

**REMOVAL OF NITROGEN COMPOUNDS
IN THE PROCESS OF AUTOTROPHIC
DENITRIFICATION IN A SEQUENCING BATCH
BIOFILM REACTOR (SBBR)**

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Key words: autotrophic denitrification, inorganic carbon, hydrogen gas, sequencing batch biofilm reactor (SBBR), synthetic wastewater.

Abstract

The paper discusses the impact of the C/N ratio of inorganic carbon (KHCO_3) and its interaction with carbon dioxide liberated by the oxygenation of a carbon electrode and with gaseous hydrogen produced by water electrolysis, on the concentration of oxygenated forms of nitrogen, during the process of autotrophic denitrification in a multi-cathode reactor with immobilized biofilm. The experiment was set under anaerobic conditions, at the electric current density of 79 mA/m^2 and the C/N ratios of 0.5, 0.75 and 1.0. The results showed that a higher dose of inorganic carbon (KHCO_3) significantly decreased the concentration of nitrate. The concentration of this form of nitrogen was even lower in a reactor additionally loaded with CO_2 and H_2 , in which the physicochemical parameters of sewage sludge (the redox potential and electrolytic conductivity) were therefore better for the denitrification process. Nitrate was not completely consumed by dissimilation reduction in either of the reactors. Some small amount of this compound was converted to the ammonium form on the assimilation pathway. In addition, the carbon electrode served as an acceptor of electrons, in the process of external oxygenation of organic compounds.

**USUWANIE ZWIĄZKÓW AZOTU W PROCESIE DENITRYFIKACJI AUTOTROFICZNEJ
W SEKWENCYJNYM REAKTORZE Z BŁONĄ BIOLOGICZNĄ (SBBR)**

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Słowa kluczowe: denitryfikacja autotroficzna, węgiel nieorganiczny, gazowy wodór, sekwencyjny reaktor z unieruchomioną błoną biologiczną (SBBR), ścieki syntetyczne.

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Abstrakt

W pracy przedstawiono wpływ stosunku C/N węgla nieorganicznego (KHCO_3) oraz jego współdziałania z dwutlenkiem węgla wydzielanym w wyniku utleniania elektrody węglowej i gazowym wodorem powstającym w procesie elektrolizy wody, na koncentrację utlenionych form azotu, w procesie autotroficznej denitryfikacji z wykorzystaniem reaktora wielo-katodowego z unieruchomioną błoną biologiczną (SBBR). Badania prowadzono w warunkach anaerobowych, przy gęstości prądu elektrycznego – 79 mA/m^2 i stosunku C/N = 0.5, 0.75, 1.0. Przeprowadzone badania pokazały, iż wzrost dawki węgla nieorganicznego (KHCO_3), istotnie wpłynął na zmniejszenie stężenia azotu azotanowego (V). W reaktorze wspomaganym dodatkowo CO_2 i H_2 ze względu na korzystniejsze, dla procesu denitryfikacji, parametry fizyko-chemiczne oczyszczanych ścieków (potencjał redox i przewodność elektrolityczna) odnotowano najniższą jego koncentrację. Azot azotanowy (III) w obu reaktorach, nie został całkowicie wykorzystany w redukcji dysymilacyjnej. Niewielka jego część przeszła w szlaku asymilacyjnym do formy amonowej. Elektroda węglowa pełniła dodatkowo rolę akceptora elektronów, w procesie zewnętrznego utleniania związków organicznych.

Introduction

Nitrates belong to compounds that dissolve very well in aquatic environment, which they enter from uncontrolled discharge of municipal and industrial wastewater, from landfill leachate and as a result of inadequate sewage and wastewater management solutions. A high concentration of this oxygenated form of nitrogen in surface waters has a negative impact on water, mostly by stimulating eutrophication. Moreover, nitrates can be transformed to nitrites in the digestive tract of animals and humans. In reaction with hemoglobin in blood, they cause methemoglobinemia, which inhibits the oxygen transport in cells. Bio-denitrification, both the heterotrophic and autotrophic one, has been known as the most frequently applied method in this respect (KARANASIOS et al. 2010, ZHAO et al. 2011). The heterotrophic denitrification, commonly applied in technological systems, consists in the consumption of organic compounds like methanol, ethanol or acetate by denitrifying bacteria as sources of carbon and electrons. Despite its high efficiency, this method has significant drawbacks that include the presence of carbon residues and process by-products in treated wastewaters and greater biomass growth from 0.6 to 0.9 g/gN- NO_3 (MATEJU et al. 1992, KULIKOWSKA et al. 2008).

Hence, it is essential to continue search for new technological solutions, which will ensure highly effective removal of these compounds. Recently, researchers have paid more attention to a possible combination of biological and physicochemical processes affected by an electric current. (KRZEMIENIEWSKI and RODZIEWICZ 2005, RODZIEWICZ et al. 2011, RODZIEWICZ et al. 2011a, RODZIEWICZ et al. 2011b, KŁODOWSKA et al. 2013). In order to reduce costs of providing a hydrogen donor (ex situ), researchers have postulated the use of an electro-biochemical reactor (in situ). The implementation of a bio-electrochemical reactor (SBBR), in which microorganisms are used to catalyze an

electrolytic process has been proven to be an effective solution. The biomass immobilized on the surface of a cathode (discs) acts as a biocatalyst accepting electrons, which is a better solution than expensive chemical catalysts. The process of electrolytically supported denitrification relies on the use of gaseous hydrogen biofilm, serving as substrate for microorganisms. This biofilm is produced on the surface of a cathode during the hydrolysis of water, which functions as a donor of electrons in the reduction of nitrates to gaseous nitrogen. Less biomass is generated during autotrophic denitrification than in the course of heterotrophic denitrification supplied with different carbon sources. The reason is that microorganisms expend more energy on digesting the compounds in which the degree of carbon oxygenation is higher than in biomass or which contain fewer C atoms (KULIKOWSKA et al. 2008). Low solubility of hydrogen (1.6 mg/L/bar at 20°C), no need for the removal of its excess and lack of process by-products make this method a good alternative to other electron donors for example: iron, sulfur, etc. (KULIKOWSKA et al. 2008, KARANASIOS et al. 2010, ZHAO et al. 2011). Autotrophic denitrification with a hydrogen donor can be additionally stimulated by inorganic substrate in the form of carbon dioxide, carbonates or bicarbonates. Carbon dioxide produced inside the system in the course of oxygenation of the carbon electrode (anode) and dosed bicarbonates may serve as an additional source of inorganic carbon for microorganisms. These compounds neutralize the reaction by capturing hydroxyl ions, while CO₂ ensures that appropriate anaerobic conditions are maintained for the growth of biofilm (ZHOU et al. 2007, SUKKASEM et al. 2008, ZHAO et al. 2011). Ions HCO₃⁻ and CO₃²⁻ produced by the dissociation of bicarbonates and carbon dioxide can improve the conditions for the course of hydrogenotrophic denitrification by stimulating a higher electrolytical conductivity of sewage and wastewater.

The available literature does not mention the possibility of supporting hydrogenotrophic denitrification with inorganic carbon in the form of potassium bicarbonate, or its interaction with carbon dioxide released by oxygenation of a carbon electrode and gaseous hydrogen generated during the electrolysis of water. This has encouraged the authors to examine the effect of the C/N ratio of inorganic carbon (KHCO₃) on changes in the concentration of oxygenated nitrogen forms, in the process of autotrophic denitrification, using a multi-cathode reactor with immobilized biofilm (SBBR).

Material and Methods

The research was conducted on synthetic wastewater characterized by a low concentration of organic compounds and a high concentration of

oxygenated forms of nitrogen. Synthetic wastewater was produced as follows: 2.0 dm³ of tap water was mixed with enriched broth (0.08 g/dm³), NaNO₃ (30.36 g/dm³), KCl (21 g/dm³), MgSO₄ · 7H₂O (307.5 g/dm³) and CaCl₂ (21 g/dm³).

The average values of physicochemical parameters are given below:

- concentration of nitrates – 52.09 [mgN_{NO₃}/dm³],
- concentration of organic compounds (ChZT_{Cr}) – 70.57 [mgO₂/dm³],
- concentration of total organic carbon (TOC) for C/N ratio of 0.5, 0.75 and 1.0 – 31.89, 33.70, 35.29 [mgC/dm³],
- concentration of inorganic carbon (IC) for C/N ratio of 0.5, 0.75 and 1.0 – 49.70, 69.50, 83.20 [mgC/dm³],
- pH value – 7.5 [-],
- redox potential – 131.14 [mV],
- electrolytic conductivity – 1.58 [mS/cm].

The experiment was run in parallel, in 2 sequential batch biofilm reactors (SBBR) with vertical cylinders of the capacity of 3.0 dm³, working under anaerobic conditions (Figure 1). Each reactor contained a set of 12 stainless

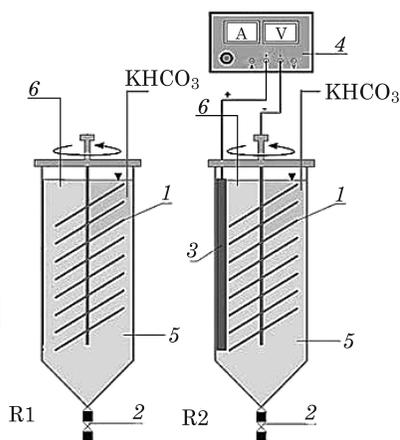


Fig. 1. The scheme of an experimental installation with anaerobic rotating multi-disc reactor: 1 – stainless steel cathode – discs with biomass, 2 – outlet, 3 – carbon anode, 4 – electric current source, 5 – reactor: R1 – control reactor 1 (KHCO₃), R2 – reactor 2 (CO₂ + KHCO₃ + H₂), 6 – inlet

steel cathodes (discs) with immobilized biofilm, each of the diameter of 0.10 m and total surface of 0.19 m². The discs fitted axially on a vertical shaft rotated at a speed of 10 rev./min, and were 100% submerged. Reactor 1 (R1) was a control system, unaffected by an electric current. In contrast, reactor 2 (R2) worked under an electric current flow. It contained a carbon steel anode, whose total surface area was 0.003 m². Both reactors (R1 and R2) were fed

potassium bicarbonate (KHCO_3) at the C/N ratio of 0.5, 0.75 and 1.0. The cathode and anode were connected to a laboratory power supply to ensure the required electric current, i.e. 15 mA, 3.0 V (electric current density of 79 mA/m^2). Prior to the proper tests, the reactors had been working until an adequate structure of the biofilm and a stable concentration of nitrates in the outflow were achieved, which took three months. Samples for analyses were taken once a day (1.0 dm^3). Afterwards, the reactors were emptied (1.0 dm^3) and refilled with sewage (2.0 dm^3).

The following physicochemical analyses were made in raw and treated wastewater:

- nitrates – with the colorimetric method [ISO 7890-3:1988],
- nitrites – with the colorimetric method [ISO 6777:1984],
- ammonia – with the colorimetric method [PN – 73/C-04576/01],
- organic compounds (COD) – with the dichromate method [ISO 6060:1989],
- total organic carbon and inorganic carbon – with an IL 550 TOC-TN analyzer by Hach,
- pH value – with an HI 123 pH meter by Hanna Instruments,
- electrolytic conductivity – with an HI 99301 conductivity meter by Hanna Instruments,
- redox potential – with an pH 211 meter by Hanna Instruments.

Results and Discussion

The experiments have demonstrated that an increased dose of KHCO_3 resulted in a decreased concentration of nitrate in treated wastewater, but did not guarantee a complete run of the autotrophic denitrification process. The said process proceeded through two steps: to ammonia nitrogen on the assimilation path and to molecular nitrogen by the dissimilation reduction. According to KLIMIUK and ŁEBKOWSKA (2008), the assimilation path is catalyzed by nitrate reductase type B (constitutive enzyme), while nitrate reductase type A (inductive enzyme) is the catalyst of the dissimilation.

In the R2 system, where – apart from potassium bicarbonate – the reactor was supplied with carbon dioxide produced by the anodic oxygenation of a carbon electrode and with gaseous hydrogen generated by water electrolysis, at the initial value of $52.09 \text{ mg NNO}_3/\text{dm}^3$, the final concentration of nitrate decreased to $12.38 (\pm 0.64) \text{ mg NNO}_3/\text{dm}^3$ at C/N equal 0.5, $8.44 (\pm 0.88) \text{ mg NNO}_3/\text{dm}^3$ at C/N = 0.75, and $4.46 (\pm 0.26) \text{ mg NNO}_3/\text{dm}^3$ at C/N = 1.0 (Figure 3). At the same time, the consumption of KHCO_3 decreased as its dose increased from 31.51% (± 5.86) (C/N = 0.5) to 15.67% (± 3.37) (C/N = 1.0), while

the percentage of used of organic carbon rose from 73.77% (± 2.98) (C/N = 0.5) to 76.19% (± 2.50) (C/N = 1.0) (Figure 2).

In the R1 system fed with KHCO_3 alone, a much higher concentration of the examined nitrogen form was recorded in the discharge: 32.66 (± 3.47) mg NNO_3/dm^3 (C/N = 0.5), 24.63 (± 2.38) mg NNO_3/dm^3 (C/N = 0.75) and 18.34 (± 0.58) mg NNO_3/dm^3 (C/N = 1.0) (Figure 3). Same as in reactor 2, the percentage of used potassium bicarbonate fell from 21.23% (± 2.32) (C/N = 0.5) to 10.73% (± 2.49) (C/N = 1.0) to the advantage of organic carbon, rising from 67.67% (± 2.94) (C/N = 0.5) to 67.74% (± 3.69) (C/N = 1.0) (Figure 2). This substantial decrease of the total carbon in the outflow of treated raw sewage is attributed to the fact that some of the organic form of carbon was converted to inorganic form; another reason is the participation of autotrophic and heterotrophic bacteria in the wastewater treatment process.

The nitrite in the outflow from both reactors, shown in figure 3, was not completely used up on the dissimilation pathway and a small amount of this compound changed into the ammonium form by the assimilation reduction. According to SZEWCZYK (2005), ammonium ions are used by microorganisms to build their cells. Thus, denitrification was participated by “*true denitrifying*” bacteria, which used nitrite as an acceptor of electrons in the conversion of nitrate to gaseous nitrogen, and by “*nitrate respiring*” bacteria, which did not take part in the transformation of atmospheric nitrogen (GLASS and SILVERSTEIN 1998). These microorganism are characterized by a much faster growth than “*true denitrifying*” bacteria (WILDERER et al. 1987). The lowest concentration of nitrite was recorded in reactor 1. On average, it was 2.26 (± 1.18) mg NNO_2/dm^3 at the C/N ratio of 0.5; a large decline, down to 0.17 (± 0.34) mg NNO_2/dm^3 , was observed at C/N = 0.75, and a rise to 0.40 (± 0.27) mg NNO_2/dm^3 appeared at C/N = 1.0.

The ammonium nitrogen concentration was low. An increase in the dose of inorganic carbon (KHCO_3) caused an increase in the outflow of this nitrogen form. On average, it reached 0.52 (± 0.46) mg NNH_4/dm^3 at C/N = 0.5, 2.42 (± 0.36) mg NNH_4/dm^3 at C/N = 0.75 and 2.85 (± 1.00) mg NNH_4/dm^3 at C/N = 1.0. A much higher nitrite concentration was obtained in reactor 2. At the lowest carbon to nitrogen ratio (C/N=0.5), it was 5.88 (± 0.68) mg NNO_2/dm^3 on average. A small decrease was noticed at the C/N ratio of 0.75 (5.60 (± 0.31) mg NNO_2/dm^3), but then again the level of this nitrogen form increased at a higher C/N proportion (6.43 (± 0.35) mg NNO_2/dm^3 at C/N = 1.0). Compared to reactor 1, reactor 2 had slightly higher concentrations of ammonia nitrogen at the C/N ratios of 0.5 and 0.75 (1.08 (± 0.61) mg NNH_4/dm^3 , 3.06 (± 0.54) mg NNH_4/dm^3) and a lower concentration of this compound at C/N = 1.0 (1.16 (± 0.19) mg NNH_4/dm^3).

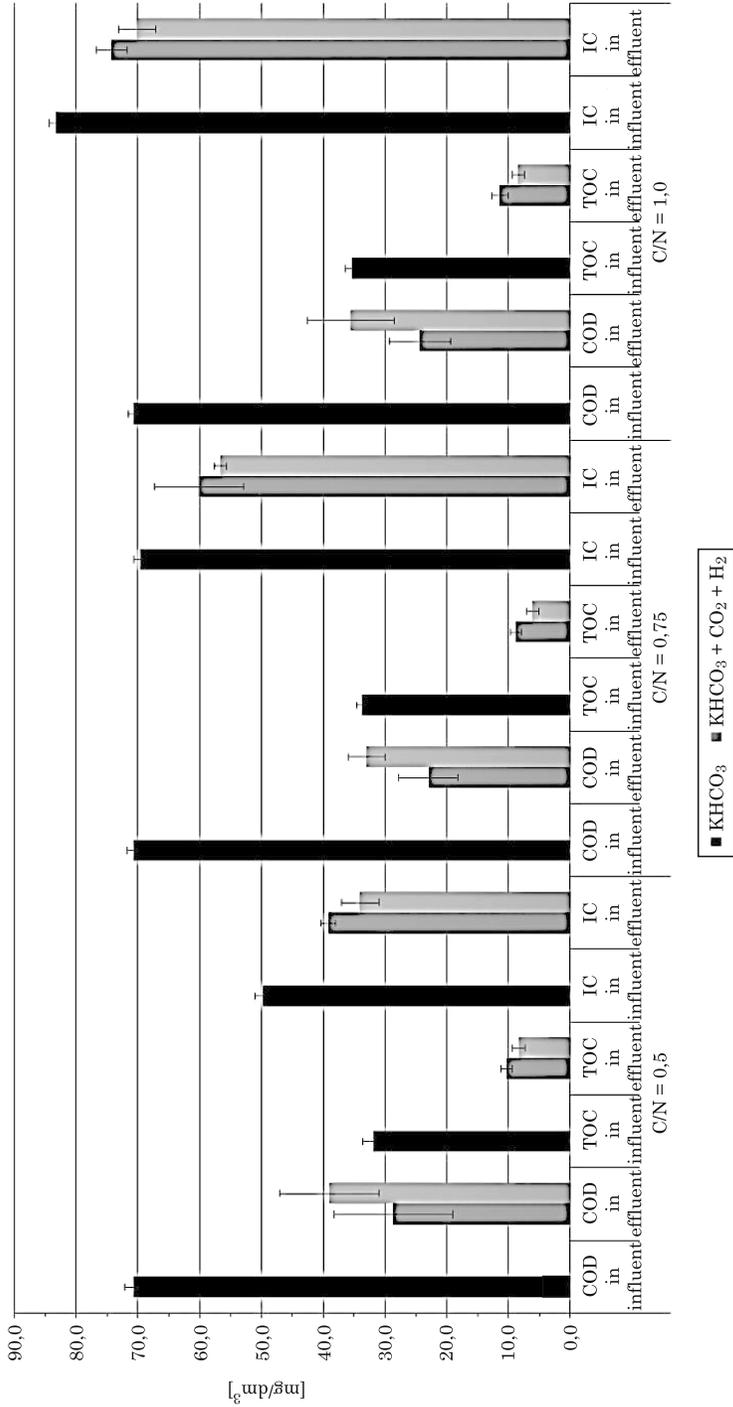


Fig. 2. Effect of C / N ratio on the concentration of organic compounds (COD), total organic carbon (TOC) and inorganic carbon (IC)

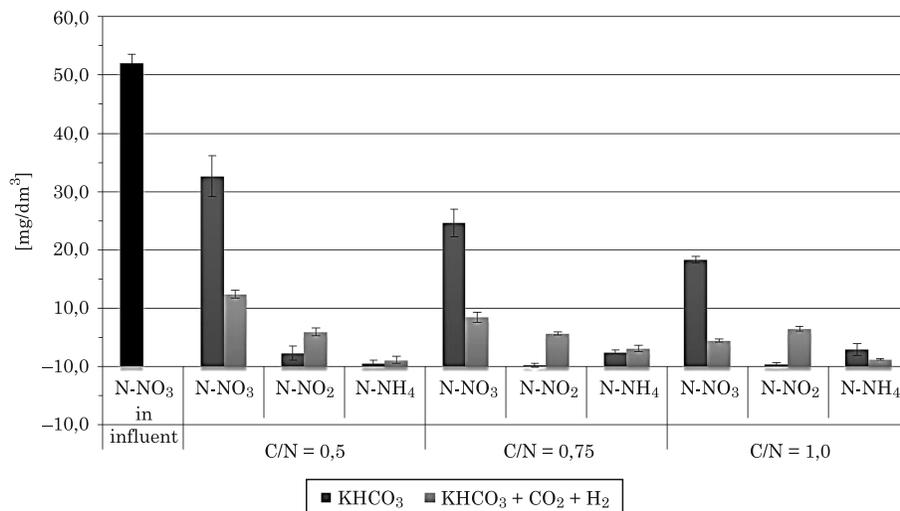


Fig. 3. Effect of C/N ratio on the concentration of mineral forms of nitrogen

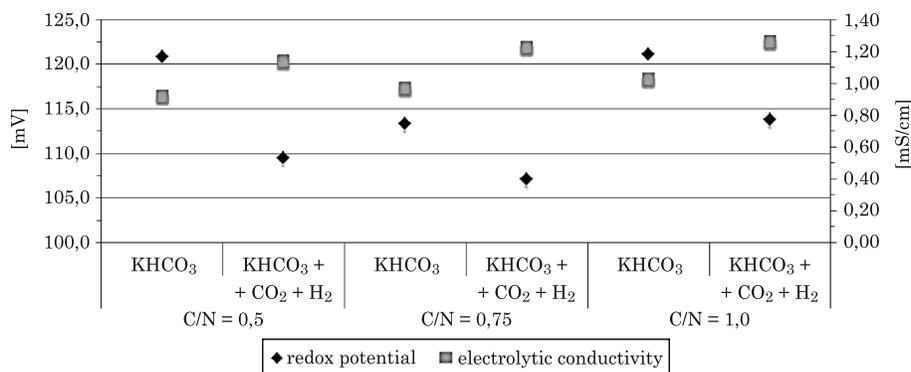


Fig. 4. Effect of C / N ratio to change the electrolytic conductivity and redox potential

Analogously to other studies (KURODA et al. 1996, KURODA et al. 1997, ZHOU et al. 2009), the COD value decreased (Figure 2). In reactor 2, organic substances were oxygenated by both microorganisms and hydroxyl radicals, generated by the oxygenation of water on the surface of the anode. During the flow of an electric current, the carbon electrode played the role of an electron acceptor in the process of external oxygenation of organic compounds, and was a source of CO₂ in the process of hydrogenotrophic denitrification. In raw sewage, organic compounds expressed by the COD index value, made up 70.71 mgO₂/dm³. In the discharge from the control system (R1), the average concentration of COD at C/N = 0.5 was 28.68 (±9.61) mgO₂/dm³; at the higher value of C/N = 0.75,

it fell to 22.93 (± 4.81) mgO_2/dm^3 , and for the highest C/N ratio of 1.0, it equalled 24.30 (± 4.94) mgO_2/dm^3 . A higher concentration of the discussed parameter was observed in the R2 system. At the C/N ratio of 0.5, it was 39.04 (± 8.19) mgO_2/dm^3 , it equalled 32.97 (± 3.64) mgO_2/dm^3 at C/N = 0.75 and reached 35.53 (± 7.80) mgO_2/dm^3 at the highest C/N ratio of 1.0.

Under the increased dose of potassium bicarbonate and availability of an additional source of carbon in reactor 2, such as carbon dioxide released from the oxygenation of a carbon electrode, an increase in the conductivity of wastewater and a large decrease in the redox potential appeared, which was in contrast to the process in reactor 1 (Figure 4).

A much larger decrease in the redox potential in reactor 2 was mainly caused by a much more intensive course of the denitrification process in that reactor. The higher conductivity was a result of the dissociation of potassium bicarbonate and hydrolysis of HCO_3^- :



as well as the oxygenation of the carbon electrode. The carbon dioxide generated during the oxygenation of the anode underwent further dissociation according to the following reaction:



As suggested by the course of the above reactions, amounts of soluble ions HCO_3^- and CO_3^{2-} in treated wastewater in R2 were higher than in R1.

Effect of inorganic carbon of C/N ratio and electric current density on the number of denitrifying bacteria (MPN), will be subject for further research.

Conclusions

1. An increase in the dose of inorganic carbon (KHCO_3) had a significant influence on the decrease in nitrate. In reactor 2, additionally loaded with CO_2 and H_2 , the concentration of nitrate was the lowest.

2. In neither of the reactors, nitrite was completely used up during the dissimilation reduction. A small amount of this compound was converted to the ammonium form on the assimilation pathway.

3. The substantial decrease in the discharge of total organic carbon available in raw sewage proves that some of the organic form of carbon has been converted to the inorganic form; it also indicates the participation of autotrophic and heterotrophic bacteria in the process of wastewater purification.

4. Increasing the C/N ratio of potassium bicarbonate (KHCO_3) as well as the presence of larger amounts of CO_3^{2-} and HCO_3^- ions originating from the dissociation of KHCO_3 and CO_2 , prevented any larger decrease in the electrolytic conductivity in reactor 2.

5. The carbon electrode served as an acceptor of electrons during the process of oxygenation of organic compounds (COD).

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