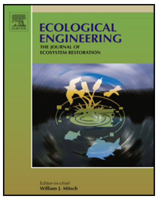
Ecological Engineering 90 (2016) 12–24

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Influent COD/TAN ratio affects the carbon and nitrogen removal efficiency and stability of aerobic granules

Irem Kocaturk, Tuba H. Erguder*

Environmental Engineering Department, Middle East Technical University, Ankara, Turkey

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form
21 December 2015
Accepted 26 January 2016
Available online 10 February 2016

Keywords:
COD/N ratio
Extracellular polymeric substance
Granulation
Nitrification
SBR
Simultaneous denitrification

ABSTRACT

Two identical sequencing batch reactors (SBRs) seeded with aerobic granules were operated with varying chemical oxygen demand/total ammonia nitrogen (COD/TAN) ratios (1–30). R1 was operated at increasing COD/TAN ratios (7.5, 10, 20, 30), while R2 was operated at decreasing COD/TAN ratios (7.5, 5, 3.5, 2, 1). The results indicated that high COD/TAN ratios (7.5–30) provided high COD removal efficiency (around 92%) and low TAN removal (33%), favoring heterotrophs that form white, fluffy flocs and large granules. Maintenance of high treatment efficiency and granular stability is hard due to high growth rate of heterotrophs. On the other hand, low COD/TAN ratios (2–5) provided high TAN removal efficiency up to 100%, while COD removal was relatively low (60%); leading to small, dense, orange granules enriched in nitrifiers with slow-growing but stable characteristics. The optimum COD/TAN ratio in terms of high COD and TAN removal and granular stability was found as 7.5. It was found out that bacterial population distribution among nitrifiers and heterotrophs can be adjusted by changing influent COD/TAN ratios. This study also proposed the polysaccharide to protein (PS/PN) ratio in the extracellular polymeric substance of the granules as indicators of their stability and value of 0.6 as threshold level for stable granulation.

1. Introduction

Aerobic biogranulation is a significant and up-to-date technology for carbon and nitrogen removal (Liu and Tay, 2004; de Kreuk et al., 2006; Adav et al., 2008a). Microbial aggregates including aerobic heterotrophic bacteria, autotrophic nitrifiers and heterotrophic denitrifiers are immobilized in the form of aerobic granules in SBRs (sequencing batch reactors) (Liu and Tay, 2004; de Kreuk et al., 2006; Adav et al., 2008a). Aerobic granules are advantageous over the conventional activated sludge in terms of high biomass content, high settleability, ease of separation from the effluent and tolerance for high-strength wastewater, shock loadings and toxicity (Liu and Tay, 2004; Adav et al., 2008a). In addition, aerobic granules eliminate the drawbacks of anaerobic granules such as long start-up period requirement, a relatively high operation temperature and unsuitability for low-strength wastewater treatment (Liu and Tay, 2004). However, aerobic granule instability, often occurring in long-term operation and mainly caused by granule disintegration, remains unresolved (Luo et al., 2014). Possible factors associated with granule disintegration might be defined as filamentous over-growth (Liu and Liu, 2006), role of extracellular polymeric substance (EPS) (Zhu et al., 2012), limited mass transfer and formation of anaerobic zones in the granules (Zheng et al., 2006) and the decreased aggregation tendency of cells (Adav et al., 2010). However, the exact causes for disintegration of aerobic granules are still unknown (Luo et al., 2014).

Despite the instability problems, aerobic granules are widely investigated for the treatment of domestic and industrial wastewaters due to above-mentioned advantages (Liu and Tay, 2004; Kocaturk and Erguder, 2015). Successful applications of aerobic granules require better understanding of wastewater compositions and their effects. One significant parameter is carbon/nitrogen (C/N) ratio or chemical oxygen demand/nitrogen (COD/N) ratio of the wastewater. The influent COD/N ratio adjustments may lead to microbial selection pressure in order to enrich either heterotrophic bacteria (aerobic or anaerobic) or nitrifying species contributing to granulation (Wu et al., 2012); thus, affect the treatment performance of granules. Previous studies demonstrate that C/N and/or COD/N ratios in the influent varying from 1 to 20 allowed aerobic granulation (Yang et al., 2005; Liu et al., 2003; Wu et al., 2012). High COD/N ratios such as 20 were found to enhance the relative amounts and activities of heterotrophs compared to nitrifiers

---

* Corresponding author at: Environmental Engineering Department, Middle East Technical University, Dumlupinar Bulvari, 06800 Ankara, Turkey.
E-mail addresses: iremkocaturk@hotmail.com (I. Kocaturk), etubahan@metu.edu.tr (T.H. Erguder).

http://dx.doi.org/10.1016/j.ecoleng.2016.01.077

in aerobic granules, while the enrichment of slow-growing nitrifiers in the granular structure were reported at low COD/N ratios such as 3.3 and below (Liu et al., 2003; Yang et al., 2005; Cydzik-Kwiatkowska and Wojnowska-Baryla, 2011; Wu et al., 2012). For affecting the relative abundance of microbial types in granules, COD/N ratio might also affect granular stability (Luo et al., 2014). COD/N ratio of 1 and 2 were found to adversely affect the stability of the granules, even lead to granule disintegration at COD/N ratio of 1 (Luo et al., 2014). This disintegration was attributed to the reduction in net tyrosine production in EPS and microbial community shift.

EPS, which is defined as sticky substance secreted by microorganisms, contributes to cell adhesion and microbial matrix formation of granules, promoting their stability and structural integrity (Liu et al., 2004). Biogranular EPS contains protein (PN), polysaccharides (PS), nucleic acids, humic-like substances and lipids. The amount and contents of EPS are influenced by type, growth phase and physiological state of microbial species, including the shift of microbial species during granulation (Etchebehere et al., 2003; Yi et al., 2003), as well as operational conditions such as oxygen and nutrient limitations, ionic strength, temperature and shear force (Liu et al., 2004). Granules cultivated on protein-rich substrate were reported to secrete protein-rich EPS, whereas EPS with high PS content is produced by granules fed by other types of substrates (Batstone and Keller, 2001). Deterioration of strength and settleability and granular disintegration were reported for granules with low PS/PN ratios (Liu et al., 2004; Tay et al., 2004; Wu et al., 2012). In contrast, low PS/PN ratios of 0.16–0.29 and of 0.6 were reported for mature granules (Adav and Lee, 2008) and stable aerobic granule formation (Li et al., 2008), respectively. Despite the debate on the role of PS/PN ratio in granular EPS (Zhu et al., 2012), it seems that the COD/N ratio and its change during operational period might result in changes in EPS concentration, content and PS/PN ratio in the granules and, in turn, the granular stability.

This study, therefore, aims to investigate the effect of varying influent COD/total ammonia nitrogen (COD/TAN) ratios on COD and nitrogen removal efficiency and stability of aerobic granules. For this purpose, a wider range (1–30), with both decreasing (from 7.5 to 1) and increasing (from 7.5 to 30) COD/TAN ratios were researched. The optimum influent COD/TAN ratio that allows the highest possible COD and TAN removal efficiencies without destroying the structural stability of the granules was examined. In order to determine the changes in granular stability, the effects of varying COD/TAN ratios on physical characteristics, EPS concentration and PS/PN ratios of granules were also investigated.

2. Material and methods

2.1. Seed sludge and nutrient solution

Aerobic granular sludge with 6050 ± 500 mg/L total suspended solids (TSS), 4760 ± 450 mg/L volatile suspended solids (VSS) and 36 mL/g sludge volume index (SVI) developed in a previous study (Erşan and Erguder, 2013) was used as seed sludge in SBRs. SBRs were fed with synthetic wastewater with the composition given in Table 1 (Smolders et al., 1994; Erguder and Demirer, 2005; Fang et al., 2009; Shi et al., 2010). Synthetic wastewater contains acetic acid for heterotrophic bacteria and bicarbonate for nitrifiers as carbon source. The nitrogen source for denitrifiers is $NaNO_3$. The carbon and TAN (NH_4^+-N + NH_3) content of the wastewater stands for 1500 mg/L COD and 200 mg/L TAN, corresponding to a COD/TAN ratio of 7.5. COD concentration of wastewater was further changed in a range of 200–6000 mg/L in order to provide the desired COD/TAN ratios of 1–30. The wastewater with (initially adjusted) pH of 7 was stored at +4 °C during the study.

Table 1
Composition of the synthetic wastewater.

Solution		[a] Content of micronutrient solution	
Acetic acid	1.34 mL/L	$FeCl_2 \cdot 4H_2O$	1.5 g/L
NH_4Cl	765 mg/L	H_3BO_3	0.15 g/L
$NaNO_3$	486 mg/L	$CuCl_2 \cdot 2H_2O$	0.02 g/L
$NaHCO_3$	1800 mg/L	KI	0.18 g/L
$MgSO_4 \cdot 7H_2O$	180 mg/L	$MnSO_4 \cdot H_2O$	0.1 g/L
$CaCl_2 \cdot 2H_2O$	160 mg/L	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.044 g/L
$Na_3PO_4 \cdot 12H_2O$	244 mg/L	$ZnCl_2$	0.057 g/L
Yeast extract	2 mg/L	$CoCl_2 \cdot 6H_2O$	0.15 g/L
Micronutrient solution[a]	0.6 mL/L	EDTA	10 g/L

[a] The content of micronutrient solution used in synthetic wastewater for necessary microbial growth.

2.2. Acclimation period and operation of SBRs

In order to acclimate the previously developed aerobic granular seed sludge to operational conditions, one week of acclimation period was performed. A plexiglass SBR with 8 cm inner diameter, 60 cm depth, effective volume of 2.45 L and volumetric exchange ratio (VER) of 50% was seeded with aerobic granular seed sludge. The reactor was operated continuously with 6-h cycles consisting of 5 min-feeding, 43 min-anoxic, 305–309 minute-aerobic, 1–5 min-settling and 2 min-withdrawal periods. The hydraulic retention time (HRT) of the SBR was 12 h. During aerobic periods, the reactor was aerated at an airflow rate of 180 L/h by air pumps. The experiments were conducted at 20–26 °C laboratory medium. The synthetic wastewater with a COD/TAN ratio of 7.5 (i.e. 1500/200 (mg/L/mg/L)) was fed to the reactor (Table 1). The organic loading rate (OLR) and nitrogen loading rate (NLR) were 3 g COD/L day and 0.4 g TAN/L day, respectively. When stable treatment efficiency (79 ± 4% TAN and 74 ± 1% COD) and sufficient VSS concentration (an average of 5743 ± 1165 mg/L) were obtained (in one week), the reactor content was divided equally into 2 identical SBRs, namely, R1 and R2.

R1 and R2, which had same dimensions, working volume and VER as the acclimation SBR, were operated under the same SBR conditions as in the acclimation period. Both SBRs were fed with synthetic wastewater at a COD/TAN ratio of 7.5 for the initial 10 days (Tables 1 and 2). For the following days, in order to provide the desired COD/TAN ratios, only COD (acetic acid) concentrations were changed. The feed NH_4Cl concentration was constant at 200 mg/L NH_4-N throughout the operation. The feed $NaNO_3$ concentration of both R1 and R2 was halved by Day 20 after achieving around 90% nitrification and producing enough amount of nitrate. R1 experienced gradually increasing COD/TAN ratios as 7.5, 10, 20 and 30; whereas R2 experienced gradually decreasing ratios as 7.5, 5, 3.5, 2 and 1. COD/TAN ratios were changed, when there was less than 3% difference between the three consecutive removal efficiencies, i.e. after achieving almost stable efficiency.

2.3. Analytical methods

The treatment efficiencies were determined by analyzing the samples taken from feed (influent), at the end of feeding period (initial sample) and at the end of the anoxic and aerobic periods (See Supplementary Information (SI) for equations). The removal efficiencies were calculated by percent difference between the initial and aerobic samples for total removal efficiency, the initial and anoxic samples for anoxic period removal efficiency, and anoxic and aerobic samples for aerobic period removal efficiency determinations (Kocaturk and Erguder, 2015). The samples were filtered through 0.45 μm filter papers before soluble COD (sCOD), TAN, NO_2^--N and NO_3^--N analyses. sCOD, NO_2^--N, and NO_3^--N were