



Quantitative dynamics of ammonia-oxidizers during biological stabilization of municipal landfill leachate pretreated by Fenton's reagent at neutral pH



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ABSTRACT

The application of multi-stage systems including biological step, for the treatment of leachate from municipal landfills, is economically and technologically justified. When microbial activity is utilized as 2nd stage of treatment, the task of 1st stage is to increase the bioavailability of organic matter. In this work, the effect of advanced oxidation process by Fenton's reagent for treatment efficiency of landfill leachate in the sequencing batch reactor was assessed. The quantitative dynamics of bacteria taking a part in ammonia removal process was evaluated by determination of number of DNA copies of *16S rRNA* and *amoA*. Products of neutral pH chemical oxidation, had a definite positive impact on the quantity of β -proteobacteria *16S rRNA*, whereas the same gene specified for *Nitrospira* sp. as well as *amoA* did not show a significant increase during the process of biological treatment, regardless of whether the reactor was fed with raw leachate or chemically pre-treated.

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1. Introduction

Although, landfilling is not the best solution from the environmental risk point of view, it is still the commonest method of municipal waste management in most of European countries, including Poland, due to both technological and economic reasons (Cossu, 2010). One of the major adverse effects of waste dumping is formation of the leachate. This dark liquid is produced as a result of dissolving, mainly in rainwater percolating the landfill, products of physical, chemical and biological reactions occurring in the layers of dumped waste (Aziz et al., 2010). Properly designed municipal landfill should be effectively insulated from the ground to collect the entire volume of leachate by drainage system, and, where it's possible, direct it into a treatment system. There are numerous methods and modifications proposed for efficient treatment of landfill leachate (Renou et al., 2008), which could be divided in three main groups; first covers the techniques based on physical, chemical or combined processes, as: reduction of volume by ultrafiltration and reverse osmosis (Peters, 1998; F. Li et al., 2009; Pi et al., 2009; Renou et al., 2009), or evaporation (di Palma et al., 2002), ammonia stripping (Cheung et al., 1997; Ferraz et al., 2013), decomposition of refractory organic compounds by radiolysis or ozonolysis (Cataldo and Angelini, 2012), advanced chemical or electrochemical oxidation (Amiri and Sabour, 2014; Fernandes et al., 2015, 2016; Moreira et al., 2016) or supercritical water oxidation (Wang et al., 2011; C. Gong et al., 2015; Y. Gong et al., 2015), coagulation and flocculation or adsorption (Labanowski et al., 2010; Oloibiri et al., 2015) or combination of those methods. The second group concerns methods of biological treatment, based on microbial activity of suspended sludge or biofilm, maintained in miscellaneous types of reactors (Koc-Jurczyk, 2014). If landfills are located close enough to WWTP, some authors also propose dilution of leachate in municipal wastewater, sometimes with additional supplementation with external carbon source (Ye et al., 2014). In practice the problem of leachate is often only temporarily suspended by mechanical recirculation and spattering the liquid back on the surface of landfill with or without additional processing (Xing et al., 2013; Talalaj and Biedka, 2015). In recent years it is becoming more common to combine above mentioned groups of methods in multi-stage systems concerning physio-chemical and biological treatment in various order (Silva et al., 2013; Abood et al., 2014).

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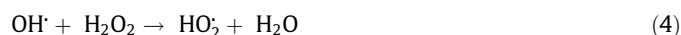
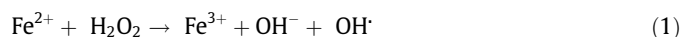
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The age of landfill is considered as a main factor determining choice of the method of leachate treatment, because it influences the most chemical characteristics of leachate. Leachate from young landfills, during the acidic phase, are characterized by low pH, average concentrations of ammonium nitrogen (500–2000 mg L⁻¹),

high concentrations of organic substances expressed as biological oxygen demand (BOD_5 , 4000–13,000 $mg\ L^{-1}$) and chemical oxygen demand (COD, 30,000–60,000 $mg\ L^{-1}$), so the value of BOD_5/COD ratio is high and reaches even over 0.7. Whereas, during the methanogenic phase, in chemical composition non-biodegradable organic substances appear, the degradation of volatile fatty acid (VFA) occurs, and pH value increases to approximately neutral. Along with time of the waste storage and increasing advantage of anaerobic processes taking place in the landfill bed, the concentrations of ammonia nitrogen could rise up to 3000–5000 $mg\ L^{-1}$, at the same time the value of COD falls to 5000–20,000 $mg\ L^{-1}$, so BOD/COD ratio is much lower than 0.1 (Foo and Hameed, 2009; Guo et al., 2010; Lee et al., 2010; Ahmed and Lan, 2012; Kalcikova et al., 2012; Müller et al., 2015). Such conditions are especially disadvantageous for maintenance of microbial communities in technological systems.

Thus, biological treatment is suggested mainly for the “young” leachate, whilst for stabilized ones, rather physio-chemical methods are proposed. However, low-cost and much more environment friendly biological methods are still useful for mature leachate disposal when designed in multistep combinations, as a pre-treatment (biological stabilization) or, *vice versa*, as a final stage, when proceeded by physical or chemical decomposition of non-biodegradable organic compounds. These methods are also viable, when considered as a remedy for reduction of high load of ammonia nitrogen (Peng et al., 2008; Shalini and Kurian, 2012). When the microbial activity is used in 2nd stage of treatment, the main task of 1st is usually the raise of organic matter assimilability (Cheremisinoff, 1996).

One of the methods proposed in combination with biological processes, as applicable for preliminary degradation of refractory organic compounds, is chemical advanced oxidation by using Fenton’s reagent. The mechanism is known for over a century, but adapted as a technological method for leachate neutralisation only in the 90s (Huang et al., 1993). In this process the hydrogen peroxide H_2O_2 is used as oxidiser, ferrous (Fe^{2+}) as a catalyst, and the result of chemical reaction is formation of highly active hydroxyl radicals (OH^\bullet), according to Eqs. (1)–(5) (Singh and Tang, 2013).



In presence of the radicals the refractory organic compounds are efficiently mineralised or converted to biodegradable forms, which consequently can be removed in following biological processes. During vigorous reaction between substances dissolved in leachate and Fenton’s reagent, compounds of high molecular mass are converted into compounds of low mass, and complex aromatic or aliphatic hydrophobic chains are transformed to hydrophilic structure (Umar et al., 2010; Zhao et al., 2013; Koc-Jurczyk, 2014). Effectiveness of the process depends, among other, on the pH, doses and molar ratio of reactants (Fe^{2+} and H_2O_2) and H_2O_2/COD , as well as Fe^{2+}/COD ratios.

In two-stage treatment systems with a biological reactor, a crucial role has controlling of the quantitative dynamics of microbial community which taking a part of biochemical processes, such as ammonia removal. From the one side, low-mass hydrophobic compounds can be absorbed by heterotrophic bacteria that interact with autotrophs, however, unspecific character of the reaction

between radicals, numerous various compounds dissolved in leachate and reaction intermediates, allow to suspect that, at least, a part of products could be potentially adverse to ecosystem of the bioreactor. Also, the advanced oxidation process could release or transform the nitrogen compounds. Thus, the main goal of this study was to determine the influence of chemical oxidation of municipal landfill leachate by Fenton’s reagent in neutral conditions, on quantitative dynamics of chosen groups of microorganisms inhabiting suspended activated sludge inside sequencing batch biological reactor.

2. Materials and methods

2.1. Leachate sampling

Samples of leachate used in this study were collected from the landfill located near Kozodrza (Subcarpathian Province, Poland, 50°07′07.8″N 21°37′19.1″E) which has a status of RIPOK (regional instillation for municipal waste treatment) and is supplied by over 92,000 Mg of municipal waste per year (others than hazardous and inert, but with separated quarter for asbestos). Landfill is still operated since 1990 and currently has an area of 18 ha, including ten closed and one active quarter.

Samples were collected once from leachate retention pond, with a capacity of $\approx 2900\ m^3$, to 10 L plastic tanks, and immediately transported to laboratory of waste management in University of Rzeszow, where were stored in 4 °C and darkness until further analysis. Before treatment, the temperature of probes were equilibrated to $\approx 25\ ^\circ C$ by being left overnight in warm room. Raw leachate was characterized by concentration of organic compounds, expressed as COD – 6092 $mg\ L^{-1}$, and as BOD_5 – 311 $mg\ L^{-1}$, thus low BOD/COD ratio, at level of 0.05, was typical for stabilized landfill, and very unfavorable from the point of view of biological treatment. Concentration of ammonium nitrogen was 896 $mg\ L^{-1}$, and organic nitrogen 333 $mg\ L^{-1}$, both nitrates and nitrites were not detected.

2.2. Experimental setup

Leachate was treated in two-stage system including advanced chemical oxidation by Fenton’s reagent (1st stage) and sequencing batch reactor with suspended activated sludge (2nd stage). Fenton’s reaction was conducted in laboratory scale, in glass jar reactor, which was filled with 1.5 L of raw leachate and simultaneously supplemented by iron(II) sulfate and 30% hydrogen peroxide in molar ratio $H_2O_2:Fe(II) = 5$ (1g H_2O_2). The reactionary solution was stirred (200 rpm) for 30 min, then left for another 30 min for sedimentation (Fig. 1A). 1 L of the reaction product was decanted and then supernatant transferred into biological reactor. The pH value was not equilibrated during and after the reaction, and a dose of leachate was always freshly prepared before each feeding of the biological reactor.

The second, biological stage was conducted in two parallel, independent glass jar reactors of 2 L of working volume. First (SBR1) was filled with chemically pre-treated leachate, the second one, as a comparative reactor (SBR2), with the same volume of raw leachate. Hydraulic retention time (HRT) was set at 2 days, and 24 h cycle consisted of: 0.1 h of refilling, 3 h of mixing (60 rpm), 20 h of mixing/aerating, 0.7 h of sedimentation, and 0.2 h of decantation (Fig. 1A). Organic loading rate (OLR) was 2.35 $kg\ m^{-3}\ d^{-1}$ in SBR1, and 3.04 $kg\ m^{-3}\ d^{-1}$ in SBR2, whilst nitrogen loading rate (NLR) after Fenton’s pre-treatment amounted to 0.51 $kg\ m^{-3}\ d^{-1}$, and 0.61 $kg\ m^{-3}\ d^{-1}$ in SBR2. Both reactors were equipped with fine bubbles aeration system, but during aeration phase dissolved oxygen (DO) did not exceed 1 $mg\ L^{-1}$. The temperature was stabi-

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