0273-2289/89/2021—0731$02.40

Biosolubilization of Lignite

RICHARD T. MOOLICK, JAMES C. LINDEN,* AND M. NAZMUL KARIM

Department of Agricultural and Chemical Engineering, Colorado State University, Fort Collins, CO 80523

ABSTRACT

The rates at which lignite is solubilized by the extracellular products of a *Penicillium* fungus designated as RWL-5, four substrains isolated from RWL-5 and *Streptomyces flavovirens* were examined. The effects of lignite surface oxidation were also examined. Comparison of data generated from studies with the two microorganisms suggested that *S. flavovirens* solubilized coal in a more alkaline environment than that of the *Penicillium* strain RWL-5. The rate of biosolubilization by RWL-5 in an oxygen-enriched atmosphere was almost twice that in air. Two substrain isolates exhibited biosolubilization rates approximately two times as great as that of the parent strain.

Index Entries: Biosolubilization; lignite; *Penicillium; Streptomyces.*

INTRODUCTION

Some strains of microorganisms, including several *Streptomyces* and *Penicillium* species that are capable of degrading lignin, have been found to solubilize lignites and other low-ranked coals (*1–3*). Strandberg and Lewis have shown that these organisms excrete a substance capable of solubilizing the coal (*4*). The solubilization of lignite by *Streptomyces* species is related to the excretion of alkaline substances by the cells (*4–6*). The pH has been found to have a large effect on the rate and degree of solubilization of coal by microbial as well as nonbiological systems.

The products obtained through biosolubilization of coal are highly water soluble with low solubility in organic solvents. The high oxygen

*Author to whom all correspondence and reprint requests should be addressed.

content and complex aromatic structures of the biosolubilized coal makes the process a potential source for chemical feedstocks and for relatively clean burning fuel.

In order to develop the coal biosolubilization process, an understanding of the kinetics and mechanisms of the solubilization process is needed. Some preliminary kinetic data are presented in this paper that may be useful in modeling and eventual scaleup of the process.

METHODS

Pretreatment of Lignite

For evaluation of surface oxidation effects, both oxidative and non-oxidative pretreatment methods were used. Untreated Texas lignite (TXL-UT) was crushed and screened to −20 mesh.

Nitric acid pretreated lignite (TXL35W) was prepared by adding 25 grams of −10 by +20 mesh untreated Texas lignite to 75 mL of 35% (w/w) (8 *M*) nitric acid according to the conditions set forth by Strandberg and Lewis (*4*). The slurry was allowed to stand for 48 h with periodic swirling. The slurry was then placed in a separatory funnel. The coal was washed with an upflow of deionized water until the pH was greater than 6.0. The coal was filtered, washed with 100 mL of distilled water, and dried.

Hydrochloric acid pretreated lignite (TXL*37W) was prepared as above with the substitution of 37% (w/w) hydrochloric acid. Hydrogen peroxide pretreated lignite (TXL030W) was prepared as above with the substitution of 30% (w/w) hydrogen peroxide. Ammonia Freeze Explosion Pretreated Lignite (TXL-AFEX) was prepared by subjecting Texas lignite (−20 mesh) to the AFEX (Ammonia Freeze Explosion) process developed by Dale (*7*). The reactivity of this material was described by Henk et al. (*8*). After drying, the coal was crushed to −60 mesh.

A solubilized fraction of lignite (Lignite Extract) was prepared by digesting 50 g of untreated lignite (−10 mesh) in 250 mL 0.5 *M* NaOH with continuous vigorous agitation for 2.5 h. The extract was clarified by centrifugation at 3000×g for 1.0 h. The concentration of the extract was determined by washing, drying, and weighing the sludge. The extract concentration was 45.6 g of lignite solubles per liter. The solution had a pH of 9.8, which was later neutralized to pH 6.64 and 35.58 g/L concentration with nitric acid.

Media Preparations

For the majority of the experiments, Difco yeast maltose agar was used. In order to select for strains capable of utilizing lignite directly, the following media formulations were used.

A modified yeast maltose agar was prepared by substituting various amounts of lignite extract for equivalent amounts of glucose in the base

formula of yeast extract (3.0 g/L), malt extract (3.0 g/L), peptone (5.0 g/L), agar (20.0 g/L), and glucose (20.0 g/L). The media was designated with the letters YML- followed by the percentage of the glucose replaced by an equivalent (dry wt basis) amount of lignite extract (e.g., YML-75 contained 15.0 g/L (dry wt) lignite extract and 5.0 g/L glucose). The lignite extract raised the pH of the media from 6.2 to 7.3 ± 0.2.

In order to eliminate the possibility of microbial utilization of the non-glucose components of the Difco yeast maltose agar as substrates, a modified Czapek agar was prepared. A base formula of 2.0 g/L $NaNO_3$, 1.0 g/L K_2HPO_4, 1.0 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L KCl, 0.02 g/L $FeSO_4 \cdot 7H_2O$, 15.0 g/L agar, and 30.0 g/L glucose was used. Lignite extract containing media were prepared by substituting lignite extract for equivalent amounts of glucose, as above. These media were identified by the letters LISA- followed by the percentage of the substitution (e.g., LISA-100 contained 30.0 g/L (dry wt) of lignite extract).

A second series of media was prepared by substituting glucose, cellobiose, amylose, or lactose for the glucose in the Czapek agar formula. These media were identified by the letters MCA- followed by G, CB, A, or L for the respective substrate. In order to isolate strains capable of living directly on the coal as a sole carbon source, a modified Czapek Dox broth (MCDB-TXL35W) was prepared. The broth consisted of 3.0 g/L of $NaNO_3$, 1.0 g/L of K_2HPO_4, 1.0 g/L of $MgSO_4 \cdot 7H_2O$, 0.5 g/L of KCl, 0.02 g/L of $FeSO_4 \cdot 7H_2O$ and 30.0 g/L of TXL35W. The broth had a pH of 7.3.

Culture Procedures

The cultures were grown in Difco yeast-maltose agar to form a uniform lawn on which 0.45 micron membrane filters (47 mm diameter) were placed. Coal was distributed evenly on the filters to prevent direct contact between the coal and the organisms and to facilitate recovery of solubilized products and residual coal. The coal used in these experiments was autoclaved 15 min at 121°C and dried at 100°C.

Six culture plates of *Streptomyces setonii* #75 and six of RWL-5 all on YM agar were prepared and grown for 6 d. Then, 200 mg of sterile TXL35W were placed on the membrane filter on 2 plates each of *S. setonii* and RWL-5. Similarly, 200 mg amounts of sterile TXL*37W and TXL030W were added to pairs of plates of *S. setonii* and RLW-5. Following various times of incubation in a humidified chamber at 30°C for periods up to 16 d, the residual coal was washed, dried, and weighed to determine the degree of solubilization as a function of time. Culture surface pH was measured with pH paper immediately after harvesting the solubilized coal.

Stress Selection and Isolation of New Substrains

Initially, sterile plates of YML-75 were inoculated with RWL-5 and incubated at 30°C. The fungal mat that grew had very short white mycelia

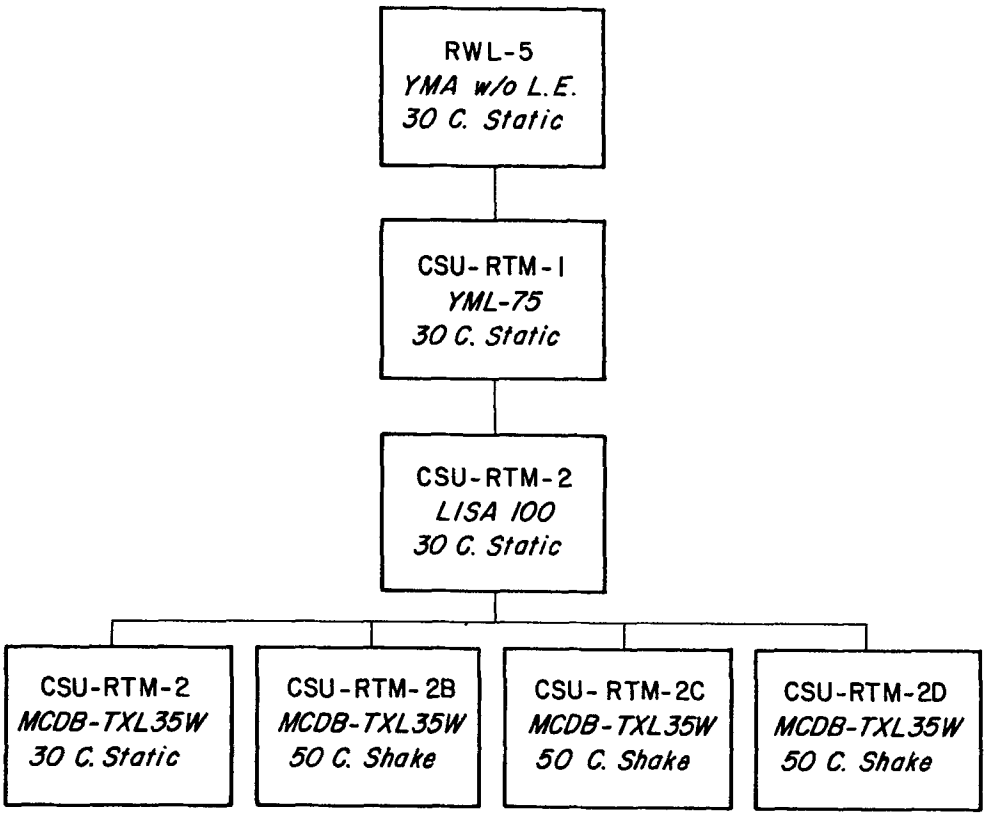

Fig. 1. Selection map. Stress selection of substrains.

with gray-green spores when plated on Difco yeast maltose agar and was designated RTM-1 for identification purposes. A plate of YML-100 (see media preparation) was inoculated with RTM-1 and incubated at 30°C for 7 d. The fungal colony morphology had changed and now had white mycelia and green spores when plated on YM agar and was designated RTM-2.

In the stress selection experiments, the organisms were cultured in two flasks with 100 mL of the inorganic salts solution and 3.0 g of pulverized lignite. Both flasks were inoculated with RTM-2. One flask was placed in the humidified incubator at 30°C. The other flask was placed in a shaker incubator and was incubated at 50°C and 150 rpm for 24 h followed by 9 d at 35°C. After a total of 10 d, the static flask had several colonies of green-spored fungus. The shake flask had no visible growth. The shake flask was incubated statically in the humidified chamber at 30°C for 8 d, after which several colonies of a fungus with tan spores, one colony of a fungus with gray spores, and one colony of a fungus with dark green spores were evident. A selection diagram representing these activities is given in Fig. 1.

Table 1
Lignite Material Balance

Material	Sample wt	Percent H_2O	Percent ash	Percent total C
TXL35W	0.1019	—	14.16	100.00
Solid residue	0.0371	—	14.37	36.32
Solubilized fraction	0.1889	67.33	14.12	60.59
Organic balance				96.91

Analytical Procedures

The degree of solubilization of the coal was determined by a simple separation process. First, the solubilized coal liquid was filtered and reserved for future experiments. The membrane filter with the residual coal was transferred to the filter apparatus. The coal was carefully washed from the membrane on to the filter used earlier for the solubilized fraction. The residue was washed thoroughly, dired at 100°C, and weighed. The difference between the initial and final masses of coal was used to determine the percentage of coal solubilized. The ash content of TXL35W, the solid residues after solubilization, and the dried solubilized fractions were approximately equal (Table 1). Thus, the degree of solubilization in the following equation was calculated without considering ash analyses.

$$\%_{sol} = M_i - M_f / M_i$$

where $\%_{sol}$ is percent solubilized, M_i is initial mass of coal, and M_f is final mass of coal residue.

In order to determine the material balance, the liquid fraction was dried at 100°C and weighed. The ash content of the untreated Texas lignite, TXL35W, TXL*37W, TXL030W, and solubilized coal solids were determined by difference following heating in an ashing furnace at 425°C for 6 h.

RESULTS AND DISCUSSION

Effect of Surface Oxidation on Biosolubilization

The pretreatment processes were accompanied by significant losses caused by dissolution and physical degradation of the coal. TXL35W lost 13.2%; TXL*37W lost 14.9%, and TXL030W had an excessive loss of 87.3% on a dry wt basis owing to pretreatment. The high losses from H_2O_2 pretreatment are consistant with the use of H_2O_2 and H_2SO_4 to dissolve coal for nitrogen analysis (9).

Table 2
Effect of Surface Oxidation on Biosolubilization

Organism	Pretreatment	Percent solubilized
S. setonii	TXL35W	41.94
	TXL*37W	0.29
	TXL030W	18.73
Penicillium RWL-5	TXL35W	16.71
	TXL*37W	1.03
	TXL030W	6.30

The relative degree of solubilization of TXL35W, TXL*37W, and TXL-030W by *Streptomyces setonii* and *Penicillium* strain RWL-5 were compared after 6 d of incubation (Table 2). The results confirm previous results of Henk et al. (*8*), in which nitric acid treated lignite (TXL35W), AFEX treated lignite (TXL-AFEX), and untreated lignite (TXL-UT) were solubilized in suspension culture. The surface oxidation of the coal had a positive effect on the rate of biosolubilization. These data confirm the observations of Strandberg and Lewis (*4*). The lignite balance closed to within 3.09%, as shown in Table 1. These data were obtained by ashing samples of TXL35W, drying, and ashing the black liquid formed during solubilization and the unsolubilized coal from six experiments with RWL-5.

Bacterial vs Fungal Biosolubilization Characteristics

Determination of the rates of TXL35W coal solubilization by *S. flavovirens* and RWL-5 revealed significant differences in the kinetics exhibited by each organism. In Fig. 2, *S. flavovirens* exhibited a 3-d lag followed by a very rapid biosolubilization rate. RWL-5 exhibited a similar rate of solubilization between 1–3 d; eventual degree of solubilization by RWL-5 was approximately 65%. The pH profiles of the surface cultures in Fig. 3 indicate that *S. flavovirens* produced a very alkaline substance soon after inoculation. Only after the pH had attained a value of greater than 8.5 did solubilization occur. This agrees with the results reported by Quigley et al. (*5*), which was originally described by Strandberg and Lewis (*4*). In contrast, only transitory increases to approximately pH 7.5 were observed in two studies conducted with RWL-5. The pH increases were coincident with the rapid rate of solubilization (Fig. 2). Moderation of the pH in these cultures may have been a titration effect by acidic groups released during solubilization of the acid treated lignite.

Oxygen Enrichment Study

The effect of oxygen partial pressure on the rate of biosolubilization of TXL35W by RWL-5 was significant. Figure 4 shows both the rate of solubilization in humidified oxygen and the solubilization rate in humidified

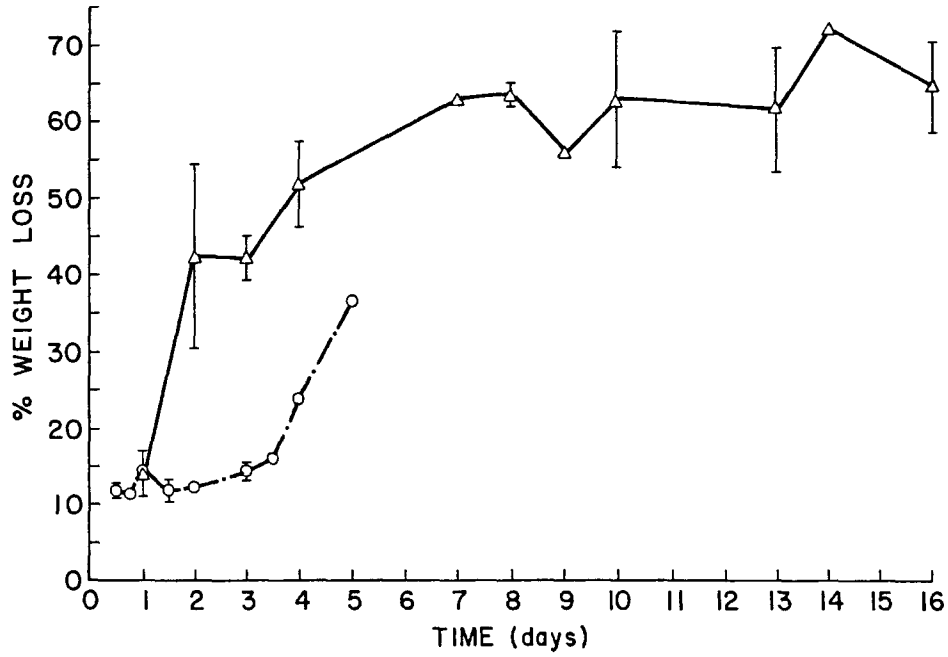

Fig. 2. Biosolubilization of Texas lignite, comparison of rates between *S. flavovirens* and *Penicillium* RWL-5. RWL-5 (△), *S. flavovirens* (○).

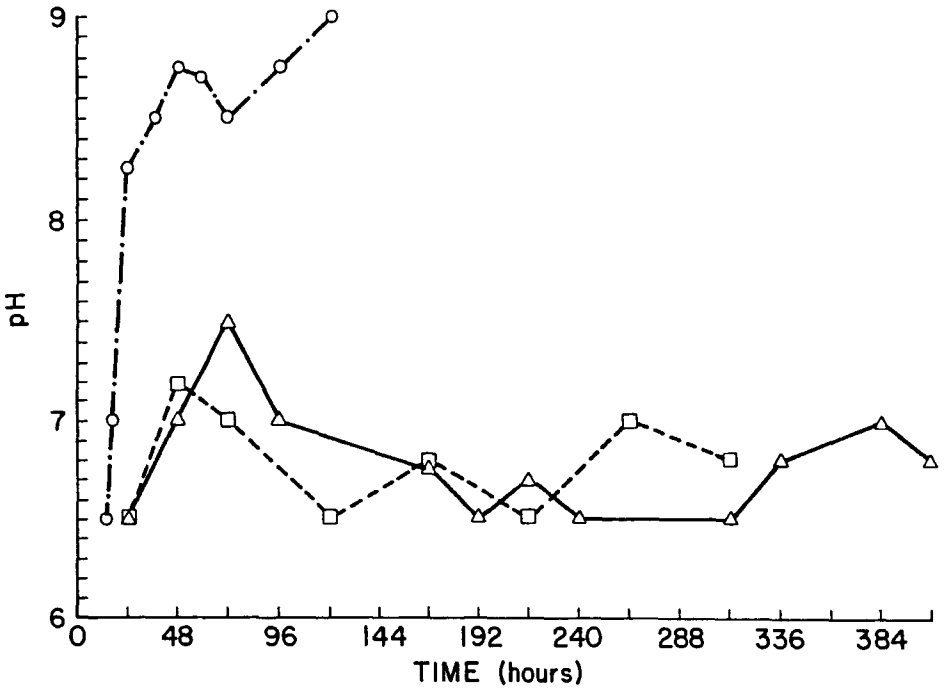

Fig. 3. Biosolubilization of lignite, pH profile. *S. flavovirens* (○), RWL-5 in humidified air (△), RWL-5 in humidified oxygen (□).

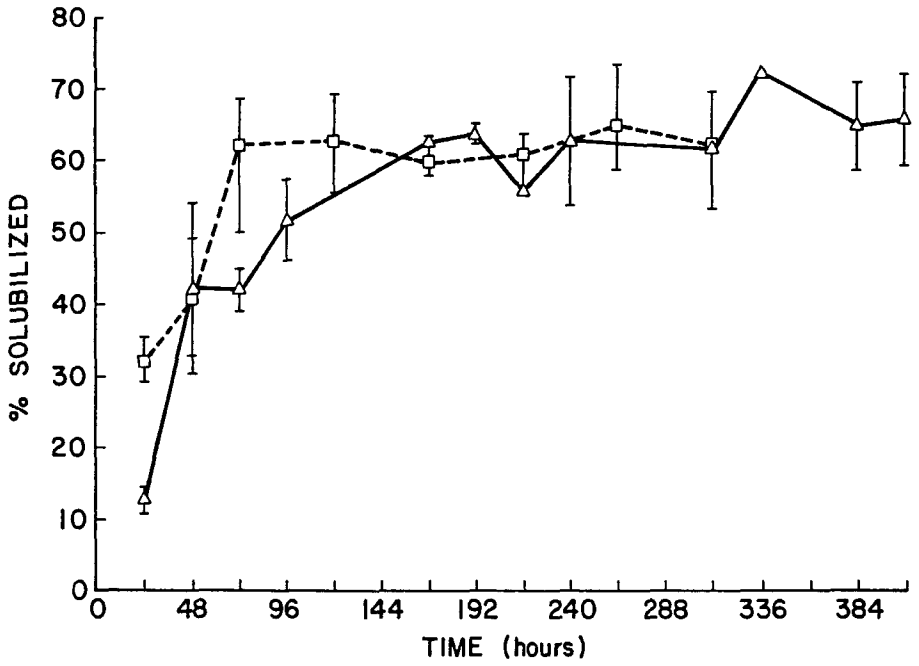

Fig. 4. Biosolubilization of lignite, effects of oxygen enrichment. Humidified air (•), humidified oxygen (□).

air. The average rate measured during the first 72 h of incubation in a humidified 99.6% oxygen atmosphere was roughly one and a half times that observed in humidified air. The influence of oxygen enrichment on biosolubilization was reflected in the pH dynamics only in that maximum pH values coincided with periods of the greatest biosolubilization rates in both studies (*see* Fig. 3).

Substrate Utilization by New Isolates

The selection experiments produced isolates of five substrains capable of utilizing oxidatively pretreated coal as a carbon substrate. These isolates may be normal variants originally present in the genetic makeup of the RWL-5 (*10*). For the purpose of identification, the substrains were designated as follows.

RTM-1. Produces very short white mycelia with gray-green spores on yeast maltose agar.

RTM-2. Produces a thick mat of white mycelia topped with green spores on yeast maltose agar.

RTM-2B. Produces a thick mat of mycelia with tan spores on yeast maltose agar.

RTM-2C. Produces a wrinkled mat of short white mycelia topped with gray spores on yeast maltose agar.

RTM-2D. Produces a thick mat of white mycelia topped with dark green spores on yeast maltose agar.

The heterotrophic capability of RWL-5 substrain isolates was investigated in a series of experiments. First, because modified yeast maltose agar contained a variety of potential carbon substrates, a series of plates using a modified Czapek agar (LISA 75), which contains no yeast extract, were prepared. The strains RWL-5, RTM-1, and RTM-2 were inoculated and incubated 7 d at 30°C. Only RTM-1 and RTM-2 grew. Second, plates in which lignite extract completely replaced glucose as the carbon source in the modified Czapek agar (LISA 100), were inoculated with every lignin degrading organism in our collection. The organisms tested were *Streptomyces setonii, Streptomyces flavovirens, Streptomyces viridosporous,* ACL-13, YML-21, and RWL-5 (provided by Idaho National Engineering Laboratory) and substrains RTM-1 and RTM-2. After incubation for 10 d at 30°C, only the plate inoculated with RTM-2 had any growth.

The capability of RTM-2 to utilize coal with varying degrees of surface oxidation as sole carbon sources was investigated. To four Cazpek Dox inorganic salts solutions was added TXL-35W, TXL-AFEX, TXL-UT, and no carbon source. All four flasks were inoculated with RTM-2 and incubated statically at 30°C for 21 d. RTM-2 grew in all but the control flask, indicating that the fungus was not a strict autotroph. Growth in the liquid medium was sparse in each case and the colonies were similar in appearance.

The ability of the new substrains to grow on other substrates was investigated. Plates of MCA-G, MCA-CB, and MCA-A media were inoculated with RWL-5. Good growth occurred on all substrates. After 11 d, TXL35W was placed on a membrane filter on each culture. No visible solubilization was detected after 3 wk, but after 6 wk, the cultures exhibited solubilization. Those on glucose and cellobiose solubilized approximately 66% of the lignite. Amylose media produced 77% solubilization. Similarly, a series of MCA-G, MCA-CB, and MCA-L was inoculated with RWL-5, RTM-2, RTM-2B, RTM-2C, and RTM-2D and incubated 6 d at 30°C in the humidified incubator. Only the strain RTM-2C grew on the lactose substrate. After the d 6, 100 mg of TXL35W was added to a membrane filter placed on each culture. After 6 wk of incubation, solubilization had occurred on all grown cultures, as indicated in Fig. 5.

The relative rates of solubilization of TXL35W by isolated strains were studied. A series of YM agar plates were inoculated with RWL-5 and the new isolates RTM-2B and RTM-2C. In Fig. 6, data shows both RTM-2B RTM-2C solubilized more coal in the first 5 d than the strain RWL-5, suggesting a possible improvement in solubilization rate. An additional experiment comparing RWL-5 and RTM-2C verified the differences in the relative initial rates of biosolubilization. RTM-2C solubilized coal more rapidly and to a greater extent than RWL-5 (Fig. 7). The length of a lag phase before initiation of solubilization was primarily responsible for the difference in rates.

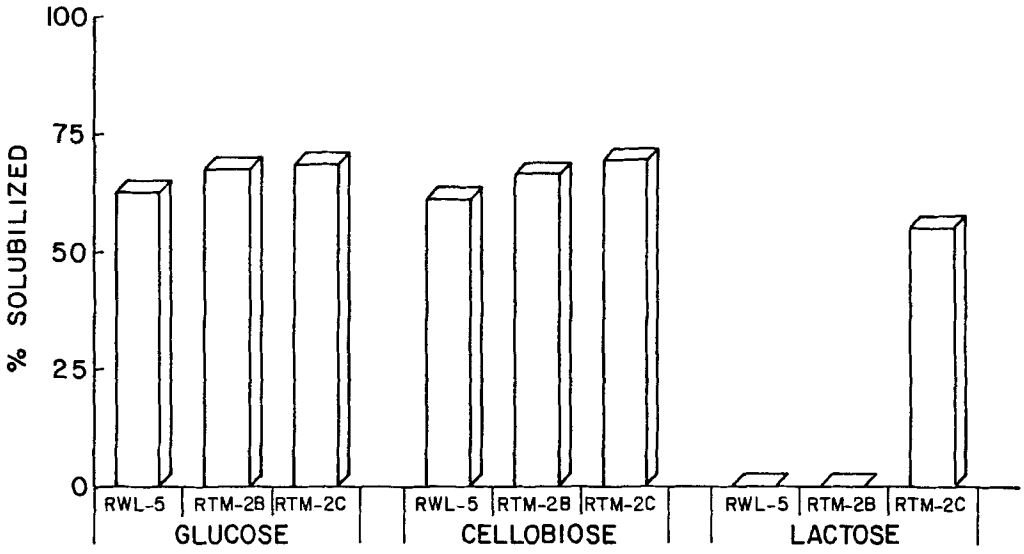

Fig. 5. Effect of substrate on lignite biosolubilization.

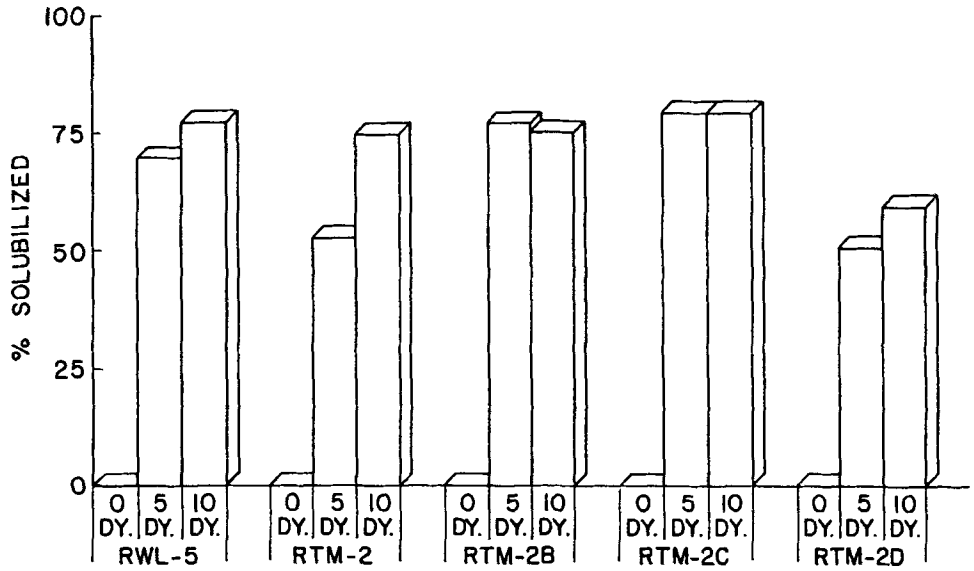

Fig. 6. Relative rates of biosolubilization by RWL-5 and four substrains.

CONCLUSIONS

Surface oxidation was shown to have a major effect on biosolubilization. At \$195 per ton of 100% HNO_3 (*11*) and an abundance of cheap nutrients (e.g., waste sulfite liquor), the chemical value of the products of the fermentation would have to be greater than the value of the coal by at least \$100 per ton plus separation costs to justify oxidatively pretreating coal. Hence, biological processing of coal would be restricted to weathered coal deposits.

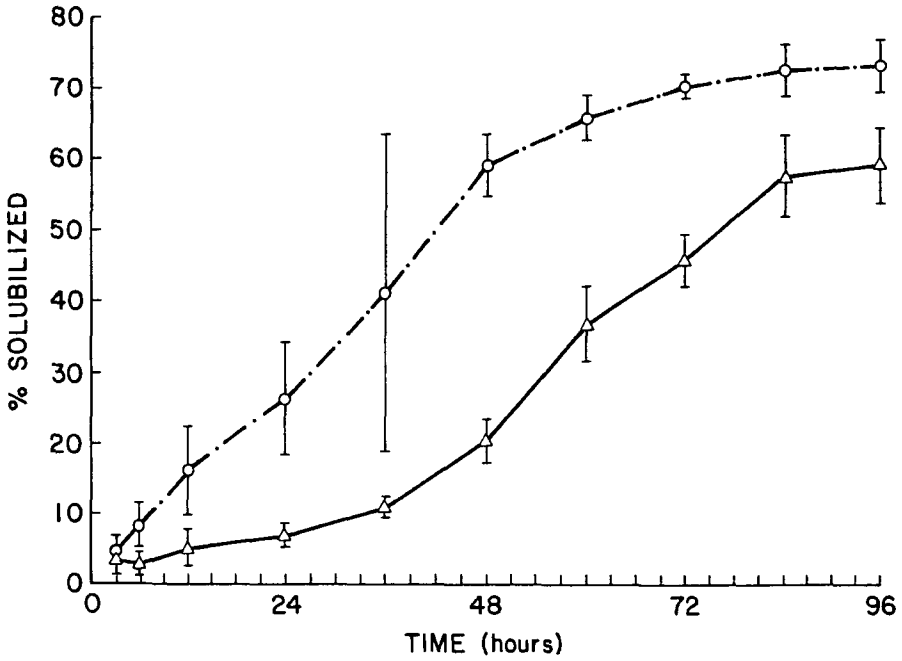

Fig. 7. Biosolubilization of lignite. Initial biosolubilization rates by RWL-5 and RTM-2C. RWL-5 (△), RTM-2C (○).

Oxygen partial pressure was shown to be important in the biosolubilization process. In submerged culture, aeration becomes an important cost and technical consideration. The use of purified oxygen in airlift or pressurized fermentors may ultimately be necessary. However, preliminary experiments with an airlift fermenter have been unsatisfactory because of excessive wall growth blocking circulation. Solubilization has been slow in suspension cultures. However, on solid medium, RTM-2C showed solubilization rates of 300 g/kg/d. Hence, RTM-2C may be suitable for processing the coal by solid substrate fermentation. Aeration distribution will be a critical parameter in solid state fermenter design.

ACKNOWLEDGMENTS

This work was supported by the Idaho National Engineering Laboratory and the US Department of Energy under Contract No. DE-AC07-761D01570. Additional support was provided by the Colorado Agricultural Experiment Station.

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