Degradation of n-valeric acid by alginate-entrapped *Alcaligenes denitrificans*

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**Summary.** *Alcaligenes denitrificans* was isolated from sewage sludge and showed a strong degradative ability towards volatile fatty acids. The organism was tested both as free cells and immobilised in calcium alginate, for the ability to degrade the sodium salt of a typical volatile fatty acid, valeric acid.

In shake flask culture the immobilised cells could be used to fully degrade 18 mM valerate over ten 48 h runs before bead break up occurred. The use of beads in conventional stirred tank fermenters, and a bubble column reactor was also investigated, with a 50 ml bubble column containing 5 ml of beads giving the highest overall degradation rate of 1.8 mmol/h, for 40 h in a fed batch mode of operation.

**Introduction**

Volatile fatty acids (VFAs) are important chemicals in the field of odour control. Early investigations into sewage chemistry revealed that there is a high occurrence of VFAs in sewage sludge (e.g., see Montgomery et al. 1962). Work by Williams and Evans (1981) has shown a close correlation between VFA concentration and the odour offensiveness of stored and fresh pig slurries.

There is considerable interest in the removal of odorous compounds from wastes, and in particular the use of isolated microbial strains which are able to degrade the compounds specifically.

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Ohta and Ikeda (1978), isolated strains of Actinomycetes from pig faeces, which were able to deodorise piggery wastes. In later work, (Ohta and Sato 1985) a deodorant organism, *Corynebacterium* sp. no 922, was found to have a broad specificity for growth on fatty acids varying from formic acid to valeric acid.

Favourable results on chemical degradation by immobilised microorganisms have been obtained by Rehm and co-workers in a series of studies. Bettmann and Rehm (1984) used a *Pseudomonas* sp. immobilised in alginate and polyacrylamide hydrazide to degrade up to 2 g/l phenol in an air lift fermenter. Degradation of phenol was also studied using two organisms, a *Pseudomonas* sp. and a *Candida* sp., immobilised on activated carbon particles (Erhardt and Rehm 1985), resulting in an increased tolerance of the organisms to phenol. Westmeier and Rehm (1985) studied the biodegradation of chlorophenol by alginate entrapped *Alcaligenes* sp. A7-2. Several reactor configurations were tested, and degradation rates of 1.44 mmol/day were obtained in stirred tank reactors. However, problems were encountered using this configuration, namely pH instability in Tris buffered medium and bead disintegration in phosphate buffered medium.

This study reports an investigation of the potential use of a newly isolated strain of *Alcaligenes denitrificans* for VFA degradation. The aims of the study were to investigate the substrate specificity of the strain, and the ability to metabolise concentrations of VFA typically found in waste streams. In particular it was intended to compare the use of the strain in two forms; as free growing cells or as cells entrapped in alginate beads. A variety of bioreactor designs were used to study the potential of using entrapped cells for VFA degradation in liquid wastes.
Materials and methods

Culture isolation and media

The organism was isolated during screening procedures of soil, sewage and water samples. Two different media were used during the study. Both were made up from concentrated stock solutions.

(A) Tris buffer. 1.25 M Tris-HCl, pH 7.2, containing 50 mM KH_2PO_4

(B) Phosphate buffer, 1 M KH_2PO_4, adjusted to pH 7 with sodium hydroxide

(C) Nitrogen source. 0.85 M (NH_4)_2SO_4

(D) Trace metal solution. MgCl_2·6H_2O (63.5 g); CaSO_4 (1.25 g); ZnO (0.25 g); FeNO_3·9H_2O (3.3 g); MnCl_2·4H_2O (0.62 g); CuCl_2·2H_2O (0.11 g); CoCl_2·2H_2O (0.15 g); H_2BO_4 (0.04 g); H_2SO_4 (10 ml) in 1 l.

The media were made up as follows: a phosphate buffered medium was used for the isolation of the organism, consisting of 40 ml of solution B, 12 ml of solution C in 1 l of distilled water. All other experiments with free cells and with alginate beads were carried out using a Tris buffered medium, consisting of 40 ml solution A, 12 ml solution C in 1 l of distilled water. Both media were sterilised by autoclaving for 30 min at 121°C and then sterile trace metal solution D was added to a final concentration of 2 ml/l.

Cells were stored on agar plates at room temperature, either on nutrient agar (Oxoid), or a minimal agar. This was prepared by the addition of 1.5% Oxoid agar No. 1 to the salts medium and then addition of sterile sodium valerate solution to the cooling agar at a final concentration of 18 mM.

Substrate

N-valeric acid was obtained from BDH Chemicals (Dagenham, Essex), quoted purity 98%. The other acids used in this study were from the same source, and had purities ranging from 97.5 to 99%. Sodium valerate was prepared by the addition of 1 M sodium hydroxide solution to valeric acid, and monitoring the neutralisation using a pH electrode. The valerate solution was autoclaved and added to the medium prior to incubation.

Fermentation conditions

All fermentation experiments were carried out at 37°C.

I Shake flask incubations were used for isolation of the culture and initial experiments. 250 ml and 2000 ml baffled Erlenmeyer flasks were used, containing 50 and 250 ml of medium, respectively. A standard 10% (v/v) inoculum was used throughout. Flasks were shaken in an orbital incubator at 100 rpm.

II Fermentations were carried out in a LH series 500 fermenter (LH Engineering, Stoke Poges, UK). The working volume of the tank was 750 ml. The fermenter could be stirred at 500 rpm either by the use of a under mounted magnetic stirrer, or by a paddle connected to an overhead motor. The aeration rate was set at 500 ml/min.

III A bubble column reactor was constructed, consisting of a Pyrex tube of internal dimensions 300×25 mm (working volume 50 ml). The ends were sealed with silicon rubber bungs and air was supplied through a sintered glass plate via a flow controller and rotameter. Temperature was maintained by the use of an external heating coil and a circulating water bath.

Analytical methods

Cell growth was monitored by optical density at 660 nm using a Pye-Unicam PU8610 spectrophotometer. Valerate concentrations were monitored by gas chromatography. A Pye Unicam PU4500 chromatograph was used, with a Porapak P column. Oven temperature was 195°C, the carrier gas was nitrogen at 30 ml/min, and detection was by flame ionisation detector. Small aliquots (0.5 μl) of whole broth or buffer were injected without extraction.

Immobilisation of cells

Cells were immobilised using the method of Bettmann and Rehm (1984). Flasks were inoculated with a small quantity of Alcaligenes denitrificans removed from an agar plate. After incubation for 20 h, corresponding to early stationary phase of growth, cells were harvested by centrifugation (10 min, 10000g) and washed in Tris buffered medium. The cells were centrifuged again and resuspended in a 4% solution of sodium alginate (BDH Chemicals Ltd.) in Tris buffered medium. During the formation of beads, the cell concentration was maintained so that direct comparisons between free and immobilised cells could be carried out. Typically, 50 ml of cell culture were centrifuged and resuspended to a final volume of 50 ml in alginate solution. Beads were formed by dripping the alginate solution through a 0.5 mm diameter syringe needle into sterile 2% calcium chloride solution, stirred magnetically in a beaker. The beads were hardened for 1 h in the calcium chloride solution, washed with water and stored in the Tris buffered medium.

Oxygen electrode

Oxidation of various substrates by free cell suspensions was followed using a polarographic oxygen electrode (Rank Brothers, Bottisham, UK), to obtain information on the substrate range of the strain. Experiments using the various acids as sole carbon source in shake flask culture were used to confirm the substrate utilising ability of the strain.

Results

Substrate specificity

Information on the substrate specificity of the strain was obtained using the oxygen electrode. The results are shown in Table 1. Cells were grown in shake flasks on valerate, and were then prepared and tested for oxygen uptake rates with various other volatile fatty acids.

Simultaneous experiments with the various acids as sole carbon source in shake flask culture were used to confirm the electrode studies.