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ORIGINAL RESEARCH ARTICLE

Influence of dissolved organic nitrogen on surface waters☆

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KEYWORDS
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Summary The aim of this study was to determine the susceptibility of dissolved organic nitrogen (DON) contained in biologically treated wastewater disposed from municipal wastewater treatment plants (WWTPs) to biodegradability and bioavailability in a water environment. Additionally an evaluation was performed of the participation of this organic nitrogen fraction, including bioavailable DON (bDON), in the nitrogen balance for the Baltic Sea.

Based on the samples of secondary effluent taken from two large municipal WWTPs located in Northern Poland DON bioavailability and biodegradability tests were carried out. It was concluded that DON concentration in the tested samples was on average from 1.5 to 2.0 g N m^{-3}. This fraction constituted as much as 50% of organic nitrogen and 15—18% of total nitrogen contained in treated wastewater.

The participation of biodegradable DON (brDON) in activated sludge tests was on average 24—35%. In the bioavailability tests *Selenastrum capricornutum* were able to use from 19 to 26% of DON, however taking into account the results of the control test, these values are reduced to 3—4%. On the other hand, taking into account the combined effect of bacteria and algae it was possible to reduce the DON concentration by nearly 40%.

The estimated annual bDON load introduced to Baltic Sea waters from Poland through disposal of treated biological wastewater in 2010 reached up to 1.7 thousand tons of N $year^{-1}$.

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1. Introduction

European Union legal regulations in terms of disposal of treated municipal wastewater are specified particularly in the Council of European Communities directive 91/271/EEC dated May 21st, 1991. It imposes the obligation on member states to ensure at least a good condition of surface waters by the year 2015. In Poland the priority task in protecting surface waters, flowing waters and the Baltic Sea waters against pollution caused by municipal wastewater is to ensure complete biological treatment of wastewater and increased removal of biogenic compounds in urban centers above 15,000 PE. This should at minimum provide 75% reduction of the total nitrogen (TN) and phosphorus load in municipal wastewater from all over the country. The achievement of the intended nitrogen load reduction effect is associated with reducing the concentration of inorganic forms of nitrogen contained in secondary effluents and primarily involves improving the effectiveness of the nitrification and denitrification processes carried out in bioreactors of municipal wastewater treatment plants (WWTPs). However, in biologically treated wastewater, organic nitrogen (ON) may constitute a significant participation of the TN. This directly influences the functioning of large municipal WWTPs in Poland (above 100,000 PE), for which the admissible TN concentration in sewage disposed to the receiving bodies is 10 g N m^{-3}. The results of studies conducted so far domestically and abroad show that the participation of dissolved organic nitrogen (DON) in treated wastewater is less than 2% up to as much as 85% of the total nitrogen (e.g. Pagilla et al., 2008). In such a case, the origin, fate and degree of bioavailability of the dissolved fraction of ON in treated wastewater constitutes a significant issue in the perspective of protecting waters against eutrophication. If the degree of this fractions availability in wastewater receiving bodies is high, the goal should be to develop wastewater treatment technologies taking into account removal of DON. If, however, the fraction is not bioavailable also outside of wastewater treatment plants, this fact should be reflected in regulations on the quality of treated wastewater. In the treated wastewater receiving bodies as a result of ammonification the increase of the ammonia concentration may occur. The limiting factor of this increase is the ammonification process rate. Ryzhakov et al. (2010) have identified this value at 0.004—0.035 mg N dm^{-3} d^{-1} based on the studies of the four lakes. In a typical municipal wastewater treatment plants ammonification rate was higher, and amounted above 50 mg N dm^{-3} d^{-1} (Katipoglu-Yazan et al., 2012). However, the studies concerning the impact of wastewater discharge (containing DON) on the Chesapeake Bay Lake did not show a significant increase of ammonia concentration, which was completely consumed within 2 days (Filippino et al., 2011).

Organic nitrogen is disposed into ground waters from natural sources (atmospheric precipitation, swamp areas, infiltration) and as a result of the human activity (agriculture, intensive animal farming and treated wastewater). It accesses the water as a result of a single point disposal (e.g. from treatment plants), surface flows and atmospheric precipitation (Seitzinger and Sanders, 1997, 1999). DON may have a significant participation in the total amount of nitrogen available in most water systems, also including oligotrophic waters (lacking in biogenic compounds), in which original production is limited by the availability of nitrogen (Bronk et al., 2006). In such cases ON may constitute a significant source of this element for the growth of microorganisms. It should be, however, taken into account that DON is created by compounds of varying molecular weight, lability and bioavailability. Literature features publications regarding the possibility of using DON by the water ecosystem, including bacterioplankton, cyanobacteria and phytoplankton (Berman, 1997; Berman and Chava, 1999; Bronk et al., 2006). Results of experimental studies show that in river and lake waters DON constitutes an average of 40—50% of TN, however its participation may exceed 85% (Kroeger et al., 2006). An inverse relation between DON concentration and the concentration of dissolved inorganic nitrogen was observed, which indicates that DON may be an alternative source of nitrogen for microorganisms. According to Seitzinger and Sanders (1997) the participation of DON varies between 20 and 90% of the TN load in estuaries.

The participation of DON biologically utilizable for microorganisms primarily depends on its characteristics. It has been concluded that compounds of a low molecular weight (LMW) are more easily available in sea waters, as well as fresh-waters compared to the high molecular weight (HMW) compounds, whereas a significant part of DON consists of compounds not susceptible to biodegradation (Stepanauskas and Leonardson, 1999). The sources of DON origin and environmental conditions may also be of particular significance. Based on the results of tests incorporating bacteria and algae it was concluded that the degree of DON use also depends on the season. During spring floods Seitzinger et al. (2002) have observed an increase in DON bioavailability despite its concentration being maintained at a stable level. The authors point out that DON released from the soil is less utilizable for microorganisms than DON originating from other sources, e.g. discharged from a treatment plant. This may be due to the fact that DON from agriculture and forest areas contains aromatic compounds, while municipal wastewater DON contains primarily aliphatic compounds. Aliphatic compounds are more easily utilizable for microorganisms compared to aromatic compounds, which may indicate the existence of a correlation with the availability of nitrogen. The degree of DON usage was between 0 and 73%, whereby the higher values were observed for ON originating from municipal, rather than natural sources (like forest area runoff). Also, in the opinion of Wiegner and Seitzinger (2004) DON from municipal sources influences higher bacteria growth. The degree of DON usage by microorganisms in a water environment (rivers, streams, swamp areas and seas) is variable and fluctuates depending on the author from 0 to 80% (Bronk et al., 2006; Stepanauskas and Leonardson, 1999; Wiegner and Seitzinger, 2004).

Berman (1997) presents data indicating that LMW compounds included in DON may be directly or indirectly digested by microorganisms. In the Kinneret lake (Israel) studied by him the concentration of DON decreased from 0.371 to 0.125 g N m^{-3}, and the concentration of dissolved inorganic nitrogen decreased from 0.065 to 0.013 g N m^{-3}, as a result of the development of *Aphanizomenon ovalisporum* Cyanobacteria. These results indicate, that compounds which comprise DON are an important direct and indirect source of nitrogen for microplankton. This conclusion has been confirmed by laboratory studies, in which pure cultures of bacteria developed well in a medium supplemented with ON compounds (such as urea, hypoxanthine, lysine, guanine and glucosamine). Later studies conducted by Berman and Chava (1999) have not only confirmed that DON constitutes an important direct and indirect source of nitrogen for phytoplankton, but have also shown that different types of algae may use this source to a different degree. This means that DON may selectively impact the type of algae types dominant in a given environment.

The bioavailability of DON in natural waters depends primarily on the differences in composition and molecular weight of compounds which form this fraction of ON. Compounds such as free amino-acids, urea and nucleic acids are easily taken in by heterotrophic bacteria, as well as sea and fresh-water algae. Veuger et al. (2004) have shown that apart from urea and free amino-acids also dissolved linked amino-acids may serve as an important source of nitrogen for heterotrophic bacteria and phytoplankton. Further, HMW compounds may constitute an alternative source of nitrogen needed for the development of microorganisms. Pehlivanoglu-Mantas and Sedlak (2008) have stated, that dissolved free amino-acids may be used directly by algae as a source of nitrogen, while dissolved linked amino-acids must be initially subjected to the process of hydrolysis to the form of monomers before they become available to algae. Also inorganic compounds created as a result of DON hydrolysis and ammonification are easily utilizable for algae.

Hasegawa et al. (2001) have conducted studies on the intake and release of DON by microorganisms based on the measurement of ^{15}N isotope concentration. The authors have concluded that nitrogen released by microorganisms feeding on phytoplankton was easily utilizable by bacteria. This indicates the occurrence of a significant nitrogen flow from phytoplankton to bacteria through micro-consumers of the former.

The evaluation of bioavailability of dissolved organic matter routinely uses biological studies based on the growth of bacteria. In this approach a very important, yet often omitted notion is the achievement of environment samples with limited access to nitrogen during incubation. This stems from the fact that microorganisms are more likely to use inorganic nitrogen than DON. This DON bioavailability evaluation method is based on three types of measurements: the usage of dissolved oxygen and the associated mineralization of DON (Moran et al., 1999), decrease of DON concentration overtime (Wikner et al., 1999) and increase of biomass concentration (Stepanauskas and Leonardson, 1999).

The aim of this study was to determine the susceptibility of DON fraction contained in biologically treated wastewater on biodegradability and bioavailability, as well as evaluate the participation of this ON fraction in the TN load discharged into the Baltic Sea.

2. Material and methods

2.1. Study sample

The biologically treated wastewater originated from the Gdańsk and Gdynia WWTPs. They are the largest municipal WWTPs located in Northern Poland carrying out disposal of wastewater to the Gdańsk Bay which forms a part of the Baltic Sea. They enable biological removal of biogenic compounds and their detailed description has been presented in prior publications (Czerwionka et al., 2012). The test samples were taken as daily average, in proportion to time. The characteristic of biologically treated wastewater is presented in Table 1.

In order to limit the availability of inorganic forms of nitrogen, their concentration was reduced below $1\ g\ m^{-3}$. Due to the fact that in all biologically treated wastewater samples studied the concentrations of ammonia nitrogen and nitrite nitrogen were very low, in practice the concentration of nitrate nitrogen was reduced. For that purpose denitrification with an external source of organic carbon in the form of sodium acetate was used (at a dose of 6 gCOD/gNO_3-N).

The activated sludge used in the studies was taken as an immediate sample directly from the nitrification chamber of the studied WWTP. After transportation to the laboratory the sludge was intensely aerated (oxygen concentration approx. $6\ g\ O_2\ m^{-3}$) for 1 day in order to mineralize the organic contaminants contained in the sludge's biomass. Afterwards the sludge was separated from the supernatant water through spinning, the sludge was flushed two times with distilled water and again condensed by spinning. After the last spinning session, the sludge was dissolved in distilled water in order to receive a solution with the desired concentration ($1\ cm^3$ of solution contained a sludge mass discharged to $80\ cm^3$ of wastewater).

The bioavailability tests incorporated a pure cultivation of *Selenastrum capricornutum* algae on a standard microbiological medium.

2.2. Experimental procedure

Four variants of performed tests were planned:

- secondary effluent without additions (control test);
- secondary effluent + algae (bioavailability test);
- secondary effluent + activated sludge (biodegradability test);
- secondary effluent + activated sludge + algae (evaluation of interaction with bioavailability and biodegradability).

The analyzed samples were mixed using a mechanical mixer (250 rpm). The algae tests were irradiated with a fluorescent

Table 1 The average concentration of nitrogen forms (±standard deviation) in secondary effluent in the studied municipal WWTPs.

WWTP	NH_4-N [g N m^{-3}]	NO_3-N [g N m^{-3}]	NO_2-N [g N m^{-3}]	TN [g N m^{-3}]	ON [g N m^{-3}]
Gdańsk	0.73 (±0.31)	6.43 (±0.90)	0.17 (±0.08)	11.24 (±0.82)	3.91 (±0.82)
Gdynia	0.37 (±0.32)	6.28 (±0.92)	0.23 (±0.22)	9.61 (±0.91)	2.73 (±0.43)

lamp with a 12 h light/dark cycle, while the samples without algae were isolated from light. Tests were carried out at a temperature of 20°C. The initial concentration of activated sludge biomass in the biodegradability tests amounted 20 g m^{-3}, and the initial content of algae suspended matter in the bioavailability tests was 5 g m^{-3}. The samples for analysis were taken in the first day of measurements and after 1, 2, 4, 7, 10, 14 and 21 days, respectively.

2.3. Analytical methods

The samples were filtered through membrane Millipore nitrocellulose filters (Billerica, MA) with different pore sizes (1.2 and 0.1 μm, respectively). The filtrates were analyzed for TN using a TOC analyzer (TOC-VCSH) coupled with a TN module (TNM-1) (SHIMADZU Corporation, Kyoto, Japan), and inorganic forms of nitrogen (NH_4-N, NO_3-N and NO_2-N) using Xion 500 spectrophotometer (Dr Lange GmbH, Berlin, Germany). The analytical procedures, which were adapted by Dr Lange GmbH (Germany) and SHIMADZU (Japan), followed the Standard Methods for Examination of Water and Wastewater (APHA, 2005). The DON concentrations were estimated from the difference between TN after filtration and the sum of inorganic N concentrations (Eq. (1)):

$$DON = TN_{0.1\,\mu m} - (NH_4\text{-}N + NO_3\text{-}N + NO_2\text{-}N). \qquad (1)$$

Total suspended solids (TSS) were measured by the gravimetric methods in accordance with standard methods (APHA, 2005).

3. Results and discussion

Studies on the bioavailability and biodegradability of dissolved fractions of organic nitrogen contained in biologically treated wastewater were carried out during the period from March 2009 to June 2010. Five series of tests were carried out on each of the two selected municipal treatment plants.

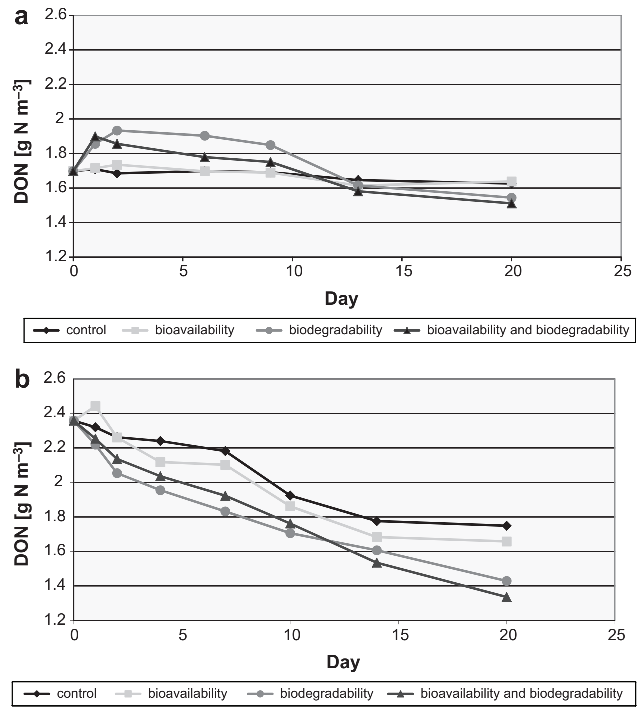

Figure 1 DON concentration changes during test 1 for secondary effluent from the Gdynia WWTP (a) and test 4 for secondary effluent from the Gdańsk WWTP (b).

Table 2 presents the DON removal efficiency values achieved during individual tests.

During the tests it was possible to observe two primary trends in the progress of DON changes. The first (most often occurring) was characterized by an initial period in which the DON content would increase (especially in samples with added activated sludge). In subsequent days of the tests a decrease in the content of this organic nitrogen fraction was observed. An example of such a process is presented in Fig. 1a

Table 2 Efficiency of DON removal from secondary effluents originating from the analyzed municipal WWTPs.

WWTP	Value	DON [g N m^{-3}]	Efficiency of DON removal [%]			
			Control	Bioavailability	Biodegradability	Bioavailability and biodegradability
Gdańsk	Test 1	1.45	20.87	22.56	35.78	38.89
	Test 2	1.78	17.67	23.23	28.67	32.78
	Test 3	2.56	19.45	24.87	31.09	37.59
	Test 4	2.36	25.83	29.69	39.40	43.34
	Test 5	2.08	23.89	28.97	38.75	42.54
	Average	**2.05**	**21.54**	**25.86**	**34.74**	**39.03**
	SD	0.40	2.96	2.94	4.22	3.80
Gdynia	Test 1	1.70	4.24	3.53	9.07	11.01
	Test 2	1.14	14.56	16.32	21.92	24.08
	Test 3	1.56	18.88	20.56	25.71	29.17
	Test 4	2.02	21.23	26.08	30.11	37.67
	Test 5	1.13	11.15	14.02	19.76	23.58
	Average[a]	**1.46**	**16.46**	**19.25**	**24.38**	**28.63**
	SD[a]	0.37	4.59	3.94	5.66	3.89

[a] The values of test 1 were not taken into account when calculating the mean and standard deviation.

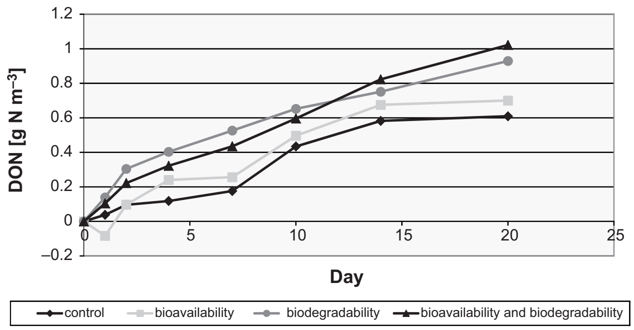

Figure 2 Changes to the content of biodegradable/bioavailable DON during test 4 of secondary effluent from the Gdańsk WWTP.

(test 1 for wastewater from the Gdynia WWTP). In certain tests a trend of continued decrease of DON value during the entire incubation period was observed. An example of such a process is presented in Fig. 1b (test 4 for wastewater from the Gdańsk WWTP).

The amount of DON susceptible to biodegradation/utilizable by Chlorophyta can also be presented in the form of a chart similar to the BOD test. Fig. 2 presents an example of such a chart (test 4 for wastewater from the Gdańsk WWTP).

The amount of DON susceptible to biodegradation with the participation of activated sludge microorganisms (brDON) varied between 20 and 40%. Simultaneously in the control sample (containing filtered biologically treated wastewater containing bacterial biomass, which went through a filter with a pore size of 1.2 μm) a reduction of DON was observed within 10—25%. The achieved results show that a significant increase in biomass concentration (from trace values to approx. 20 g m^{-3}) resulted in an approx. double increase in the amount of DON which was removed through biodegradation. Simultaneously these are values close to the ones presented by Pagilla et al. (2006), who in his studies achieved a value of brDON at a level of approx. 26%. At the same time, the initial increase of DON concentration in samples with activated sludge occurring in most tests may be associated with the release of soluble microbial products (SMP) by microorganisms. It was also observable during earlier studies of DON conversions in bioreactors with activated sludge, at a laboratory scale as well as at a full scale. Each time an increase of DON was observed in the nitrification zone, where the processes of final wastewater purification in aerobic conditions occurred (Czerwionka et al., 2012).

The amount of DON digested by *S. capricornutum* varied between 12 to nearly 30%. Pagilla et al. (2006) have shown that a participation of bioavailable DON (bDON) was approx. 21%. Also the results of the 30-day test with the participation of those Chlorophyta have shown that 26% of DON was bioavailable (Litman et al., 2008). However, the results of studies by Porro et al. (2008) were less conclusive and have shown a participation of bDON at a level of 25—50%. All the while, the participation of bDON in studies conducted by Sedlak and Jeong (2011) was significantly higher and reached approx. 50%. At the same time, based on the results of the DON composition analysis, they have shown that only 10—29% of DON is biologically not utilizable to algae (inert). Therefore, the results received in the presented studies are within the lower ranges of bDON participation contained in secondary effluent.

Such an interpretation, however, raises doubts due to the usage method of nitrogen contained in DON by Chlorophyta which are unable to break down organic compounds. An explanation of this situation can be found by comparing the value of removed DON in the control sample and the bioavailability test. Based on the values presented in Table 2 it can be concluded that a significant portion of DON undergoes decomposition with the participation of bacteria contained in wastewater, and is only available to algae in that form. A confirmation of this situation is the very quick decrease in concentration of inorganic forms during the bioavailability tests. It should be noted however, that in all of those tests the achieved values of bDON were higher than the amount of removed DON in the control sample. A clarification of such a situation comes in the form of study results presented by Litman et al. (2008), in which the incorporation of an algae inoculum varied in composition contributed to the increase in the bDON amount to 68% compared to 26% achieved for the pure *S. capricornutum* culture. In the analyzed studies the biologically treated wastewater samples were not disinfected and could have contained other types of algae, whereby some of them could have the ability to decompose organic compounds in order to gain nitrogen (e.g. blue algae). A significant influence on the availability of inorganic forms of nitrogen on the development of algae is shown by the results of a test in which activated sludge and Chlorophyta cultures are simultaneously added to the biologically treated wastewater. In these types of tests the decrease in DON concentration was the highest, ranging from 21 to 43% of the initial value. Even larger decreases, up to nearly 58%, were recorded in studies by Pagilla et al. (2006).

The fertilizing degree of the Baltic Sea with biogenic compounds (including nitrogen) is very important for living conditions of organisms in these waters. An excess of biogenic compounds contributes to the intense eutrophication. Due to its specificity (the time period of total water exchange is very long, approx. 25—30 years) the Baltic Sea is particularly sensitive to the increased flow of nutrients. Based on the analysis of data from the year 2000 regarding the origin of biogenic compounds disposed to the Baltic Sea basin it was concluded, that up to 58% of nitrogen compounds originated from area sources, 32% constituted natural ambience, and 10% originated from point sources (treated municipal and industrial wastewater discharge) (HELCOM, 2005). In the year 2000 the total nitrogen load disposed to the Baltic Sea was 822 thousand tons of N year^{-1}, including Polish participation accounted for approx. 28%. The load disposed by rivers and point sources from the area of Poland was approx. 186.2 thousand tons of N year^{-1}, including 36.8 thousand tons of N year^{-1} as a discharge of treated municipal wastewater. As a result of constructing new and modernizing existing main and local treatment plants as a activated sludge, trickling filter or wetland systems (Obarska-Pempkowiak and Gajewska, 2003), in 2010 the load from municipal wastewaters was reduced by nearly 40% (to 22.4 thousand tons of N year^{-1}).

Reported effluent ON contributions vary widely in municipal WWTPs from less than 2% to 85% of the effluent TN (Gajewska, 2011; Pagilla et al., 2006; Pehlivanoglu-Mantas and Sedlak, 2008). Czerwionka et al. (2012) based on similar studies carried out in eight large municipal treatment plants with biological nutrients removal located in Northern Poland have concluded that the average DON concentration in treated wastewater varied from 0.5 to 1.3 g N m^{-3}. At the same time DON constituted 12—45% of ON contained in such wastewater. Within the scope of this study it was concluded that in the 2 analyzed WWTPs, the DON constituted 15—18% of TN contained in secondary effluents up to 50% of ON, which is within the variable range specified in prior studies. Assuming similar proportions for treated wastewater discharged from other municipal WWTPs in Poland it can be estimated, that the annual DON load discharged into the Baltic Sea waters in 2010 ranged from 3.4 to 4.0 thousand tons of N $year^{-1}$, whereby the annual load of bDON may reach up to 1.7 thousand tons of N $year^{-1}$.

This was approx. 7.5% of the total nitrogen load entering with secondary effluent from municipal wastewater treatment plants. It is also only 4 times lower load in relation to estimated submarine groundwater discharge for the entire Baltic Sea (7.1 thousand tons of N $year^{-1}$) (Szymczycha et al., 2012). This indicates the great importance of bDON on nitrogen balance for this basin.

4. Conclusions

An analysis of literary data and results achieved during conducted research shows that the use of the method involving determination of brDON based on a test similar to measurement of dissolved biodegradable organic carbon is a reliable method of determining its contribution. The only value which requires clarification in further studies is the recommended duration of the test which, depending on the authors, varies within a range between 7 and 30 days (Khan et al., 2009; Simsek et al., 2013).

The situation is drastically different in terms of determining the bDON fraction. The application of a standard test with Chlorophyta does not guarantee reliable and repeatable results of bDON content. Literature presents results of studies involving various methods, whereby currently it cannot be concluded which of them should be considered appropriate.

The results achieved during the performed studies indicate, that it is possible to significantly decrease the DON concentration at a biological level, ranging from 20 to 40% for the studied wastewater samples. Simultaneously, up to 43% of the DON load discharged into the water environment with biologically treated wastewater may be used by algae. Therefore, the DON should be considered as a form of nitrogen, which may have a significant and direct impact on the conditions obtaining at the treated wastewater receiving bodies.

The estimated annual bDON load deposited into Baltic Sea waters from Poland through discharge of biologically treated wastewater in the year 2010 reached 1.7 thousand tons of N $year^{-1}$.

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