Biodegradation of nonylphenol by novel bacterial strains isolated from sewage sludge

E. Morillo*, M. Rubio-Bellido, J. Villaverde, F. Madrid

[1] COIMed Research Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC). Sevilla, 41012. Spain.
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*Presenting author e-mail:morillo@irnase.csic.es

Introduction

Nonylphenol (NP) and nonylphenol mono (NP1EO) and diethoxylates (NP2EO; referred as NPE) are used as nonionic surfactants in a large variety of industrial and domestic applications. Although the use of NPEs has been banned under Council Directive 2003/53/EC (European Union 2003), their environmental presence and risk is still high because of its historical and pervasive widespread use. In the waste water treatments plants (WWTPs) the biodegradation of NPEs leads to an increase in the concentration of NP, much more hydrophobic. As a consequence, NP has high affinity for sludge flocs, and is recalcitrant to wastewater and sludge treatment. This is why NP is the main alkylphenol associated with sewage sludge (90%; Soares et al. 2008).

Sewage sludge is rich in organic matter and nutrients, and can be utilized in land applications as fertilizer, soil conditioner and composting material. NP in sludge may harm the environment due to its endocrine disrupting properties. It can be accumulated in agricultural soils and contaminate surface water and groundwater, and even concentrate in plants and animals.

Different methods have been proposed to remove organic contaminants from sewage sludge (Semblante et al., 2015), such as ozonation, Fenton treatment, UV oxidation, ultrasonication or thermal treatment, but these are expensive treatments. On the contrary, bioaugmentation, which is a procedure that involves the addition of exogenous or endogenous microorganisms to enhance the biodegradation of contaminants, is being considered as a relatively cheap and ecological treatment to further improve organic contaminants biodegradation in sewage sludge and biosolids.

The aim of this work was to add NP amendments on sewage sludge to obtain degrading bacterial consortia, and then to isolate bacteria from the NP enrichment cultures in order to test for their ability to degrade it, in a search for strains appropriated for bioremediation uses. The better NP-degrading bacteria obtained will be tested for NP biodegradation in solution and on sewage sludge. The final objective of this research is to use specific NP-degrading bacteria during the period of sewage sludge composting to reduce the NP content in the final biosolids obtained.

Materials and Methods

Microbial consortia were isolated from a sewage sludge sample and from a compost obtained from sewage sludge. NP degrader enrichment was carried out adding NP as the only source of carbon and energy to 1 g of sludge, together with 20 ml of a mineral salt medium (MSM) containing also micronutrients. Cultures were incubated with orbital shaking at 30ºC and every 15 days an aliquot of the culture was transferred to another flask containing the same sterile mineral medium and incubated again. After four enrichment transfers (60 days), an aliquot of the culture was plated in R2A-agar medium and incubated for 72 h at 30ºC. The different isolated consortia which potentially showed NP degrading activity were stored in Microbank™ cryovials and kept at -80ºC.

Strains isolation was performed on solid medium prepared in Petri dishes following standard microbiological protocols. Degrading bacteria were isolated on MSB medium plus NP. Different strains were obtained, and their identification accomplished by extracting DNA from the liquid culture and amplifying the 16S rRNA genes by PCR using universal oligonucleotide primers. The PCR products were cloned in a T/A vector (PGEMT easy vector from PROMEGA). After strains were analyzed by PCR, plasmid DNA from selected strains was purified and the insert was sequenced with T7 and SP6 universal primers. Finally, the 16S rRNA gene sequences (1450 bp) were compared by BLAST searching with the EzBioCloud database.

Biodegradation experiments of NP in solution were carried out using the sludge and compost consortia. NP biodegradation in solution was carried out in semi-closed glass containers at 20ºC for 30 days. Together with NP 10 ppm, the flasks were inoculated with the consortia in mineral salt medium (MSM) and a trace of nutrient solution (NS). Just at the beginning of the experiment and at periodic intervals (2, 5, 8, 12, 15, 20 and 30 days) the herbicide residues that remained in solution were determined by HPLC. Analysis of 1:1 (v/v) hexane extract of the aqueous supernatant solutions was performed (NP was completely recovered in the separated hexane phase due to it much higher solubility in the organic solvent). Calibration curves were also prepared in hexane. The concentration of NP was determined by HPLC coupled to a fluorescence detector (Shimadzu RF-10APXL) with excitation and emission wave lengths of 222 and 315, respectively. In order to observe the metabolic activity of the consortia and isolated strains and the biodegradability of the contaminant the respiration activity was determined by OxiTop (WTW), where the CO_2 produced is adsorbed by NaOH granules and the oxygen demand measured, as well as the percentage of NP remaining in solution after 20 days.

Results and Discussion

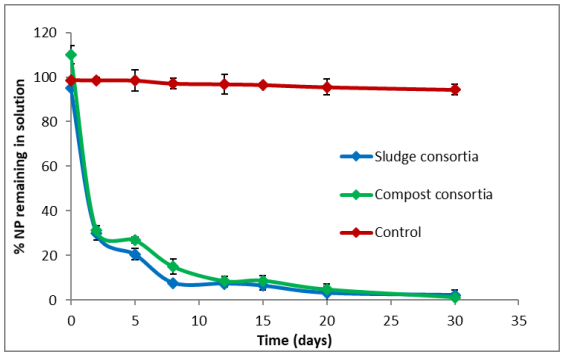

Fig. 1. Biodegradation of nonylphenol in the presence and absence of sludge and compost bacterial consortia.

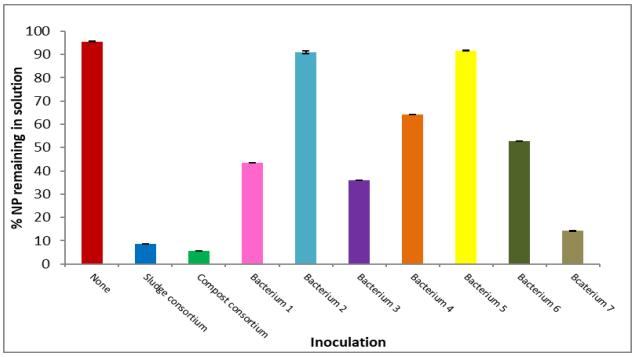

Figure 2. Percentage of nonylphenol remaining in solution after 20 days of incubation with sludge and compost consortia and some isolated strains.

About 70% of NP initially added was degraded after only two days (Figure 1), both with sludge and compost consortia, and the complete degradation was reached after 20-30 days. It indicates that some of the strains in these consortia are active to biodegrade NP. Strains capable to use NP as the only carbon source were isolated in the laboratory. Their metabolic activity was determined by OxiTop (figures not shown) and the percentage of NP remaining in solution after 20 days was calculated (Figure 2). The isolated bacteria seem to have in general a reduced degrader capacity than the natural consortia. The identification of the more active bacteria is currently being carried out. The next step will be to combine the most effective bacteria in groups composed by two, three of four of them in order to obtain an artificial consortium as effective as the natural consortia isolated from sludge. It would offer interesting perspectives to develop mixed inoculants for the bioaugmentation, facilitating the implementation of effective protocols for sewage sludge decontamination.

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References

European Union (2003) Council Directive 2003/53/EC of the European Parliament and the Council of 18 June 2003 amending for the 26th time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (nonylphenol, nonylphenol ethoxylate and cement). Official Journal of the European Union 17.7.2003. L 178/24.

G.U. Semblante, F.I. Hai, X. Huang, A.S. Ball, W.E. Price, L.D. Nghiem (2015) Trace organic contaminants in biosolids: Impact of conventional wastewater and sludge processing technologies and emerging alternatives. J Hazard Mater 300, 1-17.

A. Soares, B. Guieysse, B. Jefferson, E. Cartmell, J.N. Lester (2008) Nonylphenol in the environment: a critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ Int 34, 1033-1049.