Journal of Industrial Microbiology, 13 (1994) 292–299

Published by The Macmillan Press Ltd

Bioconversion of coal, lignin, and dimethoxybenzyl alcohol by *Penicillium citrinum*

J. Kevin Polman, Daphne L. Stoner and Karen M. Delezene-Briggs

Industrial Biotechnology and Process Engineering, Idaho National Engineering Laboratory, EG&G Idaho, Inc., Idaho Falls, ID 83415-2203, USA

(Received 15 January 1994; revised 22 April 1994; accepted 29 April 1994)

Key words: Penicillium citrinum; Coal; Lignin; Dimethoxybenzyl alcohol; Veratryl alcohol

SUMMARY

Bioconversion of alkali-soluble coal, sulfonated lignin, and dimethoxybenzyl alcohol (DMBA) by *Penicillium citrinum* was investigated with respect to the effects of (1) these compounds on growth and metabolism, and (2) the organism on the chemical nature of coal and DMBA. Alkali-soluble coal caused a slight enhancement of growth and metabolism; DMBA and lignin partially inhibited growth and metabolism. Both whole cells and cell-free extracts were capable of oxidation of DMBA to dimethoxybenzaldehyde. Whole cells demonstrated the capability of modifying alkali-soluble Beulah Zap and Ugljevik lignite coals by producing compounds that were of lower and higher molecular weight than the original coal. In vivo conversion of alkali-soluble Ugljevik coal resulted in a substantial decrease in the sulfur content of the coal (52% decrease). Cell-free extracts were able to degrade alkali-soluble Ugljevik lignite coal. The results suggest a potential usefulness of this microorganism for coal bioprocessing.

NOMENCLATURE

A	light absorption
DMBA	3,4-dimethoxybenzyl alcohol
HPSEC	high performance size exclusion chromatography
MW	molecular weight

INTRODUCTION

Coal is the most abundant fossil fuel resource in the United States [2]. The combustion of coals, such as lignite and leonardite, produces gaseous nitroxides and sulfoxides that pose environmental problems [13]. One of the most notable of these problems is acid rain. Consequently, the treatment of coal in order to decrease its potential for producing pollution is very important to the United States for both economic and environmental reasons.

Coal is a complex and biologically recalcitrant material whose organic portion is composed of: (1) macromolecules which are similar in structure to lignin and humic acid, and (2) low molecular weight organic molecules [7,10]. Most coal organic carbon in lignite and leonardite is macromolecular and can be dissolved in dilute aqueous alkali. This dissolved material is known as alkali-solubilized coal [20].

Microorganisms interact with coal in different ways, which include biological depyritization, coal solubilization by biologically produced alkaline materials, and coal solubilization by biological chelators [1,6,8,9,11,12,24]. At the Idaho National Engineering Laboratory there is a particular interest in identifying microorganisms that can convert coal to liquid fuels and chemicals. One of our primary research goals is to convert alkali-solubilized coal biologically into clean liquid fuels such as ethanol. This effort requires the depolymerization and fermentation of a predominantly macromolecular material. Due to the complex chemical nature of coal, the depolymerization and fermentation stages of coal processing are likely to be mediated by different types of microorganisms, i.e. those that are adept at degrading recalcitrant chemicals, and those that can efficiently ferment the less recalcitrant products of degradation. Our initial goal has been to identify microorganisms that are capable of depolymerizing recalcitrant macromolecules in coal [22].

Penicillium citrinum is a well known, airborne, hyphomycete fungus that is found in aquatic environments, rice seeds, the phylloplane of wheat, packaging materials, and forest soils, as well as in many other environments [5,23,26,29,30,32]. The fungus can degrade cellulose, organophosphates, the pesticide pendimethalin, aromatic petroleum hydrocarbons, polyurethane, starch, pectin, and the fungicide carboxin, and produces hydrolytic enzymes (many of which are extracellular) such as cellulase, amylase, protease, lipase, and esterase [3–5,14,17–19,25,27,33]. *P. citrinum* is also capable of using humic and fulvic acids as carbonaceous and nitrogenous nutrients [16]. Based on these characteristics, *P. citrinum* is a good candidate for a coal-degrading microorganism.

The following questions were asked in the present study:

Correpondence to: Kevin Polman, Industrial Biotechnology and Process Engineering, Idaho National Engineering Laboratory, EG&G Idaho, Inc., Idaho Falls, ID 83415-2203, USA.

(1) How do alkali-soluble coal, sulfonated lignin, and 3,4-dimethoxybenzyl alcohol (DMBA, also known as veratryl alcohol) affect the growth and metabolism of *P. citrinum*? Lignin is structurally similar to alkali-soluble coal, and DMBA is a substrate of lignin-degrading ligninases [13,15].
(2) Does *P. citrinum* enzymatically oxidize DMBA in a fashion similar to previously reported ligninases? Ligninases oxidize DMBA to 3,4-dimethoxybenzaldehyde (veratraldehyde) [15].
(3) Does *P. citrinum* enzymatically modify alkali-soluble coal?
(4) How does conversion of alkali-soluble coal by *P. citrinum* affect the elemental composition of the coal? Are troublesome elements such as sulfur decreased?

MATERIALS AND METHODS

Effects of coal, lignin, and DMBA on growth and metabolism

The strain of *Penicillium citrinum* used in this study (strain 26) was isolated as a laboratory contaminant at the Idaho National Engineering Laboratory and was subsequently identified to species level by the American Type Culture Collection (Rockville, MD, USA). Stock cultures of *P. citrinum* on nutrient agar (DIFCO, Detroit, MI, USA) were used to inoculate twelve 50-ml flasks each containing 10 ml of nutrient broth (DIFCO). These were shaken at 200 r.p.m., 30 °C, for 4 days. Six of the flasks were then autoclaved and used as dead cell controls. Cells from all 12 flasks were washed with water, and resuspended in 10 ml LPM broth in 60-ml serum bottles, containing basal salts [21], dextrose (0.65% w/v final concentration), and nutrient broth (0.085% w/v final concentration). One or more of the following were added to the LPM broth: alkali-solubilized coal mix (1 ml, see below), sulfonated lignin (1 ml, see below), and 3,4-dimethoxybenzyl alcohol (DMBA, 0.03% v/v). Controls that did not receive any of these compounds and six additional serum bottle cultures which were abiotic controls were prepared at this time. All 18 serum bottles were sealed with rubber caps and seals and were shaken at 30 °C, 200 r.p.m. for 10 days, at which time the headspaces were analyzed by gas chromatography for oxygen and carbon dioxide content (CTR I column, Alltech, Deerfield, IL, USA). Cell material was separated and dry biomass weights were determined, and final culture pHs were measured.

Alkali-solubilized coal mix was prepared by mixing 0.2 g of North Dakota Beulah Zap lignite [31], 0.2 g of Mississippi Wilcox lignite (donated by Dr Bailey Ward, University of Mississippi), and 0.2 g of North Dakota leonardite (collected by INEL researchers) in separate 100-ml portions of 0.1 N NaOH overnight, neutralizing the suspensions with HCl, then filtering them through a nitrocellulose, 0.45-μm pore size filter. The three solutions were then mixed so that 1 ml of alkali-solubilized coal mixture (used in media preparation above) contained 0.4 ml soluble Beulah Zap lignite, 0.4 ml soluble Mississippi Wilcox lignite, and 0.2 ml soluble leonardite. Sulfonated lignin solution was prepared by mixing 0.8 g REAX 100M (sodium salt of sulfonated modified lignin, Westvaco, Charleston Heights, SC, USA) in 100 ml water for 30 min, neutralizing the suspension, and filtering it through a nitrocellulose filter, 0.45-μm pore-size.

In vivo oxidation of DMBA

Stock cultures of *P. citrinum* were used to inoculate four 50-ml flasks each containing 25 ml of nutrient broth. These were shaken at 200 r.p.m., 30 °C, for 3 days. Two of the flasks were then autoclaved and used as dead cell controls. Cells from all four flasks were washed with water, and resuspended in 20 ml LPM broth in 50-ml flasks; some flasks contained DMBA (0.03% v/v). Two additional flask cultures which were abiotic (no cell) controls were prepared at this time. All six flasks were shaken at 30 °C, 200 r.p.m. Samples (1 ml) were removed periodically and centrifuged (5 min, room temperature, 12700 × *g*). Light absorption at 310 nm was measured for the supernatant fluid as an indication of DMBA oxidation to dimethoxybenzaldehyde (activity is indicated by an increase in absorption) [15].

In vitro oxidation of DMBA

Stock cultures of *P. citrinum* were used to inoculate two 50-ml flasks each containing 20 ml of nutrient broth. These were shaken at 200 r.p.m., 30 °C, for 3 days. Cells were washed with water and resuspended (in 50-ml flasks) in either 20 ml of nutrient broth or 20 ml LPM broth. Flasks were shaken at 30 °C, 200 r.p.m. Samples (2 ml) were removed periodically from each flask and filtered (Acrodisc HT Tuffryn, 0.2-μm pore size, Gelman, Ann Arbor, MI, USA). Portions (90 μl) of filtrates were added to two 1.5-ml microfuge tubes, to which 10 μl of a solution containing 19 mM DMBA and 1 mM hydrogen peroxide were added. One of each pair of filtrate mixtures was frozen immediately at −80 °C (time zero control); the other sample was incubated overnight, stationary, 29 °C, then frozen. Samples were thawed and light absorption at 310 nm was measured [15].

In vivo conversion of alkali-soluble Beulah Zap lignite

Stock cultures of *P. citrinum* were used to inoculate two 50-ml flasks each containing 20 ml of nutrient broth. These were shaken at 200 r.p.m., 30 °C, for 3 days. Cells were washed with water, and resuspended in 20 ml of LPM broth in 50-ml flasks. Alkali-soluble Beulah Zap lignite (0.8 ml) was added to one of the flasks. Two additional flask cultures which were abiotic (no cell) controls were prepared at this time. Flasks were shaken at 30 °C, 200 r.p.m. Samples (1 ml) were removed after 10 days and centrifuged (5 min, room temperature, 12700 × *g*). Supernatant fluids were analyzed by high performance size exclusion chromatography (HPSEC) in order to detect changes in coal macromolecular size. Supernatant samples (50 μl) were eluted through a SynChropak GPC300 HPLC column (260 × 4.6 mm, SynChrom, Lafayette, IN, USA) using 20 mM KH_2PO_4 buffer (pH 7.1) containing 0.5% Tween 80 as eluant (0.3 ml min^{-1}) [20]. Light absorption by the eluant was measured at 200–500 nm with a photodiode array detector (resolution = 20 nm). Globular proteins (bovine gamma

globulin, 158000 MW; ovalbumin, 45000 MW; myoglobin, 17000 MW) and cyanocobalamin (1350 MW) were used as molecular weight (MW) standards [20]. Molecular weights for coal components that are reported in this study do not represent absolute molecular weights, but rather, relative molecular weights compared with the molecular weight standards. Absorption maxima for different coal components were derived from component peak areas calculated at different wavelengths ranging from 200 to 500 nm.

In vivo conversion of alkali-soluble Ugljevik lignite

Stock cultures of *P. citrinum* were used to inoculate two 50-ml flasks, each containing 20 ml of nutrient broth. These were shaken at 200 r.p.m., 30 °C, for 3 days. One of the flasks was then autoclaved and used as a dead cell control. Cells in both flasks were washed with water, and resuspended in 20 ml of LPM broth in 50-ml flasks. Alkali-soluble Ugljevik lignite (2 ml, solid Ugljevik lignite was provided by C.-S. Wang, US Bureau of Mines) was added to each flask. Flasks were shaken at 30 °C, 200 r.p.m. Samples (1 ml) were removed after 15 days and centrifuged (5 min, room temperature, 12 700 × *g*). Supernatant fluids were analyzed: (1) by HPSEC in order to detect changes in coal macromolecular size (see above), and (2) for changes in coal elemental composition. Light absorption by the HPSEC eluant was measured at 240–460 nm with a photodiode array detector (resolution = 20 nm). Absorption maxima for different coal components were derived from component peak areas calculated at different wavelengths ranging from 240 to 460 nm. Supernatant samples were also treated with HCl to precipitate coal. Precipitated coal was collected by centrifugation and subjected to total carbon, hydrogen, sulfur, and nitrogen analyses using a Carlo Erba model EA1108 elemental analyzer (Fisons Instruments, Woburn, MA, USA). Phenanthrene and isothiourea were used as standards. Data are averages of triplicate analyses (live cell sample) and single analyses (dead cell control) of single samples.

In vitro degradation of alkali-soluble coal

A stock culture of *P. citrinum* was used to inoculate a 1-L flask containing 100 ml of nutrient broth. This was shaken at 200 r.p.m., 30 °C, for 5 days. Cells were washed with water, and resuspended in 84 ml of GC1 broth in a 1-L flask. GC1 broth contains (per liter of water): 2 g KH_2PO_4, 2 g $MgSO_4 \cdot 7H_2O$, 1 g $(NH_4)_2SO_4$, 10 g dextrose, 0.3 g yeast extract (DIFCO), and 10 ml alkali-soluble Ugljevik lignite solution. The flask was shaken at 30 °C, 200 r.p.m. Samples (1–2 ml) were removed and frozen at −80 °C after 0, 1, 2, 3, 4, 7, 8, 9, 10, 11, 15, 16, and 17 days. Each time a sample was removed, 1–2 ml of sterile GC1 broth was added to the culture. The samples were thawed, pooled (approximately 26 ml total volume), and filtered (cellulose acetate, 0.2 μm).The filtrate was concentrated using Centriprep no. 3 tubes (Amicon, Beverly, MA, USA) according to manufacturer's specifications, which allowed a five-fold concentration of molecules greater than 3000 MW; the final volume of the concentrate was 5 ml. Two and a half ml of the concentrate was autoclaved to provide a non-enzymatic control. Fifty μl of either water (no-coal control) or alkali-soluble Ugljevik lignite was placed in four 1.5 ml microfuge tubes, and 50-μl portions of either enzymatic or autoclaved concentrates were added to these tubes every 15 min for 120 min, for a total of 400 μl concentrate per tube. During this time, the tubes were incubated at 30 °C, stationary. After incubation the mixtures were frozen, later thawed, then mixed with 500 μl HPSEC elution buffer, allowed to sit for 30 min at room temperature, then centrifuged (2 min, room temperature, 12700 × *g*). Portions (20 μl) of the supernatant phases were analyzed by HPSEC (see above). The supernatant phases were also examined for light absorption at 400 and 600 nm in order to assess degradation of coal.

RESULTS AND DISCUSSION

Effects of coal, lignin, and DMBA on growth and metabolism

Data in Table 1 indicate that alkali-solubilized coal may slightly enhance growth and metabolism; DMBA and lignin partially inhibit growth and metabolism. These conclusions are based on Enhancement of Metabolism Index (EMI) values, which generally reflect the overall effect of added compounds (see note i, Table 1). The slight enhancement of growth by coal is supported by previous observations that *P. citrinum* is capable of utilizing humic acid components as nutrients; the chemical nature of the alkali-soluble portion of coal is similar to humic acid [7,10,16,20]. While DMBA tends to inhibit metabolism overall, it appears to enhance oxygen utilization; this may indicate reaction-specific utilization of oxygen rather than utilization for general respiratory purposes.

Oxidation of DMBA

Whole cells of *P. citrinum* are capable of oxidizing DMBA to dimethoxybenzaldehyde, since only live cell cultures with DMBA showed a significant increase in absorption at 310 nm (Table 2). In vitro DMBA oxidation activity was also demonstrated (Table 3). DMBA oxidation was not observed for cell-free preparations from nutrient broth cultures, and was not observed to a significant extent for cell-free preparations from LPM cultures until day five. These osbervations suggest that DMBA oxidation activity may be repressed by rich nutrient compositions; DMBA oxidation is affected similarly in *Phanerochaete chrysosporium* [15].

Degradation of alkali-soluble coal

Whole cell cultures of *P. citrinum* exhibited coal degradation capabilities with alkali-solubilized Beulah Zap and Ugljevik lignites (Tables 4 and 5). HPSEC data indicated that both higher and lower molecular weight products were derived from the coals. These products were observed only when coal was present in the cultures (data not shown). Peak areas indicate that a significant portion of the original coal was converted to coal-derived products. The absorption maxima for products showed that the aromaticity of the original coal was conserved in many of the products. Some

TABLE 1

Oxygen consumption, CO_2 production, biomass production, and pH changes associated with the addition of coal, lignin, and DMBA to *P. citrinum* cultures

Substrate	Sample	Final O_2[a]	O_2 consumed[b]	Final CO_2[c]	CO_2 produced[d]	Final dry wt[e]	Growth dry wt[f]	Final pH[g]	ΔpH[h]	EMI[i]
None	No cells	10.07	–	0	–	–	–	6.56	–	–
	Dead cells	12.05	–	0	–	5.4	–	6.82	–	–
	Live cells	2.99	9.06	10.69	10.69	22.0	16.6	3.14	−3.68	40.03
Coal	No cells	9.79	–	0	–	–	–	7.33	–	–
	Dead cells	12.29	–	0	–	4.6	–	7.16	–	–
	Live cells	3.00	9.29	10.63	10.63	24.6	20.0	4.44	−2.72	42.64
DMBA	No cells	12.43	–	0	–	–	–	6.46	–	–
	Dead cells	12.45	–	0	–	5.9	–	6.76	–	–
	Live cells	3.17	9.28	9.64	9.64	18.8	12.9	3.08	−3.68	35.50
Lignin	No cells	11.67	–	0	–	–	–	6.32	–	–
	Dead cells	9.97	–	0	–	5.6	–	6.59	–	–
	Live cells	4.97	5.00	7.46	7.46	23.6	18.0	3.38	−3.21	33.67
Coal +	No cells	12.42	–	0.01	–	–	–	7.21	–	–
DMBA	Dead cells	12.57	–	0	–	6.4	–	7.18	–	–
	Live cells	3.09	9.48	9.69	9.68	24.1	17.7	4.44	−2.74	39.60
Lignin +	No cells	9.76	–	0	–	–	–	6.21	–	–
DMBA	Dead cells	12.40	–	0	–	3.7	–	6.55	–	–
	Live cells	5.88	6.52	6.38	6.38	17.8	14.1	3.33	−3.22	30.22

[a] Final oxygen concentration, μmol ml^{-1} of headspace.

[b] Oxygen consumed by metabolism, μmol ml^{-1} of headspace; dead cells final oxygen concentration minus live cells final oxygen concentration.

[c] Final CO_2 concentration, μmol ml^{-1} of headspace.

[d] CO_2 production due to metabolism, μmol ml^{-1} of headspace; all values are uncorrected data, except for coal plus DMBA culture (which was live cell CO_2 concentration minus no cell CO_2 concentration).

[e] Final dry weight of cell material, mg.

[f] Dry weight of live cells due to growth, mg; live cells final dry weight minus dead cells final dry weight.

[g] Final pH of culture.

[h] Change in pH due to metabolism; live cells final pH minus dead cells final pH.

[i] Enhancement of Metabolism Index (EMI) = O_2 consumed by metabolism (μmol ml^{-1}) + CO_2 produced by metabolism (μmol ml^{-1}) + dry weight of live cells due to growth (mg) + (−)ΔpH. EMI value is directly proportional to the degree that a substrate enhances metabolic activity since carbonaceous substrate utilization by aerobic acid-producing microorganisms involves the utilization of oxygen, production of carbon dioxide, increase in biomass weight, and decrease in culture pH. This study is the first to describe the use of EMI to indicate general effects of a substrate on metabolism.

TABLE 2

In vivo oxidation of DMBA by *P. citrinum*

Culture	A_{310} of culture medium after incubation for (h)					Highest rate of increase in absorption (ΔA_{310} h^{-1})
	0	8	19	24	45	
No cells, + DMBA	0.304	0.306	0.303	0.308	0.307	0.001
Live cells, + DMBA	0.307	0.371	0.570	0.627	1.179	0.026
Dead cells, + DMBA	0.310	0.314	0.321	0.319	0.327	0.001
No cells, no DMBA	0.214	0.206	0.207	0.209	0.202	0.000
Live cells, no DMBA	0.217	0.242	0.243	0.232	0.252	0.003
Dead cells, no DMBA	0.229	0.236	0.231	0.229	0.233	0.001

TABLE 3

In vitro oxidation of DMBA by *P. citrinum* cell-free extracts

Medium used for growth	Time after inoculation that extract was prepared and assayed (days)	A_{310} at time zero	A_{310} after overnight incubation	ΔA_{310} (A_{310} overnight minus A_{310} time zero)
Nutrient broth	0	0.158	0.159	0.001
	1	0.184	0.177	−0.007
	2	0.200	0.194	−0.006
	3	0.197	0.195	−0.002
	4	0.202	0.199	−0.003
	5	0.209	0.207	−0.002
LPM	0	0.038	0.038	0.000
	1	0.041	0.040	−0.001
	2	0.042	0.042	0.000
	3	0.043	0.045	0.002
	4	0.045	0.048	0.003
	5	0.046	0.058	0.012

TABLE 4

HPSEC data for in vivo conversion of Beulah Zap lignite by *P. citrinum*

Culture containing[a]	MW of coal derived HPSEC peaks	Normalized MW[b]	Peak area (A_{350}·min·10^{-6})	Normalized peak areas[c]	Absorption maxima (nm)
No cells, + coal	25 130	1.000	144 531	1.000	240
	6139	0.244	24 171	0.167	280
Live cells, + coal	222 597	8.858	4991	0.034	240, 280
	70 958	2.824	5893	0.041	240, 440
	25 298	1.007	10 610	0.073	240
	13 627	0.542	102 625	0.710	260
	5388	0.214	211 778	1.465	300
	185	0.007	35 759	0.247	300, 400

[a] Analyses of live and no cell cultures without coal were also performed; no peaks were observed which were similar (with respect to retention time and peak area) to those observed when coal was present.
[b] MW of 25 130 peak was set at 1.000; other MW were normalized accordingly.
[c] Peak area of 25 130 peak was set at 1.000; other peak areas were normalized accordingly.

of the products showed maxima characteristic of pigment-like compounds; this is not unusual since coal is geologically derived from plant material which would be expected to contain pigments [7,13].

In vitro coal degradation was also observed (Tables 6 and 7). Spectrophotometric data from assay mixtures indicated that 14–25% (400 and 600 nm data, respectively) degradation of Ugljevik lignite occurred within 2 h of incubation. HPSEC analyses of cell-free preparations also indicated coal degradation; however, the product spectrum of in vitro degradation was considerably less diverse than that observed for in vivo degradation. In vivo interactions with coal are likely to involve more than one enzyme; preparation of cell-free culture fluids may not preserve all the enzyme activities involved in modifying a complex chemical conglomerate such as coal. Nonetheless, peak areas indicate that most or all of the original coal was altered in enzymatic cell-free culture fluids.

Elemental composition of coal after in vivo treatment

Data in Table 8 indicate that carbon composition changed only slightly, that hydrogen and nitrogen content increased significantly, and that sulfur content decreased by 52%. Since alkali-soluble Ugljevik coal contains sulfur only as organosulfur, these data suggest considerable degradation of coal organosulfur with a minimum of carbon degradation [28]. This is a valuable observation, since coal organosulfur is difficult to remove from coal without extensive degradation of the coal carbon skeleton [28]. Coal organosulfur gives rise to polluting sulfoxides upon combustion of coal [13].

TABLE 5

HPSEC data for in vivo conversion of Ugljevik lignite by *P. citrinum*

Culture containing	MW of coal derived HPSEC peaks	Normalized MW[a]	Peak area (A_{350}·min·10^{-6})	Normalized peak areas[b]	Absorption maxima (nm)
Dead cells, + coal	52 900	1.000	485 195	1.000	260
Live cells, + coal	213 200	4.030	13 406	0.028	260
	72 900	1.378	266 450	0.549	260
	24 100	0.456	4208	0.009	260
	11 000	0.208	42 816	0.088	260
	6200	0.117	142 238	0.293	280
	200	0.004	3047	0.006	240, 360
	100	0.002	50 977	0.105	240, 300, 400

[a] MW of 52 900 peak was set at 1.000; other MW were normalized accordingly.
[b] Peak area of 52 900 peak was set at 1.000; other peak areas were normalized accordingly.

TABLE 6

Spectrophotometric analysis of *P. citrinum* cell-free culture fluids assayed for coal degradation

Sample	Wavelength (nm)	Absorption of whole extract after 2 h	Normalized absorption values
Autoclaved plus coal	400	1.115	100[a]
	600	0.194	100[a]
Enzymatic plus coal	400	0.963	86
	600	0.146	75
Autoclaved no coal	400	0.165	100[a]
	600	0.073	100[a]
Enzymatic no coal	400	0.178	108
	600	0.087	119

[a] Autoclaved values set at 100; enzymatic values normalized accordingly.

TABLE 8

Changes in elemental composition of Ugljevik lignite coal after in vivo treatment with *P. citrinum*

Element(s)	Coal elemental percentages and ratios after treatment with live and dead cells	
	Dead cells	Live cells
Carbon	50.198 (100)[a]	48.064 (96)
Hydrogen	5.212 (100)	6.596 (126)
Nitrogen	5.147 (100)	6.325 (123)
Sulfur	3.541 (100)	1.706 (48)
H/C	0.104 (100)	0.137 (132)
N/C	0.102 (100)	0.132 (129)
S/C	0.070 (100)	0.035 (50)

[a] Values outside parentheses are actual composition data; values inside parentheses are dead cell values that were set at 100 and live cell values that were normalized accordingly.

TABLE 7

HPSEC analysis of *P. citrinum* cell-free culture fluids assayed for coal degradation

Sample	MW of coal and coal products	Normalized MW	Peak area (A_{400}·min·10^{-6})	Normalized peak area
Autoclaved plus coal	60 968	100[a]	62 106	100[a]
Enzymatic plus coal	52 252	86	58 879	95
	360 081	591	1958	3

[a] Autoclaved values set at 100; enzymatic values normalized accordingly.

CONCLUSIONS

P. citrinum possesses enzymatic capabilities for modifying coal and DMBA. The capabilities do not appear to benefit the organism significantly with respect to growth and general metabolism. Since DMBA is a substrate for ligninases in other microorganisms, and coal is similar to lignin in chemical structure, the possibility exists that coal and DMBA modification by *P. citrinum* are at least partially related. This is supported by the observation that coal and DMBA modification occur during the same time frame in whole cell cultures (data not shown). Coal degradation capabilities of *P. citrinum* suggest that the organism may be useful in pretreatment of coal used as a fermentation substrate. Additionally, observed changes in coal elemental composition imply that this organism has some long-term potential in terms of desulfurizing coal. Future studies will clarify these potentials.

ACKNOWLEDGEMENTS

Funding for the work described herein was provided by the United States Department of Energy (Office of Advanced Research and Technology Development; Office of Fossil Energy; contract # DE-ACO7-76IDO1570).

REFERENCES

1 Andrews, G.F., C.J. Stevens, A. Glenn and K.S. Noah. 1993. Microbial coal depyritization: why and how? In: Biohydrometallurgical Technologies, vol. 1 (Torma, A.E., J.E. Wey and V.I. Lakshmanan, eds), pp. 381–391, Mineral, Metals and Materials Society, Warrendale, PA.

2 Anonymous. 1990. Clean coal technology: energy and the environment. In: The PETC Review, March 1990 (Chun, S.W., ed.), pp. 26–33, Pittsburgh Energy Technology Center, Pittsburgh.

3 Balasubramanya, R.H. and R.B. Patil. 1980. Heterotrophic nitrification by microorganisms capable of degrading carboxin and oxycarboxin. Indian J. Microbiol. 20: 294–297.

4 Barua, A.S., J. Saha, S. Chaudhuri, A. Chowdhury and N. Adityachadhury. 1990. Degradation of pendimethalin by soil fungi. Pestic. Sci. 29: 419–426.

5 Chary, M.P. and S.M. Reddy. 1988. Amylase production by different seedborne fungi of rice. Acta Bot. Indica 16: 92–94.

6 Cohen, M.S., K.A. Feldman, C.S. Brown and E.T. Gray, Jr. 1990. Isolation and identification of the coal-solubilizing agent produced by *Trametes versicolor*. Appl. Environ. Microbiol. 56: 3285–3291.

7 Derbyshire, F., A. Marzec, H.-R. Schulten, M.A. Wilson, A. Davis, P. Tekely, J.-J. Delpuech, A. Jurkiewicz, C.E. Bronnimann, R.A. Wind, G.E. Maciel, R. Narayan, K. Bartle and S. Snape. 1989. Molecular structure of coals: a debate. Fuel 68: 1091–1106.

8 Faison, B.D. 1991. Biological coal conversions. Crit. Rev. Biotechnol. 11: 347–366.

9 Fredrickson, J.K., D.L. Stewart, J.A. Campbell, M.A. Powell, M. McMulloch, J.W. Pyne and R.M. Bean. 1990. Biosolubilization of low-rank coal by a *Trametes versicolor* siderophore-like product and other complexing agents. J. Ind. Microbiol. 5: 401–406.

10 Green, T., J. Kovak, D. Brenner and J.W. Larsen. 1982. The macromolecular structure of coals. In: Coal Structure (Meyers, R.A., ed.), pp. 199–282, Academic Press, New York.

11 Gupta, R.K., J.K. Spiker and D.L. Crawford. 1988. Biotransformation of coal by lignolytic *Streptomyces*. Can. J. Microbiol. 34: 667–674.

12 Klasson, K.T., M.D. Ackerson, E.C. Clausen and J.L. Gaddy. 1993. Direct bacterial conversion of coal to liquid fuels. In: Microbial Transformations of Low Rank Coals (Crawford, D.L., ed.), pp. 93–110, CRC Press, Boca Raton.

13 Klein, J., M. Beyer, M. van Afferden, W. Hodek, F. Pfeifer, H. Seewald, E. Wolff-Fischer and H. Jüntgen. 1988. Coal in biotechnology. In: Biotechnology, Vol. 6b (Rehm, H.-J. and G. Reed, eds), pp. 497–567, VCH Verlagsgesellschaft, Weinheim.

14 Kuhad, R.C. and A. Singh. 1993. Enhanced production of cellulases by *Penicillium citrinum* in solid state fermentation of cellulosic residue. World J. Microbiol. Biotechnol. 9: 100–101.

15 Linko, S. 1992. Production of *Phanerochaete chrysosporium* lignin peroxidase. Biotech. Adv. 10: 191–236.

16 Macor, M. 1979. Decomposition of humic acids from peat soil by micromycetes. Acta Fac. Rerum. Nat. Univ. Comenianae. Microbiol. 7: 1–24.

17 Maliszewska, I. and P. Mastalerz. 1992. Production and some properties of lipase from *Penicillium citrinum*. Enzyme Microb. Technol. 14: 190–193.

18 Olutiola, P.O. and O.A. Akintunde. 1979. Pectin lyase and pectin methyl esterase production by *Penicillium citrinum*. Trans. Br. Mycol. Soc. 72: 49–56.

19 Pathirana, R.A. and K.J. Seal. 1985. Studies on polyurethane deteriorating fungi. 4. A note on the spectrochemical changes during fungal deterioration. Int. Biodeterior. 21: 123–126.

20 Polman, J.K. and D.R. Quigley. 1991. Size exclusion chromatography of alkali-solubilized coal. Energy Fuel 5: 352–353.

21 Polman, J.K., C.R. Breckenridge, P.R. Dugan and D.R. Quigley. 1991. Growth of aerobic bacteria on alkali-solubilized lignite. Appl. Biochem. Biotechnol. 28/29: 487–494.

22 Polman, J.K., C.R. Breckenridge and D.R. Quigley. 1991. Degradation of lignite coal by bacteria. In: Proceedings 1991 Second International Symposium on the Biological Processing of Coal (Yunker, S., ed.), pp. P/63-P/77, Electric Power Research Institute, Palo Alto, CA.

23 Prabu, S.K., B. Subramanian and A. Mahadevan. 1991. Mycoflora of sediment and waters of the Madras Coast Bay of Bengal Indian Ocean. Indian J. Mar. Sci. 20: 226–228.

24 Quigley, D.R., B. Ward, D.L. Crawford, H.J. Hatcher and P.R. Dugan. 1989. Evidence that microbially produced alkaline materials are involved in coal solubilization. Appl. Biochem. Biotechnol. 20/21: 735–763.

25 Sharma, O.P. and K.D. Sharma. 1980. In vitro production of proteolytic enzymes by some leather deteriorating fungi. Rev. Roum. Biochim. 17: 209–216.

26 Singh, A.K. and B. Rai. 1990. Effect of sulfur dioxide and ammonia on growth behavior of some phylloplane fungi of wheat. Water Air Soil Pollut. 49: 343–348.

27 Sorkhoh, N.A., M.A. Ghannoum, A.S. Ibrahim, R.J. Stretton and S.S. Radwan. 1990. Crude oil and hydrocarbon-degrading strains of *Rhodococcus rhodochrous* isolated from soil and marine environments in Kuwait. Environ. Pollut. 65: 1–18.

28 Stoner, D.L., K.S. Miller, K.B. Barrett, J.B. Tingey, R.B. Wright and J.E. Wey. 1991. The use of water soluble coal products to examine microbial coal desulfurization. In: Processing

and Utilization of High-Sulfur Coal IV (Dugan, P.R., D.R. Quigley and Y.A. Attia, eds), pp. 549–566, Elsevier, New York.

29 Tindale, C.R., F.B. Whitfield, S.D. Levingston and T.H.L. Nguyen. 1989. Fungi isolated from packaging materials: their role in the production of 2,4,6-trichloroanisole. J. Sci. Food Agric. 49: 437–448.

30 Valmaseda, M., A.T. Martinez and G. Almendros. 1989. Contribution by pigmented fungi to p-type humic acid formation in two forest soils. Soil Biol. Biochem. 21: 23–28.

31 Vorres, K.S. 1990. The Argonne Premium Coal Sample Program. Energy Fuel 4: 420–426.

32 Wei, D.L., J.-H. Chen, S.-C. Jong and H.-D. Shen. 1993. Indoor airborne *Penicillium* sp. in Taiwan. Curr. Microbiol. 26: 137–140.

33 Zboinska, E., I. Maliszewska, B. Lejkzak and P. Kafarski. 1992. Degradation of organophosphates by *Penicillium citrinum*. Lett. Appl. Microbiol. 15: 269–272.