HYDRATED LIME AND VELOX RAPIDLY REDUCE ENTERIC MICRO-ORGANISMS OF MANURE

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ABSTRACT

A rapid and effective method for reducing the numbers of enteric micro-organisms in solid manure or slurry is often needed. Quicklime and hydrated lime have commonly been used for disinfection. The Finnish Ministry of Agriculture and Forestry recommends treatment with 30 kg hydrated lime/ton of slurry or urine for a period of one week for destroying salmonella.

Nordkalk Velox is a lime-based product containing reactive oxygen in the form of calcium peroxide. Its efficacy to destroy enteric micro-organisms was studied using hydrated lime as the control. The experiments were carried out with slurry as well as solid manure from turkeys, pigs and horses.

Doses of 3 – 30 g/kg (or g/L for slurry) were tested on the numbers of faecal coliforms, enterococci and somatic coliphages.

With cattle slurry and good stirring even doses as low as 7.5 g/L of both Velox and hydrated lime could destroy coliforms to a level below the detection limit. With solid manure, doses of 30 g/kg were necessary, perhaps because of the difficulties of obtaining a homogenous mixture between the solid manure and the disinfectant.

Velox was slightly more effective than hydrated lime. According to these results a one week treatment time would not be needed, because the disinfection effect was clear already after one to two days.

Keywords: *chemical disinfection, liming, salmonella, sanitation.*

INTRODUCTION

The efficiency of chemical disinfectant for destroying enteric micro-organisms in manure can be studied with indigenous indicator micro-organisms. Since there are many different potential pathogenic micro-organisms which can contaminate manure, it is better to study several different indicator micro-organism groups. Thus the reduction of *Salmonella* can be approximated from the reduction of faecal coliforms, since both of these bacterial groups belong to the family *Enterobacteriaceae* and exhibit many physiological and biochemical similarities. Thus salmonellae can be considered as one important group of faecal coliforms. If there are no faecal coliforms, there are unlikely to be any salmonellae. Since the quantitative determination of faecal coliform group is relatively easy and the quantitative determination of salmonellae is difficult, and expensive, coliforms are useful as indicators.

The enterococci are non-sporing and gram-positive bacteria also recognised as being faecal streptococci. They are known to be more resistant to disinfection methods than the coliforms (Rajala et. al 2003) and therefore they are often combined with coliforms in many hygiene and sanitation studies, supplementing the knowledge obtained from coliforms providing an index of the more resistant bacteria.

Somatic coliphages are viruses which infect only their hosts, mainly certain specific *E. coli*-strains. They can multiply only when their hosts are active, meaning that the nutrient status must be good and the environmental temperature must be at least near to 30^{o}C, which in the Finnish climate means that they multiply only in the intestinal tract. Since enteric viruses and coliphages have similar abilities to survive and spread in the environment, coliphages have often been used as indicators for enteric viruses.

A good disinfecting chemical should possess a wide spectrum i. e. it should have a good ability to destroy different microbes. Therefore two different bacteria and one virus type were selected for this study.

MATERIALS AND METHODS

Cattle and pig slurries and solid turkey, pig and horse manures were obtained from local farms or from a riding school. All of the manures were used within three days of collection. The dry matters (DM) of cattle slurries were from 2.9 % to 8 % and in the pig slurry it was 0.39%. Both turkey and pig manures containing some sawdust for bedding and horse manure was an almost pure horse faeces but there was also some peat used as bedding material. The DMs of turkey and horse manures were 85.1% and 20.8%. The DMs of pig manures studied were 67.3% and 17%. The manures were cleaned from the visible bedding material.

Hydrated lime and Velox were used as commercial products of Nordkalk with concentrations varying between 3 and 30 g/kg (as fresh weight). The slurries were mixed for 6 h with a magnetic stirrer after adding the disinfectants. The chemicals were added to solid manures (approximately 800 g) in plastic bags so that many thin layers of manure and chemicals were added and then mixed very carefully. The addition of chemicals was done in four parallels, except where there were three parallel runs for cattle slurry. All solid manures and pig slurries were incubated for one and two days at 4^{o}C temperature. Cattle slurries were incubated at 20^{o}C for one, two, three and seven days.

Faecal coliforms were determined on mFC agar medium (Difco 267720, SFS 3014). Enterococci were determined on bile-esqulin azide agar (Scharlau 01-592, ISO 7899-2). Both bacterial determinations were based on methods generally used in water hygiene. The somatic coliphages were detected with a double layer agar technique as described by Heinonen-Tanski et al. (1998) using ATCC 13706 as the host bacterium. The detection limits are 10 colony or plaque forming units/g. The pH was measured in the same days with Knack pH-meter.

The statistical significance was assessed by t-test calculated after log-transformation. If a result less than detection limit was encountered, then a value of half of the detection limit was used. The comparison has been made against the controls.

RESULTS AND DISCUSSION

The incubation of diluted cattle slurry (DM 2.9%) the hydrated lime or Velox as 7.5 g/L slurry destroyed all coliforms to less than the detection limit (10 CFU/g), even after one day's incubation no coliforms could be found. Both chemicals efficiently reduced coliforms at 3.7 g/L. The reduction-%s were thus 92.3, 98.0 and 97.8 for hydrated lime and 97.9, 99.4 and 98.6 for Velox in days 1, 3 and 7 when studied cattle slurry with 8% DM. Thus Velox was more potent than lime in its disinfection ability.

Both Velox and hydrated lime (30 kg/L) reduced all coliforms also in pig slurry to the level of less than the detected limit (10 CFU/g) with one or two day's incubation. Velox similarly reduced the numbers of coliphages and enterococci in two parallel tests to less than the detection limit in two days, but still some coliphages or enterococci could be shown after two days of treatment with hydrated lime. After one day of treatment the reductions of coliphages and enterococci were between 99.96 and 98.36% with the percentage being higher for Velox than for hydrated lime.

The geometric means of microbial numbers in solid manures incubated for one day with hydrated lime or Velox are presented in Tables 1a (horse), 1b (turkey) and 1c (pig).

The results from these manures when incubated for two days with these disinfectants were so similar that the results are not shown. Nonetheless, there was still some reduction and no regrowth was encountered.

Table 1a. *The effect of hydrated lime and Velox on microbial numbers / gram of solid horse manure incubated for one day at 4°C. LDL = less than detection limit in all parallel runs. (ldl)= less than detection limit at least in one parallel run. The detection limit is 10 colony or plaque forming units/g. n= 4. The statistical significance is ***when p<0.001 and ** when p<0.01 and * when p<0.05.*

Treatments	Faecal coliforms	Enterococci	Somatic coliphages
Control, no additions	**380 000**	**28 000 000**	**120**
Hydrated lime 3 g/kg	1 500 000	45 000 000	240
Hydrated lime 10 g/kg	300 000	21 000 000	100
Hydrated lime 30 g/kg	**18 (ldl)*** **	**1600*** **	**LDL*** **
Velox 3 g/kg	660 000	11 000 000	170
Velox 10g/kg	800 000	12 000 000	90 (ldl)
Velox 30 g/kg	**LDL*** **	**2000*** **	**LDL*** **

Table 1b. *The effect of hydrated lime and Velox on microbial numbers / gram of solid turkey manure incubated for one day at 4°C. See the legend in Table 1a.*

Treatments	Faecal coliforms	Enterococci	Somatic coliphages
Control, no additions	**54 000**	**12 000 000**	**17 000**
Hydrated lime 3 g/kg	9 200	560 000***	500
Hydrated lime 10 g/kg	11 000	380 000***	1200
Hydrated lime 30 g/kg	**32 000**	**1 100 000***	**45 (ldl)*** **
Velox 3 g/kg	61 000	3 200 000*	700
Velox 10g/kg	8000	1 100 000 ***	980
Velox 30 g/kg	**3 (ldl) *** **	**270 000*** **	**110***

Table 1c. *The effect of hydrated lime and Velox on microbial numbers / gram of solid pig manure incubated for one day at 4°C. See the legend in Table 1a.*

Treatments	Faecal coliforms	Enterococci	Somatic coliphages
Control, no additions	**5 300 000**	**1 300 000**	**260 000**
Hydrated lime 4 g/kg	8 000 000	890 000	260 000
Hydrated lime 6 g/kg	2 000 000*	1 400 000	130 000
Hydrated lime 10 g/kg	**850 000*** **	**71 000***	**37 000***
Velox 4 g/kg	3 800 000	540 000	350 000
Velox 6g/kg	2 000 000	510 000	94 000
Velox 10 g/kg	**1 300 000*** **	**130 000***	**74 000**

In another solid pig manure sample (17% DM) incubated with hydrated lime or Velox (30 g/kg) the reduction-%s were for coliphages 93.2 and 95.1, for coliforms 98.4 and 99.8 and for enterococci 84.9 and 85.9, respectively in two days. Thus again both disinfectants were valuable but Velox was more effective.

The pH-changes (all data not shown) were not extensive. The original mean pH in solid horse, turkey and pig manures was pH 7.53 and the mean pH of all hydrated lime additions was pH 9.44 and the mean pH of all Velox additions was pH 9.37 in the solid manures. Thus the pH change can not explain the reduction difference between the destruction efficiencies of Velox and hydrated lime. It must be assumed that the active oxygen in Velox can account for better ability to destroy enteric and metabolically anaerobic micro-organisms compared to hydrated lime.

The reduction of manure odours was evident especially with Velox. After one day of incubation there was a clear smell of ammonia, but it was weaker after two days' incubation.

CONCLUSIONS

Velox was slightly better than hydrated lime at destroying enteric micro-organisms but both chemicals are useful for instance in cases where Salmonella has to be destroyed.

The concentration of less than 10 g/L could be effective for diluted slurries, but for solid manures 30 g/kg of disinfectants were needed for almost total elimination of enteric micro-organisms, since they are unevenly distributed and they can be protected by solid particles such that the chemical cannot achieve close contact with the micro-organisms. The chemical treatments are also more difficult since the mixing of solid manure and solid chemical cannot be done by machine and thus one cannot simply do 6 hour mixings as is possible in slurry.

Both tested chemicals had a rapid inhibitory effect on the tested enteric micro-organisms. The destruction was very clear already after one or two days of incubation. No regrowth could be shown in any tests. Thus the destruction treatment times of Velox or hydrated lime could be shorter than those claimed by Finnish agricultural ministry (MMM, 1995) or those recommended by Manure Management (2003). Already a one day incubation period would have been enough and therefore the seven days proposed by the Finnish Ministry of Agriculture and Forestry (MMM, 1995) would not be needed.

The pH increase as found also in the present work may be important for destroying micro-organisms but the extent of the elevation can be rather modest and thus excessively high pH values are not needed (as discussed by Burton and Turner, 2003). Also it was not necessary to increase the temperature to guarantee good hygiene. The role of ammonia remains unclear, it may well be important, as described in Burton and Turner (2003).

Both chemicals could be used to disinfect manure in farms, food process industry, veterinary treatments, animal marketing, horse transportation or in some other activities to avoid the spreading of pathogens and to improve food or water hygiene.

In addition, both agents can increase soil pH if this manure is used as fertiliser as is normal agricultural practice in acidic soils.

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