Characterization of Anaerobic Effluent: Seasonal Influence and use of Anammox Process as Post-Treatment

Pamela Alexa Sachetto

UNICAMP

Rosana Oliveira Menezes

FEC/UNICAMP

Luana Mattos de Oliveira Cruz (✉ luanamoc@unicamp.br)

UNICAMP: Universidade Estadual de Campinas <https://orcid.org/0000-0003-3795-9111>

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Abstract

This study aimed to verify if subtropical annual seasonality variation of organic matter and nitrogen ratio facilitates the use of anammox process as a post – treatment of anaerobic effluent. We characterized the anaerobic effluent from a wastewater treatment plant (WWTP) located in Campinas/SP, Brazil. The collected composed samples were from an UASB reactor in different days, hours and months during one year. We concluded subtropical annual seasonality of COD/N ratio range (2.2 to 3.1) from anaerobic effluent indicates that the use of anammox process is appropriate as post-treatment of anaerobic effluent in subtropical areas. However, as Total COD degrades over time and as total nitrogen concentration is influenced by seasons, these factors need to be taken into account in the project and operation of the reactor over the year.

Introduction

In Brazil, most WWTP use upflow anaerobic sludge blanket (UASB) reactor to treat domestic sewage followed by a post-treatment unit (Chernicharo et al., 2015). Considering the necessity to develop and enhance new post-treatment technologies especially for nitrogen and phosphorus removal, it is essential to know the effluent composition from UASB reactor, since having greater knowledge of each fraction of the anaerobic effluent enables the development of more accurate and efficient post-treatment methods.

Since Mulder et al. (1995) has described anammox (ANaerobic AMMonium-OXidizing) process, it has been considered a promising method for biological nitrogen removal from industrial and domestic effluents (Kartal et al., 2010; Van Loosdrecht and Brdjanovic, 2014).

Anammox bacteria use nitrite as an electron acceptor during ammonium ion oxidation process. As result, nitrogen forms are converted and, consequently removed from effluent, into nitrogen gas (Lotti et al., 2014).

This process provides several advantages over traditional nitrogen removal methods, such as lower energy and oxygen demand, lower sludge production, and no need for external carbon source (Mulder, 2003).

Despite its advantages, few studies were performed to examine the application of the process as post-treatment of effluents with low ammonium ion concentration and higher COD/N ratio, which are characteristic conditions of the anaerobic treated sewage in tropical countries WWTP (Sanchez – Guillén et al., 2014; Leal et al., 2016; Chen et al., 2016; Li et al., 2019 and Wang et al., 2020).

COD/N ratio plays an important role when choosing and operating technologies and process for nitrogen removal in a WWTP. It significantly influences the fraction of microorganisms that convert nitrogen compounds (Li et al., 2019). In addition, DO concentration should be adjusted according to this ratio (COD/N) in order to enhance nitrogen removal via anammox process (Wang et al., 2020).

Studying post-treatment techniques is extremely important. For instance, although there are sewage treatment in 80% of municipalities in São Paulo state in Brazil, only 4.3% have tertiary level treatment with potential for nutrient removal (IBGE, 2008). Moreover, the release of nitrogen compounds in water bodies can lead to deterioration of ecosystems and serious consequences for the environment and public health (Ahn, 2006).

Considering the importance of developing efficient and accurate post-treatment methods and the need to know the effluent composition to develop these processes (Li et al., 2019), this work aims to verify if subtropical annual seasonality variation of COD/N ratio, in subtropical areas, facilitates the use of anammox process as a post – treatment of anaerobic effluent.

Methods

Wastewater Treatment Plant description

We have collected the anaerobic effluent (effluent from the Upflow Anaerobic Sludge Blanket – UASB reactor) from a WWTP that is located in Campinas, SP/ Brazil.

The WWTP performs biological treatment of household sewage using UASB reactors, followed by high-rate trickling filter. Currently the WWTP has 3 modules with 2 UASB reactors in each module. Each UASB reactor has useful volume of 1368.5 m³, hydraulic retention time greater than or equal to 8 hours for the mean flow rate of the station, which is equivalent to 327.6 m³h⁻¹.

Sample Collection

Composed samples used in the analyses were obtained through the collection of simple samples of effluent at different times of the day (9am, 12 pm, 3 pm, 6 pm, and 9 pm). They were storage at 4 °C until the next day, when these simple samples were mixed and the composed sample was formed to be analyzed. Analyses were performed in triplicate for each of the three days of the week (Sunday, Monday, and Wednesday) in each season of the year. COD samples were preserved at pH less than 2.0.

Since the hourly flow rates had little variation between sampling hours (Appendix A Table 1), there was no difference in the volumetric proportion of the composed sample. Thus, the final sample was composed of 400 mL of the simple sample of each hour.

Table 1
Mean flow rates per time from WWTP where anaerobic effluent samples were collected.

Time	9am	12 pm	3 pm	6 pm	9 pm
Flow rate (Ls ⁻¹)	79	118	114	96	101

Effluent Characterization

The anaerobic effluent was characterized for concentration and composition of organic and solid material. We analyzed chemical oxygen demand (COD) to evaluate sewage biodegradability and organic material concentration. In addition, we examined the concentrations of dissolved organic material ($\text{COD}_{\text{filtered}}$) – material not retained in filter with porosity of $0.45\ \mu\text{m}$ – and suspended organic material ($\text{COD}_{\text{unfiltered}}$).

The solids were also quantified so the values were correlated with the organic material. We described the concentrations of total suspended solids (TSS), fixed suspended solids (FSS), and volatile suspended solids (VSS).

In addition, the effluent was also characterized for turbidity, conductivity, pH, partial alkalinity, total alkalinity, phosphorus, ammonium, nitrite, nitrate, and total kjeldahl nitrogen (TKN) concentrations.

The analyses were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2012).

Through analyses of composed samples of anaerobic effluent, we obtained mean effluent characterization for each season of the year, with their respective standard deviation, as shown in Table 2 in Appendix B.

Table 2
Subtropical annual seasonality of analyzed variables from composed anaerobic effluent samples (mean and standard deviation values).

	Summer	Autumn	Winter	Spring
Temperature (°C)	24 ± 0.9	21 ± 1.0	20 ± 1.0	22 ± 0.5
Precipitation (mm)	92 ± 92	10 ± 9	53 ± 35	98 ± 17
Turbidity (NTU)	57 ± 9	118 ± 39	86 ± 4	69 ± 10
pH	6.8 ± 0.2	7.0 ± 0.1	7.1 ± 0.1	6.3 ± 1.3
Electrical conductivity (EC) (μS)	478 ± 53	705 ± 22	688 ± 40	485 ± 119
Partial Alkalinity (mg CaCO ₃ L ⁻¹)	155 ± 17	211 ± 2	210 ± 2	157 ± 42
Total alkalinity (mgCaCO ₃ L ⁻¹)	189 ± 13	281 ± 3	270 ± 5	208 ± 56
Total COD (mgO ₂ L ⁻¹)	65 ± 6	157 ± 16	138 ± 16	126 ± 14
Soluble COD (mg O ₂ L ⁻¹)	29 ± 6	24 ± 20	52 ± 11	25 ± 4
Phosphorus (mgPL ⁻¹)	3 ± 0.5	4 ± 0.3	6 ± 0.3	4 ± 0.6
TSS (mgL ⁻¹)	28 ± 5	84 ± 42	40 ± 8	48 ± 4
VSS (mgL ⁻¹)	24 ± 4	68 ± 34	35 ± 8	42 ± 2
FSS (mgL ⁻¹)	5 ± 1	16 ± 7	5 ± 0.8	5 ± 2
NH ₃ (mgNL ⁻¹)	25 ± 3	50 ± 1.4	47 ± 4	36 ± 9
TKN (mgNL ⁻¹)	30 ± 5	56 ± 0.7	51 ± 2	40 ± 10
COD/N	2.2 ± 0.7	2.8 ± 0.2	2.7 ± 0.7	3.1 ± 0.9

In order to obtain the seasonal influence on anaerobic effluent composition, we estimated the mean temperature and precipitation of each season of the year, based on data obtained from Somar Meteorologia (2018) for the city of Campinas from January 1st, month when the analyses began, to November 5th, date of last sampling. The information is also shown in Table 2 in Appendix B.

Results And Discussion

Subtropical annual seasonality of COD/N ratio range (2.2 to 3.1) from anaerobic effluent indicates that the use of anammox process is feasible as post-treatment of anaerobic effluent in subtropical and tropical areas.

Anaerobic effluent characterization according to subtropical annual seasonality

Precipitation standard deviations are very high (Appendix B Table 2 and in Fig. 1), which is usual for the climate zone where the country is located. Brazil is located in a region characterized by tropical climate, where the seasons are not very well defined. In the same period, there are extremely humid and extremely dry weather, which generates these large deviations from the mean precipitation in the season.

The tropical climate also justifies the slight difference between the mean temperatures of each season (difference of 4°C as shown in Appendix B Table 2 and in Fig. 1). In addition, in this climate zone there is no extremely cold season, which is consistent with the data shown in Appendix B Table 2 and in Fig. 1, as the mean temperature of all seasons were above 19 °C. This regularity in higher temperatures favors anammox activity as reported by Sanchez – Guillén et al. (2014).

When evaluating the following variables: partial alkalinity, total alkalinity, conductivity, turbidity, ammonium nitrogen (NH_3) and total kjeldahl nitrogen (TKN); we found the same behavioral profile over the seasons. The values varied from the highest to the lowest ones according to the following seasons, respectively: autumn, winter, spring, and summer. Although the difference between autumn and winter values were always very slight, we can observe that seasonality influences the effluent concentration.

The driest seasons (autumn and winter) showed higher values for the aforementioned variables, which can be justified by the fact that in the rainier seasons (summer and spring) the effluent can be diluted by rain.

Even though in Brazil there is total separation of sewer system and drainage system, we can infer that clandestine connections in the sewer system can exist and influence the effluent variables concentration.

In spite of spring being the rainiest season (6.12% higher precipitation than summer), summer presented the lowest variable values, which proves that in addition to the pluviometric factor, temperature is also a determining aspect for effluent composition.

The fact that summer presented the lowest values can be justified by its higher mean temperature compared to the mean one of spring. Thus, concentration variable (partial alkalinity, total alkalinity, conductivity, turbidity, ammonium nitrogen (NH_3), and total kjeldahl nitrogen (TKN)) values are inversely proportional to temperature. This can be explained due to people's habit during summer periods. Normally in these periods, in tropical countries, people use more water and take more showers during the days, diluting more the effluent.

Table 2 (Appendix B) shows that precipitation was very similar in summer and spring (6.12% difference), but the difference in temperature was approximately 2 °C. While in autumn and in winter the difference in precipitation was very significant (81.13%) and the temperature had no significant difference.

As we found a much larger difference between the parameters for summer and spring than for autumn and winter, we concluded that the influence of temperature is much higher than that of pluviosity on effluent composition. It may be explained by the separated sewage collection Brazil, which is not constructed to receive and transport rainwater, although some not predicted connection may be added in.

Results obtained for pH, fixed suspended solids (FSS), and phosphorus concentration were approximately constant in all seasons, indicating that these parameters are not influenced by seasonality.

Regarding the organic matter, according to the literature, the expected value for total COD should be around 150 to 170 mg O₂L⁻¹ (Saliba & Von Sperling, 2017 and Leal et al., 2016). It is approximately 60% higher than the value found for total COD in summer, which was only 65 mgO₂L⁻¹. This significant difference between theoretical and experimental values for COD may be justified by the limitation we had during summer samples collection which were not preserved with acid. Thus, there was organic matter degradation over time and the value obtained experimentally is probably below the actual value. For the other seasons, when there was no limitation to preserve the samples with concentrated sulfuric acid (pH < 2.0), values were close to the theoretical one (range of 126 to 157 mgO₂L⁻¹).

Analyzing the total COD in these three seasons (autumn, winter, and spring), we observed that the values are very close, showing a maximum difference of 13%. Therefore, it may be considered that total COD is also not significantly influenced by precipitation and temperature, being approximately constant in all seasons.

Regarding soluble COD, we obtained very close concentrations in summer Autumn and spring, 29, 24 and 25 mg O₂L⁻¹, respectively. For winter (52 mg O₂L⁻¹) higher concentration values were found. For this type of effluent, Saliba & Von Sperling (2017) obtained a concentration close to 92 mg O₂/L, and Leal et al. (2016) around 80 mg O₂/L, which is nearly the double of the highest concentration obtained. The difference is due to, probably, the filter used to analyze the soluble COD. In our study, we have used porosity equals to 0.45 µm.

Total suspended solids (TSS) and volatile suspended solids (VSS) showed the same profile, which is different from the previous profile presented by the other parameters. These solids presented a greater concentration in autumn (84mgTSSL⁻¹ and 68mgVSSL⁻¹), followed by spring (48mgTSSL⁻¹ and 42mgVSSL⁻¹), and the lowest concentration was obtained in summer (28mgTSSL⁻¹ and 24mgVSSL⁻¹). It is worth mentioning that the concentrations for all seasons and for the two analyses are close to the values found in the literature (Brito, 2006; Leal et al., 2016 and Saliba & Von Sperling, 2017).

COD/N ratio according to subtropical annual seasonality

One way to predict anammox process behavior as a post – treatment of anaerobic effluent is by analyzing the COD/N ratio, because this ratio controls the balance between heterotrophic bacteria, ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and anammox bacteria (Chamchoi et al., 2008; Meng, 2018 and Li et al., 2019). Up to a certain limit, the presence of organic matter in the reactor's feeding makes nitrogen removal more efficient, because it simultaneously causes the anammox process and the heterotrophic denitrification (Du et al., 2015; Langone et al., 2014; Ni et al., 2012 and Wang et al., 2020). However, if this limit of organic matter is exceeded, it is initiated a competition between anammox and heterotrophic bacteria, which starts to have excessive growth and leads to reduced anammox activity (Ibrahim et al., 2016; Chen et al., 2016 and Li et al., 2019).

Although an optimum COD/N ratio for anammox activity is not well established, authors have described some possible range in which anammox had activity and/or coexisted with denitrifiers. This ratio range varies from 1 to 6. The ratio value depends on COD influent concentration (Chamchoi et al., 2008; Ni et al., 2012; Chen et al., 2016 and Leal et al., 2016), dissolved oxygen concentration in the reactor (Li et al., 2019 and Wang et al., 2020), sludge retention time (Jenni et al., 2014) and the hydraulic retention time from the previous UASB treatment (Li et al., 2019).

According to Ni et al. (2012), small amounts of organic matter (from 100 to 200mgCODL⁻¹) may enhance nitrogen removal. The maximum COD/N ratio suggested by these authors are 3.1, if anammox bacteria are presented in a granular sludge. Chen et al (2016) also confirmed that COD concentration lower than 100mgL⁻¹ could enhance nitrogen removal via the coexistence of denitrification and anammox processes. However, they showed that higher COD could deteriorate the anammox activity with almost complete inhibition at the COD concentration of 285 mgL⁻¹.

Chamchoi et al. (2008) reported COD concentrations above 300 mgL⁻¹ (COD/N ratio of 2) may fully inhibit anammox reaction and concomitantly favor the activity of denitrifying bacteria.

In a short – term batch test, Sánchez-Guillén et al. (2014) added organic carbon as acetate and starch and they verified that the COD/N ratios between 2 and 6 did not influence significantly anammox bacteria. They reported that lower temperatures (less than 14°C) influences more in its activity, thus they concluded anammox is suitable for warmer temperatures as in tropical and subtropical countries, e.g. Brazil.

Jenni et al. (2014) determined the influence of different COD concentrations and hydraulic retention times on the nitrogen removal via anammox process. The COD/N ratio was gradually increased up to 1.4 gCODgN⁻¹, while the hydraulic retention time was reduced. After adding acetate and glucose (as carbon sources), the efficiency reached nearly 95%. The results showed that with a ratio of 0.8 gCODgN⁻¹ and higher there was a reduction in the abundance of anammox bacteria (estimated by FISH).

Wang et al. (2020) also showed anammox pathway might have a strong contribution in nitrogen removal routes under lower DO (0.8 mg/L) and lower COD/N ratio (6 and 2) condition. They concluded that DO

supply should be adjusted according to the influent COD/N ratio for total nitrogen removal.

When modelling a suspended growth model, Li et al (2019) could reach a total nitrogen removal of 93% by applying COD/N ratio equals to 2. The authors reported that anammox was responsible for 75% of the total removal. They also suggested that to obtain the maximum COD/N ratio (2) which allows a good performance of nitrification - anammox reactor, the HRT from a previous anaerobic pretreatment should be controlled up to 11 hours. In our studies, the UASB reactor operated with HRT equals to 8 hours and consequently COD/N was approximately 2.7.

These studies demonstrate the importance of identifying COD/N ratio in order to operate the reactors since, depending on COD/N ratio, a different operational condition should be applied.

According to Fig. 2, which shows the COD/N ratio values for each season, the studied effluent COD/N mean ratio equals to 2.7, which is close to the limit, but still within the range that favors the anammox process, according to Lackner et al. (2014) and Ni et al. (2012) – between 1.7 and 3.0. However, it is worth remembering that organic matter can gradually be degraded, so the COD/N ratio decreases over time. This factor should be taken into account in the project and operation of reactors, thus the residence time of organic matter should be lower than the time to its degradation to the point of obtaining a COD/N ratio below 1.7, in which the anammox process is no longer effective.

Another favorable characteristic from the studied anaerobic effluent is that the Total COD concentration is lower than 160mgL^{-1} , which, according to the aforementioned authors, benefits anammox process to coexist with heterotrophic bacteria.

Conclusions

Subtropical annual seasonality of COD/N ratio range (2.2 to 3.1) from anaerobic effluent indicates that the use of anammox process is appropriate as post-treatment of anaerobic effluent in subtropical areas. However, as Total COD degrades over time and as total nitrogen concentration is influenced by seasons, these factors need to be taken into account in the project and operation of the reactor over the year. Thus, it is important to verify the residence time of organic matter to avoid its degradation to the point of obtaining a COD/N ratio below 1.7, in which the anammox process is no longer effective.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: PAS analyzed and interpreted data. ROM LMOC interpreted data. LMOC mentored and supervised the work. All authors wrote, read and approved the final manuscript.

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Figures

Figure 1

Subtropical annual seasonality of precipitation and temperature values and characterization of anaerobic effluent regarding to TKN, total COD, soluble COD and suspended COD variables (mean and standard deviation values).

Figure 2

Subtropical annual seasonality of COD/N ratio and minimum (1.7) and maximum (3.0) values which favor anammox activity according to literature.

Supplementary Files

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