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# The role of biological processes in reducing both odor impact and pathogen content during mesophilic anaerobic digestion

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## HIGHLIGHTS

- Anaerobic digestion (AD) could produce annoyance for humans, i.e. odors and pathogens
- Because of bio-stabilization process, AD reduces potential odours production
- Biological process is responsible of pathogen reduction because of $NH_3$ production.
- Substrate competition, as well, is responsible for pathogen reduction.
- Plant characteristics and feedstock influence the results for pathogen reduction.

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## ABSTRACT

Mesophilic anaerobic digestion (MAD) produces renewable energy, but it also plays a role in reducing the impact of digestates, both by reducing odor and pathogen content. Ten full-scale biogas plants characterized by different plant designs (e.g. single digesters, parallel or serial digesters), plant powers (ranging from 180 to 999 kWe), hydraulic retention time (HRT) (ranging between 20 to 70 days) and feed mixes were monitored and odors and pathogens were observed in both ingestates and digestates. Results obtained indicated that MAD reduced odors (OU) from, on average, $OU_{ingestate} = 99{,}106 \pm 149{,}173$ OU $m^{-2}$ $h^{-1}$ ($n = 15$) to $OU_{digestate} = 1106 \pm 771$ OU $m^{-2}$ $h^{-1}$ ($n = 15$).
Pathogens were also reduced during MAD both because of ammonia production during the process and competition for substrate between pathogens and indigenous microflora, i.e. Enterobacteriaceae from $6.85 * 10^3 \pm 1.8 * 10^1$ to $1.82 * 10^1 \pm 3.82 * 10^1$; fecal Coliform from $1.82 * 10^4 \pm 9.09$ to $2.45 * 10^1 \pm 3.8 * 10^1$; *Escherichia coli* from $8.72 * 10^3 \pm 2.4 * 10^1$ to $1.8 * 10^1 \pm 2.94 * 10^1$; *Clostridium perfringens* from $6.4 * 10^4 \pm 7.7$ to $5.2 * 10^3 \pm 8.1$ (all data are expressed as CFU $g^{-1}$ ww).
Plants showed different abilities to reduce pathogen indicators, depending on the pH value and toxic ammonia content.



## 1. Introduction

The substitution of fossil energy fuels with renewable bioenergy biofuels has been proposed in the European Union (EU) as part of a strategy to mitigate greenhouse gas emissions, increase security of energy supply and support the development of rural communities.

Anaerobic digestion (AD) represents a well-known biotechnology able to produce renewable biofuels from organic waste and/or dedicated crops. Biogas production has been developed a lot in the EU in recent decades and in particular in Germany and Italy. In Italy, biogas has been developed considerably in the Lombardy Region in an agricultural context (Adani et al., 2013) because of the presence of intensive animal breeding (about $1.8 \times 10^6$ cows, $4.5 \times 10^6$ pigs and $36 \times 10^6$ poultry are present in the territory). As a consequence of that, about 400 agricultural biogas plants for a total of about 300 MW of installed power are now present in this region. Biogas in the Lombardy Region has developed in a sustainable way, since about 49% wet weight/wet weight (ww/ww) of the biogas feed is composed of animal slurries and only 32% ww/ww by energy crops (2 crops $y^{-1}$) being the rest, with 100% ww/ww represented by organic bio-products (Adani et al., 2013). The consequence is that the

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impact on land used because of biogas production is very limited: only 35,000 Ha of agricultural land are devoted to biogas energy crops on a total of arable land of $1 \times 10^6$ Ha (Adani et al., 2013). The wide use of animal slurry in biogas feedstocks was part of a strategy leading to the reduction of the impact caused by the use of animal slurries in agriculture, and produces both organic amendments and fertilizers which substitute for chemical fertilizers (Tambone et al., 2010). Apart from the environmental benefits derived from reducing greenhouse gas emissions and nutrient recovery, treating animal residues can also reduce the local impact and annoyance, i.e. odor impacts (Orzi et al., 2010) and the pathogen content of the digestate to be used in agriculture (Sahlström et al., 2008). These facts are very important as odors and pathogens constitute a problem when they affect the health of people exposed to them, causing a risk for the dissemination of diseases and nuisance to the surrounding population (Sahlström et al., 2008). Moreover, the biosecurity risk of MAD for animals must be considered as zoonotic infections may be introduced into the food chain, affecting public health. The EC-legislation indicated that digestates coming from biogas plants using animal by-products such as manure/slurry have the status of unprocessed manure and do not have to be analyzed for pathogens/pathogen indicator content (Commission Regulation, 2011).

Pathogens' fate during AD has been extensively studied in the past and much literature is available on the subject (Wagner et al., 2008). The principal factors affecting pathogen decay or loss of viability during anaerobic treatment are the hydraulic retention time (HRT), temperature, volatile fatty acids (VFA) present, batch or continuous digestion, bacterial species and available nutrients (Sahlström et al., 2008). The die-off of pathogens probably depends on the combination of different parameters such as: reactor configuration, microbial competition, pH value, VFA and ammonia concentrations, biogas production and chemical interactions (Smith et al., 2005; Salsali et al., 2008).

In general AD reduces pathogen content, above all when thermophile conditions are adopted (Franke-Whittle and Insam, 2013) as a consequence of high temperature which acts directly or indirectly, enhancing other effects such as, for example, ammonia toxicity (Kim et al., 2006). The mesophilic process has been reported to be less effective in reducing pathogens because of lower temperatures used than by the thermophilic process, although pathogen reduction can also be achieved with this process. Explaining the sanitation process, many parameters or combinations of them have been stressed (Battimelli et al., 2010). Less studied is the effect of the biological process on pathogen reduction in terms of substrate competition between pathogens and the microflora degrading the organic matter (OM). Skillman et al. (2009) and more recently Scaglia et al. (2014) related the pathogen reduction during AD processes to the progress of the biological process.

Odors, as well, are related to the biological process as they are volatile organic compounds (VOCs), (sulfur compounds, VFA, indoles and phenols) derived from fermentation and/or anaerobic respiration of degradable OM during the AD process (Orzi et al., 2010).

The aim of this research was to assess the effect of biological processes during mesophilic anaerobic digestion in reducing both potential odor impact and pathogen content. In particular, studying a large sample of full-scale plants characterized by the use of different biomasses and process parameters, and the implementation in the study of the detection of biological stability during the AD process, also allowed this work to investigate the role of substrate competition in pathogen survival.

## 2. Materials and methods

### 2.1. Feedstock sample collection

Ten full-scale mesophilic anaerobic digestion plants (P1–10) located in Northern Italy were considered for this study. Plants chosen were characterized by different plant designs (e.g. single digesters, parallel or serial digesters), plant powers (ranging from 180 to 999 kWe), hydraulic retention times (HRT) (ranging between 20 and 70 days) and feedstock (Table 1).

In particular, five plants (Group I: P1–5) were considered for a first sampling round, consisting of one campaign carried out in October 2012, during which both ingestates (Ing) and digestates (Dig) were sampled. Another five plants (Group II: P6–10) were considered in a second round, consisting of two different sampling campaigns performed in October 2013 and February 2014. In these latter cases not only ingestates and digestates were sampled, but, also, liquid (Sep liq) and solid fraction (Sep sol) (only for plants P6 and P10) coming from digestate solid/liquid (S/L) separation. For the plant P6 the slurry in the storage tank was also sampled before it reached before the S/L separator (Pre Sep).

Ingestates were collected directly from mix tanks feeding the digesters. The digestate and the pre-separator sample (Pre Sep) were directly taken at the output of the fermenter reactor. Liquid and solid fractions were sampled at the output of the S/L separators.

All samples were stored in containers hermetically closed, avoiding any contact with air and any volatilization of molecules. For the microbiological analyses, sterile vessels were used; and samples were brought to the microbiological laboratory within 6 h.

### 2.2. Chemical characterization of waste samples

Representative samples of both undigested and digested biomasses (Ing, Dig, Pre Sep, Sep liq, Sep sol) were used to carry out all the analytical tests. Ingestates, digestates and derived products were sampled on the same day. As no change of digester feed occurred during sampling campaigns, digestates can be considered related to ingestates.

Ingestates were collected directly from the top of the mix tank, while digestates, pre-separator samples and liquid fractions, from the inspection opening. Slurry mixing always occurred before samplings.

Solid fractions were collected directly from the pile at different points, getting a representative solid sample.

Chemical, biological stability and odor analyses were performed on samples collected by using a 500 ml jar with a telescopic bar, getting final composite samples of 5 L. Samples were then stored in a 5 L PTFE container reducing headspace presence to avoid any contact with air and any molecules' volatilization. Samples were brought to the laboratory and worked within 2 h.

Microbiological analyses were performed on samples collected by using two sterile jars of 100 ml that were kept at 4 °C and transferred to the microbiological laboratory within 6 h.

Total solids (TS) and volatile solids (VS) were determined according to standard procedures (Scaglia et al., 2014). Ammonia and total N-Kjeldahl (TKN) were analyzed on fresh samples according to the analytical method established for wastewater sludge (Scaglia et al., 2014). Volatile fatty acids (VFA); alkalinity (ALK) and pH were determined according to standard procedures (Scaglia et al., 2014). All analyses, except pH and TS, were performed in triplicate.

Toxic forms of total ammonia nitrogen (free ammonia in solution [$NH_{3aq}$, indicated as FA] plus free ammonia in the gas [$NH_{3gas}$, indicated as VFA]) and the non-toxic fraction (i.e., the $NH_4^+{}_{aq.}$ form) were calculated as reported in Scaglia et al. (2014), starting with TAN and taking into consideration the equilibrium between $NH_4^+{}_{aq.}$ and $NH_3$ (Eq. (1)).

$$NH_4^+{}_{aq.} + OH^-{}_{aq.} \leftrightarrow NH_{3aq.} + H_2O \leftrightarrow NH_{3gas} \quad (1)$$

The relationship between FA and $NH_4^+$ in solution depends on the base dissociation constant $K_a$, which is a function of the temperature's solution. In this study, $K_a$ was calculated using the following equation (Eq. (2)):

$$pK_a = 2729.92 / T + 0.090181 \quad (2)$$

where T represents the absolute temperature (*K*).