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The potential of dairy wastewater for denitrification

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Summary

In this work the potential of dairy wastewater for denitrification process by means of a microbial culture of nitrificants and denitrificants was investigated. The aim of this work was to remove nitrate by using organic compounds from the dairy wastewater as an electron donors. The minimal ratio of COD/NO_3-N of 10 (COD-chemical oxygen demand/NO_3-N-nitrate nitrogen) was required to achieve complete reduction of NO_3-N. The microbial culture of nitrificants and denitrificants, that was previously adapted on the dairy wastewater, carried out nitrate reduction with a different substrate utilization rate. The denitrification rate of 5.75 mg NO_3-N/Lh was achieved at the beginning of denitrification when the microbial culture utilizes readily biodegradable COD. Further degradation occurred with the denitrification rate of 1.7 mg NO_3-N/Lh.

Key words: dairy wastewater, denitrification, microbial culture of nitrificants and denitrificants

Introduction

The milk processing industry produces significant quantities of wastewater. Dairy industry wastewater is composed largely of diluted milk, dairy products and cleaning agents. The pollution in dairy wastewater makes organic substances, usually proteins and organic acids present in considerable quantity, and compounds with nitrogen and phosphorus. The characteristic of all wastewater flows generated in the milk processing industry is high fluctuation in the flow rate, which can be interpreted as a consequence of a various products production, and significant variations of pH-value. The organic load of dairy industry wastewater makes them a suitable substrate for the denitrification process of wastewaters rich in nitrate and of low COD/N ratio (Danalewich et al., 1998; Zayed and Winter, 1998; Bickers et al., 2003; Britz et al., 2006).

Compounds with nitrogen present in the environment can cause serious problems such as eutrophication of rivers and contamination of water sources and can have an adverse effect on human health. For example, nitrate can form nitrosamines and nitrosamides, the potentially carcinogenic components (Ono et al., 2000).

The biological denitrification is a process in which heterotrophic denitrifying bacteria in anoxic conditions reduce nitrate to nitrite and then to gaseous nitrogen. For heterotrophic denitrification it is necessary the presence of organic compounds as electron donors. In biological denitrification process the electron donor is usually one of three sources: the biodegradable COD of wastewater; biodegradable COD produced during endogenous decay and external source of carbon (Tchobanoglous et al., 2003). When the wastewater does not have enough organic compounds as electron donors for denitrification, such as wastewater with a low COD/N ratio or when organic substances have already been removed in an earlier stage of water treatment, for efficient denitrification the organic compounds as external sources of carbon must be added (Tchobanoglous et al., 2003). For each external carbon source the adaptation of microorganisms is required (Tchobanoglous et al., 2003). As a suitable carbon source, electron donor for denitrification process,

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acetate, glucose, methanol and ethanol were reported (Danalewich et al., 1998; Zayed and Winter, 1998; Bickers et al., 2003; Tchobanoglous et al., 2003; Britz, 2006). Other authors have demonstrated the possibility of using waste from agricultural and processing industries as a possible substrate for the process of denitrification, for example the wastewater from the production of ice cream and sugar (Cappai et al., 2004), from processing of potato (Rodríguez et al., 2007) and from the production of sweeteners, soft drinks and dairy products (Fernández-Nava et al., 2010). Several factors are important for the choice of carbon source such as cost price, the production of sludge, denitrification rate, kinetics, degree of utilization, the presence of the adverse/toxic substances and the potential for complete denitrification without the need for adaptation of the microbial biomass (Lee and Welander, 1996). The denitrification potential of milk processing industry wastewater was investigated using activated sludge (Cappai et al., 2004; De Lucas et al., 2005; Sage et al., 2006; Onnis-Hayden and Gu, 2008; Fernández-Nava et al., 2010) and mixed and pure microbial cultures isolated from activated sludge as suspended and immobilized biomass (Zayed and Winter, 1998). The optimal conditions for the growth of most denitrificants are the anoxic environment, the concentration of dissolved oxygen from 0.5 to 0.8 mg O_2/L, pH 7-8, and favourable temperature (Knowles, 1982; Tchobanoglous et al., 2003).

The aim of this study was to investigate the potential of dairy wastewater as an alternative carbon source for denitrification process. Denitrification was studied at various ratios of COD/NO_3-N. All the studies were performed with microbial biomass of nitrificants and denitrificants as a mixed microbial culture.

Materials and methods

Microbial culture

In this work, for investigation of denitrification potential of dairy industry wastewater a microbial culture of nitrificants and denitrificants was used. In previous studies, the activity of the microbial culture of nitrificants and denitrificants for denitrification of nitrificated mineral medium (2 g/L $(NH_4)_2SO_4$; 1 g/L KH_2PO_4; 0.4 g/L $FeSO_4$; 0.5 g/L $MgSO_4$; 0.4 g/L NaCl; 1 g/L $MgCO_3$, 1 g/L $CaCO_3$) with sodium acetate as carbon source was determined. In these studies, performed with the initial concentration of NO_3-N = 100 ± 2 mg/L, the minimum COD/NO_3-N ratio at which the microbial culture of nitrificants and denitificants achieved complete NO_3-N reduction and COD removal was 6.6 (Pavlović, 2008).

Wastewater

For investigation of denitrification potential dairy wastewater with COD of 4000 mg/L and pH of 7 was used. Dairy wastewater was produced during the various stages of milk processing and it is composed of diluted fractions of dairy products whose composition and concentration may vary from time to time (hours, days, weeks) depending on the production process. In the wastewater are present easily biodegradable components such as lactose and lactate and slowly biodegradable components such as fats and proteins. For this study the wastewater was prepared daily and it was composed of a mixture of milk and water in such proportion that the COD was 4000 mg/L, according to the literature data (Carta-Escobar et al., 2005). As a substrate rich in nitrate the nitrificated mineral medium was used.

Experimental conditions

Denitrification experiments were performed as discontinuous in reactors with working volume of 400 mL. Anoxic conditions were maintained by mixing on magnetic stirrer at 70 rpm. In reactors were placed electrodes for measuring of pH, temperature and dissolved oxygen concentration.

Analytical methods

Prior to the determination of analytical parameters the microbial biomass was separated by filtering the sample (pore size of 0.45 mm) and samples were prepared according to the protocol for the determination of analytical parameters. COD, NO_2-N, PO_4-P and biomass concentrations were determined by Standard methods (APHA, 1998). NH_4-N was determined by Nessler's method (Merck, 1974). NO_3-N and N-total were determined using spectrophotometric tests (Merck Spectroquant 1.14773 for nitrate and 1.14537 and 1.14763 for the total nitrogen). The pH and temperature were determined by pH meter WTW 330i, while the concentration of dissolved oxygen was determined using oximeter WTW Oxi 320. All experiments were conducted in triplicate and the results are presented as mean with standard deviation.

Results and discussion

The potential of carbon source for denitrification depends on its biodegradability or for a complex substrate on the content of easily biodegradable fraction of COD. In the literature as the most suitable carbon source, where the maximum rate of denitrification was achieved, acetate was reported (Gerber et al., 1986; Tam et al., 1992; Tam et al., 1994). The results of activity of microbial culture of nitrificants and denitrificants for denitrification with acetate are shown in Figure 1 and 2.

The results have shown that it is important for the efficient denitrification a suitable COD/NO_3-N ratio. So, at COD/NO_3-N ratio of 5.37 the reduction of nitrate was 84 %, and at COD/NO_3-N ratios of 7.97 and 10.47, 100 % reduction of nitrate was achieved, but the COD remained in the amount of 169 mg/L and 367 mg/L, respectively (Figure 1). The rate of denitrification was 27.83 mg NO_3-N/Lh at ratio COD/NO_3-N of 5.37 with the removal of COD at rate of 178.08 mg COD/Lh. At ratio COD/NO_3-N of 7.97 the rate of denitrification was 30.59 mg NO_3-N/Lh and COD removal rate was 208.7 mg COD/Lh. And, at ratio COD/NO_3-N of 10.47 the denitrification rate was 31.15 mg NO_3-N/Lh and COD removal rate was 176.06 mg COD/Lh (Figure 1). The minimal COD/NO_3-N ratio for complete reduction of NO_3-N and COD removal was 6.6 (Figure 2).

Sodium acetate is readily biodegradable substrate and reaches a maximum rate of denitrification (Gerber et al., 1986). The most often carbon sources investigated for denitrification are methanol, ethanol and acetic acid, but constantly are explored alternative sources of carbon (Lee and Welander, 1996; Cappai et al., 2004; Rodríguez et al., 2007; Fernández-Nava et al., 2010).

High C/N ratio in wastewater of food industry and need for the removal of COD from such wastewater before discharge into the environment makes them a suitable substrate as an alternative source of carbon for denitrification. Wastewater of dairy industry has both readily and slowly biodegradable compounds and because of that for its degradation is necessary adaptation of microorganisms. For each external carbon source the adaptation of microorganisms is required (Tchobanoglous et al., 2003). In this paper denitrification potential of dairy wastewater was investigated with the microbial culture of nitrificants and denitrificants, that was previously

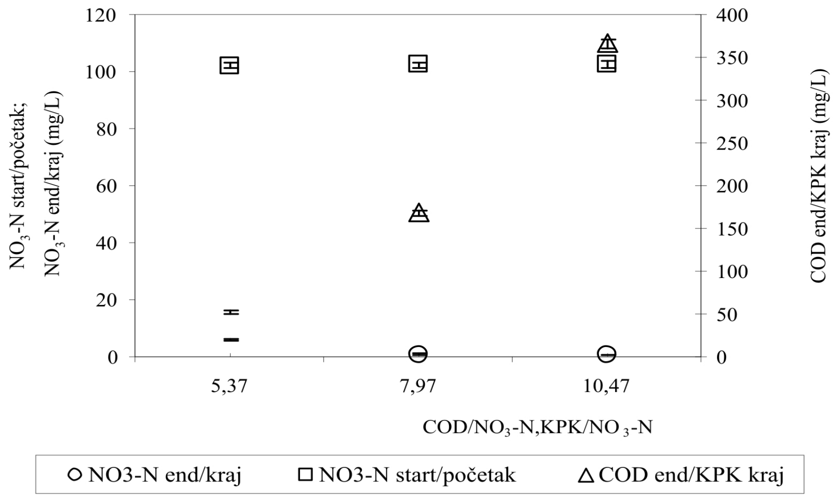

Figure 1. COD and NO_3-N at the beginning and the end of the denitrification experiment with sodium acetate as carbon source at different COD/NO_3-N ratio. Denitrification was carried out using microbial culture of nitrificants and denitrificants in anoxic conditions

Slika 1. KPK i NO_3-N na početku i kraju pokusa denitrifikacije uz natrijev acetat kao izvor ugljika pri različitim omjerima KPK/NO_3-N. Denitrifikacija je provedena pomoću mikrobne kulture nitrifikanata i denitrifikanata u anoksičnim uvjetima

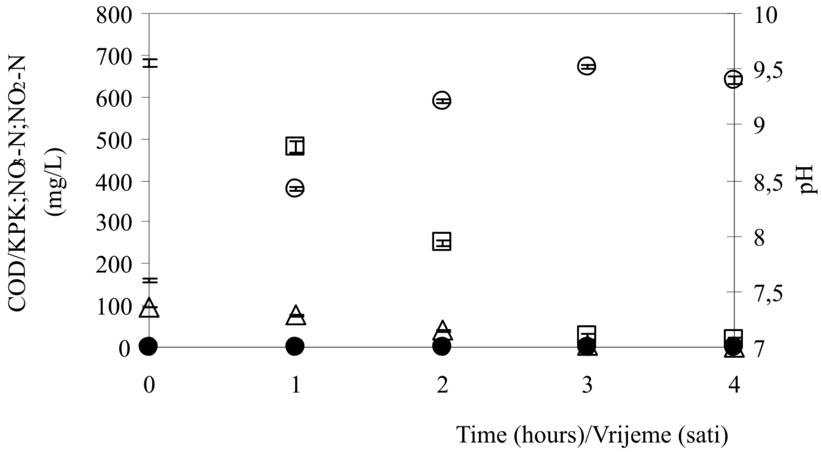

Figure 2. COD, NO_3-N, NO_2-N and pH during denitrification with sodium acetate at COD/NO_3-N ratio of 6.6. Denitrification was carried out using microbial culture of nitrificants and denitrificants in anoxic conditions

Slika 2. KPK, NO_3-N, NO_2-N i pH tijekom denitrifikacije uz natrijev acetat pri omjeru KPK/NO_3-N je 6,6. Denitrifikacija je provedena pomoću mikrobne kulture nitrifikanata i denitrifikanata u anoksičnim uvjetima

adapted on wastewater from dairy industry, and the results are shown in Figure 3 and 4.

The investigation of denitrification potential of the dairy wastewater has shown that complete reduction of nitrate (denitrification) was achieved when the ratio of COD/NO_3-N was minimally 10 (Figures 3 and 4). The results of these studies have shown that the efficiency of nitrate reduction depends on the ratio COD/NO_3-N. When the ratio COD/NO_3-N was 5 the reduction of nitrate was 59 %, and at the ratio COD/NO_3-N of 7 nitrate reduction was 86 %, during 22 hours (results not shown). Complete denitrification was achieved during the same experimental period at a ratio COD/NO_3-N of 10 (Figures 3 and 4).

These results of nitrate reduction were achieved after the adaptation of microbial culture of nitrificants and denitrificants on dairy industry wastewater, despite of its pronounced activity for the removal of compounds with nitrogen in mineral medium and with sodium acetate as a carbon source for denitrification (Figures 1 and 2). Also, the need for adaptation of activated sludge, mixed and pure microbial cultures have been reported by other authors. In addition, it was shown that greater activity in the removal of nitrate was achieved by use of mixed microbial cultures, compared to pure cultures, in which the members of the microbial communities are more resistant to intermediates and the process was performed at a shorter time (Zayed and Winter, 1998).

The literature has highlighted several factors affecting the denitrification process, such as the type and concentration of carbon source, adaptation and activity of microbial cultures (Carta-Esocobar et al., 2005). Acetate is reported as the most favourable carbon source for biological denitrification (Gerber et al., 1986; Tam et al., 1992; Tam et al., 1994; Tchobanoglous et al., 2003; De Lucas et al., 2005).

Using dairy wastewater as an alternative carbon source for the complete reduction of nitrate by microbial culture of nitrificants and denitrificants the minimal COD/NO_3-N ratio of 10 is required (Figure 3 and 4). The reason for this is probably the complexity of the dairy wastewater in which are present both easy and slowly biodegradable compounds. Initially are used easy biodegradable compounds and degradation is rapid, then slowly biodegradable compounds and degradation is slower (Figure 3 and 4).

In denitrification process performed with a complex carbon source such as agro and processing industries wastewater, the highest rate of denitrification is achieved by consumption of readily biodegradable COD, much lower denitrification rate is achieved using a slowly biodegradable COD that has

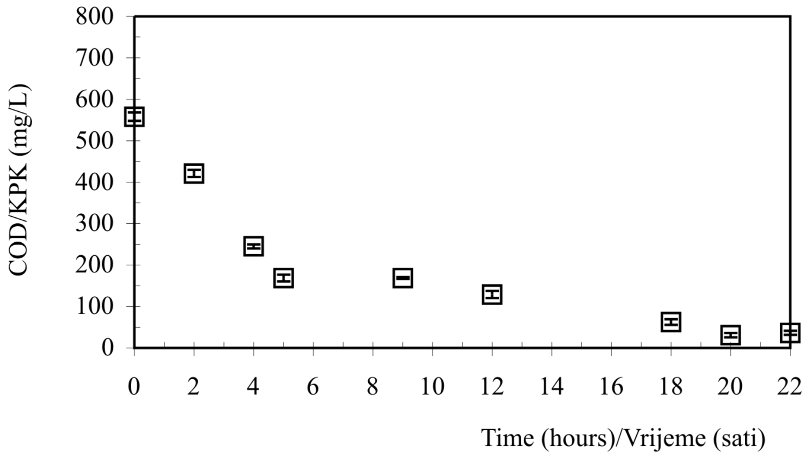

Figure 3. COD during denitrification with dairy wastewater at COD/NO_3-N ratio of 10. Denitrification was carried out using microbial culture of nitrificants and denitrificants in anoxic conditions

Slika 3. KPK tijekom denitrifikacije uz otpadnu vodu mljekarske industrije pri omjeru KPK/NO_3-N je 10. Denitrifikacija je provedena pomoću mikrobne kulture nitrifikanata i denitrifikanata u anoksičnim uvjetima

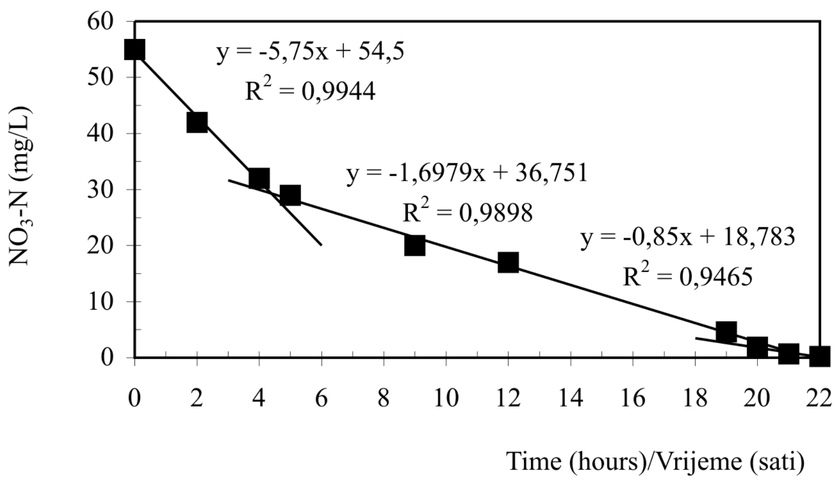

Figure 4. NO_3-N during denitrification with dairy wastewater at COD/NO_3-N ratio of 10. Denitrification was carried out using microbial culture of nitrificants and denitrificants in anoxic conditions

Slika 4. NO_3-N tijekom denitrifikacije uz otpadnu vodu mljekarske industrije pri omjeru KPK/NO_3-N je 10. Denitrifikacija je provedena pomoću mikrobne kulture nitrifikanata i denitrifikanata u anoksičnim uvjetima

to be first hydrolyzed, and the lowest rate of denitrification is achieved with an endogenous source of carbon (Sage et al., 2006). In the study of denitrification with dairy industry wastewater the removal of 500 mg/L NOx-N at a COD/N ratio of 4.6 was achieved, with remained COD at about 257 mg/L (Fernández-Nava et al., 2010). In another study was demonstrated the use of wastewater from the production of cheese and consume milk as an alternative carbon source for denitrification (De Lucas et al., 2005). During 16 hours of denitrification process nitrate reduction was achieved from an initial 60 mg NO_3-N/L to 28 mg NO_3-N/L + 7 mg NO_2-N/L. COD was reduced from 360 mg/L to 150 mg/L (wastewater from cheese production). In the process of denitrification with wastewater from the production of consume milk, for 16 hours, nitrate was reduced from 60 mg NO_3-N/L to 20 mg NO_3-N/L + 15 mg NO_2-N/L. COD was reduced from 350 mg/L to 120 mg/L (De Lucas et al., 2005). In the same work by using acetate as carbon source was achieved 60 % reduction of NO_3-N from an initial 30 mg/L to 12 mg NOx-N/L (6 mg NO_3-N/L + 6 mg NO_2-N/L) for 4 hours. The COD was reduced from an initial 360 mg/L to 200 mg/L (De Lucas et al., 2005).

In this paper, during 22 hours in the anoxic conditions 100 % removal of NO_3-N at a minimum ratio of COD/NO_3-N of 10 was achieved. There was no NO_2-N accumulation. The maximum nitrate reduction rate was achieved during the first 4 hours of the process and was 5.75 mg NO_3-N/Lh. Further reduction of nitrate (from the 5th to the 19th hour) was performed at denitrification rate of 1.7 mg NO_3-N/Lh and at the end of the process (the last 3 hours of the process) denitrification rate was 0.85 mg NO_3-N/Lh (Figure 3 and 4).

Conclusions

Dairy industry wastewater can be used as an alternative carbon source for denitrification process. Microbial culture of nitrificants and denitrificants, previously adapted to the compounds of dairy industry wastewater, during denitrification used organic compounds from dairy industry wastewater. During 22 hours in the anoxic conditions 100 % of nitrate reduction was obtained at a minimum COD/NO_3-N ratio of 10, without the accumulation of NO_2-N. Maximum nitrate reduction rate was achieved during the first 4 hours and was 5.75 mg NO_3-N/Lh with the use of easy biodegradable COD. Further reduction of nitrate (from the 5th to the 19th hour) was performed at denitrification rate of 1.7 mg NO_3-N/Lh by the use of COD that is slowly biodegradable. And, at the end of the process (the last three hour), the denitrification rate of 0.85 mg NO_3-N/Lh, was achieved probably by the using the endogenous COD.

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Potencijal otpadne vode mljekarske industrije za proces denitrifikacije

Sažetak

U ovom radu istražen je potencijal otpadne vode mljekarske industrije za proces denitrifikacije pomoću mikrobne kulture nitrifikanata i denitrifikanata. Cilj rada je bio ukloniti nitrat koristeći organske sastojke iz otpadne vode industrije prerade mlijeka kao elektron donore. Minimalan omjer KPK/NO_3-N (KPK-kemijska potrošnja kisika/NO_3-N-nitratni dušik) potreban za postizanje potpune redukcije NO_3-N iznosi 10. Mikrobna kultura nitrifikanata i denitrifikanata, prethodno prilagođena na otpadnu vodu mljekarske industrije, provodi redukciju nitrata uz različitu brzinu iskorištenja supstrata. U početku denitrifikacije mikrobna kultura troši lako razgradivi KPK i postiže brzinu denitrifikacije od 5,75 mg NO_3-N/Lh. Daljnja razgradnja zbiva se uz brzinu denitrifikacije od 1,7 mg NO_3-N/Lh.

Ključne riječi: denitrifikacija, mikrobna kultura nitrifikanata i denitrifikanata, otpadna voda mljekarske industrije

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