



## Research article

## Centrifuge separation effect on bacterial indicator reduction in dairy manure

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## ABSTRACT

Centrifugation is a commonly applied separation method for manure processing on large farms to separate solids and nutrients. Pathogen reduction is also an important consideration for managing manure. Appropriate treatment reduces risks from pathogen exposure when manure is used as soil amendments or the processed liquid stream is recycled to flush the barn. This study investigated the effects of centrifugation and polymer addition on bacterial indicator removal from the liquid fraction of manure slurries. Farm samples were taken from a manure centrifuge processing system. There were negligible changes of quantified pathogen indicator concentrations in the low-solids centrate compared to the influent slurry. To study if possible improvements could be made to the system, lab scale experiments were performed investigating a range of g-forces and flocculating polymer addition. The results demonstrated that polymer addition had a negligible effect on the indicator bacteria levels when centrifuged at high g forces. However, the higher g force centrifugation was capable of reducing bacterial indicator levels up to two-log<sub>10</sub> in the liquid stream of the manure, although at speeds higher than typical centrifuge operations currently used for manure processing applications. This study suggests manure centrifuge equipment could be redesigned to provide pathogen reduction to meet emerging issues, such as zoonotic pathogen control.

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## 1. Introduction

Large livestock farms often process manure before land application to recycle water for manure flushing, floor and sand washing (Sarkar et al., 2006). This not only reduces water usage at the farm but reduces the volume of manure that needs to eventually be land applied. Large-scale centrifugation is commonly used for dairy manure liquid/solid separation, offering high separation efficiency compared to other mechanical separators (Hjorth et al., 2010).

The emphases of most environmental studies concerning manure management have been on the effects of nutrient recovery and water quality. Nevertheless, it is important to monitor and to reduce bacteria in manure because many outbreaks of gastroenteritis related to livestock operations have been reported (Massé et al., 2011). Manure from large farms is often centrifuged for

slurry dewatering and the filtrate may be reused within barn operations (Hjorth et al., 2010). However, recycling water from manure can potentially cause disease outbreaks in both animal herds and humans unless the manure is handled and treated appropriately. More than 150 pathogens have been identified in manure which have subsequently been demonstrated to potentially cause zoonotic infections (Pell, 1997; AWWA, 2006). Pathogens may enter the human food chain as a result of land application of contaminated organic manures. The health risk is highest if manure is applied to ready-to-eat crops such as fruits and vegetables eaten raw (Nicholson et al., 2005). Therefore, management and treatment for pathogen reduction is an important practice in waste management quality control on large farms which also reduces potential risks of exporting pathogens to offsite users.

Not surprisingly, manure mechanical separators have been demonstrated in a limited number of studies to have low pathogen reduction efficiency (Hjorth et al., 2010). The most efficient and reliable pathogen reduction treatment for manure separated liquid is microfiltration, but such technology is rarely used on actual farms

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because of the complexity and cost of these systems (Burton, 2007). However, high speed centrifugation is a common laboratory practice used for harvesting suspended bacteria in the field of microbiology. In this paper, the “speed” of centrifuge refers to the relative centrifugal force which is the acceleration measured in multiples of the gravitational acceleration constant,  $g$ . Bacteria are harvested using a wide variety of forces ranging from 1000 to  $12,000 \times g$  (Peterson et al., 2012). Centrifugation at greater than  $5000 \times g$  has been reported to cause a loss of viability in some bacterial strains (Pembrey et al., 1999). Industrial-scale centrifuges used for manure liquid/solid separation are typically operated at around  $2000 \times g$ , although they can be operated at a higher centrifugal force. These centrifuges can spin at approximately 2500 to 3500 rpm (Floerger, 2003), and because of their size, result in high  $g$  forces which are functions of centrifugal radii. For example, a full-scale centrifuge with a 1 m radius of rotation operating at 3500 rpm would exert  $g$  forces over  $13,500 \times g$ . Typical decanter centrifuges can be operated at over  $4000 \times g$  and solid-bowl centrifuges can be operated at over  $10,000 \times g$  (Beveridge, 2000).

We hypothesized that a higher centrifuge speed than what is currently used in most farm centrifuges would be able to enhance manure solid separation efficiency and to further reduce the pathogen levels in the separated liquid stream. The bench-scale centrifuge used in this study has a maximum operating relative centrifugal force of  $10,000 \times g$ . Therefore, a longer retention time (10 min, which is typically used in cell harvesting in microbiology labs) than field centrifugation was used. The results obtained can be converted to the equivalent higher centrifuge speed at short retention time when applied to large-scale centrifugation separations following Eq. (1) (Cvjetkovic et al., 2014).

$$T_1/K_1 = T_2/K_2 \quad (1)$$

where  $T$  is the time of centrifugation, and  $K$  is the  $k$ -factor for the centrifuge rotor, which is the relative pelleting efficiency of a given centrifuge rotor at its maximum rotation speed.

On large farms, physical separation methods such as centrifugation are often enhanced by chemical addition (Vanotti et al., 2002; Amuda and Alade, 2006; Garcia et al., 2007). These chemicals bind and separate the smaller particles for efficient concentration of solids and nutrients (Zhang and Westerman, 1997). The use of polyacrylamide (PAM) polymers, their homopolymers, and their acrylamide/acrylic acid copolymers, alone or in combination with various inorganic salts, has proven to be effective in concentrating solids and nutrients in the separation process (Vanotti et al., 2002; Garcia et al., 2007). However, there is a lack of studies relating polymer properties such as charge density and molecular weight, as well as dairy manure characteristics such as total solid levels, chemical oxygen demand (COD), and particle size to separation efficiency. Furthermore, there is especially a lack of knowledge on their effects on pathogen sequestration into the solids or inactivation in the planktonic phase, in combination further referred to as pathogen reduction in the liquid stream.

The goal of this study was to systematically study the effects of slurry dewatering by centrifugation with and without polymer addition, to better understand the contribution these practices have on reducing the pathogen content of recycled farm water. In this study, the relationship between centrifuge speed, addition of PAM, and the microbial quality of the liquid stream after separation was performed using a factorial experimental design. Indicator microorganisms including total coliforms and *E. coli* were monitored as an index of the bacterial pathogen population in the samples. In addition, the centrifugation speed for optimal solids and pathogen reduction in the liquid stream was evaluated taking into account economic considerations.

## 2. Materials and methods

### 2.1. Farm scale study samples

The manure samples from a working centrifuge on a farm manure processing system were collected at a dairy farm in Rock County, Wisconsin where an industrial manure centrifuge separator was used. Centrifuge feed of raw manure was at a rate of 130 gallons per minute; the operation bowl speed was set at 2787 rpm. The after centrifuge liquid stream (centrate) was then sent to sand separators at a rate of 90 gallons per minute. This farm recycles the separation liquid from the centrifuge for sand bedding washing and barn flushing.

Manure samples before and after large-scale centrifugation were taken every 2 h over a 24-h period to eliminate the influence of daily farm operations and understand the short term variability. These samples were frozen in sterile containers immediately and then sent to the Marshfield Agriculture Research Station, University of Wisconsin-Madison, for total solids and total dissolved solids contents analysis according to standard manure testing protocols described by Peters et al. (2003). Additionally, samples after sand separation and washing were also collected, kept in 125 mL containers at  $4^\circ\text{C}$  and tested for bacteria indicator levels within 24 h. This farm discontinued using centrifuge separation a few weeks after samples were taken. To evaluate large-scale centrifuge effects on bacteria population, dairy manure samples before and after centrifuge separation were taken from another dairy farm in Dane County, Wisconsin, and enumerated for bacteria indicator cell numbers. These two large-scale centrifuges evaluated in this study were the same model and running under similar conditions, and the manure samples were both undigested raw manure. The sampling and testing procedures followed the protocol described by Liu et al. (2016b).

### 2.2. Bench scale study samples

The manure samples for bench scale experiments were collected at a large dairy farm in Manitowoc County, Wisconsin. The polymer reagents (cationic polyacrylamide) were supplied by Soil Net LLC, Belleville, WI. Manure samples were collected at the dairy farm from a mesophilic anaerobic digester in sterile containers and delivered in coolers. The samples were stored at  $4^\circ\text{C}$  until analysis, which was performed within 24 h.

### 2.3. Isolation of the bacteria strain

An *E. coli* strain was isolated from manure samples and used as a control organism for the pathogen reduction centrifuge experiments. The manure samples were collected at a dairy farm from the anaerobic digester in sterile containers. These samples were stored at  $4^\circ\text{C}$ , and the *E. coli* isolation was initiated within 24 h following the procedure described by Liu et al. (2016a).

### 2.4. Polymer characterization and screening

Molecular weight (MW) and charge density (CD) are two key features that determine the effectiveness of the polymers used in wastewater and dairy manure treatment industry. To understand the effect of MW and CD of the polymers on dairy manure solid separation, different polymers with varying CD and MW were compared. The cationic polyacrylamide (CPAM) tested were 1000-, 1100-, and 1400- series which varied in molecular weight from relatively low (AL), medium (SAL), and high (VAL), and in charge density levels as shown in Table 1.

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