Algae form brominated organic compounds in surface waters

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Introduction

Monitoring of organic halogen compounds, measured as adsorbable organic bromine (AOBr) revealed seasonal high concentrations of organic bromine compounds in a surface water (Lake Tegel, Berlin, Germany) *(1)*. Usually, in late summer, concentrations are up to five times higher than during the rest of the year. The AOBr of the lake inflows (throughout the year less then 6 µg/L) were always lower then those in the lake, which indicates a production of AOBr in the lake (*2*). A correlation of the AOBr and chlorophyll-a concentration (*1*) in the lake provides first evidence for the influence of phototrophic organisms.
The knowledge of the natural production of organohalogens is relatively recent. Up to now there are more then 3800 identified natural organohalogen compounds that have been detected in marine plants, animals, and bacteria and also in terrestrial plants, fungi, lichen, bacteria, insects, some higher animals, and humans (*3-9*). Halogenated organic compounds are commonly considered to be of anthropogenic origin; derived from e.g. pharmaceuticals, herbicides, fungicides, insecticides, flame retardants, intermediates in organic synthesis and solvents. Additionally they are also produced as by-products during industrial processes and by waste water and drinking water disinfection. Organohalogen compounds may be toxic, persistent and/or carcinogenic.
In order to understand the source and environmental relevance of naturally produced organobromine compounds in surface waters, the mechanism of the formation was investigated using batch tests with lake water and algae cultures.

Methods and Materials

Sample Site: Lake Tegel is located in the North-West of Berlin (Germany). It covers a surface area of 4.1 km^2 with a maximum depth of 16 m and a theoretical water detention time of 137 days. Lake Tegel is euthrophic. The lake is characterized by a typical algae bloom in late summer (*10-11*).

Samples: In November 2002 (20 L) and in September 2003 (1 L) grab samples of Lake Tegel were sampled at the south-east side of the lake near the bank side. For AOBr analysis aliquots of the samples were filtered (0.45 µm) immediately in laboratory. After filtration the samples were acidified with nitric acid to pH 2 and stored at 4ºC until they were analyzed. For the laboratory experiments, which were set up at the sampling day, unfiltered samples were used.

For the gas sparging experiment unfiltered samples were acidified as stated before and stored at 4 °C until experimental start.

Laboratory batch tests: All laboratory batch tests were carried out using open glass vessels, with artificial sun light (12 h each day, six tubes, each 18 W), aeration and room temperature (except algae tests at 25 °C in an air-conditioned chamber). Batch tests were performed with lake water and algal cultures. Lake water batch tests: unfiltered lake water (15 L) was filled into a 20 L glass vessel and sampled monthly for AOBr analysis. Nutrients and bromide were not added. Algae batch tests: two kinds of algal cultures were used: the blue-green algae *Microcystis aeruginosa* (cyanobacteria) and the green algae *Scenedesmus subspicatus* both from Culture Collection of Algae at the University of Göttingen. For each algae culture 15 L individual nutrient solutions were prepared (composition see *12-13*), with 100 µg/L bromide addition and algae inoculation (100 ml of algae suspension). For the algae batch tests with normal (optimum) nutrient concentration sampling times started approx. every two weeks until 43 d and continued thereafter weekly. The low nutrient containing algae batch tests are prepared with 15 L of a ten times diluted nutrient solutions and an addition of 100 µg/L bromide and inoculation of 100 ml corresponding algae suspension. For those batch experiments sampling times were weekly.

Adsorbable organic bromine (AOBr) / extracellular AOBr: Differentiation of AOX into adsorbable organic chlorine (AOCl), bromine (AOBr) and iodine (AOI), using ion chromatography instead of microcoulometric titration, has been used for a detailed estimation of organic bromine pollution. Individual steps of the method include enrichment on activated carbon, combustion in an oxygen stream with subsequent trapping in an adsorption solution and ion-chromatography with UV-detection (for further details see *14-15*).

Intracellular bromide: A small part of the algae sample was treated with Lugol's solution for algae conservation and kept in a refrigerator until microscopic individual count (No. of Ind.). The remaining part was filtered through 1.2 µm filters, with known filter mass for later mass determination. The filters containing the algae were dried in a desiccator at room temperature over night. Two of these filters were used for dry mass determinations at 105 °C and the rest is resuspended in water by ultrasonic treatment. The suspension was concentrated (SpeedVac Concentrator A160, Savant, Thremo LifeScience) at 55 °C to a small volume and then transferred into a small crucible, dried under nitrogen at 60 °C and weight for the determination of the samples initial volume (calculated through the dry mass concentration). The dry algae were finally used for the intracellular algae bromine determination by direct combustion such as for the AOBr measurement but without preceding enrichment on activated carbon.

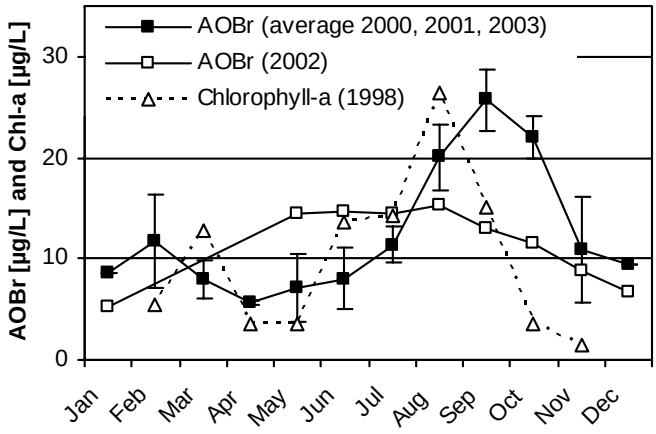
Solid Phase Extraction /Polarity: Solid phase extraction was carried out on an AutoTrace™ SPE Workstation (Zymark®) with RP-C18 and LiChrolut® EN (highly cross linked spherical polymer) cartridges (Merck). Conditioning was performed by drawing successively 10 ml ultra-pure water, 10 ml methanol and 8 ml ultra-pure acidified water (pH 2) through the cartridges with a flow of 4 ml/min. Water samples of 200 ml acidified to pH 2 were subsequently drawn through the cartridges at a flow rate of 4 ml/min. After drying the cartridges for 1 min with nitrogen, elution was done with 3 times 5 ml methanol. The eluates were reduced to a volume of 0.5 ml on a TurboVap® Concentration Workstation (Zymark®) at 55 °C and evaporated to dryness under a gentle stream of nitrogen at a maximum temperature of 60 °C.

The dry eluates were then dissolved in a small volume of hexane, transferred to a different vial and dried again as stated before. Both, the eluates residue (not soluble in hexane, methanol fraction) and the hexane soluble eluates (hexane fraction) were dissolved in a small amount of methanol (1-2 drops) transferred to a graded flask and filled up to the mark with elga water acidified to pH 2. The eluates solutions were used for AOBr measurements.

Results and Discussion

Seasonal high concentrations of organic bromine compounds, measured as AOBr, in Lake Tegel (Berlin, Germany) were observed during AOI and AOBr monitoring in the years 2000 to 2003 (Fig. 1; *1*). Except for the year 2002, late summer concentrations were up to five times higher than during the rest of the year. The AOBr of the lake inflows (throughout the year less then 6 µg/L) were always lower then those in the lake, which indicate a production of AOBr in the lake (*2*). The lake is characterized by a typical algae bloom during late summer (*10*). The year 2002 was much cooler and rainier, which resulted in a less intensive algae bloom. By considering an algae involvement during AOBr formations, the lack of a late summertime AOBr increase in 2002 can be explained by a smaller algae bloom intensity. Additionally the hypothesis of an algae involvement during the natural AOBr formation in surface waters is supported by the correlation of the AOBr and chlorophyll-a concentrations (Fig.1; *2*).

Fig.1: AOBr concentration in Lake Tegel (data 2000, 2001 are from *1*; chlorophyll-*a* data are from



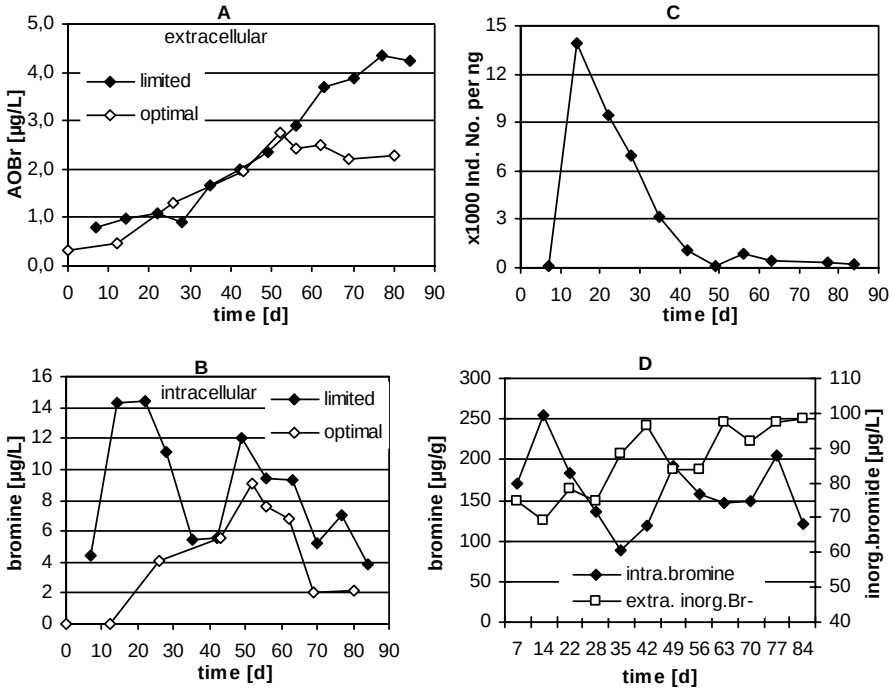
2)

In a first set of batch experiments the prerequisites for the AOBr formation were clarified (*1*). Several different batch tests were carried out with lake water and later with an algal culture. The lake water batch tests include parallel light / dark experiments, tests with and without the addition of sodium azide (abiotic control), and with and without Diuron addition (inactivation of photosynthesis). The necessity of light and phototrophic organisms for the formation of organic bromine compounds was observed during these lake water batch experiments. In a time period of 15 d an AOBr concentration of up to 35 µg/L Br were formed. The simulation of the AOBr

formation in the laboratory could also be repeated with water from a different lake (AOBr production of 50 µg/L Br in 80 d; Müggelsee, Berlin) thus, seasonal high AOBr amounts are expected to occur in all surface waters which are characterized by an intense algae bloom. Similar observations were made for a different lake in the area of Berlin (Wannsee). Additionally it was possible to simulate the natural AOBr formation with an algal culture (AOBr production of 15 µg/L Br in 15 d; *Scenedesmus subspicatus*) in the laboratory. Furthermore, it was found that stress induced by a lack of nutrients might favour the AOBr production (*1*). It appears that the AOBr formation is most probably biotic in nature and requires the presence of phototrophic organisms.

One important question arises after these first experiments: Is AOBr released from living algae or is it a degradative product of dying algae? To address that question, additional experiments with pure algal cultures were carried out and are referred to as algae batches. For these batch experiments two organisms were selected: the green algae *Scenedesmus subspicatus* and the blue-green algae (cyanobacteria) *Microcystis aeruginosa*. The green algae is widespread in fresh water lakes and cyanobacteria often proliferate in nutrient enriched lakes. Additionally cyanobacteria are known to produce a wide range of algal toxins, which have adverse effects on fish, birds and mammals. The bromine determination was carried out as extracellular (AOBr) and intracellular fraction (combustion of the separated biomass and subsequent ion chromatography of the trapped combustion gas). The most efficient evidence for an involvement of algae in the AOBr formation showed the cyanobacteria (*Microcystis aeruginosa*) batch test. These algae produced AOBr under optimal and limited nutrient supply (Fig. 2A), although the formation is higher for limited nutrients (2.5 µg/L Br, 4.3 µg/L Br respectively). The highest bromine concentration is detected in the algae cells (intracellular, Fig. 2B). A dependency can be recognized for the intracellular bromine concentrations on the nutrient supply. Under limited nutrient concentrations a higher intracellular bromine concentration is detected then under optimal nutrient supply (Fig. 2B). Furthermore, the highest intracellular bromine concentration was measured after 14 days in the test with a low nutrient amount versus 52 days under optimal nutrient supply. Figure 2C shows the dependency of the intracellular bromine content on the individual cyanobacteria number being correlated to the dry biomass. Both amounts show a decreasing trend until 35 d, followed by an independent increase of the intracellular bromine concentration thereafter, which might be due to the development of different algae species and their bromine incorporation. In contrast the extracellular AOBr does not increase in the same extent, indicating that the intracellular bromine species are volatile compounds or that algae incorporate inorganic bromine species. A possible inorganic bromine incorporation is confirmed by the correlation of extracellular inorganic bromide and intracellular bromine. The increase and decrease of both bromide/bromine measurements were opposed to each other: as the intracellular bromine increases the extracellular bromide decreases (Fig. 3D). Some time after the cyanobacteria number decrease began, a small extracellular AOBr increase is recognized (Fig. 2A and 2C), indicating that a part of the intracellular bromine could also be organic and released after cell lysis, which might require some time. After a short equilibration phase of 35 d, the mass balance of all analysed bromine species results in the amount of added bromide (100 µg/L ± 10 %).

Fig.2: Cyanobacteria (*Mycrocystis aeruginosa*) algae-batch experiment at 20 °C, with aeration and irradiation with artificial sunlight: A) extracellular AOBr concentration for limited and optimal nutrient supply; B) intracellular bromide concentration per L algae suspension for limited and optimal nutrients supply; C) individual cyanobacteria cell number per ng dry mass for limited nutrient supply; D) intracellular bromine concentration per dry mass and extracellular inorganic bromide for limited nutrient supply.



With the cyanobacteria batch test many new questions arise; concerning (1) the nature and the function of the intracellular bromine in algae, (2) the specific conditions for an intensive involvement of algae during the AOBr formation and (3) possible other biotic processes playing a role in the AOBr formation.

Initial experiments were carried out for a preliminary characterization of the AOBr. First of all the volatility was investigated by a determination of the AOBr in an original lake water sample (AOBr = 21.9 µg/L Br) and in an aliquot which was sparged with nitrogen for 1 h. The AOBr concentration in both samples did not change; implying that the AOBr formed under natural conditions is not volatile. This result is also confirmed by observations made during lake water batch tests: The AOBr always showed an increase (AOBr = 63.8 µg/L after 137 days), although they were open and aerated continuously. Secondly, preliminary experiments concerning the extractability of the AOBr were carried out. Two different solid phase extraction materials were used, RP-C18 for non polar compound and LiChrolut® EN (highly cross linked spherical polymer) for polar compounds. Only small amount of the AOBr are extractable on RP-C18 (up to 4 %),

whereas on LiChrolut® EN cartridges up to 55 % of the AOBr was extractible. Thus, the AOBr shows predominantly polar properties.

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