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Review

Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants

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HIGHLIGHTS

- Biodegradation of EOCs is implemented via metabolism and/or cometabolism mechanisms.
- Autotrophic ammonia oxidizers and nitrification play a key role in cometabolizing EOCs.
- Heterotrophic microbes can degrade EOCs via cometabolism and/or metabolism.
- Biodegradation rates of EOCs rely on microbial community structure in the environment.
- High biodegradation rates of EOCs were noted at high ammonia loading rates.

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ABSTRACT

Many efforts have been made to understand the biodegradation of emerging trace organic contaminants (EOCs) in the natural and engineered systems. This review summarizes the current knowledge on the biodegradation of EOCs while having in-depth discussion on metabolism and cometabolism of EOCs. Biodegradation of EOCs is mainly attributed to cometabolic activities of both heterotrophic and autotrophic microorganisms. Metabolism of EOCs can only be observed by heterotrophic microbes. Autotrophic ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaeal (AOA) cometabolize a variety of EOCs via the non-specific enzymes, such as ammonia monooxygenase (AMO). Higher biodegradation of EOCs is often noted under nitrification at high ammonia loading rate. The presence of a growth substrate promotes cometabolic biodegradation of EOCs. Potential strategies for enhancing the biodegradation of EOCs were also proposed in this review.

1. Introduction

Environmental pollution by emerging trace organic contaminants (EOCs) such as pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) has been increasingly gained attention due to their potential risk in causing undesirable ecosystems and human health effects (Jones et al., 2004). EOCs are frequently detected in different environmental compartments, such as wastewater, surface water, groundwater, sludge, sediments and sometimes in drinking water, at trace levels ranging from ng/L to μg/L (Kolpin et al., 2002; Lapworth et al., 2012; Stasinakis, 2012; Tran et al., 2013a,b). EOCs enter the aquatic environment in several different ways either dumped directly (i.e. industrial effluents) or from wastewater treatment plants (WWTPs) that is insufficiently treated (Kolpin et al., 2002). The presence of PPCPs and EDCs in the water environment has been reported to potentially affect aquatic organisms and produce changes that threaten the sustainability of aquatic ecosystem (Jones et al., 2004; Lapworth et al., 2012). Although the toxicity of PPCPs and EDCs to human health is relatively unknown at trace levels, continuous discharge and chronic exposure to these compounds may pose a risk to human health associated with exposure to very low concentrations of pharmaceuticals in drinking-water over a lifetime (Jones et al., 2004). The main routes for degradation of these pollutants in natural water are biodegradation and photodegradation. Biodegradation is considered to be one of the most promising clean-up technologies due to its low cost and its potential for complete degradation of pollutants.

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It has been known that biodegradation involves the use of microorganisms (such as bacteria and/or fungi) in natural soil and water that can assimilate target EOCs as growth substrates, leading to their elimination (Cajthaml et al., 2009; Stasinakis, 2012; Yang et al., 2013b). In engineered systems like WWTPs, biodegradation of EOCs have been made by exploiting catabolic activities of microbial consortia (e.g. activated sludge) or pure strains (Shi et al., 2004; Cajthaml et al., 2009; De Gusseme et al., 2011; Almeida et al., 2013). It was reported that the biodegradation of EOCs varies significantly among the different WWTPs (Joss et al., 2006; Kimura et al., 2007). Previous studies released that there were differences in biodegradation efficiency for different EOCs. For instance, Joss et al. (2006) found that only 4 out of 35 the investigated EOCs were eliminated approximately 90% in novel biological wastewater treatment systems, while 17 out of 35 were removed at least than 50%. Similarly, removal efficiencies for 14 EOCs varied from 20% (for carbamazepine) to 99% (for ibuprofen) at a WWTP in Spain (Gomez et al., 2007). These observations leads to a critical research question: why conventional WWTPs are not effective in biodegradation of EOCs, particularly with regard to slowly biodegradable EOCs?

Naturally, most WWTPs are often designed to eliminate organic matter nutrients (i.e. organic carbonaceous, nitrogenous, and phosphorus compounds) from urban and industrial wastewater streams. They do not have particular design in order to remove EOCs, particularly with regard to persistent/toxic EOCs. As a result, many persistent EOCs are frequently detected in WWTP effluents and receiving water bodies (Kolpin et al., 2002; Tran et al., 2013b). It was reported that conventional activated sludge (CAS) treatment systems are not effective in elimination of EOCs, particularly for persistent/toxic EOCs from urban/industrial effluents (Joss et al., 2004; Radjenovic et al., 2009). The main removal mechanisms of EOCs by activated sludge are biodegradation and sorption into solids/colloidals (Joss et al., 2006; Kimura et al., 2007). However, CAS treatment systems may be effective in the biodegradation of easily biodegradable EOCs, such as ibuprofen, methyl paraben, galaxolide, and caffeine (Oppenheimer et al., 2007; Roh et al., 2009; Tran et al., 2009).

In the last two decades, membrane bioreactors (MBR) have been widely used for treatment of both municipal and industrial wastewater. Previous studies reported that MBR offers several advantages over CAS in biodegrading most EOCs, particularly for biodegrading the persistent EOCs (Kimura et al., 2007; Oppenheimer et al., 2007; Cirja et al., 2008; Radjenovic et al., 2009). To date, numerous efforts have been made to elucidate why MBR systems are more effective in the EOC biodegradation than CAS systems. In addressing this question, many studies have focused on understanding biotic and abiotic factors, which control the biodegradation of EOCs during biological wastewater treatment processes such as temperature, pH, physicochemical properties and chemical structure of target EOCs, hydraulic retention time (HRT), solid retention time (SRT), presence of growth substrates, and the nature of microbial population involved in biodegradation (Kreuzinger et al., 2004; Clara et al., 2005; Cirja et al., 2008; Tran et al., 2009; Tadkaew et al., 2010; Hai et al., 2011; Helbling et al., 2012). For example, some researchers have claimed that biodegradation of EOCs is dependent on the physicochemical properties and chemical structures (Kimura et al., 2005; Cirja et al., 2008; Tadkaew et al., 2011). Some research groups have suggested that operational parameters (i.e. HRT, SRT, pH, and temperature) in biological wastewater treatment systems played a crucial role in biodegradation of EOCs (Kreuzinger et al., 2004; Clara et al., 2005; McAdam et al., 2010; Tadkaew et al., 2010; Hai et al., 2011). While, others have emphasized that the nature of microbes and their enzymes involved in the biodegradation of EOCs is of critical importance (Yi and Harper, 2007; De Gusseme et al., 2009; Roh et al., 2009; Tran et al., 2009; Khunjar et al., 2011; Helbling et al., 2012).

Numerous laboratory and field-based studies have been made to understand the biodegradation mechanisms. However, biodegradation pathways (such as metabolism and/or cometabolism), effects of environmental conditions, and the contributions of the relevant functional microbial groups (i.e. heterotrophic and autotrophic microbes) to the biodegradation of EOCs are not yet fully understood.

Therefore, the objective of this review was to summarize the current knowledge on biodegradation of EOCs to elucidate the role of metabolism and cometabolism of EOCs by microorganisms. Meanwhile, the roles of heterotrophic and autotrophic microorganisms and their enzymes in metabolism and/or cometabolism of EOCs were also discussed. Additionally, this review aimed to highlight applicability of exploiting cometabolism/metabolism in developing efficient bioremediation methods for EOCs in both natural and engineered systems.

2. Biodegradation pathways of EOCs

Biodegradation is known as the process of converting large molecular weight compounds to those with lower complexity. In the case of organic compounds, they are converted to simple inorganic molecules such as water and carbon dioxide. The biodegradation process is referred to as biomineralization. In conventional biodegradation process, microorganisms use organic compounds as primary substrates (growth supporting substrates) for their cell growth and induce enzymes for their assimilation. This process is known as metabolism. The fact is that EOCs are often present in natural waters and raw wastewater at trace concentration levels ranging from a few ng/L to a few hundred μg/L (Kolpin et al., 2002; Lapworth et al., 2012; Stasinakis, 2012; Tran et al., 2013a). Additionally, many of them are toxic and/or resistant to the microbial growth. Therefore, EOCs present in the environment may not be considered as the sole carbon or energy sources to maintain biomass growth and induce the corresponding enzymes for their assimilation. In this context, the obligatory presence of a growth substrate or another utilizable compound is critically needed to maintain biomass and induce the corresponding enzymes and/or cofactors for the biodegradation. This process is known as cometabolism (Arp et al., 2001).

The biodegradation of EOCs in both natural and engineered systems is referred to both metabolism and cometabolism by the relevant microbes. Until now, it is unclear which biodegradation pathway (i.e. cometabolism or metabolism) is dominant in the elimination of EOCs in both natural and engineered systems. Hence, this review attempts to provide an in-depth discussion on both metabolism and cometabolism of EOCs. Understanding the contributions of metabolism and cometabolism to the biodegradation of EOCs allows optimizing and designing the suitable natural and engineered systems for highest biodegradation efficiency of EOCs. The conceptual models for metabolism and cometabolism of EOCs are presented in Fig. 1a and b, respectively.

2.1. Biodegradation of EOCs via metabolism

Although numerous studies on biodegradation of EOCs have been reported, relatively little information could obtain regarding the biodegradation pathways (such as metabolism or cometabolism) as well as role of the relevant functional microbial groups (i.e. autotrophs and/or heterotrophs) in the biotransformation of EOCs. First of all, for metabolic degradation of EOCs (Fig. 1a), microorganisms utilize EOCs as the sole energy and/or carbon source to maintain their biomass and produce the relevant enzymes and cofactors for their oxidation/reduction. Therefore, to possibly metabolize an EOC by microorganisms, EOC should be

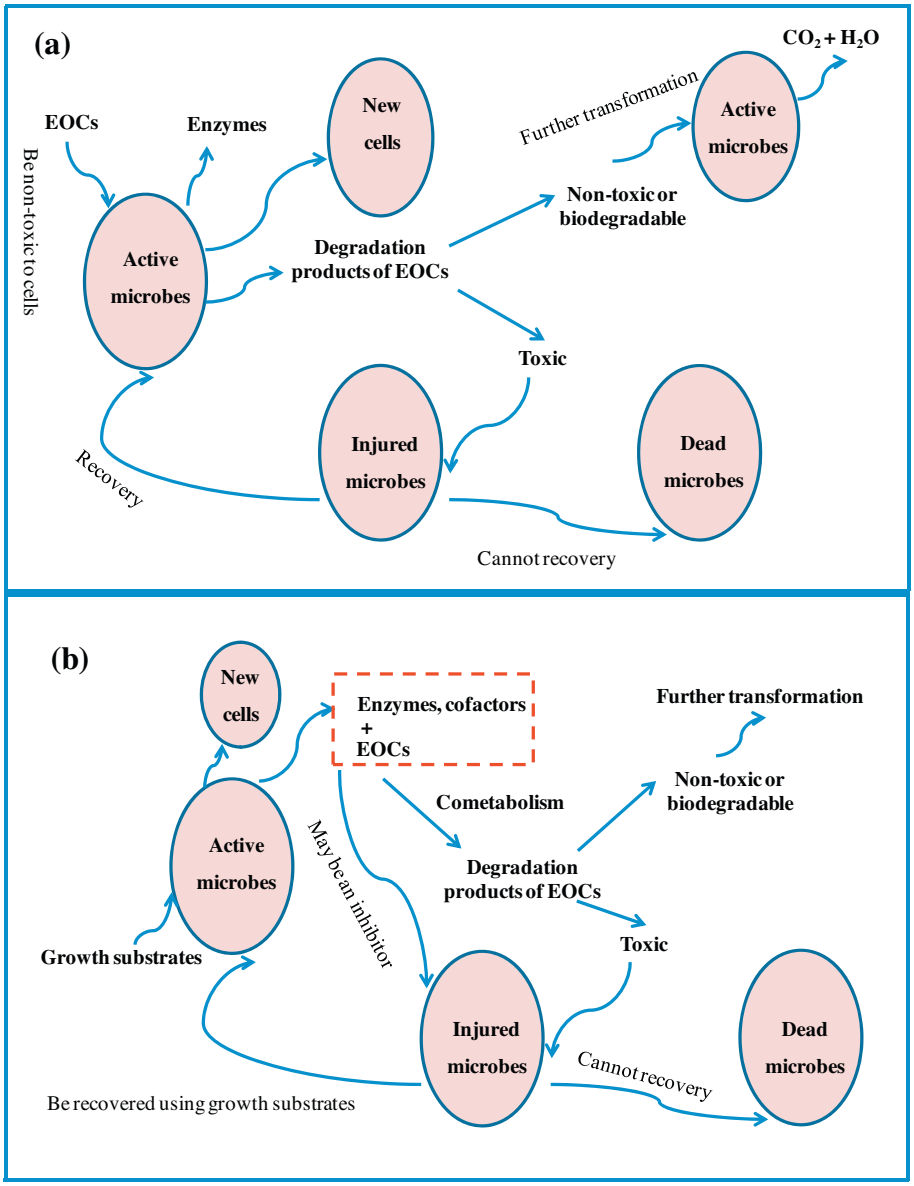

Fig. 1. Scheme of biodegradation of emerging trace organic contaminants (EOCs) via (a) metabolism and (b) cometabolism.

non-toxic or less harmful to the microbial growth, and its presence in the environment at high enough concentration levels that allow maintaining biomass and inducing the relevant enzymes/cofactors for degradation. However, it is unclear to know which concentration level of EOCs can initiate metabolic activities of microorganisms. Moreover, it has been known that almost microorganisms involved in metabolic degradation of EOCs are heterotrophic microorganisms. These microbes use EOC as the sole carbon or energy sources to maintain their biomass and induce the expression of relevant oxidative/reductive enzymes involved in metabolic biodegradation process.

To date, a number of studies on metabolic degradation of EOCs as the sole carbon sources are still limited (Quintana et al., 2005). For instance, to assess the metabolic biodegradation ability of microorganisms in activated sludge, Quintana et al. (2005) incubated fresh activated sludge as inoculums with 20 mg/L of one of the studied pharmaceuticals (naproxen, ketoprofen, bezafibrate, ibuprofen and diclofenac) as the carbon sole source in phosphate buffer under aerobic condition. The results indicated that among the investigated pharmaceuticals, only ketoprofen showed metabolic biodegradation ability, other pharmaceuticals did not degrade during the experiment course. However, this study still could not give a clear answer that whether microorganisms in activated sludge used ketoprofen as the sole carbon to induce the corresponding oxidative enzymes for ketoprofen degradation or those oxidative enzymes could be derived from dead cells. If so, the biodegradation of ketoprofen in this case cannot be considered by metabolic activities of microorganisms. Murdoch and Hay (2005) also reported that a pure culture *Sphingomonas* Ibu-2 was capable of degrading ibuprofen as the sole carbon source. Recently, Iasur-Kruh et al. (2011) found that enriched estradiol-degrading bacterium (EDB) culture or pure culture EDB-LI1 (*Novosphingobium* JEM-1) was able to degrade estradiol (E2) at initial concentration of 50 mg/L as the sole carbon source, while estriol and ethinylestradiol were not degraded by this isolate under the examined condition. Zeng et al. (2009) also released that *Pseudomonas aeruginosa* TJ1 is able to oxidize E2 via metabolic biodegradation pathway.

In a recent study, pure culture *Delftia tsuruhatensis* and *P. aeruginosa* can metabolize acetaminophen as the sole carbon source (De Gusseme et al., 2011). Interestingly, metabolism of EOCs was only observed by heterotrophic bacteria/fungi (Murdoch and Hay, 2005; De Gusseme et al., 2011; Iasur-Kruh et al., 2011; Almeida

et al., 2013). Currently, there is still no available information on metabolism by autotrophic microbes.

2.2. Biodegradation of EOCs via cometabolism

Cometabolic degradation processes were first studied in the 1950s and 60s particularly with regards to the biodegradation of chlorinated solvents, aromatic compounds and petroleum hydrocarbons (Vannelli et al., 1990; Arp et al., 2001). Cometabolism is initially defined as the ability of microorganisms to convert non-growth substrates, typically in the presence of a growth substrate. However, this definition does not allow differentiating between cooxidation and cometabolism. It has been then redefined as the transformation of a non-growth substrate in the obligatory presence of a growth substrate or another biodegradable compound (Arp et al., 2001). Unlike conventional organic pollutants (i.e. biodegradable COD/nutrients), many EOCs are toxic or resistant to microorganisms. Additionally, these compounds are often present in natural waters as well as raw wastewater at trace concentration levels (ng/L–μg/L). These two characteristics make EOCs may not enter catabolic and anabolic pathways of microbial cells. In other words, the energy derived from the biodegradation of EOCs is insufficient to support microbial growth and induce the relevant enzymes/cofactors involved in the biodegradation. Therefore, the obligatory presence of a growth substrate or another utilizable compound is critically needed for the biodegradation of EOCs.

Fig. 1b shows the proposed conceptual model for cometabolism of EOCs. Some studies suggested that cometabolism allows initiating a reaction to convert persistent compounds to their intermediates that may be more biodegradable and would participate in the central metabolic pathways for the further biotransformation (Groning et al., 2007; Yi and Harper, 2007). However, in some instances, the degradation products from both metabolism/cometabolism may appear more toxic than their parent compounds, thus the natural microbes cannot further assimilate. Haiss and Kummerer (2006) found that diatrizoate was aerobically biodegraded to its degradation product (3,5-diamino-2,4,6-triodobenzoic acid). This degradation products was not further degraded by common bacteria present in the investigated samples (Haiss and Kummerer, 2006). Similarly, Groning et al. (2007) stated that diclofenac could be biodegraded using bacteria from biofilms of river sediment to form an immediate (i.e. p-benzoquinone imine of 5-OH diclofenac), which was not further degraded in an apparent cometabolic process. Thus, cometabolic biodegradation of EOCs may offer the microbes a set of new compounds (intermediates/degradation products). Some of these are toxic to this microbe, but are mineralized by other microbes (Quintana et al., 2005; Khunjar et al., 2011; Liu et al., 2013).

So far, cometabolic degradation of organic pollutants may be taken place under either aerobic or anaerobic conditions. In aerobic cometabolic biodegradation of EOCs, the EOCs are oxidized by an enzyme or a cofactor produced during the microbial metabolism of another growth substrate with oxygen. In anaerobic cometabolic biodegradation of EOCs, the EOCs are reduced by a reductive enzyme or cofactor generated from microbial metabolism of another primary substrate in an environment with the absence of oxygen. Most of studies have demonstrated that aerobic cometabolism of EOCs was predominant than anaerobic cometabolism. For example, to examine the contribution of aerobic and anaerobic biodegradation of estrogenic steroids in the environment, Czajka and Londry (2006) studied the biodegradation of estrogens (such as 17α-ethinylestradiol [EE2] and 17β-estradiol [E2]) under both aerobic and anaerobic conditions and found that no biodegradation of EE2 (5 mg/L) was observed under anaerobic condition. In contrast, a rapid biodegradation of estrogens (i.e. estrone [E1], estriol [E3], E2 and EE2) was noted under aerobic experiments (Sarmah and Northcott, 2008). In another study, Kunkel and Radke (2008) examined the biodegradation of six acidic pharmaceuticals (clofibric acid, diclofenac, bezafibrate, ibuprofen, naproxen, and gemfibrozil) in bed sediments and observed that only naproxen was degraded efficiently in deeper and anoxic sediment layers. Inversely, other pharmaceuticals were not degraded under anaerobic conditions. Cometabolism of EOCs is apparently noted in autotrophic microbes, such as autotrophic ammonia oxidizers via the non-specific enzymes, such as ammonia monooxygenase (AMO), which oxidize a large variety of organic pollutants. This is mainly because the autotrophic oxidizers use inorganic carbon and ammonia as the sole carbon and energy sources for their growth and induce AMO and cofactors (i.e. nicotinamide adenine dinucleotide [NADH] and nicotinamide adenosine dinucleotide phosphate [NADPH]). For heterotrophic microbes, they can involve in both cometabolism and/or metabolism depending on the concentration of EOCs present in the environment and their toxicity to the microbes.

2.3. Effects of environmental conditions and chemical structures on biodegradation rate of EOCs

Biodegradation of EOCs is a multifaceted process in which many biotic/abiotic factors are involved, such as temperature, pH, oxygen content, availability of the EOCs to microorganisms, the presence of growth substrates, and physicochemical properties, molecular structures, and toxicity of EOCs (Urase et al., 2005; Cirja et al., 2008; Tadkaew et al., 2010, 2011; Hai et al., 2011; Helbling et al., 2012; Yang et al., 2013b). These factors can affect the biodegradability or metabolic activities of microorganisms by limiting or stimulating growth of microbes. Each of the factors should be optimized for the selected microbe to obtain the greatest biodegradability of the EOC of choice. It has been found that notable fluctuations in the biodegradation of EOCs are noted among biological wastewater treatment systems. Although several efforts have been made to understand the biotic and abiotic factors controlling on the biodegradability of EOCs, the reasons for such fluctuation are not yet fully elucidated. This review aimed to summarize the current knowledge to find the association between biodegradation rate of EOCs with biotic and abiotic factors.

2.3.1. Effects of temperature

In practice, temperature variation in both natural and engineered systems can result from seasonal or diurnal fluctuation. It was demonstrated that temperature can directly affect on the microbial activity as well as solubility of EOCs in aqueous phase. Each microbe has a certain temperature range for growth. For instance, previous studies reported that ammonia oxidizing bacteria (AOB) could growth at low temperature (Czajka and Londry, 2006). In contrast, ammonia oxidizing archaeal (AOA) are not actively growing at temperatures ranging from 4 to 25 °C (Wu et al., 2013). To evaluate the impacts of temperature on ammonia-oxidizing communities (i.e. AOB and AOA), Wu et al., (2013) investigated the changes in abundance and patterns of both bacterial and archaeal *amo*A genes, which encode the AMO subunit A that is capable of oxidizing not only ammonia, but also a large variety of EOCs. Consequently, relatively low abundances of both bacterial and archael *amo*A genes were noted after 4-weeks of incubation at 4 °C. This could be due to an enhanced degradation of DNA or dead cells or lower growth rate of ammonia oxidizers compared to other microbes. Conversely, significant changes in abundance of both bacterial and archael *amo*A genes were observed at 37 °C. The effects of temperature on the biodegradation of EOCs were reported in several studies (Suarez et al., 2005; Gabet-Giraud et al., 2010; Hai et al., 2011). For instance, Suarez et al. (2005) found that no

significant differences in removal of E2 and EE2 were noted between 16 and 26 °C. Gabet-Giraud et al. (2010) also released that there was no difference in the elimination of E1 and E2 under 10 and 20 °C. In a recent study, Hai et al. (2011) investigated the impacts of temperature variation on the elimination of EOCs and found the stable elimination of most hydrophobic EOCs ($\log D > 3.2$) under the temperature range of 10–35 °C. However, lower elimination of the investigated EOCs was noted at 45 °C. Lan et al. (2011) reported that sudden exposure to temperature higher than 35 °C may cause harmful effects on the bacterial enzymes that are usually responsible for the benzene ring cleavage, which is the key step in the biodegradation process of many phenolic EOCs.

Generally, the elimination of EOCs in CAS is usually more stable than MBR during seasonal temperature fluctuations. This is interpreted as being due to the larger surface of CAS than MBR allowing protecting microbial activity against temperature shock (Cirja et al., 2008).

2.3.2. Effects of pH

It has been known that pH of an aqueous environment can affect on the physiology of microbes (i.e. such as pH optima of enzyme activities), solubility of EOCs in the environment, and ionic and/or non-ionic states of an EOC leading to the influence in eliminating EOCs. It has been reported that the protonation states of EOCs are dependent on their pK_a and environmental pH. For instance, tetracyclines are not charged at pH in the range of 6–7 and are easily adsorbed onto solid phase like activated sludge. Therefore, sorption is considered as main removal mechanism for these compounds during biological wastewater treatment (Kim et al., 2005). Urase et al. (2005) showed that the removal of acidic pharmaceuticals (such as ibuprofen and ketoprofen) was strongly affected by pH. Higher elimination efficiencies of these pharmaceuticals were noted at lower pH operation. For example, more than 90% of ibuprofen amd 70% of ketoprofen were eliminated at pH in bioreactor at below 6 and 5, respectively. However, for the neutral EOCs (e.g. propyphenazone and carbamazepine), their elimination is relatively constant and independent of the mixed liquor pH (Urase et al., 2005; Tadkaew et al., 2010). To date, it has been still controversial that which removal mechanism (adsorption or biodegradation) contributes to the high removal of several EOCs in MBR at a certain mixed liquor pH (Kim et al., 2005; Urase et al., 2005; Tadkaew et al., 2010). However, it is widely accepted that extreme pH values of the mixed liquor (less than 3 or greater than 9) as well as sudden changes in pH of the mixed liquor can inhibit the microbial growth. In addition to microbial growth inhibition, pH of the environment may control the biomass composition of fungi and bacteria. Bothe et al. (2000) examined pH effects on fungal and bacterial growth in soil and found that the bacterial growth was decreased fivefold under lower acidic condition (pH 4.5), whereas the fungal growth was increased fivefold in the same lower acidic condition (pH 4.5). Low pH may be physiological disadvantageous to the bacteria, decreasing bacterial competition and thus favouring fungal growth (Bothe et al., 2000). These results indicate that pH of an aqueous environment not only affects on the sorption capacity of biomass, but also influences directly in the microbial community structure involved in biodegradation.

2.3.3. Effects of solid retention time

In engineered systems, the long solid retention time (SRT) of MBR not only benefit the removal of bulk organic matter and nutrients, but also increase the elimination of a huge number of EOCs by increasing microbial diversity, particularly with regard to slower growing microorganisms like autotrophic ammonia oxidizers (Kreuzinger et al., 2004; Clara et al., 2005; Kim et al., 2005; Helbling et al., 2012). Clara et al. (2005) and Carballa et al. (2007) reported that the elimination of easily biodegradable EOCs (i.e. bisphenol A, ibuprofen, bezafibrate, and natural estrogens) was dependent on SRT. The critical SRTs for these compounds were about 10 days. However, other studies claimed that moderate/persistent EOCs (e.g. clofibric acid, diclofenac, ketoprofen, mefenamic acid, naproxen, tetracycline, oxytetracycline, sulamethoxazole, sulfadiazine, and ampicillin) are removed efficiently in nitrifying activated sludge systems with longer SRTs ranging from 15 to 65 days (Kreuzinger et al., 2004; Kimura et al., 2007). Many efforts have been made to find a correlation between SRT and biodegradation of EOCs and it is widely accepted that longer SRTs allow enriching slow growing bacteria like autotrophic ammonia oxidizers (AOB and/or AOA), which can cometabolize a large variety of EOCs via the non-specific enzymes, such as AMO (Kreuzinger et al., 2004; Clara et al., 2005; Helbling et al., 2012). In addition, longer SRTs allow microorganisms in activated sludge system have enough time to exposure to the target EOC and to develop the corresponding enzymes for the biodegradation of this compound. Recently, it has been found that SRTs of activated sludge systems can affect directly on the biomass composition, i.e. ratio between autotrophic and heterotrophic microbes (Yi et al., 2006). Xia et al. (2012) found that bacteria belong to *β-proteobacteria,* and *γ-proteobacteria* were the dominant species in the activated sludge system with a longer SRT (>30 days). These bacteria played a key role in cometabolic biodegradation of antibiotics in wastewater treatment process. Taken together, all the results discussed above suggest that the composition of microbes in activated sludge systems and their relevant enzymes induced may play a decisive role in the biodegradation of EOCs, which can control the biodegradation pathways of EOCs by cometabolism and/or metabolism.

2.3.4. Effects of chemical structure of EOCs on their biodegradability

Numerous studies have been made to elucidate the correlation between the chemical structures of EOCs and their removal efficiencies in biological wastewater treatment processes (Kimura et al., 2005; Joss et al., 2006). Kimura et al. (2005) released that low removal efficiencies of clofibric acid, diclofenac, and dichloprop in biological wastewater treatment processes might be attributed to the presence of chlorine groups in their chemical structures. Similarly, Tran et al. (2009) investigated the biodegradation of ten pharmaceuticals by enriched nitrifier culture and found poor biodegradation efficiencies of pharmaceuticals that contain at least one chlorine group in their molecular structures (i.e. clofibric acid, diclofenac, and indomethacin). More recently, Tadkaew et al. (2011) elucidated the relationship between specific molecular characteristics of EOCs and their removal efficiencies during a wastewater treatment process in a lab-scale MBR and found that: (i) poor removal efficiencies were noted for all hydrophilic and moderately hydrophobic ($\log D < 3.2$) EOCs possessing strong electron withdrawing functional groups (i.e. chlorine or amide groups); (ii) high removal efficiency was observed with compounds bearing electron donating functional groups such as hydroxyl groups and primary amine groups; and (iii) no significant differences in removal efficiencies between heterocyclic or non-heterocyclic compounds was noted. However, these conclusions from the above studies still have limitations which cannot explain for several cases. For instance, diclofenac and indomethacin are poorly removed by bacterial consortia (i.e. mixed culture/activated sludge), whereas these are easily biodegraded by fungi and their extracellular enzymes such as laccase and manganese peroxidase (Tran et al., 2010; Suda et al., 2012).

Taken together, these results suggest that chemical structures and characteristics of target enzymes involved in the biotransformation can play decisive roles in biodegradation efficiencies. Further degradation studies using bacteria, and fungi/their enzymes

are recommended to elucidate the connection between chemical structures of EOCs and their biodegradation efficiencies.

3. Microbes and their enzymes involved in cometabolism/metabolism of EOCs

Many lab and field-based studies have emphasized that the composition of biomass (i.e. microbial community structure) affects directly the biodegradation of EOCs in both natural and engineered systems like WWTPs. The changes in environmental conditions (such as pH, temperature, SRT, and concentration of growth substrates or target EOCs) can directly or indirectly influence in the composition of microbial population and their enzymes involved in biodegradation of EOCs. Consequently, biodegradation efficiencies and biodegradation pathways of EOCs are also affected. It is widely accepted that MBR systems offer several advantages over CAS systems in biodegradation of many EOCs, particularly for persistent EOCs. The reasons for the higher biodegradation efficiencies is suggested to be due to more diverse microbial community in activated sludge in MBR system compared to that in CAS system (Joss et al., 2006; Fernandez-Fontaina et al., 2012; Helbling et al., 2012). This may be true as MBR systems allow enriching slow growing microbes (such as autotrophic ammonia oxidizers) leading to changes in the composition of biomass (i.e. ratio between autotrophs and heterotrophs). Higher nitrifying activity of activated sludge in MBR is noted (Kimura et al., 2007; Fernandez-Fontaina et al., 2012; Helbling et al., 2012). Previous studies found that autotrophic ammonia oxidizers (AOB and/or AOA) could cometabolize a vast variety of EOCs. Interestingly, several previous studies demonstrated that heterotrophs are more favorable in biotransformation of biodegradable EOCs, such as ibuprofen, acetaminophen, methyl paraben, galaxolide, and caffeine than autotrophic nitifiers (Oppenheimer et al., 2007; Cajthaml et al., 2009; Tran et al., 2009; Larcher and Yargeau, 2013). The question is then arisen: which microbial group (heterotrophs or autotrophs) is mainly involved in the biodegradation of EOCs. In this review, we have just focused on discussing the roles of autotrophic ammonia oxidizers and heterotrophic bacteria/fungi and their relevant enzymes involved in cometabolism and/or metabolism of EOCs.

3.1. Nitrification and autotrophic ammonia oxidizers

A natural process considered for biodegradation of EOCs, for many years is nitrification (Arp et al., 2001; Shi et al., 2004; Yi and Harper, 2007; Roh et al., 2009; Tran et al., 2009; McAdam et al., 2010; Helbling et al., 2012). Nitrification is the biological oxidation of ammonium to nitrite and nitrate, which initiate the removal of reduced nitrogen compounds in the nitrogen cycle. In engineered systems, nitrification often takes place during biological wastewater treatment processes and is part of the inorganic nutrient elimination chain. Several groups of microorganisms are involved in the two-step process, including AOA, AOB, and nitrite-oxidizing bacteria (NOB) (Leininger et al., 2006; Gao et al., 2013). In the first step of nitrification process, both AOA and AOB are the key ammonia-oxidizers to convert NH_4^+ to NO_2^-. In the second step, NOB oxidizes NO_2^- to NO_3^-. Although some heterotrophic bacteria/fungi and autotrophic annammox bacteria (Suneethi and Joseph, 2011; Zhang et al., 2012) can also oxidize ammonia to nitrite, AOA and AOB are reported to be the main contributors for environmental ammonia oxidation in both natural and engineered systems (Leininger et al., 2006; Gao et al., 2013; Zeng et al., 2013). Leininger et al. (2006) reported that AOA predominate among ammonia-oxidzing prokaryotes in soils. Recently, Helbling et al. (2012) investigated the contribution of AOA and AOB in the ammonia oxidation in WWTPs using the corresponding specific primers for *amo*A genes in AOA and AOB. The results found that both bacterial and archael *amo*A were detected at significant levels in activated sludge systems in WWTPs compared to the negative control in all samples, indicating that both bacterial and archael *amo*A genes are abundant in WWTPs. It has been found that there is a significant association of relative abundances of archael *amo*A genes with ammonia oxidation rate in WWTPs, whereas no significant association was identified between bacterial *amo*A genes and ammonia oxidation rate (Helbling et al., 2012). Taken together, the results suggested that archael *amo*A is an important contributor to ammonia oxidation in WWTPs, as has been shown for soil/agriculture composting communities (Leininger et al., 2006; Sarmah and Northcott, 2008; Zeng et al., 2013). To date, the bacteria responsible for the nitrification have been widely known to belong to the genera *Nitrosomonas, Nitrosopira* (*β-Proteobacteria*), and *Nitrosococcus* (*γ-Proteobacteria*) (Bothe et al., 2000). In general, AOB and AOA are obligatory chemolithoautotrophs, but some of them can use organic compounds (i.e. acetate and pyruvate) for mixotrophic growth (Bothe et al., 2000).

Previous studies showed that many toxic/recalcitrant organic pollutants, such as halogenated hydrocarbons (Arp et al., 2001) and EOCs (Vader et al., 2000; Shi et al., 2004; Yi and Harper, 2007; De Gusseme et al., 2009; Tran et al., 2009; Khunjar et al., 2011; Fernandez-Fontaina et al., 2012; Helbling et al., 2012) can be effectively biodegraded under nitrification. There is circumstantial evidence linking nitrifying bacteria to a unique capability to biologically degrade EOCs. It was demonstrated that AOB and/or AOA can oxidize these recalcitrant pollutants in the presence of ammonia (Helbling et al., 2012), which plays as a growth substrate to induce enzyme AMO and supply reductant that is critically needed for the oxidative cometabolism of these pollutants.

3.2. Role of ammonia monooxygenase in cometabolism of EOCs

In autotrophic ammonia oxidizers, two key enzymes are involved in energy conservation during the oxidation process of ammonia, including AMO and hydroxylamine oxidoreductase (HAO). In vivo both enzymes are codependent since they generate the substrate and electrons, respectively, for each other. It is obvious that AMO catalyzes the oxidation of ammonia to hydroxylamine (NH_2OH). The two electrons required in this reaction are derived from the oxidation of NH_2OH to nitrite by HAO. Thus, one of the oxygen atoms in nitrite comes from O_2 and the other one derives from H_2O. Like many monooxygenases, AMO has a remarkably board substrate range and is capable of oxidizing a large variety of organic pollutants, such as methane and longer alkanes, alkenes, halogenated and aromatic hydrocarbons (Arp et al., 2001) and numerous EOCs (Vader et al., 2000; Shi et al., 2004; Yi and Harper, 2007; De Gusseme et al., 2009; Roh et al., 2009; Tran et al., 2009; McAdam et al., 2010; Khunjar et al., 2011; Helbling et al., 2012). Previous studies found that AMO is consisted of three subunits, α, β, and γ, also known as AMO-A, AMO-B, and AMO-C with different sizes and structures and arrangements within the membrane/periplasmic space of the cells (Rotthauwe et al., 1997; Bothe et al., 2000). It has been reported that the three subunits of AMO from autotrophic AOB are encoded by the corresponding genes: *amo*A, *amo*B, and*amo*C of the *amo* operon. These genes were cloned and sequenced (Bothe et al., 2000; Arp et al., 2001).

It was demonstrated that the active site of AMO contains metal ions such as copper or iron ions (Gilch et al., 2010). The metal content of AMO has still been controversial; however, the active site of AMO is generally believed to contain copper ions (Yi et al., 2006; Yi and Harper, 2007; Gilch et al., 2010). It has also been suggested that an active site of AMO includes two face; one face of the AMO contains an active site with oxygen-activating region, and the other has a hydrophobic pocket. Normally, organic pollutants like EOCs may be positioned by the hydrophobic pocket for easy

reaction with oxygen-activating site (Vannelli et al., 1990; Yi and Harper, 2007). Yi et al. (2006) found that both NH_4^+ and EE2 are simultaneously oxidized by an AMO-containing extract. Similar to the oxidation of primary substrate (ammonia), the oxidation of nongrowth substrates (such as EOCs) by AMO also requires reductant and electrons. The oxidation of NH_2OH by HAO is generated NO_2^- and 4 electrons. Two out of these 4 electrons enter a catalytic cycle involving a binuclear copper site (Cu^{2+}–Cu^{2+}) located in the active site of AMO. Yi and Harper (2007) suggested that under aerobic condition, oxygen will react to convert (Cu^{2+}–Cu^{2+}) into (Cu^{+}–Cu^{+}) while the oxygen remains bound as peroxide ion (O_2^-). This oxygenerated form of AMO then reacts with nongrowth substrates like EOC to produce a binuclear copper site (Cu^{2+}–Cu^{2+}) form and EOC degradation products.

Arp et al. (2001) indicated that nongrowth substrates (EOCs) themselve sometimes can inhibit AMO, while degradation products of them also can cause extensive cell damage. Moreover, there may be a certain competition between the growth substrate (NH_4^+) and nongrowth substrates (e.g. EOCs) for the active site of AMO resulting in decreased oxidation rates of both two kinds of substrates. In the case of noncompetitive inhibition, NH_4^+ and EOCs bind to different active sites on the AMO and result in decreased overall rate of oxidation.

However, AMO has not been purified with sustained activity until now. The only purification of an AMO as active enzyme was reported from heterotrophic nitrifier *Paracoccus denitrificans*, but the cell-free preparations loss enzymatic activity within hours (Arp et al., 2001). As a result, almost kenetic studies for AMO have been often based on whole-cell experiments. Several studies reported the relationship between ammonium oxidation and removal of EOCs (Shi et al., 2004; Yi and Harper, 2007; Roh et al., 2009; Tran et al., 2009; Helbling et al., 2012). Shi et al. (2004) found that the biodegradation rate of EE2 was 1.5 μM/gVSS/h at a nitrification rate of 0.1 mM NH_4–N/gVSS/h. In another study, Yi and Harper (2007) characterized the relationship between nitrification rate and EE2 transformation rate and observed that a linear relationship between ammonium oxidation and EE2 biodegradation rates. The biodegradation rate of EE2 increased from 1.1 to 4.1 μM EE2/gVSS/h, while the ammonium oxidation rate enhanced from 0.3 to 5.1 mM NH_4/gVSS/h. These data taken together indicate a linear between relationship between nitrification and biodegradation rate of EE2 in enriched nitrifier culture (Yi and Harper, 2007). Helbling et al. (2012) found that ammonia oxidation associates with biodegradation rates of EE2, and only archael *amo*A genes are associated with both ammonia oxidation and biodegradation rates of EOCs. They also released that either AMO or acetylene-inhibited monooxygenase significantly contributes to the biotransformation of EOC (Helbling et al., 2012). This implies that monooxygenases from AOA can play an important role in the biodegradation of EOCs.

The results suggest that AMO enzymes produced from both AOB and AOA play a key role in the cometabolic biodegradation of EOCs. However, the contributions of each kind of AMO (i.e. bacterial or archael AMO) in the cometabolic biodegradation of EOCs have not been elucidated yet. Hence, further studies to differentiate the contribution of bacterial and archael AMO in the cometabolism by using a specific inhibitor of ammonia oxidation in AOB and AOA would be critically needed. Moreover, further investigations on the roles of other AMO subunits (such as AMO-B and AMO-C) in the biodegradation of EOCs is necessary.

3.3. Role of heterotrophic bacteria in cometabolism/metabolism of EOCs

As discussed above, nitrification and autotrophic ammonia oxidizers probably play important roles in the biodegradation of EOCs. So far, the observation of enhanced biodegradation of EOCs in nitrifying activated sludge/enriched nitrifier culture is suggested to be attributed to AMO activity and autotrophic AOB and/or AOA.

To elucidate the contribution of autotrophic ammonia oxidizers and heterotrophic bacteria to the biodegradation of EOCs, previous studies have used allylthiourea (ATU) as a specific AMO inhibitor to suppress ammonia oxidizing activity of autotrophic ammonia oxidizers (Shi et al., 2004; Roh et al., 2009; Tran et al., 2009; Khunjar et al., 2011). Interestingly, some EOCs are significantly biodegraded in the presence of ATU. Tran et al. (2009) found that high degradation of ibuprofen and partial degradation of other pharmaceuticals were observed during the biodegradation in the presence of ATU (10 mg/L), while no nitrification was noted. This implies that biodegradation of these pharmaceuticals is probably attributed to the activities from heterotrophic microorganisms. Similarly, Roh et al. (2009) also reported that complete degradation of bisphenol A and ibuprofen was observed in the presence of AMO inhibitor. They also demonstrated that pure culture *Nitrosomonas europea* – a typical autotrophic ammonia oxidizing bacterium was unable to degrade ibuprofen (Roh et al., 2009). This result indicates that non-ammonia oxidizers also play an important role in biodegradation of EOCs, particularly with regard to biodegradable EOCs (i.e. ibuprofen) in biological wastewater treatment processes.

More recently, Khunjar et al. (2011) assessed the relative contribution of AOBs and heterotrophs in EE2 and trimethoprim (TMP) biotransformation using both pure culture and mixed culture experiments. Those experiment results showed that AOBs could biodegrade EE2 but not TMP, whereas heterotrophs mineralized EE2, transformed TMP, and biomineralized EE2-derived metabolites generated from autotrophic AOBs. Interestingly, it was found that autotrophic AOBs and heterotrophic microbe could cooperatively enhance the reliability of treatment systems where efficient removal of EE2 is desired (Khunjar et al., 2011). Larcher and Yargeau (2013) have also examined the biodegradability of EE2 using the seven pure heterotrophic bacterial cultures (i.e. *Bacillus subtilis, Pseudomonas aeriginosa, Pseudomonas putida, Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus rhodochrous, and Rhodococcus zopfii*). As expected, all these seven heterotrophic bacteria are capable of degrading EE2. Particularly, *R. rhodochrous* completely degraded EE2 after 48 h of incubation. Other strains achieved 21–61% EE2 removal. These observations help explain the variable EE2 removals by different activated sludge samples since there are the differences in microbial community structure among activated sludge systems. Additionally, these results allow confirming that the biodegradation of EE2 or similar compounds in biological wastewater treatment can be attributed to the activity of heterotrophs and may not rely solely on nitrifying bacteria as previous suggested in the literature (Yi and Harper, 2007; De Gusseme et al., 2009; Roh et al., 2009; Khunjar et al., 2011)

3.4. Roles of heterotrophic fungi and their extracellular ligninolytic enzymes in cometabolism/metabolism of EOCs

It has been known that some white rot fungi show catabolic pathways that are distinct from those of aerobic bacteria, thus allowing for the catabolic breakdown of highly persistent organic pollutants, such as halogenated aromatics, PAHs, pesticides, and industrial wastes in a variety of environmental compartments (Cabana et al., 2007). The action of white rot fungi is attributed to extracellular ligninolytic enzymes (i.e. oxidases and peroxidases), such as lignin peroxidase (LiP), and manganese peroxidase (MnP), and laccase (Lac). A white rot fungus can produce one or more extracellular ligninolytic enzymes whose low substrate specificity, which makes them suitable for degrading a broad spectrum of organic pollutants (Cabana et al., 2007; Cajthaml et al., 2009; Tran et al., 2010; Yang et al., 2013b). Their superiority in many instances

over bacteria is attributed to the extracellular nature of ligninolytic enzymes. Furthermore, white rot fungi can secrete low molecular weight mediators that allow enlarging the oxidation of a broad range of pollutants. As fungi are eukaryotes, they allow to be operated over wide ranges of temperatures and pHs. Due to the filamentous nature of white rot fungal growth by hyphal extension in soils and other environmental compartments, which allows fungi reach organic pollutants better than that by bacteria.

Although white rot fungi are able to breakdown many recalcitrant organic pollutants including EOCs, they are seldom selected as biological agent of choice for biodegradation of EOCs in WWTPs (Cajthaml et al., 2009). One of the drawbacks in the application of white rot fungi in real WWTPs is the contamination of other microorganisms (i.e. bacteria) in the wastewater where sterilization may not be feasible. The contamination may hinder the biodegradation of pollutants by white rot fungi. Recent studies have developed novel fungal bioreactors and have optimized operational conditions for enhancing the elimination of EOCs (Zhang and Geissen, 2012; Yang et al., 2013a) under non-sterile conditions. Zhang and Geissen (2012) reported that high elimination of carbamazepine (60–80%) was achieved in a novel plate bioreactor. Similarly, Yang et al. (2013a) have demonstrated that relatively stable removal of bisphenol A (80–90%) and diclofenac (≈55%) was observed in a novel fungal-based bioreactor when applying a hydraulic retention time (HRT) of 2 days at the bisphenol A and diclofenac loadings of 475 ± 25 and 345 ± 112 μg/L d, respectively.

However, the application of fungal culture in these bioreactors still shows drawbacks as the operation at HRT of greater than one day. Other reasons hinder the application of white rot fungi in WWTPs, such as the filamentous nature can clog biological wastewater treatment facilities, and mechanical homogenization of fungi treated matrices inhibit the growth of fungal mycelia (Harms et al., 2011). Under practical point of view, the potential use of extracellular ligninolytic enzymes (e.g. LiP, MnP, and Lac) for cleaning up organic pollutants in general and EOCs in particular appears as a solution to overcome the above drawbacks. It was found that the outstanding extracellular ligninolytic enzymes, such as LiP, MnP and Lac, appear low substrate specificity and strong oxidative ability while enabling to oxidize a large variety of highly persistent organic compounds (Cabana et al., 2007; Yang et al., 2013b). Recently, numerous studies have examined the elimination of EOCs using either crude culture extract or purified enzymes (Cabana et al., 2009; Wen et al., 2009; Tran et al., 2010; Suda et al., 2012). It is obvious that either purified or commercial laccase can efficiently degraded most phenolic EOCs without any assistance of mediators, such as bisphenol A, triclosan, E1, E2, E3, and EE2 (Auriol et al., 2007; Cabana et al., 2009). Interestingly, some nonphenolic EOCs (i.e. diclofenac and indomethacin) can be oxidized by laccase alone (Tran et al., 2010). However, EOCs with complex structures like carbamazepine and clofibric acid are resistant to laccase even in the presence of redox mediators (Tran et al., 2010). In addition to laccase, other ligninolytic enzymes (such as MnP and LiP) were also demonstrated to oxidize a variety of EOCs (Cajthaml et al., 2009; Eibes et al., 2011; Yang et al., 2013b), but both LiP and MnP enzymes are unstable in environmental conditions. Thus, this is one of the drawbacks in the use of them for real wastewater treatment systems.

Obviously, laccase offers some advantages over LiP and MnP in the application to wastewater treatment. For example, enzymatic activity of laccase is relatively stable under a wide range of operating conditions (e.g. pH and temperature). In particular, laccase does not require cofactors in reaction process. However, it is unfortunate that laccase are not effective in the oxidation of un-functionalized aromatic compounds and nonphenolic structures with their high redox potentials without having the assistance of redox mediators. Thus, this is also a drawback for application of laccase in elimination of nonphenolic EOCs. Therefore, the addition of redox mediators in laccase reaction is critically needed, allowing laccase overcome kinetic barrier to oxidize the many EOCs with redox potential higher than laccase solution.

4. Potential strategies for enhancing the biodegradation of EOCs

As discussed above, EOCs are often present in both natural and engineered systems at trace concentration levels, and many of them are toxic and/or resistant to microbial growth. Therefore, in many cases EOCs cannot serve as the sole carbon or energy sources to maintain biomass and induce the expression of relevant enzymes and/or cofactors for the assimilation by microbes. This suggests that the main biodegradation pathway of EOCs is probably attributed to microbial cometabolic activities via metabolizing a growth substrate or another utilizable compound. The obligatory presence of a growth substrate is critically needed, which helps to maintain biomass and produce the relevant enzymes/cofactors involved in the cometabolic biodegradation of EOC. Therefore, one of the proposed strategies for enhanced cometabolic biodegradation EOCs is the maintenance of the growth substrate during cometabolism of EOCs.

4.1. Enhanced cometabolism of EOCs by maintaining the presence of growth substrates

It has been known that two functional microbial groups (heterotrophic and autotrophic microbes) are mainly involved in the biodegradation of EOCs in both natural and engineered systems. Heterotrophic microbes can use both metabolic and cometabolic activities in the biodegradation, whereas autotrophic microbes only biodegrade EOCs via metabolizing other inorganic substrates. This has been noted in autotrophic ammonia oxidizers, they use inorganic salts (i.e. HCO_3^-, CO_2, and NH_4^+) as their growth substrates for building up microbial cells and inducing the non-specific enzymes, such as AMO that is known to catalyze the oxidation of a large variety of organic pollutants (e.g. halogenated aromatics, PAHs, as well as EOCs). The biodegradation efficiency of EOCs suggested to be dependent on the amount and characteristics of relevant enzymes involved in biodegradation (Tran et al., 2009; Helbling et al., 2012). The amount of relevant biodegradative enzymes induced may rely on the concentration of a growth substrate/inducer during cometabolism process. To enhance the cometabolic activities of microbes in biodegrading EOCs, the presence of relevant growth substrates for each functional microbial group must be ensured. Numerous studies reported that higher cometabolic biodegradation efficiencies of EOCs were noted with nitrifying activated sludge/enriched nitrifier culture at high ammonium loading rates (Yi and Harper, 2007; Tran et al., 2009; Fernandez-Fontaina et al., 2012; Helbling et al., 2012). Fortunately, growth substrates are available in engineered systems like wastewater treatment plants. Therefore, there is no need to add the external substrates (such as conventional organic substrates or ammonium salts), but the frequent monitoring the concentrations of macropollutants (i.e. NH_4^+, BOD, COD, etc.), biomass concentration are critically needed to ensure that organic oxidation and nitrification is being taken place during the biodegradation course of EOCs.

For the real WWTPs, Joss et al. (2004) postulated that low biodegradation efficiencies of EOCs was usually noted at higher organic substrate loading (i.e. at high F/M). This may be attributed to preferential substrate selection (Joss et al., 2004; McAdam et al., 2010). However, this may be the case. It is obvious that high F/M ratios are usually associated with short SRTs of activated sludge system. The microbes in activated sludge system do not have

enough time to exposure to an EOC and develop the relevant enzymes/cofactors for the degradation of this compound. This is a reason explain why low biodegradation efficiencies are observed at higher *F*/*M* ratios. In addition, there may be the differences in microbial community structure of the activated sludge under low and high organic substrate loading conditions. The nature of organic substrate loading may also affect the diversity of microbes in the activated sludge. Indeed, Racz et al. (2012) have recently demonstrated that there are the more diverse heterotrophic and autotrophic ammonia oxidizing bacterial population in the peptone fed activated sludge system compared to the glucose fed activated sludge system. As a consequence, higher removal efficiencies of estrogens, particularly for EE2, were observed in the peptone fed activated sludge.

In the natural systems, both EOCs and other growth substrates are usually present at low concentrations that may not support biomass growth and induce the relevant degradative enzymes. It is therefore required to add the external substrates to promote the biodegradation of target EOCs. The different growth substrate sources added can influence on cometabolic degradation efficiency as well as the formation of by-products.

Zhong et al. (2007) investigated the influence of different growth substrate sources on the cometabolic degradation of PAHs by a heterotrophic bacterial strain (*Sphingomonas* sp. Strain PheB4). Results showed that a more rapid cometabolic degradation rate of single or mixed non-growth substrates (PAHs) was observed in the medium containing peptone, beef extract, and glucose as the growth substrates compared to that in the medium containing mineral salts with 10 mg/L phenanthrene as the sole carbon source. In addition, Zhong et al. (2007) also reported cometabolic pathways of mixed PAHs were more diverse in culture cultivated in the medium peptone, beef extract, and glucose than that in culture cultivated in the medium with only phenanthrene.

In a recent study, Liu et al. (2013) also demonstrated that when adding different utilizable substrates (i.e. sucrose and succinate) in biodegradation experiments of imidacloprid (IMI)-an insecticide, they obtained different biodegradation pathways and found that the main cometabolic biodegradation product was 5-hydroxyl IMI in the presence of sucrose as a growth substrate. This degradation product could not be further cometabolic dehydrated to convert into olefin IMI, whereas this degradation product was further cometabolized to olefin IMI. It is interesting to note that IMI could not be transformed to 5-hydroxy IMI using the cell-free extract of the same strain (*Stenotrophomonas maltophilia* CGMCC 1.1788) in the presence of either sucrose or succinate without the external addition of NADPH or NADH to reaction mixture. Therefore, sucrose and succinate in that study not only provided carbon source or energy to the cell growth, but also produced the relevant enzymes and cofactors (i.e. NADPH or NADH) for the cometabolic biodegradation of IMI. In the natural water treatment systems (such as riverbank filtration and aquifer recharge sites), the structure and function of microbial community are mainly affected by the concentrations of different biodegradable dissolved organic carbon (BDOC) concentrations (Li et al., 2013).

Taken together, all the direct/indirect evidences suggest that biodegradation kinetics of EOCs is dependent on the nature of microbial community structure (i.e. ratio between autotrophs and heterotrophs), organic oxidation and nitrification rates of macropollutants.

4.2. Enhanced biodegradation EOCs via controlling the microbial community structure

It is widely postulated that biodegradation of EOCs is attributed to cometabolic activities of autotrophic microbes and cometabolic/metabolic activities of heterotrophic microbes. Thus, biodegradation of EOCs depends mainly on the characteristics and amount of the relevant degradative enzymes, which are produced from the relevant microbes in the environment. For example, autotrophic ammonia oxidizers, the typical autotrophic microbes in the environment, are able to cometabolize EOCs via the non-specific enzyme AMO, while heterotrophic microbes are also capable of degrading a large variety of EOCs via their various monooxygenases and/or dioxygenases (Arp et al., 2001; Khunjar et al., 2011). Due to the difference in enzyme systems, the biodegradation rates of EOCs by autotrophic and heterotrophic microbes may be different. Khunjar et al. (2011) have demonstrated that biodegradation rate of EE2 by AOB is five time faster than that by heterotrophic bacteria. In addition, it has been found that there may be the cooperation between AOB and heterotrophs in enhancing the degradation of EOCs (Rotthauwe et al., 1997; Tran et al., 2009; Khunjar et al., 2011). In wastewater treatment processes, the use MBRs in place of CAS systems is simple to control the biomass composition. The application of MBRs in wastewater treatment allows minimizing the washout of microbes, particularly with regard to slow growing microbes like autotrophic ammonia oxidizers. Microbial community in MBR activated sludge system is controlled using the changes of SRT. Additionally, the microbial community structure can be controlled via changes of HRT and the addition of external growth substrates as required. One proposed possibility to restructure microbial community of activated sludge is the addition of external target pure strains or enriched cultures, which can be directly involved in the biodegradation of EOCs. Particularly, it has been demonstrated that the key driver for biodegradation of EOCs in the subsurface environment (i.e. groundwater, aquifer recharge, riverbank filtration, etc.) is the composition of the microbial community (Li et al., 2013). In short, more insight into the contributions of heterotrophs and autotrophs involved in the biodegradation of EOCs is of critical importance in design an effective natural or engineered treatment processes for biodegradation of EOCs.

4.3. Combination of fungal laccase and bacterial oxygenases in biodegradation of EOCs

The extracellular laccase from white-rot fungi appear low substrate specificity and have strong oxidative/reductive ability to transform a large variety of persistent phenolic compounds (Tran et al., 2010; Yang et al., 2013b), which may be resistant to bacterial growth. However, laccase is challenging to attack un-functionalized aromatic compounds or EOCs with nonphenolic structure with no the assistance of synthetic or natural mediators present in the environment (Tran et al., 2010; Yang et al., 2013b). Fortunately, bacterial monooxygenase and dioxygenase have been demonstrated to be able to hydroxylate many un-functionalized aromatic compounds and nonphenolic pollutants like most EOCs (Arp et al., 2001; Khunjar et al., 2011). Nevertheless, biodegradation products derived from bacterial hydroxylation by oxygenases have been known to be more resistant to bacteria and can inhibit the biotransformation of bacteria (Haiss and Kummerer, 2006; Khunjar et al., 2011; Liu et al., 2013). In contrast, by-products produced from bacterial hydroxylation usually contain phenolic groups, which may be favourable for fungal laccase attack. Hence, the combination of fungal laccase and bacterial culture may have an advantage in biodegrading EOCs in both engineered and natural systems. However, it should be noted that all the proposed strategies discussed above are only suggestions. Further studies on these strategies are required to verify the feasibility of the proposed strategies.

5. Conclusion

Biodegradation of EOCs is attributed to cometabolic and/or metabolic activities of microorganisms. Autotrophic microbes show cometabolic activities in biodegrading EOCs, while heterotrophs degrade EOCs via cometabolism and/or metabolism mechanisms, depending upon the nature of target EOCs and their bioavailability in the environment. Autotrophic ammonia oxidizers and nitrification play a key role in cometabolizing EOCs, particularly for slowly biodegradable compounds. Heterotrophs play other important roles in the biodegradation of EOCs, particularly with regard to fast biodegradable EOCs. A better understanding mechanisms and factors controlling the biodegradation rates are critically needed to design bioreactors or natural systems for enhancing biodegradation of EOCs.

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