EVALUATION OF A PRACTICAL END EFFICIENT METHOD FOR DETERMINING ALTERNATIVE TIME-TEMPERATURE REGIMES FOR EFFECTIVE SANITATION IN DIGESTION TREATMENT PLANTS

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ABSTRACT

Anaerobic digestion plants that treat category 3 materials (and/or manure) have to comply with the European regulations concerning treatment of Animal By-Products (ABP) EU (No) 1069/2009 and EU (No) 142/2011. As an alternative for operating an expensive, energy-consuming pasteurization unit operating at 70°C , 1 hour batches with particle size <12mm, a process validation (*Prozessprüfung*) can be executed. This includes a study in which an alternative time-temperature regime is defined by demonstrating that under these conditions adequate reduction of biological risks is also ensured. In the Netherlands we developed a standardized measurement protocol (NTA8777:2011) to measure reductions of pathogens during exposure inside the reactor by introducing an *Enterococcus faecalis* culture as a test-organism in a suitable test body. This procedure is fully in line with the ABP regulation and has been applied for official validation studies in 13 digestion plants in The Netherlands and Belgium. This paper evaluates the results obtained from these 13 studies. Results show that the inactivation rate of *E. faecalis* is in this dataset not responsive to temperature changes between 51,3°C to 54,3°C. Observed D-values averaged 0,8 hrs, but fluctuated significantly between 0,09 to 1,53 hrs. The only value obtained below 51,3°C (at 50,5°C) indicates that the inactivation rate reduces rapidly at lower temperatures, resulting in a needed minimal guaranteed retention time (MGRT) of 40 hrs. Fluctuations in D-values were not determined by reactor type (wet slurry vs. dry plug-flow) nor by main input regime (organic household waste vs. manure with co-substrates). Our results indicate that *E. faecalis* inactivation measured using the NTA8777:2011 is repeatable and applicable to different reactor types and diets. Nevertheless, between and within reactors, D-values can still fluctuate considerably, indicating the necessity for location-specific studies at each site. Therefore we conclude that NTA8777:2011 is a very effective tool to safeguard a biologically safe end product at thermophillic temperature regimes.

KEY WORDS: *Enterococcus faecalis, process validation, sanitation, digestion plant, thermofillic time-temperature regime*

INTRODUCTION

Anaerobic digestion has in recent decades been effectively developed into a reliable technology to produce 'green' energy from organic materials. The organic inputs may include animal by-products (category 3 materials) like catering waste, over-date supermarket products, organic household waste and/or manure (category 2 material). If manure and/or cat 3 materials are applied, these plants have to comply with the European regulations concerning safe treatment of Animal By-Products (ABP) EU (No) 1069/2009[1] and EU (No) 142/2011[2]. This involves primarily the elimination of (spreading) biological hazards linked to transport of these substrates. Pathogenic micro-organisms in these processes are mainly inactivated by their exposure to high temperatures inside the reactor. To guarantee effective hygienization of the substrate a controlled temperature regime has to be implemented in the treatment process. The ABP regulation has defined standard transformation parameters for a pasteurization unit in which material must be submitted to a minimum guaranteed retention time (MGRT) of 1 hour at 70°C with particle size ≤12mm. The implementation of such a hygienization unit is often unwanted as it represents a high investment, high maintenance and exploitation costs and a reduction of overall availability of the installation due to an increased risk of operational failure.

The ABP regulation allows also for a pathogen reduction during the treatment process using alternative transformation parameters, when this is properly studied, documented and audited by the national competent authority. The demonstration of adequate reduction of biological risks is defined as a process validation (*Prozessprüfung*) which includes measuring a reduction of viability/infectivity of indicator organisms during the process. The minimal risk reduction that needs to be achieved in a treatment process is a reduction of 5 log10 of *Enterococcus faecalis* or *Salmonella Senftenberg* (775W, H2S negative) and, when identified as a relevant hazard a 3 log10 reduction of infectivity titre of thermoresistant viruses. When manure is part of the input of the digester, the ABP regulation states that only *E. faecalis* used as indicator, if the digestate is to be applied as 'treated manure' and transport to other member states is an option.

Standard spot-test analyses are sometimes possible focusing on endogenous indicator organisms provided that they are consistently present in the raw material in high numbers. In reality this is rarely the case as input composition (qualitatively and quantitatively) fluctuate based on market prices and seasonal availability. Besides, suitable indicator organisms are rarely present in high numbers. Therefore the ABP regulation allows for a measurement of die-off of pathogens during exposure by introducing a test-organism in a suitable test body.

As no further description of the measurement procedure is formulated in the ABP regulation, we initiated the development of a standard test procedure for quantifying the inactivation of a well-characterized test organism together with the Dutch Standardization Institute (NEN) and a board of stakeholders including the Dutch Food and Drug Authority (NVWA). This resulted in a practical procedure published as a Netherlands Technical Agreement (NTA 8777:2011).

This paper evaluates the results from 13 validation studies using the standardized method for determining the inactivation rate of *E. faecalis* in anaerobic digestion facilities in The Netherlands. Evaluation of the dataset focuses on the repeatability of results, the applicability of the procedure at different temperature levels, reactor types and substrates.

MATERIAL AND METHODS

Thirteen anaerobic digestion plants were included in this evaluation carried out from 2007-2012 in the Netherlands and Belgium. The plants can be categorized in 4 groups based on their diet and mode of operation (see table 1).

Table 1: overview of digestion units included in the study

	co-digestion	mono-digestion	*total*
Slurry	8	1	*9*
plugflow	1	3	*4*
total	*9*	*4*	**13**

Most plants (9) are typified as co-digesters that are typically fed with a diet of a minimum of 50% manure complemented with mainly maize and other agricultural waste. Also small quantities of glycerine and other category 3 materials may be added to elevate biogas production. Also four industrial mono-digesters were included, that are fed exclusively (source separated) organic household waste. Within these groups two types of reactors are distinguished: (i) a stirred tank reactor with a dry matter content of around 12% and a mean retention time of 20-40 days and (ii) a plug-flow reactor with a dry matter content of around 20-25% and a mean retention time of 15-20 days.
The pathogen reduction studies were carried out conform the standardized procedure NTA8777:2009[3]. The principle is based on the exposure of substrate spiked with *Enterococcus faecalis* to the reactor conditions inside specific test body. A string of test bodies is placed inside the reactor after which at specific time intervals, test bodies are removed and analyzed for *E. faecalis* levels. The pathogen die-off rate over time is measured as the D-value, which represents the number of hours it takes to reduce *E. faecalis* with one logunit (i.e. by 90%). To our opinion, this procedure is even more in line with the requirements of EU (No) 1069/2009 and EU (No) 142/2011 compared to the procedure formulated in the '*Bioabfallverordnung*'. The NTA8777:2009 demonstrates more accurate if a reduction of 5 log10 of the indicator organism is taking place under the given process conditions but also demonstrates which MGRT is needed. But at the same time it is very targeted and efficient to execute. However, to be sure of reliable test results, it is of high importance that

the inspecting organization is certified according to ISO/IEC 17020 (inspection)

RESULTS AND DISCUSSION

Results show that the inactivation rate of *E. faecalis* is not highly responsive to temperature fluctuations in the range of 51,3°C to 54,3°C (maximum temperature level in the dataset). Observed D-values averaged 0,8 hrs, but still fluctuated significantly between 0,09 to 1,53 hrs (see figure 1). This is in line with results shown in a review article of Sörqvist in 2003[1] reporting D-values of *E. faecalis* in a comparable medium and temperature of 1,06 hrs. This results in MGRT's (time required for a 5 logunit reduction) of 4 hours (ranging from 0,45 to 7,7 hrs).

The only value obtained at a temperature below 51,3°C indicates that the inactivation rate can reduce rapidly at lower temperatures, increasing the D-value to above 8 hr resulting in an MGRT of 40 hrs. Fluctuations in D-values were not significantly influenced by reactor type (wet slurry vs. dry plug-flow digester) nor main input regime (mainly organic household waste vs. manure with agricultural co-substrates).

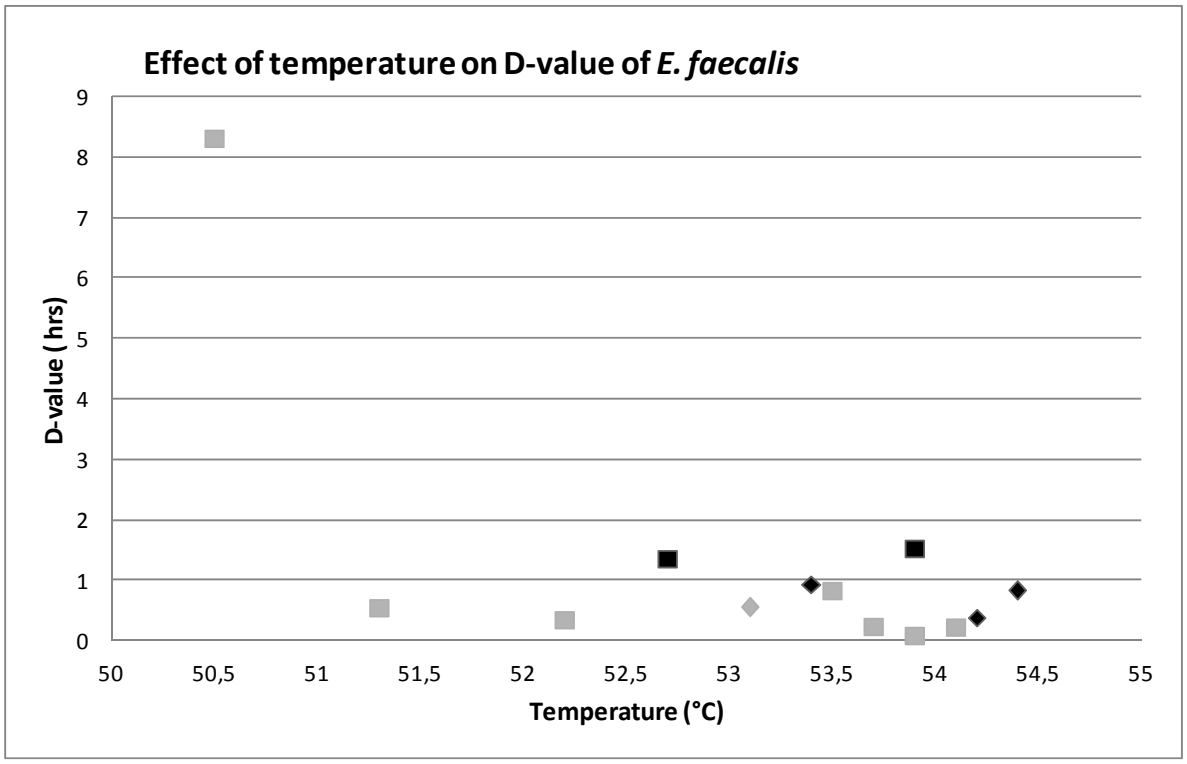

Figure 1: The effect of temperature inside the reactor and inactivation rate of the indicator organism *E. faecalis*. Black symbols represent results obtained from dry digestion in plug-flow reactors , gray symbols represent results from slurry reactors (wet digestion). Squares and diamonds represent reactors with major input components represented by manure (co-digestion) and organic household waste respectively.

The results were compared to with other results obtained studies to verify the repeatability of our methodology. Studies were selected based on test organism (*E. faecalis*) and growth conditions (liquid medium; anaerobic digestion (see figure 2). The figure indicates that our results are much in line with those of other findings proved by the 88% of the variation explained by the trendline. For a clearer evaluation the log D-values were plotted on the y-axis, showing the linear relationship of temperature and log D-values in the range of 30°C to 72°C. The relationship makes it possible to give an indication of the MGRT inside the reactor that are required at different temperature levels to obtain the reduction of 5 logunits (see table 2).

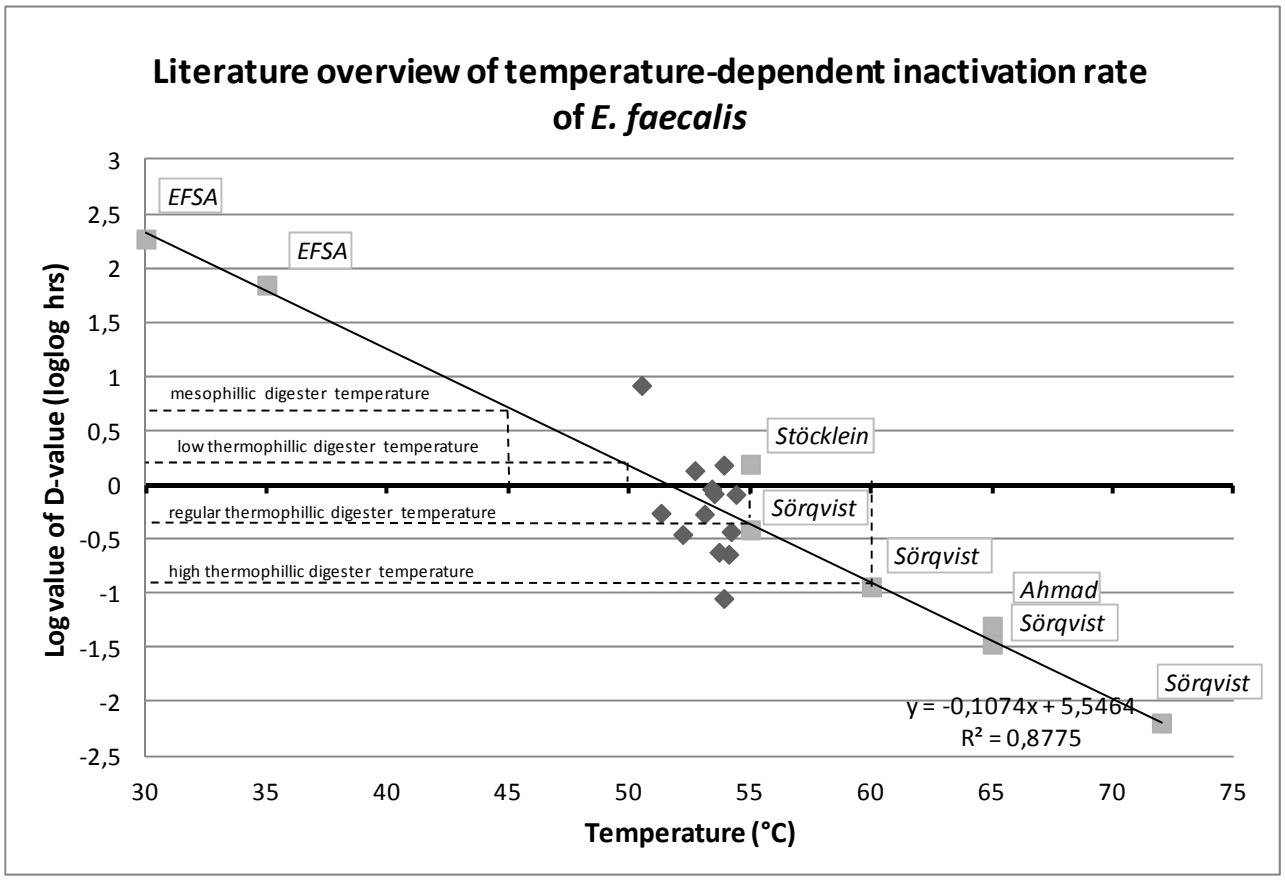

Figure 2: A comparison of the inactivation rates of *E. faecalis* of our studie (dark diamonds) with results found in the literature from comparable study conditions (light squares) of different sources including Stöcklein[7] , Sörqvist[8] , Ahmad et al[9] and three reports of the EFSA[456]. Values are plotted on a logarithmic scale of the D-values measured at different temperatures. The trendline is plotted over all values.

Table 2: Indication of the minimal guaranteed retention time (MGRT) required attaining pathogen die-off required in the European Animal By-Products regulation at different temperature levels.

Temperature *(°C)*	**log D-value** *(log hr)*	**D-value** *(hr)*	**MGRT** *(hr)*
45	0,7	5,0	25,1
50	0,2	1,6	7,9
55	-0,3	0,5	2,5
60	-0,9	0,1	0,6

CONCLUSIONS

Although the dataset is far from complete, our study indicates that *E. faecalis* inactivation measured using the standardized measurement procedure (NTA8777:2011) is repeatable and applicable to different reactor types and diets. Nevertheless, between and within reactors, D-values still fluctuate considerably, indicating the necessity for location-specific studies at each site. Apparently there are other (not measured) parameters that influence the reduction rates in anaerobic digesters like pH, conductivity etc. Therefore we conclude that process validation using the standardized inactivity test NTA8777:2011 is a very effective tool to validate elimination of biological hazards under well defined, alternative, more suitable time-temperature regimes. Our results combined with those of comparable studies with *E. faecalis* show additionally a log-linear relationship between die-off rates (D-values) and temperature. Judging from this relationship we recommend

the process validation approach only to reactors at thermophylic temperature regimes above 50°C to stay within suitable MGRT's.

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