The assessment of the coke wastewater treatment efficacy in rotating biological contractor

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ABSTRACT

Coke wastewater is known to be relatively difficult for biological treatment. Nonetheless, biofilm-based systems seem to be promising tool for such treatment. That is why a rotating biological contactor (RBC) system focused on the Anammox process was used in this study. The experiment was divided into two parts with synthetic and then real wastewater. It was proven that it is possible to treat coke wastewater with RBC but such a procedure requires a very long start-up period for the nitritation (190 days), as well as for the Anammox process, where stable nitrogen removal over 70% was achieved after 400 days of experiment. Interestingly, it was possible at a relatively low (20.2 ± 2.2 °C) temperature. The polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) based monitoring of the bacterial community showed that its biodiversity decreased when the real wastewater was treated and it was composed mainly of GC-rich genotypes, probably because of the modeling influence of this wastewater and the genotypes specialization.

Key words | biofilm, coke wastewater treatment, PCR-DGGE, rotating biological contractor

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INTRODUCTION

The state of the art wastewater treatment trends are focused on biological methods. They are effective in municipal wastewater treatment but, in the case of industrial sewage, biology-based methods can be difficult or, in some cases, impossible to use. The substances in industrial wastewater can be harmful to the bacteria performing biological treatment, causing bacterial community impoverishment and even its total destruction. Coke wastewater belongs to the industrial wastewater which is difficult for biological treatment due to its complex and changeable composition and the presence of such toxic substances as ammonia, thiocyanides, cyanides, sulfides, phenols and polycyclic aromatic hydrocarbons (Park *et al.* 2008; Chu *et al.* 2014). In Poland, especially in areas with heavy industry, development in coke production, as well as coke wastewater volume, is increasing. To fulfill European Union regulations towards high quality effluent obtainment, modern technological systems are required. This has led to the search for effective wastewater treatment technologies.

When considering wastewater treatment, nitrogen is one of two main biogens which should be eliminated from the wastewater effectively. In a typical wastewater treatment plant (WWTP) system a combination of nitrification and denitrification is used. However, in the case of coke wastewater, the high concentration of toxic pollutants can heavily inhibit the biological activity of nitrifying and denitrifying bacteria (Amor *et al.* 2005; Eiroa *et al.* 2005; Kim *et al.* 2008). Nevertheless, there is an effort to develop biological treatment methods for such streams as they are cheaper and more environmentally friendly than chemical methods. For biological treatment of coke wastewater, various reactor types and configurations have been suggested, such as anoxic–aerobic reactors, anaerobic–anoxic reactors, sequencing batch reactors, fixed bed biofilm reactors, membrane-based reactors and others (Zhao *et al.* 2009; Gu *et al.* 2014). Among various proposed processes, the pre-denitrification process deserves particular attention due to its simplicity and economic benefits (Kim *et al.* 2008; Park *et al.* 2008). More recently, the ammonia oxidation (nitritation)–Anammox process has been developed for treatment of nitrogen rich streams (Strous *et al.* 1997). The main advantages of this process compared to the conventional nitrification/denitrification are: low sludge production, a decrease in aeration costs by almost 60% (only half of the ammonia is oxidized to nitrite in the nitritation process without further oxidation to nitrate), and no need for an external organic carbon source addition (in the Anammox process)

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(De Clippeleir *et al.* 2011). Additionally, Anammox enables a lowered CO_2 emission in comparison to nitrification/denitrification. The disadvantage of the Anammox process is slow biomass growth and difficulties in creating favorable technological conditions. The Anammox process is also sensitive to various factors such as substrates (ammonia and nitrite), organic matter, salts, heavy metals and many others (Jin *et al.* 2012). There are also reports showing that phenol, which is present in coke wastewater, inhibits the Anammox process (Toh & Ashbolt 2002; Yang *et al.* 2013; Pereira *et al.* 2014). The addition of phenol not only suppresses the Anammox activity but also changes the stoichiometrics ratios and the microbial community structure and composition (Yang *et al.* 2013; Pereira *et al.* 2014). What is more, due to the presence of refractory and toxic compounds in coke wastewater, the nitrifying bacteria and other specialized microbes (as Anammox) can be easily washed out of the system due to their inhibited growth. Moreover, when the activated sludge is operated under high loading rates, problems with poor settleability were reported (Gu *et al.* 2014). As it has been previously described (Xiao *et al.* 2009; Langone *et al.* 2014), industrial wastewater such as coke wastewater can cause damage to activated sludge – a mixture of *bacteria*, *metazoa* and *protozoa* functioning in WWTP's bioreactors as suspended flocs. That is why biofilms, three dimensional structures containing extrapolimeric substances as a protective matrix, can be useful in difficult wastewater treatment without harming the bacterial community (Hall-Stoodley *et al.* 2004). The technological system containing biofilm which could be used in coke wastewater treatment is the rotating biological contractor (RBC).

The aim of this work was to estimate the possibility and the effectiveness of phenol and nitrogen rich wastewater treatment in laboratory-scale rotatory biological contractor. The experiment was divided into two parts: with synthetic phenolic wastewater and with real coke wastewater as an example of the industrial wastewater containing phenol and high concentration of nitrogen. The biofilm bacterial community was monitored with polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) in order to present the impact of the medium change on the bacteria performing the removal processes.

MATERIALS AND METHODS

Technological setting of the experiment

The experiment was performed with a laboratory-scale RBC divided into three chambers in a series. The RBC unit was covered by polystyrene foam to prevent the growth of algae through light exclusion and to help maintain a stable temperature. Figure 1 presents the RBC scheme, Table 1 presents RBC technological parameters. The first chamber was fed constantly with wastewater by peristaltic pump (ISMATEC reglo digital), then the medium was directed gravitationally to the second and third chamber.

The experiment was performed for 719 days with synthetic medium (Table 2), then for 192 days with real coke wastewater from Jadwiga coke plant in Zabrze Poland (Table 3).

During the research, samples were collected from the influent, effluent and each stage of the contactor. The efficiency of the biological treatment was followed in terms of

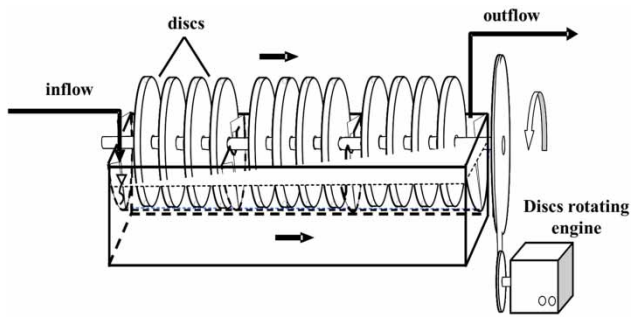

Figure 1 | RBC scheme.

Table 1 | RBC technological parameters

Parameter	Unit	Value
Chamber number	–	3
Number of discs per chamber	–	4
Total disc number	–	12
Disc diameter	m	0.225
Total disc surface	m^2	2.61
Disc immersion	%	41
Working volume	m^3	0.014

Table 2 | Synthetic medium characteristics

Compound	Concentration [g/L]
NH_4Cl*	3.45
KH_2PO_4*	0.006
CH_3COONa*	2.0
C_6H_5OH	0.15
$NaHCO_3$	7

*$N\text{-}NH_4$ – 900 mg/L; P – 1 mg/L and theoretical COD of sodium acetate was 1,560 mg/L.

Table 3 | Real coke wastewater characteristic

Compound	Concentration [mg/L]
$N\text{-}NH_4$	475.7 ± 56.8
COD	692.5 ± 219.5
C_6H_5OH	148.8 ± 80.6

the general parameters such as: chemical oxygen demand (COD) (dichromate method), phenol concentration (Merck tests), ammonia nitrite, and nitrate nitrogen forms (Merck tests). The process was monitored by measuring other parameters: flow rate temperature, pH (WTW pH 340i), and dissolved oxygen (WTW Oxi 340i). No specific heating was applied and the temperature was kept at the level of 20.2 ± 2.2 °C.

Inoculum characteristics

The RBC used in this experiment was previously performing nitrogen removal from landfill leachate (Cema 2010) and then reject water derived from municipal WWTP in Zabrze, Poland was treated (data not published). The influent NH_4-N and COD concentrations were on the level of 1,010 mg/L and 1,427 mg/L, respectively. Microbial analysis (fluorescence *in situ* hybridization (FISH)) confirmed the coexistence of nitrifiers and the Anammox bacteria belonging to *Candidatus* Brocadia anammoxidans and/or *Candidatus* Kuenenia stuttgartiensis in the RBC.

Biofilm sampling, DNA isolation and PCR-DGGE monitoring of bacterial community

Biofilm samples (volume of 50 ml) were collected from all three of the RBC chambers, vortexed and stored at −20 °C until DNA isolation.

Total genomic DNA was extracted from 0.2 g of the biofilm samples using the mechanical method. The samples were washed three times with 1× phosphate-buffered saline (PBS) buffer (Sigma) and disintegrated with bead beating (Roth, Germany) in lysis buffer (Tris–HCl 100 mM, EDTA 100 mM, NaCl 1.5 M; pH = 8.0). The samples were incubated 20 minutes in 1,400 rpm and 200 µl 10% sodium dodecyl sulphate (SDS) was added. After 30 minutes of incubation in 65 °C samples were centrifuged twice at 13,000 rpm and placed on spin filters (A&A Biotechnology). DNA attached to the filter was washed twice with 70% ethanol solution (A&A Biotechnology). The amount of DNA was measured spectrophotometrically using Qubit (Invitrogen) and stored at −20 °C until PCR amplification.

Partial 16S rRNA gene amplification of all the bacteria was performed using primers: 338f-GC and 518r gene fragment (Muyzer *et al.* 1993). PCR reaction was performed in 30 µl mixture and the amplification was performed in thermocycler T-1000 (Bio-Rad) as previously described (Ziembińska *et al.* 2009).

The DGGE of the PCR products obtained in reactions with 338F-GC/518R primers underwent electrophoretic separation in the DCode Universal Mutation Detection System (BioRad). Polyacrylamide gel (8%, 37:1 acrylamide–bisacrylamide, Fluka) with a gradient of 30–60% denaturant was prepared according to the manufacturer's instruction. The gel was run for 17 h at 40 V in a 1× TAE buffer at a constant temperature of 60 °C and stained as previously described (Ziembińska *et al.* 2009).

The analysis of DGGE fingerprints was performed using Quantity One 1D software (BioRad). Bacterial biodiversity was estimated on the basis of densitometric measurements and Shannon diversity index as previously described (Ziembińska *et al.* 2009). Dendrogram was constructed on the basis of the neighbor-joining algorithm with Dice coefficient.

RESULTS AND DISCUSSION

In this experiment, the RBC was fed for 719 days with synthetic phenolic wastewater (period I) and for 192 days with real coke wastewater (period II) derived from Jadwiga coke plant in Zabrze, Poland. The temperature during the experiment was 20.2 ± 2.2 °C and was much lower than the temperature of 37 °C which is usually reported as an optimum value for the Anammox process (Schmidt *et al.* 2003). The pH value in the inflow did not exceed 8.3 ± 0.3. During the operational period I, a slight increase in the pH value between the influent and chamber I was observed (to 8.6 on average) and then a slight decrease to 8.0 on average. This phenomenon can be explained by three different processes overlapped in the contactor. During the nitrification process, a decrease in pH value is normally observed due to alkalinity reduction. The alkalinity depletion is partially recovered during denitrification process by production of one equivalent of alkalinity for one equivalent of NO_3-N reduced. Additionally, an increase in pH value is expected in the Anammox process due to consumption of hydrogen ions during cell synthesis. In the part of the experiment utilizing real coke wastewater treatment, the pH value dropped to 6.5 in the first chamber. The average hydraulic

retention time was 4 days and the average wastewater inflow was 3.5 L/d.

In the first part of the experiment (period I) when the synthetic wastewater was directed to the system, the average COD value in the inflow was 1264.6 ± 454.4 mgO_2/L, COD removal was 62.5 ± 22.5%. From day 719 of the experiment real coke wastewater was directed to the system with organic load ranging between 350–675 mg O_2/L (the average COD value in the influent was 495 mg O_2/L, median was 425 mg O_2/L). During the total length of the real wastewater treatment (period II), the organic matter removal was unstable and it fluctuated between 1 and 80%, with an average value of 45.3%.

The average phenol concentration in the inflow was equal to 151.1 ± 44.1 mg/L (median 169.5 mg/L). In the beginning of the experiment on synthetic wastewater, stable nitrogen removal was difficult to maintain. Pereira *et al.* (2014) observed the reduction of nitrogen removal efficiency from 96 to 47% with phenol concentration equal to 300 mg/L and no negative with phenol concentration up to 200 mg/L. However, in their experiment, the Anammox process was already established. In our case, we started to add phenol from the beginning of the reactor operation. That is why we decided to decrease phenol concentration in the wastewater from 150 to 20 mg/L. From day 230 of the experiment the concentration of the phenol was increasing to a value of 239 mg/L at day 405. In the first phase and during the entire length of the experiment performed on synthetic wastewater, phenol was removed with high effectiveness (average 96.8 ± 5.5). The concentration of ammonia nitrogen directed to the system was maintained between 310–1,207 mg NH_4^+-N/L, with an average value 748.5 ± 224.9 mg N-NH_4/L. In the beginning stage of the experiment NH_4^+-N removal was disturbed and the average removal was 67%. From 190 day to the end of the period I of the experiment performed on the synthetic wastewater, high and stable ammonia nitrogen removal (96.7 ± 4.4%) was maintained (Figure 2).

In the beginning of period II, a breakdown in ammonium nitrogen removal was observed. It seems that toxic substances present in real coke wastewater seriously affected the nitrification process. After 15 days of real wastewater dozing, the ammonium nitrogen removal dropped to only 10.9%. However, after that time, a gradual process of restoration was observed and after 50 days the ammonium oxidation exceeded 50%.

In the RBC effluent NH_4-N was present until 370 day of the experiment, after this time its concentration was not higher than 10 mg N-NH_4^+/L. Within the first 36 days of the experiment the main nitrogen form present in the effluent was ammonium whereas nitrite and nitrate concentration did not exceed 2 mg N/L. From day 36 of the experiment nitrite was cumulated in the system even to a value of 300 mg NO_2^--N/L (average value for days 36 to 220 of the experiment was 202.6 mg NO_2^--N/L) (Figure 3). Such a high concentration for a longer period of time did not favor the Anammox process in the RBC. However, according to bibliographic data there are discrepancies about the level of nitrite nitrogen concentration toxic towards Anammox, some authors assumed that a

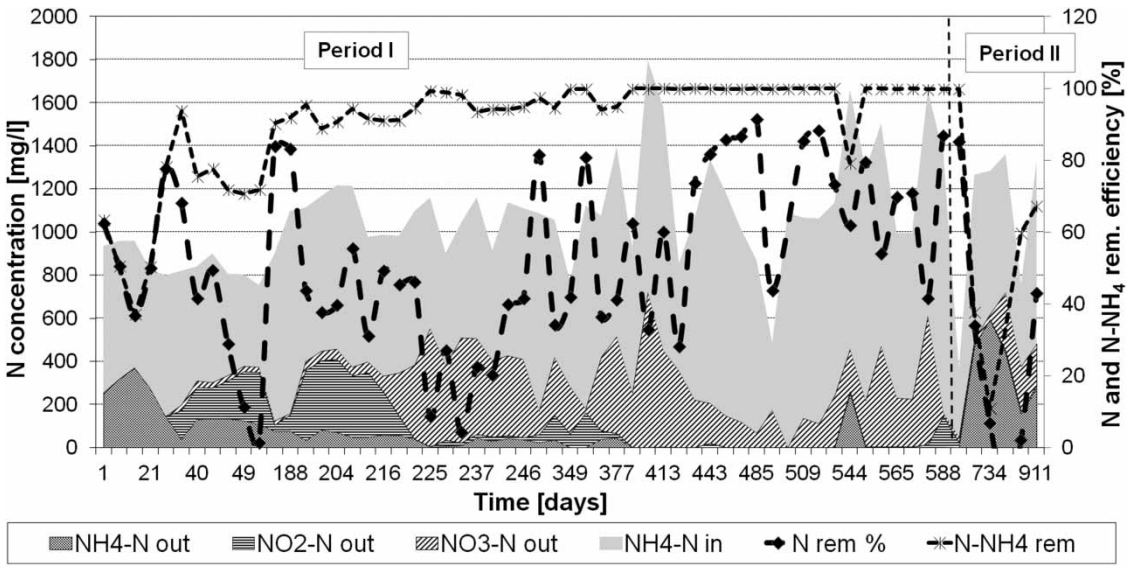

Figure 2 | Nitrogen compounds concentration changes in RBC during synthetic (period I) and real (period II) coke wastewater treatment and nitrogen removal efficiency.

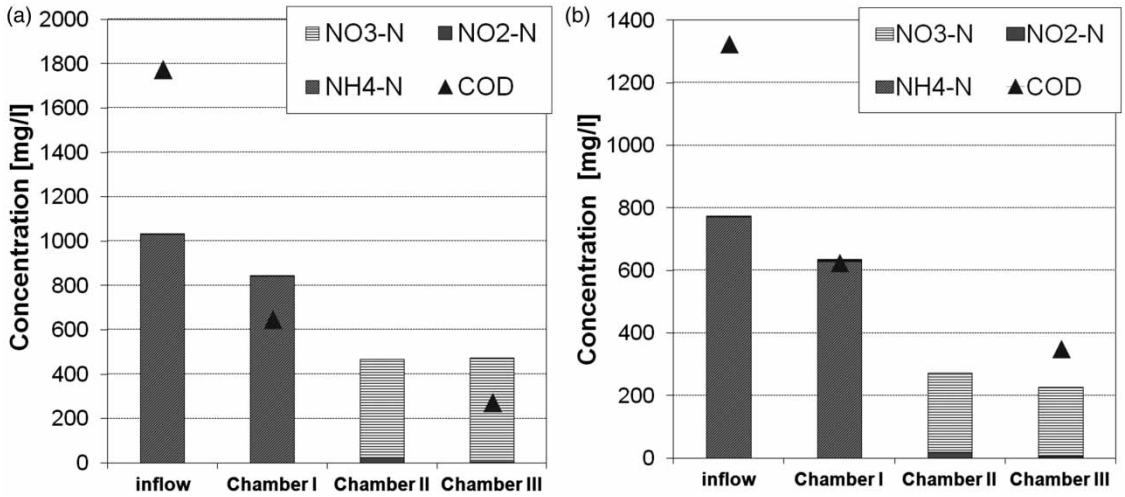

Figure 3 | The examples of COD and nitrogen removal values for synthetic wastewater on days 558 (a) and 573 (b) of the experiment.

concentration above 70 mg/L for a longer period of time inhibits Anammox completely (Op den Camp *et al.* 2007). On the other hand, Dapena-Mora *et al.* (2007) reported nitrite concentrations as high as 350 mg/L causing only 50% inhibition. Also, Lotti *et al.* (2012) claimed that nitrite was responsible for 50% process inhibition at a concentration of nitrite equal to 400 mg/L. In the next step of the research (after 220th day of the experiment), the decrease in nitrite in the effluent was observed in which nitrate was also observed.

The average total nitrogen removal was $53.9 \pm 24.8\%$ but its removal was not stable in both periods of the experiment (Figure 4). At day 430 of the experiment, nitrogen removal reached an average level of $43.6 \pm 21.4\%$. Here, the nitrogen removal was caused mainly by the denitrification process. Anammox was inhibited by very high nitrite concentration within the RBC. From day 429 of experiment to the end of period I, the nitrogen removal reached an average level of $75.5 \pm 16.2\%$.

The COD requirement for nitrate denitrification is 2.9 g COD/g N-NO_3. Courtens *et al.* (2014) showed, that for denitrification with acetate as carbon source, the ration of 3.6 $gCOD_{removed}/gN_{removed}$ was measured in mesophilic condition, and additionally for lower temperatures, this ratio was even higher. In our reactor, the ratio of $COD_{removed}/N_{removed}$ was equal to 1.5 (between days 430 and 719 of the experiment) so it may be stated, that denitrification could not be the main process responsible for nitrogen removal. Especially if we consider that the main part of

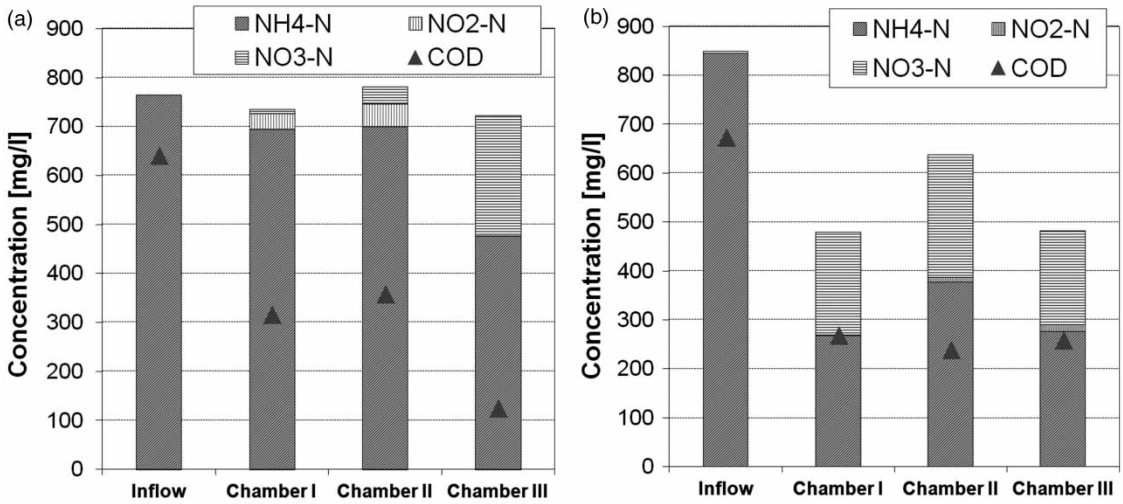

Figure 4 | The examples of COD and nitrogen removal values for real wastewater on days 719 (a) and 911 (b) of the experiment.

COD was removed in the first chamber (COD removal at the level of 55% with ca. 20% of nitrogen removal), whereas the nitrogen was removed mainly in the second chamber of RBC (effectiveness ca. 61%) (Figure 3(a) and (b)). In the first chamber, there was additionally a very high concentration of free ammonia (even over 200 mg NH_3/L) which is considered an Anammox process inhibitor even at low concentrations of 20–25 mg NH_3/L for continuous operation (Fernandez *et al.* 2012). High free ammonia concentration is also an inhibitor of the nitrification process, thus, the main nitrogen conversion took place in the second chamber with the Anammox process responsible for the nitrogen removal as COD being removed mainly in first chamber. Generally, according to Jenni *et al.* (2014) it should be stated that in a single-stage process with partial nitritation and Anammox with high COD/N ratio, it is not possible to assess the activities of the different bacterial groups based on mass balances. Additionally, in some cases the process instabilities were observed in systems, where elevated COD/N ration were in the influent to the system (Jenni *et al.* 2014).

The change from synthetic to real coke wastewater caused the nearly complete inhibition of nitrogen removal. Directly before medium change from synthetic to real wastewater, the nitrogen removal averaged over 75%, while in the first day after the real coke wastewater addition, it plummeted to 34%, and by day 15, a further drop to only 6% (Figures 4(a) and 5). At the end of the experiment, the nitrogen removal increased slightly to a value of 43%. This process was performed mainly in the first RBC chamber as opposed to the synthetic wastewater where nitrogen removal predominated in the second RBC chamber (Figure 4(b)). Such a decrease in the nitrogen removal effectiveness can most probably be explained by the toxic compounds present in the real coke wastewater influencing bacterial performance in RBC biofilm.

The nitrogen load varied from 0.44 to 2.12 g N/m^{2}d, with an average of 1.11 g N/m^{2}d (Figure 5). The lowest load was maintained in the first period of the experiment (to day 400 of the experiment) when the problems with effective nitrogen removal were observed. The load increased and from day 430 of the experiment it averaged 1.3 g N/m^{2}d. Nitrogen removal effectiveness was fluctuating between 0.03 and 1.53 g N/m^{2}d and during the first period of the experiment it stabilized at a level of 0.7 g N/m^{2}d. From day 430 of the experiment nitrogen removal settled and its average removal rate value was 0.98 g N/m^{2}d. In the second period of the experiment, the nitrogen load changed; it gravitated between 0.5 and 1.1 g N/m^{2}d, with an average value of 0.8 g N/m^{2}d, lower than in the case of synthetic wastewater (average 1.1 g N/m^{2}d).

Biological wastewater treatment is performed by microorganisms living in the technological system, linked together with ecological relationships as in natural habitats. Understanding the factors influencing the stability of such industrial ecosystems is a topic for years of research (Fernández *et al.* 1999). The mechanisms maintaining the stability of the ecosystem have not precisely been explained

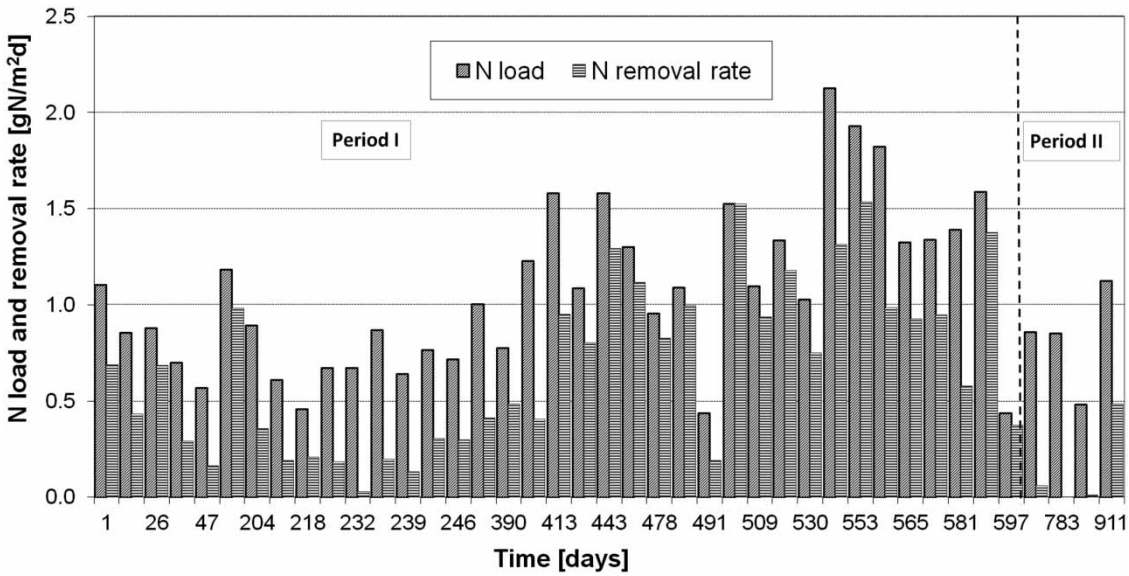

Figure 5 | Nitrogen loads and its removal in RBC during synthetic (period I) and real (period II) coke wastewater treatment.

and ecosystem destabilization could be linked with measurable parameters describing the total ecological system or only the structure and dynamics of its biocenosis. In this experiment the stability of the biofilm bacterial biocenosis in the RBC was influenced by a change in feeding medium. In period I (until day 719 of the experiment) it was synthetic, and in period II (after day 719) it was real coke wastewater. The composition of the synthetic wastewater was relatively simple, while real wastewater brought unknown substances and often toxic compounds to the system. Such a change influences the community composition. The analysis of the community structure was performed on the total DNA isolated from RBC biofilm (samples were collected from all RBC discs and mixed before isolation). PCR-DGGE was performed on a partial 16S rRNA coding gene. As it can be seen in Figure 6, the qualitative community shift is observed from day 719, when real wastewater was directed to the system. The genotypes, relatively weak in period I of the experiment, seem to be amplified at a larger degree in period II of the experiment. The biodiversity, maintained at the same level as it had been in the biocenosis during synthetic wastewater treatment, increased from a value of 3.18 to 3.35 in the beginning of the real wastewater treatment (Figure 7(a)). Such a situation is intriguing because in an average biological system such as activated sludge, the introduction of toxic wastewater to the technological system usually reduces the number of the genotypes and removes those less adaptable from the reactor. In this case, the biocenosis diversity increased as the period with real wastewater treatment began, which could be explained in two ways: either the real wastewater contained easily removable substances which support particular bacteria multiplication or the real wastewater contained additional allochronic microflora easily adaptable to a biofilm ecosystem. However, the situation occurred only in the first 63 days of the second part of the experiment; afterwards, the biodiversity decreased (Figure 7(a)) and the samples collected in days 800 and 823 present lower biodiversity, caused mainly by the GC-poor genotypes' removal from the system. Fingerprints obtained for the samples collected in days 800 and 823 also differ drastically from the rest of the samples collected in period II of the experiment on real wastewater. Their dissimilarity to the rest of the samples is underlined by their location in the dendrogram as a separate branch (Figure 7(b)). It is possible that, in the beginning of the experiment done on real wastewater, the bacterial community was protected from the toxic influences by the biofilm matrix and after 60 days of the second part of the experiment, the protective sheet of matrix substances was saturated with harmful

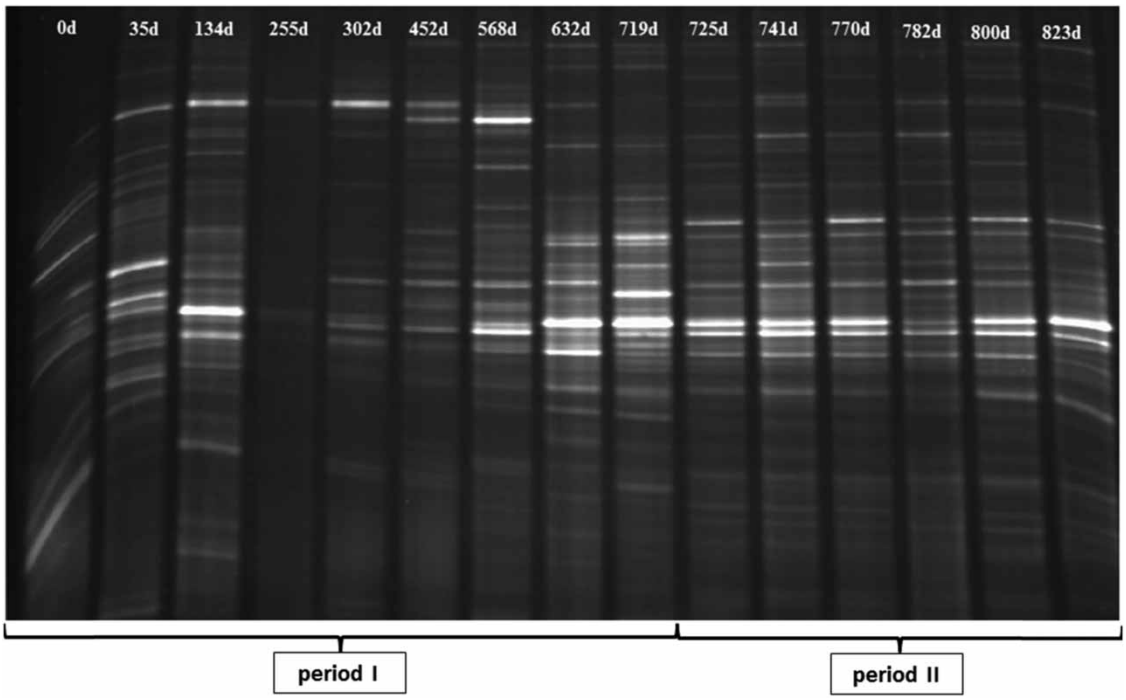

Figure 6 | PCR-DGGE analysis of biofilm bacterial community structure in RBC during total length of experiment; period I – samples collected from the RBC biofilm during synthetic wastewater treatment; period II – samples collected from the RBC biofilm during real coke wastewater treatment.

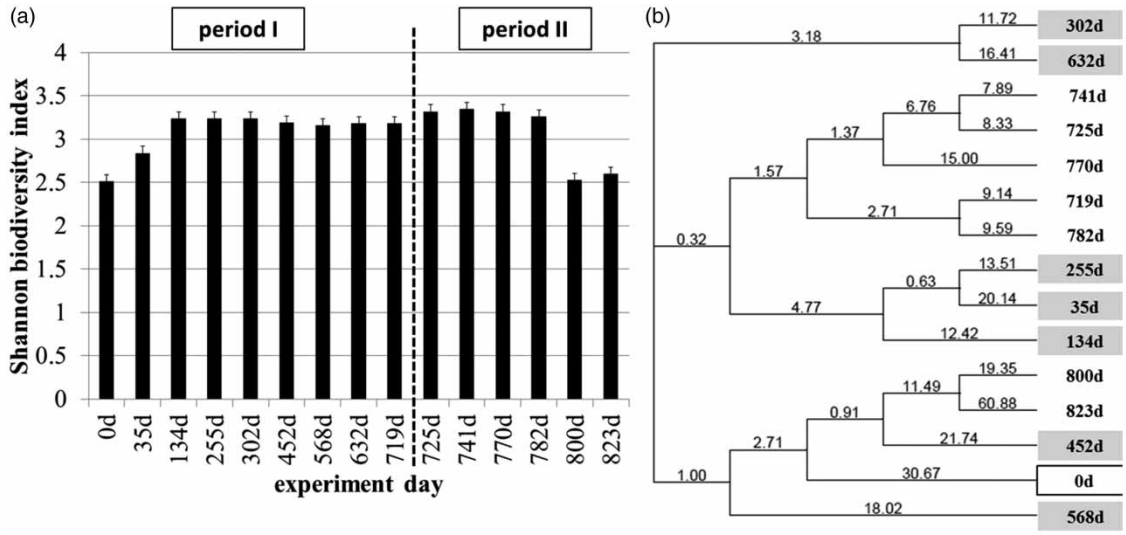

Figure 7 | Biofilm bacterial community analysis performed on densitometric measurements in RBC during total length of experiment; (a) Shannon biodiversity index; (b) dendrogram presenting samples similarity constructed on the basis of the neighbor-joining algorithm with Dice coefficient (0d – inoculum, white boxes – samples collected during the synthetic wastewater treatment (period I), grey boxes – samples collected during real wastewater treatment (period II)).

compounds, thereby allowing its toxic influence on the bacterial biocenosis to be visible. Interestingly, in the time of the experiment on real wastewater the biodiversity decreased simultaneously with the nitrogen removal restoration. Thus, it could be suspected that after 2–4 months of adaptation, the RBC system would be able to treat coke wastewater effectively and the biocenosis in the biofilm would become specialized to such sewage purification.

CONCLUSION

During the synthetic wastewater treatment period, ammonium removal efficiency was at the level of 96.7 ± 4.4%; however, in order to reach this high efficiency, a very long start-up period for the Anammox process (190 days) was required. Stable nitrogen removal of over 70% was achieved after more than 400 days of the experiment; however, the process could be successfully operated even at a relatively low temperature of around 20.2 ± 2.2 °C. The change of synthetic to real wastewater caused a temporary process break down but, at the end of the experiment, a process restoration was observed. The research performed on the RBC revealed that coke wastewater is a difficult type of sewage to be treated using biological methods; nonetheless, most likely due to the protective role of biofilm matrix, such a biocenosis could be a tool in such treatment. On the basis of the research performed in this experiment, we could state that the biodiversity of the specialized community for coke wastewater treatment is lower than the one treating synthetic wastewater and the community is composed mainly in GC-rich bacterial genotypes. Probably, the GC-rich group of bacteria would be the community specialized enough to treat recalcitrants (e.g. phenols, polycyclic aromatic hydrocarbons (PAHs)) present in real sewage.

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