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Short communication

Two-stage mesophilic anaerobic–thermophilic digestion for sludge sanitation to obtain advanced treated sludge

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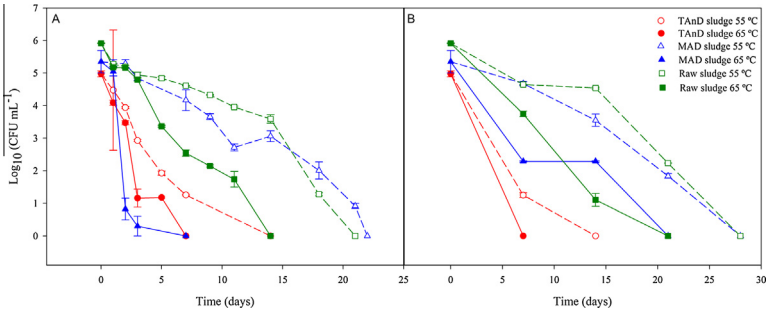
HIGHLIGHTS

- Pathogen content was analysed in three different types of sludge.
- Mesophilic and thermophilic processes produced Class B and Class A Biosolids.
- The presence of *C. perfringens* prevented the obtaining of advanced treated sludge.
- A two-stage digestion process is suggested for fully sanitation of the sludge.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, raw, mesophilic anaerobic, and thermophilic anaerobic sludge were analysed to evaluate whether the pathogen content limits established in the "Proposal for a Directive of the European Parliament and of the Council on spreading of sludge on land" were satisfied. *Salmonella* spp., *Escherichia coli*, and *Clostridium perfringens* spores were cultivated and pathogenity genes *invA* and *cpa* PCR-amplified. Thermophilic anaerobic digestion produced Class A biosolids by eliminating *E. coli* and *Salmonella* spp. but did not accomplished the microbial requirements of the future Directive due to the presence of *C. perfringens* spores (9.6×10^4 CFUs mL^{-1}). Hence, the final goal of this work was to propose a two-stage process capable of removing the spores of *C. perfringens* to obtain an advanced treated sludge that could be land-applied with no environmental risks. The first stage of the process suggested in this study involved the mesophilic anaerobic digestion of the sludge while the second stage of operation consisted of an aerobic or anaerobic thermophilic digestion.

1. Introduction

The addition of organic matter has proven to be a suitable technique for soil restoration or conservation [1]. Among organic amendments, the use of sewage sludge as soil fertilizer is gaining importance for both soil properties improvement and waste disposal. Most sewage wastes contain valuable nutrients that could be used to improve soil fertility, crop production, and some soil physical and chemical properties as well as promoting its biological activity [2,3]. However, the agricultural use of sewage sludge may introduce some risks associated with its potential content of heavy metals, toxic compounds, pathogenic bacteria, viruses and parasites that pose direct or indirect hazards to human, animal and plant health [4]. In this respect, the Environmental Protection Agency of the United States establishes treatment goals or post-application measures designed to reduce these risks and defines Class A and Class B biosolids with different pathogen content limits and use restrictions [5]. In contrast, the current European legislation only requires that the sludge is subjected to a process of

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stabilisation before land application and only establishes limits regarding the content of heavy metals [6]. In order to improve this situation, the European Union is promoting a new regulation through the Proposal for a Directive of the European Parliament and of the Council on spreading of sludge on land [7]. This provides stricter parameters for the content of pathogenic microorganisms and introduces the concept of advanced and conventional treatments. In the future legislation, limits for *Escherichia coli*, *Clostridium perfringens* spores, and *Salmonella* spp. are established for the sludge to achieve an advanced treated status.

Many studies have focused on sludge digestion processes with the aim to fulfill Class A biosolids conditions [8,9]. However, there is a lack in the existing literature regarding the requirements to achieve an advanced treated sludge.

In view of the above, in the present work, raw sludge (mixed primary and secondary sludge), sludge produced after a mesophilic anaerobic digestion (MAD sludge) and sludge produced after a thermophilic anaerobic digestion (TAnD sludge), were analysed to assess whether they fulfilled the microbiological standards established for advanced treated sludge in the future European legislation. Mesophilic anaerobic digestion is one of the most widely used processes for the stabilization of sludge in wastewater treatment plants and is defined as a conventional treatment in the future European Directive. On the other hand, thermophilic anaerobic digestion is considered an advanced treatment of sewage sludge in the future European Directive.

2. Material and methods

2.1. Sewage sludge sampling

Sewage sludge was collected from a waste water treatment plant (WWTP) in Molina de Segura (Murcia, Spain) with a population equivalent of 290,000 p.e. The main characteristics of the MAD process were: a digester effective volume of 7612-m^3, an organic loading rate (OLR) of 0.8 kg m^{-3} d^{-1} volatile solids, an average temperature of 38.1 °C, a sludge retention time (SRT) of 59.3 days and a volatile solids destruction (VSD) of 45.9%. On the other hand, the principal characteristics of the TAnD process were: a digester effective volume of 15-m^3, an OLR of 1.6 kg m^{-3} d^{-1} volatile solids, an average temperature of 53.9 °C, a SRT of 20.3 d and a VSD of 42.0%. Both the mesophilic and the thermophilic anaerobic digesters were fed with the same raw sludge. The main chemical characteristics of raw, MAD and TAnD sludge are described in Table 1.

2.2. Batch laboratory incubations of sewage sludge

500 mL of raw, MAD and TAnD sludge were placed in 1 L flasks in a rotatory shaker both at 55 and 65 °C and under aerobic and anaerobic conditions. The incubations were performed to determine the time needed to eradicate *C. perfringens* spores for each combination of type of sludge, temperature and atmosphere. Raw and TAnD sludge were previously incubated at 40 °C during 24 h. The experiment was carried out per triplicate. Anaerobic conditions were achieved by introducing each flask in a thermo-sealed bag containing anaerobic sachets (AnaeroGen Compact, Cambridge, UK). Aerobic treatments were sampled on days 1, 2, 3, 5, 7, 9, 11, 14, 18, 21 and 22 and anaerobic treatments were sampled weekly on days 7, 14, 21 and 28.

2.3. Cultivation and molecular detection of pathogens

After sampling, sludge was immediately cooled (4 °C) and microbial analysis were performed within the following 24 h. Total coliforms, *E. coli*, *Salmonella* spp. and *C. perfringens* spores were cultivated and pathogenity genes *invA* and *cpa* PCR-amplified as described by Lloret et al. [10]. Briefly, the presence/absence detection method of *Salmonella* spp., consisted of four steps: (1) non-selective enrichment of sludge using buffered peptone water, (2) selective enrichment with Rappaport–Vassiliadis' soy broth, (3) plating in the chromogenic media Colorex Salmonella Plus, and (4) DNA extraction of positive colony-forming units (CFUs) and PCR-amplification of the *invA* gene enconding for invasine. The quantification of *E.coli* and total coliforms was performed by plating 1:10 (w/v) serial dilutions of sludge in sterile Ringer solution in Chromocult coliform agar. To quantify *C. perfringens* spores, vegetative cells were first eradicated subjecting the sludge to a thermal shock at 75 °C for 20 min. Then, 1:10 (w/v) serial dilutions of sludge in sterile Ringer solution were inoculated into Tryptose sulphite cycloserine agar, and DNA extraction and PCR-amplification of the *cpa* gen enconding for the α-toxin were performed. All sludge suspensions were mixed by shaking for 15 min and a minimum of three replicates per dilution were assayed. The detection limit for both culture-dependent and molecular methods was 1 CFU mL^{-1}.

2.4. Statistical analysis

For the analysis of pathogens in raw, MAD and TAnD sludge, data (log-transformed) were subjected to one-way ANOVA with

Table 1
Chemical characteristics of raw, MAD and TAnD sludge (values on dry weight basis).

Parameter[a]	Raw sludge	MAD sludge	TAnD sludge	UE limits[b]
Dry matter content (%)	5.9	3.5	2.9	
pH	6.3	7.6	8.0	
EC (dS m^{-1})	5.3	13.6	13.7	
TOC (g kg^{-1})	801	621	690	
Total N (g kg^{-1})	117	93.1	89.7	
Total P (g kg^{-1})	62.1	55.2	55.2	
Total K (g kg^{-1})	3.4	3.4	3.4	
Total Cd (mg kg^{-1})	BDL[c]	BDL	BDL	10
Total Cr (mg kg^{-1})	24	59	55	1000
Total Cr_{VI} (mg kg^{-1})	BDL	BDL	BDL	10
Total Cu (mg kg^{-1})	108	266	197	1000
Total Fe (g kg^{-1})	17.1	46.0	34.09	
Total Hg (mg kg^{-1})	BDL	BDL	BDL	10
Total Ni (mg kg^{-1})	15	31	24	300
Total Pb (mg kg^{-1})	21	45	38	750
Total Zn (mg kg^{-1})	376	738	821	2500

[a] EC: electrical conductivity; TOC: total organic carbon.
[b] Limits for advanced treated sludge [7].
[c] BDL: below detection limits.