Haloacetic acids (HAAs) are byproducts formed upon the addition of chlorine to water for disinfection purposes. HAAs pose a potential human health risk and their concentrations in drinking water are regulated in many countries. HAAs can be biodegraded under aerobic conditions through a hydrolysis-oxidation pathway. There is experimental and modeling evidence that HAA biodegradation is a potentially effective approach for drinking water treatment in order to reduce HAA concentrations in drinking water and comply with regulations. HAA-degrading bacteria have been isolated from different environments such as soil, activated sludge and drinking water systems. There is, however, very little overlap in the species obtained from water systems and those from other environments, suggesting that the specific conditions in water systems (oligotrophic environment, chlorine residual) select for unique bacteria. As more information becomes available about the diversity of HAA-degrading bacteria and the halocarboxylic acid dehalogenase genes that are involved in the HAA biodegradation pathway, researchers will be able to develop nucleic acid-based quantification methods for determining the HAA-degrading biomass in drinking water systems. This would allow water utilities to predict and possibly control the rate of HAA biodegradation taking place in their systems.

Key words: haloacetic acid, disinfection byproduct, halocarboxylic acid dehalogenase, drinking water systems, maximum contaminant level.

HALOACETIC ACIDS (HAAs): PREVALENCE AND CONCERNS

Haloacetic acids (HAAs) are ubiquitous environmental contaminants and their presence in the environment is due both to natural processes and to human activities. HAAs are naturally formed through the biodegradation of organic matter in forest soil (1) and the photodegradation of some herbicides (2). However, much higher amounts of HAAs are released in the environment by human activities. Thus, HAAs were used as food preservatives (3), trichloroacetic acid (TCAA) was used

*Corresponding author (E-mail: grigo009@umn.edu; Phone: (612)625-3581; Fax: (612)626-7750).
as herbicide (4) and monochloroacetic acid (MCAA) is still used as precursor for the synthesis of various chemicals (3). High quantities of HAAs are produced from industrial chlorination processes such as pulp bleaching (5) and the chlorination of drinking water and wastewater (6, 7).

HAAs have been detected in many places such as soil (8, 9), conifer needles and lichens (10), snow and ice in Antarctica (11), fog and rainwater (3, 12), sea water (13), lake water (14), wastewater (7) and drinking water (6). Terrestrial concentrations of HAAs range from 1.4 to 120 µg/kg of dry weight in soils (9) and from 1 to 180 µg/kg of dry weight in conifer needles (10). HAA concentrations in aquatic environments range from parts per million (ppm) in wastewater (7) to parts per billion (ppb) in drinking water (6), and to parts per trillion (ppt) in surface waters (15).

HAAs are generally phytotoxic (16, 17) and toxic to green algae (18), while some HAAs (e.g., dichloroacetic acid (DCAA) and TCAA) have hepatocarcinogenic potential (19). HAAs, however, are not phytotoxic at typical environmental concentrations but their effect on aquatic life is poorly understood, as HAAs often occur as mixtures that could enhance their toxicity (20).

HAAs IN DRINKING WATER

HAAs represent the second most prominent class of halogenated disinfection byproducts (DBPs), after trihalomethanes, in drinking water (21). HAAs are formed in water treatment plants and distribution systems due to reactions between free chlorine, added for disinfection purposes, and the organic matter that is present in natural waters (22, 23). HAAs can also form in the distribution systems as a result of the hydrolysis of other DBPs (e.g., haloacetonitriles) (24). Several researchers have reported, however, that HAA concentrations decrease along the distribution systems (25–27). Due to the potential adverse health effects of HAA consumption (28–30), the United States Environmental Protection Agency (USEPA) began regulating HAAs in tap water in 1998 under the Stage 1 Disinfectants/DBPs Rule (D/DBPR) (31). At present, the maximum contaminant level (MCL) in the United States is 60 µg/L for the sum of five HAAs (HAA₅: MCAA, DCAA, TCAA, monobromoacetic acid (MBAA) and dibromoacetic acid (DBAA)). The European Union, on the other hand, does not regulate HAAs (32). Studies done prior to the implementation of the USEPA D/DBPR showed that HAA₅ concentrations in finished waters were sometimes higher than the proposed MCL (33).

DEGRADATION OF HAAs

HAA degradation in aquatic environments and soils is typically attributed to microbial activity (34–36). Microbial degradation of HAAs was also detected in water treatment systems such as biologically-active filters (37–41) and in water
distribution systems (42–44). HAAs are also capable of undergoing abiotic degradation reactions such as reductive dehalogenation in the presence of zero valent iron (45, 46) or iron minerals (47) and hydrolysis (i.e., decarboxylation) (48). The abiotic degradation reactions, however, are not likely to be important because either the reactions are slow at environmental pH and temperature values (e.g., the half-life of TCAA at room temperature and neutral pH is 2,190 days according to Zhang and Minear, 2002) or they require a specific combination of conditions (e.g., rapid reductive dehalogenation requires a potent reductant, such as zero valent iron and trihalogenated HAAs that contain one or more bromine atoms, according to Hozalski et al., 2001). Thus, biodegradation is likely to be a more rapid and efficient degradation process than abiotic processes, which could be applied as a way of reducing HAA concentrations in drinking water.

**Aerobic biodegradation of HAAs.** HAAs are biodegraded aerobically via a hydrolysis-oxidation pathway (Fig. 1). This pathway involves an initial substitutive dehalogenation step in which the halogen atom is replaced by a hydroxyl group (Fig. 1) (35). This step is catalyzed by enzymes called halocarboxylic acid dehalogenases.

![Fig. 1. – Potential biodegradation pathway for MCAA (adapted from Ellis et al., 2001). The resulting biodegradation intermediate, glycolic acid, enters into the general metabolism and gets readily mineralized.](image)

Information concerning the aerobic biodegradation of HAAs (i.e., pathway, kinetics and the genes involved) largely comes from work with enrichment cultures and isolates obtained from soil and wastewater environments and from studies with other α-halocarboxylic species like monochloro- and dichloropropionic acids (MCPA and DCPA). HAA-degrading bacterial strains such as *Burkholderia* sp., *Xanthobacter* sp., *Sphingomonas* sp. and *Chryseobacterium* sp. were isolated from soil and activated sludge (49, 50). Recent studies by Zhang et al. (43, 44) revealed some of the HAA degraders from drinking water systems, including previously unknown HAA degraders, such as *Afipia* spp. Marchesi and Weightman (51) demonstrated that the α-halocarboxylic degraders isolated by enrichment cultures are not necessarily the environmentally-relevant organisms due to the culturing bias (52). Kerr and Marchesi (53) further showed that the culturing conditions (i.e., temperature, pH, oxygen supply and choice of batch cultures or direct plating) are
the key elements in isolating novel bacteria, which could lead to the future isolation of a broader range of HAA degraders.

McRae et al. (50) was the first group to perform batch experiments to evaluate the HAA biodegradation kinetics at the low HAA concentrations similar to those found in surface waters and drinking water systems (<< 1 mg/L). MCAA and TCAA degradation followed pseudo-first order reaction kinetic models with MCAA being degraded faster than TCAA. The bacteria used in these experiments were enriched from a wastewater activated sludge inoculum where HAA concentrations are generally higher than in drinking water distribution systems. Recently, Zhang et al. (43, 44) showed that isolates from drinking water systems have a wide range of HAA-degrading abilities at drinking water HAA concentrations, with the faster DCAA degrader, an *Afipia* species, having a 4-fold faster DCAA biodegradation rate than the slowest DCAA degrader, a *Methylobacterium* species. The drinking water isolates were able to degrade DCAA faster than MCAA and MCAA faster than TCAA. There was little overlap in the bacterial species isolated from wastewater and drinking water enrichment cultures, suggesting that drinking water distribution systems select for unique HAA-degrading bacteria, perhaps because of the oligotrophic conditions present therein (43, 44, 50).

**HALOCARBOXYLIC ACID DEHALOGENASES AND CORRESPONDING GENES**

Halocarboxylic acid dehalogenases catalyze the initial step in the biodegradation pathway of HAAs. Several genes encoding those enzymes have been sequenced and grouped into two phylogenetically unrelated groups, called *dehI* and *dehII* (49). These two groups of *deh* genes are characterized by high intra-group genetic diversity and are divided into several phylogenetic subgroups (Figures 2 & 3) (49). Studies on the genetic localization of the *deh* genes from different bacterial strains showed that many of these genes are found on the chromosome (54–57), which explains their high genetic variability. Some of the *deh* genes, however, are carried by plasmids (58–61) and can be found on transposable genetic elements (58, 62).

The genetic diversity between the two *deh* groups is also reflected in the mode of action of the corresponding enzymes on the substrate. The dehalogenases from group II proceed via a nucleophilic attack resulting in a covalent ester-enzyme link between an aspartate residue and the dechlorinated substrate (63), while the dehalogenases from group I do not form any ester bond with the substrate (64). Additionally, the two groups of halocarboxylic acid dehalogenases have stereospecificity towards optically active substrates such as 2-monochloropropionic acid (2MCPA) (49). Thus, the group I of halocarboxylic acid dehalogenases is active with both L- and D-isomers of 2MCPA, while the other group is only active with the L-isomer. Moreover, the dehalogenases from group II are members of the
haloacid dehalogenase (HAD) superfamily and are structurally more closely related to other enzymes like phosphatases and epoxidases (49).

Fig. 2. – Dendrogram illustrating the phylogenetic relationships between several group I deh sequences (adapted from Zhang et al., 2009a (43) and Hill et al., 1999). DNA sequences of ~ 270 bp were aligned with the DNAMAN version 7 software and the phylogenetic trees were constructed with the same program using the Jukes-Cantor algorithm (85). Bootstrap values are shown for nodes with > 50% probability of 500 replicates. The scale bar indicates an estimated change of 5%. A, B, C, and D are subdivisions based on nucleotide sequence identities that are higher than 55%.

Fig. 3. – Dendrogram illustrating the phylogenetic relationships between several group II deh sequences (adapted from Zhang et al., 2009a (43) and Hill et al., 1999). DNA sequences of ~ 420 bp were aligned with the DNAMAN version 7 software and the phylogenetic trees were constructed with the same program using the Jukes-Cantor algorithm (85). Bootstrap values are shown for nodes with > 50% probability of 500 replicates. The scale bar indicates an estimated change of 5%. A, B, C, and D are subdivisions based on nucleotide sequence identities that are higher than 55%.
Bacterial isolates carrying halocarboxylic acid dehalogenase genes can often metabolize a broad substrate range of mono-, di- and trihalogenated short chain carboxylic acids (43, 44, 53). Although there are no studies relating the presence of either dehI or dehII genes with the degradation of specific halocarboxylic acid substrates, there is some evidence to suggest that only the drinking water isolates having a dehI gene could degrade trihalogenated acetic acids (43). Also, organisms that possess both a dehI and dehII gene had a broad substrate range and were capable of biodegrading all chlorinated and/or brominated HAAs that were tested (i.e., up to 7 different HAAs) (43).

**DETECTION AND ENUMERATION OF HAA-DEGRADING BACTERIA IN DRINKING WATER SYSTEMS**

_Drinking water distribution systems as microbial “black boxes”_. Drinking water systems are intentionally harsh environments for microorganisms, in order to protect public health by inhibiting the survival of pathogens. This is achieved by maintaining high disinfectant residuals and very low nutrient concentrations (65–67). Nevertheless, many bacteria adapted to live in low nutrient conditions (68) and became resistant to the disinfectant residuals present in drinking water systems by growing in biofilms on the pipe walls (69–73). Bacterial densities on the pipe walls can be up to $10^7$–$10^8$ cells/cm$^2$ of pipe (69). Although tap water bacterial concentrations are generally very low (e.g., < 500 CFU/mL to comply with USEPA drinking water regulations), bacterial numbers can increase substantially (e.g., over two orders of magnitude) in the distribution systems at locations where chlorine residuals are very low (e.g., high residence time locations) (74, 75), due to a preference of bacterial cells to detach from surfaces (76). Information regarding the phylogenetic diversity of the bacteria found in drinking water systems has only recently started to appear in the literature, showing that Gram positive and α-, β- and γ-Proteobacteria are the predominant bacterial groups (77, 78). Microbial ecology data is still very scarce for drinking water systems and the general understanding about microbial life in such aquatic environments is very limited (79).

_Biologically active filters as controlled systems for HAA removal_. Sand and granular activated carbon (GAC) filters, which are frequently used in the process of drinking water treatment, can accumulate high densities of bacterial cells. For example, typical bacterial densities in biologically active GAC filters are between $10^7$ and $10^{10}$ cells/cm$^2$ of filter grain (80, 81). Moreover, if prechlorination is used in the water treatment process, generating high HAA concentrations, GAC filters are expected to allow the development of HAA-degrading bacteria because the residual chlorine is readily dissipated in the GAC filter (82). In fact, biodegradation of HAAs in biologically-active filters has been confirmed in pilot-scale experiments (40, 41) and full-scale monitoring studies (37, 38). In addition, such filters have the advantage
of controlling operating parameters (e.g., empty bed contact time and temperature), which can result in a better removal of HAAs (39).

**Enumerating HAA degraders in drinking water systems.** No precise information is currently available regarding the abundance and the diversity of HAA-degrading bacteria in drinking water systems. Zhang et al. (43, 44), however, obtained several HAA-degrading bacterial isolates from pipe wall and granular activated carbon biofilms. The isolation of those HAA-degrading bacteria from water systems was very difficult and time-consuming (43, 44). Moreover, it is possible that the obtained HAA-degrading isolates are not the environmentally relevant HAA degraders in drinking water systems, due to the cultivation bias (51, 52). Thus, more research is needed to find the predominant HAA degraders in drinking water systems, which could be used as indicators for quantifying the HAA-degrading biomass in these systems.

Leach et al. (83) attempted to obtain information on the abundance of HAA degraders in drinking water systems by developing a quantitative polymerase chain reaction (qPCR) method targeting the \textit{dehI} and \textit{dehII} gene groups in tap water samples. The authors showed that \textit{dehI} and \textit{dehII} genes could be amplified from various drinking water samples using the highly degenerate primers developed by Hill et al. (49). These degenerate primers, however, could not be used for quantification purposes due to nonspecific amplification. Thus, the authors developed specific \textit{deh} primers for qPCR using HAA degraders isolated from wastewater as targets. Those isolates carried only the \textit{dehII} gene and could be detected in drinking water samples only if an additional nested PCR step was used. Therefore, the qPCR method developed by Leach et al. (83) needs further optimization as more information about the presence of HAA degraders in drinking water systems becomes available.

**APPLICABILITY OF THE HAA BIODEGRADATION PROCESS FOR DRINKING WATER TREATMENT**

The biodegradation of HAAs is a potential useful process for decreasing HAA concentrations in drinking water systems. A recent study by Grigorescu and Hozalski (84) proposed a kinetic model for the estimation of HAA loss due to biodegradation in biologically-active filters and distribution systems. The authors showed that the quantity of HAA-degrading biomass is a critical parameter controlling HAA removal (Fig. 4). The authors, however, pointed out that HAA-degrading biomass in drinking water systems are not known because of the difficulty in obtaining biofilm samples, especially from distribution system pipes, and because of the lack of a rapid and reliable method for quantifying HAA degraders in the samples. It is therefore important to have more precise information about HAA-degrading bacteria levels in drinking water systems. Thus, more
research is needed concerning the identification and enumeration of major HAA degraders in drinking waters systems.

![Fig. 4. – Effect of HAA-degrading biomass density on the biodegradation of MCAA, DCAA and TCAA in drinking water distribution systems (A) and biologically activated filters (B) (adapted from Grigorescu and Hozalski, 2010) (84). The grey boxes represent HAA removals obtained with calculated HAA-degrading biomass densities based on the typical ranges of total bacterial cells in US drinking water systems (i.e., $10^4$–$10^6$ cells/cm$^2$ of pipe wall and $10^8$–$10^{10}$ cells/cm$^2$ of filter grain).]

CONCLUSIONS

There is concern over the potential deleterious health effects of chlorinated disinfection byproducts (DBPs), such as HAAs, in drinking water. As HAAs are easily biodegraded, modeling studies suggest that biodegradation could be exploited as a means to effectively remove HAAs during water treatment. In fact, biodegradation of HAAs in biologically-active filters has been confirmed in pilot-scale experiments and full-scale monitoring studies. Furthermore, HAA bio-degradation is likely the major loss process occurring in drinking water distribution systems. Although drinking water distribution systems are harsh environments for microorganisms, studies have shown that HAA degraders are present in such environments. Future research is needed to develop quantification methods for estimating the HAA-degrading biomass in drinking water systems to improve estimates of the HAA biodegradation potential in these systems.

REFERENCES


