Bacterial Degradation of Detergent Compounds

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A survey for surfactant degradation among aerobic bacteria has been undertaken. Tests have been made in peptone medium where such a degradation, if it occurs, will be gratuitous. Tallow-alkyl-sulfate, alkyl-ethoxylate-sulfate, and linear-alkyl-benzene-sulfonate were used. Forty-five strains of 34 species in 19 genera degrade one or more of these detergent compounds. With some species, the surfactant inhibits degradation without inhibiting growth, whereas with one species slight degradation took place even at a toxic concentration of surfactant.

Interest centers on microbial degradation of environmental pollutants. Detergent compounds have become an important component in wastewater. The conditions which favor microbial degradation in nature or in some future controlled process will reflect particular needs of the important species. Our research has sought, therefore, to identify the species capable of degrading detergent compounds. Although some work has been done in other laboratories, a systematic survey has been needed. This report summarizes findings with aerobic bacteria. Ordinarily, much organic matter will be available to the bacteria; therefore, we have carried out our tests in peptone medium in which the potentialities of species are more fully expressed and where degradation will be gratuitous. Very few species grow in defined medium with surfactant as sole carbon/energy source.

Huddleston and Allred (2) demonstrated gratuitous degradation of lauryl sulfate by pure cultures of Escherichia coli, Serratia marcescens, Proteus vulgaris, and Pseudomonas fluorescens. Payne and co-workers (3-5) and Hsu (1) have done extensive quantitative work with the genus Pseudomonas. One study extends these investigations. We examined 45 bacterial strains on 3 detergent compounds. Assays for degradation during growth in various concentrations of detergent were made. At the same time, assays for viable cell numbers were made to detect toxicity.

MATERIALS AND METHODS

Surfactants. Tallow-alkyl-sulfate (TAS), alkyl-ethoxylate-sulfate (AES), and linear alkyl-benzene-sulfonate (LAS) were generously provided by the Procter & Gamble Co., courtesy of J. R. Duthie, Environmental Water Quality Research Department, Ivydale Technical Center, Cincinnati, Ohio. TAS is CH₃(CH₂)₇OSO₂ONa, where x = 17. The stated purity was 92.7%, the balance being mostly sodium sulfate and water. AES is CH₃(CH₂)₉(CH₂CH₂O)VSO₂ONa, where x ≈ 11 and y ≈ 3. The stated purity was 24.4%, the balance being mostly water. LAS is R(CH₂CH₂O)VSO₂OH where R ≈ 11.8 carbons. The stated purity was 4.24%, the balance being mostly water. We are grateful to R. N. Sturm and J. R. Duthie for this information.

Bacteria. Nineteen genera, 34 species, and 45 strains of bacteria were analyzed (Tables 1-3). The sources of the cultures are given in a footnote to the tables. Most of the bacteria are well-authenticated strains; some are widely known through their use in current experimental fields. Anaerobic bacteria or species lacking respiratory pathways have not been investigated in detail because the few examined in preliminary tests were found to be nondegraders.

Media. Tryptase soy (TS) broth without glucose, purchased from Baltimore Biological Laboratory, was used as a general purpose growth medium and as growth medium in the analytical experiments. Stock cultures were maintained on TS broth enriched with 0.3% glucose and solidified with 1.6% agar (TSGA) and was also used in the plate counts.

Procedure. Bacteria from a stock culture were inoculated into 50 ml of TS broth in a 250-ml Erlenmeyer flask. The flask was incubated on a rotary shaking apparatus at 31øC until heavy growth occurred, usually 24 hr, and the viable cell count had risen to 10⁸ to 10⁹/ml. This culture was used as inoculum for three 500-ml Erlenmeyer flasks containing TS broth medium to test a given bacterial strain in a particular concentration of surfactant. One flask, containing 99 ml of TS broth without surfactant, served as a blank and received 1 ml of inoculum. The second flask contained 99 ml of TS broth with surfactant and also received 1 ml of inoculum (experimental flask). The third flask contained 99 ml...
of TS broth with surfactant, but received 1 ml of sterile medium in lieu of cells (standard flask). All flasks were incubated at 31°C on the shaker. After 24, 48, and 72 hr, samples were taken from all three flasks and assayed for viable cells per milliliter and for surfactant degradation. The “blank” flask provided the blank for the methylene blue photometric assay to detect surfactant degradation and also served as a reference to detect growth inhibition in the “experimental” flask caused by the surfactant therein. The “standard” flask provided surfactant standard for use in the photometric assay. A refrigerated, aqueous surfactant standard was also employed, on occasion, to verify that the incubation of surfactant with TS broth did not, by itself, affect the assay. Also, on occasion, cells were added to a sample from the standard flask immediately before assay to verify that the cells contributed no interfering substances. The standard flask also served as a sterility control to monitor our microbiological technique.

The methylene blue test was used to measure degradation. Since anionic surfactant binds methylene blue, the dye partitions with the surfactant in chloroform extracts. Suitably degraded surfactant, on the other hand, no longer binds methylene blue so the dye remains in the aqueous phase. Under controlled conditions (6), the quantity of dye in the chloroform extract is proportional to surfactant property. One can monitor degradation by photometric absorbance measurements at 652 nm (methylene blue absorption peak) on the chloroform extract. For a given surfactant, to which methylene blue binds, the method possesses a limitation. Loss of methylene blue binding is proportional to loss of surfactant property and not necessarily to full surfactant degradation. LAS which contains a benzene ring may lose surfactant property prior to losing the benzene moiety. The percentage of degradation summarized for LAS in Table 3 does not provide evidence for full degradation, but evidence only for degradation of that portion of the molecule which confers surfactant property. Nevertheless, the methylene blue test has value and is the only convenient method to measure gratuitous degradation without special equipment. We are interested only in that degree of degradation which leads to the loss of surfactant property, and for this the methylene blue test is suitable. (Where the surfactant can be used as sole carbon/energy source in growth medium, growth itself measures degradation. Few bacterial species can grow under such a circumstance.) The equation employed to calculate percentage of degradation is given in a footnote to the tables.

Growth was measured by plate counts. A suitably diluted 0.1-ml sample was spread on duplicate TSGA plates which were incubated at 31°C overnight or longer as required by the particular species. Assays were expressed as viable cells per milliliter. The cell yield was determined when the culture attained the maximum stationary phase of growth and will be defined as follows: cell yield = viable cells per milliliter after incubation – viable cells per milliliter at time of inoculation.

Estimates of surfactant toxicity were made by comparing cell yield in the experimental flask (containing surfactant) with that in the blank flask (lacking surfactant). These estimates are recorded as percentage of growth. The equation used to calculate growth is given in a footnote to the tables. In all experiments the bacterial inoculum had no previous exposure to surfactant. Our results were from unadapted cells.

The tables herein summarize only a few of our assays. Many preliminary assays were required to determine tolerances and the particular surfactant concentrations to employ. In most cases, we have presented results with only the highest surfactant concentration, permitting an appreciable and reproducible degradation. As examples, we have summarized results with two concentrations of surfactant with six of the strains. In a few instances negative degradation is tabulated for comparative purposes.

RESULTS AND DISCUSSION

Table 1 summarizes results with TAS at concentrations of 0.01, 0.05, and 0.5 g/liter. Table 2 summarizes results with AES at 0.01 and 0.1 g/liter. Table 3 summarizes results with the relatively toxic LAS where lower concentrations had to be used: 0.004, 0.01, and 0.02 g/liter.

Wide differences in ability to degrade surfactants may occur within a particular genus. For example, all three surfactants are degraded within 24 hr by Acetobacter suboxydans, whereas A. peroxidan had degraded no TAS in 72 hr and had degraded only 41 and 42%, respectively, of the available AES and LAS. Several species of Azotobacter were strong degraders, whereas A. beijerinckii was unable to withstand or appreciably degrade the three surfactants.

High concentration of surfactant may inhibit degradation to a greater degree than growth: Chromobacterium iodinum in TAS (Table 1), Pseudomonas species in LAS (Table 3), Sarcina lutea in LAS (Table 3). On the other hand, at toxic levels of surfactant some degradation may occur with killing: A. beijerinckii in TAS (Table 1). Usually, however, the result typified by Microbacterium flavum was observed; no degradation occurred at a surfactant concentration which was sufficiently toxic to kill the inoculum (Table 3).

The finding that the genus Azotobacter comprises powerful degraders was unexpected. This suggests that Azotobacter may be capable of a dual service: degrading detergent and fixing nitrogen while doing so. In preliminary work using defined medium in which nitrogen fixation may be easily measured, we have been unable to replace the usual carbon/energy
Table 1. Degradation of tallow-alkyl-sulfate (TAS) by bacterial cultures

| Bacterial strain* | Conc (g/liter) of TAS in culture | TAS degradation\(^t\) (%) in culture after incubating for (hr) | Growth\(^t\) (%) |
|-------------------|----------------------------------|-------------------------------------------------------------|-----------------|
|                   |                                  | 24              | 48              | 72              |                   |
| Acetobacter peroxydans ATCC 838 | 0.01                             | 0               | 0               | 0               | 20               |
| Acetobacter suboxydans ATCC 621  | 0.05                             | 100             | 100             | 100             |                   |
| Azotobacter agilis ATCC 7494     | 0.05                             | 87              | 100             | 100             | 70               |
| Azotobacter beijerinckii ATCC 19860| 0.01                            | 0               | 10              | 19              |                   |
| Azotobacter chromoocum ATCC 490  | 0.05                             | 91              | 100             | 100             |                   |
| Azotobacter insignis ATCC 12523  | 0.5                              | 100             | 100             | 100             |                   |
| Azotobacter vinelandii ATCC 12518| 0.5                              | 88              | 100             | 100             |                   |
| Azotobacter species AZ           | 0.05                             | 72              | 96              | 100             |                   |
| Bacillus cereus M                | 0.05                             | 72              | 93              | 100             | 60               |
| Bacillus subtilis M              | 0.05                             | 70              | 91              | 100             | 80               |
| Chromobacterium amethystinum ATCC6915| 0.05                        | 0               | 53              | 77              | 2                |
| Chromobacterium iodinum B-141    | 0.01                             | 66              | 85              | 100             |                   |
| Chromobacterium iodinum B-141*   | 0.05                             | 0               | 0               | 0               | 70               |
| Chromobacterium lividum ATCC 12473| 0.05                            | 68              | 79              | 96              | 100             |
| Chromobacterium violaceum ATCC 12472| 0.06                           | 56              | 80              | 91              | 30               |
| Cytophaga kremziewskiae M        | 0.05                             | 69              | 87              | 100             | 100             |
| Corynebacterium aquaticum ATCC 14665| 0.01                           | 43              | 89              | 100             | 100             |
| Corynebacterium hydrocarboclastus ATCC 15592 | 0.05                        | 0               | 59              | 96              | 100             |
| Corynebacterium hydrocarboclastus (oleophilus) ATCC 15108| 0.01                             | 59               | 59              | 100             |                   |
| Corynebacterium hydrocarboclastus (oleophilus) ATCC 15108* | 0.05                        | 11              | 67              | 100             | 100             |
| Corynebacterium mediolanum ATCC 14004| 0.01                            | 29              | 62              | 98              | 100             |
| Escherichia coli B/r             | 0.01                             | 0               | 0               | 0               | 100             |
| Escherichia coli G3404           | 0.05                             | 34              | 40              | 50              | 100             |
| Escherichia coli K-12            | 0.05                             | 6               | 12              | 20              | 30               |
| Escherichia coli Ma              | 0.05                             | 12              | 63              | 90              | 100             |
| Escherichia coli A20a            | 0.05                             | 6               | 30              | 67              | 30               |
| Flavobacterium dehydrogenans ATCC 13830 | 0.05                        | 6               | 100             | 100             |                   |
| Microbacterium flavum ATCC 10340 | 0.06                             | 38              | 82              | 100             |                   |
| Micrococcus cinnabareus LA 2.1   | 0.05                             | 100             | 100             | 100             |                   |
| Micrococcus flavus ATCC 400      | 0.01                             | 19              | 76              | 95              | 100             |
| Mima polymorpha ATCC 9967        | 0.01                             | 5               | 19              | 23              | 50               |
| Mycobacterium phlei M            | 0.05                             | 57              | 90              | 90              |                   |
| Mycobacterium smegmatis M        | 0.05                             | 93              | 93              | 2               |                   |
| Nocardia corallina M             | 0.05                             | 94              | 94              | 80              |                   |
| Proteus vulgaris E               | 0.06                             | 94              | 94              | 100             |                   |
| Pseudomonas aeruginosa 60L-4     | 0.05                             | 93              | 93              | 90              |                   |
| Pseudomonas fluorescens Md.U.    | 0.05                             | 51              | 60              | 87              | 50               |
| Pseudomonas species 40.1         | 0.05                             | 65              | 93              | 100             | 30               |
| Sarcina lutea ATCC 9341          | 0.05                             | 49              | 64              | 89              | 40               |
| Serratia marcescens ATCC 274     | 0.05                             | 94              | 94              | 100             |                   |
| Sew\(A\) (Pseudomonas species)   | 0.05                             | 29              | 40              | 53              | 80               |
| Sew\(N\)(Pseudomonas species)    | 0.05                             | 100             | 100             | 100             |                   |
| Sew\(E\)                       | 0.05                             | 100             | 100             | 100             |                   |
| Sew\(I\)                       | 0.05                             | 100             | 100             | 100             |                   |
| Staphylococcus albus M           | 0.05                             | 48              | 81              | 91              | 100             |
| Vibrio commaus ATCC 6972         | 0.05                             | 57              | 90              | 98              | 100             |
| Vibrio paralaeus ATCC 8461       | 0.05                             | 78              | 78              | 40              |                   |

* Strains designated ATCC were purchased from the American Type Culture Collection, Rockville, Md. Azotobacter strain AZ was isolated by us from soil. The Sew strains were isolated by us from activated sludge acquired from the local sewage treatment plant. Strains designated M were provided by Frank Engley, University of Missouri Medical School. The other strains were from our own collection.

\(^{t}\) Determined by the methylene blue test and calculated by means of the following equation, where the symbol \(A_{652}\) represents absorbance at 652 nm: percentage of degradation \(= \frac{100 - (\frac{[A_{652}]\text{experimental} - \frac{[A_{652}]\text{blank}}{[A_{652}]\text{standard}})\times100}}{100}\).

\(^{c}\) Determined by the following equation: percentage of growth \(= \frac{\text{(cell yield in experimental/ cell yield in blank)}}{x}\) 

\(^{d}\) Inoculum killed. No growth.

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sources such as mannitol with surfactant. These studies continue and will be reported elsewhere. Nitrogen fixation using "spent" detergent as energy source by Azotobacter alone or by Azotobacter in conjunction with biochemically complementary species is an attractive idea from the ecological point of view.

The Sew strains were isolated from mixed culture, growing together in defined, mineral salts medium in which TAS was sole carbon/energy source. Micrococcus cinnaba- reus, Pseudomonas aeruginosa, and P. fluorescens will also grow in defined medium with TAS as sole carbon/energy source. AES will serve as carbon/energy source but LAS will

| Bacterial strain | Conc (g/liter) of AES in culture | AES degradation (%) in culture after incubating for (hr) | Growth (%) |
|------------------|----------------------------------|--------------------------------------------------------|------------|
| Acetobacter peroxidan ATCC 838 | 0.01 | 40 | 40 | 41 | 30 |
| Acetobacter suboxydans ATCC 621 | 0.01 | 96 | 96 | 96 | 90 |
| Azotobacter agilis ATCC 7494 | 0.01 | 53 | 91 | 100 | 100 |
| Azotobacter beijerincki ATCC 19360 | 0.1 | 0 | 0 | 0 | - |
| Azotobacter chroococcum ATCC 480 | 0.1 | 39 | 74 | 93 | 100 |
| Azotobacter insignis ATCC 12523 | 0.1 | 37 | 75 | 100 | 80 |
| Azotobacter vinelandii ATCC 12518 | 0.01 | 62 | 95 | 2 | 2 |
| Azotobacter species AZ | 0.01 | 10 | 50 | 100 | 100 |
| Bacillus cereus M | 0.01 | 100 | 100 | 100 | 100 |
| Bacillus subtilis M | 0.01 | 98 | 98 | 98 | 80 |
| Chromobacterium amethythinum ATCC 6915 | 0.01 | 14 | 73 | 100 | 100 |
| Chromobacterium iodinum B-141 | 0.01 | 21 | 89 | 100 | 70 |
| Chromobacterium luidum ATCC 12473 | 0.01 | 24 | 94 | 7 | 7 |
| Chromobacterium violaceum ATCC 12472 | 0.01 | 93 | | 100 | 100 |
| Cytophaga kuzumiewskae M | 0.01 | 65 | 96 | 90 | 90 |
| Corynebacterium aquaticum ATCC 14665 | 0.01 | 23 | 49 | 99 | 100 |
| Corynebacterium hydrocarbonoblastos ATCC 15592 | 0.01 | 10 | 25 | 49 | 30 |
| Corynebacterium hydrocarbonoblastos (oleiphilus ATCC 15108) | 0.01 | 55 | 93 | 99 | 100 |
| Corynebacterium mediolanum ATCC 14004 | 0.01 | 89 | 89 | 89 | 100 |
| Escherichia coli B/r | 0.01 | 24 | 67 | 70 | 100 |
| Escherichia coli G3404 | 0.01 | 46 | 84 | 90 | 90 |
| Escherichia coli K-12 | 0.01 | 71 | 72 | 72 | 60 |
| Escherichia coli Ma | 0.01 | 67 | 90 | 100 | 60 |
| Escherichia coli A20a | 0.01 | 79 | 97 | 97 | 90 |
| Flavobacterium dehydrogenans ATCC 13930 | 0.01 | 75 | 74 | 1 | 1 |
| Micrococcus flavum ATCC 10340 | 0.01 | 64 | 82 | 84 | 50 |
| Micrococcus cinnabaureus LA.2.1 | 0.01 | 97 | | 100 | 100 |
| Micrococcus flavus ATCC 400 | 0.01 | 70 | 89 | 100 | 70 |
| Mina polymorpha ATCC 9957 | 0.01 | 13 | 15 | 15 | 50 |
| Mycobacterium phlei M | 0.01 | 25 | 99 | 100 | 100 |
| Mycobacterium smegmat M | 0.01 | 89 | | 100 | 100 |
| Nocardia corallina M | 0.01 | 61 | | 100 | 70 |
| Proteus vulgaris E | 0.01 | 100 | | 100 | 100 |
| Pseudomonas aeruginosa 60L-4 | 0.01 | 100 | | 90 | 90 |
| Pseudomonas fluorescens Md.U. | 0.01 | 42 | 91 | 1 | 1 |
| Pseudomonas species 40.1 | 0.01 | 95 | | 100 | 100 |
| Sarcina lutea ATCC 9341 | 0.01 | 30 | 84 | 100 | 100 |
| Serratia marcescens ATCC 274 | 0.01 | 96 | | 100 | 100 |
| Sew (Pseudomonas species) | 0.01 | 86 | | 70 | 70 |
| Sew | 0.01 | 99 | | 100 | 100 |
| Sew | 0.01 | 100 | | 100 | 100 |
| Sew | 0.01 | 94 | | 100 | 100 |
| Staphylococcus albus M | 0.01 | 41 | 88 | 100 | 100 |
| Vibrio commaetus ATCC 6972 | 0.01 | 95 | | 100 | 100 |
| Vibrio paralutus ATCC 8461 | 0.01 | 100 | | 100 | 100 |

*See footnotes, Table 1.*
### Table 3. Degradation of linear alkyl-benzene-sulfate (LAS) by bacterial cultures

| Bacterial strain          | Conc (g/liter) | LAS degradation (%) | Growth (%) |
|---------------------------|----------------|---------------------|------------|
|                           | of LAS in culture | in culture after incubating for |             |
|                           |                | (hr)                |            |
|                           |                | 24 | 48 | 72 |             |
| *Acetobacter peroxydans* ATCC 838 | 0.004 | 40 | 42 | 42 | 60          |
| *Acetobacter suboxydans* ATCC 621 | 0.004 | 100 |    |    | 50          |
| *Azotobacter agilis* ATCC 7494 | 0.02 | 70 | 88 | 100 | 1          |
| *Azotobacter beijerinckii* ATCC 19380 | 0.004 | 0 | 0 | 0 | _d |
| *Azotobacter chroococcum* ATCC 480 | 0.02 | 64 | 87 | 95 | 100        |
| *Azotobacter insignis* ATCC 12523 | 0.02 | 51 | 68 | 100 | 90         |
| *Azotobacter vinelandii* ATCC 12518 | 0.02 | 23 | 45 | 89 | 50         |
| *Azotobacter species* AZ | 0.004 | 87 |    |    | 90          |
| *Bacillus cereus* M | 0.004 | 100 |    |    | 100         |
| *Bacillus subtilis* M | 0.004 | 97 |    |    | 100         |
| *Chromobacterium amethythinum* ATCC 6915 | 0.01 | 69 | 82 |    | 100         |
| *Chromobacterium iodinum* B-141 | 0.01 | 24 | 37 | 93 | 30         |
| *Chromobacterium luidum* ATCC 12473 | 0.01 | 75 | 88 |    | 100        |
| *Chromobacterium violaceum* ATCC 12472 | 0.004 | 80 |    |    | 60         |
| *Cytophaga kuzmieniewskae* M | 0.004 | 83 |    |    | 100        |
| *Corynebacterium aquaticum* ATCC 14665 | 0.004 | 100 |    |    | 100        |
| *Corynebacterium hydrocarboclastus* ATCC 15592 | 0.004 | 0 |    |    | _d  |
| *Corynebacterium hydrocarboclastus* (oleophi- lus) ATCC 15108 | 0.004 | 61 |    |    | 30         |
| *Corynebacterium mediolanum* ATCC 14004 | 0.004 | 99 |    |    | 100        |
| *Escherichia coli* B/r | 0.004 | 38 | 68 | 97 | 100        |
| *Escherichia coli* G3404 | 0.004 | 76 | 77 |    | 100        |
| *Escherichia coli* K-12 | 0.01 | 33 | 47 | 61 | 10         |
| *Escherichia coli* Ma | 0.004 | 41 | 68 | 100 | 100        |
| *Escherichia coli* A20a | 0.004 | 62 | 93 |    | 60         |
| *Flavobacterium dehydrogenans* ATCC 13930 | 0.004 | 50 | 93 |    | 100        |
| *Microbacterium flavum* ATCC 10340 | 0.004 | 10 | 51 | 78 | 70         |
| *Microbacterium flavum* ATCC 10340 | 0.01 | 0 | 0 | 0 | _d  |
| *Micrococcus cinnabareus* LA.2.1 | 0.004 | 67 |    |    | 100        |
| *Micrococcus flavus* ATCC 400 | 0.004 | 63 | 85 |    | 100        |
| *Mima polymorpha* ATCC 9957 | 0.004 | 32 | 33 | 32 | 100        |
| *Mycobacterium phlei* M | 0.004 | 0 | 0 | 0 | _d  |
| *Mycobacterium smegmatis* M | 0.004 | 10 | 60 | 81 | 100        |
| *Mycobacterium smegmatis* M | 0.01 | 0 | 0 | 0 | _d  |
| *Nocardia corallina* M | 0.01 | 67 |    |    | 100        |
| *Proteus vulgaris* E | 0.01 | 98 |    |    | 90         |
| *Pseudomonas aeruginosa* 60L-4 | 0.01 | 97 | 0 |    | 90         |
| *Pseudomonas fluorescens* Md. U. | 0.01 | 36 | 70 | 94 | 30         |
| *Pseudomonas species* 40.1 | 0.004 | 87 |    |    | 100        |
| *Pseudomonas species* 40.1 | 0.01 | 0 | 0 | 0 | _d  |
| *Sarcina lutea* ATCC 9341 | 0.004 | 69 | 80 | 87 | 60         |
| *Sarcina lutea* ATCC 9341 | 0.01 | 14 | 53 | 74 | 60         |
| *Serratia marcescens* ATCC 274 | 0.01 | 88 |    |    | 100        |
| *Sewo* (Pseudomonas species) | 0.004 | 87 |    |    | 100        |
| *Sewo* | 0.004 | 93 |    |    | 80         |
| *Sew* | 0.01 | 71 |    |    | 100        |
| *Sew* | 0.01 | 50 |    |    | 70         |
| *Staphylococcus albus* M | 0.01 | 87 |    |    | 80         |
| *Vibrio cuneatus* ATCC 6972 | 0.01 | 94 |    |    | 100        |
| *Vibrio paralae* ATCC 8461 | 0.01 | 73 | 84 |    | 100        |

* See footnotes, Table 1.
not. LAS is very toxic in mineral salts medium.

Most of the 19 genera tabulated are strong gratuitous degraders of surfactants under our laboratory conditions. Their relative importance in soil, water, and sewage remains to be assessed.

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