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Effect of fermentation liquid from food waste as a carbon source for enhancing denitrification in wastewater treatment



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Yongmei Zhang ^a, Xiaochang C. Wang ^{a, *}, Zhe Cheng ^a, Yuyou Li ^b, Jialing Tang ^a

^a Key Lab of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an, 710055, PR China ^b Graduate School of Environmental Studies, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi, 980-8579, Japan

HIGHLIGHTS

- Food waste was used for anaerobic fermentation to prepare carbon sources.
- Fermentation liquid of high organic acid was used for enhancing denitrification.
- High denitrification rate and potential were gained with fermentation liquid.
- Fermentation liquid showed similar denitrification result as sodium acetate.

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ABSTRACT

Food wastes were used for anaerobic fermentation to prepare carbon sources for enhancing nitrogen removal in wastewater treatment. Under anaerobic conditions without pH adjustment, the fermentation liquid from food wastes (FLFW) with a high organic acid content was produced at room temperature (25 °C) and initial solid concentration of 13%. Using FLFW as the sole carbon source of artificial wastewater for biological treatment by sequence batch operation, maximized denitrification (with a denitrification rate of V_{DN} = 12.89 mg/gVSS h and a denitrification potential of P_{DN} = 0.174 gN/gCOD) could be achieved at a COD/TN ratio of 6. The readily biodegradable fraction in the FLFW was evaluated as 58.35%. By comparing FLFW with glucose and sodium acetate, two commonly used chemical carbon sources, FLFW showed a denitrification result similar to sodium acetate but much better than glucose in terms of total nitrogen removal, V_{DN}, P_{DN}, organic matter consumption rate (V_{COD}) and heterotrophy anoxic yield coefficient (Y_H).

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1. Introduction

Nitrogen removal is one of the main objectives to meet increasingly stricter discharge requirements in some wastewater treatment plants (WWTP). However, nitrogen removal is limited by the shortage of available organics in domestic wastewaters; presently, external carbon sources have attracted more attention for improving the COD/TN ratio of influent wastewater and enhancing nitrogen removal efficiency. Research on the utilization of carbonaceous organic materials as alternative carbon sources for denitrification has been conducted for over 20 years (Sage et al., 2006). Additional literature has verified the effectiveness of common carbon sources (acetate, methanol, ethanol and glucose), but the

* Corresponding author. *E-mail address:* xcwang@xauat.edu.cn (X.C. Wang). cost of these sources causes them to be difficult to apply in practical wastewater treatment plants (Kargi and Uygur, 2003; Peng et al., 2007). Consequently, various results have been reported regarding industrial wastewater and food industry effluent and their use as external carbon sources for denitrification due to their practical characteristics such as cleanliness (i.e., relatively free of metals and other contaminants), stability in terms of composition and content of readily biodegradable organic compounds, and availability in the required quantities on a consistent schedule. Several different organic mixtures have been tested under lab-scale conditions for denitrification performance, e.g., crude syrup and hydrolyzed starch, industrial wastewaters from the ice cream production industry and beet-sugar factories, dairy effluents and agro-food wastewater (Lee and Welander, 1996; Cappai et al., 2004; Sage et al., 2006; Rodriguez et al., 2007; Swinarski et al., 2009; Crzerwionka et al., 2012; Lee et al., 2013). The characteristics of



the external carbon sources exhibited significant effects on the denitrification process such as denitrification rate, denitrification potential, COD demand and biomass yield (Lee and Welander, 1996; Fernandez et al., 2010). Thus, the cost and denitrification characteristics that have been considered when choosing a carbon source have been highlighted.

Food waste is an ideal substrate for anaerobic digestion due to its high moisture content and organic matters. The process of anaerobic digestion consists of four steps: hydrolysis, acidogensis, acetogenesis and methanogenesis; the fermentation products in the first two steps are viable alternative carbon sources for the denitrification process due to the high level of dissolved organic matters (composed of organic acids, primarily acetate, propionic and lactic acid) in the effluent. In this case, food wastes could be economically converted into a carbon resource via anaerobic digestion, which is considered a resourceful way to transform "waste" into "treasure". Therefore, the reuse of food waste can reduce environmental issues with the valorization of products as carbonous organics.

Recently, some literature have evaluated the effects of pH, temperature, substrate concentration, hydraulic retention time (HRT) and organic loading rate (OLR) on the hydrolysis and acidification of food waste (Kim et al., 2006; Chu et al., 2008; Nagao et al., 2012; Jiang et al., 2013). Most previous research has focused on the effects of the aforementioned factors on VFAs production from food waste and then selected the operational parameters that produced the maximum VFAs, all of which were based on the adjusted pH values in the above-mentioned research. However, so far the fermentation products, as external carbon source have failed to gain practical application in WWTPs due to the specific drawbacks of time consuming (5-10 days), consumption of large amount of chemicals (especially for pH adjustment), and particularly larger degree degradation of organic substrates (more than 50%) in the form of gas release (Chu et al., 2008; Liu et al., 2013b; Jiang et al., 2013). In fact, by free hydrolysis and acidogenesis, which usually takes short time and retains most of organics, the carbohydrate and protein of food waste can be transformed to intermediates readily or slowly utilizable by bacteria in the biological reactors (Elefsiniotis et al., 2004), though they are not really in the form of VFAs or lactic acid that are commonly recognized as high quality carbon sources for denitrification (Elefsiniotis and Li. 2006; Sage et al., 2006). Therefore, a short-term free anaerobic fermentation may become suitable for the preparation of FLFW as external carbon source to enhance nitrogen removal in wastewater treatment.

This study aims to select suitable operational temperature and solid concentration from campus food wastes in a short-term free anaerobic fermentation experiment and then to obtain the maximum concentration of total organic acid content in the fermentation liquid. Furthermore, as a complex carbon source, the denitrification performance of FLFW is also assessed.

2. Materials and methods

2.1. Food waste collection and pretreatment

Food waste is available everywhere, but it is easily collected from public restaurants, such as student canteens at a university campus. Therefore, food waste was collected at such a location; it was mainly composed of leftovers of cooked foods, such as rice, noodles, meats, eggs and vegetables. In each collection, bones and insert material (paper and plastic) were discarded; only the fermentable materials were preserved. After selecting the waste, it was crushed and homogenized in a blender to ensure particle sizes of less than 5 mm. The physical and chemical properties of the food waste used in this study are presented in Table 1.

2.2. Seed sludge

The anaerobic sludge obtained from anaerobic zone of a fullscale wastewater treatment plant, whose operation mode is an anaerobic-anoxic-oxic (A^2O) biological unit followed by a submerged MBR and continuous operation for more than two years, was used as the seed organism in the anaerobic fermentation reactors after natural sedimentation for 2 days prior to working.

2.3. Experimental methods

2.3.1. Batch anaerobic fermentation of food wastes

The anaerobic fermentation experiments were conducted using a batch fermenter with a working volume of 10 L. The temperature effect was investigated by operating the fermenter at 25 °C, 35 °C and 55 °C through water jackets connected to a water bath; then, the effect of the initial substrate concentration was controlled by adding different mass ratios of raw food wastes and tap-water (w:w = 1:1, TS = 9%; w:w 2:1, TS = 11%; w:w 3:1, TS = 13%; w:w 4:1, TS = 15%) at a temperature of 25 °C. The fermenter was run for 60 h when the soluble COD (SCOD) and organic acid effluent content were stable without any adjustment of the pH. Each batch experiment was triplicated for obtaining reliable results.

At the beginning of the experiment, the seed organism was added to the reactor to fill one-third of the effective volume, and the remaining volume was filled with food wastes. For the mixing condition, the bladed reactors were operated at 80 rpm with a cycle of 1 h on and 1 h off throughout the experiments. As fermentation liquid production was the main objective of this study, biogas was not collected. The reactors headspace was flushed with nitrogen gas to maintain the anaerobic condition. The reactors were operated in batch mode of one feeding and draw-off. At the end of batch fermentation, the mixture was moved to a centrifugal separator to remove most of the solids, and the liquid was filtered by a 0.45 μ m membrane filter to obtain the fermentation liquid.

2.3.2. Nitrate uptake rate (NUR) tests

The denitrifying sludge was obtained from the anaerobic zone of the anaerobic-anoxic-oxic ($A^{2}O$) process in a practical wastewater treatment plant for acclimation with the external carbon sources, FLFW, glucose or sodium acetate. The synthetic wastewater supplied in the study, containing nutrient and trace element solutions, was prepared for the denitrifying sludge following the methods proposed by Cao et al. (2013). The initial nitrate concentration was maintained at approximately 50 mg/L using 5 M NaNO₃ solution, and the COD/NO₃⁻-N ratio at around 8 with corresponding three carbon sources (FLFW, glucose, and sodium acetate) (Ge et al., 2012). After 15 cycles of continuous operation of the

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Physical and chemical property of food wastes.

| Parameter | units | Value ^a |
|--------------------------------|------------------------|--------------------|
| Total solids content (TS) % | % of wet weight | 17.4 ± 1.6 |
| Volatile solids content (VS) % | % of wet weight | 16.1 ± 2.6 |
| VS/TS | _ | 97.3 ± 1.1 |
| T-carbohydrate | % of TS | 61.5 ± 11.1 |
| T-protein | % of TS | 9.8 ± 2.6 |
| T-lipid | % of TS | 9.2 ± 1.2 |
| Nitrogen content | % of TS | 2.0 ± 0.2 |
| Phosphorus content | % of TS | 0.2 ± 0.01 |
| Total COD (TCOD) | g-COD _{cr} /L | 229.7 ± 15.9 |
| Soluble COD (SCOD) | g-COD _{cr} /L | 55.4 ± 4.2 |

^a Average of 20 testes ±standard deviation.

denitrification process, the effluent nitrogen (nitrate and nitrite) was less than 1 mg/L. Further batch experiments were conducted as follows.

Sealed conical flask reactors (1 L) were used in the batch experiments to investigate the effect of COD/N ratios on the denitrification process. The denitrifying sludge was first loaded into five reactors to ensure the concentration of the denitrifying sludge for all tests was 2500 ± 200 mg VSS/L. Then, different quantities of carbon sources and NaNO₃ solutions were added into the reactors with synthetic wastewater, with initial COD/N ratios of 2, 4, 6, 8 and 10, respectively. The nitrogen gas was immediately pumped into the reactor to establish an anoxic environment. All of the reactors were placed on stirring plates in a constant temperature incubator at 25 ± 1 °C. The initial pH values in all of the batch experiments were adjusted to 7.5 \pm 0.2 using a 0.5 M NaOH and 0.5 M HCl solution.

2.4. Sampling and chemical analysis

All of the mixed fermentation samples were measured by stepwise dilution methods, and the fermentation liquid was analyzed after it was centrifuged and filtered through 0.45-µm pore size Millipore filter units. The carbohydrate was determined according to the phenol-sulfuric acid method, using glucose as the standard (Herbert et al., 1971). The protein concentration was measured by the Lowry-Folin method with BSA as standard (Lowry et al., 1951).

The VFA concentration was determined using a gas chromatograph (GC, Agilent 6890N) equipped with a flame ionization detector (FID) and a WAXETR column (30 m × 0.25 mm × 0.25 µm). N₂ was the carrier gas, and the flux was 20 ml/min. The injection port and the detector were maintained at 200 °C and 250 °C, respectively. The GC oven process was conducted according to the column temperature ramp program (100 °C, 2 min; 3 °C/min; 160 °C; 2 min; 250 °C). The sample injection volume was 1 µL.

The lactic acid concentration was determined using a liquid chromatograph (LC, Shimadzu Co., Ltd.) equipped with an ultraviolet detector. COSMOSIL 5C18-II was used for the column, and 0.05 mM phosphoric acid buffer liquid (50 mM NaH₂PO₄: 50 mM H₃PO₄ = 9:1) was used for the carrier liquid. The analysis was conducted at a detector temperature of 40 °C with a follow velocity of 1 ml/min and a UV value equal to 210 nm.

All soluble samples in the NUR tests were analyzed after filtrating through the 0.45- μ m pore size Millipore filter units. Quantities of NO₃-N, NO₂-N, MLSS, MLVSS were each measured according to standard methods (APHA, 1999). COD was measured using a HACH quick-analysis apparatus.

2.5. Calculation methods

2.5.1. Hydrolysis rate and acidogenesis rate

The total COD conversion ratio of the hydrolysis and acidification can be calculated according to soluble COD (SCOD), COD_{VFA} and COD_{lactic} acid, respectively. These can be expressed as the product divided by the influent COD in each reactor. All calculations were determined by previous research and the following equations were used following equations (Chu et al., 2012):

$$Hydrolysis(COD) = \frac{SCOD}{TCOD_{in}}$$
(1)

$$Acidogenesis(COD) = \frac{COD_{VFA} + COD_{lactic acid}}{TCOD_{in}}$$
(2)

where, TCOD_{in} is the total COD of the influent.

2.5.2. Denitrification rate and denitrification potential

The denitrification rate (V_{DN}) and denitrification potential (P_{DN}) were calculated using the following equations (Sage et al., 2006):

$$V_{DN}(mg/gVSS \cdot h) = \frac{NO_{x,initial} - NO_{x,S_s}}{MLVSS \cdot t_{S_s}}$$
(3)

$$PDN(gN/gCOD) = \frac{N_{initial} - N_{end_{(xs)}}}{COD_{in} - COD_{e}}$$
(4)

where t_{Ss} is the time when the biodegradable fraction (S_s) of the carbon source was consumed (h); $NO_{x,initial}$ and $NO_{x,Ss}$ are the initial NO_x -N concentration and that at t_{Ss} , respectively (mg/L); COD_{in} and COD_e are the total exogenous COD added and the effluent COD (mg/L), respectively; $N_{initial}$ and $N_{end(XS)}$ are the concentrations of NO_x -N at the initial sampling point and the final sampling point at full X_S consumption (mg/L), respectively.

2.5.3. Heterotroph anoxic yield (Y_H) and readily biodegradable fractions (S_S)

 Y_H and S_S were related to P_{DN} by the following equations (Cokgor et al., 1998; Kujawa and Klapwiji, 1999; Boursier, 2003):

$$1 - Y_{\rm H} = 2.86 P_{\rm DN}$$
 (5)

$$S_{S}(mgCOD/L) = \frac{(\Delta NO_{X} - N)S_{S}}{P_{DN}}$$
(6)

where $(\Delta NO_x-N)S_s$ was the reduced value of the nitrogen concentration in the first phase with FLFW as the carbon source.

3. Results and discussion

3.1. Selection of suitable condition for fermentation liquid production

For fermentation liquid production in this study, attention was paid to the hydrolysis and acidogenesis processes which are usually taken as the first two stages of anaerobic fermentation before the generation of large amount of biogases. Due to the acidogenesis of the hydrolyzed organic substances, pH could quickly decreased to about 3.0 and almost be kept in the whole fermentation process, which was a condition unfavorable for hydrogen and methane gas production.

3.1.1. Effect of fermentation temperature

As shown in Table 1, carbohydrate, protein and lipid are the main carbon constitutes of food waste and account for 61.5%. 9.8% and 9.2% of TCOD, respectively. It has been reported that a lower degradation rate of lipid occurs in comparison to carbohydrate or protein; thus, TCOD reduction was analyzed by focusing on the carbohydrate and protein (Sun et al., 2013). Fig. 1a shows the results of the reduction of the main organics at room temperature (25 °C), mesophilic temperature (35 °C) and thermophilic temperature (55 °C). Regarding TCOD, its reduction was less than 10% under each temperature, indicating that the short-term free fermentation without pH adjustment could bring about much less biogas production than what was reported in literature (Chu et al., 2008; Liu et al., 2013b; Jiang et al., 2013). Regarding the main organics composition, carbohydrate reduction of 6.94% at 55 °C was significantly lower than that of 24.94% and 20.33% at 25 °C and 35 °C, respectively; however, protein reduction was obtained as 18.64%, 18.3% and 14.6%, respectively, at 25 °C, 35 °C and 55 °C, indicating the maximum organics reduction at room temperature. Chu et al.



Fig. 1. Effect of temperature on reduction of organics (a) and COD transformation rate (b).

(2012) reported that carbohydrate was the main substrate to produce more organic acids through biological fermentation than proteins because protein quantity increased due to cell growth in the degradation of carbohydrate. Based on the above results, carbohydrate reduction seems to provide some significant information about the following steps during the fermentation process.

Hydrolysis occurs when insoluble organic polymers are broken into simple sugars, fatty acids and amino acids. Because most of the organics in food waste are in particulate form, hydrolysis is the first step of the anaerobic fermentation process. As shown in Fig. 1b, the hydrolysis rate of food waste reached 47% at 55 °C, which is higher than the rates of 32.8% and 32.4% at 25 °C and 35 °C, respectively. Liu et al. (2013a) also observed that a higher degree of hydrolysis occurred in thermophilic fermentation than mesophilic fermentation; however, the acidogenesis rate of 3.2% was the lowest at 55 $^\circ\text{C}$ in the study. Higher temperature can accelerate the activity of extracellular enzymes; meanwhile, acidogenesis bacterial are inhibited at higher temperature. It has been reported that the formation of various organic acids should be associated with carbohydrate reduction (Liu et al., 2013a). Based on the preceding results, the carbohydrate reduction degree was the highest at 25 °C, and the same result was obtained with the greatest acidogenesis rate of 6.9% at 25 °C.

Therefore, these data show that the hydrolysis rate at 25 °C was close to the rate that occurred at 35 °C but lower than that at 55 °C; however, the acidogenesis rate was highest at 25 °C. Hence, 25 °C was the most efficient temperature for organic acids preparation in this study.

3.1.2. Effect of initial solid concentration

The experiments on the effect of the initial solid concentration on SCOD and total organic acids (TOA) were conducted at 25 °C. As shown in Fig. 2a, the SCOD concentration in FLFW increased as the solid concentration increased; however, the SCOD concentration reached 69.1 g/L at a solid concentration of 15%, which only exceeded 6.5 g/L in comparison with that of 13%. Meanwhile, the greatest concentration of TOA obtained at a solid concentration of 13% was 16.63 g/L.

Lactic and acetic acids were most prevalent organic acids, accounting for more than 90% of TOA, and the lactic acid content was higher than the acetic acid content in both cases (Fig. 2b). It was observed that the increase in the initial solid concentration produced an increase in the acetic acid concentration. The highest value, 3.8 g/L, was obtained at a solid concentration of 15%. Ramos et al. (2012) reported similar observation. However, irregular relationship between lactic acid concentration and initial solid concentration were obtained, and the highest lactic acid value was observed at a solid concentration of 13% (12.4 g/L).

Under the above-mentioned condition, the organic components of the fermentation products included up to 75% of lactic acid, 21% of acetic acid, and about 4% of propionic acid. As most of TOA was in the form of lactic acid, it could be suggested that the predominant production of lactic acid was due to the abundance of indigenous lactic acid bacterial (LAB) in the food waste which could effectively utilize the mixed carbohydrate of hydrolyzates derived from food waste (Probst et al., 2015; Wang et al., 2015; Wu et al., 2015). Lactic acid is an intermediate metabolic product and may also be effective for denitrification because it has been considered as the precursor for propionic and acetic acids production (Elefsiniotis and Li, 2006; Li et al., 2014). Although lactic acid may not be as readily utilizable as propionic acid for denitrifiers, it is still effective for denitrification in longer biological reaction process (Sage et al., 2006).

In the process of short-term free fermentation, only about 0.125 g/L of NH_4^+ -N was released probably due to protein degradation, which was much lower than the SCOD of 62.6 g/L. Therefore, when the FLFW was used as external carbon source, it might not result in significant increase of NH_4^+ -N in the wastewater.

3.2. Performance of FLFW as a carbon source for the denitrification process

3.2.1. Effect of initial COD/N ratios on the denitrification process

Fig. 3 illustrates the transformation of NO₂⁻-N, NO₃⁻-N and COD in the denitrification process at different initial COD/N ratios when using FLFW as the carbon source. Incomplete denitrification often occurs when the carbon is limiting and is characterized by a large fraction of residual nitrate and a slight nitrite accumulation at the end of the process. Such a relationship was clearly indicated when the COD/N ratio was 2, and the accumulation value of nitrite (i.e., 12% of the total influent nitrate) in the effluent when the COD/N ratio was up to 4, though a value lower than 2 mg NO_3^--N/L was obtained in the treated effluent after 3 h of reaction. At COD/N ratios up to 6 and 8, the nitrite accumulation substantially increased to a value of 9.5 mg/L and 12.2 mg/L, respectively, at 60 min with complete denitrification (N quantities in the effluent was less than 1 mg/L), which indicated that denitrification was limited by the carbon amount rather than the carbon availability. The maximum nitrite accumulation occurred 10 min earlier with



Fig. 2. Effect of initial solid concentration on soluble organics (a) and organic acids (b).



Fig. 3. Variations of NO₂-N, NO₃-N, COD/COD₀ with FLFW as carbon source.

COD/N ratios of up to 6 and 8 but the amount did not increase when the COD/N ratio was up to 10. With respect to the residual COD in the effluent, values lower than 30 mg/L with COD/N ratios of 2, 4 and 6, and increased concentrations with COD/N ratios of 8 and 10 were obtained in the treated effluent after 4.5 h of reaction. Therefore, the optimal COD/N ratio (where both the COD and nitrogen concentrations reached minimum values in the effluent) for FLFW was 6. The following discussion about FLFW was based on the COD/N ratio of 6.

3.2.2. NUR tests

The denitrification performance of a complex carbon source is assessed by NUR tests, and then the readily biodegradable COD (S_S) and slowly biodegradable COD (X_S) in the FLFW could be evaluated

(Sage et al., 2006). During the NUR test, the variation of the NO_x-N concentration (the nitrogen provide by nitrate and nitrite) with the reaction time at COD/N ratio of 6 is shown in Fig. 4. The nitrate utilization (NOx-N) curve was obtained by calculating $NO_3^- - N + 0.6 \times NO_2^- - N$ based on the fact that reducing 1 g NO₂⁻-N to 1 g N_{2-gas} requires the same number of electrons as the reduction of 0.6 g NO3-N to 0.6 g N2-gas (Kujawa and Klapwijk, 1999). The NO_x-N curve could be divided into three characteristic stages (Fig. 4). In the first 60 min, NO_x-N decreased rapidly due to the denitrification utilizing S_S, while in the following 40 min the denitrification slowed down due to the anoxic utilization of the hydrolyzed X_s. The following stage could be viewed as endogenous denitrification because both S_S and X_S were almost completely consumed. The slopes of the NO_x-N curve corresponding to S_S and X_S consumptions could be obtained by linear regression referring to Sage et al. (2006) who pointed out that the non-additive effects of S_S and X_S would be more reliable because the denitrification rate due to the hydrolysis product of X_S might not add to the



Fig. 4. Analysis of readily biodegradable fraction (S_S) and slowly biodegradable fraction (X_S) in FLFW.

denitrification rate due to S_S.

The specific V_{DN} corresponding to the consumption of S_S (first stage of the NO_x-N curve) was obtained as 12.89 mgN/gVSS h by Eq. (3). The COD consumption rate (V_{COD}) could also be calculated as 90.39 mg COD/gVSS h in this stage. The V_{DN} value thus obtained was much higher than that reported values between 2.7 and 3.6 mgN/gVSS h when the wastewaters from ice-cream and beetsugar factories (Cappai et al., 2004), or hydrolyzed molasses (Ouan et al., 2005) were used as carbon sources in denitrification processes. The V_{COD} value obtained was also much higher than that reported by Rodríguez et al. (2007) when chemical acetic and propionic acids were used. Therefore, it could be concluded that the FLFW used in this study, as a complex organic mixture, was easily utilizable by the denitrification bacteria due to the high content of readily biodegradable substrate in the FLFW. Regarding denitrification using Xs (second stage of the NO_x -N curve), the V_{DN} and V_{COD} could be roughly evaluated as about 6.8 mg N/gVSS h and 14.5 mg COD/gVSS h, respectively, because the utilization of Xs had to go through a slow hydrolysis process which was a ratelimiting step of denitrification (Boursier, 2003; Cokgor et al., 1998). In fact, as can be seen from Fig. 4, nitrogen removal was as high as 59.2% in the first stage in contrast to 16% in the second stage.

The P_{DN} was evaluated as 0.174 g N/g COD using Eq. (4) and Y_H was then obtained as 0.5 g COD/gCOD. In principle, P_{DN} represents the quantity of oxidized nitrogen which can be reduced by the consumption of a given quantity of carbon in an activated sludge. Based on electron balances, the theoretical total consumption of COD per gram of nitrate-nitrogen converted to nitrogen gas is 2.86 g. However, in addition to denitrifying bacteria the growth and maintenance of other bacteria communities may also consume carbon source. Therefore, the Y_H is used for the description of the biomass yield in the whole biological process, and the Y_H obtained in this study was similar to previous studie (Sage et al., 2006).

Using Eq. (6), the S_S fraction was calculated as 58.35%, and Xs could then be evaluated as 41.65%, showing that the readily biodegradable organics in FLFW was very high. However, the TOA directly measured from FLFW took only 24.6% of SCOD. This indicated that in the FLFW not only organic acids but also a large portion of other organics would also be easily used by denitrifying bacteria. The Ss fraction of domestic wastewater is usually 10–20% of TCOD (Henze et al., 1994). The much higher Ss fraction in FLFW explains that it is a high quality carbon source for enhancing denitrification.

3.3. Comparison of FLFW with conventional carbon sources

3.3.1. Comparison of nitrogen removal efficiency

Glucose and sodium acetate are commonly selected as external carbon sources in wastewater treatment under a condition of insufficient carbon source. Comparative experiments were conducted regarding the denitrification performance of glucose, sodium acetate in comparison with FLFW under identical operation condition. As a result, when glucose was used incomplete denitrification was observed in 6 h of reaction time due to the higher residual concentration of nitrate (22.2 mg/L) and some accumulation of nitrite (6.6 mg/L) in the effluent. However, with sodium acetate, no nitrite accumulation occurred, and a lower concentration of nitrate (less than 1 mg/L) was measured in the effluent. The overall efficiencies of nitrogen removal using the three carbon sources are compared in Fig. 5. It can be seen that FLFW could achieve a total nitrogen removal as high as 98%, about the same as that for sodium acetate, though a longer time was required for approaching the stable removal (about 2 h for FLFW versus 1 h for



Fig. 5. Effect of carbon sources on removal efficiency of TN at COD/N ratio of 6.

sodium acetate). In contrast to FLFW and sodium acetate, the final removal efficiency was only 39.6% for glucose even after 6 h reaction time. It can thus be concluded that FLFW can be used as an alternative carbon source for sodium acetate in the denitrification process.

3.3.2. Comparison of denitrification parameters

Fig. 6 compares the special denitrification parameters such as V_{DN} , V_{COD} , P_{DN} , and Y_H with three carbon sources. The V_{DN} with FLFW was slightly smaller than that with sodium acetate but much higher than that with glucose (Fig. 6a). However, regarding the special value of V_{COD}, the greatest value was obtained with FLFW but not sodium acetate, while that with glucose was still much smaller (Fig. 6b). Higher V_{COD} often occurs in a wider variety of microbial communities when incubating with a complex organic mixture, because the COD consumption is related to the activity of the whole microbial system but not merely denitrifying bacteria. The patterns of microbial composition were found to be similar when acetate, methanol, crude syrup and hydrolyzed starch were used to stimulate the growth of different types of denitrifying bacteria, while a more heterogeneous and metabolically versatile microbial community appeared when crude syrup and hydrolyzed starch were used as the carbon sources (Lee and Welander, 1996). Due to the competition between different types of bacteria in utilizing the FLFW carbon source, V_{DN} became lower than that in the case of using sodium acetate as a mono-component carbon source solely for denitrification.

The P_{DN} values appeared similar with FLFW and sodium acetate while that with glucose was much lower (Fig. 6c). Theoretically, under anoxic conditions with biodegradable organic substrates sufficiently available, the quantity of electrons for the reduction of NO₃-N to N₂ equals to the electrons provided by the carbon sources. However, the carbon sources may also be consumed by microbial activities other than denitrification which results in a "loss" in COD and can be described by the value of Y_H. As shown in Fig. 6d, the highest Y_H value was 0.74 g COD/gCOD when glucose was used as the carbon source, because the carbohydrates had a higher sludge yield and stimulated not only denitrifying bacteria but also the fermentative and nitrite-producing bacteria. With FLFW and sodium acetate as the carbon sources, lower Y_H values (about 0.5 COD/gCOD) were obtained. The value of Y_H usually varies with carbon source types and microbial conditions, such as the Y_H values of 0.44–0.65 experimentally determined by Rodríguez et al. (2007) with agro-food wastewaters, and that of 0.66 calculated by Kujawa and Klapwijk (1999) under an assumption that internal storage of soluble COD occurred with acetate.



Fig. 6. Effect of carbon sources on denitrification parameters at COD/N ratio of 6 (V_{DN}: denitrification rate; V_{COD}: COD consumption rate; P_{DN}: denitrification potential; Y_H: heterotrophy anoxic yield).

3.3.3. Evaluation of practical applicability of FLFW

In China and maybe some other countries, a low COD/TN ratio often becomes a problem to restrict denitrification efficiency for WWTPs. Therefore, addition of external carbon source is usually required to increase the COD/TN ratio to a level sufficiently high for effective denitrification. According to this study, the denitrification potential P_{DN} of the FLFW is 0.174 g N/g COD and the productivity of carbon source from the food waste is 0.096 g SCOD/g FW. In some cases, the effluent TN concentration could be as high as 15-20 mg/L due to low COD/TN ratio (Czerwionka et al., 2012; Lee et al., 2013). If a complete TN removal is supposed to be the target of external carbon source addition, it can be calculated that 0.9-1.2 kg FW would be needed for the production of FLFW for the treatment of per m³ wastewater, which is equivalent to the food wastes generated by 2 or 3 persons (Zhang and Fu, 2010). In the case of domestic wastewater treatment, the per capita sewage discharge is usually about 0.2 m³. Therefore, the food wastes generation from domestic sources can be considered sufficient to meet the needs for FLFW production to prepare external carbon source for enhancing nitrogen removal in domestic wastewater treatment.

4. Conclusions

FLFW with high organic acid content was produced in this study under anaerobic conditions without pH adjustment and at room temperature. Using the FLFW as the sole carbon source for wastewater treatment, maximized denitrification was achieved at COD/N = 6. The readily biodegradable fraction in the FLFW was as high as 58.35% and brought about a denitrification result similar to sodium acetate but much better than glucose in terms of nitrogen removal, V_{DN}, P_{DN}, V_{COD} and Y_H. This fully showed the advantageous property of the FLFW as a complex carbon source over mono-component carbon source for enhancing nitrogen removal.

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