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A laboratory study of survival of selected microorganisms after heat treatment of biowaste used in biogas plants

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Abstract

The aim of the study was to assess the effect of pasteurisation, as set by the European regulation EC 1774/2002, on selected pathogens and indicator organisms.

Unpasteurised substrate (biowaste), including animal by-products from a full-scale biogas plant was heat treated under laboratory conditions at 70 °C and 55 °C for 30 min and 60 min.

Heat treatment at 55 °C for 60 min was not sufficient to achieve a hygienically acceptable product. Heat treatment at 70 °C for 30 min and 60 min was effective in reducing pathogenic bacteria, *Ascaris suum* eggs, Swine vesicular disease virus and indicator organisms. However, this level of pasteurisation will still not reduce the quantity of Clostridia spores, or completely inactivate heat-resistant viruses such as Porcine parvovirus or *Salmonella* phage 28B.

The results still give cause for some concern regarding the use of digested residue from biogasplants in agriculture.

Keywords: Anaerobic digestion; Fertiliser; *Campylobacter*; Enterococci; *E. coli* O157

1. Introduction

In full-scale biogas plants (BGP) in Sweden, different kinds of biowaste, though not sewage sludge, are used to produce biogas. Animal by-products (ABP) such as manure, slaughter by-products, blood, lipids and other biowaste, e.g. biological household waste separated at source and waste from food industries, can contain pathogens infectious to humans and animals. The digested residue is used as a fertiliser and soil improver, and must therefore be hygienically safe, to avoid spreading diseases through the environment to all forms of animal life and humans. Hygienically safe or acceptable is here defined as indicator bacteria and salmonella being below detectable limits with the methods used in this study or with other similar standard methods.

At the time of the present study, Swedish law requires BGPs that use animal waste to pasteurise the incoming substrate at 70 °C for 60 min before digestion, to ensure a hygienically acceptable product. In May 2003, a new European regulation (EC 1774/2002) concerning ABPs was implemented, replacing laws set by the member states of the EU that previously regulated the use of animal waste. The EC-regulation divides ABPs into three categories, depending on the expected degree of pathogenic contamination. Category 1 material, which could contain

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prions, must be incinerated. Category 2 material, including carcasses (of animals other than those in category 1 material), must be sterilised (133 °C at 3 bar for 20 min) before further treatment in a BGP. Manure is included in Category 2 material, but it is allowed to be used in BGPs without sterilisation, if not deemed contagious by the authorities. Category 3 material includes ABPs from healthy animals approved for human consumption, and requires pasteurisation at 70 °C for 60 min before use in a BGP, if it is kept separate from material in Categories 1 and 2. As Category 3 material and manure may contain agents that are potentially infectious for people and animals, it is important that the pasteurisation process is sufficiently thorough to kill pathogens. The substrate used in BGPs is rarely investigated in terms of pathogenic microorganisms and their reduction after heat treatment. The difference between this substrate and other media is the heterogeneity and variation of the mixture and the potentially rather large (maximum 12 mm) particles. The effect of pasteurisation can be studied using pathogenic microorganisms or indicator bacteria or both. *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter coli*, *C. jejuni* and verotoxin-producing *Escherichia coli* O157 (VTEC) have all been isolated from substrate in full-scale BGPs. All are zoonotic agents and important bacteria causing infectious disease amongst humans and animals and many are resistant to environmental conditions.

The aim of the present laboratory study was to assess the effect of heat treatment at 70 °C on the survival of selected pathogens and indicator organisms in substrate from a large-scale BGP in Sweden. Heat treatment at 55 °C under similar conditions was also investigated due to the well-founded commercial interest in lower treatment temperatures.

We tested the microorganisms described below, as well as indigenous indicator bacteria such as coliforms, thermotolerant coliforms, presumptive *E. coli* and enterococci in survival trials. The substrate was heat-treated and the survival of the inoculated pathogens was determined after 15 (only for *A. suum*), 30 min and 60 min.

Clostridium perfringens is commonly found in substrate from BGPs and was chosen here as an indicator for spore-forming bacteria (Carrington, 2001). Porcine parvovirus (PPV) causes reproductive failure in swine and was selected as a viral indicator (Lund et al., 1996) due to its high thermo-resistance (Haas et al., 1995; Kim et al., 2000). Swine vesicular disease virus (SVDV) is not as resistant to heat as PPV (Herniman et al., 1973; McKercher et al., 1980), but has been used in inactivation studies regarding slurry (Turner et al., 1998). SVDV is a picornavirus and belongs to the genus *Enterovirus*, together with Bovine and Porcine enterovirus (King et al., 2000). In sanitation stipulations for biogas reactors, picornavirus is proposed as an indicator (Lund et al., 1996). Various types of bacteriophages have been suggested as viral indicators and indicators of faecal contamination (Havelaar et al., 1991). They are similar to viruses in structure, but easier and cheaper to analyse and harmless to all animals. *Salmonella* typhimurium phage 28B (Lilleengen, 1948) has been successfully added to sewage and used to trace leakage from sewage to groundwater (Johansson et al., 1998; Carlander et al., 2000) and has also been used as a process evaluator for liquid composting (Eller, 1995). However, it has not previously been compared with viruses with respect to heat resistance. *Salmonella* phage 28B does not occur naturally in either environmental samples or faeces. *A. suum* eggs are one of the most heat-resistant parasitic ovas, and are therefore suitable as an indicator of parasite survival (Feachem et al., 1983).

2. Methods

2.1. Substrate

The substrate used in this study was untreated, mixed biowaste from a large-scale BGP in Sweden, consisting of waste from food industries, biological household waste separated at source, and Category 3 ABPs such as manure, blood, fat, etc. The substrate was collected in clean vessels from a tap on the homogenisation tank before pasteurisation at the BGP. It was then chilled in a water bath (+8 °C) before transportation in a cold-box to the National Veterinary Institute (SVA), Uppsala, where it was kept in a refrigerator (+4 °C) at SVA before the pasteurisation trial started within 24 h. Because several trials were performed, there were several batches of substrate; hence, the content and subsequently the structure of the substrate may have varied, as raw material used in large-scale BGP normally varies over time.

An analysis was performed before every pasteurisation trial in order to check for pathogens in the original substrate and to check that no phage was present in the substrate to interfere with the *Salmonella* serovar typhimurium type 5 host bacteria.

The supplier of media in this study was SVA, unless otherwise stated.

2.2. Bacterial strains

Pathogenic bacteria inoculated into the substrate were from the strain collection at SVA: *Salmonella enterica* subsp. *enterica* serovar typhimurium, Culture Collection, University of Gothenburg (CCUG) 31969, *L. monocytogenes* CCUG 15527, *E. coli* O157 CCUG 8018 and *C. jejuni* CCUG 11284. The bacterial strains were stored at −70 °C. They were streaked onto blood agar plates and incubated at 37 °C for 24 h, before enrichment in serum broth at 37 °C for 24 h. The final concentration of bacteria in the suspension was approx 10^8 CFU ml^{-1}, except for *C. jejuni*, which had a concentration of approx 10^7 CFU ml^{-1}. The concentration was determined based on 10-fold dilutions in buffered peptone water and colony counts on agar media, as described under Bacterial analysis, except