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Short communication

Efficiency of a Spanish wastewater treatment plant for removal potentially pathogens: Characterization of bacteria and protozoa along water and sludge treatment lines

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ABSTRACT

Biological urban water treatment plants utilize microorganisms for wastewater purification so that microbiological characterization of processes is very important. In addition, its removal in the effluent and outlet sludge for their reuse is important. This work aims to characterize the presence of bacteria and parasites along the processes in wastewater treatment plant (WWTP) and the possibility of using the purifying water and sludge in agriculture.

The results show a high level of *Escherichia coli* in the raw water. Although their total removal is not achieved, a reduction of 2.34 and 1.36 log in the concentration of *E. coli* was produced along the water and sludge treatment lines, respectively, being the trickling filters (TF) and autothermal thermophilic aerobic digestion (ATAD) the most effective processes against bacteria.

Clostridium perfringens, which is a Grampositive bacillus and fecal contamination indicator, although less usual than *E.coli*, is detected in washing water of solids which are stored in anoxic conditions and in the sludge treatment line where dissolved oxygen is absent including in the outlet of plant.

Salmonella spp, *Entamoeba* and *Cryptosporidium* were not detected in any of the samples, meanwhile *Giardia duodenalis* was identified only in two samples from washing coarse solids and sludge, but it was not identified in outlet water and sludge. *Acanthamoeba* was the most frequent protozoa isolated.

1. Introduction

Urban wastewater has high levels of microbiological contamination. These microorganisms are involved in the purifying water process, and become the treatment plant into a unique ecosystem. During the treatment, these microorganisms are not removed totally, so they are incorporated in the natural ecosystems through the treated water discharge, where the natural processes of self-purifying of water continue. Nevertheless, some of them may be potentially pathogenic to human health and animals. In Europe, the collection, treatment and discharge of urban wastewater are regulated by Directive 91/271/EEC, but it does not refer to the allowable limits for bacteria and parasites in the effluent. Moreover, sewage sludge can be used to recover agricultural soils. In this case, the current Directive 86/278/EEC does not establish the maximum concentration limit for pathogens in order to reuse the sludge, where most bacteria and parasites from water are concentrated (García et al., 2013).

Potential pathogens in wastewater and sewage sludge include various genera of bacteria, enterovirus, rotavirus, helminth eggs and protozoa, whose presence in output water and sludge may be harmful to health

The purpose of this work is to define the efficiency of each individual process in a urban wastewater treatment plant in the removal of bacteria and parasites present in raw water, with special emphasis in pathogenic microorganisms which may affect to human and animal health and they could be incorporated into the environment as a result of the treated water and sludge reuse.

2. Material and methods

2.1. Plant description and sample collection

The WWTP of study is located in a municipality of Navarra Community, inside the Ebro River Basin (design loading rate: 6.879 $kgDBO_5\ d^{-1}$). The municipality consists of 46,237 equivalent

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inhabitants and its economy is based principally on agricultural and industrial activities. Fig. 1 shows the scheme of the plant and its main processes and characteristics.

Samples (S1-S13) were taken at the input and output of each process according to the procedure ISO 5667:2003. Also, two biofilms samples were taken (B1 and B2), from trickling filter 1 (TF1) and 2 (TF2), respectively.

One liter of water and sludge were taken in April 2012, in glass bottles previously sterilized and stored at 4 °C until analysis in the laboratory (Eaton et al., 2005). Bacteriological tests were conducted within 6 h from sample collection. In addition, an aliquot of sludge samples (S11, S12 and S13) was preserved in SAF solution (sodium acetate formalin) for parasite analysis until they were observed by microscopy. Biofilm samples were taken with a cotton swab and stored in a 1.5 ml eppendorf tube with 1 ml of sterile saline solution (0.9% NaCl) at room temperature.

2.2. Analytical methodology

Table 1 shows the methodology used in this work related to physic-chemical, bacteriological, parasite and protozoa analysis. *E. coli* CFU count was performed at each dilution and the media concentration was expressed as CFU.100 ml^{-1} in the case of water samples and CFU g^{-1} (dry weight, related to Total Solids) in the case of coarse solids, grit and sludge. In the case of *C. perfringens*, *Salmonella* spp. and pathogenic protozoa the results were expressed as presence (P) or absence (A). In order to identify the presence of parasites, helminths and protozoa *in vivo*, an aliquot of samples was concentrated by centrifugation and other by formalin-ethylacetate method. Smears from all concentrated samples were examined by means of microscopy and stained by modified Zielh–Neelsen stain to detect coccidian oocysts (Henriksen and Pohlenz, 1981).

In order to confirm the presence of *Giardia* spp., *Cryptosporidium* spp. and *Entamoeba* sp, DNA from all samples concentrated by centrifugation (except B1 and B2) was extracted using the PSP ® Spin Stool kit and following the manufacturer's instructions. For molecular identification of FLA, amoebic plaque culture from all samples except S2 and S12 which were consider negative because of insufficient growth, was collected with 1 ml of saline buffer, and centrifuged for 10 min at 6000 rpm. The pellet was resuspended in 100 μl of fresh saline buffer and used for DNA isolation which was performed using Ron's Tissue DNA Mini Kit according to the manufacturer's specifications.

Additionally, PCR products were purified with GFX™ PCR DNA Gel Band Purification Kit and direct sequenced. The nucleotide sequences obtained were analyzed and compared with those registered in GenBank using Chromas, BLAST® tool and BioEdit.

3. Results and discussion

Related to physic-chemical analyses results, temperature and pH values were very similar in all samples (16–16.5 °C, pH: 7.5–8.8) except in the case of S12, where temperature was higher because it comes from the ATAD digesters, which operate at 60 °C. Regarding dissolved oxygen, values were similar in water samples (6–8 mg/l) but near zero in all sludge samples because of aerobic organic matter decomposition.

Table 2 shows the results of the quantitative analyses of *E. coli* in water (CFU·100 ml^{-1}) and solid samples (CFU · g^{-1}), and its reduction (Log_{10} (%)) along both water and sludge treatment lines. *E. coli* was analyzed as an indicator of the fecal enterobacteria present at high concentrations in the incoming water (raw water). Although their total removal is not achieved, the final reduction of *E. coli* along water treatment line was 2.34 log units (Table 2), similar to those determined by Reinthaler et al. (2003) and Muela et al. (2011) when studying secondary treatment plants they used activated sludge as secondary treatment, as well as that found by Tyagi et al. (2011) in different treatments. In this work, the greatest reduction of *E. coli* concentration in the water line was obtained in

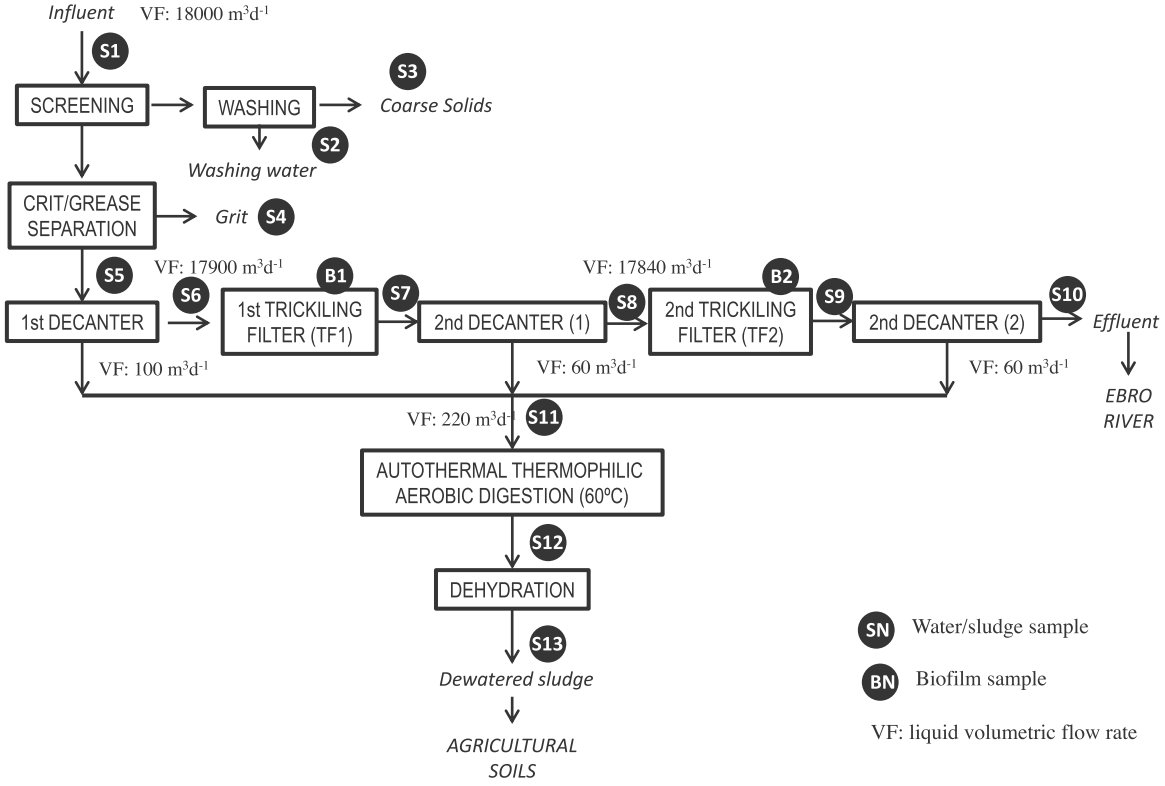

Fig. 1. Scheme of the Wastewater Treatment: main processes and characteristics.