

Letter to Editor

Preliminary Investigation of Rapid Potassium Efflux from Activated Sludge in Response to Peroxyacetic Acid Use

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Abstract

Preliminary research on activated sludge potassium efflux (GGKE – glutathione-gated potassium efflux) as a result of peroxyacetic acid (PAA) dosing were performed. Similar to the case of chlorine dosing, the PAA dosing causes initiation of bacteria defense mechanisms related to the transformations of glutathione tripeptide, resulting in the increase of potassium ion concentrations in the activated sludge environment. In the range of biomass concentration from 6.035 g_{MLSS}/l to 9.331 g_{MLSS}/l and applied peroxyacetic acid doses from 0.12 mg PAA/g_{MLSS} to 3.55 mg PAA/g_{MLSS}, the increase of potassium ions K⁺ concentration in the activated sludge environment after 10 minutes of reaction in batch varied from approximately 3 mg/l to 40 mg/l. The elementary increase of potassium ion concentrations (mg K⁺/g_{MLSS}·l) was dependant on reaction time and the PAA dose. The elementary increase values varied from 0.5 mg K⁺/(g_{MLSS}·l) for the dose of 0.3 mg PAA/g_{MLSS} and 5 minutes reaction time to 4 mg K⁺/(g_{MLSS}·l) for 2.5 mg PAA/g_{MLSS} and reaction time equal to 20 minutes.

Keywords: activated sludge, glutathione-gated potassium efflux (GGKE), peroxyacetic acid (PAA), filamentous bulking, *Microthrix parvicella*

Introduction

Application of chemical oxidants such as chlorine, hypochlorite, and hydrogen peroxide are non-specific methods of filamentous bacteria bulking control in activated sludge systems [1, 2]. The advantages of chlorine application are low cost and well documented operation effects, but its non-selective interaction with activated sludge microorganisms, negative impact on nitrification and biological phosphorus removal effectiveness [3], and formation of trihalomethanes and other chlorination by-products substantially limit usefulness of this reagent [4-6]. The results of research on disinfection of wastewater treatment plant (WWTP) effluent with peroxyacetic acid (PAA) [7-10]

prove that the formation of dangerous oxidation by-products is far smaller than in the case of chlorination. It seems that, similar to chlorine, peroxyacetic acid can be used for filamentous bulking control, although the experiences with its application have not been numerous so far [11, 12].

The results of experimental investigations, as well as many operational experiences at WWTPs with chlorine application for activated sludge bulking control prove that too high doses (8-16 mg Cl₂/g MLSS·d) can lead to significant growth of effluent turbidity, resulting from bacterial cells lysis and weakening of the activated sludge flocs structure [2, 13, 14]. Turbidity increase can also be due to deflocculation of activated sludge flocs resulting from the increase of monovalent cation concentrations followed by the increase of monovalent cations (M, mval/l) to divalent cations (D) ratio. According to Higgins and Novak [15], if

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$M/D > 2$, problems with activated sludge sedimentation may occur. In turn, Novak et al. [16] found that with M/D ratio below 3, formation of strong flocs took place. The increase of monovalent cations concentration in activated sludge environment can start up the ion exchange mechanism, causing separation of divalent cations from negatively loaded function groups present in extracellular polymeric substances (EPS) excreted by activated sludge microorganisms, according to the theory assuming bridge formation between Ca^{2+} ions and EPS (DCBT – divalent cation bridging theory). This mechanism is the reason for the decrease of sludge flocs size, effluent turbidity increase and the decrease of sedimentation ability of activated sludge [17, 18].

Higgins and Novak [15] indicated that the increase of soluble potassium concentration can lead to significant weakening of activated sludge flocs structure. Similar observations were made by Bott and Love [19], who found that sludge floc can get deflocculated at the presence of oxidant substances, activating the process of potassium leaking from the cytoplasm of bacterial cells to the extracellular environment. This phenomenon, known as GGKE (gluthathione-gated potassium efflux) is related to the self-defense reactions of most bacteria to the stress caused, among others, by the oxidants presence [20, 21]. Gluthathione (L-gammaglutamyl-L-cysteinylglycine) is a tri-peptide of the amino acids glutamic acid, cysteine and glycine. It protects DNA, RNA and peptides against destruction by free radicals [22]. Lack of GGKE mechanism can explain specific vulnerability of nitrifying bacteria to the presence of oxidants [23].

Investigating activated sludge reaction to chlorine application ($NaOCl$), Wimmer and Love [24] found that the fundamental reason for activated sludge flocs deflocculation and turbidity increase was potassium efflux causing the increase of M/D ratio inside the flocs. Similar results are reported by Henriques et al. [25] after dosing of 1-chloro-2,4-dinitrobenzen and 1-octanol.

In the article, the results of preliminary investigations of the potassium efflux from activated sludge as a result of peroxyacetic acid dosing are discussed. Assessing the relationship between PAA dosing and changes of K^+ concentration caused by the GGKE phenomenon would enable efficient control of peroxyacetic acid dosing to activated sludge.

Experimental Procedures

The samples of activated sludge were collected from an external recirculation pipe transporting the sludge from secondary sedimentation tanks to bioreactors (mUCT system) of the municipal WWTP in Gdańsk (Poland). The following parameters of activated sludge were investigated: standard sludge volume index (SVI), activated sludge concentration (MLSS, MLVSS), microscopic observations of activated sludge flocs size, shape and structure, the assessment of filamentous microorganisms spreading and identification of filamentous bacteria.

The activated sludge contained mainly quite large flocs (400-500 μm), irregular in shape and with a loose structure. High values of sludge volume index ($SVI = 200 - 210$ ml/ g_{MLSS}) were caused by filamentous bacteria growth inside the flocs and formation of filamentous structures outside the flocs.

Assessment of filamentous bacteria spreading was based upon a subjective scale worked out by Jenkins et al. [2]. The filamentous bacteria were identified after Gram and Neisser colorization on the basis of guidelines given by Jenkins et al. [2] and Eikelboom and van Buijsen [26]. *Microthrix parvicella* was identified as the dominant microorganism. Spreading of this microorganism was assessed as 4.5-5 in a 7-step Jenkins scale (0-6). Single filamentous bacteria belonging to other species were identified in the activated sludge: Type 0092, *Nostocoida limicola* and Type 021N.

In the activated sludge sampled from WWTP, concentrations of activated sludge (centrifugation – 3000 rpm, 5 minutes) and concentrations of potassium and ammonia nitrogen (supernatant filtration – 0.45 μm) were determined.

The experiments “in batch” were performed at laboratory scale bioreactors (2.0 l active volume). The experimental set was equipped with aeration and sludge mixing systems. Two investigation series at different sludge concentrations were carried out:

I series – 9.331 g_{MLSS}/l ,

II series – 6.035 g_{MLSS}/l .

Activated sludge samples (2 l) were aerated in bioreactors (1 h), maintaining the dissolved oxygen concentrations at 2.5 mg O_2/l (± 0.2), and then different doses of a substance containing peroxyacetic acid were added. The initial concentrations of PAA in bioreactors varied from 1.1 mg PAA/l to 22.7 mg PAA/l in I investigation series (9.331 g_{MLSS}/l) and from 1.6 mg PAA/l to 21.4 mg PAA/l in II investigation series (6.035 g_{MLSS}/l). The doses of peroxyacetic acid, calculated per dry matter of activated sludge, varied from 0.12 mg PAA/ g_{MLSS} to 3.55 mg PAA/ g_{MLSS} . A control sample (without PAA dosing) was examined in each series of investigations.

The concentrations of potassium were determined after filtration (0.45 μm) of samples taken from the bioreactors 2, 5, 10, 20 and 30 minutes after the addition of peroxyacetic acid to activated sludge ($T = 0$). After the last sample was collected, mixing was switched off and then, after 30 minutes of sludge sedimentation, turbidity was measured (NTU).

During the experiments, the temperature was maintained at the same level as in the bioreactors at the WWTP ($14^\circ C \pm 1$). In the experiments a Steridial P preparation, containing 1.14% of pure peroxyacetic acid, was used. Potassium was determined in a base solution with sodium tetraphenylborane, using Hach Lange test with detection limits 8-50 mg K^+/l and precision ± 5 mg K^+/l . For determination of ammonia nitrogen Hach Lange test basing on indophenol method was used. The determinations of turbidity, dissolved oxygen and pH were made with a HI98703 nefelometer (Hanna Instruments), LDO101 luminescental sonde, PHC101 electrode and meter HQ40d (Hach Lange).

Results and Discussion

Two series of experiments, with different activated sludge concentrations (I series – 9.331 g_{MLSS}/l, II series – 6.035 g_{MLSS}/l) and different initial concentrations of potassium (I series – 24.8 mg K⁺/l, series II – 23.6 mg K⁺/l), were carried out. A rapid and significant increase of potassium concentration in the activated sludge environment was observed as a result of peroxyacetic acid dosing to experimental bioreactors (Figs. 1 and 2).

The increase of potassium concentrations for similar doses of peroxyacetic acid (mg PAA/g_{MLSS}) was proportional to activated sludge concentration in experimental bioreactors. For low PAA doses (0.12 mg PAA/g_{MLSS} and 0.24 mg PAA/g_{MLSS}), the observed increase of K⁺ concentration did not exceed 8 mg K⁺/l (series I) (Fig. 1). In II series, at similar PAA dose (0.27 mg PAA/g_{MLSS}), the increase of potassium concentration was about 5 mg K⁺/l (Fig. 2). For higher doses (1.47-3.55 mg PAA/g_{MLSS}) a sig-

nificant increase of potassium ion concentrations in activated sludge environment occurred – by 35-40 mg K⁺/l in I series and by 20-23 mg K⁺/l in II series.

These results were comparable to the ones reported by Wimmer and Love [24], obtained during batch chlorination of activated sludge. At activated sludge concentration equal to 1.6 g_{MLVSS}/l and a dose of approximately 10 mg Cl₂/g_{MLVSS}, the increase of K⁺ concentration was about 5 mg K⁺/l. Assuming the typical for BNR WWTP ratio of organics to dry matter of activated sludge (MLVSS/MLSS) equal to 0.70-0.72, the increase of potassium ions per unit volume and unit MLSS was 2.0-2.2 mg K⁺/(g_{MLSS} · l). This roughly corresponds to the values obtained in the I and II series (1.5-3.5 mg K⁺/(g_{MLSS} · l)) for PAA doses 1-1.5 mg PAA/g_{MLSS} (Fig. 3). The observed potassium efflux (3.5-4.0 mg K⁺/(g_{MLSS} · l)) induced by higher PAA doses (2.0-2.5 mg PAA/g_{MLSS}) was substantially higher than the values obtained by Wimmer and Love [24] for NaOCl.

No significant increase of potassium efflux from activated sludge was observed when the peroxyacetic dose was increased over 2.5 mg PAA/g_{MLSS} (Fig. 3). These observations were consistent with the reports of Boot and Love [19], who found that exceeding the chlorine dose over the limit value did not result in increase of the GGKE effect.

The results of performed investigations proved that potassium efflux induced by peroxyacetic acid took place

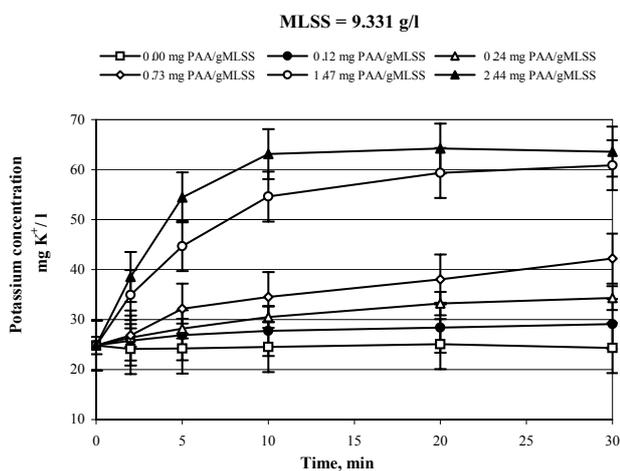


Fig. 1. Concentration of potassium ions in the activated sludge environment, depending on the dose of peroxyacetic acid and reaction time (9.331 g_{MLSS}/l).

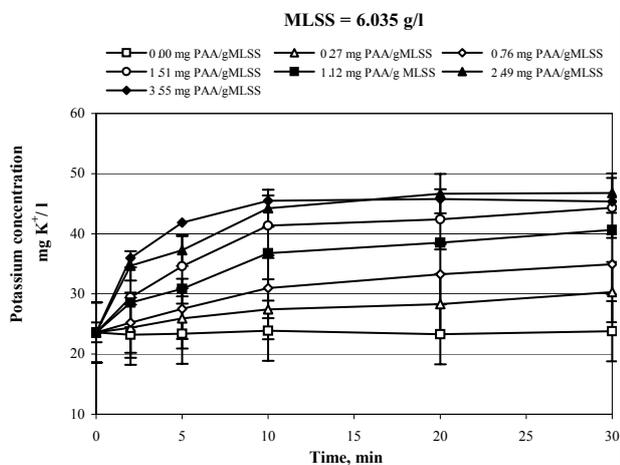


Fig. 2. Concentration of potassium ions in the activated sludge environment, depending on the dose of peroxyacetic acid and reaction time (6.035 g_{MLSS}/l).

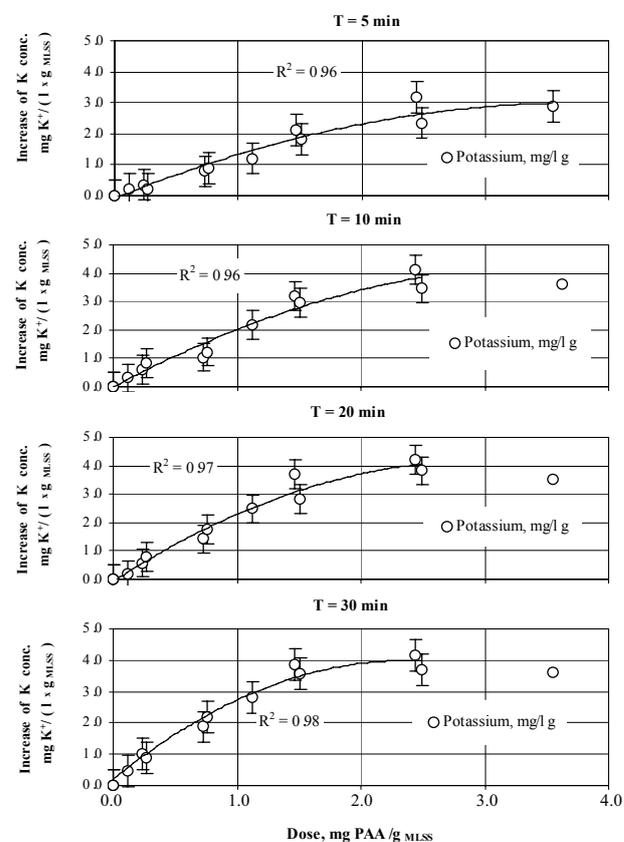


Fig. 3. The unit potassium efflux (the increase of K⁺ concentration/(g_{MLSS} · l)) depending on the dose of peroxyacetic acid and reaction time (activated sludge concentrations 6.035 g_{MLSS}/l and 9.331 g_{MLSS}/l).

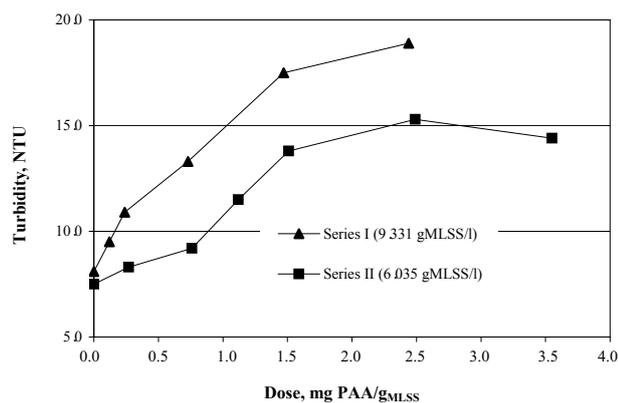


Fig. 4. Changes of sewage turbidity (30 minutes of sedimentation) depending on peroxyacetic acid dose.

within 20 minutes (Figs. 1-3). A similar rate of GGKE process was reported by Boot and Love [19], who used NEM (N-ethyl maleimide) in doses below 5 mg/l and Wimmer and Love [24] during chlorine dosing (in the form of NaOCl) to activated sludge in the doses from 1 to 16 mg Cl₂/g_{MLSS}.

In samples exposed to PAA, after 30 minutes of sedimentation, a turbidity increase was observed from 7.5-8.1 NTU (control samples) to 14.4 NTU (II series - 6.035 g_{MLSS}/l) and 18.9 NTU (I series - 9.331 g_{MLSS}/l) (in both series for the highest PAA dose) (Fig. 4). Wimmer and Love [24] reported two times turbidity increase (from 40 NTU to approximately 85 NTU) at the experiments in batch, for activated sludge concentration 1.6 g_{MLVSS}/l and chlorine dose approximately 10 mg Cl₂/g_{MLVSS}. The chlorine doses in the range from about 2.5 to 6.0 mg Cl₂/g_{MLVSS} resulted turbidity increase by about 10 NTU.

Relatively low turbidity increase (despite high biomass concentration) in the investigations with peroxyacetic acid can be due to the presence of filamentous structures of *Microthrix parvicella* inside activated sludge flocs. This phenomenon, however, requires further investigation.

Conclusions

Dosing of peroxyacetic acid to activated sludge caused rapid potassium ion efflux (GGKE effect) from bacterial cells. The process took place within 20 minutes time.

During batch experiments, carried out with two activated sludge concentrations in bioreactors (6.035 g_{MLSS}/l and 9.331 g_{MLSS}/l), the unit increase of potassium concentrations varied from 3.5 mg K⁺/(g_{MLSS} · l) to approximately 4.0 mg K⁺/(g_{MLSS} · l), for peroxyacetic doses from about 2.0 mg PAA/g_{MLSS} to 2.5 mg PAA/g_{MLSS}, and was higher than the unit values obtained during chlorination in similar experimental conditions. Sewage turbidity after activated sludge sedimentation, as an indirect parameter describing deflocculation of activated sludge, was relatively low, which

could probably result from common occurrence of *Microthrix parvicella*, forming extended structures in activated sludge (backbone theory [27]).

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