GHG emissions from algal facultative ponds under tropical conditions

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ABSTRACT
Pilot scale experiments were carried out to better understand the daytime and nighttime CH_4, CO_2 and N_2O generation dynamics from an algal facultative pond (AFP) under tropical conditions. The results showed that the AFP was a net source of CH_4 during both daytime (2,466.8±989.8 mg CH_4 m^{-2} d^{-1}) and nighttime (2,254±1,152.5 mg CH_4 m^{-2} d^{-1}). The variations in CH_4 emissions were influenced by environmental factors such as ambient temperature and total nitrogen (r^2=0.52; $p<0.05$ for all coefficients). For CO_2 emissions a strong influence of the photoperiod was observed. During the daytime the AFP was a CO_2 sink (-743±847.5 mg CO_2 m^{-2} d^{-1}) while in the night it was a CO_2 source (2,497±1,334.8 mg CO_2 m^{-2} d^{-1}). CO_2 production in the AFP was correlated positively to COD, and negatively to pH and DO. The significant difference between day and night CO_2 reflected changes in algal photosynthesis and heterotrophic respiration. N_2O fluxes from the AFP during daytime (-0.95±2.7 mg N_2O m^{-2} d^{-1}) and nighttime (3.8±7 mg N_2O m^{-2} d^{-1}) showed significant differences. According to a linear regression model the N_2O fluxes were positively correlated to air temperature, NO_3^- N, and TKN, and negatively correlated to DO and COD. This suggests that probably both nitrification and denitrification contribute to the production of N_2O in AFP.

KEYWORDS
Greenhouse Gas, Wastewater Treatment, Facultative Ponds, Ecotechnologies

INTRODUCTION
Stabilization ponds (SP) are eco-technological systems widely used for wastewater treatment mainly in developing countries due to their low operational cost and effective removal of pollutants such as organic matter and pathogens (Arthur, 1983; Mara, 2005; Peña, Madera, & Mara, 2002). However, the sustainable operation of SPs could be affected by the high rate of conversion of organic and nitrogenous loads to greenhouse gases (GHG), i.e. CH_4, CO_2 and N_2O. Especially CH_4 and N_2O are very harmful to the environment because of their high global warming potential (GWP) contributing to the human-induced greenhouse effect (Forster et al., 2007).

The emissions of CH_4, CO_2 and N_2O have been measured in various types of stabilization ponds (Singh et al., 2005; Stadmark & Leonardson, 2005; Toprak, 1995; Van der Steen, Nakiboneka, Mangalika, Ferrer, & Gijzen, 2003). In these studies, the mechanisms for CH_4, CO_2, and N_2O emissions have been related to processes that involve methanogenesis, nitrification and denitrification. In addition, the sediment, water, and air temperature are

environmental factors contributing to GHG dynamics in SPs (Singh et al., 2005; Toprak, 1995). Likewise, the availability of substrate i.e. COD or nitrate limit CH_4 and N_2O production (Johansson, Gustavsson, Oquist, & Svensson, 2004; Johansson, Klemedtsson, Klemedtsson, & Svensson, 2003; Søvik & Klove, 2007; Stadmark & Leonardson, 2005; Toprak, 1995). In the case of ponds designed for nutrient removal, the algal presence influences GHG emissions from AFPs because the oxygen produced by algal photosynthesis probably helps to limit the methane and nitrous oxide emissions (Johansson et al., 2004; Johansson et al., 2003; Stadmark & Leonardson, 2005).

However, the studies reported previously were carried out under temperate conditions and the production of CH_4, CO_2 and N_2O in algal facultative ponds operating under tropical conditions is largely unknown. The tropical conditions are characterized by high temperatures, long and stable photoperiods, changes in photosynthetic activity, high levels of bacterial and algal activity, and dynamics in dissolved oxygen and pH patterns which influence GHG production.

Thus, considering the importance of SPs from the perspective of the wastewater treatment in developing countries and their potential adverse environmental impact related to GHG emissions, the objectives of the current study are (1) to determine the CH_4, CO_2 and N_2O fluxes from algal ponds under tropical conditions; (2) to compare these fluxes for day and night conditions (light/dark influence); and (3) to determine the influence of environmental parameters such as pH, DO, and temperature on GHG fluxes from algal facultative ponds (AFP).

METHODS

Study Site and Sampling

Flux measurements were made at a pilot-scale algal facultative pond located at the experimental research station for wastewater treatment in Ginebra, Colombia (3°43'25.98 N, 76°15'59.45 W). The AFP pond received effluent from a full-scale anaerobic pond, only fed by domestic wastewater. In addition, the AFP operation occurred under steady state according to the conditions shown in Table 1.

Table 1 Design and operational characteristics of the AFP

Parameter	Units	AFP
Flow	$m^3.d^{-1}$	24
Depth	M	1.39
Volume	m^3	99
HRT	D	4.1
Organic loading	g $BOD_5.m^{-2}d^{-1}$	43.7

Wastewater Sampling

The physical and chemical characteristics of the wastewater AFP and their greenhouse gas fluxes were studied weekly under different solar radiation conditions: from 12:30 to 15:30 (daytime), which is the period of maximum solar radiation in Ginebra, and from 00:00 to 03:00 (nighttime), which is the period of minimum solar radiation.

The influent and effluent wastewater quality of the AFP was determined through 24-hour sampling campaigns. The day was divided in periods of eight hours. During each period taking fixed volume samples every hour that were added together for analysis collected a composite sample. Additional wastewater grab samples were taken in the central point of the AFP, at the spot where GHG fluxes were measured, to determine the correlation between GHG fluxes and wastewater characteristics. Biochemical oxygen demand (BOD_5), chemical

oxygen demand (COD), total suspended solids (TSS), alkalinity, total Kjeldahl nitrogen (TKN), ammonium nitrogen ($N\text{-}NH_4^+$), and nitrate nitrogen ($N\text{-}NO_3^-$) were measured according to Standard Methods (APHA, 2005). Conductivity, pH, dissolved oxygen (DO), temperature and the oxidation-reduction potential (redox) were measured with electrodes. Chlorophyll-a was measured using an ultraviolet light *Aquafluor* (Turner Designs) handheld fluorometer.

Greenhouse Gas Measurement

Greenhouse gas emissions (CH_4, CO_2, and N_2O) were determined by using the technique of closed static chambers. The chamber was designed to minimize artefacts (tubes, stopper rubber), which increase the possibility of gas leakage. The cylindrical chamber (0.48 m x 0.3 m: diameter x height) was fixed gently on the water surface. Samples for GHG (CH_4, CO_2 and N_2O) measurements were taken during 45 minutes at 15-minute intervals (0, 15, 30, 45 min).

CO_2 was measured by an infrared spectrophotometer Qubit S151 CO_2 analyser (Loligo Systems, Denmark) using 75 ml min^{-1} air as the mobile phase with a temperature of the injector set equal to the ambient temperature. CH_4 was analyzed by gas chromatography (Shimadzu Co., Japan) equipped with a flame ionic detector (FID). The column was Porapak Q (80-100 mesh), 2 m in length and 2 mm in internal diameter, and the temperature at the injector, column, and detector were 80, 70, and 320 ° C, respectively. The flow rate of the carrier gas (N_2) was 22 ml min^{-1}. N_2O concentration was analyzed by means of gas chromatography (Shimadzu Co., Japan) equipped with an electron capture detector (ECD) and a Porapak column Q 80-100 mesh 2m*2mm retention gap, using 22 ml min^{-1}. N_2 was the carrier gas, and the temperature at the injector, column, and detector were 80, 70, and 320 ° C, respectively.

The greenhouse gas fluxes were calculated according to the suggested by (Silva, Lasso, Lubberding, Peña, & Gijzen, 2015)

$$F = \frac{dC}{dt_{t=0}} \times \frac{Vc}{A} \times \frac{1440\ min}{d} \qquad Ec.1$$

F= the flux of CH_4, CO_2, N_2O ($gm^{-2}d^{-1}$); $dC/dt_{t=0}$ = slope of the gas concentration curve ($gm^{-3}min^{-1}$); V_c= volume of the chamber (m^3); A=the cross-sectional area of the chamber (m^2).

RESULTS AND DISCUSSION

The AFP influent wastewater was low-strength regarding the organic matter content (Metcalf & Eddy, 2003). In Table 2 the influent, effluent and removal of the different parameters measured in the AFP can be observed.

Table 2 Wastewater characteristics (mean ± SD) for influent and effluent AFP

Parameter ($mg.l^{-1}$)	Influent	Effluent	Removal %
$COD_{soluble}$	123 ± 59 (n=25)	87 ± 40.4 (n=25)	29.3
TSS	85.4 ± 69.8 (n=45)	64.3 ± 52.1 (n=45)	24.7
$N - NO_3^-$	1.1 ± 1 (n=30)	0.75 ± 0.42 (n=30)	31.8
TKN	40.7 ± 5.8 (n=30)	29.3 ± 3.9 (n=30)	28.0
$N - NH_4^+$	30.2 ± 4.5 (n=47)	21.4 ± 4.3 (n=45)	29.1

As can be observed, there was a significant difference between daytime and nighttime ambient temperature, ORP, and dissolved oxygen ($p<0.05$) (Table 4). The other environmental parameters did not show significant differences for the two periods studied.

CO_2 and CH_4

Figure 1 shows the day and night fluxes for CH_4 and CO_2 from the AFP. The daytime fluxes ranged from 290 to 4,510 mg CH_4 $m^{-2}.d^{-1}$ (Mean=2,466.8; SD=989.8; n=19) whereas nighttime fluxes ranged from 80 to 3,850 mg CH_4 $m^{-2}.d^{-1}$ (Mean=2,254; SD=1,152.5; n=19). The ANOVA and Wilcoxon test showed that there were no significant differences between day and nighttime CH_4 fluxes ($p=0.235$). Thus, the AFP was a net source of methane for both day and nighttime. CO_2 fluxes, however, showed a different pattern. During the day, CO_2 was taken up by the ABP with fluxes ranging from -3,100 to 300 mg CO_2 m^{-2} d^{-1} (Mean= -743; SD=847.5; n=19). In the night, ABP behaved as a source with fluxes ranging from 1,040 to 4,730 mg CO_2 $m^{-2}.d^{-1}$ (Mean=2,497; SD=1,334.8; n=19). The statistical tests showed significant differences in the AFP between the CO_2 day and nighttime gas fluxes ($p=0.001$).

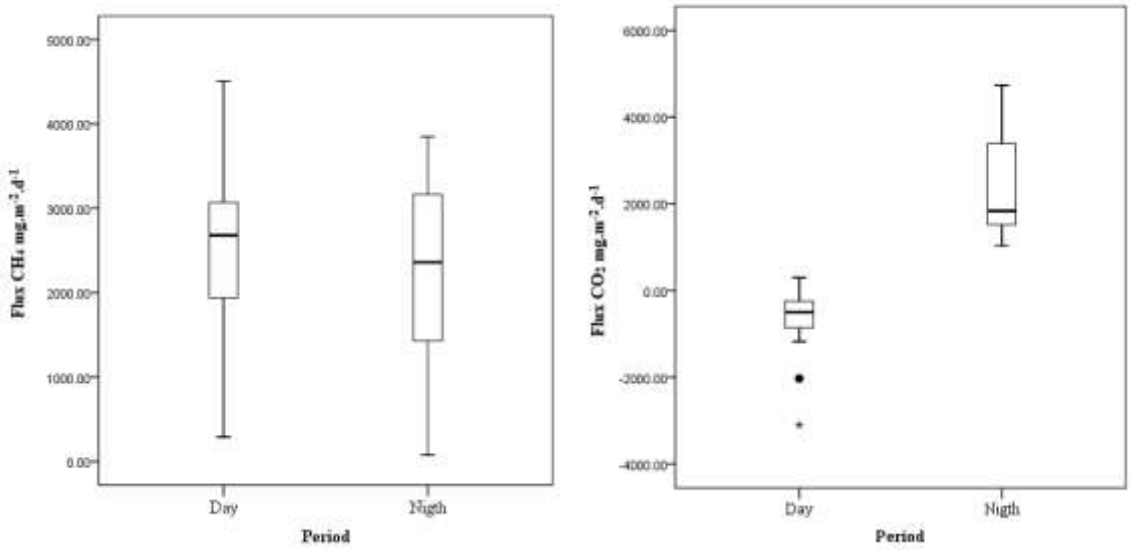

Figure. 1 Daytime and nighttime fluxes from the Ginebra`s AFP for CH_4 (a) and CO_2 (b)

The variation in the fluxes of CO_2 and CH_4 in relation to the different environmental parameters was analyzed using a stepwise linear regression (Table 3). The flux of CH_4 was positively correlated to TKN and ambient temperature. These environmental parameters could only explain 52% of the variations found for CH_4 fluxes. The statistical analysis revealed that TN accounted for a large proportion of variations in methane fluxes (38.7%), while ambient temperature explained 13.3 % of the CH_4 fluxes. The temperature changes in the water column of AFP could not explain the CH_4 flux variation.

Table 3. Models derived from multiple stepwise linear regressions of CO_2 and CH_4 fluxes

Flux	Equation	r^2
CH_4	Flux CH_4 = 65.19 TN + 100.24Ta + 2322.6 + ε	0.52
CO_2	Flux CO_2 = 4.707 COD - 834.8 pH – 255.33 DO - 4130.78 + ε	0.74

The CO_2 fluxes measured in this study were comparable to those reported in HSSF constructed wetlands treating municipal wastewater (Picek, Cizkova, & Dusek, 2007; Teiter & Mander, 2005). However, in the HSSF constructed wetland no CO_2 consumption was reported, while in our study the flux ranged from -3100 to 4730 mg $CO_2.m^{-2}.d^{-1}$. In addition the CO_2 emissions obtained in our AFP were 8 and 20-fold lower than those observed in constructed wetlands by Liikanen et al. (2006), Søvik et al. (2006), and Ström et al. (2007). On the other hand, the CO_2 fluxes measured in this study were higher than reported by Huttunen et al. (2002) in a boreal pond. In addition, the CO_2 consumption and emissions in the present study were 2 and 6-fold higher than those reported in shallow and deep lakes (Tremblay, Lambert, & Gagnon, 2004; Xing et al., 2005).

According to the linear regression model, the CO_2 variations were positively correlated to COD and negatively correlated to pH (Table 3). During maximum solar radiation, there is an intense photosynthetic activity of algae, which leads to a CO_2 deficit in the water column. The consequence of this deficit is a pH increase of the water column. At a pH higher than 8 the formation of carbonic acid and bicarbonate leads to an under-saturation of dissolved CO_2 in the water column enhancing the net transfer of CO_2 from the atmosphere into water. In contrast, when solar radiation is minimal algae switch to respiration and produce CO_2, generating a pH value close to neutrality. In the AFP, the pH during the daytime reached maximum values of 9 (Fig. 2), which could explain the CO_2 consumption observed in this period. Similarly, in previous studies with constructed wetland and natural systems, the negative fluxes have been explained by the high pH (pH up to 9) measured in the water column (Søvik et al., 2006).

The load of organic matter, represented by either COD or BOD, contributes to CO_2 emissions in wastewater treatment systems (Teiter & Mander, 2005). This was corroborated by the positive correlation found between COD and CO_2 fluxes in the AFP (Table 3). However, the model indicated that COD could only explain 5.5% of CO_2 flux in the AFP (Table 3). This suggests that the mineralization of organic matter in the ABP may have contributed to a lower proportion to net CO_2 exchange than algal photosynthesis or algal dark respiration. The results obtained in a floodplain swamp indicated that 66% of CO_2 exported is assumed to arise from live respiration than the mineralization of organic matter (Pulliam, 1993).

All the CH_4 fluxes in the AFP were positive, which means that under all conditions CH_4 was emitted. The CH_4 fluxes from the AFP were lower than in an anaerobic pond in Portugal (Toprak, 1995), because it was operated at higher organic load and constant low DO. The methane fluxes were comparable to those in a FWS constructed wetland that purifies peat mining runoff waters (Liikanen et al., 2006). However, this wetland received a higher organic load than the AFP. In general, the CH_4 emissions in this study were higher than those reported in the literature for constructed wetlands (Johansson et al., 2004; Liikanen et al., 2006; Picek et al., 2007; Tanner, Adams, & Downes, 1997; Teiter & Mander, 2005; Xing et al., 2005).

The linear regression model indicated that total nitrogen explained 38.7% of the methane variations in the AFP. Johansson et al. (2004) suggested that mineral nitrogen concentrations (ammonium, nitrite, and nitrate) reflected the general microbial activity in a FWS constructed wetland which in turn probably affected the conditions for methane formation and consumption, and thus emission. Stadmark and Leonardson (2005) and Liikanen and Martikainen (2003) found that NO_3^--N and NH_4^+-N had no effect on the CH_4 production in sediments from a pond and an eutrophic lake respectively. Søvik and Klove (2007), however, reported that the CH_4 emission was positively correlated to NO_3^-- N and NH_4^+-N. In this study it was not possible to determine whether NTK, NO_3^- -N, and NH_4^+-N individually affected the CH_4 production in the AFP. This was probably due to the smaller variation in NTK, NO_3^--N, and NH_4^+-N in the water column which affects the sensibility of the linear regression model to these parameters. This shows that it may be difficult to establish

relationships between chemical compounds in the water and gas fluxes, as was previously mentioned by Søvik and Klove (2007). The high ambient temperatures (> 30ºC) during the AFP operation, contributed to methane emissions from the AFP. These findings are consistent with previous studies in different constructed wetlands where methane emissions have been found to be significantly higher during summer than during winter (Johansson et al., 2004; Liikanen et al., 2006; Picek et al., 2007; Tanner et al., 1997; Teiter & Mander, 2005; Xing et al., 2005).

N_2O Fluxes

The N_2O fluxes (Fig. 2) during the day ranged from -6.3 to 3.6 mg m^{-2} d^{-1} (Mean= -0.95; SD=2.7; n=12) and during the night from -2.4 to 15 mg m^{-2} d^{-1} (mean 3.8; SD=7; n=12). The statistical test provided that there was a significant difference between the day and nighttime fluxes (p=0.049). A slightly trend to behave like an N_2O sink was observed in the daytime in the AFP whereas during the nighttime the AFP was a source of this gas.

The N_2O fluxes measured in the AFP were in the range reported from systems treating wastewater (Johansson et al., 2003; Liikanen et al., 2006; Singh et al., 2005; Søvik et al., 2006; Søvik & Klove, 2007; Ström et al., 2007). However, the highest N_2O flux from the AFP was lower than fluxes from nutrient-rich wetlands (Johansson et al., 2003; Søvik & Klove, 2007; Ström et al., 2007), which were observed during late spring, early summer and autumn. The negative fluxes suggesting consumption in the AFP are consistent with the negative fluxes measured in Sweden (Johansson et al., 2003; Ström et al., 2007). On the other hand, the fluxes from natural systems (Huttunen et al., 2002) were lower than from the AFP, which is probably due to both the low temperature and nutrient levels in these water bodies.

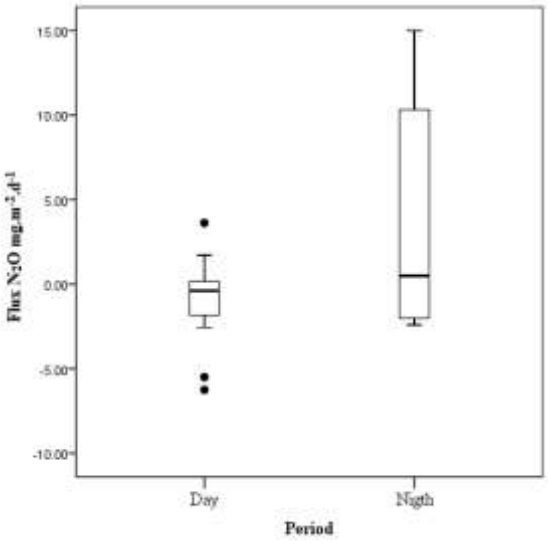

Figure 2. Daytime and nighttime N_2O fluxes from the Ginebra`s AFP

The daily N_2O emissions observed in the AFP were influenced by the algal photosynthesis (Fig. 2). During the highest solar radiation a high DO concentrations of 16.2 mg-l^{-1} was reached in the FP. This probably favoured the nitrification process and NO_3^+-N was produced. However, during the nighttime period, when the photosynthesis process is offset by respiration, the average DO concentrations in the FP reached 1.2±0.9 $mgO_2.l^{-1}$ and probably the nitrification process was limited. The probable consequence of this is that the oxidation of hydroxylamine to NO_2^-N was incomplete and N_2O was produced. Likewise, these conditions of low DO during nighttime could favour the N_2O production by nitrifier denitrification. Experiments conducted with full-scale activated sludge showed that nitrifier denitrification

contributed between 58% to 83% of the N_2O emissions for oxygen concentrations around 1 $mgO_2.l^{-1}$ (Tallec, Garnier, Billen, & Gousailles, 2006).
Linear regression was used to identify the correlation between environmental factors and N_2O fluxes. The nitrate-N (r^2=0.28), TKN (r^2=0.16), DO (r^2=0.14), COD (r^2=0.14) ambient temperature (r^2=0.12), water temperature (r^2=0.06), and ammonium-N (r^2=0.005) explained 85% of the variations in N_2O flux. On the other hand, the negative correlation observed for COD in this study reinforces the hypothesis that the N_2O was probably also produced by incomplete denitrification. The operational COD/N ratio in the AFP studied was about 3.0, which suggests a low availability of a carbon source in the AFP for the denitrification process. According to Itokawa et al. (2001), more than 20% of the influent nitrogen can be emitted as N_2O during biological nitrogen removal of high strength wastewater if the COD/N ratio is below 3.5.
The linear regression model used in this study contains all the components known to affect N_2O formation by nitrification or denitrification. The N_2O fluxes were positively correlated to air temperature, NO_3^--N, and TKN, and negatively correlated to DO and COD. These environmental parameters affecting N_2O fluxes have also been reported in the literature. In a CW planted with reed, the N_2O production was correlated positively to NO_3^- -N and soil temperature, whereas NH_4^+-N was correlated negatively (Fey, Benckiser, & Ottow, 1999). In a FSW, nitrification had strong relations to ammonia, nitrite and total mineralized nitrogen, while the denitrification was characterized by nitrite, nitrate and total mineralized nitrogen (Johansson et al., 2003).

CONCLUSIONS

This study provided information about the GHG dynamics of emissions from algal facultative ponds. In general the AFP was a net source of CH_4, CO_2, and N_2O and based on the GWP the algal facultative pond studied emitted substantial amounts of greenhouse gases into the atmosphere compared to conventional systems.

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