

Semi-Empirical Modeling of Heat Inactivation of Enterococci and Clostridia During the Hygienisation in Anaerobic Digestion Process

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Abstract : Agricultural anaerobic digestion consists in the conversion of animal slurry and manure into biogas and digestate. They need, however, to be treated at 70 °C during 60 min before anaerobic digestion according to the European regulation (EC n°1069/2009 & EU n°142/2011). The impact of such heat treatment on the outcome of bacteria has been poorly studied up to now. Moreover, a recent study¹ has shown that enterococci and clostridia are still detected despite the application of such thermal treatment, questioning the relevance of this approach for the hygienisation of digestate. The aim of this study is to establish the heat inactivation kinetics of two species of enterococci (*Enterococcus faecalis* and *Enterococcus faecium*) and two species of clostridia (*Clostridioides difficile* and *Clostridium novyi* as a non-toxic model for *Clostridium botulinum* of group III). A pure culture of each strain was prepared in a specific sterile medium at concentration of 10⁴ - 10⁷ MPN / mL (Most Probable number), depending on the bacterial species. Bacterial suspensions were then filled in sterilized capillary tubes and placed in a water or oil bath at desired temperature for a specific period of time. Each bacterial suspension was enumerated using a MPN approach, and tests were repeated three times for each temperature/time couple. The inactivation kinetics of the four indicator bacteria is described using the Weibull model and the classical Bigelow model of first-order kinetics. The Weibull model takes biological variation, with respect to thermal inactivation, into account and is basically a statistical model of distribution of inactivation times as the classical first-order approach is a special case of the Weibull model. The heat treatment at 70 °C / 60 min contributes to a reduction greater than 5 log₁₀ for *E. faecium* and *E. faecalis*. However, it results only in a reduction of about 0.7 log₁₀ for *C. difficile* and an increase of 0.5 log₁₀ for *C. novyi*. Application of treatments at higher temperatures is required to reach a reduction greater or equal to 3 log₁₀ for *C. novyi* (such as 30 min / 100 °C, 13 min / 105 °C, 3 min / 110 °C, and 1 min / 115 °C), raising the question of the relevance of the application of heat treatment at 70 °C / 60 min for these spore-forming bacteria. To conclude, the heat treatment (70 °C / 60 min) defined by the European regulation is sufficient to inactivate non-sporulating bacteria. Higher temperatures (> 100 °C) are required as far as spore-forming bacteria concerns to reach a 3 log₁₀ reduction (sporicidal activity).

Keywords : heat treatment, enterococci, clostridia, inactivation kinetics

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