## ARTICLE IN PRESS

Waste Management xxx (2016) xxx-xxx



Contents lists available at ScienceDirect

# Waste Management

journal homepage: www.elsevier.com/locate/wasman



# Inhibition on anammox bacteria upon exposure to digestates from biogas plants treating the organic fraction of municipal solid waste and the role of conductivity

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#### ARTICLE INFO

Article history: Received 15 July 2016 Revised 5 October 2016 Accepted 7 November 2016 Available online xxxx

Keywords: Anammox Conductivity Digestate Inhibition Organic wastes Salinity

#### ABSTRACT

The aim of this research was to evaluate the applicability of the anammox process for removing nitrogen from the supernatant originating from the anaerobic digestion of the organic fraction of municipal solid waste (OFMSW).

The short term inhibitory potential of this concentrated wastewater was evaluated by means of batch tests in terms of maximum specific anammox activity reduction. A total of 20 real wastewater samples were tested originating from 4 different full scale anaerobic digestion plants treating OFMSW.

Activity reduction between 73% and 89% was observed in the presence of undiluted real wastewaters. The specific activity remained stable for 6–7 days after the initial reduction, thus suggesting its treatability even without dilution.

The inhibitory effect of both the real and synthetic saline media tested could be modelled as a function of conductivity. IC50 of 6.1 mS/cm was obtained for exposure to the tested liquid fraction of biowaste digestate.

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#### 1. Introduction

Anaerobic digestion (AD) is a commonly applied technology for the valorisation of the organic fraction of municipal solid wastes (OFMSW), both in terms of energy (biogas) and material with a digested cake that can be further stabilized and turned into a soil conditioner or high quality compost (De Baere and Mattheeuws, 2015). At European level, approximately 70 Mt/y of OFMSW are produced (475 kg municipal waste per capita per year, Eurostat 2014, 503 million of EU27 population and assuming a 30% of OFMSW content in the municipal waste). The recovery of organic material by composting and anaerobic digestion has grown with an average annual rate of 5.3% from 1995 to 2014 (Eurobserver 2014). In 2014, 244 anaerobic digesters were in operation in Europe treating a total of 7.75 Mt/y of OFMSW, i.e. around 10% of the biodegradable waste produced (De Baere and Mattheeuws, 2015). Among them, 49 AD plants are located in Italy, treating more than 1 Mt/y of OFMSW (ISPRA, 2015).

About 40% of the AD plants treating OFMSW are operated under wet conditions (De Baere and Mattheeuws, 2015), the digestate being separated into a solid and a liquid fraction, with the latter

being usually treated in dedicated wastewater treatment systems prior to its final disposal into the public sewerage or into receiving water bodies (Malamis et al., 2014). The physical/chemical characteristics of the digestate vary substantially according to the operating conditions of the AD plant (e.g. SRT, temperature, etc.), the season and the pre-treatment technologies, being the physical/chemical characteristics of the liquid fraction also highly dependent on the solid capture efficiency of the solid/liquid separation devices in use. Ammonia concentration in the liquid fraction is reported in literature in the range 0.5–5.6 gN/L (Malamis et al., 2014) with conductivity in the range 17–35 mS/cm (Malamis et al., 2014). The high costs related to the treatment of the liquid fraction of the digestate (mainly conventional biological processes) are a major bottleneck limiting the potentiality of energy recovery from the OFMSW via anaerobic digestion.

The anaerobic ammonium oxidation (anammox) process is quoted as a cost-effective nitrogen removal process for the treatment of ammonium-rich wastewaters (Hu et al., 2013). Anammox based technologies have been applied for the treatment of waste sludge digester liquors as well as of several types of industrial wastewaters. To date, about 100 full-scale installations are in operation worldwide (Lackner et al., 2014).

In addition to digestates, other saline effluents have been treated successfully with the anammox process, such as landfill

http://dx.doi.org/10.1016/j.wasman.2016.11.014 0956-053X/© 2016 Elsevier Ltd. All rights reserved.

Please cite this article in press as: Scaglione, D., et al. Inhibition on anammox bacteria upon exposure to digestates from biogas plants treating the organic fraction of municipal solid waste and the role of conductivity. Waste Management (2016), http://dx.doi.org/10.1016/j.wasman.2016.11.014

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leachate (Ruscalleda et al., 2010) and fishcanning industry effluents (Val del Rio et al., 2015).

Studies on the effect of salinity on the anammox metabolism are more and more numerous, since salinity is recognized as one of the major inhibitory factors when dealing with industrial wastewaters (Jin et al., 2012). The majority of the experimentations were performed in batch by exposing the anammox biomass to increasing salinity levels and by monitoring the reduction in the maximum specific anammox activity (SAA) in the test (Dapena-Mora et al., 2007; Carvajal-Arroyo et al., 2013). Most of the literature studies have been performed using single salts (usually NaCl) dissolved in synthetic media at concentrations from 3 to 34 g/L, corresponding to conductivities from 5.7 to 52.6 mS/cm.

In the literature, variable inhibition levels were found with  $IC_{50}$  (concentration corresponding to the 50% activity reduction) values ranging from 5.4 gNaCl/L (corresponding to 11.4 mS/cm, Carvajal-Arroyo et al., 2013) to 13.5 gNaCl/L (corresponding to 22.9 mS/cm, Dapena-Mora et al., 2007).

In this paper, results of activity batch tests conducted to assess the inhibitory potential on supernatants originating from 4 full scale AD plants treating the OFMSW will be presented. The AD plants had different conditions of feeding, pre-treatment and operating parameters.

Considering the complexity of the wastewater tested, conductivity has been selected as an aggregate parameter to quantify the strength of the potential inhibition capacity on anammox biomass. In order to verify the effectiveness of this strategy, a series of test with saline mineral medium at different salinity concentrations has been also performed.

To the best of the author's knowledge this is the first time that the treatability of the liquid fraction of digested OFMSW with the anammox process has been tested.

#### 2. Materials and methods

### 2.1. Origin of the supernatant

Samples of OFMSW (hereafter referred to as real\_WW) for the lab-scale experimentations were taken from four full scale AD plants located in the North of Italy. The main plants characteristics are summarized in Table 1 and a scheme is reported in Appendix D. The four AD plants are different in terms of feeding composition (OFMSW only or co-digestion with waste sludge), dilution mode in the pre-treatment section, hydraulic retention time (HRT), operational temperature (mesophilic or thermophilic) and solid/liquid separation technologies applied to the digestate. Some of the plants have a dedicated wastewater treatment plant (WWTP) for the treatment of the liquid fraction, while others send the liquid fraction to a nearby municipal WWTP. For AD plant n.1, three samples were taken at different sections of the conventional WWTP currently treating the liquid fraction of the digestate: (i) at the

inlet, (ii) at the outlet of the secondary treatment (pre-denitrifica tion/nitrification), (iii) at the plant outlet (after post-denitrification and final sedimentation stage). For AD plant n. 2, two samples were taken, (i) at the inlet and (ii) at the outlet of the existing biological treatment (nitrification-denitrification in a SBR reactor). AD Plants n. 3 and 4 did not have any treatment for the liquid fraction that is currently treated in a nearby municipal WWTP and therefore sampling was performed on the untreated liquid fraction. AD plant n.3 operated in a first period (3a) in co-digestion mode with waste activated sludge and, in a second period (3b), with the OFMSW only. Finally, AD plant n.4 was in operation in co-digestion mode with a larger fraction of waste sludge compared to the OFMSW (80% and 20% of the organic load, respectively).

#### 2.2. Saline mineral media

The saline mineral media were prepared in order to obtain an ions concentrations and composition similar to the ones of the real liquid fractions (real\_WW) (reported in Appendix A), by dissolving the following salts in micronutrient of the mineral medium described in Lotti et al. (2012a): CaCl<sub>2</sub>·2H<sub>2</sub>O (0-300 mg/L), KCl (1893-5679 mg/L), NaCl (2600-7430 mg/L), NaHCO<sub>3</sub> (1840-2014 mg/L), MgSO<sub>4</sub>·7H<sub>2</sub>O (247–916 mg/L). Details of the ions concentrations for the saline mineral media are reported in Appendix A. Two stock-solutions were prepared, having conductivity of 9 and 26 mS/cm, respectively. These stock-solutions were then diluted with Milly-Q water to obtain several saline solutions to be tested, with conductivity in the range 4-26 mS/cm (4, 7, 9, 10, 11, 12, 13, 14, 16, 26 mS/cm) as detailed in Table 2. All samples were characterized by analysing pH, conductivity, alkalinity, ammonium, Total Kjeldahl Nitrogen (TKN), Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Biochemical Oxygen Demand (BOD<sub>5</sub>), Chemical Oxygen Demand (COD), sulphate, phosphate, and metals concentrations (chloride, Al, As, Ag, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mo, Mn, Na, Ni, Pb, Se, V, Zn).

#### 2.3. Origin of the inoculum

Granular anammox biomass used in this experimentation originated from the full-scale anammox reactor of Dokhaven-Sluisjesdijk municipal WWTP, Rotterdam (Van der Star et al., 2007). The dominant anammox bacteria belonged to the genus *Candidatus* Brocadia (Lotti et al., 2014). The specific anammox activity was in the range 0.5–0.6 g N<sub>2</sub>-N/gVSS/d and the VSS/TSS content of the granular biomass was 75%.

The anammox biomass used as inoculum for the inhibition tests in Sections 3.1 and 3.2 was initially socked in mineral medium at 4 mS/cm; later, the salinity on the mineral medium was increased during 4 months up to 11 mS/cm by dosing KNO<sub>3</sub> which also

**Table 1**Main design and operational parameters of the full scale AD plants.

Plant	OFMSW load (t/y)	Feed	Dilution in pulping stage	HRT (d)	Temp (°C)	Digestate solid/liquid separation	real_WW current treatment
1	285,000	100% OFMSW	Water	20	57	Centrifuge + floatation	Conventional dedicated biological WWTP
2	20,000	100% OFMSW	Ricirculation of digestate supernatant	60	55	Centrifuge	Biological SBR + evaporation
3a	10,000	80% OFMSW - 20% sludge <sup>a</sup>	Waste water from civil WWTP	30	40	belt-press	Municipal WWTP
3b	15,000	100% OFMSW	Waste water from civil WWTP	30	40	Belt-press	Municipal WWTP
4	5000	20% OFMSW - 80% sludge <sup>a</sup>	Water	25	35	Centrifuge	Municipal WWTP

a As fraction of VS load.

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