Plasma-based nitrogen enrichment and the hygienisation effect on organic slurries

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Sammendrag

Plasma-basert nitrogenanrikning og hygieniserende effekt på organisk substrat. Plasma-basert nitrogenanrikning er en teknologi som tar sikte på lokal gjødselproduksjon med luft og elektrisitet som innsatsfaktorer. Produsert NOx absorberes i organisk avfall og gir en gjødsel anriket med plante-tilgjengelige nitrogenforbindelser. Produktet, Nitrogen Enriched Organic fertiliser (NEO), har økt konsentrasjon av mineralsk nitrogen og redusert pH. I tillegg er biologisk aktivitet tilsynelatende fullstendig inhibert i produsert NEO. Denne observasjonen la grunnlag for idéen om å bestemme kritiske prosessparametere for å deaktivere spesifikke indikatororganismer beskrevet i forskrifter med krav til hygienisering av organisk avfall: «*Salmonella*, *E.coli* og *A.suum*.»

Nylig produsert NEO prosessert til ulike pH-nivåer i maskin ble tilsatt testorganismene. Etter ulike eksponeringstider [0-48 timer] ble levende organismer kvantifisert for å bestemme grad av inaktivering. Resultatene viser at prosessen inaktiverer alle kravspesifikke indikatororganismer til under grensene i forskriftene på pH-nivåene testet (≤ pH 5.0). Kritisk eksponeringstid varierer for ulike substrater, pH-nivåer og type indikatororganisme, og spenner fra 1-26 timer.

Summary

Plasma-based nitrogen enrichment process is a novel technology targeting local fertiliser production using air and electricity as single inputs. Produced NOx is absorbed into organic waste producing a manure enriched in plant available nitrogen. The product, Nitrogen Enriched Organic fertiliser (NEO) , has an increased content of mineral nitrogen and a lower pH. Additionally, biological activity is inhibited in produced NEO. This observation gave rise to the idea of determining critical operating conditions to inactivate indicator organisms described in legislations for hygienisation of organic waste; "*Salmonella*, *E. coli* and *A. suum*".

Test organisms were added to freshly produced NEO processed to specific pH-levels. After different exposure times [0- 48] hours, viable cells were counted to determine degree of inactivation. The results show that all indicator organisms are reduced to below limits set in

legislations at tested pH-levels (≤ pH 5.0). Critical exposure time varies for different substrates, pH-levels, and indicator organisms, ranging from 1-26h.

Introduction

Utilising the nutrients in organic slurries from municipal sludge and manure as fertiliser is important to global food-systems, as it returns valuable nutrients to agricultural land. Livestock is inefficient in converting plant protein into milk and meat, and a large share of the nutrients they consume end up in the organic waste. Reusing these nutrients will therefore limit the need for external fertiliser inputs and ensure optimal resource efficiency on the farm. However, several challenges arise from the use of these organic fertilisers:

A large share of the ammonium nitrogen present will volatilize, resulting in value loss and local pollution.

- Methane from manure management is a major source of agricultural greenhouse gas emissions.
- Organic waste contains pathogens that enter the broader environment when applied to farm fields.

Plasma-based nitrogen enrichment is a novel processing method addressing the challenges associated with organic fertilisers. The method uses electricity to generate air plasma that produces a reactive nitrogen gas (NO_x) which is subsequently absorbed in the liquid fraction of organic substrate (Graves, et al., 2019), forming nitric- (HNO_3) and nitrous acid (HNO_2), see Figure 1. As a result, the nitrogen content in the organic waste increases, and the elevated acidity prevents ammonia (NH_3) from volatilisation. The treatment has also been shown to eliminate methane (CH_4) formation, an effect ascribed to the antimicrobial properties of the plasma treatment (Nyvold & Dörsch, 2024). If the antimicrobial effect is transferrable to persistent pathogens, the resultant Nitrogen Enriched Organic fertiliser (NEO) will not only be efficient and environmentally beneficial but also meet the legislative requirement for hygienisation. The process operates semi-continuously to ensure that pH in the system is always close to the target pH. Outfeed from machine occurs when pH-setpoint is reached, followed by refill of new substrate.

In this work we assessed the impact of plasma-based nitrogen enrichment on the presence of potential pathogens in organic waste.

Two different Norwegian legislations will apply to the use of organic slurries on farmland, depending on the origin:

- Organic waste originating from animals; Animal By-Product (ABP) law for category 3

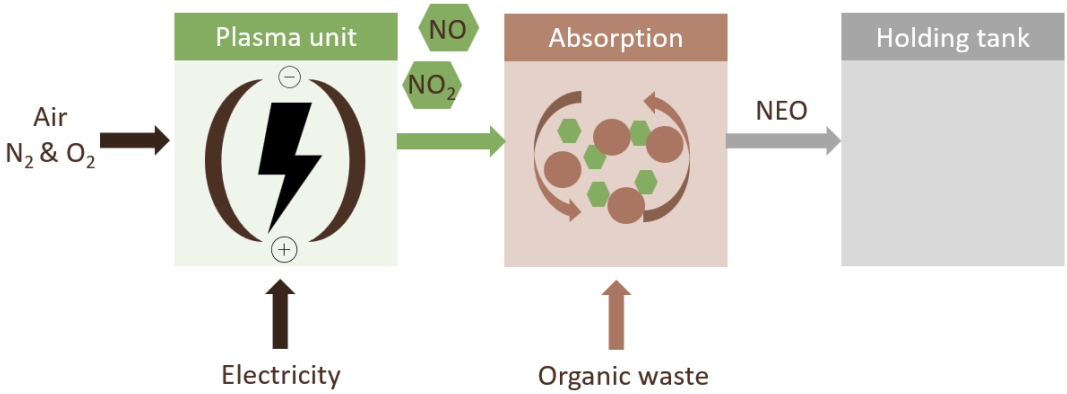


Figure 1. The N2 process. Air and electricity are entering the plasma unit, producing NOx, and air rich in NOx enters the absorption system, where it is absorbed into organic liquid slurry. The product, NEO, has an elevated concentration of mineral nitrogen and a reduced pH.

Table 1: A summary of Norwegian and European legislations; type of indicator organisms and requirements for absence of organisms after treatment.

Animal by-product regulation		Fertiliser regulations	
Indicator organism	**Requirement**	**Indicator organism**	**Requirement**
Salmonella	5 $\log_{10}$ reduction	*Salmonella*	>5 $\log_{10}$ reduction
E. coli	<1000 CFU/g wv.	*E. coli*	<1000 CFU/g
		A.suum eggs	Zero infectious eggs

materials' (Animaliebiproduktforskriften, 2016) (Regulation (EC) No. 1069/2009)

- Organic slurries with human origin; 'EC/ Ecolabel' (Forskrift om organisk gjødsel, 2003).

The legislations describe limitations for abundance of two and three organisms respectively, to fulfil requirements of hygiene, referred to as 'hygienisation'. These organisms are used as indicators due to their prevalence in organic wastes and resistance to thermal and chemical hygienisation methods and are chosen in this work to quantitatively measure the impact of the plasma-based nitrogen enrichment process: *Salmonella* Senftenberg (*Salmonella*), *Escherichia coli* (*E. coli*) and *Ascaris suum (A.suum).* The strain *Salmonella* Senftenberg w775 H_2S negative is the recommended test organism described in the animal by-product legislation. *E. coli* is a primary indicator microorganism described in regulations for disposal of biosolids in many countries (Pascual-Benito, et al., 2014). *A.suum* is a parasitic roundworm in pigs. Inactivation of parasite eggs is an absolute requirement in legislation regarding organic waste with human origin. *A.suum* eggs are recognised as one of the hardest types of helminth ova that can be found in wastewater sludge and pig slurry (US EPA, 2003). If conditions are such that *A.suum* eggs cannot survive, the probability for inactivating all other types of parasites is high and this is therefore a good indicator organism to prove the hygienisation efficiency of a process.

Table 1 describes the Norwegian requirements for documenting the hygienisation of soil improver growing media.

The aim of this work was to investigate the ability of the plasma-based nitrogen enrichment process to meet the requirements for hygienisation and to identify critical control points (pH levels and exposure time) to fulfil the requirements, referred as 'Critical Control Points' (CCP).

Methods and material

Processing and test method

Processing of NEO

All experiments were carried out in full-scale pilot unit, schematically illustrated in Figure 1. This set of experiments investigated the antimicrobial properties of NEO after it was processed, illustrated as 'Holding tank' in the figure. Any reduction in pathogen abundance would consequently be the result of substrate properties, and not the exposure to plasma gas.

Sampling

For each experiment, the NEO-process was operated overnight before sampling into two 1 L bottles for testing of *Salmonella* and *E. coli*, and a third 2 L bottle for testing of *A.suum* eggs.

Samples were immediately brought to the laboratory for nitrogen characterisation and microbial analysis. Microbial analyses were repeated at exposure time 2h -4h - 6h and 24h (and 48h for *A.suum* tests).

Nitrogen characterisation

Samples were centrifuged at 3400 rpm for 10 minutes. The supernatant was diluted in distilled water and added to standardised LCK cuvettes containing reagents for reactions with, respectively, ozone (O_3), ammonium (NH_4-N), nitrite (NO_2-N), nitrate (NO_3-N) and total nitrogen (tot-N). Photo spectrometric method was used

to determine the concentrations, using a DR3900 split beam spectrophotometer supplied by Hach®.

Expression of rate reduction of viable cells

The experimental results for viable *Salmonella* and *E. coli* cells were used to derive the survival expression, by fitting an exponential curve to the viable cell values, N, above the detection limit.

Equation 1 $$N = N_0 e^{\alpha t}$$

N_0 is the number of viable cells at t=0, α is a rate constant and t is the exposure time.

Indicator organisms

Salmonella **Senftenberg**

Salmonella Senftenberg w775 H_2S negative strain was cultivated in a nutrient broth overnight at 37° and the same volume was added to NEO and the controls. Start concentration of bacterial cells was 10^9 to 10^{12} CFU/mL. Samples were diluted in series (10^{-1} – 10^{-10}) in peptone water (Oxoid UK) and incubated overnight at 37°C, followed by an overnight cultivation in RVPB medium at 42°C. The samples were further transferred to BGA and XLD medium plates for visual count of *Salmonella* colonies. For confirmation a blue-latex agglutination test kit was performed (Salmonella test kit, DR1108A, OXOID). The method analyses 1 mL of sample and the detection limit for the culturing method is therefore ca 1 CFU/mL, determined by the statistical accuracy.

Escherichia coli

Inactivation of *E. coli* strain ATCC 11775 in samples were analysed by two methods; Most Probable Number (MPN) method (NS-EN ISO 9308-2) and a cultivation method following the same principles as for *Salmonella* testing: The samples were diluted in series ranging from 10^{-1} – 10^{-10} in peptone water and cultured overnight at 37°C, followed by an overnight incubation on a selective TBX-medium, where *E. coli* forms blue colonies.

Ascaris suum

A.suum eggs were prepared in permeable nylon bags at "Swedish University of Agricultural Sciences" (SLU) in Uppala. This laboratory also quantified the eggs after in testing. About 10 000 eggs were filled in permeable nylon bags of 30µm pore size, and transported to test centre where the actual test was performed. Bags were put into treated slurry for 2 - 48 hours, and then rinsed carefully in water to remove remaining slurry before incubation at 28°C and transport back to SLU. Analysis was done after 21 days total incubation time. Each exposure time was run as duplicates, results are presented as the average. Each experiment includes a positive control (no treatment but transported back and forth between SLU and test centre stored in the incubator), the NEO samples at different exposure times and a negative control (addition of *A.suum* to untreated digestate). Survival of *A.suum* is determined by counting developed larvae and undeveloped eggs, where viability is calculated as

Equation 2
$$Survial\ [\%] = \frac{n_{larvae}}{\Sigma(n_{larvae} + n_{undevopled\ eggs})} \times 100\%$$

Results

Test matrix

The initial phase of this project aimed to identify the upper pH-limit where sufficient inactivation of microorganisms is obtained. Untreated slurries normally have a pH between 7-8 and pH is gradually lowered during the plasma-based nitrogen enrichment process, while concentrations of reactive compounds increase. The final pH of NEO is a setpoint established by the operator and can theoretically be set as low as desired, but to retain great fertiliser properties of NEO the lower limit for accepted operational pH is set to 4.5. pH-setpoints of 5.5, 5.0 and 4.5 were investigated in these experiments.

The identified pH-levels were further used in the validation tests. A summary of all tests conducted is presented in Table 2.

*Table 2: Summary of experiments; substrate, operational-pH, what organisms that were included in the test and exposure time when sample is analysed. *The two marked IDs are the same operation. **The two marked IDs are from the same operation but divided into two bottles and followed up at different laboratories.*

Test protocols	Test-ID	Substrate	pH	Organism	Exposure time [h]
Initial tests	NEO pH 4,5	Cow manure	4.5	*Salmonella*	2, 4, 6, 24
	NEO pH 5,0*	Cow manure	5.0	*Salmonella*	2, 4, 6, 24
	NEO pH 5,5	Cow manure	5.5	*Salmonella*	2, 4, 6, 24
Validation tests	NEO C-1*	Cow manure	5.0	*Salmonella*	2, 4, 6, 24
	NEO C-2	Cow manure	5.0	*Salmonella*	2, 4, 6, 24
	NEO C-3	Cow manure	5.0	*E. coli*, *Salmonella*	2, 4, 6, 24
	NEO C-4**	Cow manure	5.0	*E. coli*, *Salmonella*	2, 4, 6, 24
	NEO C-5**	Cow manure	5.0	*E. coli*,	2, 4, 6, 24
	NEO D-1	Digestate	4.6	*E. coli*, *Salmonella* *A.suum*	2, 4, 6, 24 (A.suum 48)
	NEO D-2	Digestate	4.5	*E. coli*, *Salmonella* *A.suum*	2, 4, 6, 24 (A.suum 48)

The robustness of the *Salmonella* culture was first tested to evaluate robustness towards acidification. A standard growth medium was acidified to the same pH-levels as the most likely operational pH of the tests, and presence of viable cells was determined by Optical Density (OD). In addition, different likely salinity levels [0%; 2%] were investigated.

The *Salmonella* strain grow at all pH-levels tested and has robustness towards pH levels down to pH 4.5, as well as robustness towards salt concentrations up to 2%.

pH-adjustment by acid to pH 4.5 is not inhibiting growth of *Salmonell*a.

NEO pH dependent effect on hygienisation

The range of acceptable pH-levels when producing NEO for use as fertiliser is wide, and we wanted to establish the hygienisation effect of pH of NEO on Salmonella at different pH levels. Subsequently, we added 10^9 cells/mL of Salmonella to freshly produced NEO at three different pH; 5.5, 5.0, and 4.5 and monitored the viability of salmonella in the samples by MPN over 24h. The results presented in Figure 2 show that the concentration of *Salmonella* remained unchanged in the cow manure during the 24-hour period, whereas a clear pH dependency emerged in the NEO-samples samples: In NEO pH 5.5, an estimated 10^6 viable cells per ml remained, while the NEO at pH 5.0 and 4.5 demonstrated a complete elimination. The detection limit for the cultivation method used implies there are no viable cells after four hours of storage.

Although NEO at all pH levels tested seem to affect growth of Salmonella compared to untreated cow manure, at pH 5.0 and lower a complete inhibition of Salmonella was observed after 4 hours of exposure.

Validation tests

We next sought to test the hygienisation performance of NEO towards *E.coli* in addition to

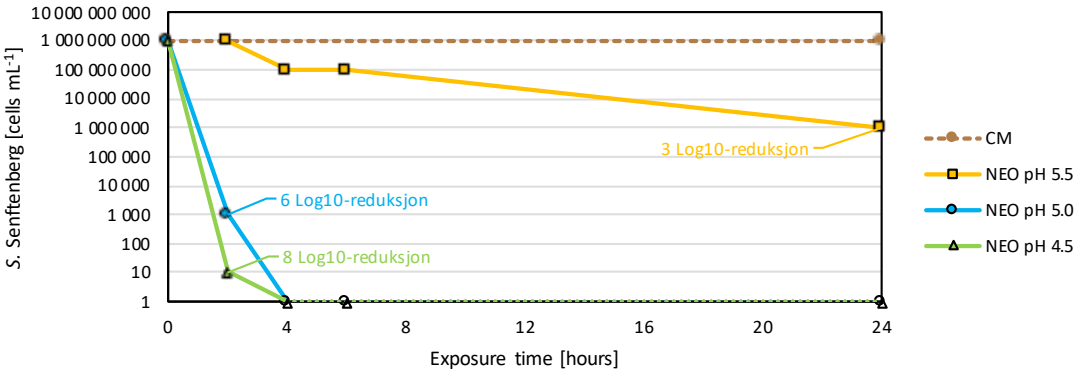


Figure 2. Viable Salmonella cells in untreated Cow Manure (CM), NEO treated to pH 5.5, pH 5.0 and pH 4.5. Exposure time is the time between sampling and addition of Salmonella, and analysis.

Salmonella. We decided to use a pH 5.0 NEO as it was the highest pH level with a documented hygienisation effect on *Salmonella*, and was close to the pH-level threshold for obtaining hygienisation and thus interesting to investigate for the other organisms.

Consequently, pH 5.0 was considered an appropriate pH to investigate as a CCP for the validation tests that followed on cow manure.

The next step was therefore to perform repetitions of the same test setup as used for pH 5 for both *Salmonella* and *E. coli* in NEO produced from cow manure. Measured viable cells of *Salmonella* and *E. coli* added to NEO is presented in Figure 3 as a function of exposure time in sample. For *Salmonella*, a significant reduction in viable cells in the first hours of exposure time is observed in 3 out of 4 parallels (Figure 3a). After 24 hours no viable cells are detected in any of the parallels. *Salmonella* inactivation in sample NEO C-3 shows a deviating behaviour compared to the other three experiments; there is no detected decrease in the first hours of exposure, while after 24 hours no viable cells are counted. Same pattern is not found in the same sample for *E.coli*.

At pH 5.0 NEO showed a hygienisation effect on both *Salmonella* and *E.coli.*, with no viable cells detected after 8 and 6 hours exposure, respectively.

Digestate is the product from anaerobic digestion, often using municipal wastewater sludge as substrate. For organic matter originating from humans, the regulation requires that also *A.suum* eggs needs to be inactivated before digestate slurries are allowed to be spread onto farmland.

We subjected this substrate to hygienisation testing using the same approach as for cow manure. We chose to run the process at a lower pH to increase the chance of success inhibiting development of *A.suum* eggs, due to the greater resilience of *A.suum* (US EPA, 2003). Therefore, pH 4.6 and pH 4.5 was chosen as setpoints for, respectively, NEO D-1 and NEO D-2 test samples. Figure 4 shows the number of viable cells of both *Salmonella* and *E. coli* and percentage survival of *A.suum* eggs as a function of exposure time. Figure 4. All *Salmonella* cells were inactivated after 24 hours, but interestingly, the reduction of viable *Salmonella* cells in NEO produced from digestate was slower compared to the equivalent for NEO pH 4.5 produced from cow manure. All *E.coli* cells were inactivated after 6 hours exposure time. The survival in the positive control (transported between SLU and the N2 Applied test centre, not exposed to any slurry) was found to be lower compared to the negative control sample (exposed to untreated digestate), indicating that the environment in digestate supports viability. Compared to the control developed eggs of *A.suum* were decreasing as a function of exposure time in NEO in both NEO test samples, and at 24 hours exposure time no larvae were found. There is little difference between operation at pH 4.5 and pH 4.6.

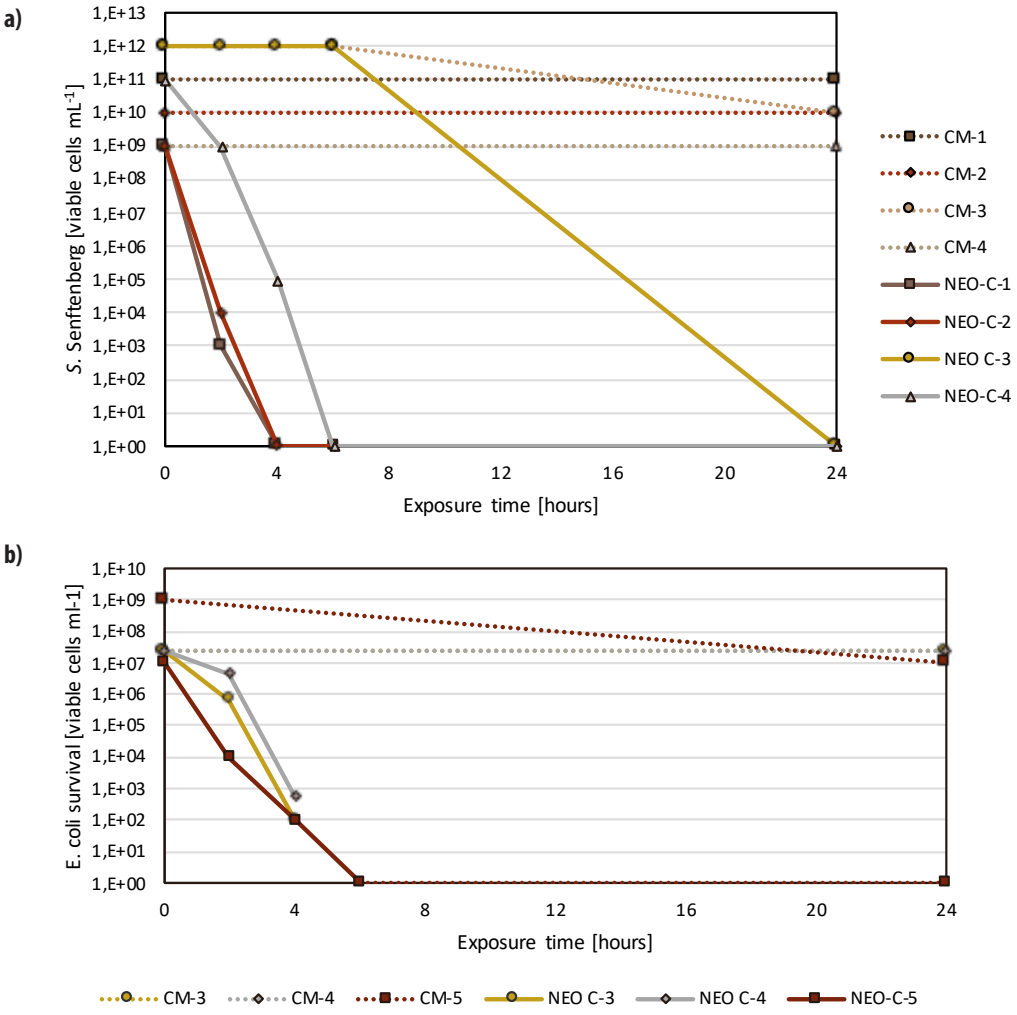


Figure 3. Measured viable cells as a function of exposure time in NEO pH 5 for a) Salmonella and b) E. coli. Dashed lines show concentration of viable cells in untreated cow manure (The control sample).

Low pH (<4,6) NEO produced from digestate has a hygienisation effect for *Salmonella* and *E.coli* (after 24h) similarly as pH 4.5 NEO produced from cow manure. In addition, NEO from digestate at this pH is also hygienising against the intestinal parasite *A suum*.

Calculation of rate constants

Calculation of rate constants enables the calculation of exposure time to reach a certain target of reduction. The experimental data presented in Figure 2, Figure 3 and Figure 4 are fitted to an exponential expression for viable cells as described in Equation 1 for *Salmonella*, *E.coli* and *A.suum*. Table 3 lists the experimentally derived values for rate constant, , and the calculated exposure time necessary to achieve respective requirements.

Nitrogen content

Nitrogen characterisation for cow manure and digestate and NEO produced from each of the substrates at, respectively, pH 5.0 and pH 4.5, at time of sampling are presented in Figure 5. A significant increase in nitrite and nitrate is observed in NEO compared to raw substrates.

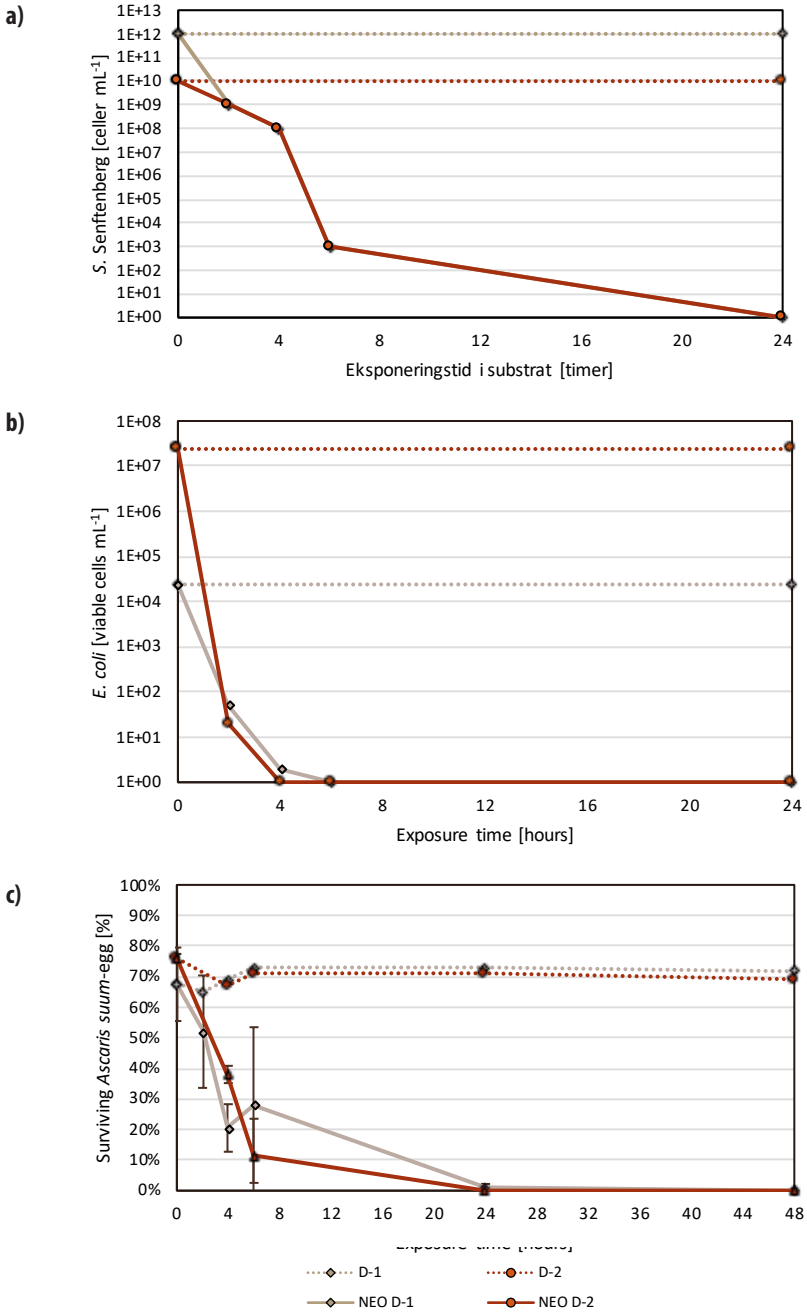


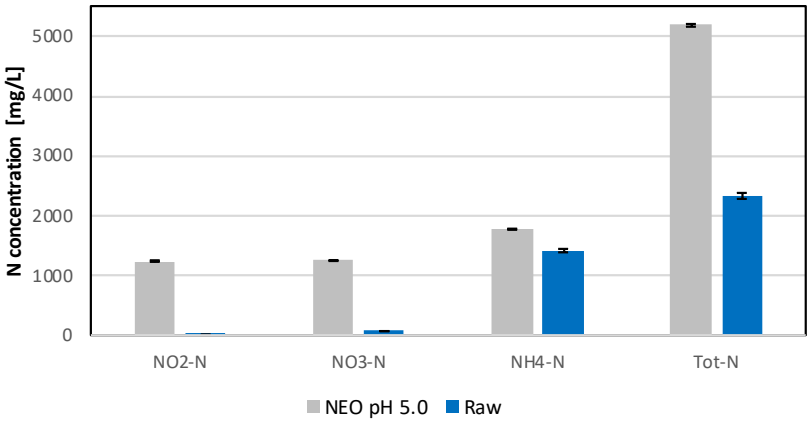
Figure 4. Viable Salmonella (a), viable E.coli (b) and percentage survival of A.suum eggs (c). Survival of Ascaris suum eggs in c) is presented as average of duplicates, error bars show the standard deviation of the duplicates.

Oxidation-Reduction Potential (ORP) is measured in NEO pH5.5, ozone is measured in samples from initial phase, presented in Figure 6. The figure shows a significant change in measured ORP between cow manure and NEO pH 5.5 after 2.5 hours. After 24 hours measured ORP-value in NEO pH5.5 sample has dropped.

*Table 3. Rate constants and time to meet respective requirements for viable cells for Salmonella (>5-log_{10} reduction), E.coli (<1000 viable cells/mL) and A.suum (no viable organisms in NEO-C (cow manure) and NEO-D (digestate). Presented rate constant for NEO-C pH 5 is the average of three experiments. *NEO C-3 is not included.*

	NEO-C			NEO-D		
	pH	α [h^{-1}]	Time to target [h]	pH	α [h^{-1}]	Time to target [h]
Salmonella (5log_{10} reduction)	4.5	-5.18	2.22	4.6	-2.53	4.6
	5.0	-4.87 ± 0.75*	2.4	4.5	-3.22	3.6
	5.5	-0.28	>24			
E.coli (<1000 viable cells mL^{-1})	5.0	-2.81 ± 0.36	3,28	4.6	-2.35	1.35
				4.5	-4.25	2.37
A.suum (>99.9% removal)				4.6	-0,18	25.9
				4.5	-0,30	15.6

a) Cow manure substrate, pH 5.0



b) Digestate substrate, pH 4.5

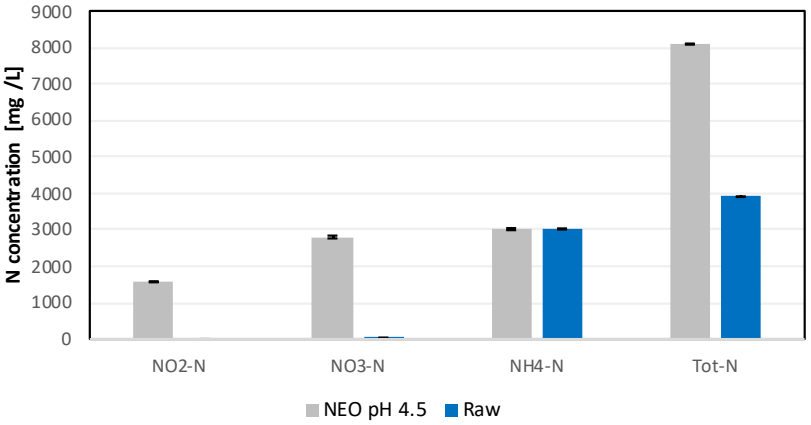


Figure 5. Nitrogen composition in untreated and NEO for a) Raw Cow Manure and NEO pH 5 and b) digestate and NEO pH 4.5 at time of sampling.

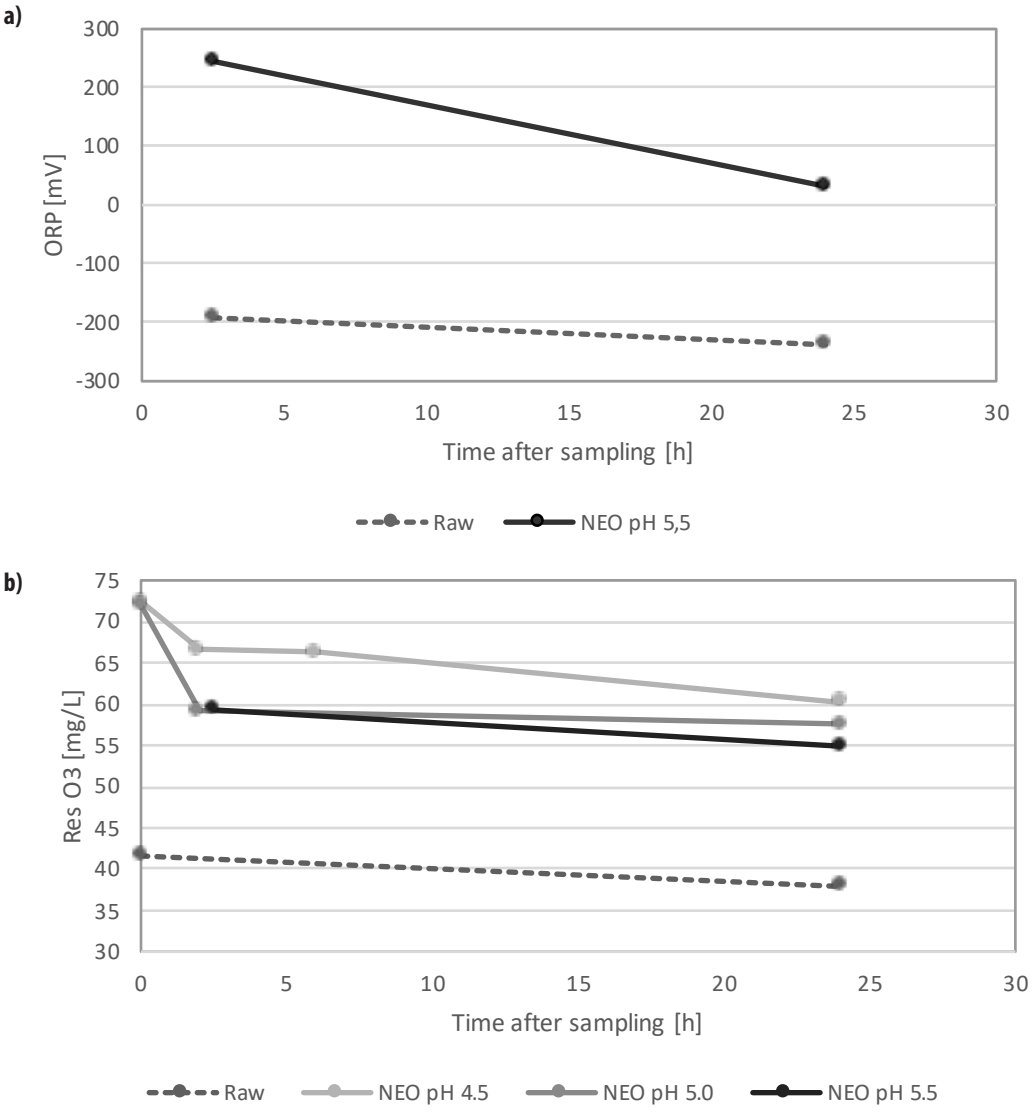


Figure 6. a) Oxygen Reduction Potential (ORP) in samples from experiment in NEO at pH 5.5 at time 2,5h and 24h after sampling. b) Residual ozone measured in samples treated to pH 5.5, pH 5.0 and pH 4.5.

Discussion

Results show that all organisms are reduced to below respective regulatory limits for hygienisation, for pH-levels and exposure times determined as Critical Control Points (CCP). It is important to note that the herein conducted experiments present a conservative estimate of the sanitising effect, as the plasma-based nitrogen enrichment treatment itself was not part of the test, only the antimicrobial effect of the freshly produced NEO. It is an assumption that the concentration of the factors causing inactivation of microorganisms, formed in slurry when exposed to plasma-activated gas, are decreasing after treatment. Thus, the required exposure time is likely to be less if microorganisms are added to slurry prior to the plasma-based nitrogen enrichment process.

Only *Salmonella* was tested at several pH levels, and only pH 5 was operated multiple

times, thus the pH 5 result is by far the most robust. More replicates are recommended in future test setups to improve robustness of results, and also to allow for including the NEO process in the tests.

The needed exposure time and pH (CCP) vary for the different indicator organisms and does also seem to be dependent on type of substrate. Comparing samples operated at pH 4.5, cow manure (NEO pH4.5) and digestate (NEO D-2), rate constants are, respectively, -5.18 and -3.22 h^{-1}. That is a considerable difference, a longer exposure time is needed in digestate (3.26 h) compared to cow slurry (2.22 h). A potential explanation is the difference in retention time. Since the NEO-D has a significantly longer retention time than NEO-C, its reactivity may be expended in the absorption system, leaving less reactivity to act in the holding tank, which results in a lower deactivation rate.

The deviating behaviour of sample NEO C-3 is distinct. No obvious explanation for this deviating result is found. The same NEO is used in *E. coli* are the same, and the *E. coli* results behave similarly to the other series. No datapoints exist for exposure times between 6 and 24 hours to verify any gradual decrease.

Only single experiments are run at different pH-levels with *Salmonella*, but the trend is consistent; Rate constant is decreased for decreasing pH (shorter exposure times needed), supporting the hypothesis of more efficient microbial deactivation activity at lower pH. At pH 5.5 24hours is not sufficient to reach the 5 log_{10}-reduction for *Salmonella.*

Mechanism

The robustness tests of the *Salmonella*-strain showed that pH itself is not sufficient to inactivate *Salmonella*, even at a pH of 4.5, other factors added to NEO in the plasma-based nitrogen enrichment process must cause inactivation.

Exposing slurry to the plasma-activated gas has similarities to an emerging disinfection method called 'Plasma-Activated Water (PAW) (Hadinoto, et al., 2022). PAW produce Reactive Oxides and Nitrogen Species (RONS), such as superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydroxyl radicals ($OH^\cdot$), hydrogen peroxide (H_2O_2), peroxynitrite (ONOOH), nitric oxide (NO) and nitrite ions (NO_2^-). These are reactive species, which due to high oxidation potentials, can damage cell components and trigger inactivation of pathogens. (Hu, et al., 2023), (Xu, et al., 2021).

Most of the listed RONS are not determined in these experiments, but some indications of involvement from RONS exist (Figure 5, Figure 6). Nitrite and ozone concentration in NEO is significantly elevated compared to cow manure, although ozone is drastically reduced within the first 2 hours after sampling. An increase in Oxidation-Reduction Potential (ORP) is one of the observed changes in a solution exposed to the PAW-process; a higher ORP leads to more efficient inactivation of microorganisms (Kasih & et al. , 2022). ORP is measured in one of the experiments in this work, NEO pH 5.5 (Figure 6). pH 5.5 is the highest pH-level tested and the experiment with least effect, and even at this pH level the increase in ORP is significant compared to cow manure. ORP is hereby seen as a possible indication for an increase of RONS in NEO and should be included in future experiments.

An assumption of steady decrease of components actively involved in inactivation of microorganisms leads to an undefined time limit of when exposure in NEO stops having effect. The inactivation must occur in the machine or in first time after production.

Comparison to existing hygienisation methods

Existing hygienisation methods in Norway involve either thermal hygienisation or a combination of thermal and chemical treatment (lime + temperature >55°C). Thermal hygienisation range from rank composting, with total residence time of several years, to thermal hydrolysis, with required residence time of minutes (Ødegaard, et al., 2009). Pasteurisation is among the most abundant methods for hygienising organic slurries requiring a residence time of 30

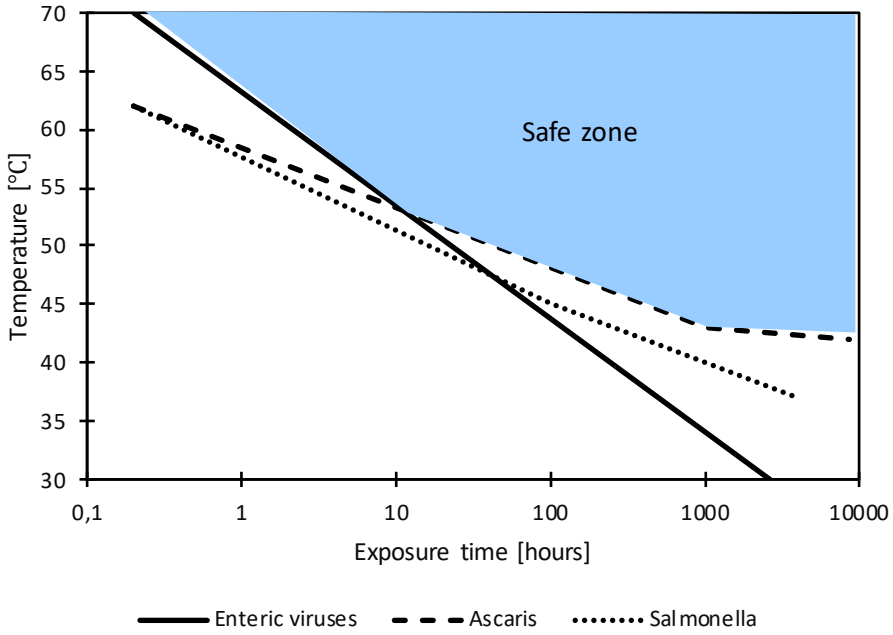


Figure 7: Temperature vs required exposure time to obtain inactivation of Enteric viruses, Ascaris suum eggs and Salmonella. Data are collected from (Feachem, et al., 1983) .

minutes at temperature >65°C (Ålund & Gentile, 2021), and is likely the most relevant comparison to nitrogen enrichment process with respect to exposure time. Figure 7 shows the relation between temperature and required exposure time in thermal hygienisation to obtain inactivation of enteric viruses, *A.suum* eggs and *Salmonella* (Feachem, et al., 1983). Exposure time to inactivate *Salmonella* and *E. coli* in this work is in the range 1.35h to 4.6 hours at pH 4.5-5.0, *A.suum* requires 16-26 hours at pH 4.5-4.6.

With respect to exposure time the thermal hygienisation method is faster, however, other factors such as reduced odour, reduced GHG-emissions, reduced loss of ammonium and increased fertiliser value from final product are positive contributions when using the plasma-based nitrogen technology for hygienisation.

Conclusion

This set of experiments have demonstrated a hygienisation effect from the plasma-based-nitrogen enrichment process, when treating substrate at low pH (<=5) and a minimum exposure time. The CCPs differ for different substrates, the required exposure times for hygienisation of the studied substrates were:

- 4 hours for NEO produced from cow manure treated to pH 5.0 (tested on *Salmonella*, *E. coli* according to the Animal by-product regulation)
- 26 hours for NEO produced from digestate at pH 4.6 (tested on *Salmonella*, *E. coli* and *A.suum*) according to the Fertiliser regulation. *A.suum* is the test organism requiring long exposure time, *Salmonella* and *E.coli* needs 4hours on this pH-level.

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References

Animaliebiproduktforskriften, 2016. *Forskrift om animalske biprodukter som ikke er beregnet på konsum (animaliebiproduktforskriften),* s.l.: Landbruks- og matdepartementet, Nærings- og fiskeridepartementet.

Chauvin, J., Jdigestateée, F. & Yousfi, M., 2017. Analysis of reactive oxygen and nitrogen species generated in three liquid media by low temperature helium plasma jet.. *Science Rep.*, 7, p. 4562.

Espey, M. et al., 2002. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species.. *Annals of the New York Academy of Sciences*, 962, pp. 195-206.

Feachem, R., Bradley, D., Garelick, H. & Mara, D., 1983. *Sanitation and disease - Health Aspects of Excreta and Wastewater Management,* s.l.: World Bank Studiesin Water Supply and Sanitation 3.

Forskrift om organisk gjødsel, 2003. *Forskrift om gjødselvarer mv. av organisk opphav,* s.l.: Helse- og omsorgsdepartementet, Klima- og miljødepartementet, Landbruks- og matdepartementet.

Graves, D., Bakken, L., Jensen, M. & Ingels, R., 2019. Plasma Activated Organic Fertilizer. *Plasma Chemistry and Plasma Processing*, 39, pp. 1-19.

Hadinoto, K., Rao, N., Barrales, J. & Zhou, R., 2022. Hybrid plasma discharges for enegy-efficient production of plasma-activated water. *Chemical engineering Journal*, 25 08, p. 10.1016/j.cej.2022.138643.

Hu, X., Feng, J., Ding, T. & Lv, R., 2023. Correlation analysis of reactive oxygen and nitrogen species (RONS) components in plasma activated water (PAW) and its inactivation of Bacillus cereus endospore. *Journal of water process engineering*, 12, p. 104332.

Kasih, T. & et al. , 2022. *Effect of plasma activated water (PAW) in maintaining the quality of cherry tomatoes.* s.l., s.n., p. 012063.

Liu, X., Lendormi, T. & Lanoisellé, J.-L., 2019. Overview of hygienization pretreatment for pasteurization and methane potential enhancement of biowaste: Challenges, state of the art and alternative technologies. *Journal of cleaner production*, 236, p. 117525.

Lukes, P., Dolozalova, E., Sisrova, I. & Clupek, M., 2014. Aqueous-phase chemistry and bactericidal effects from an air discharge plasma in contact with water: Evidence for the formation of peroxynitrite through a pseudo-second-order post-discharge reaction of H2O2 and HNO2. *Plasma sources Science and Technology*, 23(01) 02, p. 015019.

Nyvold, M. & Dörsch, P., 2024. Complete elimination of methane formation in stored livestock manure using plasma technology. *Front. Sustain. Food Syst*, 8, p. 1370542.

Pascual-Benito, M. et al., 2014. Effect of hygienization treatment on the recovery and/orregrowth of microbial indicators in sewage sludge. *Journal of Applied Microbiology*, 10 11, pp. 1364-5072.

Radi, R., 2013. Peroxynitrite, a Stealthy Biological Oxidant. *Journal of Biological Chemistry*, 13 09, Issue 288(37), pp. 26464-26472.

Regulation (EC) No. 1069/2009, 2009. Regulation (EC) No. 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No. 1774/2002 (Animal by-products Regu. *Official Journal of the European Union L 300*, 14 11, pp. 1-33.

Saveyn, H. & Eder, P., 2014. End-of-waste criteria for biodegradable waste subjected to biological treatment (compost and digestate): Techincal proposal.. *EC. JRC Scientific and policy reports.*

United states Environmental Protection Agency, 2003. *Environmental Regulations and Technology - Control of Pathogens and Vector Attraction in Sewage Sludge.* [Internett] Available at: https://www.epa.gov/sites/default/files/2015-07/documents/epa-625-r-92-013.pdf

Xu, H. et al., 2021. Subcellular mechanism of microbial inactivation during water desinfection by cold atmospheric.pressure plasma. *Water research*, Issue 188, p. 116513.

Ødegaard, H., Rusten, B., Storhaug, R. & Paulsrud, B., 2009. *Veiledning for dimensjonering av avløpsrenseanlegg,* Hamar: Norsk Vann.

Ålund, I. & Gentile, V., 2021. *Behandling og disponering av avløpsslam og annet organisk avfall i Norge,* s.l.: Carbon Limits AS/ Miljødirektoratet.