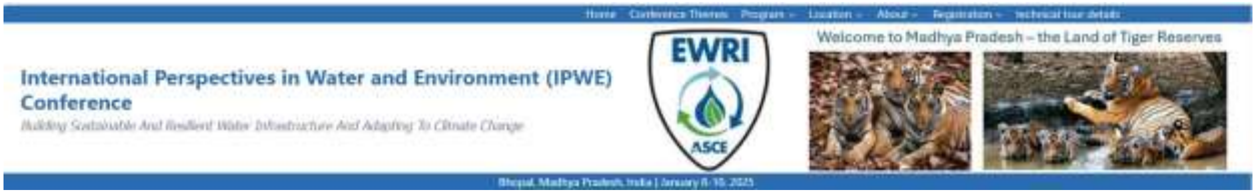


# Evolution of agricultural wastewater treatment technology implementation and dairy industry for controlling pathogens and food security

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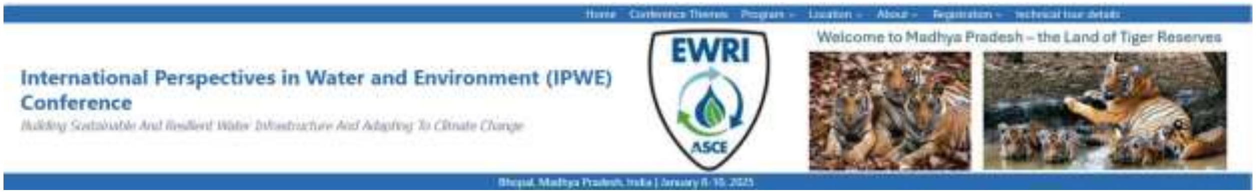
## ABSTRACT

Agriculture accounts for more than 40% of California's total water use, and state's dairy industry uses substantial amount of water for flushing the dairy sludge (i.e., manure), and often effluent from dairy farm is considered to pose microbial contamination risk. Relatively advance flush systems in dairy, which collects and transports dairy sludge, uses 200-600 gallons of water/cow/day, and generates wastewater with solid content of 1-2%. Subsequently, this dairy wastewater is stored in anaerobic lagoons as a standard practice, which causes the emissions of greenhouse gases (GHG) by biological processes responsible for climate change. This study examines the dairy wastewater technologies and analyze the functionality of the technology. Further, it evaluate the impacts of air injection and ozone injection treatment methods on *E. coli* reduction. In addition, this research provides overview of innovative policies of California Department of Food and Agriculture (CDFA), which are assisting to control the pollution from dairy waste.

## INTRODUCTION

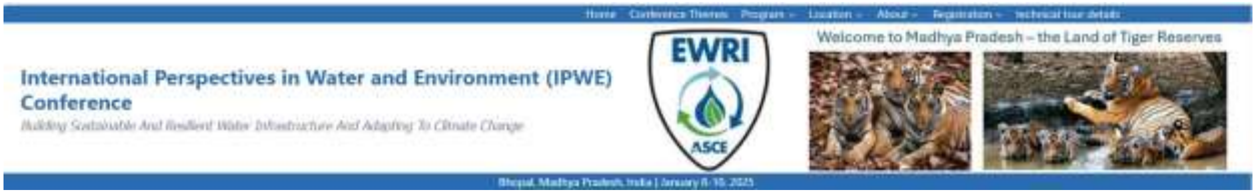
Improvement in the management of dairy wastewater have transformed the dairy industry in the way how dairy farms manage manure. More than 2 billion tons of manure is generated per year in the USA on concentrated animal feeding operation (i.e., dairy farms) (Figure 1), and majority of this dairy manure is used as a fertilizer to support plant growth (Pandey et al., 2018). In general, manure is a valuable source of nutrients, which supports the growth of plants. A relative comparison of macronutrients and micronutrients of different type of manure, and various waste material is shown in Table 1 indicates that manure is rich with many nutrients including carbon, nitrogen, phosphorous, potassium, sulphur, calcium, magnesium, iron, zinc, copper, and sodium. Raw manure (feces) contains more than 70-80% water, and 20-30% solid. The carbon content of




dairy manure can vary between 30-40% and C:N ratio varies from 15-35% (Pandey et al 2024a; Pandey et al., 2024b; Shetty et al., 2023; Shetty et al., 2024).


**Figure 1. Cattle waste production and storage in a dairy farm**

Recently substantial emphasis is given to improve manure management in dairy farms to control the emissions of greenhouse gases from manure stored in dairy farms, particularly by California State Agencies such as California Department of Food and Agriculture (CDFA), which produced promising results in fields and provided numerous benefits to dairy farms and dairymen. Dairy manure management involves handling, capturing, collecting, separating, storing, and utilization of manure as soil amendment (Figure 1). Dairy farms at large mostly use flush system to collect and capture manure from dairy barn. In a flush system, water is pumped 3-4 times per day to clean dairy farm alleys and cow holding areas using the flush valves, which facilitates labour reduction in manure handling (Pandey et al., 2024a; Pandey et al., 2024b). The flush system, which is an alternative to historical scrap system (i.e., scrapping of manure from freestalls or holding areas), collect and transport manure from holding areas through the use of water (Harner [Harner 1996]. The water requirement in a flush system vary depending on the slopes (1-2%) of holding areas, number of cows, flush lane lengths and width. Previous study showed that about 175 gal per ft or 240-620 gal of water per cow per day could be needed (Hamer, 2003). In most cases, water




from lagoon is recycled for flushing the holding areas (Pandey et al., 2018; Pandey et al., 2024a; Shetty et al., 2024).

While manure provides valuable nutrients for plants and soil (Table 1), multiple issues related with manure management related issues exist such as excessive use of water, loss of nitrogen to environment, air pollution, water pollution, microbial risks to food, and public health risks (Oliver

**Table 1. Comparative macronutrients and micronutrients of livestock and food waste**

| | Macronutrient (%) | | | | | | | Micronutrients (ppm) | | | | Process | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feedstock | C | N | P | K | S | Ca | Mg | Fe | Zn | Cu | Na | | |
| Municipal Solid Waste | 19.5 | 1.9 | 0.65 | 0.67 | 0.99 | 6.5 | 4.5 | 5930 | 184 | 80 | 5520 | Composted | Hargreaves et al. (2009) |
| Ruminant manure | 23.8 | 2 | 0.58 | 0.39 | 0.66 | 1.6 | 4.3 | 7280 | 302 | 25 | 326 | composted | Riahi et al. (2009) |
| Swine manure | 4.9 | 0.2 | 0.68 | 0.22 | 0.21 | 1.6 | 0.55 | 1899 | 106 | 74 | | Composted | Chien et al. (2011); Varma et al., 2021 |
| Green waste | 20.6 | 1.2 | 0.38 | 0.44 | 0.19 | 2.2 | 0.29 | 14300 | 190 | 85 | 1600 | Composted | Chien et al. (2011) |
| Green waste kitchen waste, paper | 25.5 | 1.7 | 0.02 | 0.53 | 0.02 | 0.04 | 0.01 | 157 | 2.9 | 1.2 | 632 | Composted | Dimambro et al. (2007), |
| Green waste,fruit, vegetable waste | 11.8 | 1 | | 0.27 | 0.04 | 0.02 | 0.01 | 108 | 0.7 | 0.3 | 432 | Composted | Dimambro et al. (2007); |
| Municipal, Kitchen, commercial waste | 37.3 | 1.9 | 0.01 | 0.31 | 0.07 | 1 | 0.09 | 70 | 39 | 13 | 2512 | Composted | Dimambro et al. (2007), |
| Biogas slurry | 18.6 | 2.8 | | 0.27 | | 0.49 | | 989 | 26 | 33 | | Vermicomposted | Sekar et al. (2010) |
| Cattle manure | 52.2 | 1.3 | 0.34 | 0.3 | | | | | | | | Vermicomposted | |
| Food waste | 19.5 | 1.3 | 2.7 | 9.2 | 2.6 | | 4.4 | | | 50 | 842 | Vermicomposted | |
| Chicken manure | | | | 0.04 | 0.007 | 0.006 | 0.012 | | | | | Anaerobic composting | Welke (2005) |
| Cattle manure | | | | 0.02 | 0.002 | 0.001 | 0.001 | | | | | Aerobic composting | Welke (2005) |
| Papermill waste | 50 | 0.48 | | 0.01 | | 0.25 | 0.03 | | | | 218 | Biochars | van Zwieten et al. (2010) |
| Farmyard manure | 39.4 | 2.2 | 4.6 | 21.6 | | | | | | | | Biodynamic composts | Zaller and Köpke (2004) |
| Dairy manure and bedding | 33.2 | 1.2 | 0.03 | 0.76 | | | | | | | | Biodynamic composts | Carpenter-Boggs et al. 2000 |

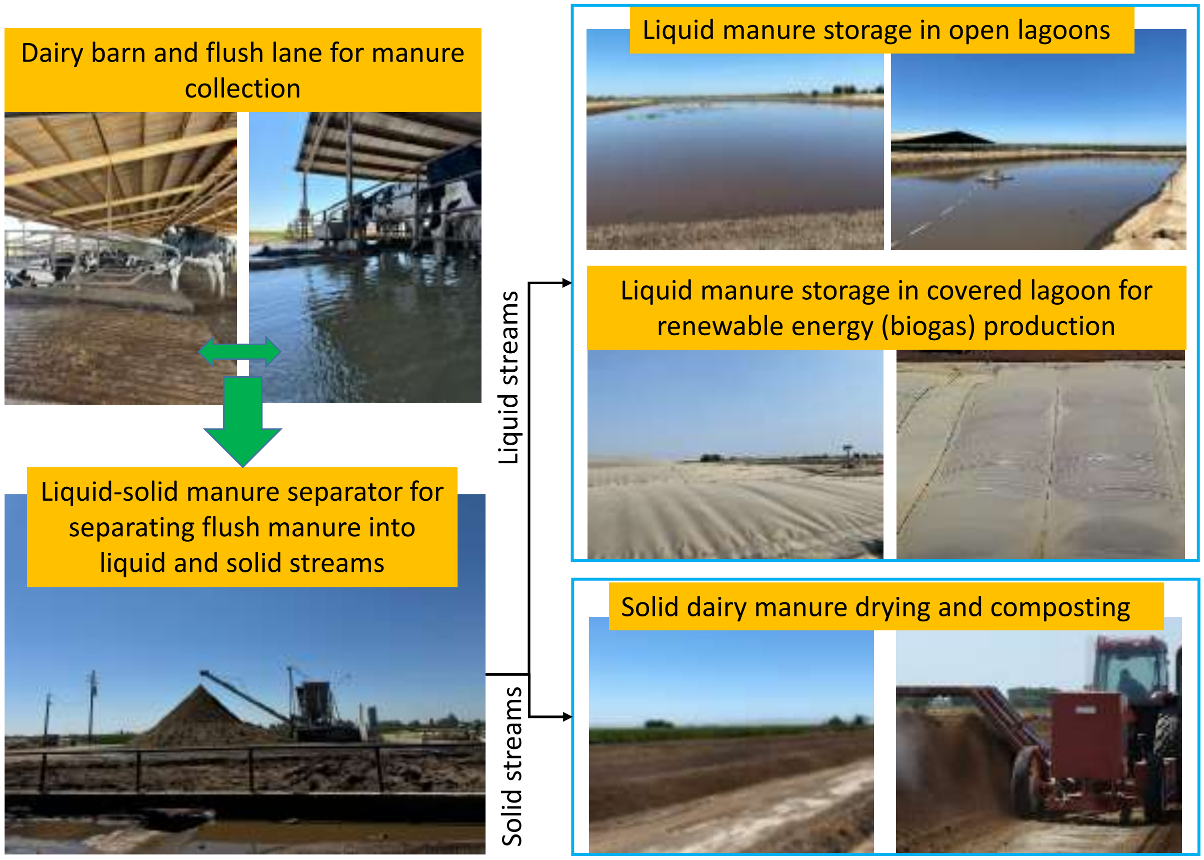



**Figure 2. Multistep liquid-solid manure separation in a typical dairy farm using flush system**

et al., 2020). Dairy waste contains bacteria including pathogens, and antibiotic residues, which has potential to cause food plant contamination (Pandey et al., 2018; Black et al., 2021; Varma et al., 2021). Further, GHG emissions from manure is a serious issue and considerable efforts are in place to control GHG emission from dairy manure (Chang et al., 2023; Ba et al., 2020; Uddin et al., 2020; El Mashad et al., 2023). Recently substantial efforts are put by state and federal agencies to control GHG emissions from livestock waste. As an example, California Department of Food and Agriculture invested hundreds of millions USD to implement the improved manure management technologies in dairy farms which are proven to control GHG emissions from cattle manure (El Mashad et al., 2023).

In comparison to human waste (feces), cattle feces contains less number of disease causing human pathogens, and more cellulosic and fiber material. A single dairy cow can produce more than 60 liters of urine and feces per day, however, an average excretion of feces and urine from human is 1.5 liters per day. Comparing the global population of human and livestock, Figure 3 shows the total livestock and human waste production. Because of public health risks, the majority of human feces goes through some sort of treatment (Giusti, 2009; Hargreaves et al., 2018).

However, in the case of livestock, majority of the waste is untreated or insufficiently treated. Further, livestock produces substantially higher amount of waste, and the economic feasibility and limited available options for treatment are prohibitive. Majority of the existing treatment methods used for animal waste treatment are yet to be perfected. In addition, over the time the size of farms (number of animals per farm) increased substantially, and number of farms reduced considerably. What it means that a large amount of dairy waste is stored in limited number of farms. One of the major issues with this is that untreated manure causes microbial contamination, which causes risks to public and animal health. The goal of this research is to provide overview of different steps involved in dairy waste handling, current efforts on improvement of manure management, and potential treatment options, which can be implemented to improve manure management.

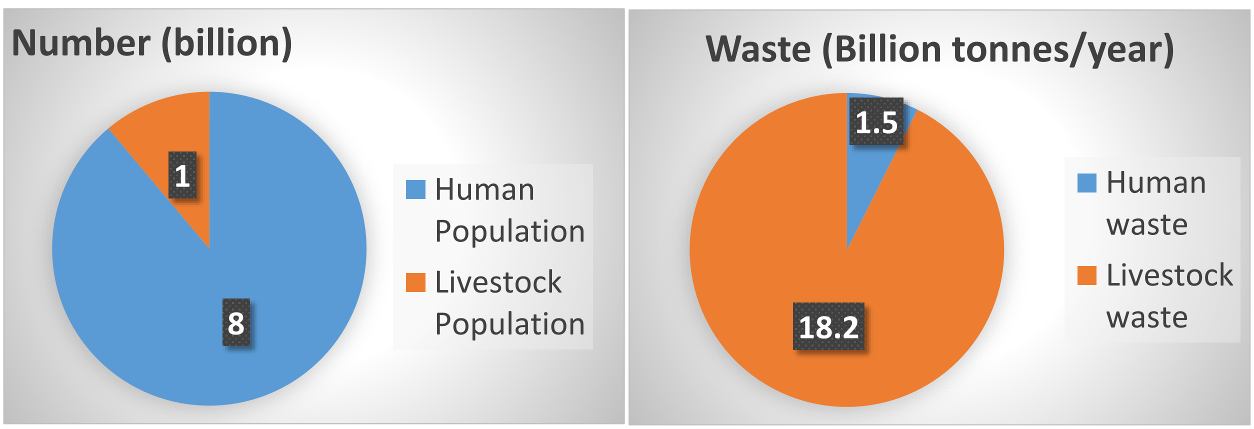



**Figure 3. Global livestock and human population and comparative waste production (average numbers are for 2021-2023)**

## MATERIAL AND METHODS

### Manure Testing Methods

This research involved dairy farm visits, understanding manure management in dairy farms, evaluating manure characteristics, literature review on existing technologies, lab experiments, and data analysis. In terms of dairy farm visit, we focused on evaluating multiple dairy farms in Central Valley of California. Current literature on animal waste treatment technologies was synthesised to understand the various manure management options available and implemented across developing and developed countries. In order to test manure characteristics such as total solids, moisture content, and volatile solids, we used standard methods of measurement published by American

Public Health Association (APHA). To evaluate the degradation during ozone and air injection (i.e., *E. coli* in manure), we used EPA Method 1603, which uses modified mTEC agar for detecting indicator organisms in liquid. The methods are published in details elsewhere (Pandey et al., 2024a; 2024b). To test the *Salmonella* in manure sample collected from dairy farms, we used XLT-4 Agar plates. In order to test presumptive *E. coli* in manure samples, we used MacConkey Agar plates (Pandey et al., 2024a).

**Batch Experiment for Ozone and Air Injection**

To test the impacts of air and ozone injection, an experiment setup was designed using ozone generator, ozone injection, ozone distributer, ozone regulation, and ozone exposure system. This experiment setup was previously used for poultry litter and manure experiment, and setup details are described in previously published research (Chang et al., 2020; Pandey et al., 2024 a).

## RESULTS

***E. coli* and *Salmonella* prevalence**

To test the prevalence of presumptive *E. coli* and *Salmonella*, we collected 177 samples from lagoons located in dairy farms. These samples were processed using method described in the method section for determining the prevalence of pathogens. Results showed that out of 177 samples, 142 samples were positive to *E. coli*. In contrast, only 4 samples were positive to *Salmonella* (Table 2). These results showed that *E. coli* is more common in dairy manure than *Salmonella*, and identification of treatment technology, which can control *E. coli* in manure is needed. Approximately 80% of samples were positive to *E. coli*, while only 2.2% of samples were positive to *Salmonella.* In general, *E. coli* is a group of bacteria (Figure 4) that may cause infections in human gut, and urinary parts. Many of *E. coli* strains are not excessively harmful to human but strains such as Shiga-toxin producing *E. coli* (STEC) can cause severe illness.

**Impact of Air Injection**

In dairy farm, recirculation and air injection is often used to create aeration in manure. Aeration is often considered to be useful for reducing bacterial and organic loads in waste water, however, the extent of aeration is needed for bacteria control in dairy manure is not well established. In order to test the effectiveness of short-term aeration on *E. coli* reduction, we used specially designed setup to inject air into liquid manure for 240 minutes. Samples at regular intervals were collected and tested using modified mTEC agar to determine the reduction in *E. coli*. This short term experiment showed that air injection was not a very effective solution. As shown in Figure 5, *E. coli* in initial samples were 7 order of magnitude. In this figure, the results of control (no air and ozone injection), air injection (only air injection), and ozone injection (only ozone injection) are shown. At the end of experiment, there was slight reduction in *E. coli*, however, the reduction was minimal. Final samples also showed *E. coli* level in 7 order of magnitude.

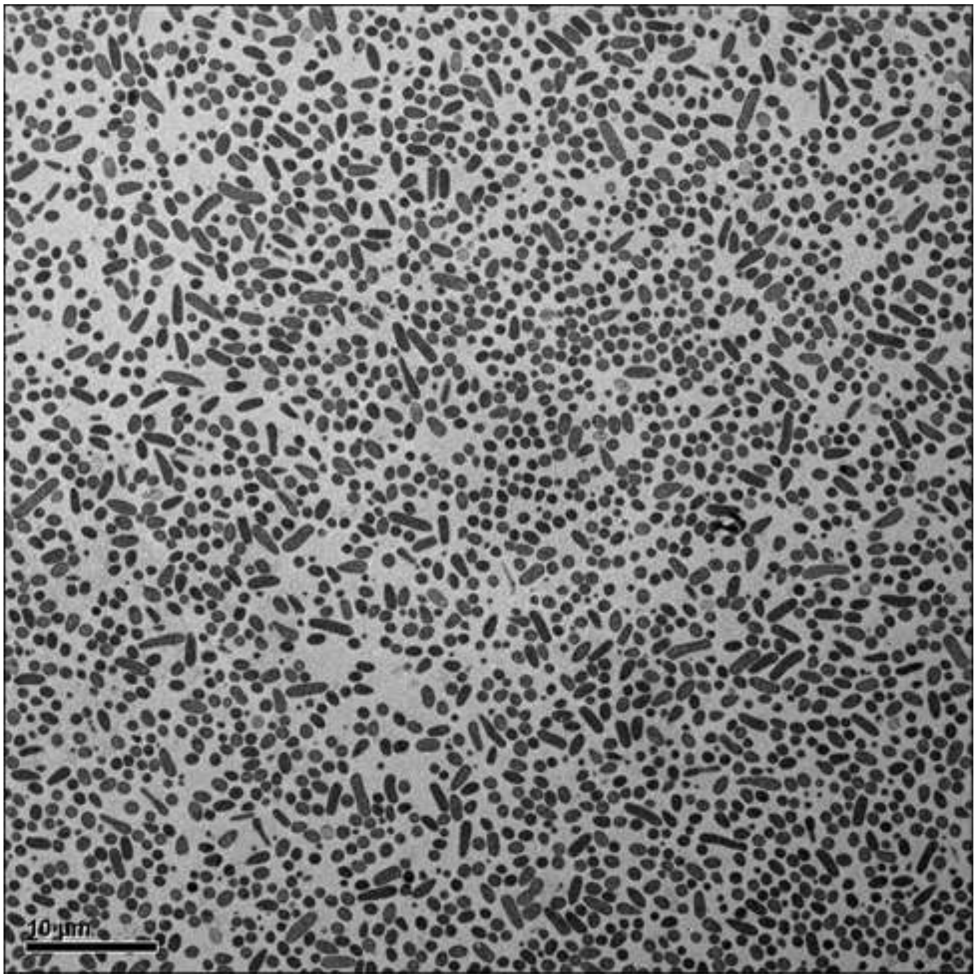
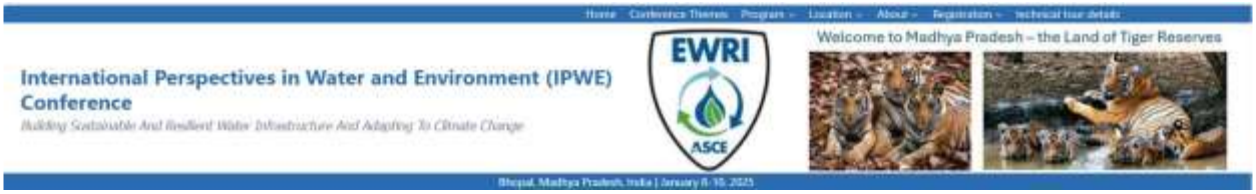



**Figure 4. Transmission Electron Microscopy (TEM) of *E. coli***

## Impact of Ozone Injection

Another method, which is relatively novel, is ozonation, and experiments were conducted to test this method on dairy manure. Ozone gas was produced using ozone generator, and the concentrations of ozone was monitored in real time to control the concentrations by adjusting the flow of oxygen and air. Oxygen was passed through the ozone generator, which converted oxygen into ozone. Air was mixed with ozone to dilute the ozone concentrations, and for the experiment,

**Table 2. *E. coli* and *Salmonella* prevalence in dairy waste water**




| Sample Type | Sample collection method | Sample no. | Bacteria test | Positive sample no. |
|---|---|---|---|---|
| Liquid from lagoon | Grab samples | 177 | *E. coli* | 142 |
| Liquid from lagoon | Grab samples | 177 | *Salmonella* | 4 |

ozone concentration was set to 2.38% wt of oxygen. Interestingly, ozone injection provided promising results, and *E. coli* level was reduced substantially. In initial sample, *E. coli* level was 7 order of magnitude, and within 200 minutes, *E. coli* was reduced to no-detectable levels. This 7 order of *E. coli* reduction can be considered an excellent outcome, and showed that ozone injection can be an option for reducing *E. coli* from dairy waste water rapidly. Currently, this technology (i.e., ozonation) for dairy waste treatment is not well established, and we anticipate that the results of this research enhanced our understanding of bacteria removal in dairy waste water using ozone and air injection. In comparison to air injection, ozone injection was significantly more effective for *E. coli* removal. In general, conversion of oxygen into ozone is relatively a simpler method and technology is readily available.

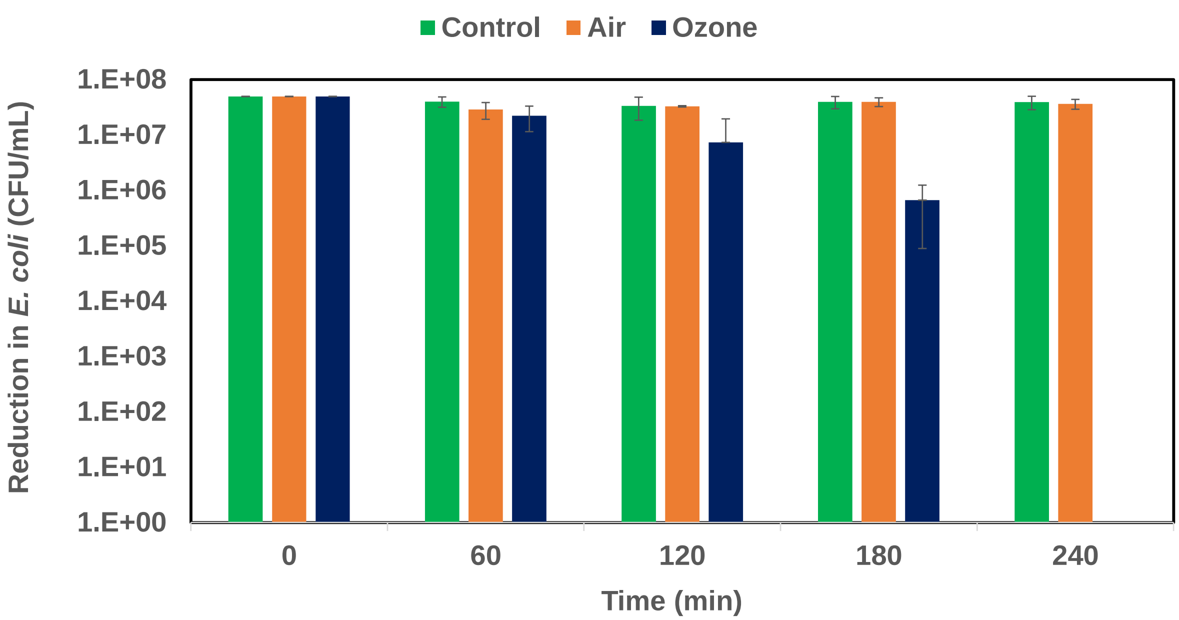



**Figure 5. Impacts of ozone and air injection on *E. coli* removal.**

**DISCUSSION**

Currently enormous amount of manure is produced in dairy farms, and over the time microbial contamination caused by manure borne bacteria became a significant issue potentially due to multiple outbreaks, which were linked with dairy manure application as soil amendment to fertilize the crop. Currently, large scale technology, which are robust and simple are needed to treat dairy waste water. Historically, the herd size in dairy farms were smaller, however, over the time the average herd size in dairy farms increased substantially. As an example, dairy farm numbers in California were 4,500 in 1991, and total number of cattle were 1.13 million. The average herd size per farm was 252. In contrast, by 2021, the average herd size was 1,438 (Figure 6). The total number of dairy farms reduced from 4,500 to 1,195. However, the total cattle population slightly increased from 1.13 million in 1991 to 1.71 million in 2021. This increase in herd size per farm was potentially needed to enhance the productivity of dairy farms and reduce the cost of production. However, this also poses challenges in manure management because an enormous amount of manure is stored in limited numbers of farms. In order to treat manure in these large-scale farm, improved methods are needed, especially to control bacteria. This research showed that aeration may not be a rapid solutions, and ozonation could provide a rapid solution for *E. coli* reduction in manure.

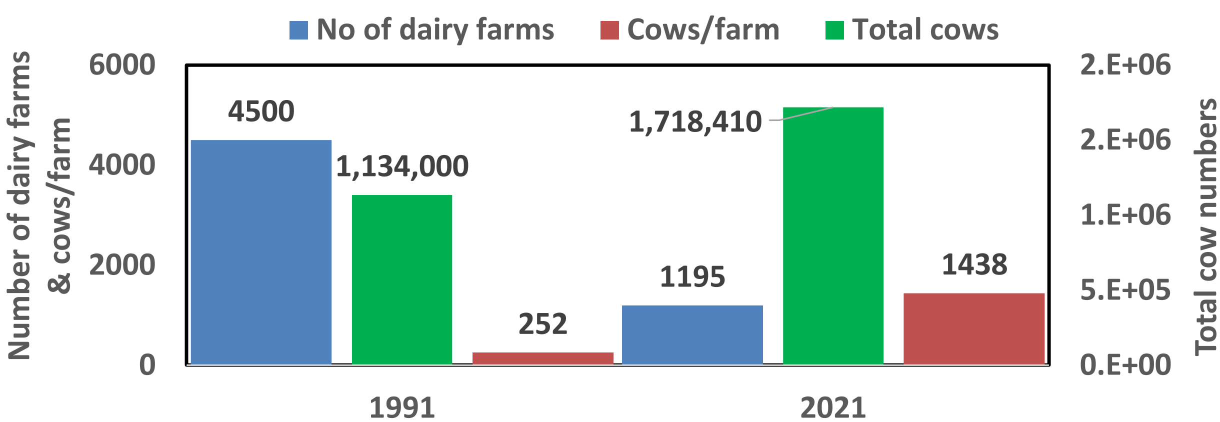



**Figure 6. Evolution of dairy farm numbers and herd size in California**

## CONCLUSIONS

From years of field trips and research in dairy farm, it was observed that the flush system of manure management (Fig. 1) is the most common type of manure removal methods in dairy farms. Further, mechanical liquid-solid separation technique was found to be the most common technique in California for separating liquid and solid streams of manure. Majority of the farms in California are using open lagoons to store liquid manure. A significant interest among farmers was observed recently towards using anaerobic digester for treating dairy manure, which produces biogas (a


source of renewable energy). Currently limited options are available for pathogen removal from liquid manure, and this study tested air injection and ozone injection for controlling microbial pollution from dairy waste water. This method can also be useful for removal of viruses, however, further tests are needed. Results of this research showed that the impact of ozone on *E. coli* removal was rapid, and this process removed *E. coli* from manure in less than 240 minutes. The air injection method for controlling *E. coli* was not found to be very effective. The ozone injection process disinfect dairy waste water effectively. Further, the ozone injection is a scalable process and can be designed for any size farms depending on the amount of manure needs to be treated, and it can be a potential option for both types of farms (small size and large size), especially when microbial contamination and outbreak risks exist.

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