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Studying species composition of microbial communities with the use of gas chromatography-mass spectrometry: microbial community of kaolin

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Abstract

The composition of microbial communities has been investigated avoiding conventional cultural techniques by chromatography-mass spectrometric analysis of chemical signature markers. Concentrations of fatty acids, hydroxy acids, aldehydes, sterols, and methanolysate of the biomass lipid fractions were used to determine the population size of the individual community members. The calculation is based on the information stored in a data bank about the chemical composition of the probable members of the community. An algorithm for rapid assessment of the genus or species composition from the total biomass GC-MS data is developed for a quantitative analysis of this community, treating them as a combination of chemical profiles of individual members. The microbial community in kaolin slurry was analyzed; it includes the following genera (in 10^5 cells/g): *Nitrobacter* (4), *Bacillus* (0.9), *Pseudomonas* (0.3), *Burkholderia* (0.1), *Nocardia* (0.4), *Caulobacter* (3), *Deinococcus* (4), *Arthrobacter* (0.7), *Clostridium* (4), *Bacteroides* (0.08), *Desulfovibrio* (1), *Desulfobacter* (0.3). Analyses of the profiles of unassigned components revealed the presence of two as yet unknown organisms, one being an iron-reducing bacterium designated strain FeRed, the other a microscopic fungus.

Keywords: Microbial community; Species composition; Gas chromatography-mass spectrometry; Kaolin

Contents

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1. Introduction

Classical microbiological methods are based on culturing the microorganisms in a sample on selective media and determining the cell number in each taxonomic group by the serial dilution method. Such methods are useful to isolate microbial strains but have serious limitations when the composition of microbial communities is to be determined. These assays take weeks and their results cannot be used to monitor processes going on within the microbial community. Furthermore, only a very small number of microorganisms in environmental samples (0.1–10%) can be accounted for by enumeration of colony-forming units (CFU) [1].

Current chemical methods find increased use to distinguish microorganisms in applied research, they are by far more rapid [2,3]. The two chiefly used methods are gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), which, when combined, provide extensive information on monomeric chemical components of microbial cells and metabolites [4]. Specific cellular chemical components (or markers) are detected among other chemical constituents of the total biomass, indicating the presence of the corresponding microbial genus or species in the community under study [5–7]. Different applications of the biomarker method were surveyed by Morgan [8].

The marker method was a starting point for the research of the group of D.C. White who later turned to the analysis of microbial community composition: "As certain fatty acids are specific to certain bacteria, it has been the practice to determine microbial community structure by the use of fatty acid markers" ([9], reviews in [10,11]). Here we present an extension of White's approach: in addition to markers full lipid profiles of microorganisms are included to calculate the community composition at the genus and, in some cases, even the species level [12–16].

Chemodifferentiation is a widely used method of determining and verifying the taxonomic position of microorganisms. It is usually applied for identification of microorganisms isolated in pure cultures and based on large databases containing the fatty acid composition of a few thousand strains of bacteria and microscopic fungi, e.g. the Microbial Identification System (MIS), marketed by MIDI, Delaware [17]. In our approach, we use available as well as new methods of identification and enumeration of microorganisms in native samples. These methods do not require preceding growing the organisms or isolating colonies on selective media. Local databases were developed for different ecological niches to store our own and published data on the chemical composition of microorganisms inhabiting this particular niche. This eliminates the ambiguity of species identification inherent in the use of general data banks since the fatty acid compositions of some microorganisms from different ecosystems are very close.

We herewith develop a theory, methodology and computer implementation of calculations for the rapid determination of the community structure yielding population numbers of each species. It is based on the use of high resolution, specific and selective GC-MS analysis. The approach is illustrated by describing the structure of the microbial community inhabiting kaolin.

2. Methodology and mathematical treatment for the quantitative determination of the species composition of microbial communities from GC-MS data

Chemodiagnostics combines a number of techniques for detection, identification and enumeration of microorganisms on the basis of their chemical features, independent of their physiological characteristics and type of ecological niche.

A wide range of cellular components (lipids, sugars, proteins and volatile metabolites) form the basis of chemodifferentiation. These components uniquely identify a single species despite their diversity in nature. In practice, however, it is unrealistic to obtain a complete chemical composition of the given sample of microorganisms.

In order to distinguish microorganisms by their chemical properties it is necessary to define a sufficient set of chemical constituents of microorganisms by which the species can be identified.

A limited set of cellular chemical components (e.g. lipids, fatty acids, or sugars) seems to be inappropriate for the infinite diversity of microbial strains. In reality, however, and for each particular case, only a restricted number of microorganisms occurs under the given chemical and biological conditions of the environment; e.g. microbes from salt lakes, thermal vents, and oil wells are missing in the bodies of mammals.

Since microorganisms are differentiated by their trophic properties and habitat conditions, and only a limited number of taxonomic units are expected in each given case, all such units can be detected and identified by their chemical properties.

Correlation of profiles of chemical components of microorganisms can only be done on a computer and must incorporate pattern recognition methods. All other approaches from expert-based similarity judgements to compiling diagnostic matrices and using comparison prescriptions are inherently subjective and fail to produce a quantitative measure of the quality of fit to the standard.

The lipid composition of microorganisms contains ample components to form the basis for a comprehensive diagnostic system useful to the strain level; distinctions between strains may even extend beyond the species limit. Variations observed at this level, however, reflect the biological variability of species and result from the existence of a strain-species biological continuum at the molecular level. Cross-identifications at the species level with chemodiagnostics is thus not a shortcoming of the method (provided it was consistently implemented).

The difference between taxons on the basis of their lipid composition is sufficient for chemodifferentiation, but as a result of individual strain-dependent changes the distinctions between taxons overlap and we observe a continuous quantitative variation of chemical parameters of the cell, which, at some point, translates into a qualitative change, the change of taxon.

3. An algorithm for calculating microbial community composition

The following m linear equations with n unknowns are written for m biomass components and n bacterial genera (species), where i is the subscript for the lipid components of the total biomass and j of the microorganisms.

$$\begin{aligned}
A_1 \cdot q &= X_1 R_{1,1} + X_2 R_{1,2} + X_3 R_{1,3} + \dots\dots\dots + X_n R_{1,n} \\
A_2 \cdot q &= X_1 R_{2,1} + X_2 R_{2,2} + X_3 R_{2,3} + \dots\dots\dots + X_n R_{2,n} \\
A_3 \cdot q &= X_1 R_{3,1} + X_2 R_{3,2} + X_3 R_{3,3} + \dots\dots\dots + X_n R_{3,n} \\
&\vdots \\
A_m \cdot q &= X_1 R_{m,1} + X_2 R_{m,2} + X_3 R_{m,3} + \dots\dots\dots + X_n R_{m,n}
\end{aligned} \tag{1}$$

A_i (i = 1, 2,..., m) is the peak area of the i_{th} compound in the chromatogram of the total biomass, X_j (j = 1, 2,..., n) the number of cells of the j_{th} microorganism, q the scale coefficient depending on analytical conditions and instrumental sensitivity, and R_{ij} the content of the i_{th} compound in the fatty acid profile of the j_{th} microorganism. This set of equations has a single solution provided that m = n, and that the equations are independent.

The solution of Eq. 1 is given by

$$X_j = D_j / D \tag{2}$$

where D_j is the determinant of a partial matrix of coefficients for the j_{th} component and D is the determinant of Eq. 1. The number of equations in this set can be as large as the number of components of the cellular fatty acid pool determined by GC-MS. There is information on as many as 150 components in our data bank. At the same time, one single microorganism contains only 10–20 compounds from this list, hence most compounds from this set are not present in any individual microorganism. Thus, most of the coefficients R_{ij} are zero and the system is degenerate and can be reduced to subsystems of smaller rank (and fewer unknowns). Assuming the number of members in actual microbial communities between 30 and 40 and the number of fatty acids in the total biomass 70–80, we select 30–40 equations to obtain a rank of minimal subsystems. Finally, many microbial constituents act as biomarkers, i.e. are specific to only one taxon. Such microorganisms can be enumerated from the concentration of their marker, $X_i = A_i q / R_{ij}$. This makes it possible to eliminate the corresponding unknowns and to reduce the rank of the entire equation set.

q in Eq. 1 is calculated as follows: the number of microorganisms in 1 g of sample material is

$$X_j = X_{jsample}/m_{sample} \tag{3}$$

where $X_{j\ sample}$ is the number of microorganisms in the sample and m_{sample} is the weight of the sample. Then:

$$X_{jsample} = (V/v)(m_{jsample}/m_0) \tag{4}$$

where V is the volume of the silylated solution prepared for GC, v is the solution volume injected in the chromatograph, $m_{j\ sample}$ is the total mass of cells of the j_{th} microorganism in the sample, and m_0 is the mass of a single bacterial cell. The amount of fatty acid m_j in the sample is related to the number of microorganisms by

$$m_j = (QR_{ij}/100)m_{jsample} \tag{5}$$

where Q is the percent content of lipid monomers in the cell. The amount of acid is related to the peak area by

$$m_j = A_j/S \tag{6}$$

where S is the sensitivity coefficient of the instrument. Using Eqs. 5 and 6, we find

$$m_{jsample} = (10^2 m_j)/(QR_{ij}) = (10^2 A)/(SQR_{ij}). \tag{7}$$

Substituting Eqs. 7 and 4 into Eq. 3, we obtain

$$X_j = (A_i/R_{ij})(V/(vm_{sample}))(10^2/(Qm_0))/S.$$

It follows that

$$q = (V/(vm_{sample}))(10^2/(Qm_0))/S = 1/(q_1 q_2 S).$$

In this equation, $q_1 = m_{sample}\ v/V$ accounts for the part of the sample injected into the chromatograph, and $q_2 = Qm_0/10^2$ expresses the mass of fatty acid constituents of a single cell of a microorganism. If 1 g of bacteria contains 5.9×10^{12} cells and each cell contains on average 3% of lipid monomers (fatty acids and aldehydes), $q_2 = 3/(5.9 \times 10^{14}) = 5.08 \times 10^{-15}$ g. S can be found as A_{stand}/m_{stand}, where A_{stand} is the peak area of a standard used (a compound with a defined concentration) and m_{stand} is the amount of the standard used in instrument calibration.

The value of q in Eq. 1 is assumed to be equal for all compounds, inferring equal sensitivity of the GC-MS system for all compounds in the fatty acid fraction. This is reasonable, since the actual difference in the sensitivity of the system is essentially eliminated by the use of the coefficient R_{ij} from the data bank of the fatty acid profile of pure cultures obtained under similar conditions.

The second step involves the test of the mass balance of the lipid components. We took the difference between the calculated and actual concentrations of biomass compounds that were not included before when solving Eq. 1:

$$\Delta A_i = A_i - RX_j R_{ij}/q.$$

For marker compounds $\Delta A_i = 0$. For compounds present in most or several microorganisms ΔA_i will not necessarily vanish. Compounds with a positive balance, $\Delta A_i > 0$, indicate the presence of microorganisms unaccounted for in the data bank. A negative balance is a signal that not all microorganisms were correctly enumerated, due to errors introduced in sample preparation and analysis. Such errors amount to about 20%, larger errors may arise as a result of differences between the fatty acid profiles of microorganisms in the data bank and in the community. The third step of calculation is to formulate and solve the following equation set, which applies to compounds with a negative balance:

$$A_i = Rk_j X_j R_{ij}/q$$

where k_j is the correction factor compensating for the error in the calculation of the population density of microorganisms. Microorganisms with $k_j = 0$ are then dropped from the tentative list of community members.

4. Results of investigation of the bacterial community of kaolin

The investigation of the microbial community inhabiting kaolin from the Prosyanovskoe deposit is of interest in the context of modelling the transformation of minerals in alluvial kaolin deposits. The community was maintained by passages in a sterile kao-

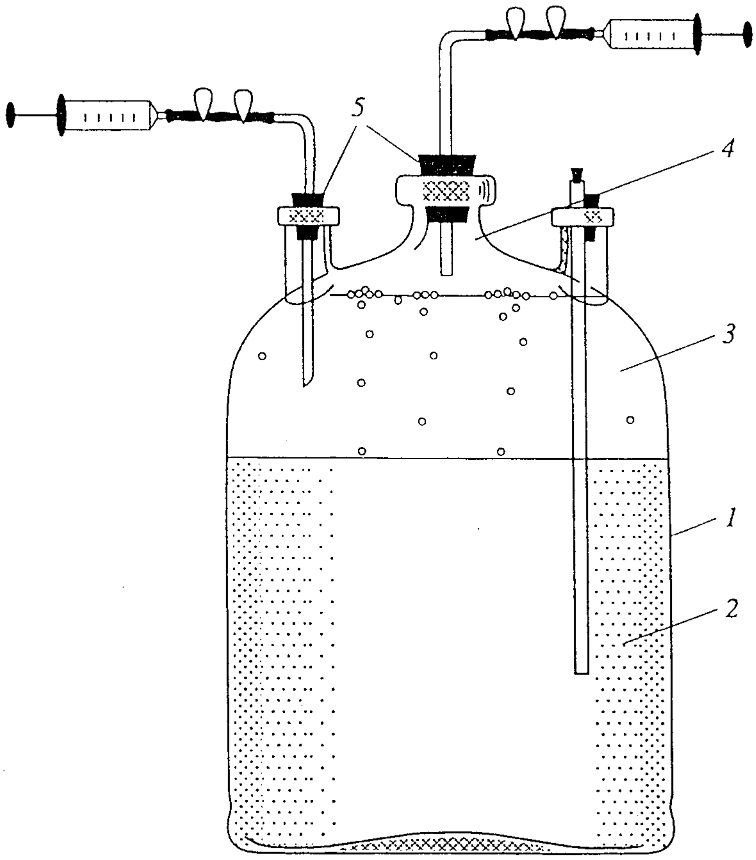


Fig. 1. Experimental vessel. (1) Glass bottle; (2) kaolin sediment; (3) supernatant; (4) gas phase; (5) rubber stoppers with tubes for sampling.

lin suspension (solid phase/liquid phase = 3:1) supplemented with 20 vol % of the nutrient medium composed of (g/l): $(NH_4)_2SO_4$ (0.2); $MgSO_4 \cdot 7H_2O$ (0.2); KH_2PO_4 (1); and glucose (5). The experiment was carried out at 30°C in a glass vessel (Fig. 1). The gas phase was not changed. The bottle necks were closed with rubber stoppers equipped with tubes for sampling solid, liquid, and gaseous phases. Samples of the sediment were taken for community structure analysis after incubation for 14 days.

4.1. *Community analysis by conventional microbiological and biochemical methods*

After 2 weeks, glucose in the medium was practically exhausted and metabolites accumulated were volatile fatty acids (butyrate, 0.2 g/l; acetate, 0.12 g/l; propionate, 0.03 g/l; lactate, 0.02 g/l, and formate, 0.01 g/l), hydrogen sulfide (6.9 mg/l), and soluble iron (0.5 mg/l) (Fig. 2). The number of aerobic heterotrophic microorganisms was estimated at 10^6 cells/g, anaerobic fermentative bacteria included 10^5 cells/g, sulfate-reducing bacteria 10^5 cells/g, and iron-reducing organisms 10^2 cells/g (Fig. 3). The main physiological groups of bacteria were enumerated in the sediment by the end-point 10-fold dilution method. The following media were used: 1:10 diluted nutrient broth, for aerobic microflora; Winogradsky agar [18], for fermentative bacteria; Postgate medium [19] supplemented with lactate, for sulfate-reducing bacteria; and Lovley medium [20], for iron-reducing bacteria.

Our data on the enumeration of microorganisms in the community indicate the presence of aerobic and facultatively anaerobic heterotrophic bacteria of the genera *Bacillus, Pseudomonas*, and *Arthro-*

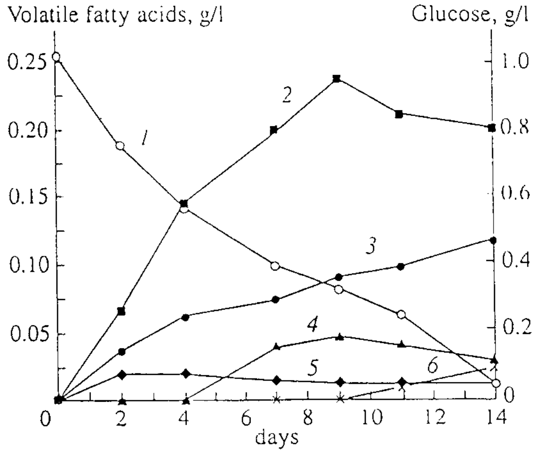


Fig. 2. Dynamics of the chemical composition of the kaolin suspension. (1) Glucose; (2) butyrate; (3) acetate; (4) lactate; (5) formate; (6) propionate.

bacter, as well as anaerobic bacteria belonging to clostridia and sulfate- and iron-reducing bacteria.

4.2. Fatty acid profile of the total community biomass

GC-MS analysis on a HP-5985B gas chromatograph-mass spectrometer (Hewlett Packard, USA) showed the presence of 56 compounds belonging to fatty acids, hydroxy acids, and aldehydes after methanolization of the lipid fraction of the total biomass (Table 1).

4.3. Creating a limited data bank of fatty acid profiles of bacterial cells

The analysis of the lipid composition of pure microbial cultures shows that, if a very large number of taxa were considered, most markers of individual genera or species lose absolute specificity. The restriction of the data bank to presumable members of a specific microbial community significantly reduces the probability of misidentification. Data banks listing microorganisms and their chemical compositions have previously been formed for the microbial communities of activated sludge, river sediment, methane tanks, and peat [13]. For the kaolin community, we started from the members revealed by conventional physiological and biochemical methods. This list was supplemented by the microorganisms whose markers were present among fatty and hydroxy acids, as well as aldehydes revealed in the total kaolin biomass (Table 1). Organisms unlikely to be present in the given ecological niche, thermophiles, halophiles, and representatives of specific human and animal microflora in particular, were excluded. As a result, the following putative members of the kaolin community were included in the limited data bank: *Pseudomonas putida*, *Ps. stutzeri*, *Burkholderia cepacia*, *Shewanella putrefaciens*, *Bacillus cereus*, *B. subtilis*, *Methylococcus capsulatus*, *Nocardia*, *Enterobacter*, *Arthrobacter globiformis*, *Caulobacter*, *Deinococcus*, *Bacteroides ruminicola*, *Desulfovibrio*, *Desulfobacter*, *Clostridium butyricum*, and *Nitrobacter*.

4.4. Calculating the kaolin community structure

After solving Eq. 1 in its general form and analyzing the balance equations, the calculation and its results were optimized. The value of ΔA_i was negative for several fatty acids. For these compounds, the equation system $A_i = \Delta k_j X_j R_{ij}/q$ was solved for k_j, the correction coefficient compensating for an error in the calculation of the population density of microorganisms. The microorganisms with $k_j = 0$ were deleted from the list: *Shewanella putrefaciens*, *Pseudomonas stutzeri*, *Bacillus cereus*, *Methylococcus capsulatus*, *Enterobacter*, and *Bacteroides ruminicola*. The correction coefficients obtained for the popula-

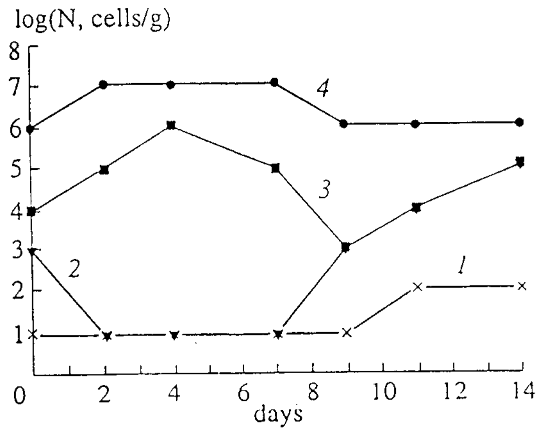


Fig. 3. Dynamics of microbial populations. (1) Iron-reducing, (2) sulfate-reducing, (3) fermentative, and (4) aerobic heterotrophic bacteria.

Table 1
Fatty acids, hydroxy fatty acids and fatty aldehydes in kaolin and assigned microorganisms

No.	Substance	Quantity (mol %)	Microorganism	No.	Substance	Quantity (mol %)	Microorganism
1	C12:0	2.85	Nonspecific	29	19:0	0.88	*Nitrobacter, Pseudomonas cepacia, Bacillus, Serratia*
2	iC13	0.06	*Bacillus subtilis*	30	20:0	1.22	*Actinomyces bovis*
3	a13	0.24	*Bacillus cereus*	31	24:0	1.49	*Francisella, Mycobacterium*
4	13:0	0.56	*Bacteroides, Bacillus, Nocardia*	32	i19	0.3	*Bacillus subtilis, Bacterotdes hypermegas*
5	i14	0.1	*Actinomyces, Bacillus, Bacteroides, Legionella, Kurthia*	33	a19	0.3	*Staphylococcus*
6	14:1w5	1.27	*Sphaerotilus, Clostridium*	34	10Me16	0.1	*Desulfobacter*
7	14:0	10.18	Most microorganisms, *Clostridium*	35	10Me18	0.05	*Mycobacterium, Nocardia, Corynebacterium, Actinomycetes*
8	i15:1	0.08	*Desulfovibrio, Flavobacterium*	Hydroxy acids			
9	i15	0.45	Most microorganisms	36	h10	0.01	*Pseudomonas, Thiobacillus, Leptothrix*
10	a15	0.56	Most microorganisms	37	h12	0.01	*Pseudomonas, Neisseria, Beggiatoa, Vibrio*
11	15:1	0.99	*Nitrobacter, Deinococcus, Microeoccus*	38	hi13	0.01	*Pseudomonas maltophila*
12	15:0	5.45	Most microorganisms	39	h13	0.01	*E. coli, Bacteroides hypermegas, Selenomonas*
13	16:1w9	0.37	*Methanobacteriaceae, Deinococcus, Desulfotomaculum acetoxidans*	40	h14	0.03	*Enterobacteriaceae, Fusobacterium, Haemophylus, Wolinella, Vibrio*
14	16:1w7	9.42	Most microorganisms	41	hi15	0.01	*Flavobacterium, Capnocytophaga, Bacteroides melaninogenicus*
15	16:1w5	0.37	*Bdellovibrio, Vibrio, Fusobacterium, Bacteroides amylophilus*	42	ha15	0.01	*Bacteroides ruminicola*
16	i16:0	0.26	*Actinomyces, Nocardiopsis, Bacteroides, Micromonospora, Brevibacterium, Corynebacterium, Curtobacterium, Oerscovia, Cellulomonas*	43	h16	0.07	*Erwinia, Brucella, Pseudomonas cepacia, Ps. pseudomallei, Bacteroides, Wolinella, Cytophaga, Flexibacter*, methanogens, *Campylobacter fetus, C. sputorum, C. fecalis, Fusobacterium, Bordetella*
17	16:0	27.24	*Desulfovibrio, Flavobacterium*	44	hi17	0.02	*Bacteroides, Flavobacterium, Cytophaga, Flexibacter*
18	i17:1	0.83	*Nocardiopsis, Clostridium, Pseudomonas putrifaciens, Methanobacteriaceae*	45	ha17	0.02	*Bacteroides ruminicola*
19	17:1	0.41	*Methanobacteriaceae, Nocardiopsis, Clostridium, Pseudomonas putrifaciens*	46	h17	0.01	*Bacteroides*
20	i17:0	0.46	*Butyrivibrio, Bacillus, Prevotella, Propionibacterium*	47	10h18	0.06	*Clostridium*
21	a17:0	0.77	*Corynebacterium, Bacteroides, Nocardiopsis, Actinomyces, Nocardia, Micromonospora*	48	2h12	0.02	*Pseudomonas putida*
22	17cyc	0.22	*Enterobacteriaceae, Pseudomonas, Desulfobacter*	49	2hi15	0.02	*Flavobacterium, Flexibacter*
23	17:0	2.04	Most microorganisms	Fatty aldehydes			
24	18:2	2.79	Fungi, yeasts, protozoa	50	14a	0.68	*Butyrivibrio, Spirochaeta, Clostridium fimetarum, Bifidobacterium*
25	18:1w9	15.25	Most microorganisms	51	i15a	0.05	*Butyrivibrio, Lactobacillus, Propionibacterium*

Table 1 (*continued*)

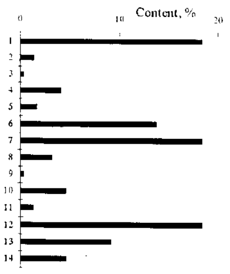
No.	Substance	Quantity (mol %)	Microorganism	No.	Substance	Quantity (mol %)	Microorganism
26	18:1w7	3.91	*Nitrobacter, Bdellovibrio, Penicillum, Succinivibrio, Fusobacterium, Enterobacteriaceae, Pseudomonas,* sulfate-reducing bacteria	52	16a	2.55	*Clostridium beijerinckii, C. fallax, Lachnospira, Butyrivibrio, Lactobacillus*
27	18:0	0.76	Most microorganisms	53	18:1a	0.32	*Lachnospira, Butyrivibrio, Bifidobacterium, Selenomonas, Clostridium butyricum*
28	19cyc	0.04	*Lactobacillus, Enterobacteriaceae, Pseudomonas, Brucella, Campylobacter*	54	18a	4.06	*Clostridium*

tion number of *Arthrobacter globiformis*, *Bacillus subtilis*, *Deinococcus*, and *Desulfovibrio vulgaris* were introduced into the balance equations. With the contributions of all likely members of the community, the residual profile of unassigned components of the total biomass calculated from the concentration balance reflected the presence of inexplicable microorganisms and components of the background. Quantitative determination of bacteria of major physiological groups by conventional microbiological methods showed that the kaolin sediment contained 10^2 cells/g of iron-reducing bacteria. However, no patterns of iron-reducing bacteria of known fatty acid profiles (*Shewanella putrefaciens* [21] and *Geobacter metallireducens* [22]) were identified. From an enrichment culture in the Lovley medium [20] we isolated a strain of an iron-reducing bacterium FeRed. This strain formed magnetite from amorphous iron hydroxide. The fatty acid composition of FeRed was identified in the residual profile of the total biomass by the presence of oxy acids h11 and h13 and unsaturated acids 15:1 and 17:1, which are not common among bacteria. Strain FeRed was added to the final list of the kaolin community members. Furthermore, in the residual fatty acid profile, linolenic acid was determined, which is specific to fungal lipid profiles. Since no known microorganism could be correlated with the set of components remaining after the scaled fungal lipid profile had been subtracted, the final residual components were attributed to a nonspecific biological background.

Thus, the analysis based on the existence of specific patterns in the lipid composition of the total biomass made it possible to determine the generic and in some cases even the species composition of the community (Table 2), which matched the results of conventional physiological and biochemical determinations.

Table 2
Genus and species composition of kaolin microbial community as measured by GC-MS whole cell lipid components analysis

N	Microorganism	Number of cells/g	Content, %
1	*Nitrobacter sp.*	$4*10^5$	18,4
2	*Pseudomonas putida*	$3*10^4$	1,4
3	*Burkholderia cepacia*	$1*10^4$	0,4
4	*Bacillus subtilis*	$9*10^4$	4,1
5	*Nocardia sp.*	$4*10^4$	1,7
6	*Caulobacter sp.*	$3*10^5$	13,8
7	*Deinococcus sp.*	$4*10^5$	18,4
8	*Arthrobacter globiformis*	$7*10^4$	3,2
9	*Bacteroides ruminicola*	$8*10^3$	0,4
10	*Desulfovibrio sp.*	$1*10^5$	4,6
11	*Desulfobacter sp.*	$3*10^4$	1,4
12	*Clostridium butyricum*	$4*10^5$	18,4
13	Strain FeRed	$2*10^5$	9,2
14	Fungi	$1*10^5$	4,6
	Total		100



5. Conclusions

A theory of rapid analysis of the generic (species) composition of communities of microorganisms was developed using the chemical composition of the total biomass (no pure cultures have to be isolated) and relying on biomarkers and analysis of profiles of fatty acids, hydroxy acids, and aldehydes obtained by gas chromatography-mass spectrometry. A complete determination of the community composition from three samples of the community total biomass takes only 2 days. Any member microorganism can be monitored. The method is patent pending.

The essential advantage of the GC and GC-MS diagnostics is that isolation of viable cells from the sample and their cultivation on selective media is not required. The species composition of a microbial community is determined by the analysis of the chemical composition of the total biomass (lipids, polysaccharides, and metabolites of different chemical origin). This method can be equally applied for the characterization of microorganisms belonging to different taxonomic, physiological and trophic groups, e.g. bacteria, fungi, protozoa or anaerobes.

The method is only possible with the availability of a large data bank on the chemical composition of microorganisms and their metabolites. This data bank is subdivided into local banks corresponding to various natural ecological niches. By this way cross-identification is reduced to a minimum. Biomarkers allow the detection of microorganisms in biological fluids or in natural samples against high environmental background.

The calculation of the bacterial community composition can be limited to the use of markers for detection of certain microorganisms, like monitoring specific microorganisms active in the given ecological niche or during a bioengineering process, or for detection of a single pathogen or several important organisms in body fluids of humans. In more complex situations, such as control of disbiosis in humans with a large group of microorganisms, the profile and marker methods are to be used in combination and, in addition, the biological fluid background is to be accounted for.

Instrumentally, gas chromatography and gas chromatography-mass spectrometry are two highly sensitive methods which need as little as 10 ng (10^{-8} g) of bacteria-derived components against the background of a biological matrix for biomass determination, equivalent to population densities of 10^4 cells/ml.

In summary, with phospholipid fatty acid (PLFA) biomarkers [9–11] viable and nonviable parts of biomass are quantified from the ratio of phospholipids and glycerides in the native sample; the nutritional status of the community is determined from the ratio of PLFA and polyhydroxyalkanoates; the stress status of the community is estimated from the *trans/cis* PLFA ratio; and the presence of specific groups of microorganisms is established on the basis of particular chemical marker compounds. With the method described in this paper the species composition of the microbial community is determined from the overall lipid components profile; minor microbial community components are determined from specific markers by GC-MS single ion monitoring; and determination of microbial associations in environmental samples, biotechnology, and medicine are possible quantitatively in real-time.

The two methods complement each other perfectly and, when used in parallel, constitute a powerful tool for studies of microbial populations.

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