Project Report: **Inactivation of *Escherichia coli* O157:H7 in drinking water of cattle by sodium caprylate**

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INTRODUCTION

E. coli O157:H7 is a major food-borne pathogen in the United States. Cattle serve as the principal reservoir of *E. coli* O157:H7, excreting the pathogen in feces, thereby contaminating food, water, and the environment (Chapman *et al* 1993, Laegreid *et al*., 1999, Shere *et al*., 1998; Zhao *et al*., 1998). Environmental persistence of *E. coli* O157:H7 is critical in its epidemiology on farms (LeJeune *et al*., 2001). Several researchers have isolated *E. coli* O157:H7 from cattle water troughs, indicating that water troughs on farms could serve as a potential long-term reservoir of the pathogen. Persistence of *E. coli* O157:H7 in cattle water troughs can potentially act as a source of re-infection of cattle, birds, flies, and rodents, which, in turn can act as vectors of the pathogen (McGee *et al*., 2002). Thus, there is a need for an effective and practical method for killing *E. coli* O157:H7 in cattle water troughs. Inactivation of *E. coli* O157:H7 in water at farm will potentially shut down one source of infection to cattle, thereby leading to a reduced carriage of *E. coli* O157:H7 in cattle. This in turn will translate into improved farm and animal hygiene, and a reduced contamination of beef products with *E. coli* O157:H7. Finally, a safe supply of beef products is critical for the economic viability of the beef industry.

Caprylic acid is a natural, eight-carbon fatty acid present in breast milk, bovine milk (Jensen *et al*., 2000), and coconut oil (Jensen *et al*., 1990, Sprong *et al*., 2001). Caprylic acid is a food-grade chemical approved by the FDA as GRAS (CFR 184.1025). Previous research conducted by the PI indicated that caprylic acid was highly effective in killing *E. coli* O157:H7 in bovine rumen fluid (Annamalai *et al*., 2004). The objective of this research is to determine the potential of sodium caprylate as an antibacterial additive

to kill *E. coli* O157:H7 in cattle drinking water. Specifically, the antibacterial effect of sodium caprylate (75, 100, and 120 mM) on *E. coli* O157:H7 in water in the presence and absence of 1% bovine feces or feed at 4, 10, and 21°C will be determined.

MATERIALS AND METHODS

Bacterial strains and media:

Four strains of green fluorescent protein (GFP)-labeled *E. coli* O157:H7 (E0143, C7927, K262, C0083) were used in the study. The cultures were obtained from Dr. Michael P. Doyle at the Center for Food Safety, University of Georgia, Griffin, Georgia. The four strains of GFP-labeled *E. coli* O157:H7 were individually cultured in 10 ml of Tryptic soy broth (TSB, Difco) containing 100 µg/ml of ampicillin (Sigma-Aldrich Chemical) at 37°C for 24 h with agitation (150 rpm). Following incubation, the cultures were sedimented by centrifugation (3600 X *g* for 15 min), washed twice, and resuspended in 10 ml of sterile deionized water. Equal portions from each of the four cultures were combined, and 100 µl (approximately 10^8 CFU) of the four-strain mixture was used as the inoculum. The bacterial population in each culture and in the four-strain mixture was verified by plating 0.1-ml portions of the appropriately diluted culture on Tryptic soy agar (TSA, Difco) plates containing 100 µg/ml of ampicillin, with incubation at 37°C for 24 h.

Sample inoculation and treatments:

The efficacy of sodium caprylate for killing *E. coli* O157:H7 was determined in water with and without bovine feces or feed. Water was obtained from a local dairy farm, and aliquots of 100 ml each of water were dispensed in 250-ml wide-mouthed, sterile plastic containers. Appropriate quantities of sodium caprylate (Sigma-Aldrich Chemical)

were added to each water sample to obtain a final concentration of 75 mM, 100 or 120 mM. Samples without sodium caprylate (0 mM) were used as controls for the study. In addition, a set of water samples containing bovine feces (1% w/v) (McGee et al., 2002) or feed (1% total mixed ration, TMR) were also included to determine the effect of feces/feed on the antibacterial property of caprylate. Each treatment and control water sample was inoculated with the four-strain mixture of *E. coli* O157:H7 to obtain an inoculation level 10^6 CFU/ml of water. The containers were loosely covered with plastic lids to enable free passage of air. The samples were incubated at 21^oC, 10^oC or 4^oC (average summer, fall and spring temperature of northeastern U.S. National Climatic Data Center, Ashville, North Carolina) for 21 days. Water samples were analyzed for surviving pathogen population and pH on days 0, 1, 3, 5, 7, 14 and 21. Triplicate samples of each treatment and control were included at each of the specified temperatures, and the entire study was duplicated.

Enumeration of *E. coli* O157:H7:

The population of surviving *E.coli* O157:H7 in each water sample was determined by plating 0.1-ml portions of the samples directly or after serial dilutions (1:10 in phosphate buffered saline, PBS, pH 7.4) on duplicate TSA plates containing 100 µg/ml of ampicillin. The plates were incubated at 37°C for 24 h and viewed under ultra violet light to enumerate *E. coli* O157:H7 (Vialette *et al*., 2004). At each sampling time, 1 ml of water from each container was also transferred to separate 250-ml flasks containing 100 ml of sterile TSB for enrichment at 37°C for 24 h. When growth was observed in TSB, the culture was streaked on TSA containing 100 µg/ml of ampicillin. Representative, fluorescent colonies from TSA plates were confirmed as *E. coli* O157:H7 by API-20E

bacterial identification kit (BioMeireux) and *E. coli* O157 latex agglutination assay kit (Oxoid). The pH of each treatment and control sample was determined using an Accumet pH meter (Fisher Scientific, Pittsburgh, PA).

Statistical analysis:

For each treatment and control, the data from independent replicate trials were pooled, and analyzed using a split-plot design with repeated sampling over time. The model included the treatment, concentrations, storage temperature and days. Significant differences ($P < 0.0001$) in bacterial counts due to treatment, concentrations, storage temperature and days were determined.

RESULTS AND DISCUSSION

The initial pH of water samples without feces or TMR was 6.8 ± 0.3. Addition of sodium caprylate at 75 mM concentration increased the water pH to 7.1 ± 0.07, with no further rise in pH with subsequent increase in caprylate concentration. The pH of water samples containing 1% feces or TMR were 7.1 ± 0.28 and 5.5 ± 0.06, respectively. Addition of sodium caprylate (75 mm) to water samples with feces or TMR increased the pH to 7.8 ± 0.12 and $6.80 \pm. 03$, respectively. It was also observed that there was no significant change ($P > 0.0001$) in the pH of water samples during the duration of the study.

The average initial population of *E. coli* O157:H7 in the water samples was ~ 6.0 log CFU/ml. The antibacterial effect of sodium caprylate on *E. coli* O157:H7 in water at 21°C is illustrated in Figures 1, 2, and 3. Sodium caprylate at 75, 100 and 120 mM completely inactivated *E. coli* O157:H7 (~6.0 log CFU/ml reduction) on days 3, 5 and 14, respectively (Figure 1). However, in the control samples containing no sodium caprylate,

E. coli O157:H7 population decreased to ~ 4.0 log CFU/ml on day 21 of incubation. Although sodium caprylate had a slightly reduced antibacterial effect on *E. coli* O157:H7 in water containing 1% bovine feces, in comparison to that in water with no fecal matter (Figure 2), the pathogen declined steadily over time in all the samples containing caprylate. *E. coli* O157:H7 populations were decreased to undetectable levels on days 5, 14, and 21 by 75, 100, and 125 mM of sodium caprylate, respectively. However, the control water samples demonstrated no significant reduction in pathogen counts on day 21 of incubation. The addition of sodium caprylate to water containing 1% TMR brought about rapid decline in *E. coli* O157:H7 counts, killing the pathogen completely by 24 hours of incubation (Figure 3). In the control samples containing TMR, *E. coli* O157:H7 population increased, reaching ~ 7.0 log CFU/ml on day 21.

The inactivation of *E. coli* O157:H7 in the water samples stored at 10^oC is depicted in Figures 4, 5, and 6. Similar to the results observed at 21^oC, sodium caprylate was very effective in killing *E. coli* O157:H7 in water (Figure 4) or water containing 1% TMR (Figure 6). However, a significantly ($P < 0.0001$) reduced antibacterial effect of sodium caprylate on *E. coli* O157:H7 was noticed in the samples containing feces (Figure 5). Caprylate at 75 and 100 mM levels decreased the pathogen load by 1.0 log CFU/ml and 2 log CFU/ml, respectively on day 21, where as 120 mM sodium caprylate brought about complete inactivation of *E.coli* O157:H7 on day 14 of storage. The control samples without any sodium caprylate demonstrated 3.0 log CFU/ml reduction in the pathogen counts after 21 days of incubation.

The antibacterial effect of sodium caprylate on *E. coli* O157:H7 in water at 4°C is shown in Figure 7. Although sodium caprylate at 120 and 100 mM levels completely

inactivated *E. coli* O157:H7 by day 3 and 5, respectively, the samples with 75mM sodium caprylate reduced the bacterial load only by 1.5 log CFU/ml at the end of the 21-day period. In the control water samples, *E. coli* O157:H7 population exhibited a mere reduction of ~ 0.5 log CFU/ml on day 21 of incubation. The inactivation of *E. coli* O157:H7 by caprylate in water containing feces at 4°C is depicted in Figure 8. As observed at 21 and 10°C, sodium caprylate had a reduced antibacterial effect on *E. coli* O157:H7 in water containing 1% feces, in comparison to that in the water samples containing no fecal matter. None of the tested concentrations of sodium caprylate significantly ($P > 0.0001$) killed *E. coli* O157:H7 when compared to that in the control samples. Similar to the results noticed at 21°C and 10°C, sodium caprylate in water samples containing TMR, killed *E. coli* O157:H7 rapidly, reducing the pathogen load by 6.0 log CFU/ml after 24 h of incubation (Figure 9). In the control samples containing TMR, *E. coli* O157:H7 population did not decrease significantly over the 21-day period.

The magnitude of *E. coli* O157:H7 inactivation by sodium caprylate in water was found to be dependant on the concentration of caprylate at all the three incubation temperatures. For example, at 21°C, 120 mM of sodium caprylate completely inactivated *E. coli* O157:H7 (6.0 log CFU/ml reduction) on day 3 of storage, whereas the samples containing 100 and 75 mM caprylate tested negative for the pathogen on days 5 and 14, respectively (Figure 1). Similarly at 10°C, complete killing of *E. coli* O157:H7 (enrichment negative) was observed on day 3 in water containing 120 mM sodium caprylate, whereas samples containing lower concentrations of caprylate yielded more than 3.0 log CFU/ml of the pathogen on same day of storage. A similar trend in *E. coli*

O157:H7 reduction was also observed in water samples containing 1% feces at the three storage temperatures.

Temperature had a significant effect (P < 0.0001) on the antibacterial activity of sodium caprylate, especially at 75 mM. For example, 75 mM sodium caprylate reduced *E. coli* O157:H7 population to undetectable levels in 14 days of storage at 21°C (Figure 1). However, the same magnitude of reduction in the pathogen counts at 10°C was observed only on the last day of storage (Figure 4). At 4°C, ~ 4.0 log CFU/ml of the pathogen was recovered even on 21 day of storage (Fig. 7). The increased antibacterial effect of caprylate at higher temperatures could be attributed to the higher metabolic rate of *E. coli* O157:H7 at 21 and 10°C, compared to that at 4°C. The significantly reduced antibacterial effect of sodium caprylate at 4°C could also be attributed to its decreased solubility at lower temperatures. The reduced antibacterial effect of caprylate at lower temperatures could also be due to the changes in the fatty acid profile and fluidity of bacterial cell membrane at cold temperatures. The plasma membrane is the primary target of fatty acids and monoglycerides (Bergsson *et al*., 1998, Bergsson *et al*., 2001, Reese *at al*., 1973), and changes in cell membrane lipid composition, including alterations in the relative proportions of different fatty acid classes, and increased lipid unsaturation and fluidity up on exposure to cold are reported (McElhaney *et al*., 1976 and Russel *et al*., 1984). These changes in bacterial cell membrane may potentially interfere with the action of sodium caprylate, thus resulting in a reduced antibacterial effect.

Feces or feed also had a significant effect (P < 0.0001) on the antibacterial property of sodium caprylate on *E. coli* O157:H7 in water. At all the three storage temperatures, bovine feces at 1% level substantially reduced the killing of *E. coli*

O157:H7 by caprylate, whereas inactivation of the pathogen was rapid in presence of feed (TMR). The reduced antibacterial of caprylate in water containing feces could be attributed to binding of the antimicrobial by organic matter present in these samples. The marked increase in the antibacterial effect of sodium caprylate in the water samples containing feed could be substantiated by the organic acids present in the silage portion of the TMR. Analysis of the acid profile of TMR by a commercial laboratory (Dairy One Inc., New York) revealed a concentration of 5.6% lactic acid and 3.7% acetic acid with 9.6% total acids in the feed (on dry matter basis). The antibacterial effect of sodium caprylate on *E. coli* O157:H7 might have been potentiated by the inherent organic acids in the feed, thereby resulting in rapid decline of the pathogen in water.

Results of this study indicate that sodium caprylate (125 mM) is effective in killing *E. coli* O157:H7 even in water containing bovine feces. The antibacterial effect of caprylate was more pronounced at higher environmental temperatures (21 and 10°C) than at 4°C. This is important since fecal excretion of *E. coli* O157:H7 by cattle has been reported to be higher in summer months than in winter (Heuvelink et al., 1998; Jackson et al., 1998). Our future studies will focus on the palatability of water containing sodium caprylate to cattle.

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Fig. 1 Inactivation of *E.coli* O157:H7 in water by sodium caprylate at 21ºC
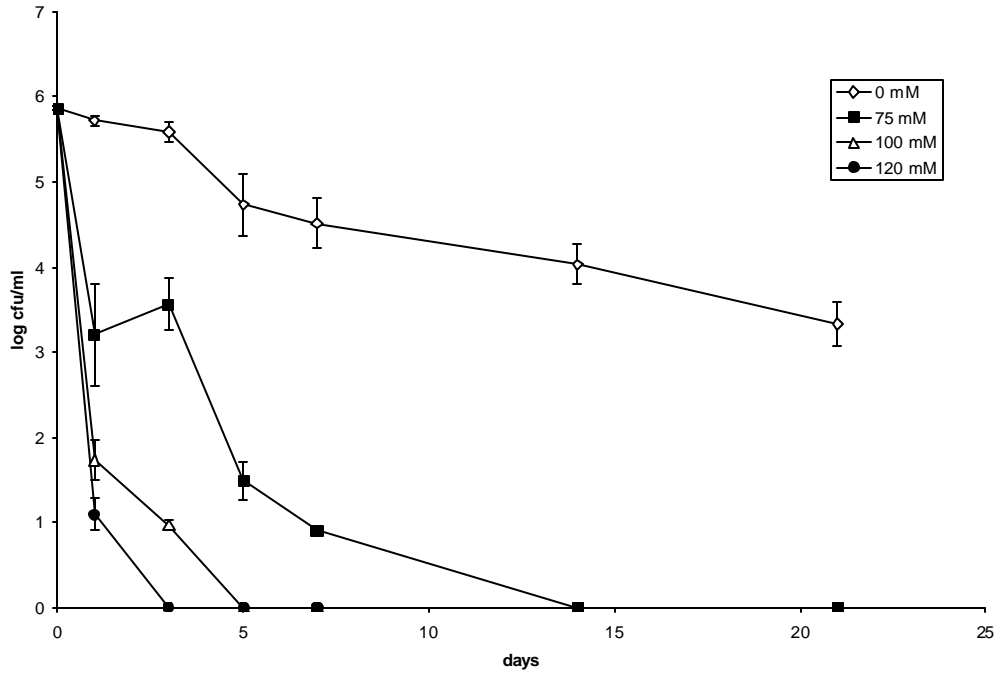
Fig. 2. Inactivation of *E.coli* O157:H7 in water with 1% feces by sodium caprylate at 21ºC
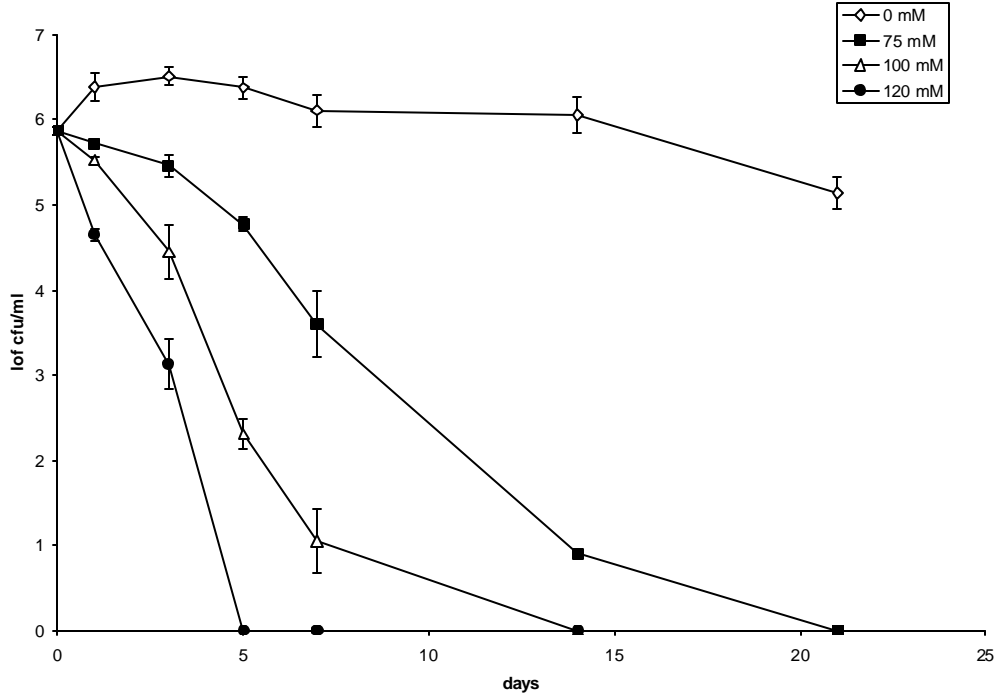
Fig. 3. Inactivation of *E.coli* O157:H7 in water with 1% TMR by sodium caprylate at 21ºC
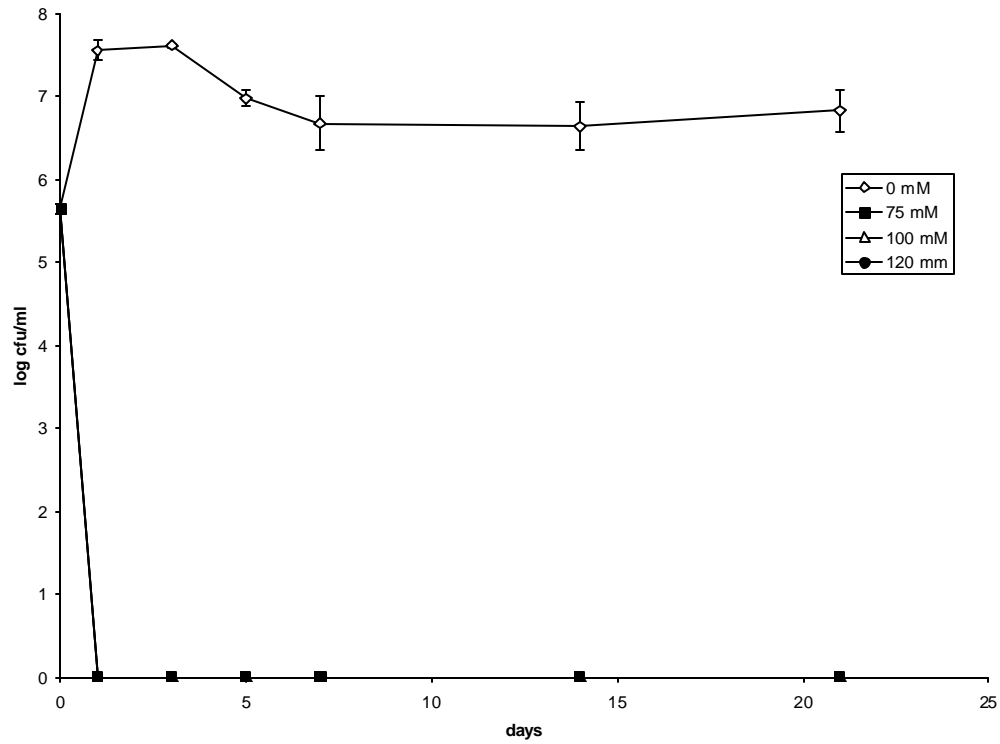
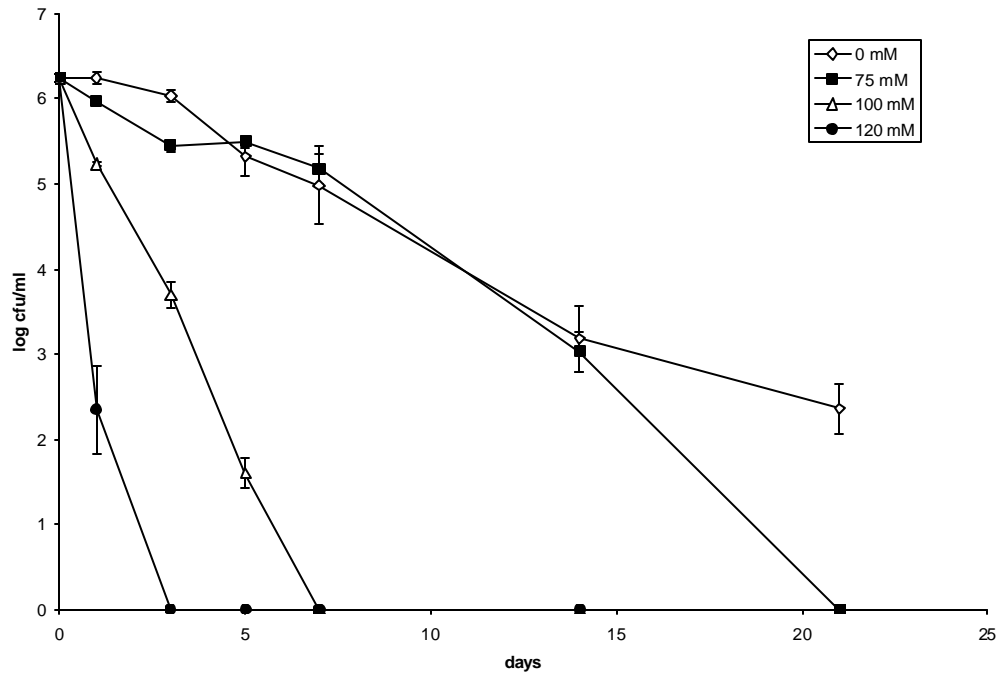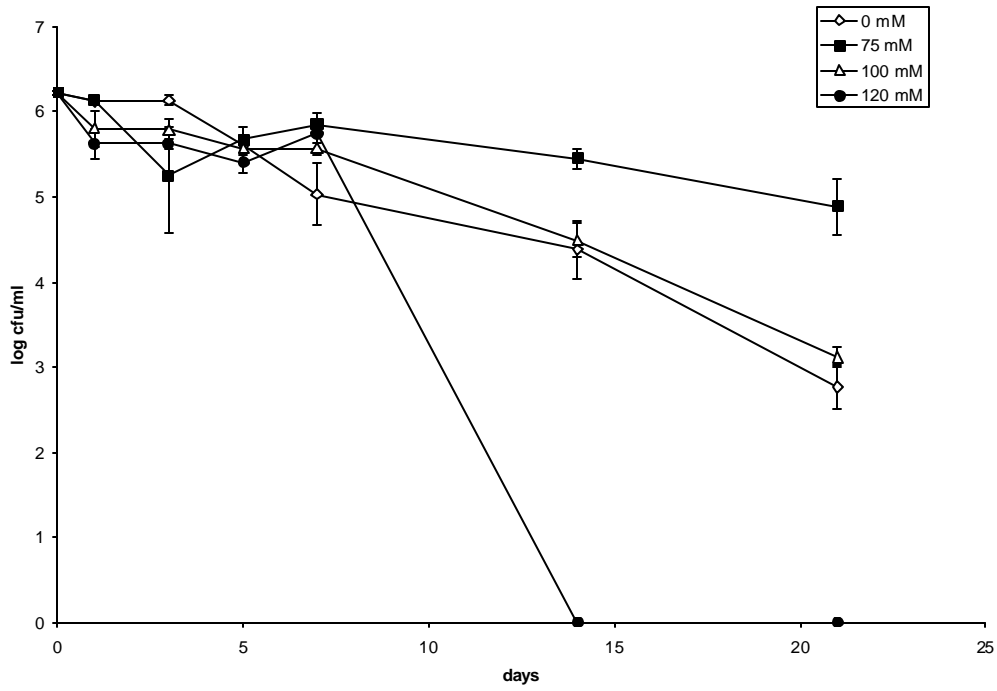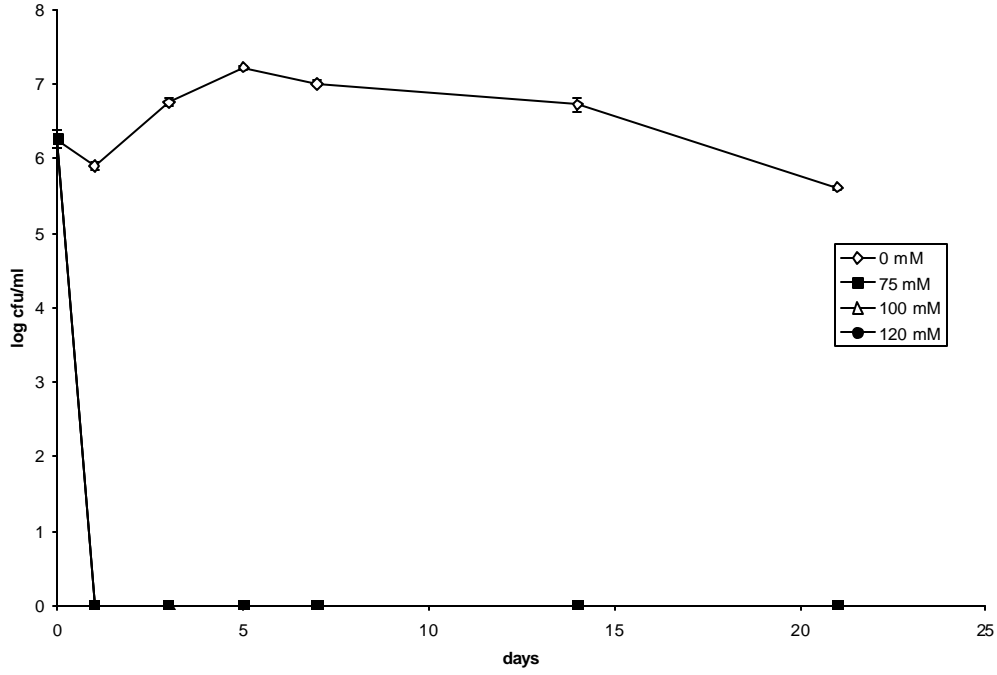**Fig. 4. Inactivation of *E.coli* O157:H7 in water by sodium caprylate at 10ºC**

Fig. 5. Inactivation of *E.coli* O157:H7 in water with 1% feces by sodium caprylate at 10ºC

Fig. 6 Inactivation of *E.coli* O157:H7 in water with 1% TMR by sodium caprylate at 10ºC

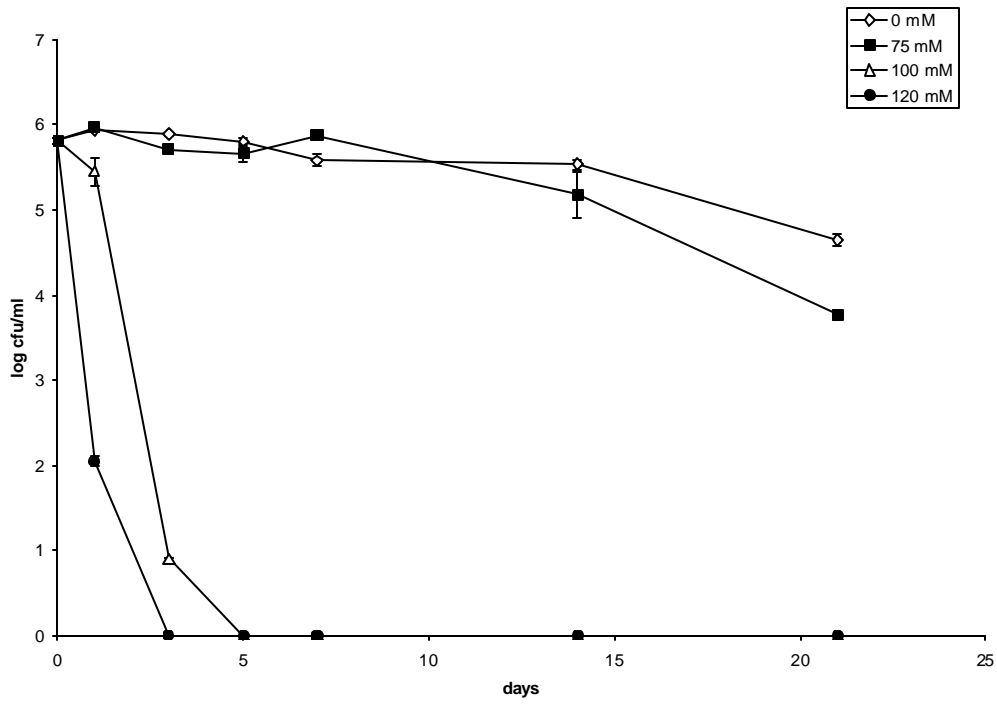**Fig. 7. Inactivation of *E.coli* O157:H7 in water by sodium caprylate at 4ºC**

Fig. 8. Inactivation of *E.coli* O157:H7 in water with 1% feces by sodium caprylate at 4ºC
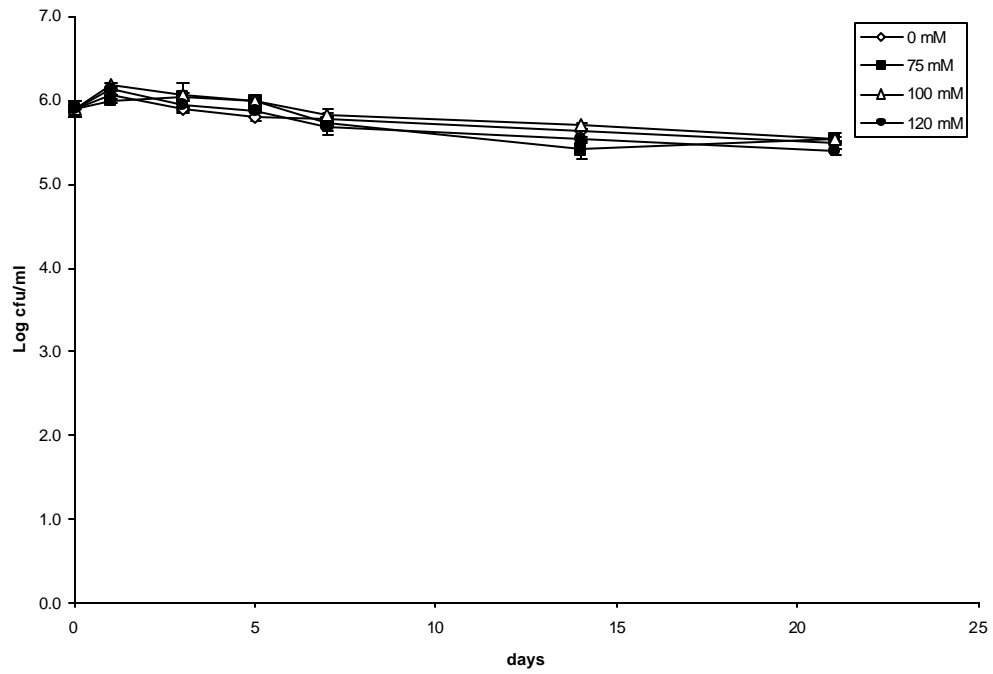
Fig. 9. Inactivation of *E.coli* O157:H7 in water with 1% TMR by sodium caprylate at 4ºC