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PHOTOCATALYTIC DEGRADATION OF TOXINS SECRETED TO WATER BY CYANOBACTERIA AND UNICELLULAR ALGAE AND PHOTOCATALYTIC DEGRADATION OF THE CELLS OF SELECTED MICROORGANISMS

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Excessive algal growth in drinking water sources is responsible for toxin generation, and desinfection-by-product formation. In the photocatalytic degradation of organic contaminants, titanium dioxide has been found to be highly efficient in the generation of hydroxyl radicals, which aree considered responsible for degradation of toxins and inactivation of water-borne microorganisms. The paper reviews the investigation about photocatalytic degradation of hepatotoxins and inactivation of bacteria, viruses and protozoan parasites.

INTRODUCTION

Waterworks encounter difficulties connected with exploitation of water intakes and water mains. This results from an increasing level of pollution of waters used as the source of drinking water.

One of many reasons triggering off the deterioration of water quality and causing difficulties in making water drinkable and in forwarding it are living organisms, namely bacteria, fungi, plants and animals. They influence many features of water quality, including its smell, colour, turbidity, pH value, the content of organic substances, the content of nitrogen, and among other things they also influence the concentration of toxic organic compounds. Phytoplankton is an ecological group exerting the most unfavourable influence on the drinking water quality. Detergents and fertilizers cause eutrophication of the surface waters, which results in lush development of phytoplankton. As regards taxonomy, among phytoplankton there are cyanobacteria, which belong together with bacteria to the kingdom of non-nucleated (Prokaryota) and algae, belonging to nucleated (Eukariota). Cyanobacteria are particularly troublesome organisms because apart from producing large amount of biomass, they secrete to water very toxic substances. High pH of water, scarcity of carbon dioxide solved in water and high amount of phosphorus is conducive to the production of the biomass of cyanobacteria and algae (Carmichael, 1994; Gajdek, 2000).

Cyanobacteria and algae can survive the period of unfavourable conditions in the form of spores and then, in favourable conditions quickly achieve quantitative mass development. In this time they give water distinct colour, referred to as bloom. The authors are not unanimous about the number of algae or cyanobacteria cell population, which results in water bloom (Fawell, Hart, James & Parr, 1993; Carmichael, Jones, Mahmood & Theiss, 1985).

During the bloom the number of their cells can reach the number of 10 million per one millilitre (10 mln/ml) (Starmach, Wróbel & Pasternak, 1976). Sudden dying of the bloom causes a number of negative after-effects for a water tank. The release of toxins to water from undamaged cells of cyanobacteria and algae is rather small, only the disintegration of these cells releases toxins occurring mainly in the cell walls.

Cyanobacterial toxins are the group of compounds with very diverse chemical structure. They are divided into two groups: cytotoxins and biotoxins. Cytotoxins are not lethal for people and animals, but they are relatively more toxic for algae and the cells of mammals. They are enzymes, antibiotics and anticarcinogenic factors with very complicated chemical structure. Biotoxins are very toxic for people and can even cause lethal effects. They are divided into neurotoxins (affecting nervous system), hepatotoxins (affecting liver) and dermatotoxins.

Hepatotoxins (liver toxins) occur more often than neurotoxins. Until now the chemical structure

Table 1. Lethal doses (LD50) for different types of toxins converted to one kilogram of body mass (according to Nawrocki *et al*., 2000).

Toxins	LD_{50} [μg/kg]	Toxins	LD_{50} [μg/kg]
Sodium cyanide	15 000	Mikrocystin-LD and -YR	68
Potassium cyanide	10 000	Toxin of rattlesnake *(Crotalus sp.)*	60
Toxin of toadstool *(Amanita muscaria)*		Mikrocystin-LR	
Mikrocistin -RR	1 100	Nodularin	50
Strychnine	600	Anatoxin-a (s)	30-50
Anatoxin-(a)	500	Saksitoxin and neosaksitoxin	20
	200		10

of about 60 microcystins and nodularins, the most toxic representatives of hepatotoxins was characterised and specified. Hepatotoxins belong to peptides with cyclic structure; they consist of 5 (nodularins) or 7 (microcystins) amino acids.

They inhibit the activity of protein phosphatase, which leads to contraction of hepatocytes (liver cells) (El Saadi & Cameron, 1993; Falconer, 1996).

The cells start to separate, and blood which retains between them leads to local hepatocellular damage and a shock. Lethal dose leads to death within a few hours, however the intake of small doses leads to chronic disorder of digestive system and liver (Osiecka, 1995).

Hepatotoxins are very durable chemically; they react neither with acids nor with alkali and boiling in water cannot decompose them. In 1996 in Brazil 50 cases were recorded of lethal poisoning of people hospitalised in haemodialysis centre. The people died because toxins from the polluted water got into their blood system (Jochimson, Carmichael, An, Cardo, Cookson, Holmes, Autines, Demelo, Lyra, Barreto, Azevedo & Jarvis,1998).

Water plays a very important role in transportation of many pathogenetic microorganisms, including bacteria, viruses and protozoa (Nawrocki & Biłozor, 2000; Kawecka & Eloranta, 1994). Viruses are more resistant to exterior factors than bacteria and illnesses caused by viruses are very often acute infections, with high mortal ratio. Moreover, cysts or oocysts of *Giardia muris* and *Cryptosporidium parvum* protozoa can appear in drinking water. They characterise with high immunity (resistance) to environmental factors and the process of making water drinkable.

Classical methods of removing toxins secreted by bacteria and algae and of degradation of microorganism cells

Toxins secreted by bacteria and algae are removed in processes of coagulation, adsorption, biodegradation and microfiltration (Nawrocki *et al*., 2000). Removing microcystins is the most difficult problem, and although the adsorption on activated carbon is an effective process, it does not lead to complete removal of these compounds. There has been described an adsorption of nodularins on natural alusilicates (Miller *et al*., 2001), with reference to microcystins-LR adsorption on activated carbon (Lawton *et al*., 1998; Lambert *et al*., 1996) and the process of its biodegradation

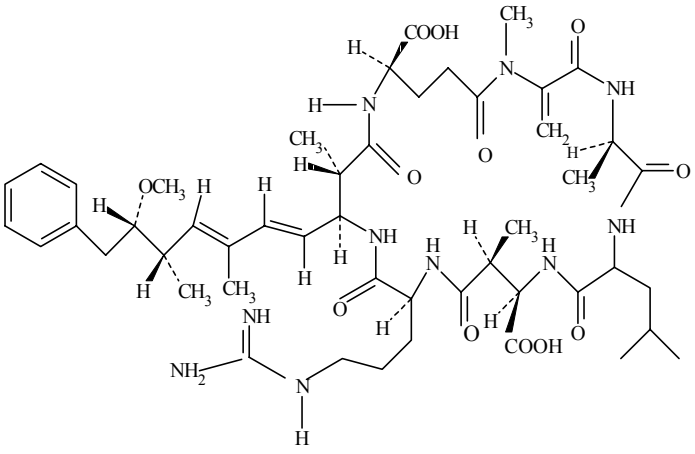

Fig. 1. Structural pattern of microcystin -LR produced by *Microcystis aeruginosa* (according to J. Nawrocki *et al.,* 2000)

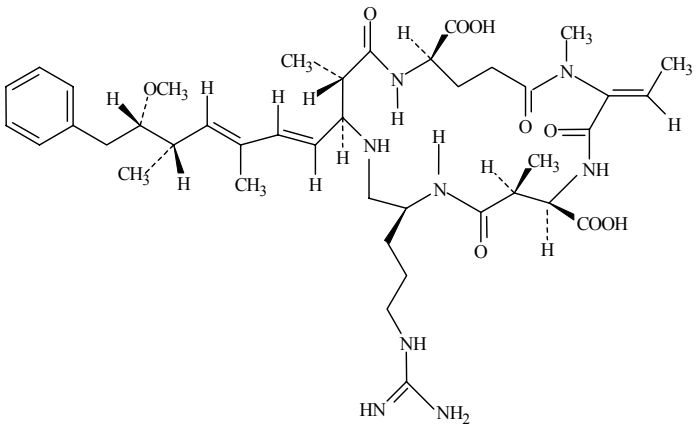

Fig. 2. Structural pattern of nodularin produced by *Nodularia spumigena* (according to Nawrocki *et al.,* 2000)

(Cousins, Bealing, James & Sutton, 1996). It turned out however, that both methods of nodularin adsorption, microcystin-LR adsorption and the discussed biodegradation do not lead to their complete decomposition. In connection with this there has been researched an influence of a number of different methods and their combinations, namely flocculation, filtration, adsorption on activated carbon and ozonation and chlorination on the removal of hepatotoxins from water. The best results were obtained from the connection of the adsorption on activated carbon with ozonation (Himberg, Keijola, Hiisvirta, Pyysalo & Sivonen, 1989). The degradation of hepatotoxins under the influence of chlorination with chlorine and chloramines was also researched by Nicholson, Rositano and Burch (1994).

The removal of microorganisms from water, that is its disinfection, is a serious technological problem. With this end in view we use chlorination, ozonation, irradiation with UV rays, coagulation, biodegradation, microfiltration and so-called slow filtration. Slow filtration removes most of bacteria from *Coli* group and *Giardia lamblia* cysts and it is more efficient than coagulation. Chlorination of water is efficient because chlorine, apart from its disinfection abilities, inhibits also the growth of algae. Free chlorine, chlorine dioxide or chloramines are added to water. The amount of chlorine needed to kill bacteria and protozoa ranges from 0.2 mg/l to 0.5 mg/l. algae are very resistant to the effects of chlorine, e.g. *Chlorella vulgaris* can survive the dose of chlorine 5 mg/l working for 1.5 hour. Application of large doses of chlorine is

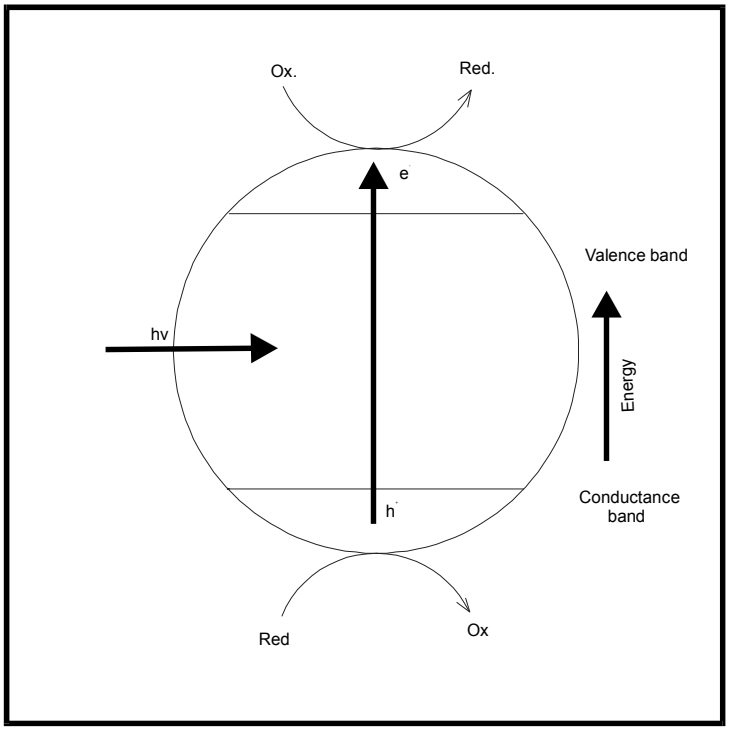

Fig. 3. The scheme of TiO_2 activity in the solution containing oxidative and reductive factors (according to Rajeshwar & Ibarez, 1995)

inadvisable because of hazardous side effects. The process of chlorination of water containing phytoplankton is a significant source of trihalometanes and chloriderivatives of other organic compounds, which means that this process cannot be carried out in case of water bloom (Fawell *et al*., 1993). To destruction of bacteria, viruses and the cysts of protozoa, UV radiation in wide range UV A-C is used. A bactericidal and mutagenic effect of UV radiation is connected with changes in the structure of nucleic acids, mainly DNA.

The dose of radiation is important because application of a smaller dose than the lethal dose can have a stimulating or bacteriostatic effect. Doses of UV – C radiation for destruction of 90% of bacteria range from 10 to 200 units per square meter (J/m^2), however in the process of making water drinkable, the doses of about 400 units per square meter are used (Nawrocki *et al*., 2000). It has been proven that the majority of bacteria and viruses and cysts of protozoa need a higher dose of UV radiation in comparison with *Escherichia. Coli* (Hang, Ossoff, Lobe, Dorfman, Dumais, Qualls & Johson, 1985). The influence of UV radiation on cysts of *Giardia lamblia* and *Cryptosporidium parvum* (Craik, Weldon, Finch, Bolton & Belosevic, 2001; Craik, Finch, Bolton & Belosevic, 2000) and on cyanobacteria *Microcystis aeruginosa* was also researched into (Alam, Otaki, Furumai & Ohgaki, 2001).

Photocatalytic oxidation processes – application to the degradation of microcystins and cells of the selected microorganisms

In the processes of making water drinkable, apart from the aforementioned ozonation, chlorination and photochemical methods, there are also other methods, which are constantly used and developed. These are processes in which, in the initial stage, slow and highly reactive hydroxyl radicals are generated. Among these methods there are both photolytic processes O_3/UV, H_2O_2/UV, O_3/H_2O_2/UV and Fenton's process (used also in technological aims) and also catalytic processes H_2O_2/TiO_2/UV, TiO_2/UV that now undergo pilot research.

Photocatalytic, heterogenic oxidation processes can take place in water solutions in which there are compounds, mainly oxides with semi conductive properties, namely TiO_2, ZnO, Fe_2O_3, WO_3 and CdSe (Fox & Dulay, 1993; Legrini, Oliveros & Braun, 1993). TiO_2 is most widely used, because apart from its satisfactory catalytic properties, it is also chemically passive in wide pH range. In order to initiate the photocatalytic process it is necessary irradiate the solution with radiation absorbed by the catalyst. Semiconductors have a valence band filled with electrons and an unfilled conductance band. These bands are separated with a bandgap, called an energetic gap. Adsorption by the semiconductor of photons with energies with the range of bandgap energy (>3.2 eV for TiO_2) causes transfer of an electron from valence band to conductance band. An electron moving to the conductance band, leaves in the valence band an unfilled energetic level called h^+ hole.

$$TiO_2 + h\nu \rightarrow e^- + h^+$$

Radiation with the wavelength (λ) smaller than 400 nm, and that is also sunlight (in the range from 300 nm to 400 nm), can induce valence electrons. However, the participation of this radiation in the solar energy is only 3% of its total energy. Positively charged holes can generate hydroxyl radicals in the following reactions:

$$OH + h^+ \rightarrow {}^{\bullet}OH_{ads}$$
$$H_2O_{ads} + h^+ \rightarrow H^+ + {}^{\bullet}OH_{ads}$$

In the presence of molecular oxygen (in water the concentration of dissolved oxygen is in the order of mmols/l) the electrons are caught in the following reactions:

$$O_2 + e^- \rightarrow O_2^-$$
$$OH + h^+ \rightarrow {}^{\bullet}OH_{ads}$$
$$O_2 + 2e + 2H^+ \rightarrow H_2O_2$$
$$H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^-$$

Hydroxyl radicals have the highest oxidation potential (2.8 V with relation to NHE) in comparison with other substances used for water disinfection, namely ozone (2,07 V), H_2O_2 (1,78 V), HOCl (1,49 V) and chlorine (1,36 V).

If the oxidation potential of the molecule adsorbed on the surface of the semiconductor is lower than the potential of the hole, then what follows is the transfer of the electron to an unfilled energetic level in the valence band and the molecule is transformed into a cation-radical $S^{+\bullet}$. Otherwise the molecule undergoes reduction to anion-radical $S^{\bullet -}$. Ion-radicals, as reactive chemical individuals, can undergo further reactions leading in consequence to complete mineralisation of, e.g. the discussed toxins. Generating of hydroxyl-radicals and arising of ion-radicals are the main mechanisms causing photocatalytic degradation of organic compounds.

The photocatalytic method has found its application also in the degradation of toxins secreted to water by bacteria and unicellular protozoa and in the degradation of bacteria and algae cells (Lawton, Robertson, Cornish & Jaspars, 1999; Robertson, Lawton, Münch & Rouzade, 1997).

The photocatalytic method of degradation of *Escherichia coli* exposed to UV irradiation in the presence of TiO_2 was reported for the first time in 1988 (Matsunaga, Tomada, Nakajima, Nakamura & Komine, 1988). Next, it was proven that the degradation of *Escherichia coli* in these conditions, within 16 minutes occurred in 99% (Zhang, Scrudato & Germano, 1994). In this connection *Escherichia coli* was proposed to the assessment of effectiveness of degradation of microorganisms that underwent the photocatalytic processes. It was also proven that hydroxyl-radicals play a significant role in the processes of degradation of *Escherichia coli*; by adding to the reaction environment a sodium thiosulfate, which is a strong scavenger of radicals, it was observed that this process was dying away (Ireland & Valinierks, 1992; Ireland, Klostermann, Rice & Clark, 1993). Other authors, who by irradiating the suspension of *Escherichia coli* and TiO_2 with sunlight observed the bactericidal effect within a few minutes, also confirmed this view (Wei, Lin, Zainal, Wiliams, Zhu, Jruzic & Rajeshwar, 1994). In the quoted research it was also stated that oxygen is a significant substrate in the discussed process – the photo catalyst used in the absence of oxygen was inactive. The speed of degradation was proportional to the intensity of light (in range 180-1660 $\mu Es^{-1}m^{-2}$). The authors of the discussed research work indicate that bactericidal properties of TiO_2 are connected with the influence of hydroxyl-radicals on the replication of DNA in a cell and also with modification by these radicals of cell membranes radicals (by oxidation of membrane lipids). There was also suggested another mechanism of bactericidal effects of TiO_2 — this mechanism assumes direct oxidation of intracellular coenzyme A (Matsunaga *et al*., 1988).

In the majority of the presented research work, the authors used crystalline TiO_2 with anatase structure. In one of the works the authors used titanium dioxide deposited in the form of a thin layer on the glass (Sunada, Kikuchi, Hashimoto & Fujishima, 1998). TiO_2 prepared in this way also had bactericidal properties with reference to *Escherichia coli.* Moreover, the authors observed that with the decrease of the amount of living bacteria in the culture, the concentration of pyrogenic bacterial endotoxins freed from cell-walls of the dead bacteria increased. During the reaction of photocatalytic degradation, as the result of TiO_2's activity, the cell-walls of bacteria started breaking and the endotoxins were getting out into the environment of the reaction. The bacteria were neutralised within two hours, however the disappearance of endotoxins was observed only after four hours of the process. Deactivation of the discussed endotoxins is unsuccessful with the use of membrane filtration and adsorption on the activated carbon (Issekutz,1983), so they are a serious problem in medicine and pharmacy (production of medicines).

Photocatalytic degradation of *Coliform* bacteria and *Polio 1* virus irradiated with sunlight was also researched into. It was observed that the degradation of bacteria proceeded in the time five-times longer than the degradation of virus. This fact was associated with greater speed of diffusion to the surface of photocatalyst of viruses that are much smaller than bacteria (Watts, Kong, Orr, Miller & Henry, 1995).

It was stated that the discussed photocatalytic processes influence (among others) iron ions (II) which stimulate the speed of arising of hydroxyl-radicals (Legrini *et al*., 1993). The stimulating influence of these ions was also observed in the process of photocatalytic (with participation of TiO_2) degradation of MS2 bacteriophage (Sjorgen & Sierka, 1994).

The process of the growth of *Oedegonium* algae culture in the presence of TiO_2 irradiated with sunlight was also researched into (Linkous, Carter, Locuson, Quellette, Slattery & Smitha, 2000). The presence of TiO_2 caused a 60% inhibition of the growth of the culture with relation to the control

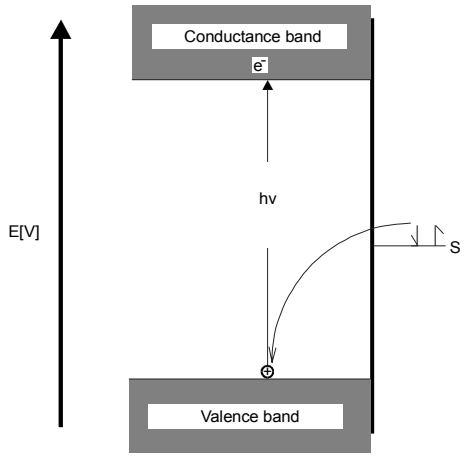

Fig. 4. Transfer of an electron from an organic molecule S (which is in the solution) to valence band of a semiconductor (according to Pearl *et al*.,1997)

culture. The opposite effect of culture growth stimulation was observed with relation to *Chlorella* algae culture in the presence of silica aerogel (Janikowska, Makowski, Wardas & Jarzębski,1996).

The arising of highly reactive hydroxyl-radicals with unspecific activity can lead both to removal of the toxins from water and to neutralisation of algae, bacteria, viruses and protozoa which also are in water. However, practical application of the method can be connected with numerous technological difficulties, which is why there are so few reports on this subject. The possibility of the application of the aforementioned method for disinfection of municipal sewages was only mentioned (Melian, Rodriguez, Suarez, Rendon, Campo, Arana & Pena, 2000). In spite of that, the photocatalytic degradation of toxins secreted to water by cyanobacteria and unicellular bacteria and the degradation of the cells of selected microorganisms is an interesting alternative as the supplement of the methods applied so far.

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