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Degradation of the surfactant Cocamidopropyl betaine by two bacterial strains isolated from activated sludge

Marketa Merkova, Michal Zalesak, Eva Ringlova[1], Marketa Julinova, Jan Ruzicka*

Department of Environmental Protection Engineering, Tomas Bata University in Zlin, Faculty of Technology, Vavreckova 275, 760 01, Zlin, Czech Republic

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ABSTRACT

Cocamidopropyl betaine (CAPB) is an amphiphilic surfactant commonly used in a variety of personal care products and in some technical applications. The aim of the study was to obtain bacteria that utilized CAPB from a sample of municipal activated sludge, and to investigate the possible role such bacteria performed in surfactant degradation. The CAPB (300 mg l^{-1}) degradation experiments involved the application of two isolated strains. Whilst tests in a mineral medium containing ammonium salt as a nitrogen source revealed almost complete mineralization of the compound in both strains during 4 days, the same process required more than 29 days of incubation under nitrogen-free conditions. Degradation assays and a series of growth tests with and without the source of nitrogen showed that *Pseudomonas* sp. FV proved to be the primary degrader of CAPB, capable of utilizing the alkyl chains of the surfactant. The other strain, *Rhizobium* sp. FM, ensured the degradation of intermediates originating from the primary biodegradation stage and, in the absence of ammonium salt, provided a supply of nitrogen to its microbial partner.

1. Introduction

The frequent use of personal care products is a typical aspect of a modern lifestyle. Huge amounts of these enter municipal wastewater systems daily, most of which are subjected to microbial degradation during wastewater treatment processes.

Cocamidopropyl betaine (CAPB) is a zwitterion primarily employed as a surfactant in cosmetic products due to its great utility as an antistatic and hair conditioning agent, skin conditioning agent, surfactant-cleansing agent, surfactant-foam booster and viscosity increasing agent (Jacob and Amini, 2008). In addition, some engineering or biological operations can utilize the properties of CAPB for certain purposes, e.g. to shorten the stabilization process of waste activated sludge (Zhou et al., 2017) and to mitigate harmful algal blooms (Sun et al., 2004).

CAPB belongs to the amidopropyl betaine group, and its chemical structure is usually derived from coconut oil and dimethylaminopropylamine. The predominant fatty acid in coconut oil is lauric acid (C_{12}), although other fatty acids (C_8–C_{18}) are also present in minor proportions. CAPB is usually marketed as a clear, pale yellow liquid, which is soluble in water, ethanol and isopropyl alcohol (Anonymous, 2005). The structure of prevailing lauramidopropyl betaine is given in Fig. S1.

Several scientific studies have demonstrated rapid primary aerobic biodegradation of CAPB over a duration of 24–168 h, depending on the given microbial community (Eichhorn and Knepper, 2001; Sun et al., 2004; Vonlanthen et al., 2011; Zhou et al., 2017). As for ultimate aerobic biodegradation of CAPB, Gheorghe et al. (2012) recorded 80–81% biodegradability after 28–30 days. Despite this, little is known about the bacterial species actually responsible for aerobic degradation of CAPB during the wastewater treatment process. Consequently, this study set out to isolate and describe the bacterial members involved in aerobic CAPB degradation, as well as to study the potential roles of such bacteria in surfactant mineralization and to evaluate the importance of the available nitrogen source in the degradation process.

2. Materials and methods

2.1. Chemicals and biological materials

The CAPB was obtained from Enaspol (Czech Republic) in the form of Flavol KDA, which is a 30% solution containing approximately 5% NaCl. According to the data provided by the producer, the fatty acid profile of Flavol KDA comprises (in %): lauric a. (acid) 44–51, myristic a. 13–18, palmitic a. 7–10, caprylic a. 5–9, oleic a. 5–8, capric a. 4–10, stearic a. 1–4 and linoleic a. 1–3.

* Corresponding author. Tel.: +420 576 031 221.
E-mail addresses: merkmark@centrum.cz (M. Merkova), michal.zalesak@iaeste.cz (M. Zalesak), eva.ringlova@inspiringbeauty.cz (E. Ringlova), julinova@ft.utb.cz (M. Julinova), ruzickaj@ft.utb.cz (J. Ruzicka).
[1] Present address: Inspiring Beauty, Bratislavska 1488/6, 102 00, Praha, Czech Republic.

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Lauramide and betaine anhydrous were purchased from TCI Europe, sodium dodecanoate (laurate sodium) from Sigma-Aldrich and the remaining chemicals from Penta, Czech Republic.

The sample of activated sludge originated from a municipal wastewater treatment plant in Zlin, the Czech Republic. In brief, the sludge was washed and decanted three times with tap water and aerated for 24 h. Afterwards, the biomass was harvested by centrifugation (3000 *g*, 10 min, 20 °C) and suspended to the final concentration of 0.5 g dry weight per litre using basic salt medium (Julinova et al., 2017).

2.2. Growth media and nutrient agars

The mineral medium (MM, in g l^{-1}, if not otherwise stated): KH_2PO_4 0.18, $Na_2HPO_4{\cdot}12H_2O$ 1.92, NH_4Cl 0.3, $MgSO_4{\cdot}7H_2O$ 0.1, $FeSO_4{\cdot}7H_2O$ 0.01, $CaCl_2$ 0.01, NaCl 0.5, trace element solution 0.5 ml (Muchova et al., 2009).

The nitrogen-free mineral medium (NFMM) mirrored the MM detailed above, excluding NH_4Cl from its composition.

The mineral agar (MA) for strain isolation comprised: K_2HPO_4 1.0, NH_4Cl 1.1, NaCl 1.0, $MgSO_4{\cdot}7H_2O$ 0.2, $FeSO_4{\cdot}7H_2O$ 0.01, $CaCl_2$ 0.01, agar 19.0, trace element solution 1 ml.

The nutrient agars: R2A and Tryptone yeast extract agar (Himedia, India).

2.3. CAPB biodegradation in activated sludge

The CAPB was applied at the concentration of 200 mg l^{-1}. The process was monitored by the MicroOxymax respirometric system (Columbus Instruments, USA), in accordance with Muchova et al. (2009), and evaluated as the ratio of measured carbon dioxide production to carbon content of the initial sample. Blank tests without substrate were carried out in parallel and their results subtracted; the tests were performed in duplicate.

2.4. Isolation of bacterial strains

The bacterial strains were isolated from sludge suspensions sampled at the close of tests for CAPB biodegradation. Each suspension was inoculated into fresh mineral medium amended with sterile CAPB (500 mg l^{-1}) and incubated on a rotary shaker in darkness for 2 weeks at 25 °C. Afterwards, the resulting suspensions were gently disintegrated by sterile glass beads, and several dilutions were spread onto plates containing the R2A and Tryptone yeast extract agars, mineral agar (MA) and mineral agar amended with sterile CAPB (500 mg l^{-1}). Following incubation at 25 °C, the most numerous colonies growing on the R2A and TYA agars, and whichever colonies exhibited more conspicuous growth on MA with CAPB than on MA alone, were then isolated and purified.

2.5. Degradation tests

Tests to investigate CAPB degradation were carried out in 500 ml bottles containing 50 ml of sterile MM or NFMM, to which the sterile stock solution of CAPB was added to arrive at the final concentration of 300 mg l^{-1}. After inoculation with the individual strains or a combination of the strains, all bottles were incubated in the dark at 25 °C on a rotary shaker for 2–4 weeks. Samples were taken from each bottle, and an automatic analyser (Shimadzu 5000A, Japan) was used to determine the level of dissolved organic carbon (DOC), after cell removal by centrifugation (10 000 *g*, 12 min, 15 °C). Bacterial growth was monitored by the optical density at 600 nm (OD_{600}) measurements. If not otherwise stated, every test was carried out in triplicate.

2.6. Description and identification of strains

The isolates obtained were Gram-stained, characterized using common microbiological characteristics and identified by 16S rDNA sequence analysis (Krizek et al., 2015).

Testing for how the selected substrates were utilized by the strains was carried out thus:

Sterile portions of MM were individually amended with sterile lauramide and the sodium salts of caprylic, lauric, palmitic or stearic acids so as to reach the final concentration of 100 mg l^{-1}; in the case of the sterile stock solution of betaine, the final concentration equalled 300 mg l^{-1}. Each prepared medium was inoculated with a single strain and incubated in darkness at 25 °C for 2 weeks. When values for OD_{600} were gauged at approximately 0.02–0.2 or higher during incubation, they were considered positive results. All the tests were done in triplicate, and non-inoculated media and inoculated MM without any substrate were used as blanks. Moreover, the growth of the strains on lauramide and betaine in nitrogen-free mineral medium was examined.

3. Results

3.1. Biodegradability of CAPB in activated sludge and isolation of CAPB-utilizing bacteria

Fig. S2 details the course of CAPB biodegradation at its initial concentration of 200 mg l^{-1}. The data obtained showed relatively fluent degradation of the surfactant. In total, around 10 days were required to complete the process of substrate consumption, after which 62.5% of carbon mineralization was observed. This result formed a good prerequisite for obtaining CAPB-utilizing microbes, and samples of the two final sludge suspensions were applied to further enrich the desired microorganisms. Afterwards, inoculation was performed of diluted sub-samples onto the aforementioned nutrient agars. After incubating the plates, a very similar pattern in the occurrence of bacterial colonies was observed in both samples. Several types of colonies grew on the agar plates, although two types of colony predominated above all others. With respect to dilution of the samples, the counts for the two dominant bacteria in the enriched sludge suspensions reached approximately 10^7 CFU ml^{-1}, whilst the figures for the remaining bacterial members equalled 10^5 CFU ml^{-1} or lower. Therefore, quite reasonably, these two prevailing bacteria were considered the main utilizers of CAPB, hence they were re-inoculated, purified and tested in the further part of the study.

3.2. CAPB degradation by single strains

The above-mentioned predominant bacteria were designated FV and FM, and a series of tests was carried out on CAPB degradation by each single strain and their binary consortium. The process was monitored by both DOC and OD_{600} determinations and the results are graphically presented in Fig. 1A.

As is evident, contrasting results were obtained for CAPB degradation in the individual strains and in combination of the same. Whilst the FV strain alone partially used the substrate for its growth, consuming approx. 36% of the DOC, the FM strain on its own proved unable to grow in the slightest. Notably, though, combining both strains mutually ensured almost complete CAPB biodegradation in just 4 days, therein utilizing more than 90% of the organic carbon.

In order to elucidate the role of the FM strain in the degrading process, the final suspensions after 14 days of CAPB degradation by the single strains were individually collected, the cells removed by centrifugation and the supernatant fluids filtrated through sterile 0.22 μm filters. Then a sterile fluid originating from CAPB degradation by the FV strain was inoculated by the FM strain and *vice versa*. The DOC concentrations of both fluids were measured after 7 and 14 days of further incubation; the subsequent findings are given in Table 1.

Research conducted on the degradation of the supernatant fluids showed that the FM strain depleted a substantial proportion of the organic compounds present in the medium subsequent to CAPB