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Ambient alkaline hydrolysis and anaerobic digestion as a mortality management strategy for whole poultry carcasses

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ABSTRACT

Livestock mortality management is a critical factor for ensuring biosecurity, minimizing environmental impact, and maintaining public trust in livestock production agriculture. The number of technologies currently used for livestock mortality management is small, including composting, burial, incineration, landfilling, and rendering. Each technology has advantages and disadvantages which make their suitability situational. In this study, ambient alkaline hydrolysis (AAH) using 2, 4, or 8 M potassium hydroxide at ambient temperature and pressure was explored as a disposal method for whole broiler chicken carcasses. Alkaline hydrolysate ($pH > 14$) resulting from the process was neutralized by mixing with acidic corn silage, and then utilized as a substrate for anaerobic digestion in bench top continuously stirred tank reactors. All AAH treatments solubilized broiler carcasses within 20 days. Corn silage neutralized 2 M hydrolysate using a 2:1 (w/w) mixing ratio, while 4 M hydrolysate required a 4:1 mixing ratio. Anaerobic digestion of neutralized hydrolysate reduced volatile solids by >96% for all treatments. Highest methane yields were observed from the 2 M hydrolysate (607.2 ± 47.9 g mL^{-1} VS), while biogas production from the 8 M hydrolysate was totally inhibited over a total of 42 days. Ambient alkaline hydrolysis followed by silage neutralization and anaerobic digestion provides a feasible, straightforward technology to manage routine and emergency animal mortalities.

1. Introduction

During animal production, livestock mortalities can occur due to disease, injury, age, or natural disaster. In order to properly manage and dispose of these mortalities, provincial authorities in Canada have developed specific regulations and guidelines. Methods of disposal can be grouped into two broad categories: on-farm disposal and off-farm disposal. On-farm disposal methods include incineration, burial, composting, and anaerobic digestion (Laporte and Hawkins, 2009). Current mortality disposal methods often have more than one deficiency, which may include: limited biosecurity, the risk of environmental contamination, and economic or logistical infeasibility. Carcass burial has been associated with soil and groundwater contamination due to pathogens and nutrients leaching during carcass decomposition (Gwyther et al., 2011). Composting is one option for livestock mortality disposal comprising advantages due to its ability to reduce recalcitrant biomolecules such as bones and keratin-rich wastes (i.e. feathers, hooves) (Reuter et al., 2015). However, the composting process has limited effectiveness for complete inactivation of pathogens such as prions or spores (Franke-Whittle and Insam, 2013; Stanford et al., 2015; Xu et al., 2014). Beyond health and environmental concerns, many mortality management methods are costly, laborious, or rely on extensive infrastructure, which is not ideal during an emergency management situation where time and resources are limited. Current mortality management mechanisms are typically strained or overwhelmed during animal disease outbreaks, due to the high numbers of animals being culled and requiring disposal in a short period of time. For example, the 2015 Highly Pathogenic Avian Influenza (HPAI) outbreak in Iowa, USA required the slaughter and biosecure disposal of over 31 million birds, including turkeys, layers, and broilers (IDALS, 2017). This scale of emergency management situation requires the development of more efficient methods which are (i) logistically feasible, (ii) meet biosecurity demands, (iii) economical, and (iv) safeguard environmental and public health.

Alkaline hydrolysis (AH) refers to the subjection of animal tissues, carcasses, and wastes to highly basic solutions, high temperature, and pressure (El-Thaher et al., 2013). Alkaline hydrolysis is

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potentially more environmentally friendly compared to other livestock mortality management options. AH has no uncontrolled emission of gases, nutrients, or pathogens into the environment. The AH process breaks peptide bonds, causing proteins to be denatured into amino acids, fats to be hydrolyzed, and carbohydrates to be solubilized (Homer et al., 2012). Further, the reaction between an alkali and a fatty ester results in the production of long-chain fatty acid (LCFA) salts and glycerol. The hydrolysate resulting from AH is more biodegradable due to depolymerization of complex molecules and increased surface area for microbial breakdown (Battimelli et al., 2009).

As outlined by Canadian legislation, AH is an approved treatment for Specified Risk Materials (SRM), which includes the brain, eyes, tongue and spinal cord of ruminants (CFIA, 2014). The treatment requires SRM to be subjected to 180 kPa pressure for 180 min at a temperature of 150 °C (El-Thaher et al., 2013). In the United States, AH is an approved method for poultry mortality disposal after Avian Influenza infection (Khan et al., 2013). Alkaline hydrolysis is effective at eliminating pathogens such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and *Mycobacterium bovis* (Kaye et al., 1998). In addition, AH has been demonstrated to effectively inactivate thermally resistant spores of *Geobacillus stearothermophilus*, as well as highly recalcitrant prion proteins (Das, 2008; El-Thaher et al., 2013; Pinho et al., 2015).

From a financial standpoint, the low operating costs of the AH treatment (chemicals, power, labour) are undermined by the capital cost (>$1 million US dollars) of large equipment capable of digesting up to 1,800 kg per 8 h (McClaskey, 2004). Because the cost of AH systems is prohibitive, the livestock industry cannot reasonably be expected to invest in them as a preventative measure. However, if the system is not located on-site at the outset of a mass mortality event, it would need to be transported to the site (if possible) at considerable expense of both time and money. For this reason, AH has not been broadly adopted for mortality management purposes. Alkaline hydrolysis conducted at ambient temperature and pressure (ambient alkaline hydrolysis; AAH) would reduce the infrastructure and technical requirements of conventional AH. Shafer et al. (2000) investigated the effects of various concentrations of alkali solutions at a standard ambient temperature (20–25 °C) and pressure (1 atm) on euthanized broiler chicken carcasses over 10 days. After 10 days of treatment with potassium or sodium hydroxide (2 M), extensive hydrolyzation of feathers and poultry carcass was verified by visual and olfactory analysis. In addition, they noted the production of a crude saponified fat layer but no production of noxious or putrid odours.

The main drawback of AH treatment is the production of a highly caustic hydrolysate containing high chemical oxygen demand (COD) and biological oxygen demand (BOD), which is detrimental to municipal wastewater systems and soil environments if improperly land applied (Das, 2008). Therefore, the hydrolysate needs to be neutralized before subsequent treatments and/or environmental release e.g. disposal into municipal sewers (Pinho et al., 2015). Alternatively, Das (2008) investigated hydrolysate disposal by co-composting with yard trimmings (tree limbs and leaves). Mixing 0.5 L hydrolysate kg^{-1} fresh matter of yard waste showed no inhibition for the composting process.

Anaerobic digestion (AD) is a microbiologically driven process that converts organic substrates into biogas (methane and carbon dioxide), which can be used as a source of renewable energy, and nutrient-rich digestate. Industrial applications and research have shown AD as an effective method for handling slaughterhouse wastes containing rich fat and protein contents with a high capacity to produce methane (Gilroyed et al., 2010). However, there is a paucity of research pertaining to the use of livestock mortalities as feedstocks for AD since most tested systems have been laboratory scale and did not utilize whole carcasses (Chen and Wang, 1998; Chen and Huang, 2006; Massé et al., 2008; Rajagopal et al., 2014). Similar to slaughterhouse wastes, AD of entire livestock mortalities has the advantage to produce renewable energy and to recycle nutrients. In order to utilize AD, carcasses have to be pre-treated to reduce their size, and logistic concerns around storage and transportation of deadstock need to be addressed. However, by combining the technologies of AH and AD for mortality management, the potential synergism might be able to overcome the noted drawbacks of both technologies while still providing all of the benefits associated with each.

The objective of this study was to test the efficacy of three different molarities of potassium hydroxide (KOH) solution at degrading whole poultry carcasses in a static solution at ambient temperature and pressure. Following alkaline hydrolysis treatment, the hydrolysate was mixed with corn silage for neutralization and then anaerobically digested to determine biochemical methane potential.

2. Materials and methods

2.1. Ambient alkaline hydrolysis

Potassium hydroxide solutions of 2 M, 4 M, and 8 M were prepared ($pH > 14$ for all treatments) using analytical grade caustic potash KOH flakes (UNID, Korea). The concentrations of KOH were chosen based on previous research that only investigated up to 2 M (Shafer et al., 2000). The KOH was dissolved in distilled water, stirred, and left to equilibrate for 12 h before pH testing (SevenExcellence pH Meter, Mettler Toledo, USA). Deadstock from a local broiler chicken operation was used with a mean carcass weight of 2.74 ± 0.08 kg ($n = 36$). Poultry carcasses were left intact, with no physical or chemical pretreatment, and placed in 12 L, round, clear polyethylene containers (Rubbermaid Commercial, USA). For each treatment, containers were filled with alkaline solution to achieve a 2:1 (w/w) solution-to-carcass ratio in order to ensure that broiler chicken carcasses were submerged. Containers were sealed with snap-on airtight lids and stored without agitation at 20 °C and 101 kPa (atmospheric conditions). Carcass and solution weights were recorded prior to submersion, and subsamples of hydrolysate were taken every five days along with photographs to illustrate the degradation process.

2.2. Sampling and analysis

By using destructive sampling in triplicates, the impact of KOH molarity on carcass degradation after 5, 10, 15, and 20-day intervals was analyzed. On the 20th day, alkaline hydrolysate was collected and stored at 4 °C until subsequent determination of biochemical methane potential. Hydrolysate subsamples were taken that intentionally excluded any whole carcass components that remained, and then were blended (CB15N, Waring, USA) and tested for moisture, total solids (TS), and volatile solids (VS according to Standard Methods (APHA, 2012). The hydrolysate was tested for pH and electrical conductivity (EC) (SevenExcellence pH Meter, Mettler Toledo, USA). Crude fat content in the hydrolysates was determined by the Folch Method (Folch et al., 1953). Briefly, a 2:1 methanol to chloroform solvent mixture was used to extract lipids, with subsequent solvent evaporation and final weighing of residual lipids. Chemical oxygen demand (COD) of hydrolysates was determined colorimetrically (pHotoFlex colorimeter, YSI, USA) according to manufacturer's instructions using potassium dichromate and sulfuric acid (YSI Chemical Oxygen Demand Vials, YSI, USA) (APHA, 2012).