

NUMERICAL COMPARISONS BETWEEN THE PYROLYSIS MASS SPECTRA OF TWELVE U.S. COALS
AND THEIR RELATIVE SOLUBILITY IN MICROBIAL CULTURES OR ALKALINE BUFFER

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ABSTRACT

Curie-point pyrolysis mass spectra of twelve low-rank U.S. coals with various degrees of natural weathering were correlated with the results of biosolubility screening tests involving six selected microorganisms as well as with a specially designed alkaline solubility test. The main objective of this study was to determine which mass spectral characteristics, if any, correlate with the degree of biosolubility. The results indicate the possible presence of two biosolubilization trends, a main trend which correlates positively with the presence of severely oxidized aromatic moieties in the coal and apparently enables biosolubilization by all six microorganisms, and a second trend which correlates with relatively high concentrations of acid components and enables biosolubilization by only two or three of the organisms. The chemical and biological significance of these trends is not yet completely understood. Finally, in agreement with previous reports a strong positive correlation was observed between biosolubility and alkaline solubility.

INTRODUCTION

Few areas of coal science are more challenging and intimidating to analytical chemists than the study of coal bioliquefaction phenomena. In recent years, powerful chromatographic and spectroscopic techniques have begun to shed more light on the structural chemistry of coal. However, the added complexity of bioconversion phenomena puts coal bioliquefaction processes well out of the reach of most analytical methods.

A further complicating factor is the lack of standardization with regard to suitable coal samples, microorganisms and experimental conditions. Although several coal sample and data banks are in operation within the USA, none of these banks currently provides the type of relatively strongly weathered, low rank coals which have been used in nearly all coal bioliquefaction experiments reported to date. Moreover, many of the various strains of microorganisms used are not available for general distribution and/or incompletely characterized. Finally, there is no general agreement as to what constitutes "bioliquefaction" or how to measure it objectively.

In order to help solve some of these problems Idaho National Engineering Laboratory has assembled a multidisciplinary task group including researchers at several different universities. The present communication represents one of several reports detailing the results of studies carried out during the past year and primarily aimed at obtaining an improved definition and understanding of coal bioliquefaction phenomena.

A strong correlation between the bioliquefaction yields and alkaline solubilities of a series of low rank U.S. coals was reported by Quigley *et al.* [1,2]. Together with the observation that the more "successful" microorganisms tend to produce substantial alkalinity of the culture medium, this suggested that simple acid-base solution processes might play a significant role, especially in strongly acidic, e.g., highly weathered coals. Consequently, one of the key questions in any future coal bioliquefaction studies should be whether proof of covalent bond scission can be obtained or not.

In a separate paper, Ward *et al.* [3], describe a novel bioliquefaction screening and quantitation method which uses objectively measurable product diffusion zones around standard-sized coal sample "disks" imbedded in solid media. After inoculation with selected microorganisms, characteristic "bioliquefaction profiles" can then be obtained.

Here we report the results of a study of 12 low rank coal samples (representing several different U.S. provinces as well as degrees of natural weathering), each of which was submitted to the new "bioliquefaction profiling" procedure using six different strains of microorganisms. Suitable aliquots of each coal sample were further analyzed by means of Curie-point pyrolysis mass spectrometry, using techniques described by Meuzelaar *et al.* [4]. Also, alkaline solubilities were determined according to Quigley *et al.* [2].

Finally, biosolubility profiles, pyrolysis MS profiles and alkaline solubility data were compared by means of canonical correlation analysis, a powerful multivariate statistical analysis approach capable of determining common sources of variance between related data sets.

The primary objective of this study was to search for key structural features of coals (as reflected in the pyrolysis MS profiles), directly associated with bioliquefaction response (as reflected in the "bioliquefaction profiles"). A secondary objective was to obtain information on reaction mechanisms, e.g., with regard to the role of acid-base solubilization *vs.* covalent bond scission processes.

EXPERIMENTAL

Sample Preparation - Suspensions of each of the 12 coals (5 mg of -200 mesh coal per ml of spectrograde methanol) were prepared. A 5 μ l drop of each suspension (25 μ g of coal) was applied to a 610°C Curie-point wire. The methanol was evaporated under continuous rotation of the wire.

Pyrolysis Mass Spectrometry - Py-MS was performed with the MS inlet at 250°C and using low voltage (12 eV) electron ionization. For each Py-MS analysis, the total number of scans was 100 and the total scan time was 23 seconds. Each sample was analyzed in triplicate.

Data Analysis - The Py-MS data were first normalized to 100% total ion intensity. For the normalization of the total ion intensities, mass peak signals with high variance were temporarily excluded. 159 mass peaks with high "characteristicity" (outer variance/inner variance ratio) values were used for factor analysis. Furthermore, these Py-MS data were correlated with biosolubility data and alkaline solubility data by canonical correlation techniques.

Biosolubility - A set of six selected fungal strains, e.g., ACL-12, DML-12, P. Chrysosporium, RWL-40, YML-1 and YML-21, representing a diversity of taxonomic types was used for all biosolubility assays. The general methods used for standardized assays (i.e., the "diffusion zone assay") of coal biosolubility were described elsewhere [3]. In brief, 2-3 mm diameter mounds of sized coals (0.25-0.50 mm) were placed with uniform spacing on the surface of Sabouraud Dextrose 1.5% agar ("SDA" Difco Laboratories, U.S.A.). Medium depth was 5.5 mm in 100 mm diameter pyrex-glass; deep-well culture dishes. Two replicate preparations of five coal units were used for each coal type. Spores or hyphal fragments of each organism were inoculated onto the surface of the nutrient medium after which the cultures were incubated in the dark at 30°C, 70 ± 2% RH for 17 days. Circular zone diameters were measured and averaged to give values for comparisons of degree of biosolubility for each coal.

Alkaline solubility - One half gram of sized coal (0.25 - 0.50 mm) was placed in a 250 ml Erlenmeyer flask containing 50 cc of a pH 8, tris buffer. Flasks were shaken (140 rpm) for 24 hours at 25°C. Supernatants were obtained by filtering through 0.2 micron filters. Where necessary, dilutions were made using fresh TRIS buffer and absorbances at 400 nm measured.

RESULTS AND DISCUSSIONS

The wide range of different coal types included in this study is illustrated in Figure 1. At first sight the low voltage pyrolysis mass spectrum of North Dakota Hagel lignite appears to be fairly characteristic of Northern Great Plains lignites, i.e., dominated by homologous series of dihydroxybenzenes and phenols representative of fossil lignins [5]. However the relatively high peak intensities at m/z 45, 46, 60, 87 point to the presence of carboxylic moieties characteristic of oxidative changes [6]. Since the Hagel lignite was originally obtained from the Penn State Coal bank (see Table 1), these oxidative changes are probably due to long term exposure to ambient air in the laboratory. By comparison, the Mississippi Claiborne lignite (see Figure 1b) shows a much more pronounced homologous series of aliphatic hydrocarbon components. This is again in agreement with earlier Py-MS studies of lignites representing the Gulf Province [5] in which higher relative abundances of aliphatic hydrocarbons were found to correlate with aquatic depositional environments. In that study, longer chain aliphatic hydrocarbon moieties in Gulf Province coals were thought to represent liptinitic macerals primarily derived from algal materials. Finally, the spectrum in Figure 1c, obtained from an Arkansas Lower Hartshorne "bituminous" coal, appears to be totally different. Little or no aromatic compound series are seen whereas aliphatic series are not very distinct either. In fact, this spectrum is almost totally dominated by the (off scale) intensities of small mass peaks in the m/z 28-45 range. In our opinion this coal sample, collected from an old roadcut in highway 7 (see Table 1), has undergone severe chemical deterioration and should perhaps be considered as a humic acid type material rather than a coal. The wide range of structural chemical differences between just three of the twelve coals in our study illustrates the magnitude of the analytical problem.

In order to obtain a more systematic overview of the various chemical components and trends in our data set we performed a factor analysis of all 36 coal spectra (each sample was analyzed in triplicate). A description of the variance described by the first 11 factors with eigenvalue >1.0 is given in Table II whereas a score plot of factors II and III (Figure 2a) was found to display some of the most interesting chemical information. A more complete description of

the information in Factors I and IV will be provided in the final publication of the results. Chemical interpretation of the clustering trends in Figure 2a is facilitated by inspection of the so-called variance diagram plot [7] in Figure 2b which reveals the presence of at least 4 major chemical components. These components can be shown in mass spectral form using a "factor spectrum" technique described by Windig *et al.* [8]. Inspection of the factor spectra in Figure 3a-d shows two vitrinite-like patterns (component axes B and C in Figure 2b), an aliphatic hydrocarbon pattern (component axis E) and a sulfur compound pattern (component axis F). Comparison with component spectra found in earlier Py-MS studies [5,9] of U.S. lignites and coal maceral fractions, respectively, indicates that, whereas components B and C may be regarded as primarily of terrestrial origin, components E and F can be thought to represent a stronger aquatic influence on the ancient depositional environments involved.

In other words, Factors II and III appear to be primarily correlated with differences in depositional environment. Not unexpectedly, Factor I was found to be dominated by differences in the overall degree of oxidation and/or weathering (factor spectrum of component A not shown here because of space limitation). In contrast to the apparent differences in "reported rank" shown in Table I, no obvious rank dependent influence was observed other than the presence of two distinct, possibly rank dependent, vitrinite-like patterns in components B and C. However, it should be pointed out that the highest rank coal (Lower Hartshorne "bituminous") was severely degraded and that the difference in Py-MS patterns between "lignites" and "subbituminous" coals tend to be relatively minor [10].

After examining the major chemical components and trends in the Py-MS patterns of all twelve coals we undertook a thorough analysis of the "biosolubility" data obtained by systematic studies with a panel of six microorganisms (listed in Table III) using a novel technique developed by one of us (B. Ward) and reported in more detail elsewhere [3]. The biosolubility data shown in Figure 4, can be regarded as a multidimensional biological response surface obtained by measuring the diameter of dark diffusion zones of soluble coal components surrounding small disk-shaped coal pellets imbedded in solid culture media inoculated with selected microorganisms under carefully standardized conditions.

Factor analysis of the biosolubility data shown in Figure 4 reveals that the true dimensionality of the biological response surface is close to 2 with the first two factors explaining more than 97% of the cumulative variance (see Table IV). In other words, no more than two independent sources of variance can be present in the biosolubility data. Both trends are clearly identified in Figures 5a and b. All microorganisms appear to be more or less successful in solubilizing coals 3 and 9 (or, less completely, 8 and 10), whereas only organisms c and e (and to some extent b) succeed in solubilizing coals 2 and 11 (or, less completely, 4 and 12).

This can also be seen in Figure 4 where the two response patterns have been arranged to show the differences in the upper and lower rows, respectively. No significant biosolubilization response was observed for coals 1, 5, 6 and 7. It should be noted here that the two distinct biosolubilization patterns were only observed upon factor analysis of the microbiological data but were not immediately apparent in the foregoing examination of the Py-MS data.

This prompted us to resort to the powerful canonical correlation approach in order to determine which, if any, Py-MS signals correlated with the

biosolubilization trends. Moreover, in view of earlier observations by one of us of a strong correlation between biosolubilization and alkaline solubility under abiotic conditions, we decided to include the alkaline solubility values for all twelve coals in the biosolubilization data set.

Two significant canonical correlation functions were found with correlation coefficients 0.99 and 0.92, respectively. Together these two functions explained 25% of the total variance in the Py-MS data set and 92.4% of the total variance in the combined biosolubilization/alkaline solubility data. Moreover, in agreement with the earlier reports by Quigley *et al.* [1,2], alkaline solubility was found to correlate strongly (correl. coeff. = 0.93) with biosolubilization. Inspection of the scores and loadings of the two canonical correlation functions, as plotted in Figures 6a and b, reveals the same two trends already noticed in Figures 5a and 5b. This brings up the key question: what is the chemical and biological meaning, if any, of these two trends? In order to help answer this question we calculated the two factor spectra correlating with these trends (a further impression of the most important mass peak contributions can be obtained from the combined loadings plots in Figure 6b). Figures 7a and b show the two mass spectral patterns associated with good biosolubilization (and alkaline solubility) by all microorganisms (Figure 7a, 310° component axis) and with good biosolubilization by microorganisms c and e (Figure 7b, 40° component axis), respectively.

At present, we are unable to provide an unambiguous chemical interpretation of these two trends. However, spectrum 7b is dominated primarily by fatty acid and carboxylic moieties, such as commonly observed in coals weathered in a laboratory environment [6]. Spectrum 7a, on the contrary, exhibits a variety of additional aromatic or polyunsaturated aliphatic signals suggestive of oxidative products of aromatic compounds. One hypothetical explanation could be that Figure 7a represents severe oxidative destruction by natural weathering phenomena enabling a broad range of microorganisms to start solubilizing the coal matrix, e.g., by producing alkaline compounds, as suggested by Quigley *et al.* [1,2], whereas Figure 7b points to a more extreme accumulation of carboxylic acids, and thus a strongly acidic pH, possibly allowing only some of the microorganisms to produce sufficient alkaline compounds to solubilize the coal.

In line with this interpretation, the alkaline solubility vector in Figure 6b can be seen to be intermediate between the two trends. In other words, both conditions produce an equal degree of solubilization in the presence of an unlimited amount of alkaline buffer. If this explanation is correct, the lower acid concentration in Figure 7a could have been caused by secondary natural phenomena, such as the loss of water soluble acidic constituents through leaching, rather than by a different oxidative mechanisms.

Presently, we are investigating possible other explanations, e.g., involving metal chelating agents or the possible involvement of enzyme-mediated bond breaking mechanisms.

CONCLUSIONS

Multivariate analysis of pyrolysis mass spectra of twelve samples of low rank coals exhibiting different degrees of biosolubility revealed a high level of heterogeneity, apparently associated with differences in rank, depositional environment (e.g., as reflected in maceral composition) and degree of weathering.

Multivariate analysis of "biosolubility profiles", obtained by measuring the relative biosolubility response of twelve coal samples to a panel of six different microorganisms, strongly suggests the presence of at least two biosolubility mechanisms. One of these mechanisms is exhibited by all 6 microorganisms whereas a second mechanism appears to be associated with only 3 of the microorganisms.

Canonical correlation analysis of the pyrolysis mass spectrometry and biosolubility profile data sets produced two canonical variate functions with correl. coeff. 0.99 and 0.92, respectively. Together these functions explain approx. 25% of the total variance in the Py-MS data and 92.4% of the biosolubility data.

Coal samples exhibiting good biosolubility appear to have in common a pronounced oxidative degradation of aromatic structural components, thought to represent vitrinitic and related maceral constituents. Besides increased biosolubility, the most obvious effects of these oxidative changes are: (1) an apparent increase in low MW, oxygen containing pyrolysis products; (2) a notable increase in alkaline solubility; and (3) a markedly reduced abundance of characteristic vitrinitic signals in the pyrolysis mass spectrum.

The observed correlations between the biosolubility and pyrolysis MS profiles further suggest that oxidation of a coal sample (evidenced by strongly increased pyrolysis yields of CO₂ and CO) does not produce biosolubility for all six microorganisms. An additional structural change, characterized by the increased evolution of pyrolytic benzene (presumably derived from benzenecarboxylic acids) may be needed to induce biosolubility for all six microorganisms and thus achieve maximum alkaline solubility levels.

A strong linear correlation (corr. coeff. 0.91) was observed between alkaline solubility (as determined by absorbance of the solution at 400 nm) and the two-dimensional canonical variate space obtained by canonical correlation analysis of the pyrolysis MS and biosolubility profiles. This is in agreement with previous reports by Quigley et al. [1,2].

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Table I
Origin, Rank and Alkaline Solubility of 12 Coals

Sample #	seam or field (state)	reported rank	description of sampling site	alkaline ^a solubility
1	Coalmont (CO)	subbit	fresh stripmine sample (Kerr Mining CO)	-2.70
2	Coalmont (CO)	subbit	weathered chunks from abandoned mine (near site 1)	-1.09
3	Coalmont (CO)	subbit	erosion gully outcrop in pasture (near site 1)	1.45
4	Kiowa (CO)	lignite	dry gulch outcrop	-1.19
5	Green River (CO)	subbit	weathered chunks from abandoned strip mine (Black Dan, near Hayden)	-2.70
6	Hagel (ND)	lignite	fresh sample (PSOC 1482)	-1.22
7	Clairborne (MS)	lignite	creek bed outcrop near Antioch	-0.78
8	Clairborne (MS)	lignite	ibid, more weathered	-0.36
9	Wilcox (MS)	lignite	I-20 roadcut near Russell	0.91
10	Midway (AL)	lignite	I-20 roadcut between York and Cuba	0.32
11	Lower Hartshorne (AK)	bituminous	Hwy 7 roadcut of excavation near Dardanelle	-0.70
12	Brandon (VT)	lignite	fresh sample stored in H ₂ O since October, 1984	-0.35

a. Alkaline solubilities (log (absorbance)) were measured at 400 nm

Table II
Factor Analysis Results of Py-MS Data

FACTOR #	EIGENVALUE	%TOTAL VARIANCE	CUMULATIVE VARIANCE
1	88.85	57.86	57.86
2	22.60	14.71	72.57
3	14.99	9.76	82.58
4	8.06	5.25	87.58
5	6.42	4.18	91.77
6	2.85	1.86	93.62
7	2.39	1.56	95.18
8	1.84	1.19	96.38
9	1.64	1.07	97.44
10	1.28	.83	98.28
11	1.06	.69	98.96

Table III
List of Microorganisms and Description of Six Fungal Strains

Bacteria #	Code	Full Name
a	ACL-13	<i>Candida</i> sp.
b	DML-12	<i>Acremonium</i> sp.
c	<u>p. chrysosporium</u>	<u>phanerochaeta chrysosporium</u>
d	RML-40	an unidentified Basidiomycete
e	YML-1	<i>Cunninghamella</i> sp.
f	YML-21	a <i>Cunninghamella</i> - like Hyphomycete

Table IV
Factor Analysis Results of Biosolubility Data

FACTOR	EIGENVALUE	% TOTAL VARIANCE	CUMMULATIVE VARIANCE
1	5.19	86.43	86.43
2	.64	10.59	97.03
3	.14	2.34	99.37
4	.03	.49	99.86
5	.01	.12	99.99
6	.00	.01	100.00

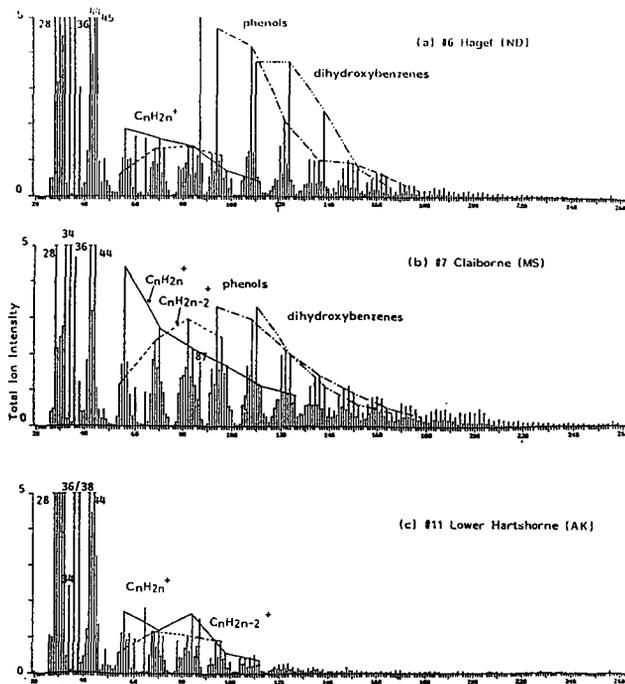


Figure 1. Pyrolysis mass spectra of (a) North Dakota lignite (b) Mississippi Claiborne lignite (c) Arkansas "bituminous" coal.

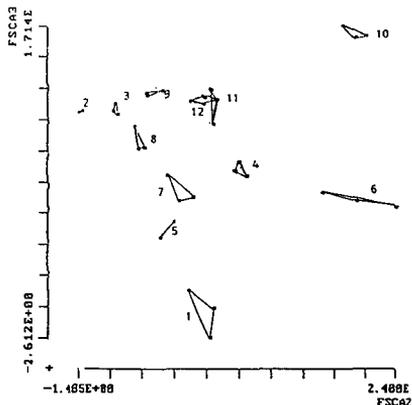


Figure 2a. Score plot of factor 2 vs. factor 3 of MS data.

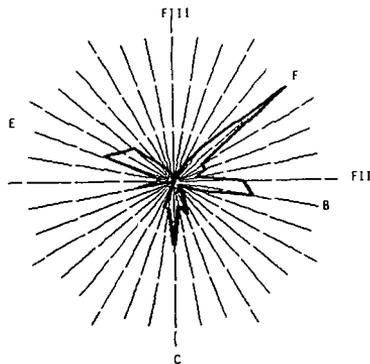


Figure 2b. Variance diagram showing the presence of four major component axes B, C, E, F, in the space spanned by factors II and III.

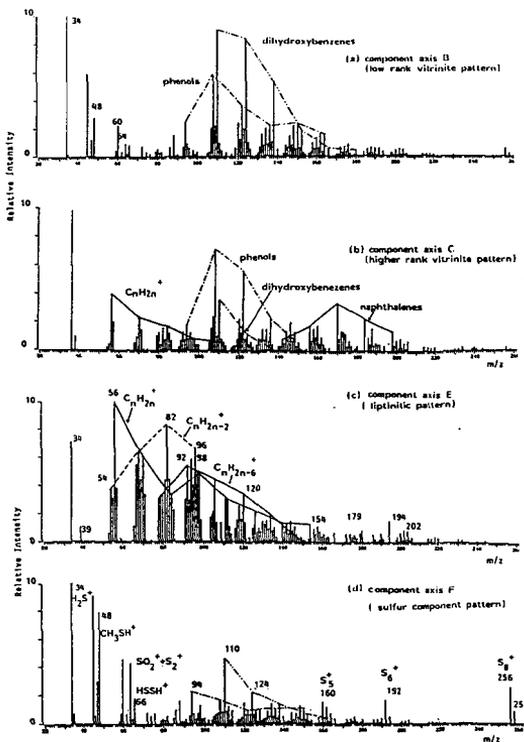


Figure 3. Comparison of numerically extracted factor spectra of (a) component B (low rank vitrinite pattern), (b) component C (higher rank vitrinite pattern) (c) component E (liptinitic pattern) and (d) component F (sulfur component pattern).

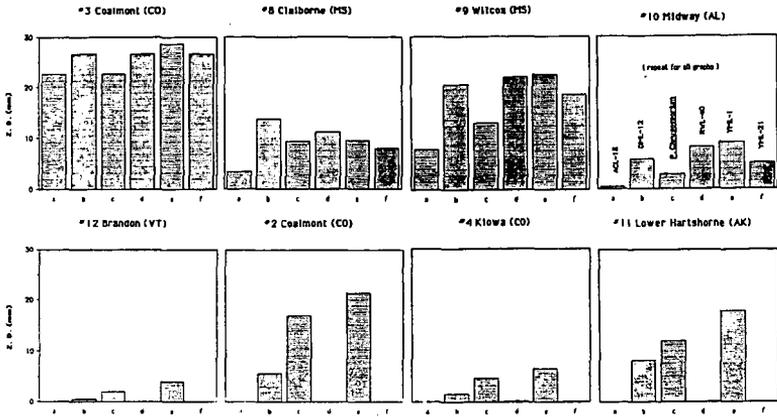


Figure 4. Biosolubility of 8 coals as a function of six microorganisms, expressed by zone diameter (mm). None of the microorganisms shows biosolubility on coals nos. 1, 5, 6 or 7.

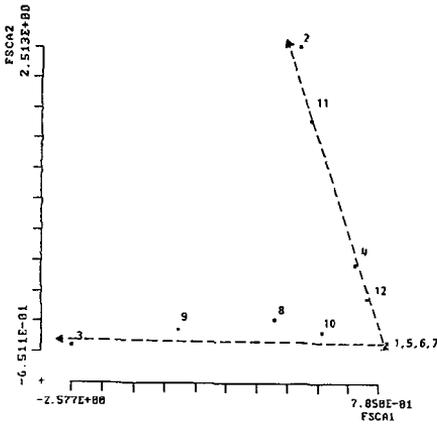


Figure 5a. Score plot of factor I vs. factor II of biosolubility data.

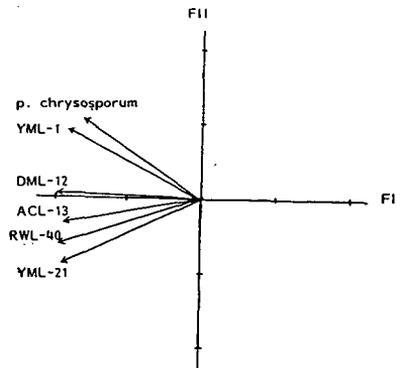


Figure 5b. Variance diagram of factor I vs. factor II of biosolubility data.

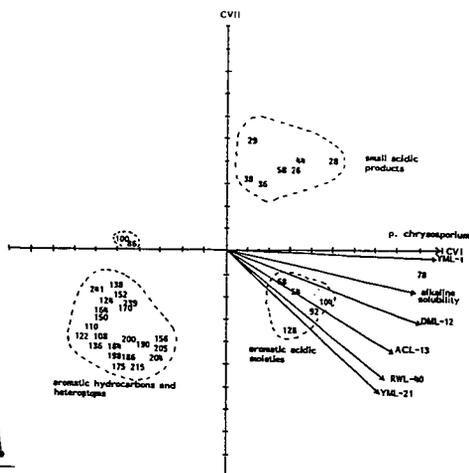
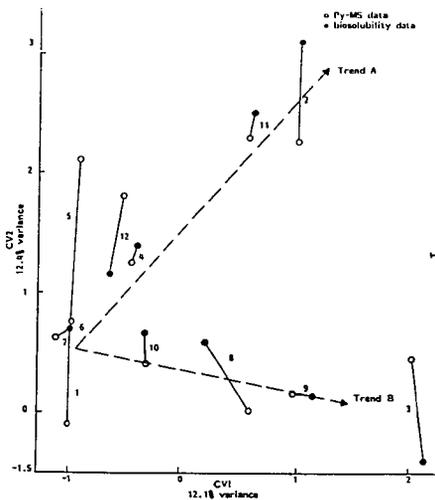


Figure 6a. Combined score plot of the first two canonical variate function of the Py-MS data and the biosolubility data, respectively.

Figure 6b. Loading plot of the mass values and biosolubility parameters contributing most strongly to the first two canonical variate functions.

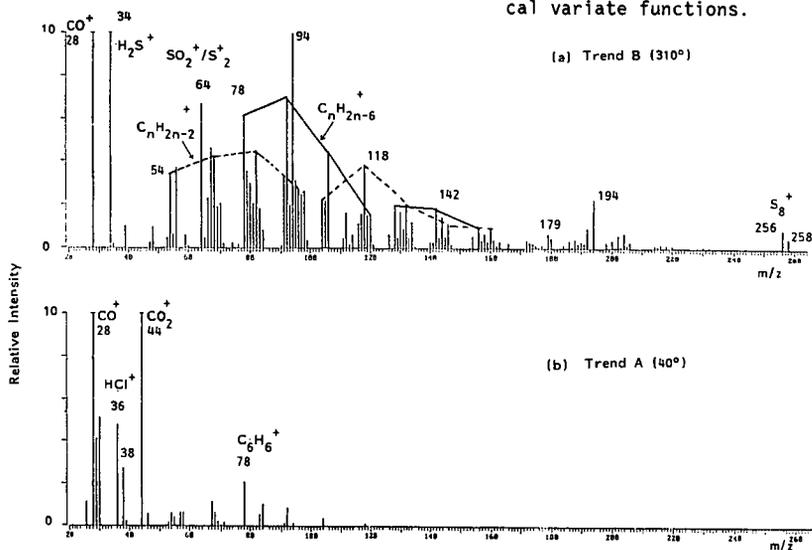


Figure 7. Numerically extracted mass spectra of trends B and A at 310° and 40° in CVI/CVII space, respectively.