Prokaryotic communities and potential pathogens in sewage sludge: Response to wastewaster origin, loading rate and treatment technology

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HIGHLIGHTS

- WWT plants differing in wastewater origin, loading rate and treatment technology.
- Technology and loading rate significantly conditioned sludge chemical properties.
- Sludge properties determined bacterial and archaeal community structure.
- Anaerobic digestion lowered down diversity and promoted potential pathogens.

GRAPHICAL ABSTRACT
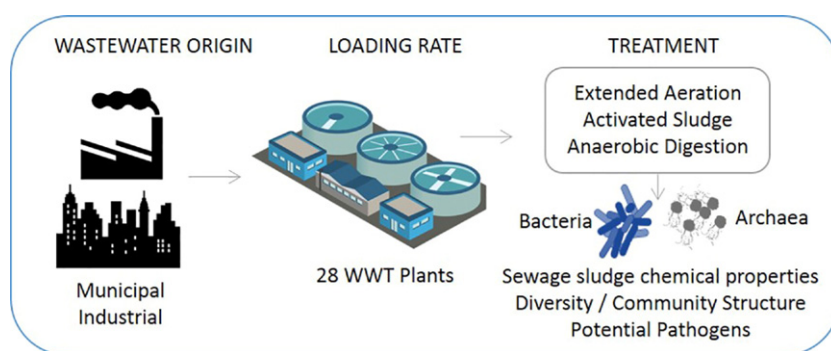
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ABSTRACT

Sewage sludge features high nitrogen and phosphorous contents encouraging its use as a biosolid in agriculture, but it bears potential chemical and microbiological risks. To tease apart the relative contribution of main factors determining the sludge chemical and microbial features, we analysed 28 treatment plants differing in the wastewater origin (municipal residues, agro-food or chemical industries), organic loading rate and treatment technology (extended aeration, activated sludge or activated sludge followed by anaerobic digestion). We found that the treatment technology and the organic loading rate are main determinants of the sludge chemical properties, including its organic load, nutrient and metal contents, and override the effect of the wastewater origin. Sludge bacterial and archaeal community structure and diversity, characterized through massive sequencing of the 16S rRNA gene, were also mostly determined by the treatment technology partly through shifts in the sludge nutrient load. The same factor conditioned the relative abundance of sequenced bacteria most closely related to potential pathogens, but not that of cultivable *Escherichia coli* or *Salmonella* spp. We did not find an effect of the geographic location of the plant on any of the variables at the regional scale of our study. Operational parameters appear as major determinants of the sludge chemical and microbial properties, irrespective of the source of wastewaters, thus leaving a broad management window for improving the agronomic value of sewage sludge.

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1. Introduction

Sewage sludge is a residue or by-product of wastewater treatment that contains high levels of organic carbon, N, P, K, S, Ca, Mg and other microelements (Torri et al., 2017), whose use in agriculture has been increasingly encouraged (European Commission, 1991; US EPA, 1994; Milieu et al., 2010). The promotion of sewage sludge as a biosolid of agronomic value partly responds to the fact that increasing crop production is exhausting the global P reservoirs (Cordell et al., 2009). Typically low soil P concentrations limit plant growth and, consequently, crop production and eventually the ecosystem's capacity to sequester carbon (Peñuelas et al., 2012). Nowadays, P fertilisation mainly relies on phosphate rock mining, an industry with simultaneous increasing costs and decreasing quality of reserves (Cordell et al., 2009; Smit et al., 2009). Alternatives that help overcome P mining dependencies include the recycling of plant and animal residues, among which sewage sludge is seen as an adequate candidate (Smit et al., 2009). Landspreading sewage sludge helps satisfying the crop demands for nutrients, while reducing the costs both of mineral fertilizers and sludge disposal (Singh and Agrawal, 2008). Sewage sludge, however, typically bears high contents of heavy metals and organic pollutants that reflect the social use and proliferation of contaminants in our society (Mininni et al., 2015; Kirchmann et al., 2017). Sewage sludge also hosts an enormous diversity of microbes, including potential pathogens, which can be released to the environment (Zhang et al., 2012). Thus, application to land of sewage sludge can shift the soil conditions and alter the community structure, activity and metabolic profiles of soil microbes, which ultimately determine plant performance (Roig et al., 2012; Lloret et al., 2016). Understanding the factors that determine the assembly of microbial communities in sewage sludge is therefore of primary importance to devise appropriate management strategies for its use in agriculture.

Microbial community composition and dynamics in wastewater treatment systems are highly linked to wastewater characteristics, operational parameters and geographic location (Wang et al., 2012; Ibarbalz et al., 2013; Hai et al., 2014). In particular, the origin of wastewater (i.e., munipal versus industrial) can largely determine the bacterial communities. Industrial wastewaters, with their more severe abiotic conditions, have been reported to be home to less diverse and unevenly distributed communities (Ju et al., 2014). Another major factor that conditions the sludge chemical characteristics, as well as the microbial community structure and composition, is the wastewater and sludge treatment technology (Singh and Agrawal, 2008; Lloret et al., 2016). Aerobic and anaerobic technologies are available for the processing of sewage sludge, which differ in their ability to stabilise and sanitise the end product (Fytili and Zabaniotou, 2008; Cieślik et al., 2015). Here, we investigated a collection of sewage sludge originating from 28 wastewater treatment (WWT) plants distributed at the regional scale, which are either fed with municipal and/or industrial wastewaters at either low, medium or high organic loading rates. Sewage sludge in these plants are either generated through extended aeration or activated sludge processing, some of which are then anaerobically digested. Activated sludge procedures are aerobic techniques that employ a diverse community of organisms that reduce the amount of nutrients in the end product (McIlroy et al., 2015). Anaerobic digestion has the added benefit of lowering the high organic load in the sludge, while generating methane that can be transformed into energy (Insam et al., 2010). Anaerobic digestion is generally thought to decrease the pathogen load (Holm-Nielsen et al., 2009; US EPA, 2017), although the level of sanitation depends both on the substrates fed into the digester and on process performance, including digestion temperature, sludge retention time, pH and ammonium concentration, among others (Sahlström, 2003; Ottoson et al., 2008). Therefore, pathogenic microbes can survive anaerobic digestion or regrow in the digested product during subsequent storage (Sidhu et al., 2001; Sahlström et al., 2004; Bagge et al., 2005; Pepper et al., 2006).

Our general objective was to untangle the relative importance of the factors underlying the community structure and diversity of prokaryotic communities in sewage sludge. We expected that the sludge chemical properties might differ depending on the origin of wastewater, organic loading rate, and the wastewater and sludge treatment technologies, with concomitant changes in the sludge bacterial and archaeal communities and particularly in the relative abundance of potential pathogens. We finally aimed to determine the links between the sludge chemical properties and the community structure of prokaryotes.

2. Materials and methods

2.1. Sewage sludge sample collection

Sewage sludge was sampled in twenty-eight wastewater treatment (WWT) plants in the Region of Murcia (SE Spain), which has a total area of 11,313 km^2 and a population of ca. 1.5 million people. The main operational parameters and the geographic coordinates of all WWT plants are given in Appendix A. We classified the plants as regards the main experimental factors considered in this study as follows: i) main wastewater origin: municipal, agro-food or chemical industry; ii) organic loading rate, measured as the mean annual Biological Oxygen Demand (BOD5) divided by the maximum flow rate, iii) treatment technology: conventional activated sludge (AS), extended aeration or AS followed by anaerobic digestion (i.e., plants using either conventional, two-phase or A2/O activated sludge procedures for wastewater treatment followed by the mesophilic anaerobic digestion of sewage sludge).

On July 2012 we collected 1 L sewage sludge from each WWT plant. Samples were immediately transported to the laboratory and stored at 4 °C during subsequent analyses. Water content was calculated as the loss of weight after oven-drying the samples (105 °C). Sewage sludge pH and electrical conductivity were measured in aqueous suspensions (1:2.5 and 1:5 *w/v*, respectively). Total Organic C (TOC) and N content were determined in air-dried ground samples using a LECO TruSpec CN analyzer (LECO Corp, MI, USA). TOC was quantified after a 55 °C acidic (HCl) treatment of the samples. Other macronutrients (P and K) and 26 microelements, including total concentrations of heavy metals, were determined in air-dried ground samples after digestion with HNO$_3$ and H$_2$O$_2$ using an Ultraclave microwave (Milestone SRL, Milan, Italy) by ICP analysis (ICAP 6500 ICP Spectrometer, Thermo Fischer Scientific, MA, USA).

Previous to the sampling reported in this study, we performed three other samplings (on November 2009, February 2010 and May 2010) and confirmed that main sewage sludge properties that potentially condition the microbial community structure (e.g. humidity, pH, electrical conductivity, and nutrients) remain relatively stable throughout the year (Murcia-Navarro, 2013).

2.2. DNA extraction

Sewage sludge DNA was extracted within 48 h after sampling and DNA extracts were stored at −20 °C. Extractions were performed from sludge suspensions in sterile water (0.5 g sludge in 500 mL water). Suspensions were homogenised using vortex (5 s) and horizontal agitation (5 min), and then disaggregated by three cycles of freezing (30 min, −80 °C) and thawing (5 min, 65 °C). DNA was extracted from 0.25 g homogenised sludge using the PowerSoil™ DNA isolation kit (MO BIO Laboratories, Solana Beach, California). Extracted DNA was electrophoresed in 1% agarose gels run in 0.5 X TAE buffer (Tris-acetate-EDTA; 100 V, 15 min) and quantified with the Quant-iT™ PicoGreen® dsDNA Kit (Invitrogen, Carlsbad, USA).

2.3. Tag-encoded FLX titanium amplicon pyrosequencing

To analyse the bacterial and archaeal communities in sewage sludge samples we used a tag-encoded FLX titanium amplicon pyrosequencing