Effectiveness of thermal sanitization of piggery slurry using heat exchangers

C. Cunault, C. Burton, A.M. Pourcher

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Cunault C.[1,2], Burton C.[1], Pourcher A.M[1,2.]
[1]Cemagref-Rennes : 17, avenue de Cucillé, 35044 Rennes cedex, France. Tel: +33 (0) 223.48.21.39.
[2] Université Européenne de Bretagne, France;
charles.cunault@cemagref.fr

1 INTRODUCTION

Livestock agriculture dominated by pig rearing is responsible for the production large volumes of wastes. Across the European Union there are over 151 million pigs that produce 300 million tonnes of slurry annually. Furthermore, intensive production linked to certain regions, leads to zones of nutrient excess where available land for land spreading is insufficient. However, even if the environmental problems that affect these zones have been long recognised and largely dealt with, the concern now lies equally with sanitary aspects. Questions are now asked on the relationship between land spreading of animal manures and the faecal contamination of groundwater (and sometimes bathing water) and the outbreaks of specific disease or general ill health. Over 150 zoonoses have been isolated from livestock slurries with a variable prevalence, but often not negligible. These include the bacteria *Campylobacter sp*, *Escherichia coli* O157:H7 and *Salmonella sp* ; the viruses enterovirus and hepatitus E; the protozoa *Cryptosporidium parvum* and *Giardia lamblia* ; and the helminths *Ascaris suum* and *Taenia solium* (Levasseur, 2007). Pathogens are today considered to be a rising barrier to recycling manures to land for crop nutrition. Furthermore, within the context of a growing demand of water quality for domestic use, it is becoming necessary (a) to find methods for sanitisation that are economically acceptable, (b) to establish effective regulations and (c) to establish and manage the hygienic risks. Within these definitions, the work presented here is set out to establish the effectiveness of sanitation of continuous thermal treatments based on the use of heat exchangers. The specific germs studied were the classic indicators (*E. coli* and enterococci), coliforms, aero-anaerobic flora and the bacteriophages F+ specific and somatic.

2 MATERIALS AND METHODS

2.1 Sanitization process and the means of evaluation

The continuous thermal treatment process developed, comprises two heat exchangers (HE1 et HE2) and a means to sustain the temperature (C) as shown in Figure 1 below:

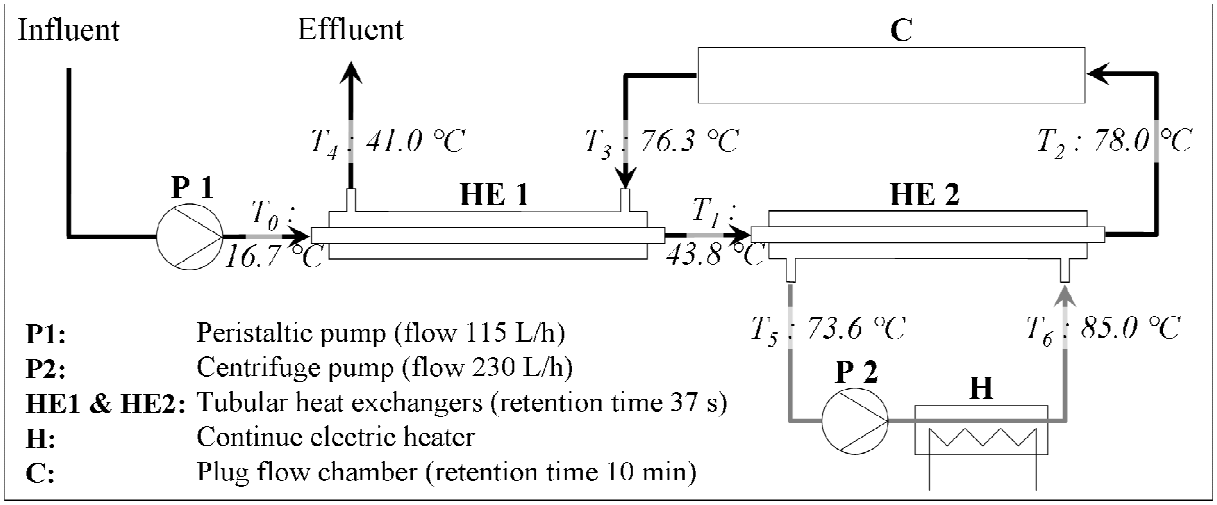

FIGURE 1 **Schematic diagram of the thermal treatment systems including heat recovery option.**

Two tubular heat exchangers made of 316 stainless steel were used for pilot studies. They comprised a series of double tube units each of a nominal 3 meters length and an interior diameter of 9.7mm. These units were joined in series to make a total length of 15 meters (5 units) for HE1 and 9 meters (3 units) of HE2. The liquid effluent stream being heated passed through the inner tube, whereas the heating fluid passed countercurrent through the external jacket formed by the two tubes. The exchanger HE1 allowed the heating of the slurry from 16.7 to 43.8°C solely from recovering the heat from the returning hot effluent in the jacket. The second exchanger, HE2 completed the heating, raising the temperature to a final set value of 78°C using an external heat source via hot water. For the slurry, the circulation flowrate was 115 litres/hour (pump P1) : for the circulating hot water (pump P2), the flowrate was 230 litres/hour. Fluid velocity was judged turbulent in both cases with a flow pattern close to "plug flow": the retention time was calculated as 75 seconds for the slurry on its heating cycle. The heating rate approximated to linearity with a mean value of 51 deg.C per minute.

Once heated to the set temperature, the slurry was held at this condition by means of passing through a retention vessel (C) made from a length of PER tube (length 125 metres and internal diameter 14.8 mm) rolled up and held in a heated chamber. The flow of slurry though this unit was deemed very close to plug flow with a calculated retention time of 11.25 minutes. The objective had been identified to achieve a minimal residence time of 10 minutes for the processed effluent. The higher actual time period ensures this condition is met noting a non perfect plug flow identified by prior tracer studies. The entry temperature of the slurry was noted as 78°C cooling slightly to 76.3°C at the exit from this unit.

Noting the small interior diameters of the tubular elements of HE1 and HE2 and of the retention unit (C), the raw pig slurry used for the trials was centrifuged before being used in trials. This pretreatment (which is not uncommon in the management of livestock effluents) allowed the removal from the liquid phase of hair, straw, fine stones and other particles larger than 5 mm that could otherwise lead to a blockage. It is of note that the separation removed no more than 50% of the total original dry matter (40 ± 1 mg/g) of which little would have been deemed reactive.

The thermal treatment carried out in these trials comprised of heating this effluent to 78°C over 75 seconds, sustaining a temperature above 76.3°C for 10 minutes then cooling to a final temperature of 41°C over 37 seconds.

2.2 Evaluation of the treatment efficiency

The sanitising performance from the thermal treatment was evaluated from the counts of selected microbiological indicators originally present in the pig slurry. Samplings were done before centrifugation (raw effluent), just after the feed pump (P1), just after the second heat exchanger (HE2) and at the exit from the retention unit (C). 50ml samples were taken and immediately put into an ice pack. The system, being continuous, allowed to take all the samples at the same time. A duplicate was taken after an interval of 15 minutes.

The counting of the coliforms at 30°C, *E.coli*, enterococci, and aero-anaerobic flora were carried out following the published standard NF EN ISO 8199:2008 on agar culture media respectively: Mac Conkey (Biokar) at 30 °C/24 hours, TBX (Biokar) at 44°C/24 hours, Slanetz (Biokar) 37 °C/48 hour then transfer to BEA (Biokar) 44°C/3 hour and finally TSYE (Biokar) 37 °C/72 hours. In order to improve the threshold of detection for the *E. coli* and the entereococci, checks for presence/absence in 10mL samples of non-diluted slurry were done with the media colilert®-18 et Enterolert™-E following the defined method of Substrat Technology® d'IDEXX.

The counting of virus indicators (phages F+ specific and phages somatics) was done by following the published standard NF EN ISO 10705:2001 parts 1 and 2.

The densities of slurry before centrifugation and following the pump, P1 were measured as 1.017 and 1.008 kg/L respectively: it was thus noted then that 1g of slurry was equivalent to 1ml. The results of viability were expressed as CFU (colony forming units) or PFU (plaque forming units) per millilitre this being closely equivalent to the same value per gram of raw effluent.

3 RESULTS AND DISCUSSION

3.1 Slurry pre treatment

The effect of centrifugation of raw pig slurry and of thermal treatment on the supernatant produced in terms of the numbers of microorganisms is set out in Tables 1 and 2. Of special note is that centrifugation had no effect on

numbers as might have been expected. A similar observation was reported by Levasseur (2007). This result justifies the choice of centrifuged effluent as model for studying effect of thermal treatment.

3.2 Resistance of indicators bacteria to thermal treatment

For the coliforms, *E. coli* and the entereococci, simply heating to 78°C is enough to achieve a reduction of 4 log10 units or more (Table 2). Holding at the elevated temperature (above 76°C) increases further the efficiency of treatment. On the other hand, the thermal treatment applied did not achieve more than 2 log10 units of reduction on aero-anaerobic flora which remained at concentrations above 10^5 CFU/mL following the period of retention at the raised temperature. It has been already established that vegetative form of spore forming bacteria are removed at temperatures of 80°C following a 10 minutes exposure (Setlow, 2006). Thus, noting the lack of distinction of the broad measures used, one might expect that the microorganisms quantified after treatment under "aero-anaerobic flora" could be spores. These could include spore formers such as *Bacillus* which have already been reported as present in animal slurries (Peu, 2006; Levasseur, 2007). Amongst these types of bacteria, some are known as pathogenic for man (Levasseur, 2007). Thus, such a treatment, even if adequate for coliforms, *E. coli* and enterococci would be insufficient for a non-negligible number of spore forming pathogens that might be present. Furthermore, the thermal shock on wastewaters are known to select and support the development of a flora including the spore formers *Bacillus* and *Clostridium* (Valdez-Vazquez, 2009). The number of pathogenic organisms in the effluent could, in some conditions, increase following an inadequate thermal treatment.

TABLE 1 **The effect of centrifugation on the concentration of selected indicators**

Microorganisms	**Mean[a] in CFU or PFU / mL**	
	Raw slurry	**Centrifuged slurry**
Coliforms at 30°C	1.6×10^5	1.4×10^5
E. coli	8.7×10^4	3.0×10^4
Enterococci	1.7×10^5	1.9×10^5
Aero/anaerobic bacteria	4.4×10^7	6.0×10^7
F+ specific phages	1.6×10^4	1.7×10^4
Somatic phages	2.2×10^5	1.9×10^5
Included morphology 1	1.5×10^5	1.1×10^5
Included morphology 2	7×10^4	8×10^4

[a] *Mean of 2 replicates*

TABLE 2 **Reduction of specific indicator organisms following thermal treatment**

Microorganisms	**Decimal reduction (log_{10})**	
	Heating only[a]	**78-76.3 °C / 10 min[b]**
Coliforms at 30°C	4	> 4
E. coli	> 5	> 5
Enterococci	> 4, < 6	> 6
Aero/anaerobic bacteria	nd[c]	2
F+ specific phages	> 4	> 4
Total somatic phages	2	2
Included morphology 1	5	> 5
Included morphology 2	2	2

[a] *after heat exchanger,* [b] *after chamber,* [c] *nd: no data*

3.3 Resistance of the virus indicators to thermal treatment

Heating alone is enough to achieve a reduction of more than 5 log10 units for the F+ specific phages (confirmed by testing for RNAase). These demonstrated substantial thermo sensitivity at 76.3°C. After 16 hours of incubation, two different morphologies were observed for the somatic phages. Morphology 1 was of large lysis plates (5-6 mm diameter) similar to those of the phage, reference ΦX 174. Morphology 2 comprised small plates (1-2 mm diameter). Both morphologies were present *before* treatment (Table 1) and in effluent leaving the exchanger (noting

just 0.5 PFU for morphology 1 and predominance of morphology 2). But leaving the retention zone, there remained only organisms giving rise to morphology 2. Without genetic typing method, it is impossible to claim whether it is a matter of one or two (or more) distinct species. However, following this reasoning, one would appreciate that the heating alone was enough to destroy 5 log10 units of morphology 1. Maintaining at the elevated temperature would increase this effect. On the other hand, passing effluent through the retention zone would have no effect on those phages giving rise to morphology 2 which have previously resisted the heating process.

Following this line of reasoning, just under 1% of the population giving rise to morphology 2 would seem insensitive to the treatment. The main part of phages responsible for morphology 2 on passing through heating showed a reduction of more than 2 log10 units. So, It could be that there are three distinct populations amongst the somatic phages such as: (i) thermo tolerant amongst phages linked to morphology 2 (ii) thermo sensitive amongst phages linked to morphology 1 and (iii) thermo sensitive amongst phages linked to morphology 2. Such an interpretation is in agreement with the results of Moce-Llivina *et al.(*2003) who observed different resistance to heat exposure between several types of somatic phage.

4 CONCLUSIONS

- Centrifugation of piggery slurry has no abatement effect on the numbers of microorganisms observed.
- Thermal treatment at 76°C was sufficient to reduce coliforms, E. coli and enterococci by more than 4 log10 units. Heating alone (prior to retention at the elevated temperature) was enough to achieve this effect.
- The broad counts on aero-anaerobic flora were only reduced by 2 log10 units even after retention at the elevated temperature for 10 minutes.
- The results for the phages used were mixed. The picture was complicated by the formation of two different types of plates during incubation suggesting tree sub-types with different thermo tolerances.

The overall conclusion is that the required conditions for sanitisation depend on the type of microorganism and regulation in question. With respect to livestock slurry, current regulations are relatively slack : the EU Directive 1774 (amendment 208/2006) specifies a maximum of 10^3 per gram for just two indicators (*E. coli* and enterococci). The French standard NFU 44-051 is more demanding but in either case a reduction of 2 or 3 log10 units from the initial concentrations observed in the raw slurry would easily be enough. Applying this standard, a treatment at a lower temperature could thus be foreseen with the objective to reduce costs. On the other hand, if the treatment aims to *guarantee* the safety of the treated effluent or slurry (that is free of pathogens), an even higher temperature for treatment must be considered. The large range of thermo resistance revealed for the selected indicators for this study underlines the importance in using more indicators in order to confirm and evaluate the efficiency of a treatment system.

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