The use of fatty acid methyl esters as biomarkers to determine aerobic, facultatively aerobic and anaerobic communities in wastewater treatment systems

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Abstract

The use of fatty acid methyl esters (FAME) as biomarkers to identify groups of microorganisms was studied. A database was constructed using previously published results that identify FAME biomarkers for aerobic, anaerobic and facultatively aerobic bacteria. FAME profiles obtained from pure cultures were utilized to confirm the predicted presence of biomarkers. Principal component analysis demonstrated that the FAME profiles can be used to determine the incidence of these bacterial groups. The presence of aerobic, anaerobic and facultatively aerobic bacteria in the communities, in four bioreactors being used to treat different wastewaters, was investigated by applying FAME biomarkers.

Introduction

The complex microbial communities present in biological wastewater treatment systems are composed of aerobic, anaerobic and facultatively aerobic bacteria. Most of the species found in such communities are nonculturable with laboratory-based culture techniques (Roszak & Colwell, 1987), hence the development of culture-independent techniques to monitor bacterial changes under particular conditions are required for improved planning of the operation of wastewater treatment plants. An effective and quantitative way to measure the microbial biomass *in situ* is to measure cellular components of the microorganisms. Lipid analysis can give some insights into the composition of microbial communities. Whereas most organisms cannot be individually identified from a lipid profile, some classes of microorganisms have distinctive fatty acids, which can serve to positively confirm their presence. The lipid profile has also been associated with the state of the cell, and can be indicative of stress or alternatively as an indicator of growth. The analysis of fatty acid methyl esters (FAME) profiles provides insight into important changes in microbial communities in wastewater treatment plants, in such a way as to avoid the microbial culture bias. FAME analysis may be used to identify isolated and pure microbial cultures (Sasser, 1997), in taxonomic studies (Yamamoto *et al.*, 1998) or for the identification of bacteria of medical importance (McNabb *et al.*, 1997).

In environmental sciences, fatty acids evaluation has allowed several authors to estimate the microbial community structure and metabolic activity in polluted soils (Zelles, 1999; Kozdrój & van Elsas, 2001; Pinkart *et al.*, 2002); to identify contamination of surface waters with polluted soil adjacent to agricultural production fields and to wooded riparian zones (Banowetz *et al.*, 2006); to quantify, characterize and compare sessile and planktonic microbial populations in wastewater treatment systems (Werker & Hall, 1998), to evaluate the adaptation of microorganisms to a particular condition, such as acid environments (Quivey *et al.*, 2000), to establish the microbial community distribution in terms of structure (Sundh *et al.*, 1997; Kozdrój & van Elsas, 2001), and to determine

the relative changes in abundance of microorganisms, such as bacteria and fungi (Zeller *et al.*, 2001). FAME analysis can be used to assess the physiological status of the microbial community. Many subsets of the microbial community respond to specific conditions in their microenvironment with shifts in lipid composition. Specific patterns of FAME can also indicate physiological stress in certain bacterial species (Pinkart *et al.*, 2002). FAME analysis has also been used to evaluate changes in microorganisms due to their exposure to toxic substances. For example, it has been shown that when the concentration of phenol is increased, *Pseudomonas putida* P8 increments the proportion of its *trans*-unsaturated fatty acids (Pinkart *et al.*, 2002).

FAME can be used to quantify and test the environmental effects on different microbial communities. In this case, Bossio & Scow (1998) concluded that fatty acid profiles were sensitive indicators of changes occurring in the structure of soil microbial communities due to agricultural management. In other cases, information obtained from lipid analysis provides insight into the community composition as well. It has been proposed that specific groups of microorganisms contain characteristic fatty acid profiles that can be used as biomarkers (Pinkart *et al.*, 2002). Mummey *et al.* (2002) applied FAME biomarkers to monitor the recovery of ecosystems following surface mine reclamation. In this study it was found that the ratio of FAME bacterial to fungal biomarkers reflected changes in other indicators of soil health suggesting that this ratio is a useful indicator of reclamation progress. Cha *et al.* (1999) identified signature fatty acids of *Nocardia amarae* (19 : 1ω8, 16 : 1ω6c, i15 : 0 2OH) and used their relative abundance to demonstrate their potential to quantitatively monitor the abundance of *Nocardia* in mixed liquor samples of activated sludge. The use of fatty acid patterns has also been applied to full-scale biological wastewater treatment plants to assess activated sludge microbial communities, demonstrating that FAME profiles could be a valuable technique in evaluating the changes in bacterial communities when a wastewater treatment system is operated in a particular way (Werker *et al.*, 2003).

The objective of this study was to evaluate the use of FAME profiles as biomarkers to determine the microbial community composition in wastewater treatment systems. In particular, FAME biomarkers were used to determine the presence of aerobic, anaerobic or facultatively aerobic bacteria comprising the community.

Materials and methods

A literature review was conducted to generate a database from which the FAME biomarkers were selected. Then, FAME profiles from 23 pure cultures were analyzed to confirm the predicted presence of biomarkers. Next, a principal component analysis was conducted with the FAME profiles of 37 pure cultures to evaluate whether they can be grouped in aerobic, anaerobic or facultatively aerobic species. To test the applicability of FAME biomarkers two control samples were prepared with a mixture of anaerobic, aerobic and/or facultatively aerobic bacteria. Finally, the selected biomarkers were used to analyze microbial communities from different wastewater treatment systems.

Biomarkers

The cellular fatty acids profiles for bacteria frequently associated with aerobic, facultatively aerobic and anaerobic treatment systems were reviewed. A qualitative and quantitative analysis of FAME was conducted and a database was generated. For the qualitative analysis, only the absence or the presence of FAME was considered and the bacteria were grouped as aerobic, facultatively aerobic or anaerobic. A fatty acid was considered a biomarker when it was present in only one group. For the quantitative analysis a multivariate analysis [principal component analysis, (PCA)] was performed. In PCA, the area percentage of each fatty acid was considered. A database was constructed with the FAME biomarkers to determine when the FAME profile is related to aerobic, facultatively aerobic or anaerobic bacteria present in the consortium. Data were statistically analyzed with Statgraphics Plus 5.0, manufactured by Manugistics (Rockville, Maryland).

Pure cultures

Microorganisms for analysis were obtained from the bacterial collection of the Biomedical Research Institute of the National Autonomous University of Mexico (UNAM). In a first experiment 23 pure cultures were used (*Alcaligenes* sp., *Gordona* sp., *Mycobacterium fortuitum*, *Mycobacterium* sp., *Nocardia asteroides*, *Nocardia farcinica*, *Nocardia otitidiscaviarum, Pseudomonas aeruginosa*, *Rhodococcus erythropolis*, *Rhodococcus globerulus*, *Rhodococcus rhodochrous*, *Rhodococcus caprophylus*, *Rhodococcus equi*, *Tsukamurella paurometabola*, *Escherichia coli, Klebsiella* sp., *Salmonella* sp., *Staphylococcus* sp., *Bacillus lichenformis, Desulfobacter* sp., *Desulfovibrio desulfuricans*, *Sarcina lutea*, *Streptomyces* sp.). For the quantitative analysis with the PCA, 37 pure cultures were studied (the former 23 species plus *Amycolaptosis* sp., *Corynebacterium* sp., *Flavobacterium* sp., *Achromobacter* sp., *Coryneform* sp., *Micrococcus* sp., *Lactobacillus* sp., *Aeromonas* sp., *Vibrio* sp., *Bifidobacterium* sp., *Streptococcus* sp., *Clostridium* sp., *Proteus* sp., *Pseudomonas* sp.).

FAME analysis

A sample of 50–60 mg of biomass, either from a pure culture or from one of the wastewater treatment systems, was harvested and transferred into sterilized test tubes. FAME

were extracted by adding 6.5 mL of 5% KOH in methanol/benzene (8 : 2, v/v), and incubated overnight under reflux (Buitrón *et al.*, 1998). After evaporation under nitrogen, samples were acidified with a 20% sulphuric acid solution (0.5 mL) and protonated fatty acids were extracted into diethyl ether. Then the fatty acids solution was treated with 1.5 mL of freshly prepared diazomethane reagent for 15 min to obtain fatty acid methyl esters. After evaporation of the samples, the FAME were dissolved in 30 μL of c-hexane and 1 μL of the sample was analyzed by gas chromatography (GC) with flame ionization detection. The GC equipment was a Varian Star 3600 CX gas chromatograph (Varian Associates, Inc.) equipped with a 30 m HP-1 capillary column (0.32 mm ID and 0.25 μm thickness). The column temperature was programmed at 50–280 °C at 10 °C min^{-1} and maintained at 280 °C for 10 min. Triplicate analyses were performed to obtain quantitative information. Behenic acid (22 : 0) and lignoceric acid (24 : 0) (Supelco) were used as internal standards. FAME were preliminary identified according to their retention time, as compared to a commercial standard mixture (47080-U, Supelco).

Samples from wastewater treatment systems

Five samples of biomass from wastewater treatment systems were analyzed. The first sample was taken from a upflow anaerobic sludge blanket system treating wastewater generated during the manufacture of terephthalic acid. The removal efficiency of the process was 95% based on chemical oxygen demand (COD). To test the FAME analysis method in an aerobic system a second sample of mixed liquor was taken from the aeration tank of an activated sludge system treating municipal wastewater in Mexico City (Cerro de la Estrella facility). The removal efficiencies of this plant were 92% based on COD. The remaining samples were obtained from two sequencing batch reactor (SBR) systems. Sample three was harvested from a system treating 100 mg L^{-1} of 4-chlorophenol (4CP) by suspended biomass. The 4CP was degraded with efficiencies higher than 99% as 4CP and 95% based on COD (Moreno-Andrade & Buitrón, 2004). The fourth and fifth samples were obtained from a SBR packed with porous volcanic rock (puzzolane). The reactor was operated as an anaerobic/aerobic sequencing batch biofilter, fed with pharmaceutical wastewater containing organic chemicals, including phenols and *o*-nitroaniline. Maximal removal loads, associated to high removal efficiencies (95–97% as COD), varied from 4.6 to 5.7 kg COD m^{-3} day^{-1} (Buitrón *et al.*, 2003). The fourth sample was obtained scraping the attached biomass from the outside layer of the rock. The fifth and last sample came from the interior of the puzzolane, and was obtained by carefully splitting open the rock, and scraping out the biomass. All FAME analyses were conducted twice.

Results and discussion

FAME as biomarkers

Table 1 summarizes the results of the FAME profile analysis in the literature to establish possible biomarkers for anaerobic, aerobic and facultatively aerobic bacteria present in wastewater treatment systems. The area percentage was obtained with respect to the total FAME. In some cases, intervals for area were reported, because data were obtained from different sources. When no FAME biomarker was found, the interval included zero.

A FAME was considered a biomarker when it was present in only one group. It was found that saturated and hydroxy FAME were exclusively associated with aerobic bacteria (Table 1), and can therefore be considered as biomarkers. For anaerobic bacteria, the biomarkers were unsaturated and branched acids. Facultatively aerobic bacteria presented several FAME biomarkers including unsaturated, branched, cyclopropane and hydroxy fatty acids. FAME 18 : 0 10Me was found in both, actinomycetes and sulfate-reducing bacteria (Sundh *et al.*, 1997). However, the percentage of this acid in sulfate-reducing bacteria, when it was found, was low ($< 0.5\%$) in comparison with the percentage present in the actinomycetes, which ranged from 1.3% to 28.7% with an average value of $13.2 \pm 3.2\%$. Therefore, the FAME 18 : 0 10Me can be considered as an actinomycete biomarker.

FAME were analyzed in 23 pure bacterial cultures and the selected FAME biomarkers were tested in order to confirm their applicability in the analytical technique employed. Because very waxy bacteria, namely *Mycobacterium* spp., have been previously identified in wastewater systems (Buitrón *et al.*, 1998), a saponification method using a methanol/benzene mixture was selected. Chromatography conditions were established to separate a commercially available FAME mixture, which was used as standard. When FAME of bacterial cultures were analyzed by this methodology, at least one of the proposed biomarkers could be easily observed in the correspondent chromatograms (Table 2), thus indicating the potential applicability of FAME biomarkers to identify specific groups of microorganisms.

The usefulness of FAME as biomarkers has previously been suggested. For instance, Vestal & White (1989) considered branched and monounsaturated FAME as biomarkers for sulfate-reducing bacteria, and polyunsaturated FAME for eukaryotes (Table 3). The use of fatty acids as biomarkers to analyze the microbial community in air biofilters and polluted soils has also been proposed (Sundh *et al.*, 1997; Zelles *et al.*, 1997). These authors considered that branched-chain fatty acids are typical in Gram-positive bacteria, monounsaturated fatty acids are common in Gram-negative bacteria, methyl branched and unsaturated branched fatty acids are common FAME in sulfate-reducing

Table 1. Biomarkers obtained in the literature analysis for anaerobic, aerobic and facultatively aerobic microorganisms

FAME biomarker	Area percentage	Type	Example of bacteria genus or group	References
10 : 0 3OH	14.6–20.3			Bøe & Gjerde (1980), Lechevalier (1981), Vestal & White (1989), Welch (1991), Rajendran *et al*. (1992), McNabb *et al*. (1997), Sundh *et al*. (1997), Bossio & Scow (1998), Tsitko *et al*. (1999), Gomez-Gil *et al*. (2003), Ivanova *et al*. (2005), Jung *et al*. (2005), Banowetz *et al*. (2006)
12 : 0 2OH	2.4–18.5		*Alcaligenes*	
12 : 0 3OH	4.6–35.2		*Pseudomonas*	
15 : 1ω5c	0–1.6	Aerobic	*Salmonella*	
18 : 0 2OH	0–3.8		*Alteromonas*	
18 : 0 10Me	1.3–28.7		Actinomycetes (group)	
22 : 0	3.0–4.0			
24 : 0	3.0–25.0			
26 : 0	0.7–7.2			
11 : 0	0–1.1			Lechevalier (1981), Vestal & White (1989), Gomez-Gil *et al*. (2003)
14 : 0 2OH	0–0.6		*E. coli*	
14 : 0 3OH	3.6–7.9		*Klebsiella*	
15 : 0cy	0–20.3	Facultatively aerobic	*Staphylococcus*	
18 : 1$\omega^{9,11}$	0–14.5			
a19 : 0	0–36.2			
i12 : 0	0.1–0.6			Lechevalier (1981), Dowling *et al*. (1986), Vestal & White (1989), Rajendran *et al*. (1992), Haack *et al*. (1994), Sundh *et al*. (1997), Yamamoto *et al*. (1998), Bossio & Scow (1998), Chihib *et al*. (1999), Mannerová *et al*. (2003), Scheldeman *et al*. (2004)
a14 : 0	0–4.5		*Bacillus*	
14 : 1ω7	0–2.7		*Cytophaga*	
15 : 1ω6	0–4.6	Anaerobic	*Sarcina*	
a16 : 0	0–5.3		*Bacillus*	
16 : 1ω5	1.3–2		*Pectinatus*	
a17 : 1	0–0.5		*Macrococcus*	
i17 : 0	0–15.5		Sulfate-reducing (group)	
i17 : 1	0.1–15.5			
a18 : 0	38.1–63.9			
18 : 1ω7c	0–3.8			

bacteria and actinomycetes and, polyunsaturated fatty acids are typical in eukaryotes.

PCA was employed to analyze FAME profiles quantitatively. In this case, FAME were obtained from 37 pure bacterial cultures. The central concept of the PCA method is to reduce the dimensionality of a data set in which there are a large number of interrelated variables, while retaining as much as possible of the variation present in the data set. This reduction is achieved by transforming to a new set of variables, the principal components, which are uncorrelated, and which are ordered so that the first few retain most of the variation present in all of the original variables. The variables were the fatty acids (53 in total) present in the 37 bacteria. The principal components were obtained to relate the FAME with aerobic, facultatively aerobic and anaerobic bacteria (Fig. 1). Results showed that eight main components are needed to explain 81.95% variability from the 37 bacteria species used. The first, second and third main components accounted for 38.04%, 10.92%, and 9.92% of the total variance, which indicates that with the first three main components, the accumulated percentage of 58.89%, was obtained.

PCA revealed that FAME analyses is a valid tool to distinguishing between aerobic, facultatively aerobic or anaerobic bacteria. This is useful to observe differences in the structure of the bacterial communities present in wastewater treatment systems. Five bacterial groups were found with this analysis (Fig. 1). Group I included Gram-positive or negative anaerobic and Gram-positive facultatively aerobic bacteria. Gram-negative anaerobic and Gram-negative facultatively aerobic bacteria formed Group II, whereas Group III contained Gram-positive or negative aerobic and Gram-positive or negative facultatively aerobic bacteria. Gram-positive or negative anaerobic and Gram-negative facultatively aerobic bacteria constituted Group IV. Finally, Group V presented Gram-positive or negative aerobic and

Table 2. Biomarkers present in different bacteria analyzed by the FAME technique

Aerobic	22 : 0	24 : 0	26 : 0	15 : 1 ω5c	10 : 0 3OH	12 : 0 3OH	18 : 0 2OH	12 : 0 2OH	18 : 0 10Me
Alcaligenes sp.					19.6	33.4			
Gordona sp.				1.5					17.8
Mycobacterium fortuitum	4.0	25.0							7.5
Mycobacterium sp.	3.5	11.5	3.8				1.9		9.3
Nocardia asteroides				0.3					14.6
Nocardia farcinica									15.2
Nocardia otitidiscaviarum				0.5					15.7
Pseudomonas aeruginosa					14.6	35.2		18.5	
Rhodococcus erythropolis				0.7					16.0
Rhodococcus globerulus									12.7
Rhodococcus rhodochrous				1.6					12.3
Rhodococcus caprophilus									9.9
Rhodococcus equi									16.4
Tsukamurella paurometabola									11.3

Facultatively aerobic	11 : 0	18 : 1 $\omega^{9,11}$	15 : 0 cy	14 : 0 2OH	14 : 0 3OH	a19 : 0
Escherichia coli			20.9			
Klebsiella sp.		14.5			3.6	
Salmonella sp.	1.1			0.6	7.9	
Staphylococcus sp.						36.2
Escherichia coli			20.9			
Klebsiella sp.		14.5			3.6	
Salmonella sp.	1.1			0.6	7.9	
Staphylococcus sp.						36.2

Anaerobic	14 : 1 ω7	15 : 1 ω6	16 : 1 ω5	18 : 1 ω7c	i12 : 0	a14 : 0	a16 : 0	a17 : 1	i17 : 0	i17 : 1	a18 : 0
Bacillus lichenformis					0.6			2.8	5.5	2.2	50.4
Desulfobacter sp.	0.2	2.3	1.7	1.9		2.3	2.7	0.7	0.8		
Desulfovibrio desulfuricans	1.7			1.9				3.3	12.6	12.6	
Sarcina lutea					0.3						63.9
Streptomyces sp.									2.3		

Values represent the percentage of biomarker in the total FAME profile.

Table 3. Cellular fatty acids usually found in different groups of microorganisms

Fatty acid	Microorganisms	References
15 : 0, i15 : 0, a15 : 0, 16 : 1ω9, 16 : 1ω5, i17 : 0, a17 : 0, 17 : 0, 18 : 1ω7t, 18 : 1ω5; i19 : 0, a19 : 0	Eubacteria	Vestal & White (1989), Sundh *et al*. (1997), Ritchie *et al*. (2000)
15 : 0, i15 : 0, a15 : 0, 16 : 1ω9, 16 : 1ω5, i17 : 0, a17 : 0, 17 : 0, 18 : 1ω7t, 18 : 1ω5, i19 : 0, a19 : 0, 20 : 0, 22 : 0, 18 : 2ω6, 18 : 3ω3	Cyanobacteria	Sundh *et al*. (1997)
i17 : 1ω7, 16 : 0 10Me, 17 : 1ω6	Sulfate-reducing	Dowling *et al*. (1986), Vestal & White (1989), Bossio & Scow (1998)
15 : 0, i15v0, a15 : 0, 16 : 1ω9, 16 : 1ω5, i17 : 0, a17 : 0, 17 : 0, 18 : 1ω7t, 18 : 0 10Me, 16 : 010 Me	Actinomycetes	Vestal & White (1989), Bossio & Scow (1998), Ritchie *et al*. (2000)
16 : 0, 18 : 1ω9, 18 : 2ω6, 18 : 3ω6, 18 : 3ω3	Fungi	Vestal & White (1989), Bossio & Scow (1998), Ritchie *et al*. (2000)
16 : 1ω8, 18 : 1ω8	Methane-oxidizing	Nichols *et al*. (1985)
18 : 2 ^{9,12}; 20 : 3ω6; 20 : 4ω6	Protozoa	Vestal & White (1989), Sasser (1997)

Gram-negative facultatively aerobic bacteria. A clear distinction between aerobic and anaerobic bacteria was thus obtained by the PCA method. However, facultatively aerobic bacteria were distributed among all groups of bacteria, probably because of the different terminal electron acceptor preferences of the species (aerobic or anaerobic).

To test the applicability of FAME biomarkers, two control samples were prepared using anaerobic, aerobic and/or

facultatively aerobic bacteria. Results showed that the biomarkers proposed in this study successfully detected the corresponding bacterial group. Control sample 1 contained *P. aeruginosa, Alcaligenes* sp., *Acinetobacter* sp. (aerobic bacteria), *E. coli*, (facultatively aerobic) *Micrococcus luteus, Bacillus subtillis* (anaerobic). The FAME biomarkers found in sample 1 were 12 : 0 2OH, 12 : 0 3OH (representing aerobic bacteria); 14 : 0 3OH (facultatively aerobic) and i17 : 0, a17 : 0 (anaerobic). Control sample 2 contained *Proteus mirabillis*, *E. coli* (facultatively aerobic) and *M. luteus, Bacillus licheniformis* (anaerobic). The observed FAME biomarkers were 14 : 0 3OH (facultatively aerobic) and i17 : 0, a17 : 0 (anaerobic). Results show that the proposed methodology is adequate.

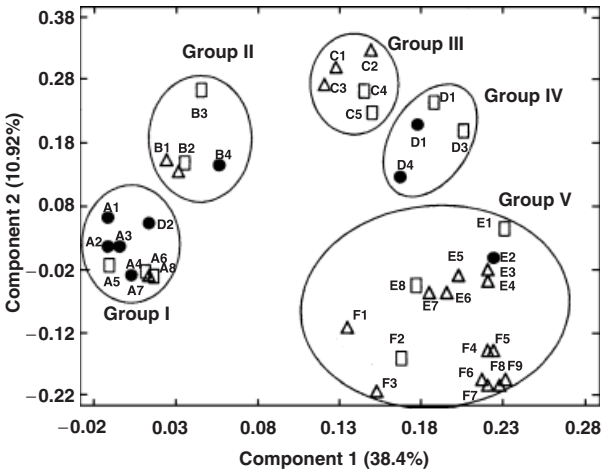

Fig. 1. Groups determined by PCA. Group I: (A1) *Bacillus lichenformis*, (A2) *Sarcina lutea*, (A3) *Amycolaptosis* sp., (A4) *Corynebacterium* sp., (A5) *Staphylococcus* sp., (A6) *Streptomyces* sp., (A7) *Desulfobacter* sp., (A8) *Desulfovibrio desulfuricans*, Group II: (B1) *Flavobacterium* sp., (B2) *Escherichia coli*, (B3) *Achromobacter* sp., (B4) *Coryneform* sp., Group III: (C1) *Alcaligenes* sp., (C2) *Micrococcus* sp., (C3) *Pseudomonas aeruginosa*, (C4) *Lactobacillus* sp., (C5) *Aeromonas* sp., Group IV: (D1) *Vibrio* sp., (D2) *Bifidobacterium* sp., (D3) *Streptococcus* sp., (D4) *Clostridium* sp., Group V: (E1) *Proteus* sp., (E2) *P. aeruginosa*, (E3) *Mycobacterium* sp., (E4) *Pseudomonas* sp., (E5) *Rhodococcus globerulus*, (E6) *Gordona* sp., (E7) *Mycobacterium fortuitum*, (E8) *Klebsiella* sp., (F1) *Rhodococcus caprophylus*, (F2) *Salmonella* sp., (F3) *Nocardia asteroides*, (F4) *Rhodococcus erythropolis*, (F5) *Rhodococcus equi*, (F6) *Rhodococcus rhodochrous*, (F7) *Tsukamurella paurometabola*, (F8) *Nocardia farcinica*, (F9) *Nocardia otitidiscaviarum*.

Microbial community analysis in wastewater treatment systems

FAME biomarkers were used to determine the bacterial groups in wastewater treatment systems. Figure 2 shows the FAME obtained for each type of bioreactor. Bars represent the relative percentage of each fatty acid. This percentage was obtained by adding all the areas under each FAME peak in the chromatographic profile of the sample. The total of the addition corresponded to 100% of the total area; the relative percentage area was obtained from these data for each one of the fatty acids present in the chromatogram.

Our results showed the presence of 12 : 0 3OH acid in the activated sludge from the aerobic wastewater treatment plant; this biomarker indicates the presence of aerobic bacteria. The 14 : 0 3OH FAME biomarker was found in all treatment systems studied and it is an indicator of the presence of facultatively aerobic bacteria. This indicated that, in the case of the discontinuous system operated under anaerobic and aerobic conditions, facultatively aerobic

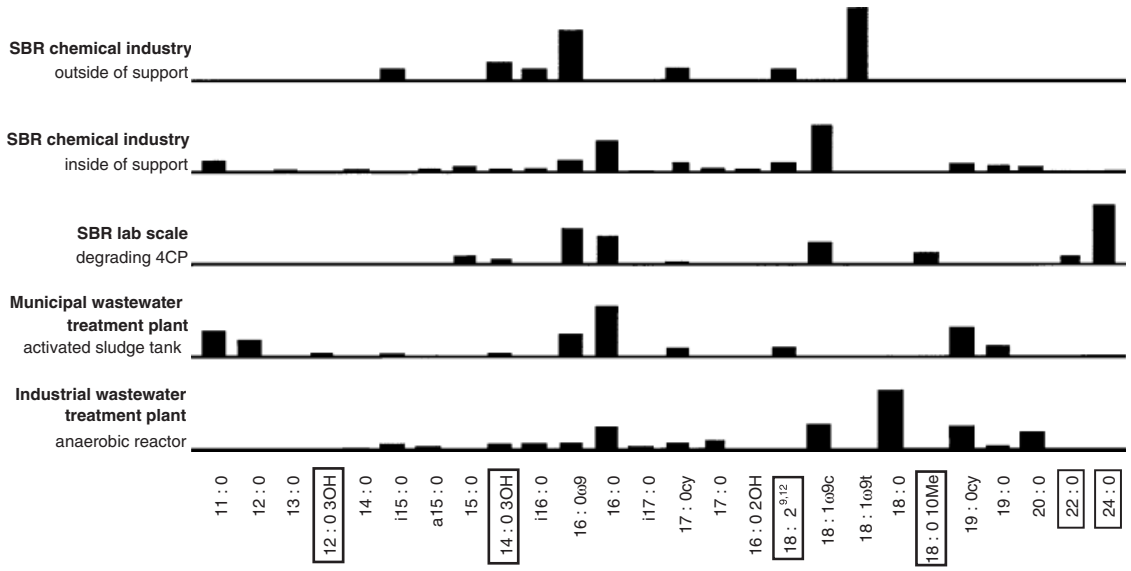

Fig. 2. FAME present in the studied wastewater treatment systems. FAME in rectangles represent biomarkers.

bacteria were selected and this type of microorganisms colonized the external part of the support.

The 18 : 0 10Me, 22 : 0 and 24 : 0 FAME biomarkers were found in the aerobic SBR system fed with 4CP, indicating the presence of aerobic bacteria such as *Nocardia*, Actinomycetes and the Mycobacterium groups (Table 3). This result is consistent with previous findings for this type of system treating 4CP (Buitrón *et al*., 1998). The anaerobic FAME biomarker i17 : 0 was observed inside the packing of the SBR system fed with chemistry industry wastewater, and in the anaerobic system that treats an effluent containing residuals of terephthalic acid production. It seems reasonable to find this biomarker for anaerobic bacteria inside the packing because low oxygen concentrations are found there. These results demonstrated that FAME methodology was useful to characterize the microbial community present in wastewater treatment systems operated under different environmental conditions.

Finally, whereas our *in-silico* study only considered bacteria, the FAME analyses of wastewater systems showed that the method can be extended to assess the presence of other microorganisms. In this study, the $18:2^{9,12}$ FAME, which is typical in protozoa (Sasser, 1997), was found in the chemical industry wastewater and in the system that treats municipal effluent. Additionally, fatty acid profiles can also be used to detect changes that occur in microbial communities. Important changes can occur due to periods of starvation, temperature variations or as a result of variations in the operating parameters in wastewater treatment systems. For example, Son & Hall (2003) demonstrated the use of fatty acid profiles to estimate the stability of a microbial community and to correlate the changes observed in the structure of the community due to variations in pH, organic loading, and chlorine addition in an activated sludge system. FAME analyses as proposed here should only be applied when the wastewater treatment process is at steady state in order to avoid changes that might purely be induced by variations to the operating parameters.

Conclusions

The present study showed that analysis of FAME chromatographic profiles is a practical tool for determining the structure of microbial communities, based on metabolism. The FAME profiles were found to be related to the terminal electron acceptor used by the microorganisms; thus, FAME biomarkers for aerobic, facultatively aerobic and anaerobic bacteria were successfully obtained. The FAME biomarkers determined for aerobic bacteria were saturated and hydroxy C:10, C:12 and C:18. Branched and unsaturated FAME were found in anaerobic bacteria. Facultatively aerobic bacteria were characterized by unsaturated, branched, cyclopropane and hydroxy C:14 FAME. The biomarkers can be used to characterize the microbial community as anaerobic, aerobic and/or facultatively aerobic bacteria, present in wastewater treatment systems.

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