Copyright © 2007 by Humana Press Inc.
All rights of any nature whatsoever reserved.
0273-2289/1559-0291 (Online)/07/141/299–320/$30.00

Microbial Growth on Pall Rings

A Problem When Upgrading Biogas With the Water-Wash Absorption Technique

ÅSA TYNELL,[1] GUNNAR BÖRJESSON,*,[1] AND MARGARETA PERSSON[2]

[1]Department of Water and Environmental Studies, Linköping University, SE-581 83 Linköping, Sweden, E-mail gunnar.borjesson@mikrob.slu.se; and [2]Swedish Gas Centre, Scheelegatan 3, SE-212 28 Malmö, Sweden

Received June 2, 2006; Revised September 1, 2006; Accepted September 30, 2006

Abstract

Biogas is upgraded using an absorption with water-wash technique by 11 of a total of 14 upgrading plants in Sweden. However, problems with microbial growth on the pall rings in the absorption column, and in one case in the desorption column, have a negative impact on the upgrading of raw gas to vehicle gas. Five of the nine biogas plants studied here have experienced problems with microbial growth. The objectives of this study were to identify such microbial growth and to determine possible factors for its control, in order to provide recommendations for process management. A questionnaire was sent out and visits were made to the upgrading plants to collect information about the upgrading process. Phospholipid fatty acid (PLFA) analysis was performed to determine microbial biomass and community structure in samples from four upgrading plants. In samples from two of the plants, methane-oxidizing bacteria (type I methanotrophs) were indicated, while samples from one of the other plants showed biomarkers indicating actinomycetes. Factors affecting development of microbial growth were found to be water quality and the pH and temperature of the process water. Plants that used wastewater in the upgrading process experienced far more problems than those using clean water of drinking quality.

Index Entries: Biogas upgrading; wastewater; actinomycetes; methanotrophs; phospholipid fatty acids.

*Author to whom all correspondence and reprint requests should be addressed. Present address: Department of Microbiology, SLU, PO Box 7025, SE-750 07 Uppsala, Sweden.

Introduction

For efficient use of biogas as vehicle fuel, a methane content of at least 95% is required. The raw gas usually contains about 55–75% methane, with the remainder consisting mainly of CO_2 and H_2S. After cleaning and upgrading (separation out of H_2O, H_2S, and CO_2), the biogas requires a lower storage volume and longer driving distances are obtained. In Sweden, the methane level is kept at 97% so that the same vehicles can be driven both on natural gas and on biogas.

In Sweden, the most common method used for upgrading biogas is water wash, which at the time of this study (September 2004) was employed by 11 plants in Sweden. There are two different types of water absorption plants: regenerating plants and single-pass plants. In both cases, condensed water and particles are first removed from the raw gas in a separator. The gas is then led to a compressor, where it is pressurized to 9–12 bar, in order to make the CO_2 more soluble. After pressurizing, the gas is cooled in a heat exchanger. In the absorption column (scrubber), a high, cylindrical column usually about 10 m high and 0.5 m in diameter, water enters from the top of the column, and the pressurized raw gas enters from the bottom of the column. When the gas and water meet, CO_2 dissolves into carbonic acid and the methane concentration of the gas is increased. To create a larger contact surface between the raw gas and the water, the absorption column is randomly filled with plastic packing called pall rings. Pall rings come in many different models but are usually cylindrical with dimensions of 25 H 25 mm^2.

In regenerating plants (Fig. 1) *(1)*, the water from the absorption column continues to a flash tank, where methane that has dissolved in water is vaporized out under an intermediate pressure of 2–4 bars. The methane gas is collected and returned to the process system with the raw gas before the raw gas enters the compressor *(2)*. The process water then continues to the desorption column, also called the stripper, which removes the dissolved CO_2 from the process water. Like the absorption column, the desorption column is randomly packed with pall rings. The pH of the process water is increased to about neutral when this occurs and the water temperature increases. The water has to be cooled in a heat exchanger to an absorption temperature of 15°C, which is the process water temperature of most regenerating water-wash plants (R. Simonsson, personal communication, October 11, 2004). The water is then ready to be reused in another loop in the regeneration process. The process water is drinking water and is replaced a little at a time.

Single-pass water-wash plants (Fig. 2) mostly use cleaned water from sewage treatment plants as process water to keep the cost of water supply low. The process water temperature is much more variable in single-pass plants, because the water temperature usually follows the seasonal variations. Water temperatures in the range of 4–21°C are common. Single-pass

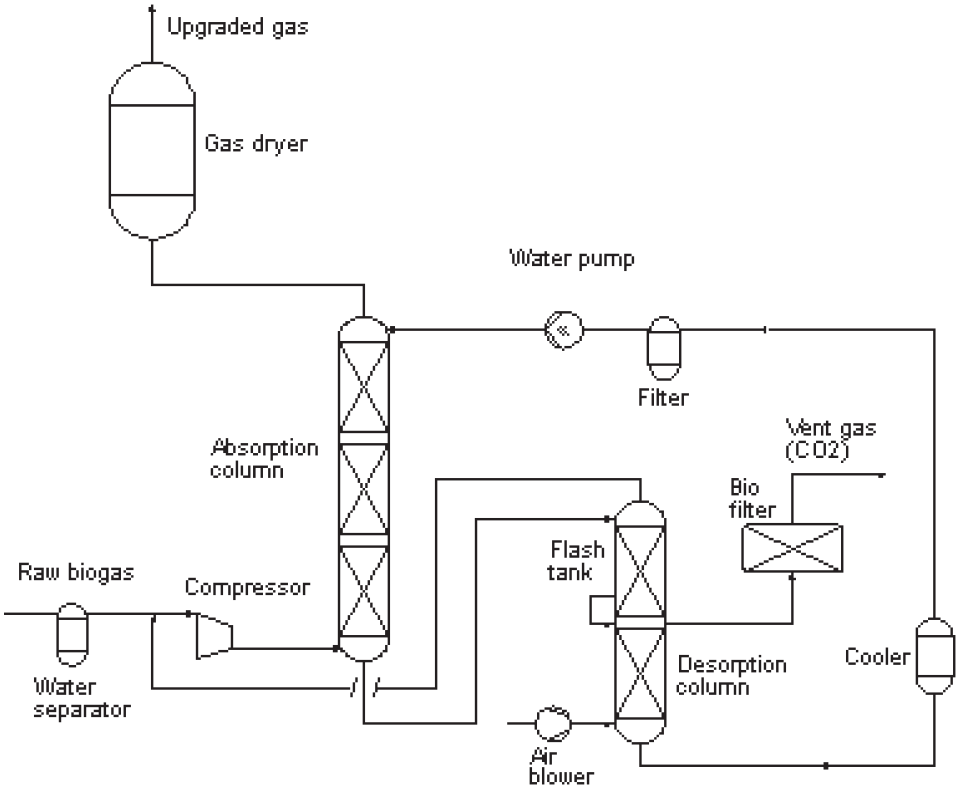

Fig. 1. Regenerating water-wash plant. (Used by permission of SGC *[1]*.)

water-wash plants are based on the same principle as regenerating water-wash plants regarding the absorption column and the flash tank. The water interacts with the raw gas in the absorption column, and methane that has been absorbed by the water is vaporized by depressurization in the flash tank. The water exits the system from the flash tank and is returned to the sewage treatment plant. The process water is not reused in the system. The gas in the flash tank is returned to the gas inlet.

Some upgrading plants experience problems with growth of biofilm on the pall rings. The appearance of this biofilm differs among upgrading plants. The growth of microorganisms lowers the efficiency of the upgrading plant, and the upgraded biogas fails to fulfill the criteria for vehicle fuel owing to its lower methane content.

The main objectives of the present study were (1) to identify the organisms in the organic material that cause microbial growth on the pall rings in the absorption column and, in some cases, in the desorption column; and (2) to investigate and determine possible factors regulating microbial growth on pall rings in the different biogas upgrading systems, in order to provide recommendations on process management.

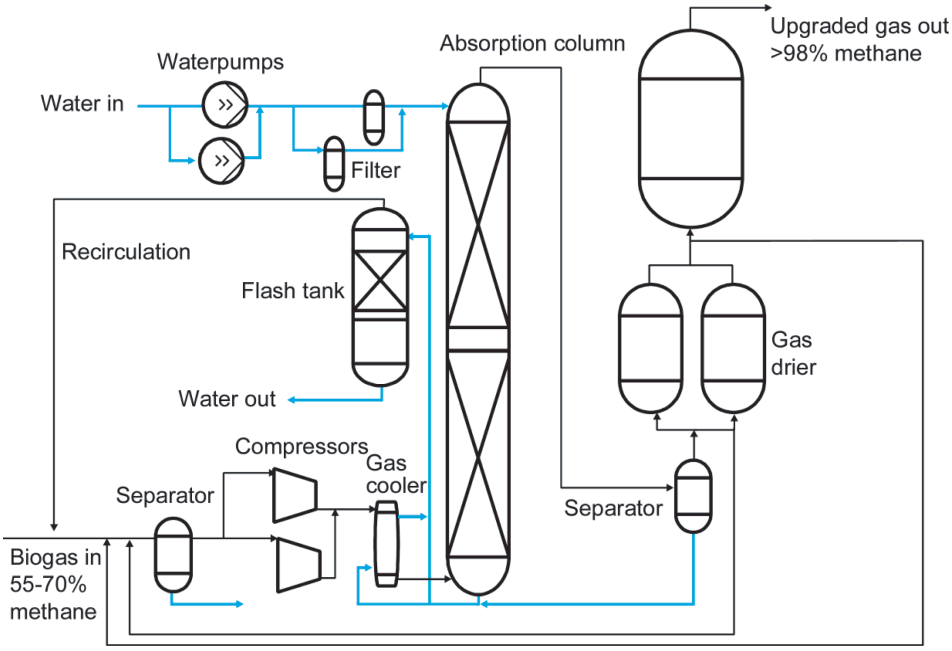

Fig. 2. Single-pass water-wash plant. (Used by permission of SGC *[1]*.)

Materials and Methods

Questionnaire

To identify the factors regulating microbial growth on the pall rings, a questionnaire for the gas plants was devised, based on findings by Persson *(3)*. The questionnaire dealt with the following parameters:

1. Raw gas quality, because depending on the material digested the raw gas content of methane, CO_2, and H_2S could be expected to vary.
2. pH, because a low pH can inhibit the growth of certain bacteria.
3. Temperature, to compare variations, because most process plants seemed to experience more growth at higher temperatures.
4. Biologic oxygen demand (BOD) and chemical oxygen demand (COD) of the process water, as measures of the amount of organic material.
5. Water velocity, because a high water velocity through the water column could perhaps prevent certain bacteria from establishing on the pall rings.
6. Dimensions of the absorption column, desorption column, and raw gas capacity, to determine whether distribution of water and process load affected the upgrading.

7. Model, size, and density of pall rings used in the absorption column, to determine whether there was any correlation between these factors and microbial growth.
8. Cleaning methods and detergents used by the plants to clean the pall rings.

The questionnaire was sent out to the upgrading plants selected for the study and was followed up by interviews and visits to some of the plants.

Phospholipid Fatty Acid Analysis

Phospholipid fatty acid (PLFA) analysis is used for quantitative determination of the fatty acids in membranes of living cells. PLFAs can be used as signature lipid biomarkers, and PLFA analysis has proved useful in detecting functional groups of bacteria in environmental samples, such as methanotrophs *(4)*. In our case, the PLFA method was chosen because the collected samples were assumed to contain methanotrophs. This assumption was based on the following reasons:

1. The environmental conditions of the absorption column, which are optimal for methanotrophs (plenty of methane and possibly some oxygen).
2. The growth of microorganisms being found in absorption columns.
3. The physical appearance of the microbial growth observed on pall-rings, because methanotrophs have the ability to produce substantial amounts of exopolymeric substances (EPSs), which can appear as both capsules and plentiful slime *(5)*. The amount of EPSs produced can vary greatly in the microbial community and under different environmental conditions.

The PLFA method was also considered advantageous for our study because no cultivation is needed before the analysis.

Culture Samples

The amount of microbial growth that could be collected limited the number of analyses performed, because not all the plants experiencing problems with microbial growth could supply enough material. Cultures were collected from all those upgrading plants that had experienced microbial growth: Jönköping, Linköping, Kristianstad, and Uppsala. In addition to these plants, the Eslöv plant has also experienced growth on pall rings, but no culture was collected in Eslöv, because the plant had operational problems during the summer of 2004.

Samples of the microbial growth on the pall rings were collected from the absorption column in all plants except for the Linköping plant, where culture was collected from the pall rings of the desorption column, because microbial growth was much less abundant in the absorption column. The samples were immediately frozen or chilled after collection.

Counting from the latest wash, the culture from Jönköping was 30 d old, that from Kristianstad was 12 d old, that from Linköping was 180 d old, and that from Uppsala was 21 d old. All samples were refrigerated (–18°C) between treatments.

For PLFA analysis, three replicates were taken from the cultures growing on the pall rings from the upgrading plants in Jönköping, Linköping, and Uppsala. Because only small amounts of growth were collected in Kristianstad, only one sample was taken. One-gram aliquots of subsamples were transferred to precleaned 50-mL glass tubes for further extraction.

PLFA Extraction

PLFA extractions were performed according to Börjesson et al. (6,7), commencing with a one-phase extraction of lipids from the samples and continuing with lipid extract dissolved in chloroform and transferred to a silicic acid column, followed by separation into neutral lipid, glycolipid, and polar lipid fractions using eluents of increasing polarity (chloroform, acetone, methanol). The polar lipid fraction containing the PLFAs was then stored at –18°C for the methanolysis step. Methanolysis was performed with hexane/$CHCl_3$ (4:1). Half of the dried samples was dissolved in hexane and placed in new glass tubes to be derivatized with dimethyl-disulfide (DMDS). DMDS derivatization was performed to determine the positions of the double bonds of 16:1 and 18:1 PLFAs.

Gas Chromatography and Gas Chromatography/Mass Spectroscopy Analysis

The fatty acid methyl ester (FAME) samples were analyzed in a Hewlett Packard 6890 gas chromatography (GC) flame ionization detector (FID) . The temperature was programmed to increase from 50 to 320°C during 60 min, according to Steger et al. *(8)*. The carrier gas was helium, and the cylindrical columns were crosslinked methyl siloxane measuring 30 m × 250 µm × 0.1 µm. To identify the FAMEs, the retention times were compared to the retention times for standard fatty acid mixtures. Selective ion monitoring was used to identify and quantify the monounsaturated, derivatized fatty acids with GC-mass spectrometry (MS) *(8)*. The instruments used were a Hewlett Packard 6890 GC-system and a Hewlett Packard 5973 Mass Selective Detector. The same temperature program as for the GC was used. The fatty acids were quantified by comparing peak areas to the peak area of the internal standard 19:0 (Larodan Fine Chemicals, Malmö, Sweden).

Fatty Acid Nomenclature

Fatty acids are named by the total number of carbon atoms followed by the number of double bonds, followed by the position of the double bond indicated from the methyl end, ω, while *c*- and *t*- refer to *cis*- and *trans*-conformation, respectively. The prefixes *i*-and *a*-signify methyl branching

in *iso-* and *anteiso-* positions. The prefix *10Me-* indicates methyl branching on the tenth carbon atom from the carboxyl end, while *cy-* refers to cyclopropane fatty acids.

Methane Oxidation Capacity

Culture aliquots of 1–3 g were transferred to 250-mL glass flasks. After adding 1 mL of methane to the gas phase, the flasks were sealed with gastight screw caps and incubated at 20°C. Three gas samples of 0.3 mL each were withdrawn on at least five occasions during 3 d and immediately analyzed for their methane content on a gas chromatograph with an FID (Packard model 428 with a Porapak T 2 m × 2 mm column operated at 125°C).

Results

Questionnaire

Information was successfully collected from all nine biogas plants, and answers to almost all of the questions were obtained.

Quality of Raw Gas

The biogas plants in Henriksdal and Eskilstuna digest sludge from sewage treatment plants together with fat from fat separators in restaurant kitchens (Table 1). In Eslöv, sludge from sewage treatment plants and sludge from starch pro-duced in the food industry are co-digested. Linköping, Uppsala, and Kristianstad co-digest mainly waste from the slaughter and food indus-tries. Jönköping, Trollhättan, and Kalmar digest mainly sludge from sew-age treatment plants, but also waste from the food industry, although Jönköping and Kalmar also digest some waste from the slaughter industry.

The methane content of the raw gas was, as expected, slightly lower in plants that digest mainly sewage sludge (Eslöv, Henriksdal, Eskilstuna, Trollhättan, and Jönköping: 55–65%) and higher in those that co-digest (Kalmar, Linköping, Kristianstad, and Uppsala: 65–70%). No correlation between a lower or higher methane content and microbial growth was observed. Only the Kalmar plant reported high levels of H_2S (650–800 ppmv), but because the plant has not experienced problems with the growth of microorganisms, H2S is not likely to affect the establishment of cultures.

Temperature and pH

One important difference between the regenerative and single-pass water-wash plants is the temperature of the process water. The regenerating plants in Trollhättan, Kalmar, and Henriksdal keep the temperature constant at 15°C during the whole year. One of two regenerating plants in

Table 1
Biogas Plants in Sweden Using Absorption with Water Wash in September 2004[a]

Plant	Substrate	Water wash technique	Type of water used
Eskilstuna	SS; fat from restaurants	R	Drinking water
Henriksdal	SS; fat from restaurants	R	Drinking water from Lake Mälaren
Linköping	SS; waste from slaughter; liquid manure waste from the food industry (degraded in two separate chambers), C	R	Drinking water from Motala River
Trollhättan	SS; wastes from fish industry, so-called draff and mash	R	Drinking water from Göta River
Kalmar	Sludge and waste from slaughter industry; liquid manure; fat from restaurants, C	R	Drinking water
Eslöv	SS; sludge of starch, C	S	Wastewater from sewage treatment plant
Jönköping	SS; food waste from industry kitchens; potato peel; draff-liquid slaughter waste	S	Wastewater from treatment plant sewage
Kristianstad	SS; waste from food and slaughter industry (degraded in two separate chambers), C	S	Wastewater from sewage treatment plant, drinking water in summer
Uppsala	SS; solid waste: waste from slaughter; liquid waste: blood, polyglucose (degraded in two separate chambers)	S	Wastewater from sewage treatment plant

[a]SS = sewage sludge; C = co-digestion; R = regenerative; S = single pass.

Linköping is a few years older (built in 1997) and has a water temperature of 10–20°C, depending on the season. The highest temperatures are recorded during the summer and the lowest during the winter. The other upgrading plant in Linköping (from 2002) has water temperatures between 15 and 20°C. The upgrading plant in Eskilstuna has a temperature range of between 5 and 20°C, depending on the season.

Because most sewage treatment plants treat their wastewater outdoors, the seasonal variations in temperature affect the temperature of the process water. Temperatures between 4 and 15°C have been measured in the Eslöv single-pass plant, 5 and 15°C in the Jönköping plant, and up to 20°C in the summertime in the Kristianstad plant. Uppsala reported a relatively constant water temperature of 10–12°C.

The plants that experience large variations in process water temperature all reported that processing problems increase during the summer when water temperatures are high. Microbial growth increases with increasing

temperatures, but problems may also arise from the fact that CO_2 does not dissolve well to H_2CO_3 at higher temperatures and, thus, the pH of the process water is kept high. At low temperatures, the CO_2 dissolves more easily to H_2CO_3 and the pH of the process water decreases. Reported pH values of incoming process water in the upgrading plants were neutral to slightly basic, ranging between 7.0 and 8.5. The pH of the water decreases downward in the column, because more CO_2 has been converted into H_2CO_3 farther down, but the pH of the process water in many upgrading plants was also reported to vary with the season. The Jönköping and Linköping upgrading plants both reported that microbial growth mainly occurs at the top of the absorption column, which could be an indication that microbial growth is favored by high pH conditions. This is in good accordance with the relatively high pH of 8.4 reported by Linköping, but not in as good agreement with the pH of 7.5 reported by the Jönköping plant.

Water Quality (BOD and COD)

For the water used in the upgrade process, all single-pass plants reported BOD values and all regenerative plants reported COD values. The BOD values were all acceptable according to Swedish regulation SNFS 1990:14 on sewage water effluent. COD values of the drinking water used in regenerating plants were all <4 mg/L and were thus all acceptable for drinking water according to Swedish National Food Administration regulation SLVFS 2001:30.

The single-pass plants in the study experienced microbial growth on pall rings. Jönköping, Kristianstad, and Eslöv, all of which experienced microbial growth, reported relatively high, but permissible, BOD values (<15, <10, and 10 mg of BOD/mL, respectively) in the process water. A high BOD value indicates that the water contains a lot of organic material that could establish and enrich microbial colonies under favorable conditions. Although Uppsala reported a lower BOD value than the other single-pass plants (4 mg/mL), of the plants studied, it experienced microbial growth and cleaned its column the most often.

The plants using drinking water reported COD values that were all under the permissible 4 mg/L. COD values could not be linked to incidence of microbial growth, because the Linköping upgrading plant was the only regenerative plant that reported microbial problems, but it had the same COD value as the other plants.

Plant Design and Water Velocity

Water velocity through the absorption column varied greatly among the different plants but was not correlated with microbial growth problems. For example, both Eslöv and Jönköping, with a relatively low and high water velocity, respectively (0.02 m/s and a minimum of 0.33 m/s at half the inflow rate of raw gas, respectively), had problems with growth on pall rings.

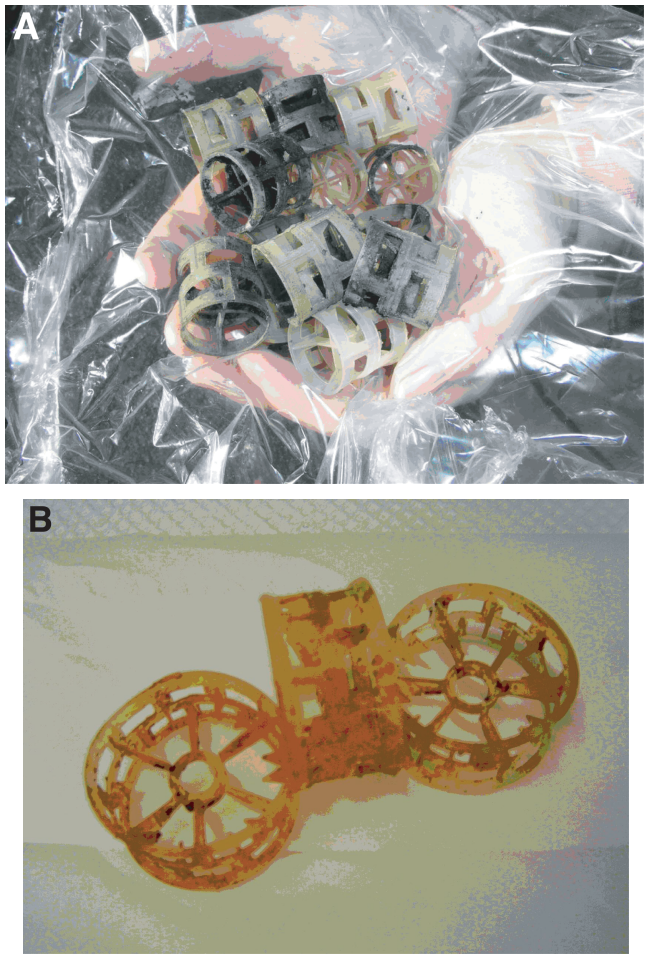

Fig. 3. Pall rings collected from different plants: **(A)** pall rings from Kristianstad plant as they looked when collected from the absorption column; **(B)** pall rings from Uppsala plant showing reddish color caused by microbial growth.

Pall Rings and Cleaning

The pall rings used in the upgrading plants differed in size and model (Fig. 3A–D). A trend is that the pall rings used today are smaller than those used a couple of years ago. The most usual dimension of pall rings at present is 25 mm in height and 25 mm in diameter. The upgrading plants that have problems with microbial growth on pall rings clean their pall rings either in the column, or by taking the pall rings out of the column (Table 2). The Henriksdal, Kalmar, and Trollhättan plants all clean their columns as a preventative measure. The Eskilstuna plant has not had any problems with microbial growth, and, therefore, it has never cleaned its pall rings. In-column wash is more convenient but not all plants are constructed in such a way that they can use this method. Taking the pall rings out of the column

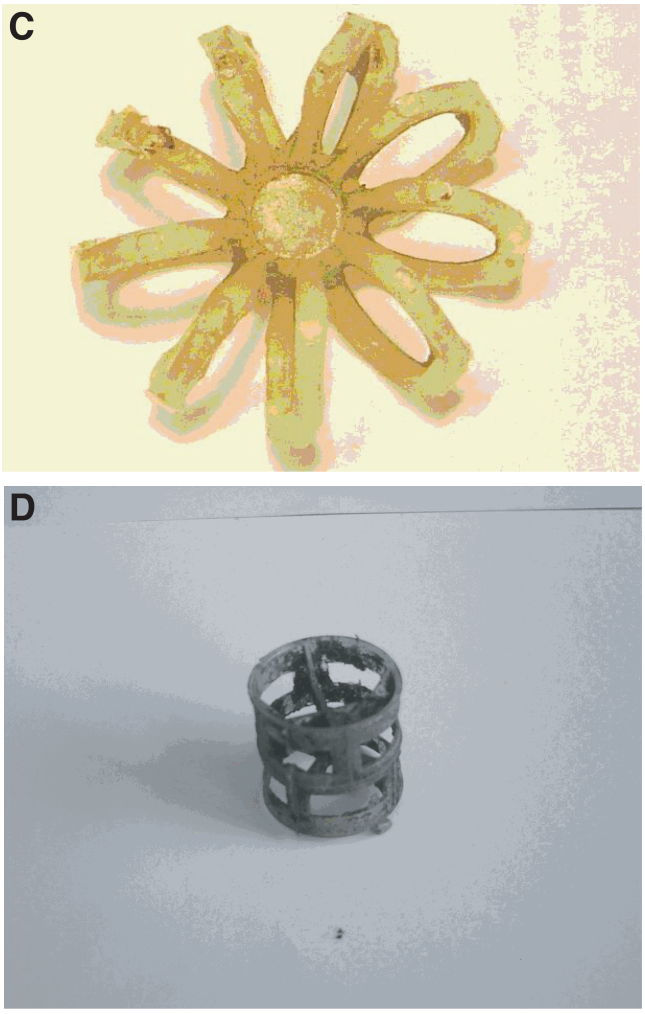

Fig. 3. *(continued)* **(C)** pall ring used in Kalmar plant showing degree of wear; **(D)** pall ring from Jönköping plant showing residual dark brown microbial growth. (Photos: [A] R. Johansson; [B] Å. Tynell; [C,D] D. Tynell.) Note that the pall rings are not shown as actual size.

is difficult, because the column usually has only two openings (Fig. 4) and the pall rings have to be removed manually.

Washing the pall rings requires a shutdown of the process varying in time between 6 and 10 h, depending on the technique used. The frequency with which a plant washes its pall rings varies. Some plants experiencing growth wash only two to four times a year, whereas others wash every 3 wk (Table 2). Each plant chooses a cleaning detergent based on information obtained through laboratory experiments, experience, and consultation with the manufacturer. A common characteristic of many detergents is that they are alkaline and designed for industrial use. Hypochlorite (NaClO) and caustic soda (NaOH) are two common ingredients in the detergents.

Table 2
Methods for Cleaning Pall Rings, Frequency of Cleaning, and Type of Detergent Used

Plant	Cleaning method	Frequency	Detergent
Eskilstuna	—	—	—
Henriksdal	In column	Once, system capacity lowered	Hypochlorite
Linköping	Outside column	Twice a year	Mechanically with water
Trollhättan	In column	Once, for preventative purposes	Hypochlorite
Kalmar	In column	Once every other year	Pineline, industrial alkaline detergent
Eslöv	Outside column	3-4 times/yr	Water
Jönköping	In column	Every other month	Liquid green, alkaline detergent
Kristianstad	In column	Once a month	Alkaclean 28, alkaline cleaning agent
Uppsala	In column	Every 3 wk	P3-asepto FL, industrial cleaning agent

Potassium hydroxide (KOH) is also an ingredient in several of the detergents reported to be in use (Table 2). The Linköping and Eslöv plants clean their pall rings mechanically using only hot water.

Microbial Growth

Visually, the cultures collected were of two kinds: (1) a yellow, slimy culture collected in Linköping that very much resembled the culture from Kristianstad in 2001 shown in Fig. 5; and
(2) a culture that looked like coffee grounds found in Jönköping, Uppsala (Fig. 3B,D), and Kristianstad in 2004 (Fig. 3A). The culture collected in Uppsala was reddish brown, the culture from Jönköping was brown and oily with a rank smell, and the culture from Kristianstad was nearly black.

Because only enough growth for one sample could be collected from the Kristianstad plant, the results should be viewed with caution. The picture of pall rings from the Kristianstad plant from the autumn of 2001 in Fig. 5 indicates that the culture then had a different physical appearance compared with that collected for our experiment (Fig. 3A). This could depend on several factors. First, the culture in Fig. 4 was older than the culture collected for the present study (2 yr compared to barely 2 wk) and had had more time to establish and more organisms had colonized. Second, the process water used in 2001 was from the sewage treatment plant, whereas the water used when the sample was collected in September 2004 was drinking water.

Fig. 4. Culture on pall rings in Kristianstad, 2001. (Photo: R. Johansson.)

Fig. 5. Lower opening of absorption column in Kristianstad plant. The column is filled with pall rings. (Photo: R. Johansson.)

Table 3
Major PLFAs Found in Samples from Four Water-Wash Systems Arranged According to GC Retention Times (% of total mol volume ± SD)[a]

PLFA	Jönköping (n = 3)	Kristianstad (n = 1)	Linköping (n = 4)	Uppsala (n = 4)
12:0	0.65 ± 0.49	0.02	0.16 ± 0.03	0.19 ± 0.12
14:0	0.91 ± 0.49	1.81	1.38 ± 0.28	2.43 ± 0.21
i15:0	3.95 ± 1.32	1.12	4.42 ± 0.80	5.49 ± 0.90
a15:0	2.67 ± 1.41	0.76	2.26 ± 0.33	1.58 ± 0.18
15:0	0.59 ± 0.21	0.44	0.93 ± 0.06	0.53 ± 0.05
br16:0	0.75 ± 0.37	0.25	0.23 ± 0.20	0.27 ± 0.31
i16:1	0.05 ± 0.08	0.39	0.05 ± 0.04	0.18 ± 0.22
i16:0	0.50 ± 0.17	0.68	0.72 ± 0.08	0.17 ± 0.17
16:1ω9c	0.59 ± 0.07	0.11	0.44 ± 0.18	1.75 ± 0.73
16:1ω9t	0.12 ± 0.17	ND	ND	ND
16:1ω8c	0.09 ± 0.02	0.60	2.05 ± 0.87	4.25 ± 1.62
16:1ω7c	7.75 ± 1.3	8.19	8.11 ± 6.50	23.02 ± 10.39
16:1ω7t	ND	ND	3.35 ± 1.34	2.08 ± 2.51
16:1ω6c	0.03 ± 0.02	0.19	2.34 ± 1.21	1.87 ± 0.57
16:1ω6t	ND	ND	0.13 ± 0.16	0.04 ± 0.08
16:1ω5c	0.24 ± 0.33	ND	4.52 ± 1.42	1.28 ± 1.52
16:1ω5t	0.19 ± 0.27	ND	2.50 ± 1.03	2.52 ± 2.81
16:0	6.94 ± 2.57	ND	15.66 ± 0.77	12.45 ± 3.87
17:1ω9	1.70 ± 1.02	7.50	0.05 ± 0.05	0.32 ± 0.27
i17:1ω8	0.92 ± 0.34	3.98	1.10 ± 0.63	3.38 ± 4.83
17:1ω7	0.12 ± 0.11	0.90	0.33 ± 0.17	0.37 ± 0.37
17:1ω6c	0.42 ± 0.19	0.67	0.30 ± 0.08	0.57 ± 0.75
17:1ω6t	ND	ND	ND	ND
10Me16:0	ND	ND	1.60 ± 1.10	0.56 ± 0.41
i17:0	0.94 ± 0.23	13.14	1.15 ± 0.19	0.59 ± 0.27
Unknown 17	0.25 ± 0.08	ND	ND	ND
a17:0	1.17 ± 0.36	1.47	1.77 ± 0.18	1.15 ± 0.70
cy17:0	0.58 ± 0.17	0.93	2.62 ± 0.51	1.75 ± 0.18
17:0	0.36 ± 0.11	5.28	0.70 ± 0.09	0.45 ± 0.34
br18:0	0.28 ± 0.07	ND	0.07 ± 0.04	0.14 ± 0.24

(continued)

Microbiologic Analyses

The assumption that the samples studied contained methanotrophs was confirmed by the results of the Linköping and Uppsala samples, in which various type I methanotroph bacteria were indicated by the specific PLFAs 16:1ω8c, 16:1ω6c, and 16:1ω5t (Table 3). In the Linköping samples, type I methanotrophs were indicated by high levels of 16:1ω8c (2.1%), 16:1ω6c (2.3%), and 16:1ω5t (2.5%). In the Uppsala samples, the presence of type I methanotrophs was indicated by 16:1ω8c (4.2%), 16:1ω6c (1.9%), and 16:1ω5t (2.81%).

Table 3 *(Continued)*

PLFA	Jönköping (n = 3)	Kristianstad (n = 1)	Linköping (n = 4)	Uppsala (n = 4)
Unknown 18	0.16 ± 0.05	ND	ND	0.39 ± 0.34
18:4	0.22 ± 0.11	0.58	0.20 ± 0.03	0.25 ± 0.07
10Me17:0	0.42 ± 0.18	0.71	0.10 ± 0.10	0.45 ± 0.08
18:2	3.31 ± 1.22	ND	1.07 ± 0.10	3.71 ± 0.29
18:3	1.19 ± 0.38	2.02	ND	ND
18:1ω11c	0.01 ± 0.01	0.04	0.05 ± 0.01	0.02 ± 0.02
18:1ω11t	ND	0.03	ND	ND
18:1ω10c	0.04 ± 0.01	0.34	0.05 ± 0.01	0.05 ± 0.03
18:1ω10t	0.01 ± 0.02	ND	ND	0.01 ± 0.01
18:1ω9c	13.50 ± 5.04	11.99	4.02 ± 0.44	10.50 ± 4.32
18:1ω9t	ND	5.97	ND	0.29 ± 0.35
18:1ω8c	0.05 ± 0.02	0.06	0.09 ± 0.01	0.13 ± 0.13
18:1ω8t	0.02 ± 0.03	0.14	0.02 ± 0.03	ND
18:1ω7c	1.95 ± 0.72	2.93	21.95 ± 1.55	5.48 ± 2.65
18:1ω7t	ND	0.90	0.55 ± 0.46	0.06 ± 0.11
18:1ω6c	0.01 ± 0.01	0.02	0.02 ± 0.01	0.02 ± 0.01
18:1ω5c	0.02 ± 0.01	ND	0.07 ± 0.01	0.01 ± 0.01
18:1ω5t	ND	ND	ND	ND
18:0	3.86 ± 1.25	0.49	2.93 ± 0.11	2.01 ± 0.79
19:1a	4.59 ± 1.23	3.10	2.10 ± 0.19	0.68 ± 0.11
10Me18:0	25.84 ± 22.09	8.81	0.77 ± 0.07	0.50 ± 0.52
Unknown 19	5.31 ± 4.36	2.65	0.14 ± 0.06	0.03 ± 0.04
cy19/19:1	0.68 ± 0.50	5.96	0.26 ± 0.07	0.56 ± 0.30
20:4	0.09 ± 0.12	ND	ND	0.31 ± 0.49
20:5	0.86 ± 0.58	0.82	0.38 ± 0.06	0.09 ± 0.06
i20:0	0.32 ± 0.29	ND	0.01 ± 0.02	0.72 ± 0.10
20:2	0.76 ± 0.34	1.49	0.28 ± 0.07	ND
20:1	0.71 ± 0.34	0.33	ND	0.30 ± 0.07
20:0	0.97 ± 0.61	1.69	1.13 ± 0.15	0.60 ± 0.11
22:0	2.32 ± 1.46	0.47	4.87 ± 4.25	3.45 ± 0.92

[a]ND, not detected.

Biomarkers for type II methanotroph bacteria (PLFAs 18:1ω8c and 18:1ω8t) were present in all samples but only in substantial amounts in the samples from Kristianstad (0.14% of total molar volume; Table 3). Gram-negative bacteria, to which both types of methanotrophs belong, could also explain some of the large numbers of the PLFAs 18:1ω7c and 16:1ω7c, regarded as typical for this group *(9,10)*. These PLFAs were present in the Linköping samples at frequencies of 21.95 and 8.11%, respectively; comprised 5.48 and 23.02% of total PLFAs in the Uppsala samples, respectively; and can also be major constituents of methanotroph PLFAs.

In the samples from Jönköping, the PLFA 10Me18:0, with 25.8% mean value of total mol volume, dominated (Table 3). This PLFA was present

in substantial amounts in all the Jönköping samples, although not evenly distributed (SD = 22.1%). PLFA 10Me18:0 is a biomarker for some actinomycetes genera (*Nocardia spp., Mycobacterium spp., Rhodococcus spp. [11]*). These strains also have unusually large amounts of the PLFA 18:1ω9c, which was very common in the Jönköping samples as well (13.5%).

Fungi were quite low in number; the specific eukaryotic PLFA 18:2 *(12)* was observed in the samples from Jönköping (3.31%), Linköping (1.07%), and Uppsala (3.71%) but was below the detection limit in the samples from Kristianstad.

Methane Oxidation Capacity

After 2 wk of incubation, no significant methane consumption could be detected in any of the samples, despite the fact that PLFA analyses indicated growth of methanotrophs in the Linköping and Uppsala samples. The samples were frozen for 2 wk before the study, which may have caused damage to the cells and their methane-oxidizing ability. Type I methanotrophs in particular are known not to survive freezing very well *(13)*.

Discussion

Raw Gas Quality

Organic material that is classed as high-risk material (in terms of the bacterial content), such as waste from the food and slaughter industries, is sanitized by heating at 70°C for 1 h before digestion. However, even though the material is sanitized, some bacteria, such as methanotrophs, can survive the high temperatures in capsules that the bacteria develop for protection *(13)* and may not be completely eliminated in the sanitation process. Natural immobilization of microorganisms on the rings may therefore lead to accumulative growth even at the low BOD and COD of the process water in the plants. Particles may also enter the absorption column with the raw gas despite the fact that the gas is filtered before entering the upgrading system. This could explain why the upgrading plant in Uppsala, which digests waste from the slaughter industry, has microbial growth in the heat exchanger, which is positioned before the absorption column. Good filters or more efficient designs than those used today might prevent (some) particles from entering the upgrading system.

Water Quality

Because all the plants studied that use single-pass water wash (water from sewage treatment plants) have experienced problems with the growth of microorganisms on the pall rings compared with only one regenerating water-wash plant (which uses drinking water), it can be presumed that poor water quality may result in microbial growth. The single-pass plant in Kristianstad has tested drinking water as process water for some periods,

with good results as the microbial growth in the column declined. This is another indicator that water quality probably contributes to the growth. Because water from sewage treatment plants easily produces foam, it is important to add a foam-reducing agent to the water before it enters the process.

Temperature and pH

Many plants have observed that the pH of the process water is lower when temperatures are high. pH is therefore probably correlated with process water temperature. A lower pH can inhibit growth on pall rings, because most organisms prefer a neutral environment to enrich. Hence, a constant temperature, or at least a lower maximum temperature, of the process water could, to a certain extent, prevent growth. In general, most microorganisms have a growth optimum at higher temperatures and therefore benefit from high temperatures.

In a laboratory study by Gordienko et al. *(14)*, increasing pH in soil samples was found to thicken the EPS capsule produced by a methanotroph type I strain.

Column Design

It can be assumed that a lower water velocity through the column would give the organisms in the process water a greater opportunity to establish in the column. A high water velocity through the column would result in most organisms being prevented from establishing on the pall rings and would flush away already established organisms. However, some bacteria, such as methanotrophs, attach strongly to surfaces and would not be flushed away by a high water speed. Because plants with both high and low water velocity through the absorption column experience microbial growth, this parameter probably does not contribute to or inhibit growth on pall rings.

All of the plants studied had randomly packed the pall rings in their columns, and most of them were packed to about 80% of the total column volume. One simple experiment would be to pack the pall rings in the column in organized cells, so-called honey cells, to determine whether the upgrading efficiency would increase.

Microbiology

Data from the Linköping and Uppsala PLFA analyses were similar; both contained biomarkers for type I methanotrophs. The Linköping and Uppsala plants digest similar materials, such as waste products from the slaughter and food industries. Some differences between the two sites are that the two Linköping plants are of the regenerating type and use drinking water as process water, whereas the Uppsala plant is of the single-pass type and uses water from sewage treatment plants.

Type I methanotrophs have the ability to produce slime or EPSs *(5,15)*. High EPS production under high oxygen concentrations (10.5%) has been observed in a study by Wilshusen et al. *(16,17)* and could explain why large amounts of EPSs are assumed to be produced in the oxygen-rich environment of the desorption column of the Linköping plant. Methanotrophs are also able to produce other polymers, such as polyhydroxybutyrates *(5,18)* which could explain the difference in color and shape between the Linköping and Uppsala samples, but a more likely reason is that the sample from Uppsala suffered from age, and other organisms grazing on methanotrophs and slime could be responsible for the difference.

Actinomycetes were indicated in the Jönköping samples. These organisms are present in sewage treatment plants and cause scumming of sludge in the aeration tanks *(19–21)*. The actinomycetes lower the effluent water quality of the sewage treatment plant, survive in the cleaned wastewater *(22)*, and probably enter the absorption column with incoming process water. Actinomycetes are able to create a fungus-like mycelium (slime) *(23)* and are also common as air contaminants in soil and agricultural/waste composts *(23)* or in any decaying vegetation, where they break down organic substances and release carbon, nitrogen, and ammonia. Some actinomycetes have a characteristic earthy odor and can also give taste and smell to water *(23,24)*. This could explain the rank smell emitted by the Jönköping samples. Like other filamentous organisms *(25)*, extensive growth of actinomycetes could give rise to particulate matter, which is in accordance with the appearance of the samples from Jönköping. Actinomycetes contribute to the stability of slime. Like fungi, they are adapted to life on solid surfaces and can produce dry spores.

Preventative Measures

In-column cleaning is much more convenient than outside-column cleaning, owing to shorter operational disturbances and easier ways for the maintenance crews to clean the column. In cleaning outside of the column, there is no good technique for taking out the pall rings, which is time-consuming. It is most important to ensure that all growth is removed by cleaning; otherwise bacteria may establish quickly again.

Detergents for Cleaning

If preventative measures are considered to reduce the microbial growth, these measures should be tested and proven not to harm the plant equipment, contaminate the gas, or contaminate the outlet process water. The detergents used today for cleaning differ among upgrading plants and their effectiveness cannot be compared because the tolerance of microbial growth is unknown. There are also economic aspects, and some plants choose to lower the capacity instead of shutting down the upgrading plant to clean.

The most efficient detergents used are hypochlorite and concentrated caustic soda. The effects of using these detergents for in-column cleaning during operation have not been studied, but a minor operational disturbance was observed in the Henriksdal plant when using hypochlorite (Lind, M. and Wiklund, O., personal communication, September 23, 2004)). Hypochlorite has also been used to control other foam-producing filamentous bacteria in sewage treatment plants and in the anaerobic tank used for the production of biogas *(26,27)*.

To eliminate microbial growth on the pall rings, Svensk Biogas, owner of the Linköping upgrading plants, has tested several detergents in a laboratory experiment. In terms of time and efficiency, the best results were obtained with high alkaline detergents with a pH of 10.0 or higher, especially concentrated caustic soda *(28)*.

Methanotrophs are sensitive to salts such as NaCl, NH_4Cl (29), $(NH_4)_2SO_4$, and K_2SO_4 *(30)*. Salts in general cause cell death by osmotic stress, but high concentrations of salt may have a corrosive effect on the plant equipment. Other well-known inhibitors of methanotrophs are ammonia and acetylene (C_2H_2) (e.g., see refs. *31* and *32*). One percent acetylene in the gas volume in the absorption column is the concentration necessary to inhibit a methanotroph population *(29)*. Acetylene is presumably the most suitable of these chemicals, because it would not harm the process equipment, contaminate the biogas, or contaminate the process water. Acetylene is easily soluble in water (2.00 g/L), a characteristic that must be considered if it is to be used as a detergent.

Other Preventative Measures

The foam caused by actinomycetes, indicated in the Jönköping sample, may be inhibited by foam-reducing detergents used at sewage treatment plants (33). In addition, a low and constant temperature could be achieved by adding a cooling system to the water inlet. Furthermore, by adding a pH-lowering substance to the process water, microbial growth could, to some extent, be prevented or slowed down. Finally, organic material in the form of particles suitable for use by microbes may also enter with the raw gas. The use of good filters could prevent this.

Conclusion

The PLFA analysis proved to be an efficient method for determining biomarkers in samples from water wash in this study. In the samples from Linköping and Uppsala, type I methanotrophs were indicated. Further evidence was provided by the fact that type I methanotrophs are able to produce EPSs, which can appear as both slime and particulate matter as a reaction to excess carbon in environments containing oxygen *(5)*.

Actinomycetes, probably from the water of the sewage treatment plant, were indicated in the Jönköping samples. Actinomycetes produce foam, so to avoid foaming in the absorption column, a foam-reducing agent could be added to the process water.

Among factors affecting the development of microbial growth, one of the most important is water quality. It affects the upgrading process, and in most cases cleaner water gives less microbial growth. All plants using water from sewage treatment plants experienced substantial growth. pH and temperature are also important factors. A low constant temperature and a low pH are beneficial for minimizing microbial growth, by improving the solubility of CO_2 and making the environment unfriendly for bacteria.

Future research should focus on the effectiveness of the recommended cleaning detergents on microbial growth, and on testing improvements to the filter that the gas passes through before entering the upgrading process.

Acknowledgments

We would especially like to thank all personnel at the biogas plants for guiding and answering questions. We are also grateful to Elisabet Börjesson for help with the GC-MS analyses.

References

1. SGC (Swedish Gas Centre). (2001), Report SGC 118, SGC, Scheelegatan 3, SE-212 28 MalmÖ, Sweden.
2. Flotech. (2004), Technical Report, 2004. Flotech Sweden, Stockholm, Sweden.
3. Persson, M. (2003), MSc thesis, Lund Technical University, Lund, Sweden. (Also available as Report SGC 142, Swedish Gas Centre, Scheelegatan 3, SE-212 28 MalmÖ, Sweden, 2003).
4. Pinkart, H. C., Ringelberg, D. B., Piceno, Y. M., Macnaughton, S. J., and White, D. C. (1999), in *Manual of Environmental Microbiology*, 2nd ed., Hurst, C. H., Crawford, R. L., Knudsen, G., McInerney, M., and Stetzenbach, L. D., eds., American Society for Microbiology Press, Washington, DC, pp. 101–113.
5. Hilger, H. A., Cranford, D. F., and Barlaz, M. A. (2000), *Soil Biol. Biochem*. **32,** 457–467.
6. Börjesson, G., Sundh, I., Tunlid, A., and Svensson, B. H. (1998), *Soil Biol. Biochem*. **30,** 1423–1433.
7. Börjesson, G., Sundh, I., and Svensson, B. H. (2004), *FEMS Microbiol. Ecol*. **48,** 305–312.
8. Steger, K., Jarvis, A., Smårs, S., and Sundh, I. (2003), *J. Microbiol. Methods* **55,** 371–382.
9. Frostegård, Å., Tunlid, A., and Bååth, E. (1993), *Soil Biol. Biochem*. 25, 723–730.
10. Bååth, E. and Andersson, T. H. (2003), *Soil Biol. Biochem*. 35, 955–963.
11. McNabb, A., Shuttleworth, R., Behme, R., and Colby, W. D. (1997), *J. Clin. Microbiol*. **35,** 1361–1368.
12. Guckert, J. B., Antworth, C. P., Nichols, P. D., and White, D. C. (1985), *FEMS Microbiol. Ecol*. **31,** 147–158.
13. Bowman, J. P. (2006), in *The Prokaryotes: An* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K-H., and Stackenbrandt, E., eds., Springer-Verlag, New York, Part 1, Section 3, pp. 266–289.
14. Gordienko, A. S., Kurdish, I. K., and Kisten, A. G. J. (1997), *Water Chem. Technol*. **19,** 40–43.

15. Huq, M. N., Ralph, B. J., and Rickard, P. A. D. (1978), *Aust. J. Biol. Sci*. **31,** 311–316.
16. Wilshusen, J. H., Hettiaratchi, J. P. A., De Visscher, A., and Saint-Fort, R. (2004), *Environ. Pollut*. **129,** 305–314.
17. Wilshusen, J. H., Hettiaratchi, J. P. A., and Stein, V. B. (2004), *Waste Management* **24,** 643–653.
18. Fassel, T. A., Vanover, J. E., Hauser, C. C., Buchholz, L. E., Edmiston, C. E., Sanger, J. R., and Remsen, C. C. (1992), Cells Mater. **2,** 37–48.
19. Lechevalier, M. P. and Lechevalier, H. A. (1974), *Int. J. Syst. Bacteriol*. **24,** 278-288.
20. Lechevalier, H. A. (1975), Actinomycetes of sewage treatment plants. Environmental Protection Technology Series Report, EPA-600/2-75-031, US Environmental Protection Agency. P.O. Box 42419, Cincinatti, OH, 1975.
21. Lemmer, H. (1986), *Water Res*. **20,** 531–535.
22. Dizer, H. and Hagendorf, U. (1991), *Water Res*. **25,** 791–796.
23. Reponen, T., Gazenko, S. V., Grinshpun, S. A., Willeke, K., and Cole, E. C. (1998), *Appl. Environ. Microbiol*. **64,** 3807–3812.
24. Oppong, D., King, V. M., Zhou, X., and Bowen, J. (2000), *Ind. Microbiol. Biotechnol*. 25, 74–80.
25. Safade, T. L. (1988), *Paper Technol. Ind*. **29,** 280–285.
26. Blackall, L. L., Seviour, E. M., Bradford, D., Stratton, H. M., Cunningham, M. A., Hugenholtz, P., and Seviour, R. J. (1996), *Water Sci. Technol*. **34,** 137–144.
27. Käppala Industries. (2001), http://www.kappala.se/admin/varor/docs/miljorapporter2001.pdf. Ac-cessed October 17, 2005.
28. Andersson, F. A. (2003), Evaluation of detergents used for dissolving microbial growth on pall rings. PM 2003-11-05. Linköping: Tekniska Verken.
29. Adamsen, A. P. S. and King, G. M. (1993), *Appl. Environ. Microbiol*. **59,** 485–490.
30. Saari, A., Rinnan, R., and Martikainen, P. (2004), *Soil Biol. Biochem*. **36,** 1037–1046.
31. King, G. M. (1990), *FEMS Microbiol. Lett*. **74,** 309–323.
32. McDonald, I. R., Hall, G. H., Pickup, R. W., and Murrell, J. C. (1996), *FEMS Microbiol. Ecol*. **21,** 197–211.
33. CDM (2005), Foamaster ENA-389. Product Information. In Swedish. http://www.cdm.se. Accessed January 17, 2005.