Fate of toxic organic compounds during bioconversion of wastewater sludge to value added products

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Abstract: Bioconversion of wastewater sludge (WWS) has made a substantial progress over last years. Traditional microbial products from WWS such as compost or methane are well studied, but other value added products (VAPs) such as biopesticides, microbial inoculants or industrial enzymes are now proposed. These WWS based VAPs are low cost biological alternatives that can compete with chemicals or other costly biological products in fructuous markets. However, when WWS is used as a raw material for VAPs production, questions still remain about the occurrence and persistence of toxic organic compounds (TxOCs) within biotransformed WWS, especially their toxic intermediates resulting from partial biodegradation. The microbial strains and techniques used for producing these VAPs can possibly remove these organic pollutants. Some literature findings concerning the impact of value added production on TxOCs removal are discussed in this paper. The potential of “value added producers” to degrade or detoxify TxOCs and toxic intermediates is also discussed. This paper proposes that future value added processes should focus to obtain TxOCs free - WWS based VAPs for preventing environmental contamination as well as favoring commercialization and public acceptance for these novel products.

Keywords: Wastewater sludge, bioconversion, value added products, toxic organic compounds, degradation products.

INTRODUCTION

Digestion and composting of WWS are biological processes that demonstrate the capacity of WWS to sustain growth of microorganisms and obtain valuable products (composts, biogas). Employing monoculture techniques, other value added products (VAPs) may be obtained from the bioconversion of WWS by industrial microbial strains or novel strains: biopesticides, microbial inoculants, industrial enzymes and bacterial biopolymers resulting from bioconversion of WWS have been studied (Ben Rebah et al., 2001; Tyagi et al., 2002; Verma et al., 2005; Yan et al., 2006; Drouin et al., 2007). These WWS based VAPs are low cost products that can compete with toxic or less expensive chemicals or other costly biological alternatives in small or big markets. There are also other advantages associated with these VAPs like sludge volume reduction, replacement of conventional raw materials and mitigation of greenhouse gases (through less transportation for WWS disposal and, to a certain extent, sequestering carbon into value added products instead of losing it during digestion or composting).

WWS is known to contain toxic metals, organic micro pollutants and pathogens that may add constraints to their beneficial uses (land application, site reclamation, use of WWS based VAPs like biopesticides or microbial inoculants). For WWS based VAPs, environmental risks related to toxic inorganics, dioxins, furans and pathogens can be controlled by: (i) selecting a WWS having low content of regulated contaminants that respect the local legislation for land application; (ii) application of decontamination process to remove toxic metals; (iii) the necessary step of sterilization for monoculture process that eliminates pathogens. However, more attention is now focused on toxic organic compounds (TxOCs) in WWS with potential carcinogenic, teratogenic and/or endocrine disrupting properties. It includes plasticizers, pharmaceuticals, personal care products, pesticide residues, flame retardants and gasoline additives which are linked to human activities. These TxOCs eventually reach sewage treatment plants (STPs) and can be concentrated in WWS. Disposal of WWS is one way for these pollutants to be introduced into environment among others. Presence of these TxOCs may add constraints to the ultimate disposal of these sludges and/or reduce the possibilities for their beneficial uses.

When WWS is used as a raw material for VAPs production, questions still remain about the fate and persistence of TxOCs within biotransformed WWS, especially the toxic intermediates that may result from partial biodegradation. These TxOCs can be a threat for ecosystem, a major constraint for product homologation and a delicate problem for public acceptance. Considering that value added product producers (microorganisms...
used for value added production through monoculture) are well equipped with panoply of enzymes, there are possibilities to detoxify or degrade partially or totally TxOCs during bioconversion of WWS. So, the WWS based VAPs could be free of contaminants and favour the use of monoculture for WWS bioconversion to achieve simultaneously sludge recycling and decontamination. Some findings concerning the impact of bioconversion of WWS on TxOCs removal are discussed in this paper. The potential of value added product producing microorganisms to degrade or detoxify TxOCs and toxic intermediates is also discussed. This paper does not attempt to make a review on the topic, but it highlights the importance of focusing on the fate of TxOCs when studying bioconversion of WWS, which means to give a particular attention on toxic intermediates and identify them when degradation pathways are poorly understood.

TOXIC ORGANIC COMPOUNDS OF CONCERN IN SLUDGE

Actually, the TxOCs having potential endocrine disrupting properties receive most of the attention from environmental agencies and the scientific community. Among them, poly aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), di-(2-ethylhexyl) phthalate (DEHP), the nonylphenol ethoxylates (NPEOs), the linear alkylbenzene sulphonates (LAS) and dibenzodioxins/furans (PCDD/Fs) were selected by European Commission for limiting their content in dried WWS (CEC, 2000). These are now considered as “priority” organic pollutants for WWS land application or land filling due to their abundance in WWS and toxicity (CEC, 2000; Abad et al., 2005). The nonylphenols (NPs) and bisphenol A (BPA) are two other TxOCs having endocrine disrupting properties and being abundant in WWS (Scrimshaw and Lester, 2003), but they are not thus far in the list of priority organic pollutants. Some of these priority pollutants like PCBs and PCDD/Fs are already under severe regulations because they are classified as “persistent” organic pollutants in environment by environmental agencies.

The priority organic pollutants are known to reach the sewer system through sewage from human activities, atmospheric deposition on the soil, urban runoff and industrial emissions. During wastewater treatment, they tend to accumulate in WWS due to their non polar and hydrophobic nature that favour adsorption onto suspended solids and organic matter. Scrimshaw and Lester (2003) presented a list of the most abundant endocrine disrupter compounds in WWS in which NPs, NPEOs, phthalates (e.g. DEHP), PAHs, BPA and PCBs came to the fore with concentrations over 10 mg kg⁻¹. Their fate during wastewater treatment is also determined by chemical and biological degradation and volatilization. Development of WWS treatment or value added processes shall consider removal of these priority organic pollutants because they are closely related to WWS beneficial uses.

It is important that removal studies include the fate of the TxOCs because toxic intermediates may be generated through partial degradation. It is especially important in the case of WWS bioconversion when biodegradation become a major removal pathway for the organic pollutants. Toxic intermediates, sometimes recalcitrant or having endocrine disrupting properties can be generated and cause a serious threat to the ecosystem once released in the environment. NPs and NPEOs are two well studied toxic intermediates that result from the biodegradation of alkylphenols, which are non-ionic surfactants used in many products like detergents, lubricants, defoamers, emulsifiers, cleaners for machinery and materials, paints, pesticides, textiles, metal working and personal-care products (Abad et al., 2005). The octylphenols (OP) and the nonylphenol carboxylates (NPCOs) are also toxic intermediates of alkylphenols with endocrine disrupting properties and they are present in WWS (La Guardia et al., 2001). It was also demonstrated that DEHP toxic intermediates, either 2-ethylhexanol, 2-ethylhexanal or 2-ethylhexanoic acid, were more toxic than their parent compounds (Nalli et al., 2002). Beauchesne et al. (2007) detected these compounds in WWS. For BPA, Kang et al. (2006) reviewed its biodegradation by different organisms and mentioned that some BPA metabolic intermediates could enhance estrogenic effect or toxicity, but BPA biodegradation generally lead to its detoxification. BPA presence in WWS is well reported (ref?), but there is a lack of information about its toxic intermediates. The degradation pathways are also poorly understood for LAS. However, it is generally accepted that sulfophenyl carboxylates are the main degradation products of LAS under aerobic conditions, but less is known about their toxicity (De Wever et al., 2004). Thus, a particular attention must be given to the toxic intermediates of the priority organic pollutants. It may also be necessary to conduct systematic studies for elucidating degradation pathways, identifying toxic intermediates and evaluate their toxicity under different biodegradation conditions.
REMOVAL OF TOXIC ORGANIC COMPOUNDS DURING BIOCONVERSION OF SLUDGE

Traditional processes for bioconversion of WWS involve an indigenous microbial flora able to totally degrade TxOCs or partially degrade them into less or more toxic intermediates. Scrimshaw and Lester (2003) reported many studies on the removal of EDCs during digestion or composting of WWS. In these studies, phthalates, PAHs and PCDD/Fs were the target priority organic pollutants. Recent works are now reporting data on removal of other priority organic pollutants like NPEOs during anaerobic digestion (e.g. Minamiyama et al., 2006). These works suggest that the WWS microbial flora produces panoply of phenol-oxidizing enzymes such as peroxidases, tyrosinases and laccases with the potential to degrade or detoxify TxOCs. The oxidases and their producers have been well reviewed for wastewater treatment (Duran and Esposito, 2002). Most works reported in these reviews were focusing on detoxification of industrial wastewaters or contaminated soils, but not for treatment of WWS. Other classes of enzymes like hydrolases are known to be involved in environmental decontamination (Whiteley and Lee, 2006). Among them, microbial esterases have been reviewed by Panda and Gowrishankar (2005) for degradation of natural materials or industrial pollutants, but no application for treatment of WWS was mentioned. Gavala et al. (2004) used pork liver esterases for pre-treatment of WWS (combined or not to a thermal pre-treatment) prior to biological treatment in order to facilitate the degradation of phthalate esters during digestion. Although esterases were not from a microbial origin, this work gives a promising perspective to the area of sludge treatment and bioconversion. As suggested by the authors, pre-treatment of WWS can be used to overcome the problem of TxOCs that negatively affect WWS quality and restrain its reuse in agriculture (discussed later). Other works on enzymatic treatment of WWS were studied to improve performance of digestion or settling processes using hydrolases (e.g. Ayol, 2005), but they were not aiming on the degradation or detoxification of TxOCs with enzymes. For bioconversion of WWS into unconventional VAPs as biopesticide and microbial inoculants, some value added product producers may be especially interesting to obtain VAPs and produce concomitantly enzymes to detoxify or degrade TxOCs. There is also a possibility that the value added producers partially degrade TxOCs into less or more toxic intermediates.

Different bacterial and fungi strains are used as value added producers for bioconversion of WWS into biopesticides, microbial inoculants, industrial enzymes and other products. No study has been conducted to date their potential to degrade or detoxify TxOCs in WWS and produce simultaneously VAPs. Table 1 presents some promising value added producers for industrial production of commercial products using WWS as raw material.

**Table 1. Value added producers and potential enzyme system for degradation or detoxification of toxic organic compounds in wastewater sludges.**

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<th>Microorganism</th>
<th>Value added products from WWS</th>
<th>Potential enzyme system</th>
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<td><strong>Bacillus thuringiensis</strong></td>
<td>Bio-insecticide (spores, insecticidal toxins and other insecticidal factors) and alkaline proteases for bleaching processes (Tyagi et al., 2002)</td>
<td>Bacillus sp. can produce esterases, tyrosinases and laccases (Claus, 2004; Panda and Gowrishankar, 2005). As example, it has been recently reported that sporulation of some species produce laccase-like enzymes in relation with their sporulation or their spore content (Claus, 2004). Also, Mohammadi et al. (2006) discovered novel and highly insecticidal B. thuringiensis isolates from WWS that show the ability to produce melanin (non-toxic), which is synthesized through the action of tyrosinases on phenol compounds (personal communication).</td>
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<tr>
<td><strong>Bacillus licheniformis</strong></td>
<td>Alkaline proteases for bleaching processes (Drouin et al., 2007)</td>
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<td><strong>Trichoderma viride</strong></td>
<td>Bio-control agent and soil enhancer for agricultural crops (Verma et al., 2005)</td>
<td>T. viride has a high potency to grow and sporulate in WWS culture media (Verma et al. 2005). It is known to produce a variety of enzymes including oxidases and hydrolases.</td>
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<tr>
<td><strong>Sinorhizobium meliloti</strong></td>
<td>Microbial inoculant for legume cultures and bio-control agent for agricultural crops (Ben Rebah et al., 2001)</td>
<td>S. meliloti is a soil microorganism that using diverse enzymes to invade the rhizosphere of legumes or crops. This strain is known to produce laccases produced by (Castro-Sowinski et al., 2000).</td>
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The works reported in Table 1 suggest that some value added producers possess the potential enzyme system to detoxify or degrade TxOCs. However, the possibility of generating toxic intermediates should be considered because they may persist in the final product. Therefore, it is required to study the fate of TxOCs during VAPs production. These studies shall focus on the fate of priority organic pollutants and their toxic intermediates. Studies on degradation pathways may be required for each value added processes in order to identify metabolic intermediates that may exhibit toxicity and especially endocrine disrupting properties.

**POTENTIAL IMPACT OF SLUDGE PRE-TREATMENT ON TOXIC ORGANIC COMPOUNDS**

As mentioned previously, application of a pre-treatment on WWS prior to bioconversion can be one solution to degrade or detoxify TxOCs that may affect the WWS quality for beneficial uses. WWS pre-treatments break suspended solids, liberate the nutrients, solubilize partially the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and/or improve the overall WWS biodegradability for subsequent biological treatment (anaerobic digestion, sequential biological reactor, bioconversion into biopesticides or microbial inoculants). Thermal hydrolysis, sonification, ozonation, enzymatic treatment, high performance pulse techniques, wet oxidation and supercritical oxidation are WWS pre-treatment methods used to improve biogas production (Rulkens, 2004). Alkaline hydrolysis and thermal hydrolysis at alkaline pH are pre-treatment methods that increase considerably yield of value added producers described in Table 1. For instance, there is a lack of information in literature about the impact of WWS pre-treatment on TxOCs. For wastewater treatment, oxidative pre-treatments are known to increase biodegradability, convert recalcitrant molecules to readily biodegradable by-products and/or detoxify the wastewater for enhancing the solubilization of the organic matter and the degradation of recalcitrant compounds during biological treatment (e.g. Contreras et al., 2003). For bioconversion of WWS, only a few studies have been published on the application of a pre-treatment (enzymatic, thermal+enzymatic, oxidation) for specifically removing organic pollutants (NPEs, PAHs, DEHP, LAS) or detoxify WWS before biological treatment (anaerobic digestion, sequential biological reactor) (e.g. Bernal-Martinez et al. 2007). These works suggest that some pre-treatment methods may facilitate TxOCs biodegradation in subsequent biological treatment. However, Gavala et al. (2004) showed that a pre-treatment at elevated temperature (70oC), without other pre-treatments, affects phthalate esters biodegradation during anaerobic digestion. Then, the authors suggested that a thermal pre-treatment could destroy some nutritional factors needed for phthalate esters biodegradation during anaerobic digestion of primary WWS. Moreover, it appears that none of the research studies on pre-treatment has considered the occurrence of toxic intermediates which may persist in the subsequent biological treatment and final product. Future studies on WWS pre-treatment methods should consider their presence after the biological treatment and in VAPs.

**CONCLUSION**

Determining the fate of TxOCs after bioconversion of WWS (with or without pre-treatment) is primordial to minimize environmental risks after WWS disposal or improve WWS quality for beneficial uses. Fate studies should focus on DEHP, BPA, NPs, NPEOs, BPCs, PAHs and PCDD/Fs, and their known toxic intermediates. These studies should also focus on the removal pathways and the identification of other metabolic intermediates with potential toxicity. Partial degradation must lead to detoxification. Such studies must be performed with value added producers such as B. thuringiensis, B. licheniformis, T. viride and S. meliloti and other promising strains. A WWS pre-treatment method can be used to detoxify WWS and to improve bioconversion, but a particular attention must be paid to occurrence of toxic intermediates. Having WWS based VAPs free of TxOCs is important to protect the ecosystem, avoid public reluctances over beneficial uses and favor commercialization of the final product. With proper researches, bioconversion of WWS can achieve simultaneously value added production and removal or detoxification of the priority organic pollutants.
REFERENCES


