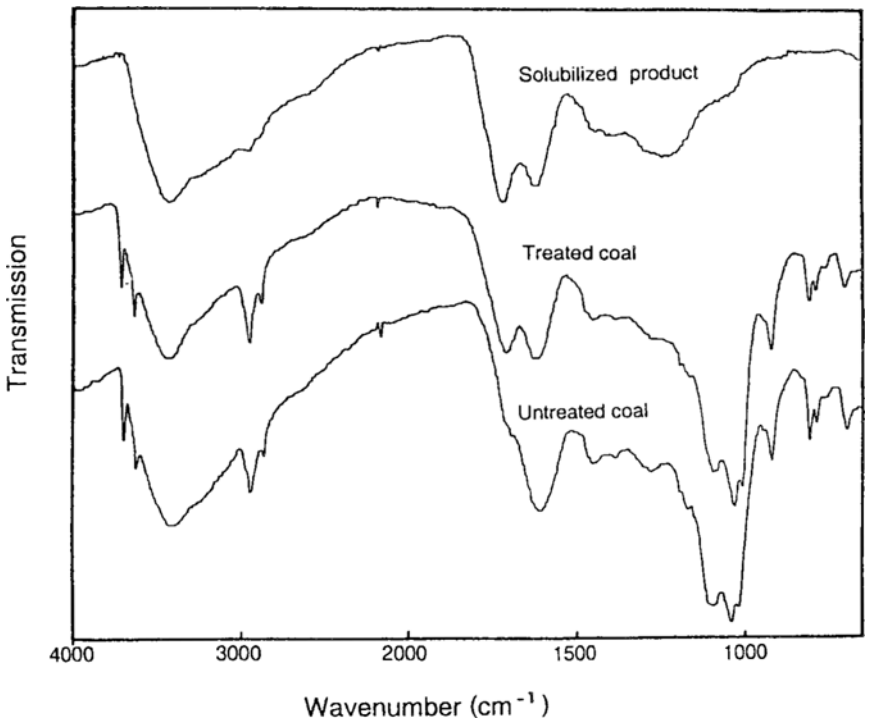

Fig. 1. Infrared spectra of coal and solubilized product. Solubilized product by strain F-10 was acid-precipitated and freeze-dried. Coal was treated with hydrogen peroxide. All samples were measured by KBr method.

Table 4
Comparison of Surface-Tension-Reducing Ability

Sample	Concentration, wt/v%	Surface tension, $10^{-3}N/m$
Solubilized product	2.0	49.8
Solubilized product	0.2	54.6
Sodium ligninosulfonate	0.2	46.8
Polyoxyethylene lauryl ether	0.2	41.1
Pure water	-	71.2

The IR spectrum of alkaline extract was similar to the microbial product (data not shown).

The surface tension of the aqueous solution containing 0.2% of microbial product was higher than the one containing the same concentration of sodium ligninosulfonate or polyoxyethylene lauryl ether (Table 4). This result indicates that microbial product is less able to decrease the surface tension of water compared to the other surfactants tested.

Table 5
Comparison of Coal–Water Mixture
Prepared with Various Substances as Additives

Additives	Additive conc., wt%	Coal conc., wt%	Viscosity, Pa·s	Stability
Solubilized product	0.7	64	0.31	[b]
Alkaline extract	0.7	64	0.31	[b]
Sodium ligninosulfonate	0.7	64	0.62	[b]
Polyoxyethylene lauryl ether	0.7	64	1.38	[c]
None	0	64	[a]	[c]

[a]Beyond the measurable range.
[b]Produce recoverable dense sediment.
[c]No fluidity.

Comparison of Microbial Product with Other Substances as an Additive for CWM

It was possible to prepare CWM with the microbial product. CWM with the microbial product or the alkaline extract had lower viscosity than CWMs with the other additives tested in this experiment (Table 5). When CWMs with the microbial product, alkaline extract, or sodium ligninosulfonate were left for 1 d, they formed dense sediments of coal in the bottoms of bottles. However, the sediments could be resuspended by mixing. CWM with polyoxyethylene lauryl ether lost fluidity after storage.

DISCUSSION

Coal-Solubilizing Microorganisms

Coal-solubilizing microorganisms were isolated from Joban coal and coal-associated samples by three methods. The isolates included 12 stains of fungi, 14 strains of bacteria, and one strain each of yeast and actinomycetes. In the previous studies, several strains of coal-solubilizing fungi and a yeast had been isolated from lignite surface (*2,3*). More than the half of all isolates from the surface of piled coal were active solubilizers of hydrogen-peroxide-treated Joban subbituminous coal. The result suggests that coal-solubilizing activity widely exists in microorganisms living in the coal-associated environment. Runnion and Combie (*17*) indicated that coal-solubilizing microorganisms were isolated from geothermal waters that had no relation with coal or lignin. These facts suggest that coal-solubilizing ability would be a peculiar expression of a common microbial function. When exposed to coal, the function if expressed.

Three bacteria and one actinomycetes from coal-enrichment culture stained medium immediately after dispersion of coal particles. This quick reaction suggests that a coal-solubilizing agent was constitutively produced without coal. Some investigators suggest that microbial coal solubilization is related to a pH increase in the culture (*5,6,13*). In our study, however, the four did not show the pH increase on a medium surface. The result shows the coal-solubilizing agent was not an alkaline metabolite. In our experiments, bacterial solubilization was only staining of agar medium and did not produce black droplets even after extended incubation, whereas fungal solubilization had a tendency to produce droplets. This difference indicates a different mechanism. The most effective fungal isolate, F-10 strain, produced profuse black droplets during incubation for 10 d. The mechanism of producing the droplets was also not related to alkaline metabolite production, since the pH of the droplets was neutral. On the other hand, Cohen et al. (*11*) isolated ammonium oxalate monohydrate as a coal-solubilizing agent of white-rotting fungus, *Trametes versicolor*, from liquid culture where coal was absent. It is possible that the quick staining by the four strains was caused by such a chemical. However, it is difficult to consider that the solubilization by F-10 was also caused by only the function of such a chemical, since profuse droplet formation does not begin immediately after the coal dispersion, i.e., the ability of the droplet production must be induced after the dispersion.

Solubilized Product

Analysis of elemental composition showed an increase in ash content by hydrogen peroxide treatment though nothing was added besides hydrogen peroxide during the treatment. In the washing process, flammable particles in the froth derived from oxygen of hydrogen peroxide were decanted out with the solution; therefore, the ash content will be increased. On an ash-free basis, the compositions of untreated and treated coal are similar. The most apparent differences are the increase in oxygen content and the decrease in carbon content. The differences would be owing to oxidation of coal by the treatment, since analytical results show a simple increase in oxygen. The nitrogen and oxygen contents in acid precipitate are less than that of freeze-dried solubilized product. This indicates the black droplets produced on the fungal mat contain not only solubilized coal, but also other nitrogenous and/or oxygenous substances derived from agar medium and/or microbial metabolite.

When compared by IR spectrum, two remarkable changes were found between untreated and treated coal substances and solubilized product. One is disappearance of absorption at 2920 and 2850 cm^{-1}; another is increase of absorption at 1710 cm^{-1} on solubilized product. According to IR absorption analysis of coal and related substances (i.e., fumic acid, lignin), the former absorptions indicate methyl and/or methylene groups, and the latter indicates carboxylic groups. These changes suggest that

methylene groups in coal substance were oxidized and produced carboxylic groups, and increased oxygen content in microbial product. There are two possibilities for the cause of the oxidation of methylene group, microbial reaction, and oxidative treatment. It could not be decided which one predominates, because recovery of microbial solubilization was only about 10%.

Molecular-weight fractionation of the solubilized substance showed that 95% of the total organic matter had a mol wt of more than 10,000, and 40% had more than 100,000. The reproducibility of this method limited further results on molecular-weight fractionation.

The black liquid produced by strain F-10 was a dilute aqueous solution that contained about 2% of a coal-derived material. Since the liquid was not combustible, it would be difficult to use the liquid directly as a fuel. Davison et al. (*18*) and Ackerson et al. (*19*) intended to change the product through anaerobic fermentation to more useful substances, such as methane or alcohol. Using the microbial product directly would be more cost-effective than the use of a substance produced through further microbial reactions.

Microbial Surfactant

Recently, advanced technology to use coal as a slurry fuel is developing, since use of coal is inconvenient. Among coal slurry fuels, CWM is expected to be one of the most important oil alternatives, since it can be used as a substitute for heavy oil. CWM consists of fine coal particles and water in the ratio 7:3, and contains about 1% of fluidity additives. Additives for CWM are usually high-moleculer-weight surfactants, and are made from natural materials or synthetic compounds.

The microbial product had a high molecular weight and was water soluble, thus exhibiting characteristics of a high molecular weight surfactant. Therefore, this product is expected to be an additive for CWM. CWM consists of coal particles that are dispersed in a small volume of water by a function of an additive. It is important for an additive for CWM to reduce viscosity and to maintain a well-dispersed condition. Our experiments indicated that the microbial product could be used to prepare low viscous and fluid CWM instead of the conventional additive, sodium ligninosulfonate. However, the surface tension of water with microbial product was not lower than the ones with the tested surfactants, indicating the ability to reduce surface tension is not related to the ability to reduce viscosity of CWM. CWM prepared with the product easily formed a dense sediment, not suitable for work as a stable CWM. This disadvantage could be compensated for the addition of the other kind of additive, such as a stabilizer that could maintain a well-dispersed condition of CWM.

It is known that solubility of coal in dilute alkaline solution correlates to microbial solubility of the coal (*15*). From the hydrogen-peroxide-treated coal, a considerable amount of black material was extracted by 0.1*N* NaOH.

The extract was similar to the microbially solubilized product in elemental composition, having the same IR spectrum and ability to reduce viscosity of CWM. Therefore, the microbial surfactant will be required to be more facile in mass production than the alkaline extract for practical use.

SUMMARY

Coal-solubilizing microorganisms were isolated from piled subbituminous coal and bituminous coal-associated samples in Japan. Strain F-10, identified as *Penicillium* sp., solubilized the hydrogen-peroxide-treated coal and produced black droplets most rapidly. Its product contained about 2% of coal-derived material, which had a high molecular weight, and gave a precipitate in an acidic condition. The product and an alkaline extract from the pretreated coal were able to prepare CWM with improved fluid characteristics over conventional surfactants.

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