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Research article

Use of cationic polymers to reduce pathogen levels during dairy manure separation

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ABSTRACT

Various separation technologies are used to deal with the enormous amounts of animal waste that large livestock operations generate. When the recycled waste stream is land applied, it is essential to lower the pathogen load to safeguard the health of livestock and humans. We investigated whether cationic polymers, used as a flocculent in the solid/liquid separation process, could reduce the pathogen indicator load in the animal waste stream. The effects of low charge density cationic polyacrylamide (CPAM) and high charge density cationic polydicyandiamide (PDCD) were investigated. Results demonstrated that CPAM was more effective than PDCD for manure coagulation and flocculation, while PDCD was more effective than CPAM in reducing the pathogen indicator loads. However, their combined use, CPAM followed by PDCD, resulted in both improved solids separation and pathogen indicator reduction.

1. Introduction

Animal manure is an excellent source of crop nutrients; when applied properly, it can improve soil structure through the addition of organic matter. However, improper application of animal manure has caused environmental issues such as excess movement of nitrogen and phosphorus to groundwater and adjacent surface waters (Ribaudo et al., 2003) and the contamination of irrigation water with pathogens (Islam et al., 2004). Solid/liquid separation of the manure produces a nutrient-rich solid and a low-nutrient, low-solids liquid stream. This is desirable in manure management. Mechanical separation alone is not suitable to remove fine suspended particles that typically contain the majority of the nutrients; thus a fair amount of nutrients remains in the liquid stream unless additives are used to enhance their removal (Vanotti et al., 2002). Chemicals to flocculate the smaller particles are used to effectively concentrate manure solids and nutrients during separation (Szögi et al., 2006). Most of the chemical additions used are polymers because of their lower dosage requirements and lower environmental impact compared to salts such as $Fe_2(SO_4)_3$ and $Al_2(SO_4)_3$. There has been a trend in using polyacrylamide (PAM), its homopolymers and its acrylamide/acrylic acid copolymers to effectively separate solids from wastewater (Garcia et al., 2007). However, whether or not PAM or other polymers have effects on pathogen reduction when applied to wastewater and animal waste is still unknown. Research has shown no significant difference in bacteria population when applying PAM treated animal waste to soil (Spackman et al., 2003), but others have reported that negatively charged high molecular weight PAM combined with inorganic salts could substantially reduce the pathogenic bacteria population in wastewater (Entry and Sojka, 2000). Polydicyandiamide (PDCD) is used as a polymer flocculant for water clarification (Meng et al., 2014), but its practical application in dairy manure is considered limited due to its low molecular weight (Zang and Li, 1994). We hypothesized that PDCD can be useful to treat the low-solids liquid stream of dairy manure after initial treatment with cationic PAM (CPAM). Thus far, most environmental studies concerning manure management have focused on the effects of nutrient recovery and on water quality. Nevertheless, the microbial quality/quantity of animal manure should not be neglected since many outbreaks of gastroenteritis related to livestock operations and manure handling practices have been reported (Massé et al., 2011).

Within large dairy farms, recycling treated dairy manure has become a common practice (Sarkar et al., 2006). The recycled water

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from the liquid stream is primarily used for floor washing and manure flushing. Additionally, large fibers are commonly separated from the solid stream and recycled as bedding (Gooch et al., 2006). The recycled materials should ideally be low in pathogen content. The most prevalent pathogenic microorganisms found in manure are the bacteria *Salmonella*, toxigenic *E. coli*, *Yersinia*, and *Campylobacter*, and the protozoa *Giardia* and *Cryptosporidium* (Bicudo and Goyal, 2003). Applying high levels of pathogens back to the barn can pose an animal health risk, such as mastitis. One study estimated that the cost of subclinical mastitis to dairy farms exceeds $35 billion worldwide (Modi et al., 2012). Beyond the needs of pathogen reduction for recycled bedding and water streams, applying manure with high pathogen levels onto crops creates a fecal-oral pathway that has the potential to increase disease outbreak. Currently, anaerobic digestion is the most widely used method for pathogen reduction for high strength municipal sludge and animal wastes, with high temperature systems creating the greatest reduction (Wright et al., 2003). In addition, with the increasing emergence of antibiotic-resistant bacterial pathogens in human/animal waste and other environmental samples (Munir and Xagoraraki, 2011), pathogen reduction at the sources using novel methods is necessary.

We investigated if cationic polymers used for flocculation of manure solids could also result in bacterial reduction. Many species of bacteria secrete polymers into their aqueous surroundings which are described as exopolysaccharides (EPS). The vast majority of EPS are anionic polyelectrolytes (Flemming and Wingender, 2001). It is known that most bacterial surfaces bear a net negative charge which is similar to other suspended particles in manure (Bos et al., 1999). Cationic polymers which are already used in manure separations on many farms are hypothesized to cause the flocculation of bacteria together with other negatively charged particles in manure. Separating these flocculated solids would therefore reduce the pathogen level in the remaining low-solids liquid stream, and the separated solids would be easier to handle. Specifically, the separated manure solids are less chemically, energetically, and operationally intensive to be disinfected than in the liquid stream (Malato et al., 2009). Thus, cationic polymers could potentially enhance the solid separation of manure and reduce pathogen levels in the aqueous stream.

It was also theorized that some level of pathogen destruction or inactivation could occur during manure solid/liquid separations. The bound microorganisms may act antagonistically towards one another, further reducing pathogen levels. Additionally, the polymers themselves may also have antimicrobial effects as polymeric biocides (Timofeeva and Kleshcheva, 2011). Tashiro (2001) reviewed antimicrobial polymer synthesis such as those for polyionenes (polymers with positively charged nitrogen atoms located in the backbone of a macrochain), cationic macromolecules containing pendant positively charged active groups including biguanide, quaternary ammonium salts, and quaternary pyridinium or phosphonium salts, as well as studies on their antibacterial activity (Tashiro, 2001). Ikeda et al. (1984) investigated the antimicrobial activity of homopolymers of polyacrylates and polymethyl acrylates with side-chain biguanide groups and their copolymers with acrylamide (Ikeda et al., 1984). These findings revealed the antimicrobial potential of polymer use in manure separation processes.

In this paper, we investigated the effects of added polymers on the reduction of indicator organisms. Furthermore, we studied the mechanism of action of the polymers on bacterial reduction to determine whether the bacteria are merely physically trapped in the flocculated solids stream or actually inactivated by the polymeric action.

2. Materials and methods

2.1. Samples and reagents

The manure samples were collected at a dairy farm in Manitowoc County, WI and the polymer reagents were supplied by Soil Net LLC, Belleville, WI. A non-nutritive but biologically gentle matrix, Phosphate buffer with Magnesium Chloride (Hardy Diagnostics, Santa Maria, CA) was used in this project, and is subsequently referred to as “dilution blank”. It contains 42.5 mg/L KH_2PO_4 and 190 mg/L $MgCl_2$, with a pH of 7.2 ± 0.2 at 25 °C. A growth medium, trypic soy broth, was also tested. Tryptic Soy Broth (TSB), Lauryl Tryptose Broth (LTB), *E. coli* (EC) Medium, and Nutrient Agar with MUG (4-Methylumbelliferyl β-D-Glucuronide) were prepared according to manufacturer specifications (BD Difco, Franklin Lakes, NJ). Water purified by reverse osmosis with resistivity ≥17.8 MΩ cm (GenPure Pro, Thermo Scientific, Waltham, MA) was used to prepare all media.

2.2. Polymer characterization

The solids content of CPAM was measured gravimetrically in accordance with Standard Methods 2540 (APHA, 2005). Solids content of the liquid polymer PDCD was determined gravimetrically using freeze drying in a vacuum dryer (VirTis, Sentry 2.0, Warminster, PA). Both polymers used in this study were analyzed for charge density using manual titration after each polymer was made into a 1000 ppm solution. Toludiene Blue was used as the indicator. These polymer charge densities were determined by electrolyte titration followed the method described by Chung et al. (Chung et al., 1993). Reverse osmosis purified water was used as control for the titration.

2.3. Isolation of the bacteria strain

Manure samples were collected from the anaerobic digester in sterile containers. The samples were stored at 4 °C until use within 24 h. *E. coli* in the sample was selectively enriched using LTB and EC Medium as described in (USEPA, 2010), and a single strain was isolated by streaking on Nutrient Agar with MUG (APHA, 2005). This strain was inoculated into a flask containing tryptic soy broth (TSB) and incubated at 35 °C for 24 h, then suspended in TSB with 20% glycerol and frozen at −80 °C. A working culture was kept on a nutrient agar slant.

2.4. Colilert method

Populations of indicator microorganisms including total coliforms and *E. coli* (a subset of total coliforms that are more closely associated with mammalian fecal matter) are usually tested to represent the overall pathogen population in animal manure as they are typically present in higher densities than any single pathogen (Garzio-Hadzick et al., 2010). The Colilert method for simultaneous detection of total coliforms and *E. coli* is approved by the USEPA for use in drinking water testing and is widely used for this purpose (APHA, 2005). It has been demonstrated to be comparable to the USEPA Method 1680 for enumerating fecal coliforms in biosolids as long as the samples are dilute enough that the turbidity does not obscure the color change (Carner et al., 2013). The Colilert reagent (IDEXX Laboratories, Westbrook, ME) was used for testing β-galactosidase and β-glucuronidase activity. One packet of Colilert reagent was added to the assayed 100 mL sample dilutions. The entire contents of each sample dilution was poured into a Quantitray/2000 (IDEXX Laboratories, Westbrook, ME) and

sealed. The Quantitrays were then incubated for 24–28 h at 35 °C ± 0.5 °C. After incubation, the Quantitrays were examined for yellow color (total coliforms) and, in a dark place under ultraviolet light, for fluorescence (*E. coli*). The most probable number (MPN) of total coliforms and *E. coli* was determined according to the manufacturer's instructions and is based on the statistical Poisson distribution of positive and negative wells in the Quantitray/2000.

2.5. E. coli enumeration

To assess the effect of polymer on *E. coli* densities, a series of experiments were conducted in high nutrient (TSB) and low nutrient (dilution blank) conditions. Briefly, an overnight culture of the isolated *E. coli* was grown in 100 mL of TSB at 35 °C ± 0.5 °C. Flasks of 100 mL TSB media and dilution blanks of 99 mL phosphate buffer with $MgCl_2$ were prepared. Two hundred microliters of the overnight *E. coli* culture was added to each flask and dilution blank. Varying concentrations of CPAM: 0 ppm, 50 ppm, 500 ppm, and 2500 ppm (final concentration) were added each into a flask or dilution blank then gently mixed. The same procedure was repeated with 0 ppm, 1 ppm, 5 ppm, 50 ppm, 500 ppm, or 2500 ppm (final concentration) of PDCD. These flasks and dilution blanks were then incubated at 35 °C ± 0.5 °C. An initial sample was taken before incubation, and additional samples were taken after 1, 4, and 24 h of incubation. One milliliter of each sample at each time interval was added into a 99 mL dilution blank, making a 10^{-2} dilution of the original sample. Subsequent dilutions were prepared as needed, up to 10^{-8}. The dilutions were analyzed using the Colilert method described previously.

Additional experiments were conducted to improve the understanding of microorganism reduction using PDCD. A 100 mL flask of TSB with 500 ppm PDCD was inoculated with *E. coli* and incubated as described previously. A sample was taken after 24 h, and then this flask was mixed vigorously for 5 min using a sterile magnetic stir bar at 1000 rpm. A second sample was taken immediately after mixing for *E. coli* enumeration analysis. Another experiment was conducted to identify the effects of buffer solutions (dilution blanks). Two hundred microliters of an overnight *E. coli* culture was inoculated to 99 mL dilution blanks and 99 mL of reverse osmosis purified water, and then 500 ppm of PDCD was added to each solution. These solutions were incubated at room temperature for 1 h. A control of *E. coli* culture in reverse osmosis purified water only was incubated as well. *E. coli* enumeration was conducted following the method described earlier. To investigate whether charge density is a main factor for pathogen reduction, 500 ppm of a high charge density polyamine (PA) and 500 ppm aluminum chloride hexahydrate ($AlCl_3$ $6H_2O$) in dilution blanks with same amount of inoculated *E. coli* culture were also incubated. The charge density of PA used in this study is 7.20 meq/g, which is higher than the CPAM but lower than the PDCD. The *E. coli* densities in each dilution blank containing 500 ppm aluminum chloride hexahydrate, CPAM, PA, or PDCD were enumerated after 24 h incubation, following the method described previously.

2.6. ATP analysis

ATP (adenosine triphosphate) analysis, which measures living microbiological activity, was conducted to confirm the results from Colilert method in this study. *E. coli* cultures incubated for 4 h in dilution blanks with and without 500 ppm PDCD addition were each aseptically collected in 125 mL sterile polystyrene bottles. Each sample was then slowly filtered through a 0.7 μm glass filter using a 60 mL syringe. The ATP was extracted from the filter using UltraLyse 7 (Hach Company, Loveland, CO) into a 9 mL UltraLute dilution tube (Hach Company, Loveland, CO). The dilution tube was well mixed and then 100 μL from the tube was pipetted into an assay tube with 100 μL of the enzyme Luminase. The tube was gently swirled and immediately inserted into PhotonMaster Luminometer (Hach Company, Loveland, CO) to measure the relative light units.

2.7. Elemental contents determination

The elemental contents of dilution blanks and TSB including P, K, S, Na, and Cl were determined by X-ray fluorescence using a Tiger S8 fluorometer from Brucker, Madison, WI. The liquid samples were prepared in 34 mm cups with 4 μm prolene film and 7.0 g standard sample weight. They were tested using the Quant-express, best-detection method. The TSB was also digested and analyzed for total nitrogen before and after each polymer was added. This was done using AQ2 following the standard procedure of SEAL Analytical, Inc. Mequon, WI.

2.8. Transmission Election Microscopy (TEM)

Four hundred microliters of overnight *E. coli* culture was inoculated into 9.6 mL of reverse osmosis purified water with and without 500 ppm PDCD added. These solutions were incubated at room temperature for 4 h and then observed under TEM.

2.9. Manure separation using polymers – jar test

For the solid settling time/velocity test, identical 400 mL low-form Griffin beakers were used. Fifty ppm of each polymer was applied to each 200 mL manure sample. Because of the lack of separation with 50 ppm PDCD, an additional 2450 ppm of PDCD was applied. The manure samples were mixed using stir plates and the settling time was measured using a stop watch. Measurement began after stopping the mixing. The settling distance was measured at fixed time intervals using a calibrated graduated scale (minimum 0.5 mm) marked on each beaker.

2.10. Manure solids content determination

The solids content of manure samples were measured gravimetrically in accordance with American Public Health Association (APHA) Standard Methods 2540 (APHA, 2005).

2.11. Statistical analysis

The statistical analysis in this study was conducted using R program version 0.98.1091 (Rstudio, Boston, MA). Comparison among different manure separation methods was done using the t-test for independent samples.

3. Results and discussion

3.1. Polymer characterizations

The CPAM used in this study was determined to be a low charge density (0.93 meq/g) high molecular weight (6000 kDa) cationic polymer with 88.8% solids content. The PDCD was determined to be a very high charge density (39.98 meq/g) low molecular weight cationic polymer (300 kDa) with 50.5% solids content.

3.2. E. coli enumeration after CPAM treatment

Two polymers were examined in this study. Commonly used animal manure coagulant CPAM (low-charge cationic, 1000SAL) was added to an isolated *E. coli* culture at a series of concentrations.

Most CPAM farm applications are between 1 and 100 ppm; 2500 ppm CPAM application in this study was also tested as an extreme condition. As shown in Fig. 1a, there was no significant effect of CPAM on changes in *E. coli* numbers in the TSB, even at the highest concentration of the CPAM used. This result suggests that low charge CPAM does not inhibit bacterial growth in nutrient-rich conditions, even at a very high application dosage.

Under low nutrient conditions (dilution blanks), the bacterial concentrations were lower when higher concentrations of CPAM were present. Earlier research has shown that *E. coli* could potentially utilize PAM as carbon and nitrogen sources to grow (Kay-Shoemake et al., 1998). Fig. 1b shows the *E. coli* enumerations when grown in dilution blanks which contain no organic source other than polyacrylamide. The *E. coli* population with CPAM added did not increase beyond the control with no CPAM added at the 24-h time point in dilution blank. There was no evidence that the *E. coli* utilized CPAM as a carbon and nitrogen source under these conditions.

A possible reason for the reduction of the *E. coli* population when 500 ppm and 2500 ppm CPAM were added is that the viscosity of the solution increased, which can slow or inhibit the duplication of the cells as well as the dispersion of new cells. In pure cultures, the bacteria are typically present in the form of microbial aggregates as a result of the presence of extracellular polymeric substances (Sheng et al., 2010). When 200 μL of the *E. coli* overnight culture was inoculated into the buffer solutions, the bacteria were highly concentrated and in the form of aggregates. In the dilution blank with no polymer addition and in the 50 ppm PAM solutions, *E. coli* cells were easily diluted and dispersed which facilitates bacterial cell separation. In the dilution blank experiments, there were limited nutrients available. As the bacterial culture was transferred from a nutrient broth, the bacteria should have been able to continue to grow to some degree even in these conditions, but this is not sufficient to explain the nearly hundred-fold increase between the 4 and 24 h time points. It is more likely that the increase in numbers demonstrated in Fig. 1b for the higher CPAM concentrations was actually a release of bacteria that were previously trapped by the polymer. This could be a result of the decreased viscosity at higher temperatures or some other effect that reduced the ability of the polymer to flocculate the bacteria over time. Under highly viscous conditions, such as in 500 ppm and 2500 ppm CPAM solutions, the cells' movement and dispersion were hypothesized to be more limited. Most of the cells were still in the aggregated forms, which may have led to a slower cell reproduction rate and higher cell death rate. Also, low charge CPAM could cause mild coagulation and flocculation of the bacteria, resulting in a slower growth rate in the clustered bacteria.

3.3. *E. coli* enumeration after PDCD treatment

Characterization in this study indicated that PDCD SL5000 is a highly charged cationic polymer. When the PDCD was added to flasks of TSB and dilution blanks at concentrations of 500 ppm or higher, both solution types turned cloudy immediately. Enumeration curves demonstrated greater than 5-$\log_{10}$ reductions of indicator bacteria populations after 4 h incubation in TSB when PDCD was added at 50 ppm or higher concentrations. From the *E. coli* enumerations of the sample in TSB with 50 ppm PDCD, an increase in bacterial numbers was observed after the 4-h time point (Fig. 2a). TSB is formulated to promote bacterial growth; it is likely that most of the PDCD added (50 ppm) became attached to the bacteria or other substances within the first 4 h, and most of the applied PDCD became sequestered in the precipitated sediments or attached to bacterial cells. Bacteria that were still unaffected by the PDCD were able to reproduce rapidly within the remaining 20 h of the experiment.

Another interesting observation that can be seen in Fig. 2a is the change in *E. coli* concentration at hour 4 in the 50 ppm experiment compared to higher concentrations. There were less *E. coli* enumerated after 4 h in the sample with 50 ppm PDCD in TSB than in the ones with 500 ppm and 2500 ppm. One possible explanation of why 50 ppm PDCD yielded a greater reduction than 500 ppm and 2500 ppm in TSB at this time point is that when excess highly charged cationic polymer applied, it may lead to a charge reversal of the cells and cell clusters. Lower concentrations of PDCD including 5 ppm and 1 ppm did not appear to have a significant effect on reducing bacteria populations in this nutrient-rich condition as shown in Fig. 2a. Also, as seen in Fig. 2b, PDCD kills or inhibits almost 100% of indicator bacteria when incubated in dilution blanks at 50 ppm or higher polymer concentrations. 1 ppm and 5 ppm of PDCD in dilution blank solutions yielded 3-$\log_{10}$ and 4-$\log_{10}$ reduction of the *E. coli* enumerations after 24 h incubation, respectively. ATP measurements of 500 ppm PDCD treated suspensions demonstrated 99% reduction compared to buffer only controls, supporting the hypothesis that PDCD inhibits bacteria activity.

This highly, positively charged PDCD may facilitate efficient flocculation and coagulation of the bacteria as well other organic matter, nutrients, or salts. The flocculated solids or “flocs” settled to the bottom and formed a film when excess PDCD (500 and

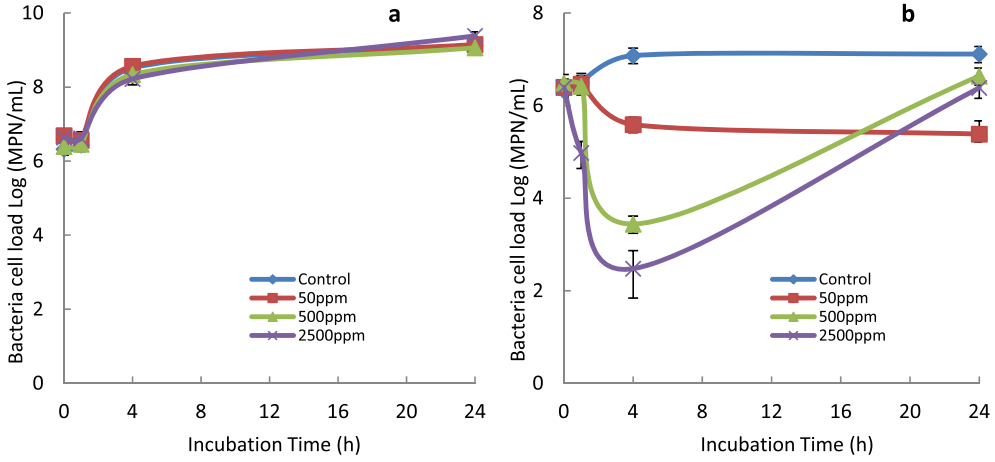

Fig. 1. *E. coli* counts in (a) TSB and (b) buffer with CPAM added.

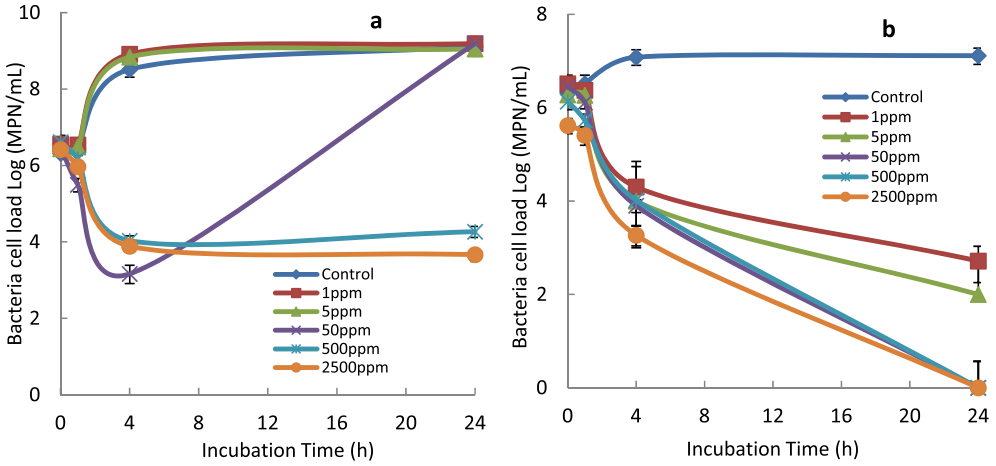

Fig. 2. *E. coli* counts in (a) TSB and (b) buffer with PDCD added.

2500 ppm) was added to both TSB and dilution blanks. The experiments with 1, 5, and 50 ppm PDCD did not form a film at the bottom of the container. A possible explanation is that when excess PDCD was applied, it reacted with organic matter, nutrients, and salts, which enhanced coagulation and flocculation. The precipitated flocs could then form a coating at the bottom of the container which bound the bacteria clusters tightly. Alternatively, Bolto and others suggested that when adding a highly charged long-chain cationic polymer, it is possible that negatively charged matter reacts with the polymer to form insoluble hydrophobic precipitates before flocculation of the solids commences (Bolto et al., 2001). Essentially, the added polymer could end up as compact insoluble aggregates incorporating the negatively charged groups (Parazak et al., 1988). Additionally, this highly positively charged polymer may act as a barrier to mass transport of nutrients contained in the growth media to microorganisms within the flocs or aggregates (Cuq et al., 1995). Either way, the bacteria are removed from the liquid stream.

To study the extent to which PDCD captured bacteria cell through flocculation or sedimentation, a flask containing TSB with 500 ppm PDCD was enumerated after 24 h incubation and then the same flask was mixed vigorously for 5 min using a magnetic stir bar at 1000 rpm. The *E. coli* enumeration was about five times greater after mixing. The results support the hypothesis that PDCD can capture bacteria in flocs that can then be disrupted by vigorous mixing.

Additional results indicate that PDCD inhibits bacterial activity in both water and buffer solutions. Cloudy precipitation was observed in the buffer solutions with PDCD added. In contrast, this precipitation was not observed in the water with PDCD added. Slightly greater bacterial reductions were seen in water with PDCD compared to buffer with PDCD added, possibly because of the coagulation of PDCD with salts in the buffer solutions.

3.4. TEM analysis

TEM images further demonstrated that PDCD formed a tight coating around bacteria cells and also caused flocculation of the bacteria cells. As seen in Fig. 3b, a thick layer of polymer has thoroughly coated a bacteria cell. This dense coating of the polymer demonstrates that charge neutralization was a possible mechanism by which PDCD reduces the planktonic *E. coli* population. This PDCD coating may reduce the ability of bacteria to reproduce by limiting the nutrient intake and retarding the cell separation. Thus, it was very likely that the cell's life cycle was disrupted and the cell activity was inhibited. On the other hand, the coated cell may have been alive after a few hours of incubation but was undetectable by the Colilert reagent. However, if the bacteria were not able to consume the Colilert reagent to initiate the color change/fluorescence, the coated cells were not expected to be metabolically active. In addition, ATP of the *E. coli* cells was also analyzed to support the Colilert results. After 24 h, cells in buffer containing 500 ppm PDCD experienced a 99% reduction in ATP levels compared to cells in buffer controls, supporting this conclusion. It was unlikely that cells in this state would be infectious unless the polymer coating was removed. In addition, the highly charged cationic PDCD can facilitate coagulation and flocculation of the bacteria to form clusters which may inhibit the bacterial growth (Fig. 3d). These clustered bacteria may form coatings on the flasks, which also made them unavailable to the enumeration method. Cationic polymers have been shown to promote cell adhesion as the highly positively charged groups such as amines interact with the negative charged groups on the cell membrane which may cause leakage of intracellular constituents (Kenawy et al., 2007). In this study, highly positively charged polymer may also have diffused through the cell wall and adsorbed onto the cytoplasmic membrane to cause the bacterial cell to burst, reducing the number of *E. coli* enumerated.

3.5. Charge density effects on pathogen reduction

PDCD has been demonstrated to be effective in reducing pathogen density in solutions. To further investigate whether charge density is a key factor of pathogen reduction, other flocculants of different change density such as $AlCl_3 \cdot 6H_2O$ (0.0124 meq/g), CPAM (0.93 meq/g), and PA (7.20 meq/g) were also tested. The results in Table 1 suggest that flocculent materials with higher charge density will result in more pathogen reduction. High charge density PA and very high charge density PDCD showed more than a 5-$\log_{10}$ and 7-$\log_{10}$ greater reduction, respectively, compared to the low charge density $AlCl_3 \cdot 6H_2O$. These results are consistent with the findings that cell-kill efficacy increases with charge density (Murata et al., 2007).

3.6. Nutrients and elements analysis

Polymers have been widely used for manure nutrient fractionation, especially with respect to nitrogen and phosphorus removal (Møller et al., 2007). Cationic polymers may also coagulate with negatively charged organic matter, nutrients, and ionic salts, which can cause depletion of available nutrients or sequestration of

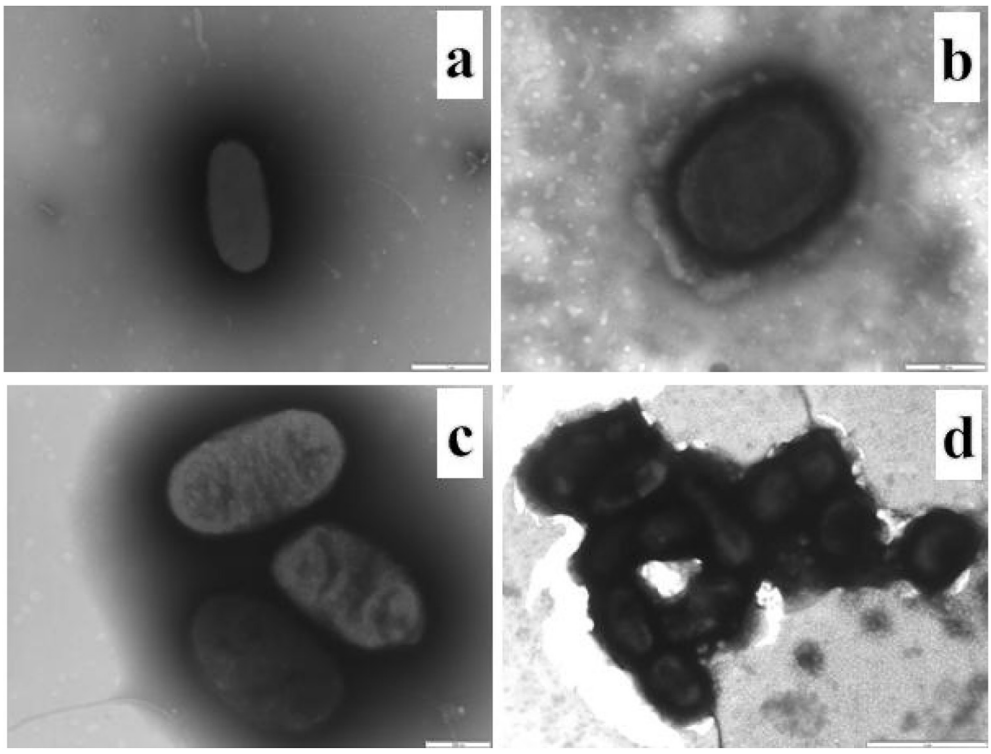

Fig. 3. *E. coli* cells after 4 h of incubation in (a, c) water alone and (b, d) with PDCD polymer (500 ppm).

Table 1
E. coli counts in dilution blanks with 500 ppm $AlCl_3$ $6H_2O$, CPAM, PA, and PDCD added after 24 h incubation.

Polymer name	Charge density (meq/g)	Log_{10} reduction
PDCD	39.98	8.01
PA	7.20	6.01
PAM	0.93	0.34
$AlCl_3$ $6H_2O$	0.01	0.06

nutrients from bacteria. To study whether polymers contribute to nutrient depletion, which could be a factor in reducing bacterial numbers, the composition of the various treatment solutions were compared. No significant differences were observed with the addition of either polymer compared to the original growth media TSB (Table 2). This result suggests that available nutrient depletion was negligible when using cationic polymer treatment in the nutrient-rich conditions of this study. The possible reason for this result is that the polymer preferentially coagulates and flocculates larger particles, which have molecular weight greater than 10 kDa (Lee and Westerhoff, 2006). Thus, the polymers used in this study appear to have been ineffective at removing dissolved nutrients, such as those present in TSB.

3.7. Applications of pathogen reduction using polymers

CPAM has been commonly used to effectively separate solids from wastewater (Vanotti and Hunt, 1999). Other highly charged cationic polymers like polyquats, polydiallyldimethylammonium chloride (polyDADMAC) and polyamines are relatively more expensive but may provide better separation efficiency in dairy manure treatment. Application of 50 ppm CPAM yielded highly efficient coagulation and flocculation of dairy manure (about 42.5% separation efficiency within 1 h), while no separation was observed when PDCD was added despite the high concentration (2500 ppm) that was applied. However, additional PDCD applied to the effluent from CPAM separation yielded further reduction of solids and bacteria indicator counts after lab-scale high speed centrifugation (15,000 × *g* for 3 min). Statistically significant decreases in total solids, total coliforms, and *E. coli* were achieved ($p < 0.05$, respectively) compared to separation solely with CPAM added. Visible separation and precipitation were observed when PDCD was added without centrifuging; this suggests it also applies in agricultural or other practical environments, such as a settling tank. Using PDCD as a second subsequent step for separation of CPAM treated manure liquid is a promising practice for solids and pathogen reduction, especially when applied to recycled water for dairy barn flushing.

Table 2
Element content in growth media and polymer solutions.

Sample	Total N (mg of N/L)	Cl (%)	Na (%)	P (%)	S (%)	K (%)
PDCD Water Solution	0.04	0.03	BDL[a]	BDL	BDL	BDL
TSB in Water	0.13	0.35	0.26	0.06	0.02	0.15
TSB with CPAM	0.22	0.39	0.27	0.06	0.02	0.15
TSB with PDCD	0.15	0.37	0.28	0.11	0.03	0.15

[a] BDL: below detection limit.

4. Conclusions

The results obtained from this study suggest that polymer addition has positive effects on manure treatment. Specifically, low charge density CPAM is effective for manure coagulation and flocculation but has a negligible effect on pathogen reduction in either nutrient-rich or nutrient-deficient conditions. In contrast, highly charged cationic PDCD does not facilitate coagulation in manure with high solids content, but it can potentially inhibit bacterial pathogens and further lower the solids content in the liquid portion of manure after CPAM separation. PDCD has potential as a new additive in manure treatment in a step subsequent to primary separation with CPAM.

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