New microwave based method forreducing human pathogensfrom agricultural waste

Aditya Pandey, Omeed Momeni
Department of Electrical and Computer Engineering, University of California-Davis, California 95616, USA
Dennis DaSilva
DaSilva Dairy Farms, L.P., Escalon, 24666 Mariposa Road, Escalon, CA 95320, USA
Pramod Pandey
Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, California 95616, USA

Abstract

In the United States of America, (U.S.), California led the nation in total annual milk production by producing around 14 million tonnes annually, which is about 19% of the total U.S. milk production. The U.S. produces around 100 million tonnes of milk every year. Comparatively, India is the highest milk producer in the world by producing 24.64% of global milk production, which is about 230 million tonnes annually. In a simplistic calculation, every 1 kg of milk production results in 2.2 kg of manure production. What it means that India is likely to be producing 460 million tonnes of manure annually, and U.S. is likely to be producing about 200 million tonnes of manure every year. While livestock waste provides valuable nutrients (i.e., carbon (C), nitrogen (N), phosphorous (P), and potassium (K)) for most agronomic and horticultural crops, livestock manure also contains human pathogens such as *E. coli, Salmonella,* and *Listeria*, which causes disease in humans. The overall goal of this project is to develop a new method for removing pathogens from livestock waste. We aimed at developing an advanced technique using microwave power to eliminate pathogens from livestock manure. Overall, results are promising, and the technique we developed here was able to reduce pathogens from livestock manure within 2- 5 minutes. In addition, the technology can remove moisture rapidly from livestock manure. The moisture removal percentages were linearly related ($R^2 = 0.98$) with the time of exposure.

Keywords: livestock manure, human pathogens, milk production, E. coli, microwave

Introduction

The global significance of the dairy industry is evident, with dairy products being integral to daily life, including milk, ice cream, yogurt, cheese, and butter. In 2023, U.S. farms produced more than 226,551 million pounds of milk. In 2022, the milk production was 226,416 million pounds, with 96.12% (217,647 million pounds) used for human consumption (USDA, 2024). This milk production also results in the enormous amount of livestock manure production. The disposal of this manure requires an environmentally favorably method to avoid the risks to public and environmental health. As an example, in 2019 the USA has over 94 million cattle that generated billions of tonnes of manure (NASS, 2024). On average, more than 200 million tonnes of manure is produced annually in the U.S. India being the largest producer of milk in the world, it is likely to be producing more than 460 million tonnes of manure annually. Currently, many livestock manure treatment methods such as anaerobic digestion, aerobic digestions, composting, and drying are used to control pathogen reduction, and greenhouse gas (GHG) emission reduction (Costa et al., 2017; Pandey & Soupir, 2011; Qian et al., 2020), however, considering the volume of livestock manure produced, and associated risks to public health, improved and rapid methods for pathogen control are needed. Pathogens of livestock manure can cause contamination to ambient water bodies, which poses risks to humans.

Currently, water-borne pathogen contamination in surface and ground water is a major water quality concern in India and many other countries including the U.S. Microbial contamination is a serious issue, especially for river waters because a substantial amount of manure is used as fertilizers, and cropland's excess manure and particle attached pathogens are transported to river through runoff(EPA, 2012; Pandey et al., 2014). Previously, the United Nations considered improving water quality as one of the eight Millennium Development Goals (MDGs) in order to protect public health and targeted to reduce the number of people without access to safe water by 50% by 2015((WHO), 2011), which was met with considerable success, but still challenges do exist. In addition, there is overwhelming scientific evidence to indicate the relationships between ongoing climate change and GHG emissions ((IPCC), 2007), and livestock manure is one of the substantial sources of GHG emissions. When it comes to water issues, water consumptions are continuously increasing, and to meet the future water demands for food, and energy, additional water storage structures, water recycling, and reduction in water contaminations are essential(Pandey et al., 2014; WorldBank, 2010).

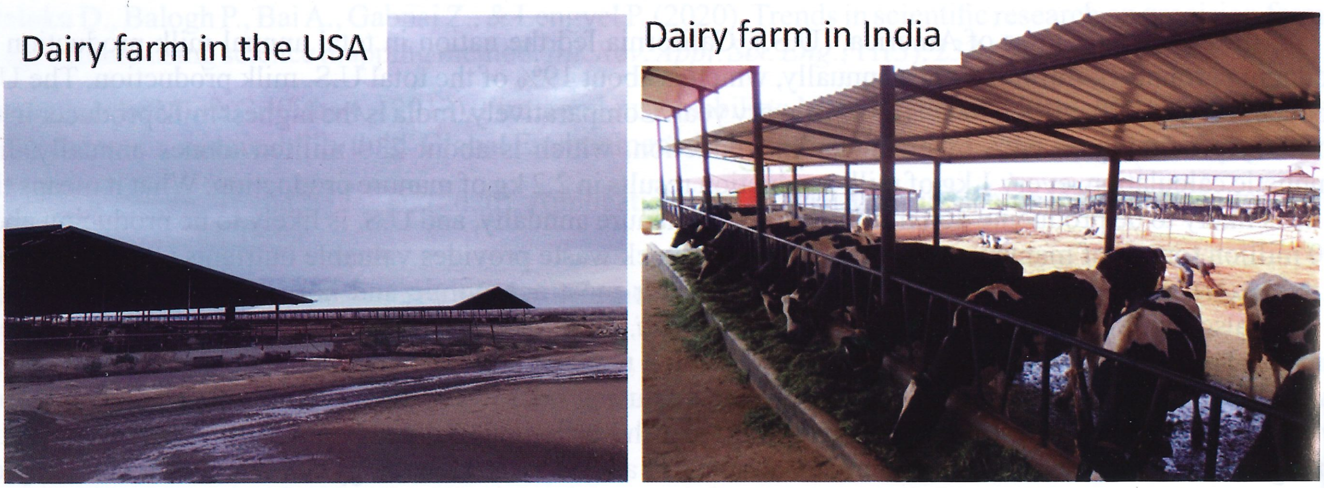

Fig. 1 Dairy fars in USA and India. In general, number of animals per farm in the US is higher than number of animals per farm in India

In terms of pathogen reductions from livestock manure, various studies have been performed to assess the risks associated with pathogens from dairy manure (Burch et al., 2017; de Matos Nascimento et al., 2020; Leach et al., 2015; Nag et al., 2021; Pell, 1997). In northern New York state, a study showed that estimated bacteria emissions from manure-amended fields were 1.27×10^5 copies $m^{-2}s^{-1}$, with a median infection risk of 1:500 at 100 meters and 1:100,000 at 1,000 meters after an 8-hour exposure, demonstrating the bioaerosol effects (Jahne et al., 2015). Composted manure is often used in land applications as organic fertilizer. One study (Pandey et al., 2018) used 16S rRNA analysis of liquid and solid manure to observe changes in microbial populations due to manure treatments, concluding that fresh and old manure piles have similar microbial populations, indicating minimal effects from composting. Another review "'(Alegbeleye & SantAna, 2020) focused on the contamination of water by manure-borne pathogens, highlighting how manure management practices impact bacterial transport. However, the key disadvantages of many of these conventional technologies, including composting, are its lengthy process, which can last several months without a well-defined time span, and often pathogen reductions are not certain during the process (Ahn et al., 2011; Brito et al., 2008; Hao et al., 2001; Inbar et al., 1993; Larney & Hao, 2007; Larney et al., 2006; Mulbry & Ahn, 2014; Owen & Silver, 2015). The goal of this research is to develop an advanced method for pathogen reduction from livestock manure to enhance the sustainability of agriculture and dairy industry, and protect public health, and ambient water.

Hypothesis & Statement of Research

Microwave heating has been widely used in households and commercial sectors for decades due to its efficiency and speed. This technology operates within the 300MHz to 30GHz range of the electromagnetic spectrum. We hypothesized that microwave radiation could be utilized for pathogen elimination from livestock manure rapidly and effectively. In addition, microwave based heating can reduce moisture content of manure rapidly, which enhances manure handling and transportation without causing biosafety risks.

Materials & Methods

From the dairy farm fresh manure is collected and stored at 4°C until the start of microwave heating experiment. The microwave utilized in the study for moisture and bacterial removal experiments was Panasonic Household Microwave (Oven Model: NN-SN968B; Newark, NJ USA) with rated cavity power of 1250W. To determine pathogen removal from livestock manure, we tested E. coli at different stages of microwave treatment. To determine the E. coli level, we used a standard protocol for bacterial enumeration, which was developed by United States Environmental Protection agency Method 1103.2(USEPA, 2023). For the bacterial (E. coli) culture plating, Avantor Difco™ Modified mTEC Agar, BD (Radnor, PA USA) plates and Advantec A045H047A Sterile MCE Gridded Filters, 0.45µm, 47mm Dia (Dublin, CA USA) were utilized. To determine E. coli levels, a known volume of manure liquid (after suitable dilution) was filtered through 0.45µm filters, and the filters were placed on agar plate. Filters placed in agar were incubated at 44.5 ± 0.2°C for 16-20 hours, and the colonies with red magenta color appeared in the agar plates were enumerated as E. coli colonies. Each sample was analyzed in triplicate, and the method used for E. coli enumeration conforms to the USEPA approved method 1103.2 (APHA, 1999).

Results and Discussion

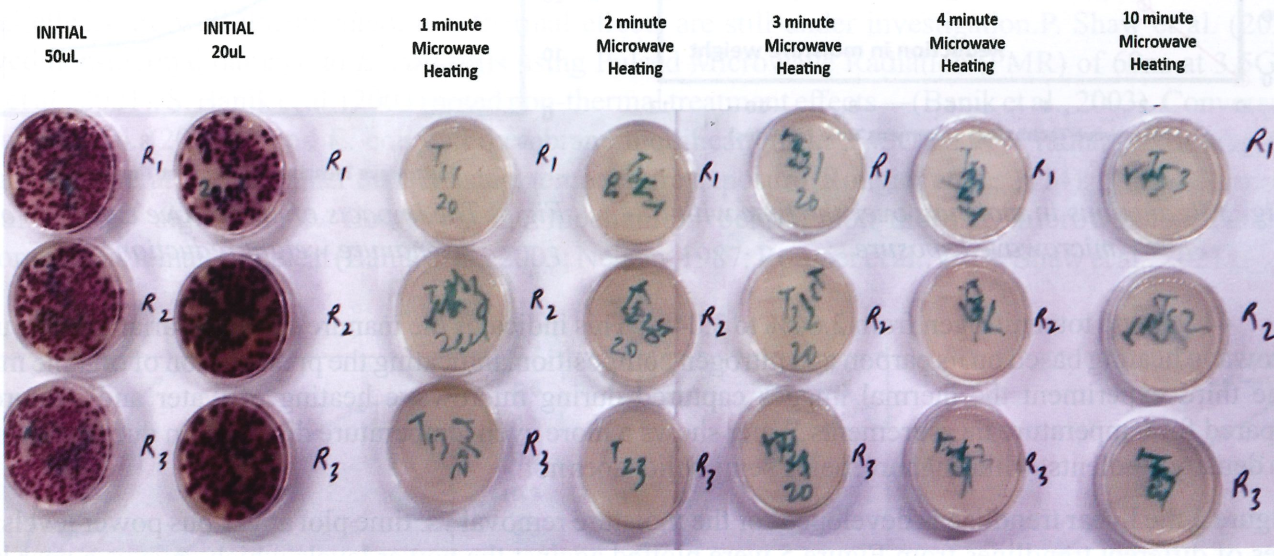

Fig. 2 E. coli enumeration in plates for investigating pathogen reduction. E. coli colonies in initial samples are shown in the left side, and right side, colonies show E. coli numbers after microwave

In the first experiment, dairy manure of 250g was exposed to microwave for 10 minutes. Manure samples were collected at regular intervals (1 min; 2 min; 3 min; 4 min; and 10 min). Solid manure before and after exposure was diluted (by 50%) in ultra-pure water produced in a Milli-Q® water filtration system. Subsequently, a known volume (50 µL) was plated in agar plates. Results are shown in Figure 2. As shown, in the initial manure (prior to exposure) we were able to see over 200 colonies in the agar plate. However, after exposure of manure to microwave, we were not able to see any bacteria in plates exposed for 1 min, 2 min, 3 min, 4 min, and 10 min. Results showed that the exposure of manure to microwave for 1-3 minutes should be sufficient for elimination of pathogens from manure. Moisture reduction over the time is shown in Figure 3 and total manure weight reduction is shown in Figure 4. In the second experiment we found that microwave heated manure loses moisture over time, showing a near-linear relationship between moisture removal and time. Higher microwave power reduces the time for total moisture removal, making the curve steeper. Reducing moisture from 87.5% to 10.8% took 93 minutes at 955W and 15 minutes to reach 5.7% at 4500W and the moisture removal curve can be seen in Figure 5. The energy consumption vs. moisture removal curves slightly overlaps for all power levels, with total energy consumption ranging from 1.12 to 1.37 kWh. The lowest energy consumption was at 4500W (1.12 kWh) and the highest at 1740W (1.37 kWh). Utilizing again the US EPA method for bacteriaenumeration we found E. coli levels went to undetectable limits within 5 minutes. The DNA extracted from treated manure samples was analyzed using a Thermo Fisher Scientific Qubit4 Fluorometer and Agilent 4200 TapeStation System. The Qubit4 Fluorometer measured DNA concentration in the elution buffer, while the TapeStation provided the DNA Integration Number (DIN). The initial DNA concentration of 92 ng/μL decreased to 8.6 ng/μL on average demonstrating DNA degradation of 90.6%. The samples from the experiment were also analyzed for total carbon and nitrogen. After removing them from 4°C storage, they were dried at 104°C

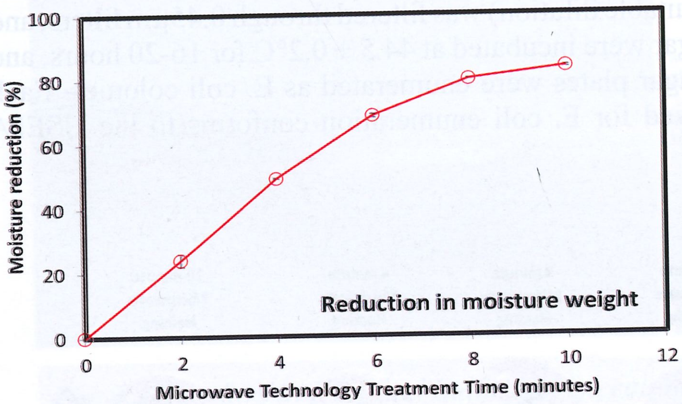

Fig. 3 Reductions in moisture over the time with microwave exposure

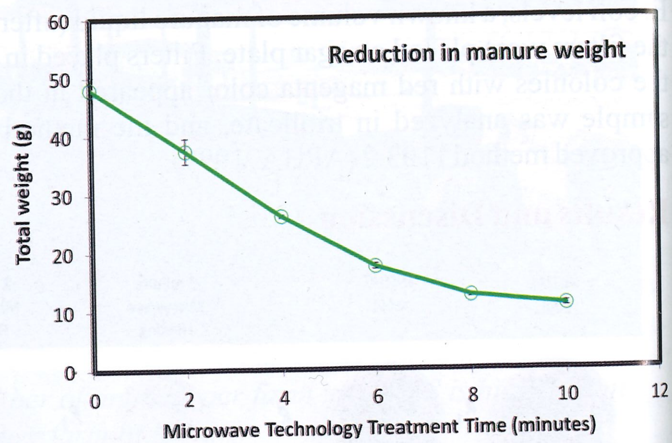

Fig. 4 The impacts of microwave exposure on manure weight reduction

44.9% and total nitrogen from 2.64% to 3.04%. This indicates the manure's near-invariant nature under microwave heating based on its carbon and nitrogen composition, indicating the preservation of organic matter. In the third experiment the thermal images captured during microwave heating of water and manure are compared for temperature measurements. Water shows a more even temperature distribution due to movement from density gradients, while manure, being semi-solid, does not.

In Figure 5 the linear trendline is developed for the moisture removal vs. time plot at various power levels. The slopes of different trendlines from Figure 5 were plotted against the power levels which gave us a near linear relation, and this helped in developing the empirical equation (1) which can be used to calculate the moisture removal with manure weight, microwave power and time as the input variables.

Similarly, we have the linear relation for the temperature rise for water and manure vs. time at various power levels and then the slopes obtained from the equations developed were used to plot against power levels in Figure 6 for water (left) and manure (right) respectively. The trendlines from were then used to develop the empirical equation (2) and (3) for calculating the temperature rise in water and manure respectively with weight, microwave power and time as input variables.

$$MR\ \% = 0.0014 \times MW(kg) \times P(W) \times T(\min) \quad .....(1)$$

$$TRW\ (^\circ C) = 0.006 \times WW(kg) \times P(W) \times T(sec) \quad .....(2)$$

$$TRM\ (^\circ C) = 0.007 \times MW(kg) \times P(W) \times T(sec) \quad .....(3)$$

Where, MR = Moisture Removal; MW = Manure Weight; TRW = Temperature Rise in Water; STRM = Slope of Temperature Rise in Manure; TRM = Temperature Rise in Manure; WW = Weight of Water; MW = Manure Weight; P = Power; T = Time.

Microwave thermal heating is crucial for increasing temperature to eliminate bacteria. Numerous studies validate these sterilization effects. K. Sasaki et al. (1998) sterilized 25,000 injection ampules using microwave heating, achieving 100% deactivation of Bacillus stearothermophilus ATCC 7953 spores(Sasaki et al., 1998). J.M. Latimer and J.M. Matsen (1977) found that Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus, and Staphylococcus aureus were eliminated in under a minute of microwave heating(Latimer & Matsen, 1977). A. Soni et al. (2020) reviewed microwave sterilization applications, predicting promising outcomes with further exploration(Soni et al., 2020). M. Novotny et al. (2013) developed a method to eradicate wood-destroying insects Hylotrupesbajulus, Anobiumpertinax, and Anobium striatum in 10 minutes using a 1200W microwave generator, requiring the wood temperature to reach 60°C (Novotny et al., 2013). Our previous study demonstrated81.6% microbial DNA degradation in E. coli cultures after 8 minutes of microwave heating at 4.5kW, with no viable colonies remaining(Pandey et al., 2024).

Sterilization via microwave primarily relies on thermal effects. At 2.45GHz frequency, microwaves effectively heat water, which inactivates or kills microorganisms upon reaching lethal temperatures. While thermal effects are well-documented, non-thermal effects are still under investigation.P. Shaw et al. (2021) observed non-thermal damage to *E. coli* cells using Pulsed Microwave Radiation (PMR) of 60ns at 3.5GHz (Shaw et al., 2021). S. Banik et al. (2003) noted non-thermal treatment effects —(Banik et al., 2003). Conversely, C. Rougier et al. (2014) found E. coli cell membrane modifications at 2.45GHz MW radiation, linking cell damage to temperature rise rather than constant temperature exposure (Rougier et al., 2014). This underscores thermal effects' significance. However, the mechanism behind non-thermal microwave effects on microorganisms remains unclear(Banik et al., 2003; Nelson, 1987; Rougier et al., 2014; Shaw et al., 2021).

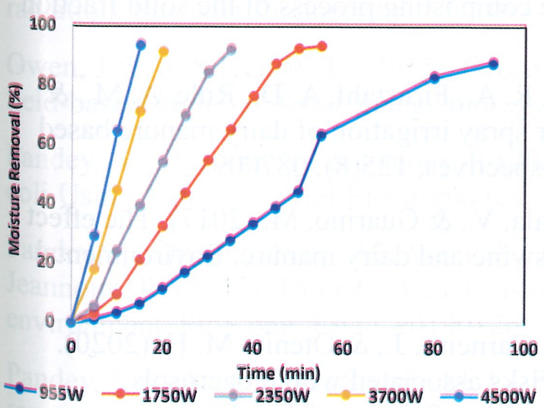

Fig. 5 The impacts of various levels of micro-wave power and exposure time on moisture

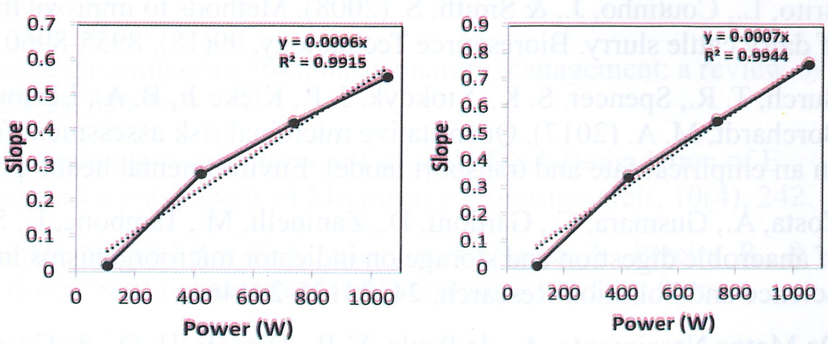

Fig. 6 Plot for slope of termperature rise vs. power level for water (left) and manure (right)

Conclusion

This study demonstrates the potential of microwave heating for treating dairy manure, effectively controlling pathogens. With moisture removal, there's a substantial 80-90% reduction in mass, enhancing storage efficiency. Organic matter remains largely intact, as indicated by total carbon and total nitrogen analyses of treated samples. Post-treatment, manure retains its organic content and nutrients, while eliminating pathogen risks. Experiment 2 establishes an empirical formula for moisture removal percentage, incorporating factors like manure weight, microwave power, and time. This equation (1) can be adjusted to consider power level by factoring in microwave conversion efficiency.

Moisture removal vs. time curves exhibit linear trends across various power levels, with R^2 values ranging from 0.98 to 0.99. Similarly, thermal imaging experiments show linear trends, with R^2 values ranging from 0.8437 to 0.9775 for water and 0.8182 to 0.9904 for manure temperature rise over time. The relationship between temperature rise in water and manure follows a linear trendline, with an R^2 value of 0.9948 and a multiplication factor of 1.1119, suggesting comparable trends which can help in estimation for manure based on water. This method, developed at the lab scale, holds promise for scaling up to field experiments, utilizing the empirical equations for better estimations.

References

(IPCC), I. P. o. C. C. (2007). AR4 Climate Change 2007: Synthesis Report - IPCC. https://www.ipcc.ch/report/ar4/syr/

(WHO), W. H. O. (2011). World Health Statistics 2011. https://www.who.int/publications/i/item/9789241564199

Ahn, H. K., Mulbry, W., White, J., & Kondrad, S. (2011). Pile mixing increases greenhouse gas emissions during composting of dairy manure. Bioresource Technology, 102(3), 2904-2909.

Alegbeleye, O. O., & Sant'Ana, A. S. (2020). Manure-borne pathogens as an important source of water contamination: An update on the dynamics of pathogen survival/transport as well as practical risk mitigation strategies. International journal of hygiene and environmental health, 227, 113524.

Banik, S., Bandyopadhyay, S., & Ganguly, S. (2003). Bioeffects of microwave—a brief review. Bioresource Technology, 87(2), 155-159.

Brito, L., Coutinho, J., & Smith, S. (2008). Methods to improve the composting process of the solid fraction of dairy cattle slurry. Bioresource Technology, 99(18), 8955-8960.

Burch, T. R., Spencer, S. K., Stokdyk, J. P., Kieke Jr, B. A., Larson, R. A., Firnstahl, A. D., Rule, A. M., & Borchardt, M. A. (2017). Quantitative microbial risk assessment for spray irrigation of dairy manure based on an empirical fate and transport model. Environmental health perspectives, 125(8), 087009.

Costa, A., Gusmara, C., Gardoni, D., Zaninelli, M., Tambone, F., Sala, V., & Guarino, M. (2017). The effect of anaerobic digestion and storage on indicator microorganisms in swine and dairy manure. Environmental Science and Pollution Research, 24, 24135-24146.

De Matos Nascimento, A., de Paula, V. R., Dias, E. H. O., da Costa Carneiro, J., & Otenio, M. H. (2020). Quantitative microbial risk assessment of occupational and public risks associated with bioaerosols generated during the application of dairy cattle wastewater as biofertilizer. Science of The Total Environment, 745, 140711.

EPA, U. (2012). Recreational Water Quality Criteria. https://www.epa.gov/sites/default/files/2015-10/documents/rwqc2012.pdf

Hao, X., Chang, C., Larney, F. J., & Travis, G. R. (2001). Greenhouse gas emissions during cattle feedlot manure composting. Journal of Environmental Quality, 30(2), 376-386.

Inbar, Y., Hadar, Y., & Chen, Y. (1993). Recycling of cattle manure: the composting process and characterization of maturity (0047-2425).

Jahne, M. A., Rogers, S. W., Holsen, T. M., & Grimberg, S. J. (2015). Quantitative microbial risk assessment of bioaerosols from a manure application site. Aerobiologia, 31, 73-87.

Larney, F. J., & Hao, X. (2007). A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta, Canada. Bioresource Technology, 98(17), 3221-3227.

Larney, F. J., Sullivan, D. M., Buckley, K. E., & Eghball, B. (2006). The role of composting in recycling manure nutrients. Canadian Journal of Soil Science, 86(4), 597-611.

Latimer, J. M., & Matsen, J. M. (1977). Microwave oven irradiation as a method for bacterial decontamination in a clinical microbiology laboratory. Journal of Clinical Microbiology, 6(4), 340-342.

Leach, K. A., Archer, S. C., Breen, J. E., Green, M. J., Ohnstad, I. C., Tuer, S., & Bradley, A. J. (2015). Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. The Veterinary Journal, 206(2), 123-130.

Mulbry, W., & Ahn, H. (2014). Greenhouse gas emissions during composting of dairy manure: Influence of the timing of pile mixing on total emissions. Biosystems Engineering, 126, 117-122.

Nag, R., Nolan, S., O'Flaherty, V., Fenton, O., Richards, K. G., Markey, B. K., Whyte, P., Bolton, D., & Cummins, E. (2021). Quantitative microbial human exposure model for faecal indicator bacteria and risk assessment of pathogenic Escherichia coli in surface runoff following application of dairy cattle slurry and co-digestate to grassland. Journal of Environmental Management, 299, 113627.

NASS, U. (2024). United States Department of Agriculture National Agriculture Statistics Service. Retrieved July from https://www.nass.usda.gov/Data_and_Statistics/index.php

Nelson, S. O. (1987). Potential agricultural applications for RF and microwave energy. Transactions of the ASAE, 30(3), 818-0831.

Novotny, M., Skramlik, J., Suhajda, K., & Tichomirov, V. (2013). Sterilization of biotic pests by microwave radiation. Procedia Engineering, 57, 1094-1099.

Owen, J. J., & Silver, W. L. (2015). Greenhouse gas emissions from dairy manure management: a review of field-based studies. Global change biology, 21(2), 550-565.

Pandey, A., Momeni, O., & Pandey, P. (2024). Quantitative Analysis of Genomic DNA Degradation of E. coli Using Automated Gel Electrophoresis under Various Levels of Microwave Exposure. Gels, 10(4), 242.

Pandey, P., Chiu, C., Miao, M., Wang, Y., Settles, M., Del Rio, N. S., Castillo, A., Souza, A., Pereira, R., & Jeannotte, R. (2018). 16S rRNA analysis of diversity of manure microbial community in dairy farm environment. PloS one, 13(1), e0190126.

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. Amb Express, 4, 1-16.

Pandey, P. K., & Soupir, M. L. (2011). Escherichia coli inactivation kinetics in anaerobic digestion of dairy manure under moderate, mesophilic and thermophilic temperatures. Amb Express, 1, 1-10.

Pell, A. N. (1997). Manure and microbes: public and animal health problem? Journal of dairy science, 80(10), 2673-2681.

Qian, H., Huang, S., Chen, J., Wang, L., Hungate, B. A., van Kessel, C., Zhang, J., Deng, A., Jiang, Y., & van Groenigen, K. J. (2020). Lower-than-expected CH4 emissions from rice paddies with rising CO2 concentrations. Global change biology, 26(4), 2368-2376.

Rougier, C., Prorot, A., Chazal, P., Leveque, P., & Leprat, P. (2014). Thermal and nonthermal effects of discontinuous microwave exposure (2.45 gigahertz) on the cell membrane of Escherichia coli. Applied and environmental microbiology, 80(16), 4832-4841.

Sasaki, K., Honda, W., & Miyake, Y. (1998). Evaluation of high-temperature and short-time sterilization of injection ampules by microwave heating. PDA journal of pharmaceutical science and technology, 52(1), 5-12.

Shaw, P., Kumar, N., Mumtaz, S., Lim, J. S., Jang, J. H., Kim, D., Sahu, B. D., Bogaerts, A., & Choi, E. H. (2021). Evaluation of non-thermal effect of microwave radiation and its mode of action in bacterial cell inactivation. Scientific Reports, 11(1), 14003.

Soni, A., Smith, J., Thompson, A., & Brightwell, G. (2020). Microwave-induced thermal sterilization-A review on history, technical progress, advantages and challenges as compared to the conventional methods. Trends in Food Science & Technology, 97, 433-442.

USDA. (2024). U.S. milk production and related data (quartely and annual). USDA Economic Research Services: Dairy Data Retrieved Feb 29, 2024 from https://www.ers.usda.gov/webdocs/DataFiles/48685/quarterlymilkfactors_1_.xlsx?v=5703.6

USEPA. (2023). Method 1103.2: Escherichia coli (E. coli) in Water by Membrane Filtration Using membrane-Thermotolerant Escherichia coli Agar (mTEC). Retrieved from https://www.epa.gov/system/files/documents/2024-06/method-1103.2-10022023_508.pdf

WorldBank. (2010). Annual Report 2010. https://pubdocs.worldbank.org/en/919451420647511562/EDS20-Annual-Report-2010.pdf