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Ammonia and indicator bacteria removal from domestic sewage in a vertical flow filter filled with plastic material

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ABSTRACT

The paper discusses the effectiveness of removing ammonium ions and indicator bacteria from domestic sewage in a vertical flow filter filled with a plastic material (PET flakes). Preliminary research has shown the effectiveness of ammonium nitrogen removal at the value of 66.74%. Principal Component Analysis was used to determine the mechanism of the removal of indicator bacteria. Three principal components were identified and they were responsible for 78,2% of input data variability. PCA analysis showed, that the effectiveness of *Escherichia coli* removal depended mainly on the filtering mechanism. In the case of coliform bacteria removal an additional factor was acidic conditions. A decrease in CFU of the coliform bacteria by one order of magnitude was observed at pH = 4.1.

1. Introduction

In the 1980s as a filling of trickling filters, plastic media (for example shredded PVC bottles, corrugated plastic shapes) started to be used. The basic concept of these filters was to provide a surface for the growth of different populations of microorganisms. They were created in such a way that a thin biofilm could oxidize the organic matter from the wastewater while generating new biomass. Compared with conventional media (quartz sand, gravel, clay, rock) the plastic filling is lightweight, has large specific surface area and less tendency to clogging. The plastic media are characterized by the highest abrasion resistance and better gas transfer due to the greater draft (Wastewater, 2000; ADF Health Manual, 2013; Wang et al., 2009). Trickling filters on small to medium sized sewage treatment stations are used for combined organic carbon oxidation and nitrification (Gujer and Boller, 1986; Pearce and Williams, 1999).

Biological nitrification is the most common method of ammonia nitrogen removal from municipal sewage. It involves two different groups of bacteria that first oxidize ammonia to nitrites in the nitritation reaction, and then nitrites to nitrates in the nitratation reaction (Biswas and Nandy, 2016; Henze et al., 2002). At the first stage of nitrification, autotrophic bacteria *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira* (Ammonia Oxidizing Bacteria) oxidize ammonium ions via an intermediate hydroxylamine to nitrite ions. The second stage of nitrification is carried out by relatively autotrophic bacteria of *Nitrobacter*, *Nitrococcus,* and *Nitrospira* (Nitrite Oxidizing Bacteria) genera. It consists in the oxidation of nitrite ions formed at the first stage to nitrate ions. Growth rate of the nitrifying bacteria depends primarily on the concentration of dissolved ammonia and oxygen that are the primary substrates of the process and on alkalinity of the sewage. Total amount of oxygen stoichiometrically required for the oxidation of 1 mg of ammonia nitrogen to nitrate nitrogen is 4.57 mg O_2. Biochemical oxidation of NH_4^+ causes a decrease in alkalinity and consequently lowers pH, which for municipal sewage is between 7 and 8 (van Haandel and van der Lubbe, 2012).

Concentration of H^+ affects the active centers of nitrification controlling enzymes (Claros et al., 2013). Optimum pH for phase I of nitrification is 7.9–8.8, and for phase II it is 7.5–8.4 (Park et al., 2007). At pH lower than 6.6, nitrification efficiency is reduced by over 50% (Sadecka, 2010), and it is completely inhibited at pH below 5.0. pH alterations affect nitrification also in indirect ways. As the reaction equilibrium changes, nitrite ions may be transformed into their non-ionized form of free nitric acid (Claros et al., 2013). HNO_2 inhibits the activity of nitrifying bacteria (Biswas and Nandy, 2016).

However, biological oxidation of ammonium ions does not remove nitrogen compounds from water but it only transforms them into a different form. Proper nitrification yields NO_3^- ions and small amounts of NO_2^- ions. Therefore, nitrification should be coupled with a process providing a removal of nitrates. This may be executed by means of biochemical reduction via assimilation or dissimilation (denitrification).

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Assimilation of NO_3^- ions, controlled by nitrate reductase, involves their reduction to NH_4^+ that may be then built up into cellular structures. González et al. (2006) reported on the presence of assimilatory nitrate reductase *Nas* typical for capsular coliforms in *coli – Klebsiella pneumoniae* group. *Escherichia coli* was also reported as capable of nitrate reduction (Cabello et al., 2004, González et al., 2006). According to González et al. (2006), in *Escherichia coli* with K19 capsular antigen the enzyme responsible for this process was respiratory nitrate reductase *Nar* (EcoCyc Database, 2017; Tiso and Schechter, 2015) found that *E. coli* reduced nitrates to ammonia.

The removal of nitrogen compounds from sewage can be carried out in a classical system of nitrification/denitrification or with the application of methods. In the Activated Sludge Process (ASP), when nitrification and denitrification are combined, ten this first stage is the most expensive, due to the high use of electric energy. Partial reduction of oxygen demand can be obtained by steering the subsequent stages of nitrification. The influence of pH to halting of the second phase of nitrification (shortened nitrification) or the differences in the speed of the bacteria growth in subsequent phases were used in non-conventional systems. Among these technologies used in the treatment of wastewater rich in ammonium the combination of processes SHARON® i ANAMMOX® were applied (Milia et al., 2016; Mumtazah et al., 2016). In SHARON® the difference of the growth rate in bacteria oxidizing ammonia nitrogen and nitrites, depending on temperature. ANAMMOX® process means anaerobic oxidation of ammonia nitrogen to gaseous N_2 with the application of nitrites as electron acceptors. In technical conditions this process must be preceded by the stage of their production (Biswas and Nandy, 2016; van Haandel and van der Lubbe, 2012). Thus the most important advantage of ANAMMOX® is the removal of nitrogen in anaerobic conditions. In this process there is also no need for external source of organic carbon (e.g., in the form of methanol), necessary for denitrification bacteria. However, due to very slow growth rate of the bacteria, the ANAMMOX® method requires the that the biomass is old enough. Thus different types of bioreactors were taken in to account for the enrichment of ANAMMOX® bacteria i.e. fixed bed reactor, gas-lift reactor, fluidized bed reactor, UASB reactor and SBR. Among them, the last one was accepted for its simplicity and efficient biomass retention (Biswas and Nandy, 2016).

Another example of anaerobic nitrogen removal is UASB reactor. Its combination with aerobic systems was investigated by Khan et al. (2011, 2012, 2013a, 2013b, 2014). Continuous flow intermittent decant type sequencing batch (CFID) reactor was found to be the most compact and flexible method for ammonia nitrogen removal.

Coliform bacteria, including e.g., *Escherichia coli* and *Klebsiella pneumoniae* are indicators of water contamination with fecal bacteria. Their presence indicates that the water may be also contaminated with other pathogenic bacteria, such as *Salmonella* sp. or *Shigella* sp. The discussed Gram-negative bacteria belong to the *Enterobacteriaceae* family. The size of these bacteria is moderate and ranges from 0.3 μm to 1.0 μm (wide) and 0.6 μm to 6.0 mm (long) (Abbott, 2007; dos Santos et al., 2015). Genus *Escherichia* ranges from 1.1–1.5 μm to 2.0–6.0 μm, Genus *Enterobacter* ranges from 0.6–1.0 μm to 1.2–3.0 μ, Genus *Citrobacter* ranges from 0.6–1.0 μm to 1.2–3.0 μm, Genus *Klebsiella* ranges from 0.5–1.5 μm to 2.0 μm (Bergey's Manual, 2005). These values represent values measured on fixed populations of bacteria and depend of their life cycle and bacterial size evolution. For example as a survival strategy of *E. coli* cells showed not only faster growth speed but also larger size to avoid grazing pressure from predators (Yoshida et al., 2014).

The removal of indicator bacteria in the systems of wastewater treatment goes with different efficiency. It depends on the kind of the applied method (aerobic or anaerobic), the duration of the process, bacterial physiology and connected succeptability to physical or chemical inactivation (Henze et al., 2008; Olańczuk-Neyman and Quant, 2015). Research literature shows that retention of Gram-negative bacteria, present in the wastewater that flows through a porous media is achieved mainly due to straining and adsorption (Langenbach, 2010; Stevik, 2004). Quartz sand as a filling of filters is an effective barrier for the indicator bacteria. Sewage treated with those filters may be eliminated with the rate of 1.9–2.6 log units of *Escherichia coli* reaching effluent concentrations of 11–142 CFU/100 cm^3 (Langenbach et al., 2009). Seeger et al. (2016) indicated mean removal for *E. coli* within 2,7–4.7 log units from the inflow containing $8.9{\cdot}10^3 \pm 8.2{\cdot}10^3$ CFU/100 cm^3. Chmielowski (2013) reported *E. coli* removal from wastewater to the mean level of $1{\cdot}10^2 - 1{\cdot}10^4$ CFU/100 cm^3 and *Salmonella* sp./*Shigella* sp. removal to the mean level of 10 CFU/100 cm^3.

However inactivation of pathogen bacteria could be caused by biotic mechanisms, for example dissolved oxygen DO concentration or pH. *Escherichia coli* and *Salmonella* sp. are facultative or obligate anaerobes that growth under aerobic conditions is inhibited (Vymazal, 2005). Khan et al. (2012) observed 97% reduction of fecal coliforms to $7.05{\cdot}10^3$ CFU/100 cm^3 for configuration of UASB and the diffusion aeration. This system was operated at 1 h hydraulic retention time (HRT), pH value 7,4–8,5 and DO concentration 5–6 mg O_2/dm^3. The prolongation of HRT to 8 h and the decrease of DO to the values 2.5–3.5 mgO_2/dm^3 allowed to remove 99% of fecal coliforms in the process combining reactors UASB and CFID (Khan et al., 2013a, 2013b).

The optimum pH range for pathogen bacteria is 5.0–6.4 (Stevik et al., 2004). *Escherichia coli* can survive at pH from 4.4 to 9.0 but acid tolerant strains are capable of surviving in highly acidic conditions. As reported by Michalska-Szymaszek (2009), viability of *E. coli* O157 strain at 36 °C and pH 2–4 was decreasing over time, and pH 5–7 was conducive to their proliferation.

To define the connections between the parameters influencing the mechanism of indicator bacteria removal and to identify common factors, Principal Component Analysis (PCA) an be applied. This is a tool of data exploration, often usefull at the assessment of the wastewater treatment process (Moon et al., 2009; Wąsik and Chmielowski, 2017; Xiao et al., 2016). By reducing the number of original parameters (so-called primary variables) and their replacement with their components, which significantly explain their variability, the PCA analysis allows us to describe the phenomena preserving maximal amount of information. Finding the main components is possible when between primary variables there are certain correlations (Jolliffe, 2002; StatSoft, 2017). Assuming that several so-called first components contain significant variability of the original dataset, they can jointly explain their variability, and thus simplify the interpretation of results.

This article discusses the possibility of using a plastic material in a vertical flow filter to ammonia and indicator bacteria removal from domestic sewage. Advanced statistical method like PCA was used to determine the mechanism of indicator bacteria removal.

2. Material and methods

To determine the efficiency of ammonium ions removal, semi-technical models of vertical flow filters filled with plastic or natural materials were prepared. Each model consisted in an identical PCV column preceded by a septic tank (Fig. 1). The main experimental column was filled with commercially available PET bottles shredded into flakes 6–12 mm in size or less (Fig. 2). The comparative columns were filled with sand, zeolite and expanded clay (Wąsik and Chmielowski, 2017).

Each filter was supplied with the same amount of sewage with similar physic-chemical and bacteriological characteristics