Safe recycling of sewage sludge on agricultural land-biowaste

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Abstract

More than 50,000 wastewater treatment plants are operating in the European Union, producing more than 7.9 millions tons of dry solids per year. The amount of sewage sludge will continue to increase as the Urban Wastewater Treatment Directives continues to be implemented in the different member countries. It is now undeniable that various toxic organic compounds, such as surfactants, hydrocarbons and residues derived from plastics are found in sewage sludge. The BIOWASTE project, under the EU 5th framework programme, offers an integrated study of xenobiotics throughout sludge recycling, using a combination of complementary approaches such as biotechnology, eco-toxicology, plant toxicology, analytical chemistry, microbiology, mathematical modelling, life cycle costing and life cycle analysis. This paper presents an overview of the results as well as their implication on the current EU regulatory work in progress concerning sewage sludge application. Particularly, two major findings are here detailed: the isolation, characterization and use of anaerobic xenobiotic-degrading microorganisms, and the modelling of the fate and impact of xenobiotics on anaerobic digestion.

Keywords: agricultural reuse, anaerobic degraders , sludge, xenobiotic, modelling

Introduction

Disposal and handling of sewage sludge is a growing problem in Europe due to the increasing quantity of sludge produced (8.3 million Tonnes in 2005 (Magoarou, 2000)). Agricultural disposal remains one of the most popular options for disposal in the EU (4.5 million Tonnes in 2005 (Magoarou, 2000)), and has long-term environmental and economic benefits, which make it competitive with other technologies such as incineration, wet oxidation, and landfill. It therefore needs to be validated as a long-term, safe, and environmentally friendly option. Sewage sludge contains small fractions of toxic organic chemicals, which may cause problems if safe use of

sewage sludge on agricultural land is to be implemented (Langenkamp and Part, 2001). The European Union has identified a number of compounds in the Legislation addressing water pollution (Directive 2455/2001/EC), listed in Annex X. These include halogenated organic compounds, metals, and a several other compound classes. The use of halogenated organics in Europe is decreasing, and they are readily removed by reductive dechlorination. The other compounds are of more concern, and include phthalic acid esters (PAEs), nonyphenols and nonylphenol ethoxylates (NPs, NPEs), polycyclic aromatic hydrocarbons (PAHs), and linear alkylbenzene sulfonates (LAS). These all readily adsorb onto solids, and are present in activated, and primary sludge (Figure 1). Most of these compounds are degraded under aerobic conditions (Spormann and Widdel, 2000), but as most sludge treatment is anaerobic, they may persist through anaerobic sludge treatment. All these compounds have environmental impacts, and are relatively surface active, which means they will follow the general path as shown in Figure 1, and are therefore a concern in the sludge. Anaerobic degradability is limited for all compounds, and assessing the impact of their presence on the process itself is also a concern.

The BIOWASTE project takes an integrated approach for xenobiotic management. This means complete cycle management from source (in this case, the treatment plant), to sink (the farm, and its impact on plant life).
First of all, analytical techniques were developed, validated and used to determine the environmental burden of the target contaminants in European sewage sludge, soil and sewage sludge amended soils. A part of the analysis involves a broad survey of treatment systems in Europe for the presence of the model compounds, as well as variation between different laboratories. Results so far have indicated a broad variation of the different compounds between countries. However, it seems that some compounds have very high level (LAS, NPE and PAE) with medium toxicity and persistence and some other low level (PAH) but high toxicity and persistence. In some cases, mild or no treatment is required to reduce below legislated levels (as assessed in countries that have legislation). In other countries where no legislated levels exist, concentrations of xenobiotics in sludge are high. Legislation at a national level would be needed to comply with new EU directives.
Bioassays for evaluating the environmental safety of using bioprocessed and non-bioprocessed sewage sludge as organic fertilisers were developed. To evaluate the impact of the xenobiotic compounds in soil, the environment and on product crops, a number of new toxicity tests have been established. One novel test assesses the end-phytotoxicity by evaluating impact on rhizospheric activity in the interface between soil and root. It was found that an impact could only be found in soil adhering strongly to the roots, and that this should be used as a standard. Alternative tests are being used to assess mutagenic tests, seed viability, and accumulation of

xenobiotics in food plants. These tests are being used to assess impacts in a large scale factorial test using lysimeters. Novel testing methods have been submitted to standards authorities for approval as standardised tests.

Life cycle assessment work seeks to determine the global and local environmental impacts of different treatment alternatives. In addition, economic impacts have been examined using life cycle costing methodology. An environmental risk assessment of untreated sewage sludge on a local scenario is also being conducted. The other package deals with micromanagement within the treatment process, and may be used to evaluate individual technologies.

Also, different packages within the project address degradation in both aerobic and anaerobic treatment, isolation and enhancement of microbes degrading the model compounds under aerobic and anaerobic conditions and modelling of the compounds through the treatment plant. Anaerobic digesters and compost reactors were developed to reflect the existing systems in use. Different strategies were investigated to find parameters of significance for detoxification of sewage sludge. These studies included the effect of hydraulic retention time, temperature, and reactor set-up on degradation of the target compound. The aerobic processes were particularly efficient. Nevertheless, the anaerobic processes showed a significant potential of biodegradation. As anaerobic digestion is worldwide used, we mainly focussed on anaerobic degraders and on in situ enhancement of the anaerobic degrading ability.

Specific Project Components

Microbial Isolation, Characterisation, and Bio-augmentation. The microbiology of aerobic and anaerobic conversion of these compounds is interesting from a fundamental, as well as practical point of view. Facultative aerobic isolates have been previously achieved on all of the model compounds, but anaerobic isolates degrading the model compounds have not been found.

Because of the difficulty involved in achieving isolates, we took a two-prong parallel approach in microbial characterisation. Enrichment procedures were tested and compared according to their potential for enhancing xenobiotic removal efficiency: one series of successive Fed-batch reactors, and one continuous CSTR reactor. For each, one abiotic control - chemically sterilized – representing the abiotic losses, and one population control - a blank without xenobiotic addition – were performed. In order to enrich the microbial ecosystems in

xenobiotic-degrading microorganisms, the reactors were continuously or sequentially fed with a specific anaerobic synthetic medium. During enrichment, isolate candidates were taken from the enrichments. During enrichment, with verified removal (99%) of target compound compared to the abiotic control (31%), the microbial community through the whole experiment was examined using SSCP (single strand conformation polymorphism; Delbes et al. 2001). A strong correlation was observed between the PAE degradation kinetics and the growth of new dominant species within the enriched ecosystems : three bacteria (*bact1*, *bact2* and *bact3*) were identified as being potentially selected by the PAE enrichment procedure, with *bact1* as the main dominant species (Figure 2). A limited clone library was then assembled from the final enrichment, and the peaks identified phylogenetically. The dominant Eubacteria species (*bact1*) was clearly identified as being very close to *Soehngenia saccharolytica,* already described as a benzaldehyde-converting bacterium. As well, the dominant Archaebacteria species was identified as *Methanosaeta concilii*, known for its hydrophobic properties. These results suggest the existence of specific relationships between these microorganisms for PAE degradation. This approach has also been extended for the characterization of microbial communities and/or the identification of bacteria involved in the biodegradation of PAH and NPE in complex environmental samples. The sequence data could also be used to design molecular probes for further *in-situ* monitoring of the abundance of the degrading consortium and describing their spatial configuration. It appears that the dominant specie (bact1) was mainly found in flocs in the enriched cultures (Figure 3). This may suggest that the dominant species of the enriched culture grows essentially where hydrophobic compounds such as PAEs are trapped, i.e. into the biofilm matrix. Moreover, this kind of spatial arrangement favoured symbiotic relationships with methanogens.

Similar work has been done with the isolates. We have had very encouraging anaerobic isolate results, achieving isolates on all target compounds except LAS. Figure 4 summarizes the ability of PAE isolates to degrade the target compound. As shown in control reactors, the abiotic losses were considered as non significant during the experiment (< 5%). Each isolate exhibited different removal performances from around 50% for isolate A for more than 90% for isolate C.

Bio-augmentation work has indicated mixed results. Very promising results has been obtained with batch augmentations, with high proportions of target microbe in the augmentation mix and with very efficient results in biodegradation as shown on Figure 5. Therefore, a continuous reactor could not be augmented for PAE degradation, due to sharp decrease of the target population indicating survival and maintenance problems (protozoa grazing for example).

Modelling of movement through the treatment plant. Behaviour of these compounds in the treatment plant is critical to the project, and modelling is an integral part of the project. The target compounds are surface active, with high K_{OW} and K_{OC} coefficients. We have created models to both assess degradation of the compounds in the treatment, as well as evaluate the impact of the compounds on the processes. The base models are the Activated Sludge Model no.1 (Henze et al. 1987), and the Anaerobic Digestion Model No. 1 (Batstone et al. 2002), together with specific extensions for adsorption, stripping, and biological conversion of the xenobiotic compounds. Parameters from these models are sourced from experiments, theoretical data (for example adsorption, and stripping), and full-scale data. So far, ADM1 model was successfully fitted to the experimental data and the parameters estimated were lower than the suggested values. Statistical analysis performed in the parameters estimated showed that xenobiotics had no apparent inhibitory effect on the anaerobic digestion process at typical concentrations (Figure 6) but that also, the xenobiotics are not readily degraded in typical non adapted anaerobic systems.

The anaerobic degradation of hydrophobic compounds in the sludge was modelled in the case of PAE based on the PAE concentration on the bioavailable sites of the sludge (Figure 7). The sites considered bioavailable are those accessible by the microorganisms. These sites are located on the surface of the biosolids and in the aqueous phase. Since PAE is considered insoluble in water, it was assumed to partition between the surface and the inner part of the biosolids through first order kinetics. Biodegradation of PAE was considered to be a co-metabolism result of the anaerobic consortium degrading sludge and took place on the surface of the biosolids through simplified Monod kinetics. The overall PAE decrease rate was limited by the desorption of the compound from the inner part to the surface of the biosolids.

We are also using a new thermodynamic modelling approach to assess favourability of potential pathways. This can also be used to assess degradability of a proposed compound. If free energy change of reaction is positive or zero in a range of reactor operating conditions, bioconversion is fundamentally not possible. All compounds examined so far have been degradable, with the exception of some high MW PAHs.

Conclusions

The study has shown so far that xenobiotics in sludge can be an issue at a national level. However, action is also relatively straightforward. Current aerobic composting or aeration as a final conditioning can remove the compounds, and we have shown in the laboratory that anaerobic digestion also has the potential to remove a significant proportion. Introduction of *de-novo* ability into existing reactors is a more difficult issue, as bio-augmentation has not worked, especially in continuous systems. However, the results are supported by new microbial isolates and enrichments, together with molecular tools that assess establishment of the target culture. The project has also resulted in a number of new tools, including easier analytical testing of the compounds, improved mutagenic and toxicity testing, mathematical models, and application of existing tools such as life-cycle assessment to this new application.

References

Batstone DJ, Keller J, Angelidaki I, Kalyuzhnyi SV, Pavlostathis SG, Rozzi A, Sanders WTM, Siegrist H, Vavilin VA. 2002. Anaerobic Digestion Model No. 1 (ADM1), IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes. London: IWA Publishing.

Henze M, Grady CPL, Gujer W, Marais GvR, Matsuo T. 1987. Activated Sludge Model No. 1. London: IAWQPRC Scientific and Technical Report.

Langenkamp H, Part P. 2001. Organic Contaminants in Sewage Sludge for Agricultural Use. Luxembourg: EU.

Magoarou, P. 2000. Urban waste water in Europe : what about the sludge?, In Langenkamp, H. and Marmo, L. (eds): Workshop Problems around sludge – Proceeding 8 p, EUR 19657 EN.

Spormann AM, Widdel F. 2000. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. Biodegradation 11:85-105.

Acknowledgements

BIOWASTE is funded by the EU 5th Framework Program under the project BIOWASTE contract no. QLK5-CT-2002-01138.

178 Figure Captions:

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181 evolution during the CSTR enrichment .

182 Figure 3 : Picture of DBP (PAE) enriched floc showing (left) all bacteria using EUB-338 probe, and (right)
183 organism responding to DBP-EN1 probe developed from SSCP results.

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185 conditions. The culture medium correspond to a mixture of synthetic medium, 2g/L yeast extract and 30 mg/l
186 DBP. CTA and CTB correspond to control reactors (non inoculated). Isolate X corresponds to PAE-non
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189 Figure 5: PAE removal in anaerobic batch tests for sludge amended or not with PAE-degrading isolates in pure
190 culture or in mixture (5 % v:v added).

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192 (uncorrelated estimates of the error) as estimated from dynamical experiments conducted in three anaerobic
193 bioreactors (two of them were fed with secondary sludge contaminated with xenobiotics at typical
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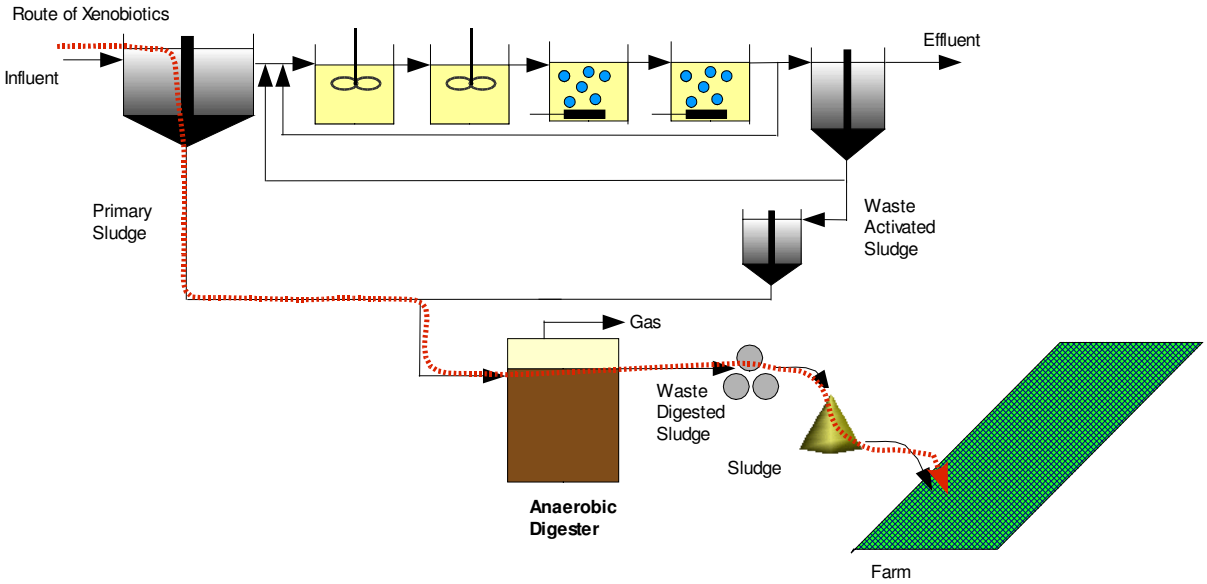

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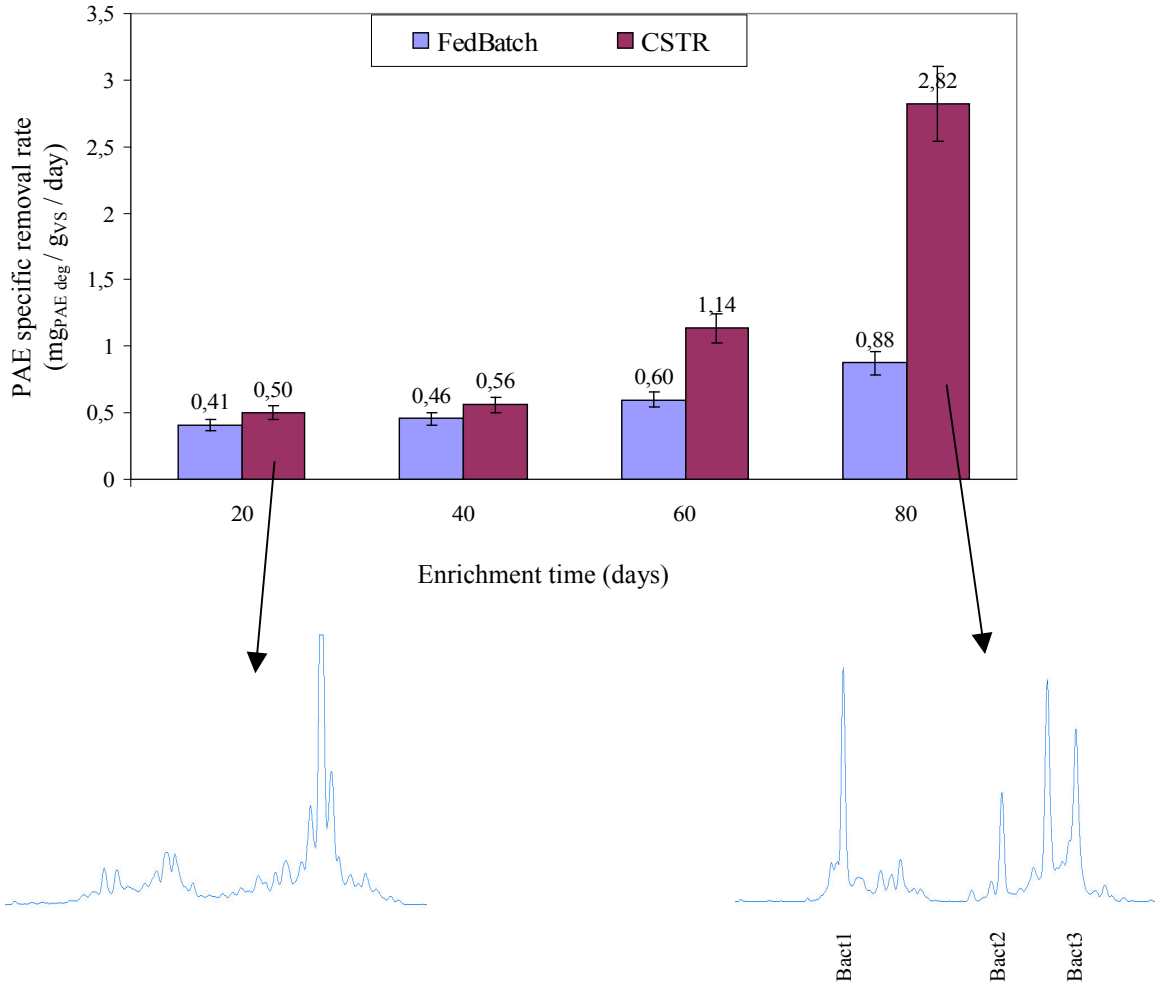

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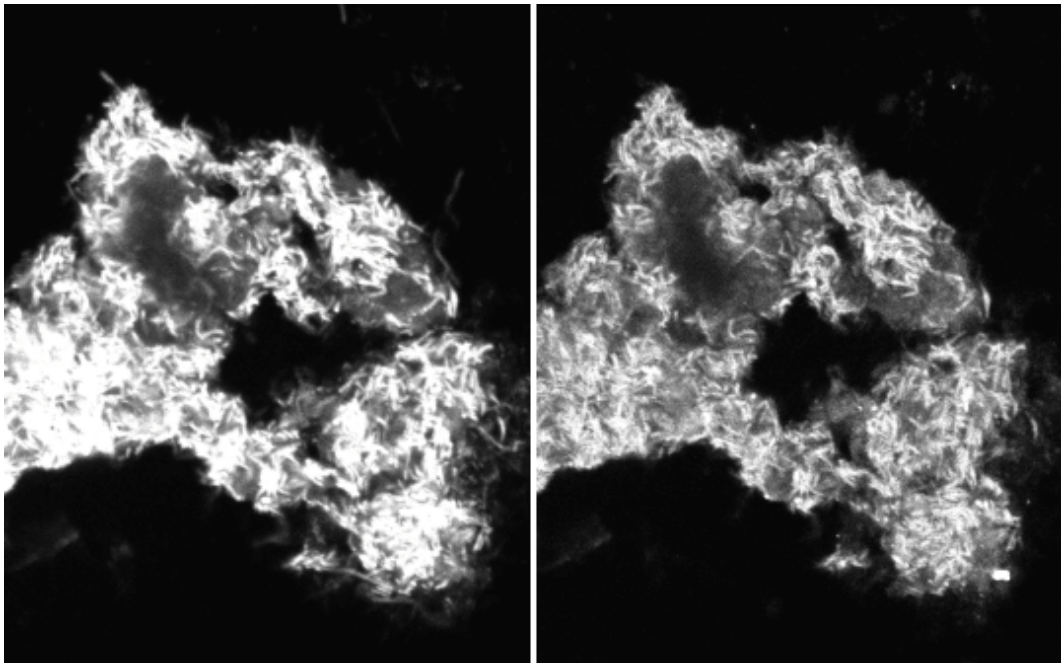

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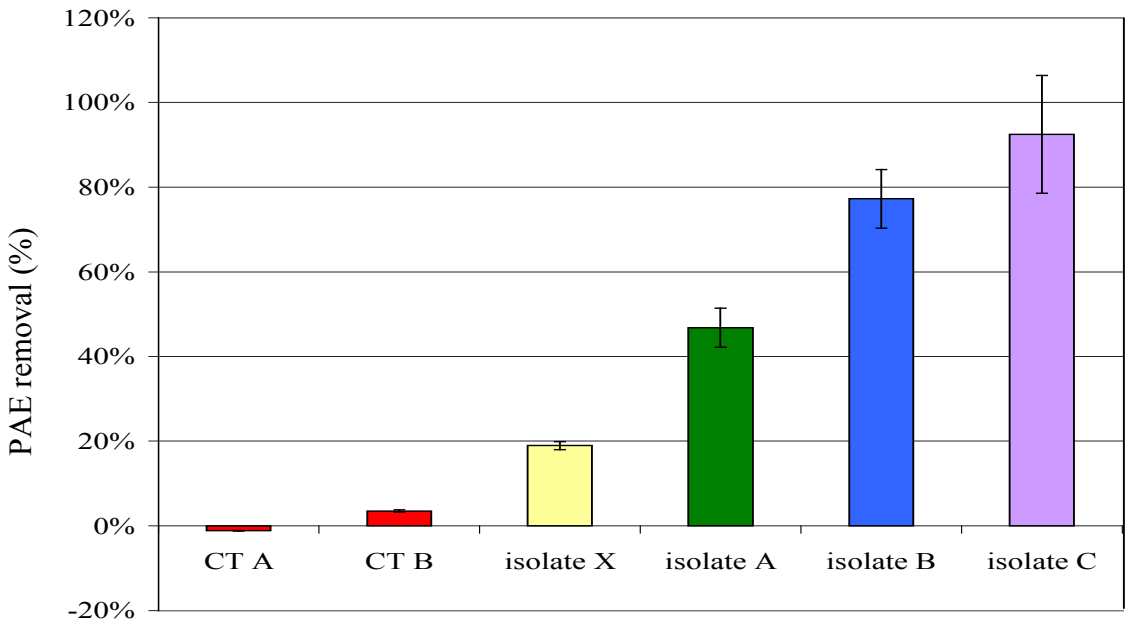

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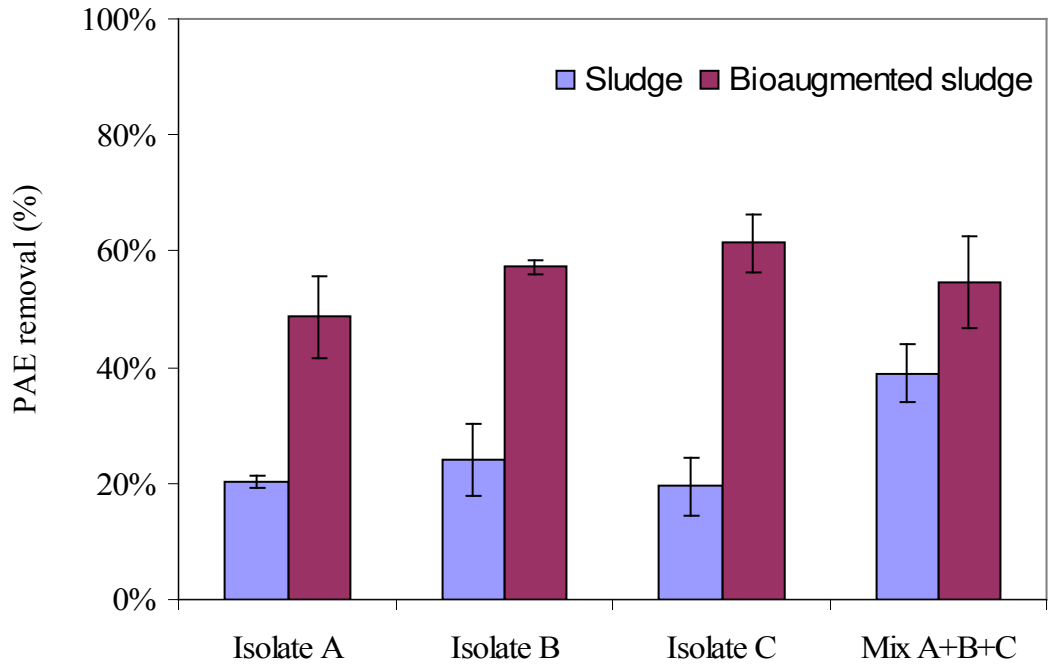

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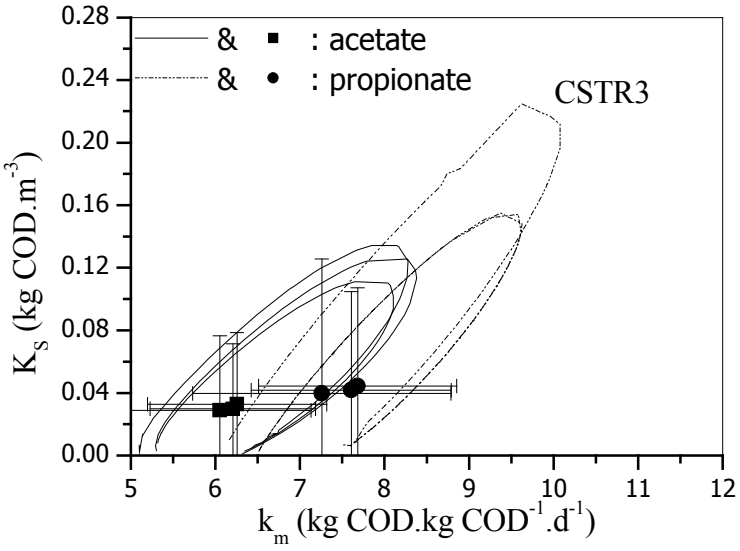

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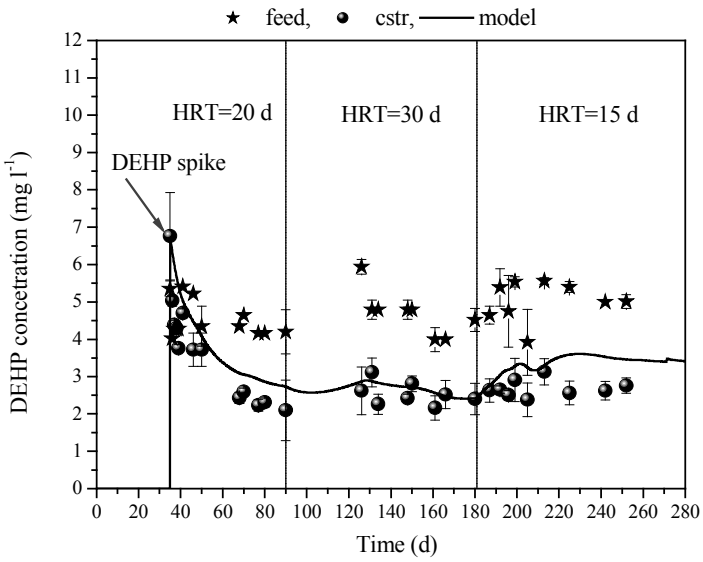

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