Microbial hydrogenation of coal and effect on liquefaction

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ABSTRACT

We have investigated the potential of hydrogenase-containing bacteria in the direct hydrogenation of different untreated coals and coal-related model compounds for improved liquefaction. Hydrogen uptake hydrogenase-possessing thermoacidophilic archaebacterium *Sulfolobus brierleyi* and mesophilic sulfate-reducing bacteria *Desulfovibrio desulfuricans* were used to study the biological hydrogenation of different coals and various model compounds such as diphenyl methane (DPM), 1,2-diquinolyl ethane, a-naphthyl hexane. The enzyme activity in *D.desulfuricans* and in *S. brierleyi* was determined by Warburg manometry with H2 as the electron donor and methylene blue as the electron acceptor under anaerobic conditions. In parallel experiments the H2 uptake by the model compounds and various coal types catalyzed by the microbial systems was also determined using G.C. The experiments with the model compounds indeed showed hydrogen uptake ranging from 0.28 µmoles H2 / µmole of DPM to 6.55 µmoles H2 / µmoles of 1,2-diquinolyl ethane in presence of *S. brierleyi* and *D. desulfuricans* respectively. However, chloroform extract of aqueous phase analysis using GC-MS did show that DPM was fragmented into lower parent fragments of M.Z values 73, 95 and 147 depending upon the microorganism used.

The biocatalyzed net hydrogen uptake by untreated coals varied from 370 to 1100 µmoles H_2 / g coal depending upon coal type and the microorganism; the highest H_2 uptake occurred in the untreated Fire Clay coal (KCER # 4677). The biohydrogention of the pretreated KY 11 coal (KCER # 91182P) and asphaltenes occured to a much greater extent and yielded the respective net H_2 uptake values of 2370 and 1800 µmoles H_2 / g coal. A net increase of 3% and 10 % in the chemical liquefaction yield was obtained respectively in case of *S. brierleyi* and *D.desulfuricans* treated coals either for hydrogenation or for in-situ catalyst formation. Therefore, our present study revealed that biotreated coals exhibited enhanced liquefaction yield.

OBJECTIVES

One of the primary objectives of our work is direct microbial hydrogenation of untreated, treated coals and model compounds for ultimate enhancement of liquefaction yield. However, the general scheme of our work includes different aspects of bioprocessing of coal and model compounds (Figure 1).

INTRODUCTION

Biological processing of coal has many inherent advantages (1), such as mild operating conditions and control of the fine crystal size of metal catalyst formation possibly due to the production and coating of biomolecules generated during bacterial growth(2). This process is considered to be one of the viable alternatives for enhanced liquefaction. However, research for improving coal liquefaction by biotreatment needs to be focussed in different directions i.e., physical and chemical pretreatment, formation of iron based metal catalysts, anaerobic hydrogenation and removal of inorganic and organic sulfur and heteroatom content. Since adaptation of microorganisms to extreme environments has been well documented (3), the success of improved liquefaction yield depends upon appropriate selection of microorganisms

and in designing suitable culture conditions. Our latest work has revealed that coal treatment in the presence of organic solvents and also in hydrogen atmosphere separately by anaerobic bacteria containing hydrogen uptake hydrogenase enzyme increased the liquefaction yield (4).

In recent times, extensive desulfurization studies have been reported in the literature (5-11). Calculation of costs of different process configurations for microbial coal desulfurization have also been reported (12). Most research has involved studies of coal-solubilizing bacteria and fungi (13-16). Evidence suggests that some microorganisms excrete oxidative enzymes which degrade coal into water soluble polymeric products (17). The detection of the ability of anaerobic bacteria possessing hydrogen uptake hydrogenase in hydrogen atmosphere is important (18-21) for direct hydrogenation of coal and model compounds.

ACCOMPLISHMENTS AND CONCLUSIONS

In-situ formation of fine FeOOH crystals:

We have investigated the potential of hydrogenase-containing bacteria in the direct hydrogenation of different untreated coals and coal-related model compounds, and of sulfur and iron metabolizing bacteria for in-situ catalist formation for improvement of the liquefaction yield (Table 1) The general process of in-situ fine crystals of FeOOH formation studies is presented in Figure 2. Our findings have shown that *Sulfolobus brierleyi* could tolerate different amounts of molybdenum which was present in culture medium (Figure 3). Though, there was increase in protein content in culture broth, we observed sudden decline of growth at the end of 12th day of the experiment (Figure 4). Mossbauer analysis of these biotreated coal samples obtained at the end of the run showed significant changes in the iron forms (Table 2). XAFS study of biotreated samples obtained with increase of time exhibted prominent increase of peak between

50-60 eV represents Mo impregnation on coal. Similarly, XPS studies revealed that Mo and Fe were deposited on coal surface of biotreated coal samples particularly in the samples collected from the growth fermentor. The liquefaction was also improved by 3% even without pre-sulfiding conditions (Figure 5). Interesting part of this work was that the reprecipitation of aqueous phase iron as FeOOH released from coal (Figure 6) and sudden decrease of protein content of the culture broth (Figure 4) might have influenced the controll of the ultra-fine size of the catalyst. Bioprocessing of coal with *S.brierleyi* lead to 5 to 10% enhancement in chemical liquefaction yield depending upon the temperature used for liquefaction of bioprocessed coal obtained under different operation conditions.

Biohydrogenation of coals:

Hydrogen uptake hydrogenase-possessing thermoacidophilic archaebacterium *Sulfolobus brierleyi* and the mesophilic sulfate-reducing bacteria *Desulfovibrio desulfuricans* were used to determine the biological hydrogenation of different coals and various model compounds such as diphenyl methane (DPM), 1,2-diquinolyl ethane, and a-naphthyl hexane (Table 1). Hydrogenase enzymes of different bacteria responsible for hydrogen uptake often contain Ni, Fe, Se, and Mo in the catalytic centers of proteins comprising the enzymes and they occur in different locations of the cells of *Desulfovibrio* species (Table 4 and Figure 7). General mechanism of reversible hydrogenase of *Desulfovibrio* species and *Clostridium thermoaceticum* is shown in Figure 8 which highlights the significance of hydrogenase enzyme present in different bacteria.

Washed cell suspensions were used from the *S.brierleyi* grown chemolithotrophically under aerobic conditions with elemental sulfur as the oxidizable energy source, and *D.desulfuricans* grown anaerobically with lactate as the energy source and sulfate as

the final electron acceptor. Both organisms possessed hydrogenase activity under these growth conditions. The enzyme activity in *D.desulfuricans* was determined by Warburg manometry at 30°C and pH 7.4, and at 60°C and pH 2.0 in *S. brierleyi* with H2 as the electron donor and methylene blue as the electron acceptor under anaerobic conditions. In parallel experiments the H2 uptake by the model compounds and various coal types catalyzed by the microbial systems was also determined using gas chromatography.

The biocatalyzed net hydrogen uptake by untreated coals varied from 370 to 1100 µmoles H2 / g coal depending upon coal type and the microorganism. Out of different coals used for biohydrogenation by *D. desulfuricans*, coal (KCER # 4677) showed the highest hydrogen uptake. However, there was significant variation in the extent of hydrogenation depending upon the complexity of the substrate (Figure 9). The biohydrogention of the pretreated (200°C in H2 atmosphere) Kentucky 11 coal (KCER # 91182P) and asphaltenes occured to a much greater extent and yielded the respective net H2 uptake values of 2370 and 1800 µmoles H2 / g coal. These results indicate that pretreatment of coals at 200°C in H2 atmosphere may significantly enhance biohydrogenation and the liquefaction yield. Liquefaction of biotreated coal sample (KCER # 4677) showed an increase of approximately 5.5% of liquefaction yield (Table 5). *Sulfolobus brierleyi* also influenced in hydrogenation of some of the coal samples and of model compounds but it was not as efficient as *Desulfovibrio desulfuricans* .

Hydrogenation of different model compounds by bacteria:

Chemical structures of different model compounds used for biohydrogenation is given in Figure 10. The experiments with model compounds indeed showed hydrogen uptake ranging from 0.28 µmoles H2 / µmole of DPM to 6.55 µmoles H_2 / µmoles of

1,2-diquinolyl ethane in presence of *S. brierleyi* and *D. desulfuricans* respectively (Table 6 and Figures 11 &12). When 1,2-diquinolyl ethane was dissolved in ethanol, it did not show any hydrogen uptake in presence of *D. desulfuricans* due to the enzyme inactivation and thus acted as a control (Figure 13). There was a wide variation in the extent of hydrogen uptake even among the model compounds (Figure 14). At present, it is not clear about the pathway of hydrogen incorporation into these compounds. However, chloroform extract of aqueous phase analysis using GC-MS did show that DPM was fragmented into lower parent fragments of M/Z values 73, 95 and 147 depending upon the microorganism used.

Sulfolobus brierleyi utilized dibenzothiophene and thiophene dissolved in coal liquid to the extent of 27 and 36% respectively (Table 7). A net increase of 6% and 7.3% in the chemical liquefaction yield was obtained in coals treated with *D.desulfuricans* in presence of various concentrations of benzene (Figure 15). Therefore, our present study revealed that biotreated coals exhibited enhanced liquefaction yield when treated with *D.desulfuricans* in presence of 50% benzene. The organism was to be metabolically active even in presence of 80% bezene..

PLANS

Our future plans will be to determine:

1.The ability of several hydrogen uptake hydrogenase-possessing thermophilic and hyperthermophilic (Table 8) bacteria to catalyze coal hydrogenation and enhanced liquefaction

2. In depth studies on anaerobic hydrogenation of different model compounds and different coal types

3.The optimal conditions for the aerobic microbial formation / precipitation of active iron catalyst on coal surface

4.The bioformation and development of different metal based catalysts on biohydrogenated coal surface as a two-stage treatment to improve liquefaction yield.

REFERENCES

1. R.M. Kelly and J.W. Deming. Biotechnol. Prog., **4** (2), 47-62 (1988).
2. R.J. Piccolini. Japan Chem. Week **31**, 5 (1990).
3. R.A. Prins, W de Vrij, J.C. Gottschal and T.A. Hansen. FEMS Microbiol. Rev.,**75**, 103-104 (1990)
4. M.I.H. Aleem, D. Bhattacharyya, R.I. Kermode, M.V.S. Murty, H. Venkatachalam and M. Ashraf. Sec. Intern. Symp. on Bioprocessing of Coal, May 1-3 (1991), San Diego, CA. Submitted for publication (1991).
5. D. Bhattacharyya, M. Hsieh, H. Francis, R.I. Kermode, A.M. Khalid and M.I.H. Aleem. Resour., Conser. Recycl., **3**, 61-86 (1990).
6. A.M. Khalid, D. Bhattacharyya, M.I.H. Aleem. Develop. Ind. Microbiol.,**31**, 115-126 (1990).
7. A.M. Khalid, M.I.H. Aleem, R.I. Kermode and D. Bhattacharyya. Resour., Conser. Recycl. **5**, 167-181 (1991)
8. T.L. Peeples, S. Hirosue, G.L. Olson and R.M. Kelly. Fuel, **70**, 599-604 (1991).
9. D. Zhou and R.H. White. J. Bacteriol. **173**, 3250-3251 (1991).
10. A. El.Sawy and D. Gray. Fuel, **70**, 591-594 (1991)
11. J.J. Kilbane and B.A. Bielaga. CHEMTECH, December, 747-751 (1990).
12. M. Beyer, J. Klein, K. Vaupel and D. Wiegand. Bioprocess Eng. **5**, 97-101 (1990).
13. C.D. Scot, C.W. Straudberg, S.N. Lewis. Bitechnol. Prog. **2**, 131-139 (1986).
14. C.D. Scot, C.A. Woodward, J.E. Thompson and S.L. Blaukenshep. Appl. Biochem. Biotechnol. **24/25**, 799-815 (1990)
15. C.W. Straudberg and S.N. Lewis. J. Indust. Microbiol. **1**, 371-375 (1987)
16. M.S. Cohen and P.D. Gabriele. Appl. Environ. Microbiol. **53**, 2840-2843 (1987).
17. D.L. Crawford and R.K. Gupta. Fuel **70**, 577-580 (1991)

18. M.W.W Adams. FEMS Microbiol Rev., **75**, 219-238 (1990).

19. M.H. Czechowski, C. Chatelus, G. Fauque, M.F.L. Coquempot, P.A. Lespinat, Y. Berlier and J. LeGall. J. Ind Microbiol., **6**, 227-234 (1990)

20 T.D. Phil and R.J. Maier J. Bacteriol., **173**, 1839-1844 (1991).

21. E. Drobner, H. Huber, K.O. Stetter. Appl Environ. Microbiol., **56**, 2922-2923 (1990).

Table 1. List of microorganisms and substrates used in this study

Microorganisms

- *Desulfovibrio desulfuricans*
- *Desulfovibrio nigrificans*
- *Rhizobium sesbanium*
- *Sulfolobus brierleyi*

Substrates

Model compounds

- diphenyl methane
- methylene blue
- asphaltenes
- 1,2-diquinolyl ethane
- α-naphthyl hexane

Coal

- KCER # 91182, 71637 and 4677
- Coal (KCER # 91182) + benzene
- Pretreated coal KCER # 91182 (200°C, 800 p.s.i H_2, 1 hour)

Table 2. Composition of iron determined by Mössbauer analysis (12 K) of coal (KCER # 91182) treated with *Sulfolobus brierleyi*

	% Fe	Phase
Control*	43 %	Pyr. + Fe^{3+} sulf.
	56 %	Jarosite
Test	61 %	Fe^{3+} sulf.(+ FeOOH)
	39 %	Jarosite

* - Not treated with *S.brierlei*

Table 3. X-Ray photoelectron spectroscopic determination of elements present on the surface (50 A°) of Coal when treated with *Sulfolobus brierleyi* at 60°C

Coal Samples (KCER # 91182)	Variation of elements (Mass %)				
	C	O	S	Mo	Fe
Raw Coal	80.69	15.4	3.54	000	0.37
Test- Shaker†	62.36	29.47	2.03	4.16	1.98
Control- "	72.98	19.64	2.07	3.79	1.53
Test - Fermentor@	56.96	25.34	2.43	8.94	6.32
Control - "	67.79	20.61	2.33	5.34	3.93

Test - With bacteria
Control - Without bacteria
† - Contains 200 ppm Molybdenum salt. Initial pH is 3 and dropped to 2.4. Contact time 20 days.
@ - Contains 300 ppm Molybdenum salt. pH maintained at 3. Contact time 21 days.

Table 4. Hydrogen production from mild steel by *Desulfovibrio* species

Organism*	Hydrogenase location	Prosthetic group metals
D.vulgaris Hildenborough	Periplasmic	Fe
D.salexigens British Guina	Periplasmic	Fe-Ni-Se
D.multispirans	Cytoplasmic	Fe-Ni

* - The organisms were grown anaerobically in acetate/sulfate medium at 32°C.

Table 5. Chemical liquefaction results of different coals (5 %) treated with *Desulfovibrio desulfuricans*

Liquefaction Products Wt. %	Coal (KCER # 4677)*		Coal (KCER # 91182)*	
	Control	Test	Control	Test
Gas	- - -	- - -	1.49	1.80
Oils	10.24	9.07	14.23	15.04
Asphaltenes	16.98	18.63	17.01	18.06
Preasphaltenes	22.00	26.83	14.51	11.93
IOM**	50.78	45.43	52.76	53.17
Conversion	49.22	54.57	47.24	46.83

* - Only test run was treated with *D.desulfuricans*. Liquefaction was carried out at the end of 144 hrs.

** - Insoluble organic matter.

Liquefaction conditions.

Temperature : 385°C
Atmosphere : 800 p.s.i Hydrogen
Reaction Time : 15 minutes
Solvent used : Tetralin

Table.6 Hydrogen uptake by different coal-related substrates in the presence of *Sulfolobus brierleyi* measured by GC

Substrate	Hydrogen Uptake (micromoles / g substrate)			
	18 hrs	48 hrs	72 hrs	144 hrs
Coal # 91182†	- - -	- - -	- - -	111
Coal# 91182P*†	- - -	- - -	- - -	423
Asphaltenes	401	1466	582	1908
DPM**	0.43	0.18	0.18	0.28
MB**	- - -	- - -	- - -	1.49

† - Denotes KCER numbering of coal.

* - Pretreated coal (200°C and hydrogen atmosphere)

** - Hydrogen uptake for Diphenyl Methane (DPM) and Methylene Blue (MB) are expressed as micromoles / micromole substrate.

Table 7. Removal of organic sulfur added to coal-liquid mixed with aqueous medium using *S. brierleyi*

Organic sulfur compound + coal-liquid	Protein in aqueous medium (µg/ml)		Org. sulfur utilized (%)	Sulfate in aqueous medium (µg/ml)	
	1st day	20th day		1st day	20th day
Dibenzothiophene	60	90	27	- - -	65
Thiophene	40	80	36	- - -	53
Coal-liquid (alone)	80	95	- - -	- - -	- - -
Methyl disulfide	38	28	- - -	- - -	- - -
Ethyl disulfide	58	54	- - -	- - -	- - -
Ethane thiole	56	65	- - -	- - -	- - -

Table 8. list of coal-related chemolithotrophic anaerobic bacteria and their metabolic significance

Desulfobacterium autotrophicum
Desulfosarcina variabilis
Desulfonema limicola
Desulfococcus niacini
Desulfobacterium vacuolatum
Desulfobacter hydrogenophilus
*Clostridium thermoaceticum**
*Themoproteus neutrophilus***
*Themoproteus tenax***
*Pyrobaculum islandicum****
*Pyrococcus furiosus****
*Pyrodictium brockii****
*Pyrodictium occultum****

Growth on H_2 + CO_2 (or CO) + SO_4^{--}
Some can grow in presence of NO_3^- instead of SO_4^{--}
Can also use a variety of organic compounds for growth e.g., cyclohexane carboxylate, butyrate, butanol, propanol, ethanol and dicarboxylic acid.
All possess hydrogenase

* - Thermophile (40-80°C)
** - Extreme thermophile (80-100°C)
*** - Hyperthermophile (100°C and above)

Fig.1 General scheme of our research activity

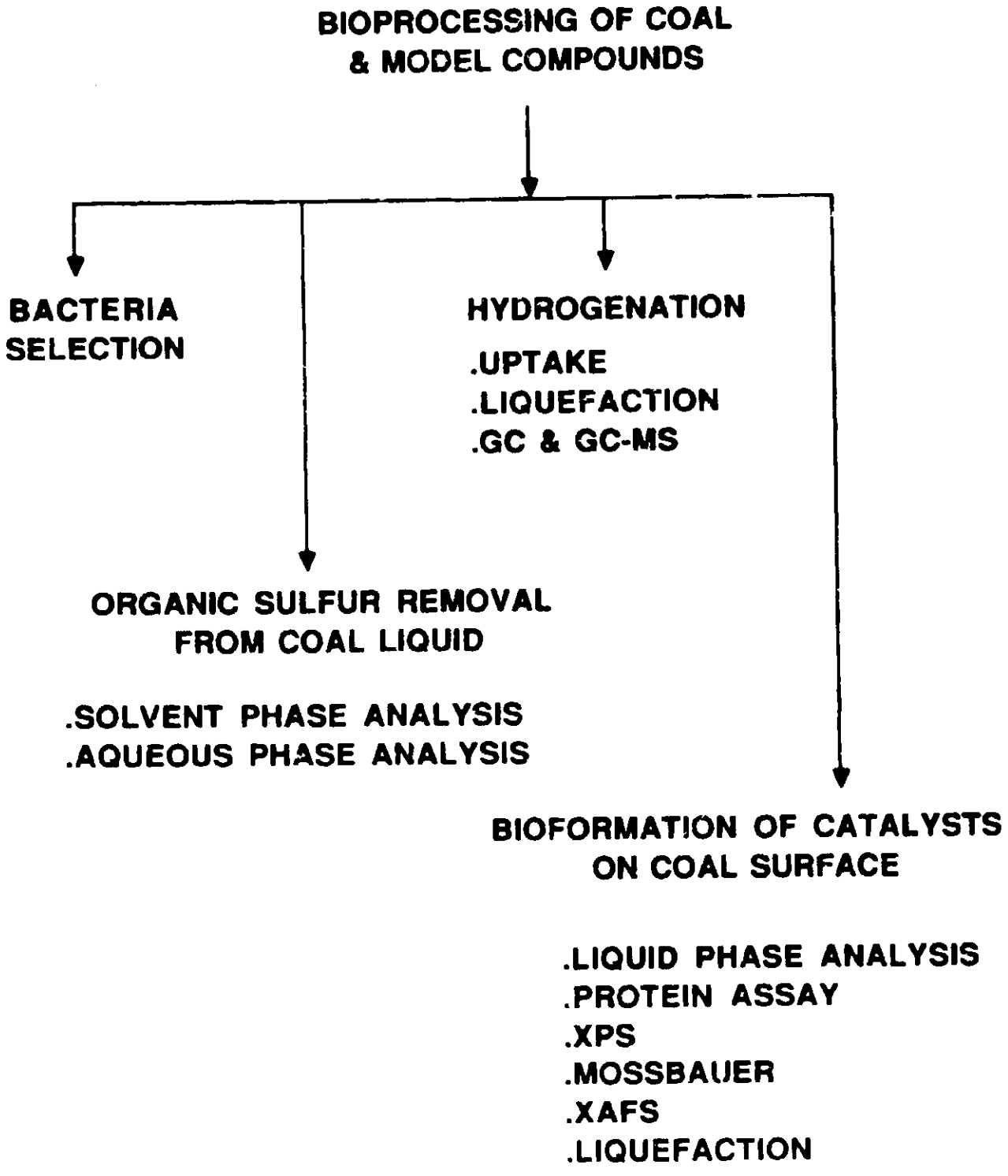


Fig. 2 SCHEMATIC REPRESENTATION OF EXPERIMENTAL PROCEDURE FOR THE PROCESSING OF COAL (KCER # 91182) WITH THERMOPHILIC BACTERIA, *SULFOLOBUS BRIERLEYI* AT 60°C AND pH 3

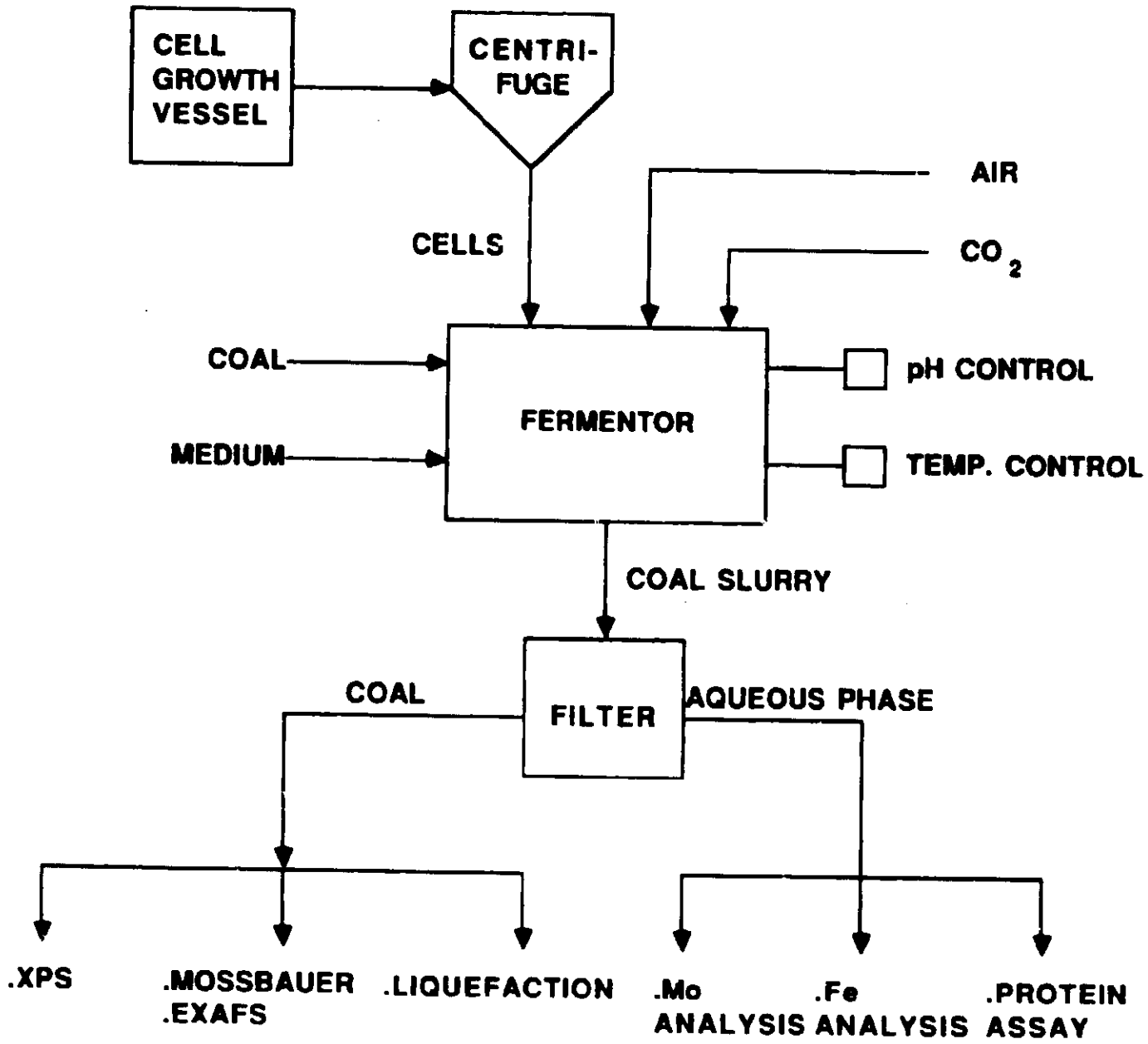


Fig. 3 Determination of Molybdenum tolerance by assay of the cell protein of Sulfolobus brierlevi when grown on 5% Coal (KCER # 91182) at 60°C and initial pH 2.5

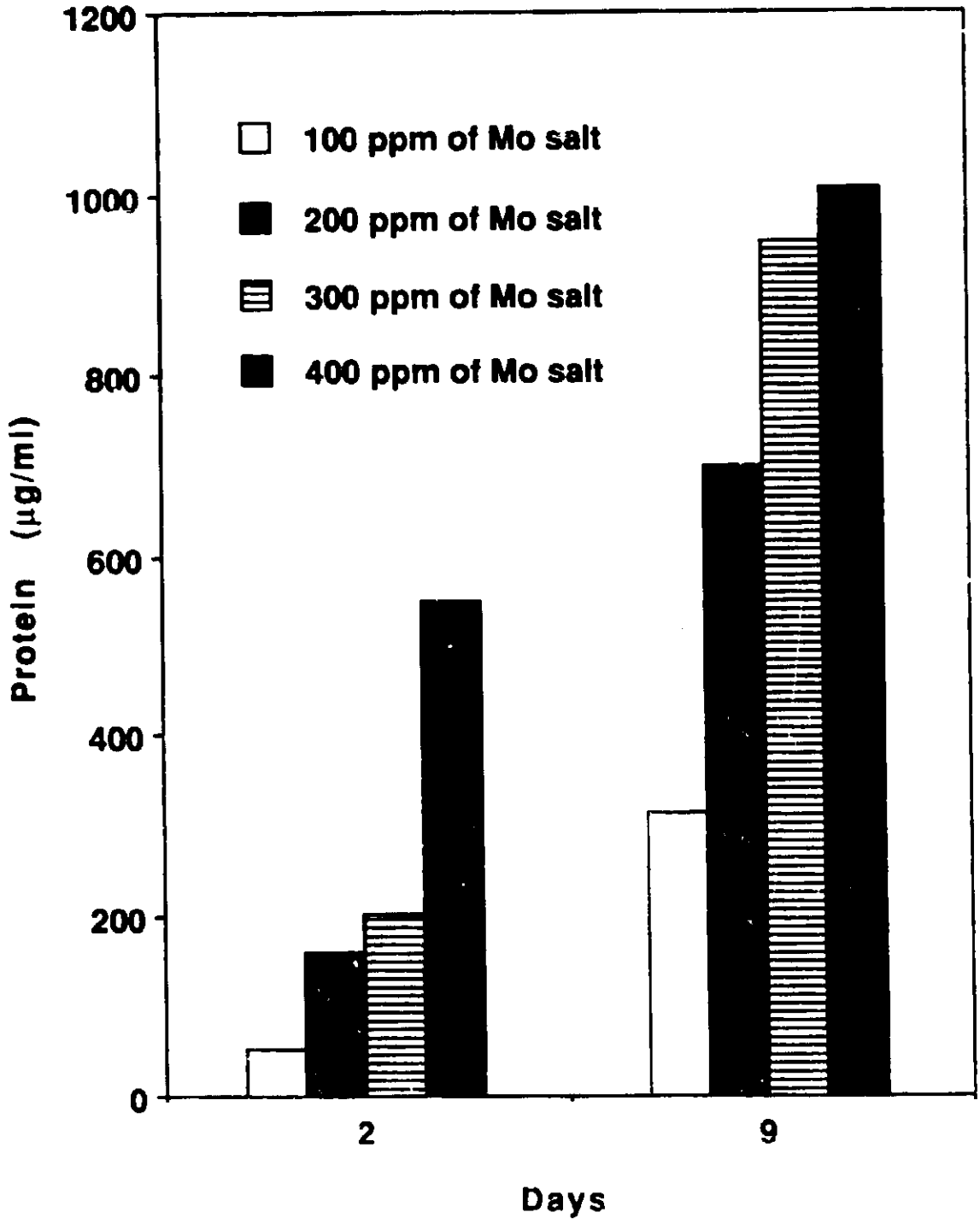


Fig. 4 Protein content of medium containing 5% Coal (KCER # 91182) and 200 ppm Molybdenum salt solution when treated with Sulfolobus brierleyi in shaker setup at 60°C and initial pH of 3.0

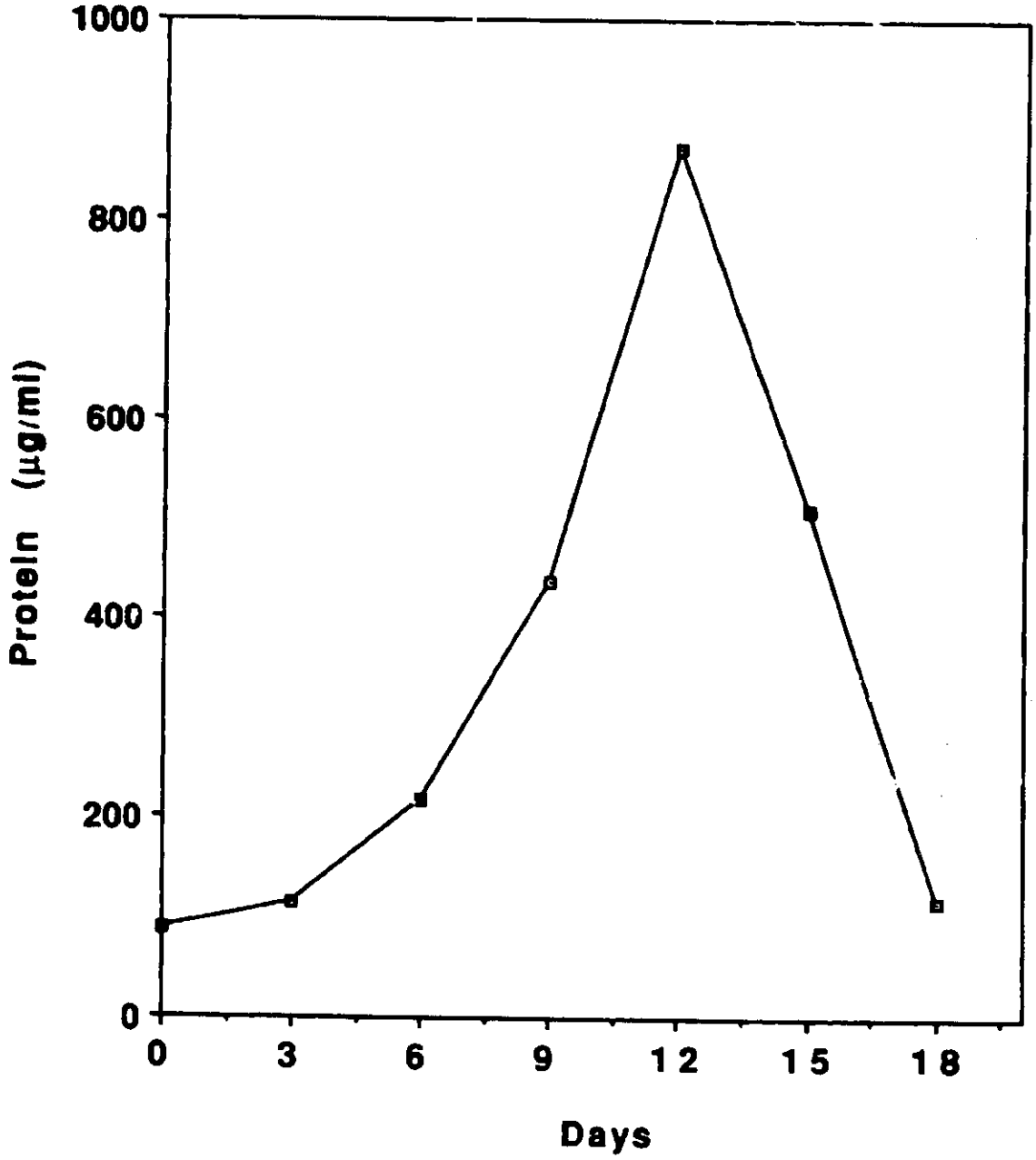
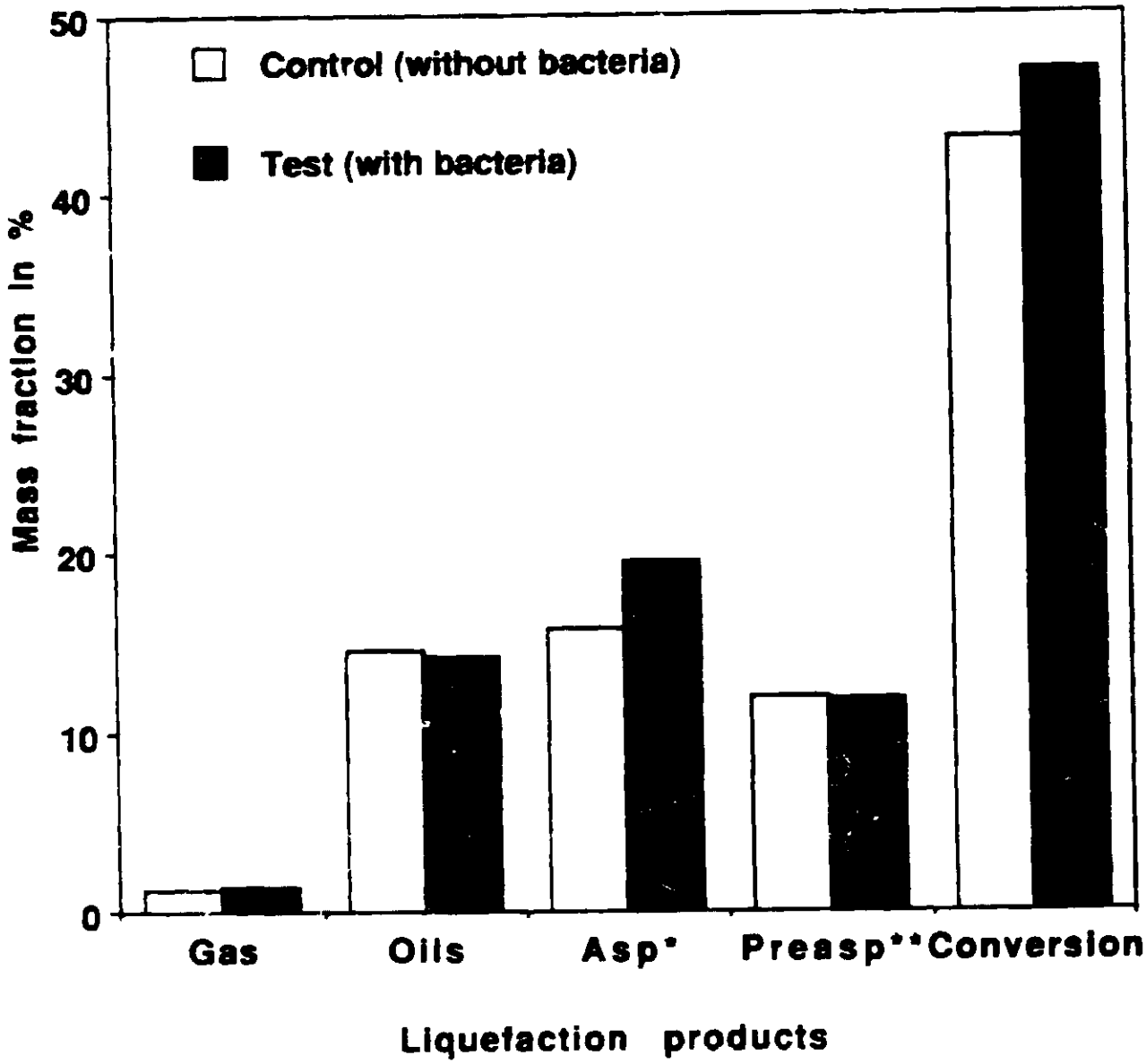


Fig. 5 Liquefaction product profile of Coal (KCER # 91182) bioprocessed with Sulfolobus brierleyi at 60°C, pH 3, in presence of air, CO2 and 200 ppm of Molybdenum salt



* - Asphaltenes
** - Preasphaltenes

Fig. 6 Variation of liquid phase iron content with time for 200 mesh Coal (KCER # 91182) of 1% slurry treated in fermentor with Sulfolobus brierleyi in the presence of CO_2 at 60°C and constant pH 2.5

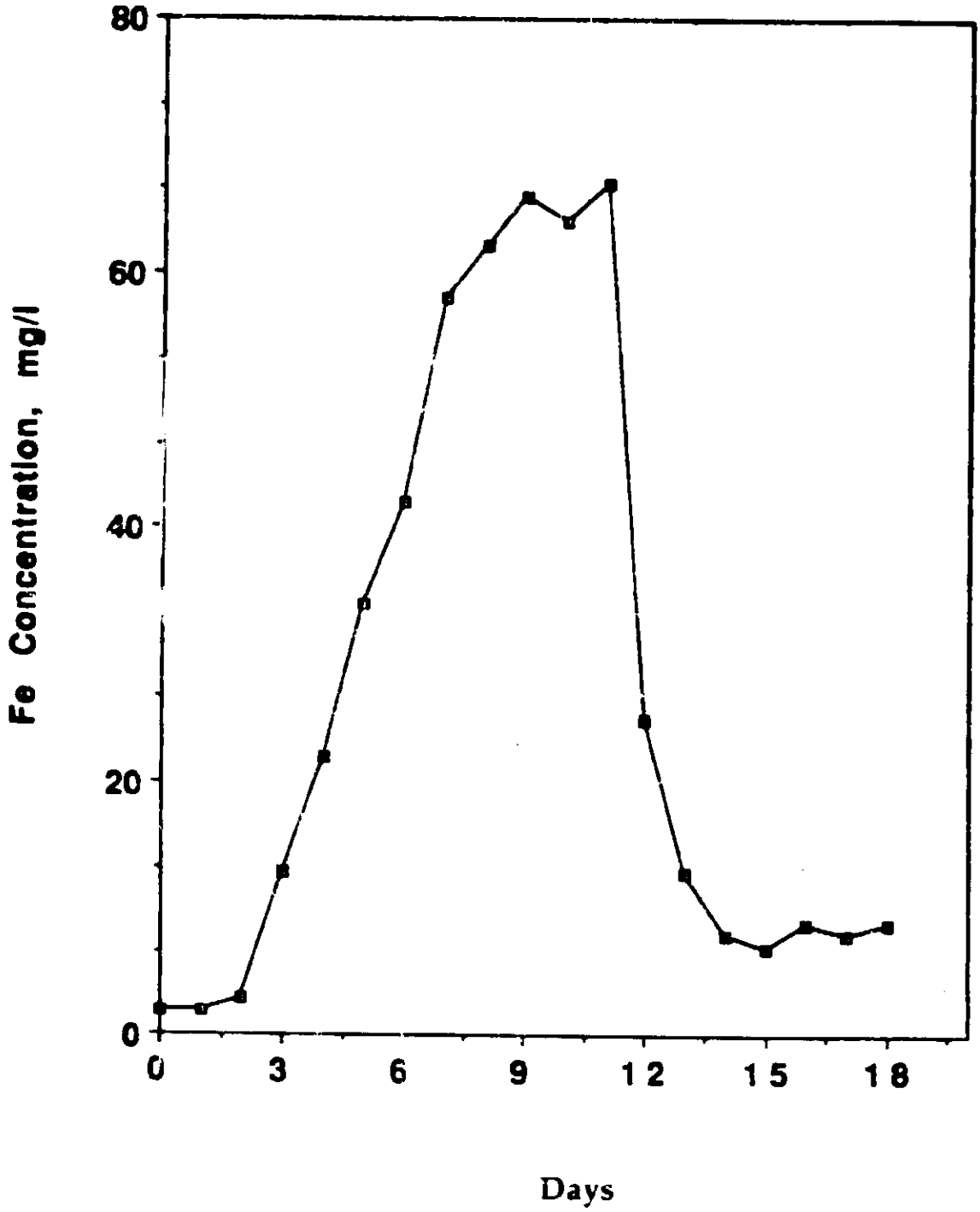
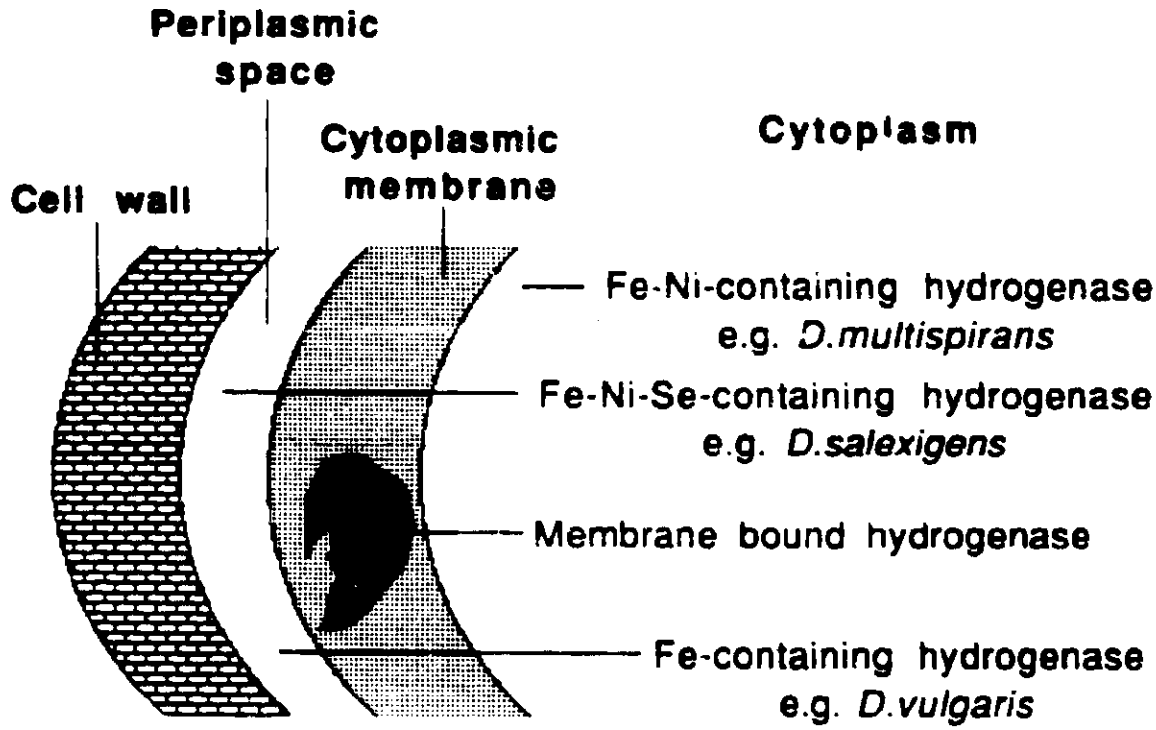


Fig. 7

A schematic representation of hydrogenase enzymes localization and different metal reaction centers present in *Desulfovibrio* species



Metal reaction centers: 1. Fe
(or prosthetic groups) 2. Fe-Ni
3. Fe-Ni-Se

Fig. 8

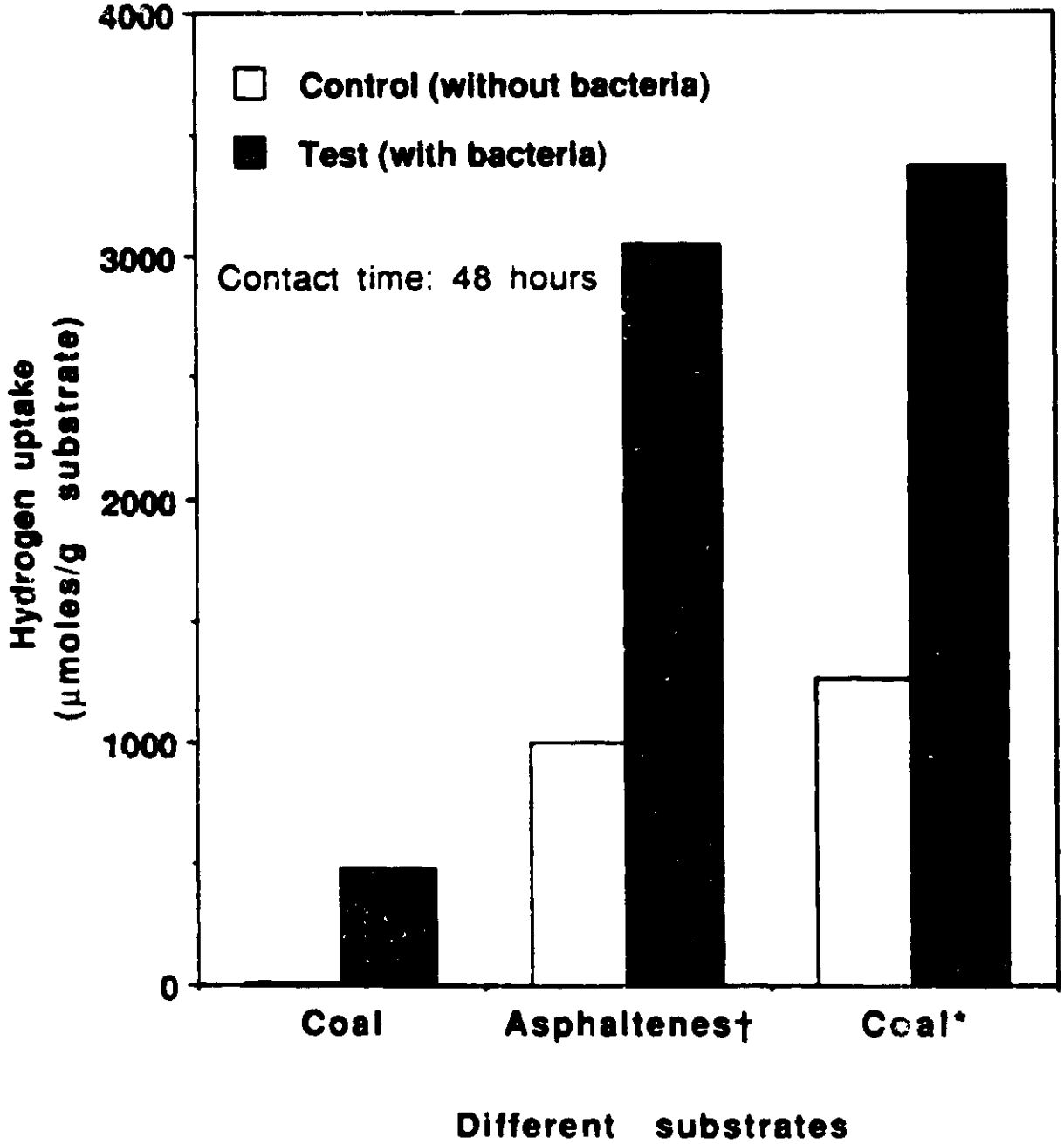
Reversible hydrogenase in *Desulfovibrio* species and *Clostridium thermoaceticum*

$$CH_3CHOHCOOH \xrightarrow[\text{dehydrogenase}]{\text{Lactate}} CH_3CO.COOH + CO_2 + 2e^- + 2H^+$$

$$CH_3CO.COOH \xrightarrow[\text{dehydrogenase}]{\text{Pyruvate}} CH_3COOH + 2e^- + 2H^+$$

$$4e^- + 4H^+ \underset{}{\overset{\text{Hydrogenase}}{\rightleftharpoons}} 2H_2$$

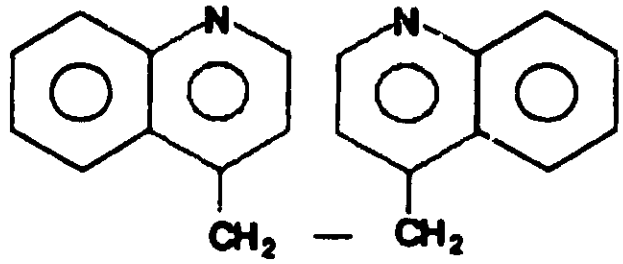
Fig. 9 Comparison of hydrogen uptake results for Coal (KCER # 91182), Pretreated coal and Asphaltenes using Desulfovibrio desulfuricans



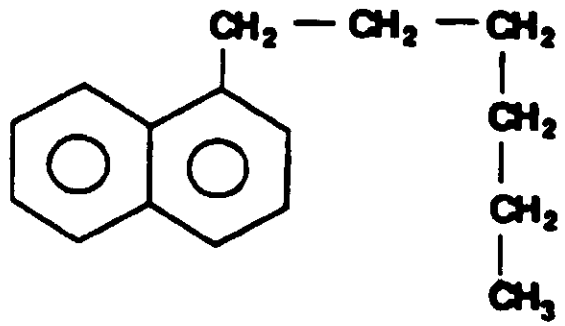
† - Asphaltenes obtained from Coal (KCER # 71637)
* - Thermal treatment (200°C, 800 p.s.i H2, 1 hr.)

Fig.10 Structures of different model compounds used for biohydrogenation

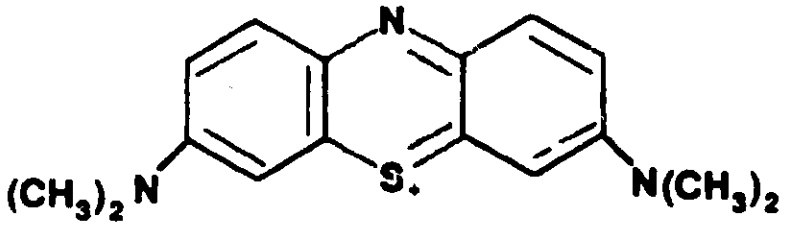
1,2-diquinolyl ethane



α - naphthyl hexane



Methylene Blue



Diphenyl Methane

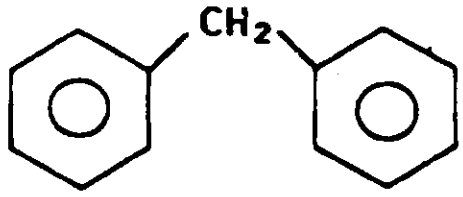


Fig. 11 Hydrogen uptake in presence of D. desulfuricans by 1,2-diquinolyl ethane subjected to autoclaving conditions

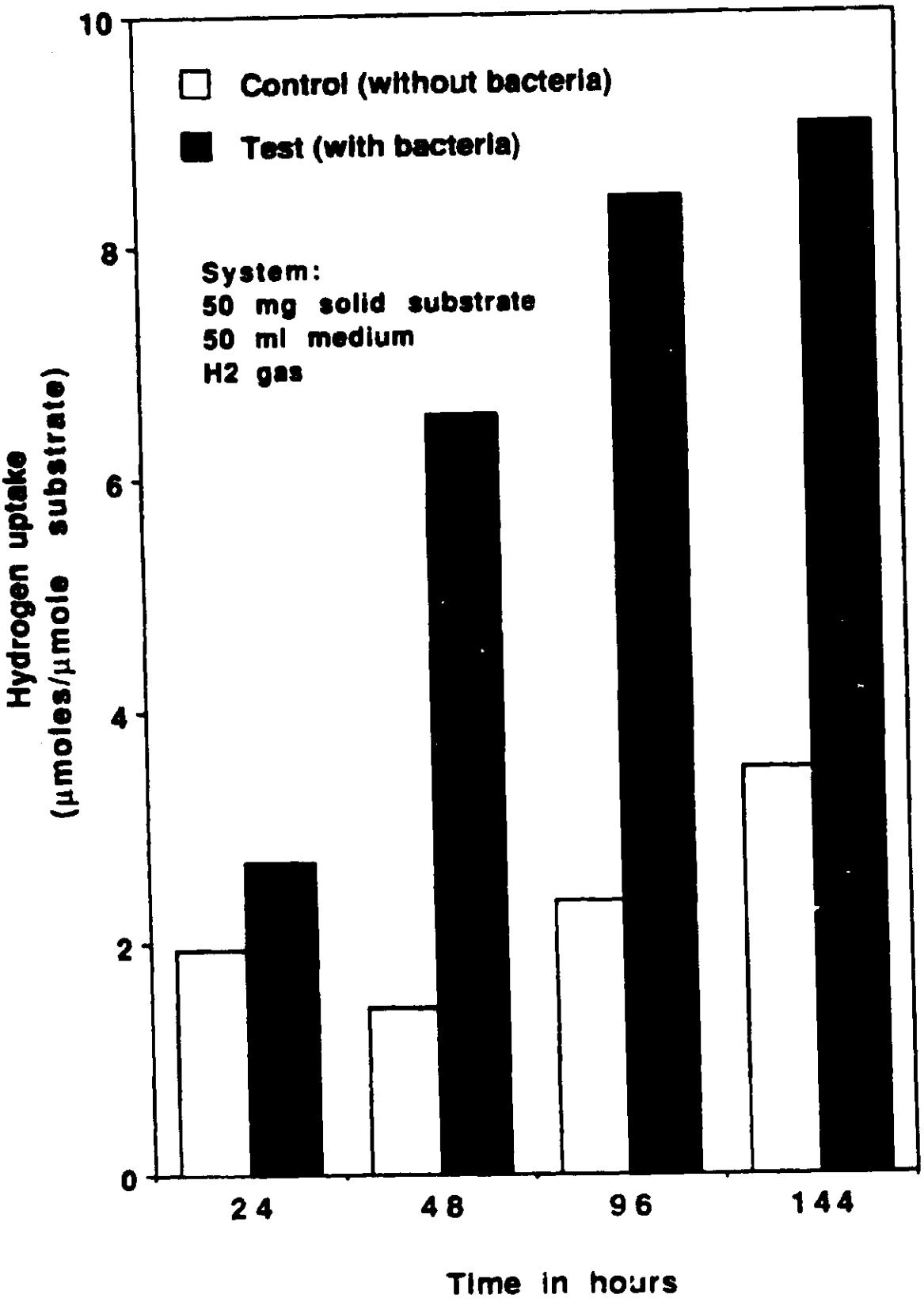


Fig. 12 Hydrogen uptake in presence of Desulfovibrio desulfuricans by α - naphthyl hexane

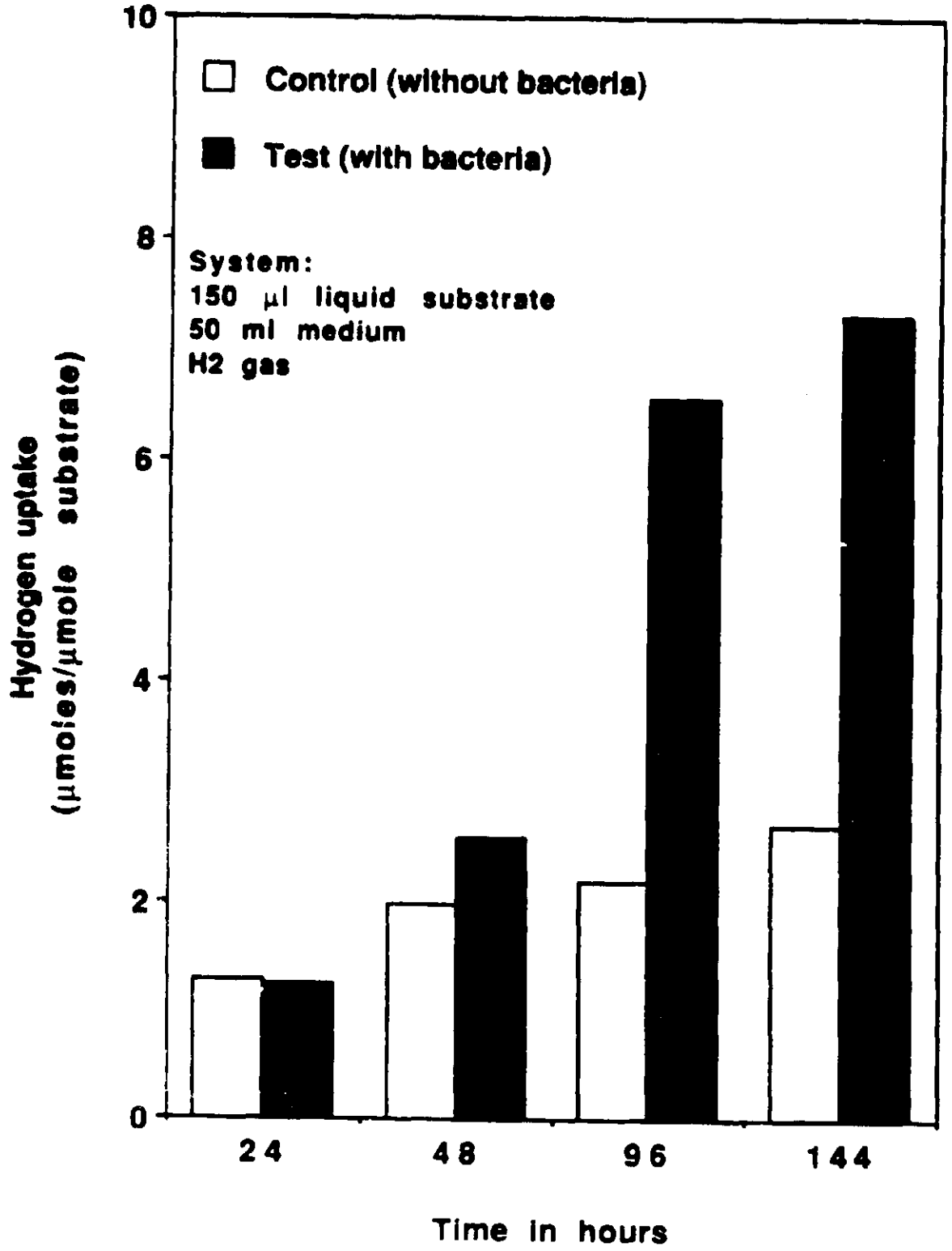


Fig. 13 Hydrogen uptake in presence of D. desulfuricans by 1,2-diquinolyl ethane dissolved in ethanol

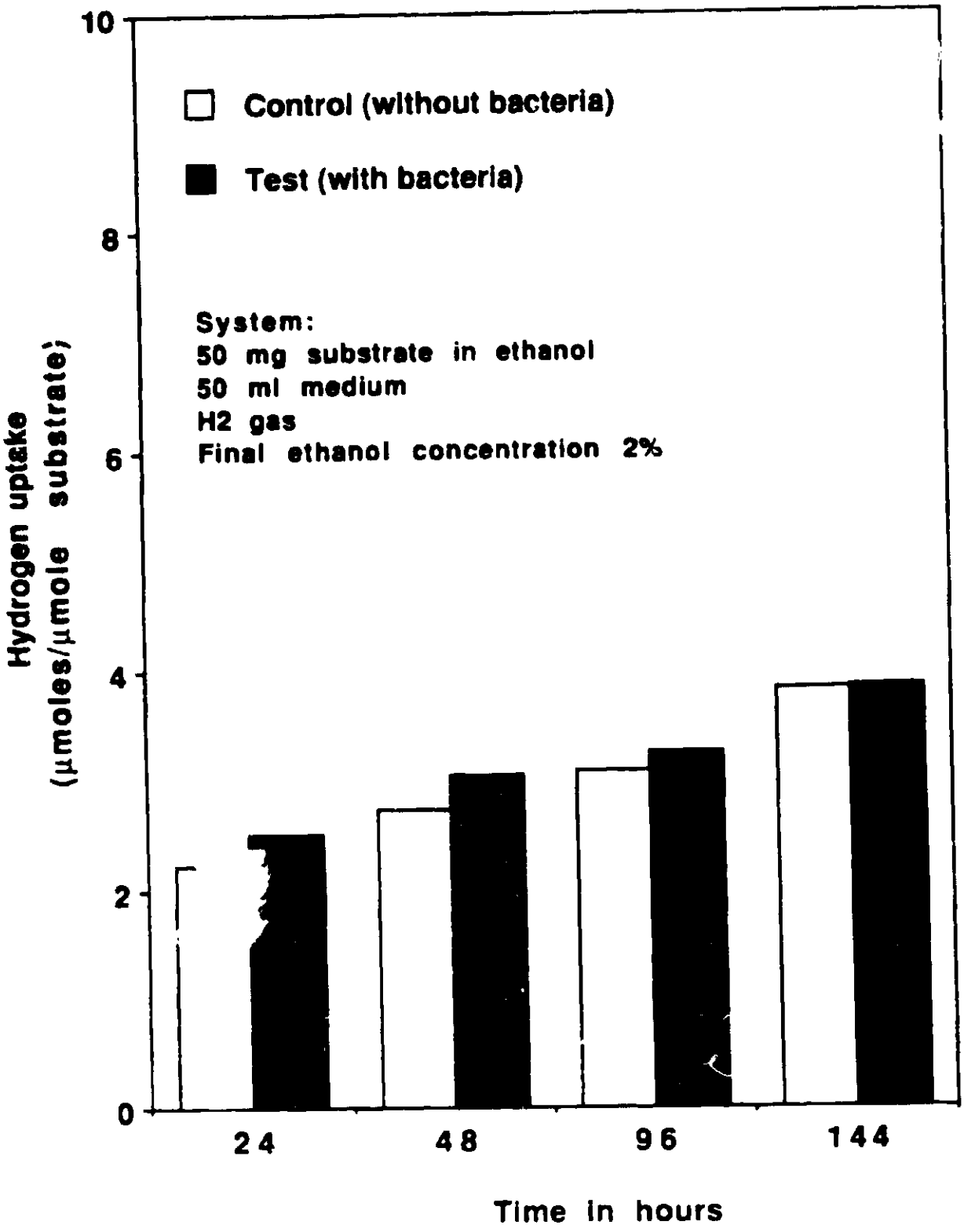


Fig. 14 Hydrogen uptake by differnet coal-related model compounds in presence of Desulfovibrio desulfuricans at the end of 144 hours

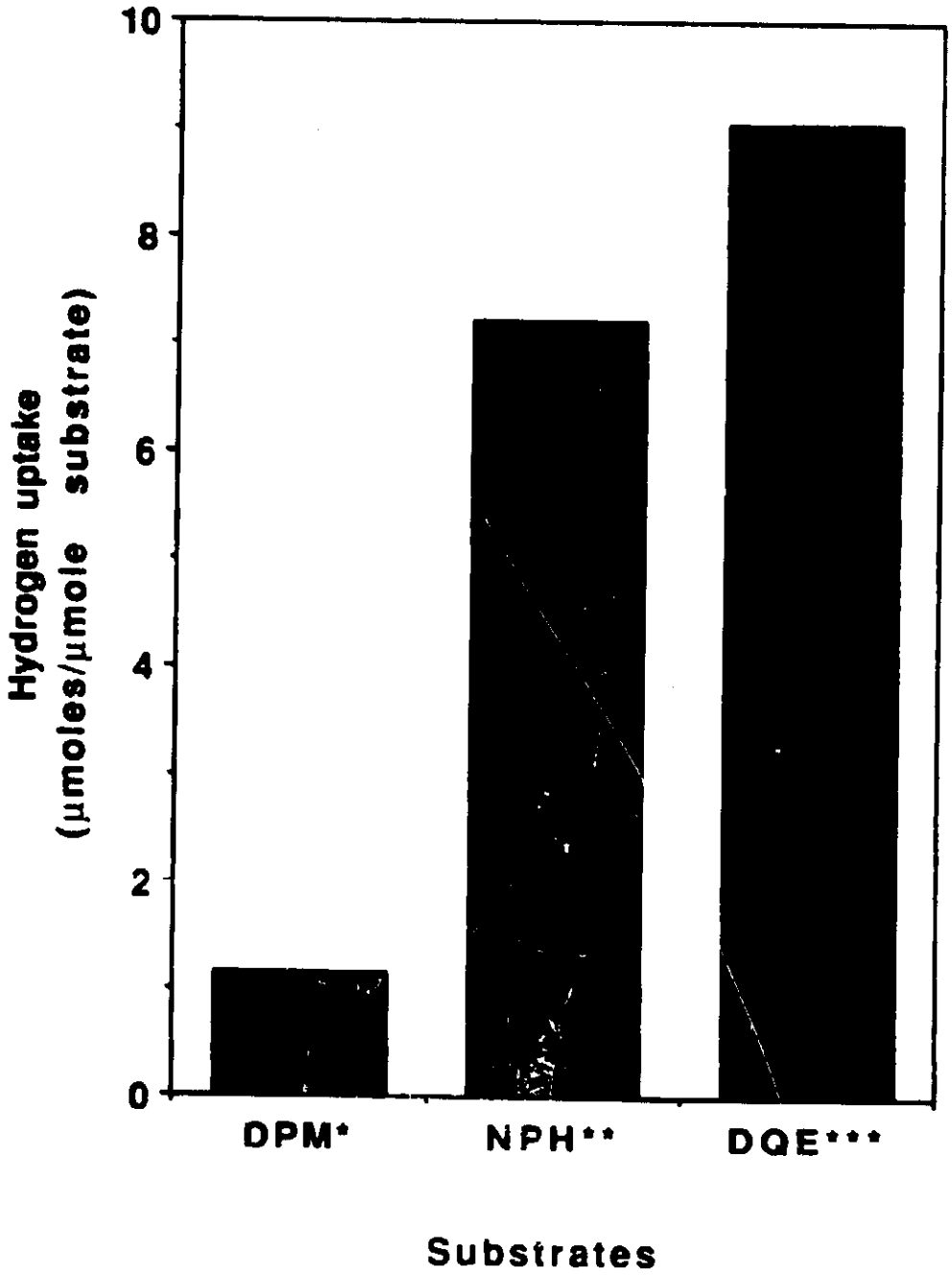
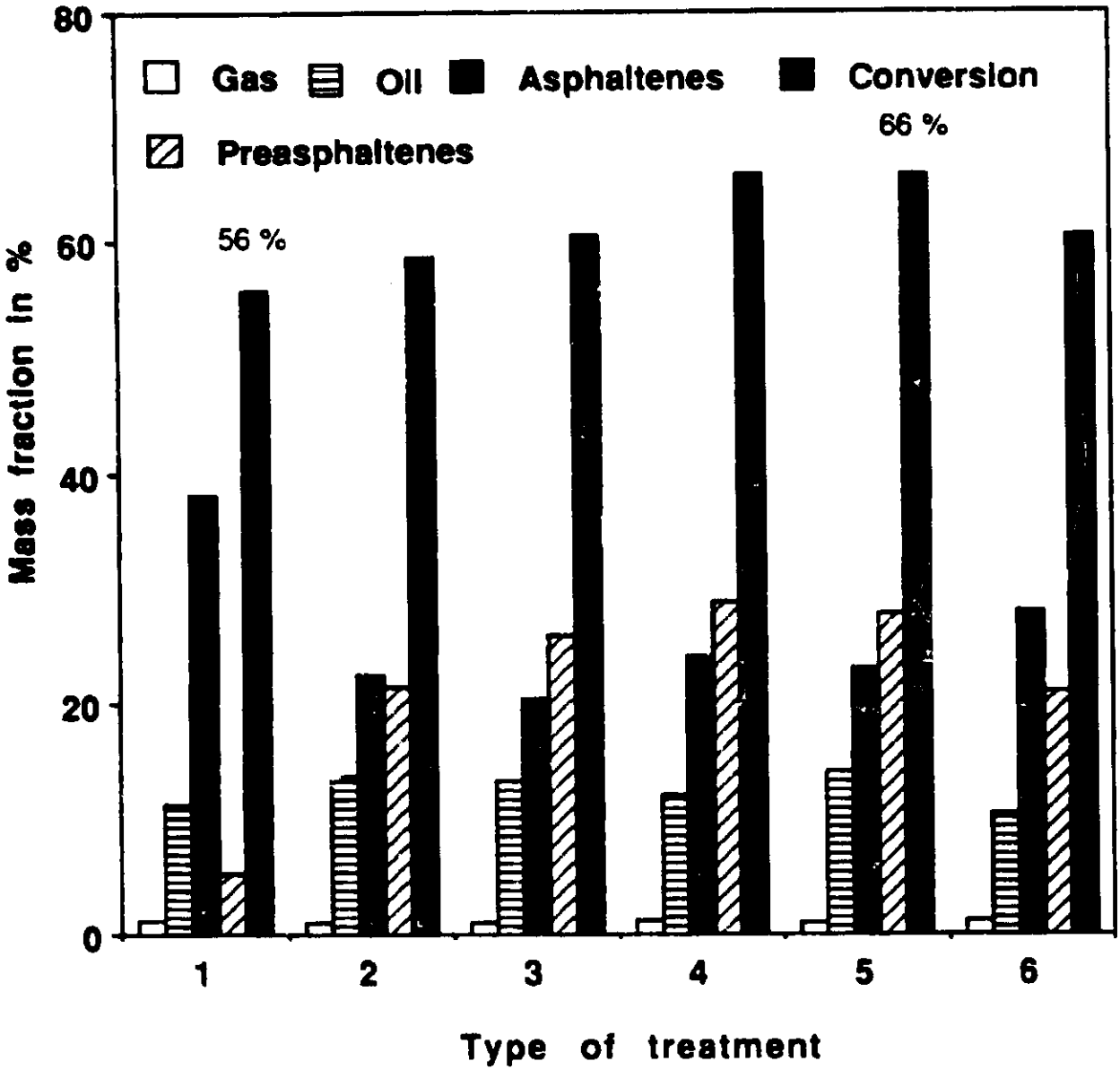


Fig. 15 Chemical liquefaction results of biotreated Coal (KCER # 91182) in presence of varying amounts of benzene



1 - No benzene no bacteria
2 - 50/50 (%), benzene/medium and no bacteria
3 - No benzene but with bacteria
4 - 20/80 (%), benzene/medium with bacteria
5 - 50/50 (%), benzene/medium with bacteria
6 - 80/20 (%), benzene/medium with bacteria