Animal Wastewater Treatment Using Constructed Wetland

Animal Wastewater Treatment Using Constructed Wetland

Chika TADA[1*], Naofumi IKEDA[1], Satoshi NAKAMURA[1], Ryu OISHI[1], Junki CHIGIRA[2], Tokuo YANO[2], Kazunori NAKANO[2] and Yutaka NAKAI[1]

[1]Laboratory of Sustainable Environmental Biology, Graduate School of Agricultural Science, Tohoku University
232-3,Yomogida, Naruko-Onsen, Osaki, Miyagi, 989-6711, Japan
[2]Ecological Engineering Laboratory, Graduate School of Engineering, Tohoku University
6-6, Aoba, Aramaki, Aoba-ku, Sendai, 980-8579, Japan
***e-Mail: tada@bios.tohoku.ac.jp**

Keywords: wastewater treatment, constructed wetland, ammonia oxidizing bacteria, anammox, E.coli

Received 24 December 2010; accepted 28 January 2011

Abstract

In this study, constructed wetlands(CWs) hybridized with vertical and horizontal flow systems treat wastewater from milking parlor. Water quality, the number of coliform bacteria and ammonia oxidizing microbes during the treatments were investigated. In the results, rate of removal COD, TN and TP from raw wastewater were more than 90% from June to September. However, these rates were decreased after October, the lowest removal was observed in December. At this time, ammonia oxidizing bacteria were $10^2 \sim 10^4$ cells/cm^3 by analysis of realtime PCR. Ammonia oxidizing archaea also found 10^2 cells/cm^3. However, ANAMMOX bacteria were not detected in December. The removal of coliform bacteria was not different from September to December, except on a single day. Furthermore, no significant differences in treatment efficiency among the three types of wetlands, were observed. In particular, the no difference in the removal of coliform bacteria by wetlands, constructed with or without plants was found. The highest removal efficiency of coliform bacteria was observed on October 22, which followed 3 days of sunny weather. This observation suggests that soil-drying due to the absence of influent was important for removing coliform bacteria.

To reuse treated water for agricultural water, concentrations of COD and TN are required under 6 mg/L and 1 mg/L respectively. In addition, reclaimed water for landscape requires that the number of coliform bacteria is under 1000 cfu/100ml. The concentration of COD, TN and coliform bacteria in the treated water in summer met the standard to reuse for agricultural water or landscape water.

In the future, more improvement of removal efficiency with the constructed wetland is required for reuse animal wastewater. In addition, it has to promote to establish the recycling system including utilization of treated water.

1. Introduction

In the world, animal meat, milk and eggs were produced 873 million ton (FAOSTAT,2007). Amount of these products was trend to increase. Runoff from animal farms where excessive nutrients are generated has been linked to downstream eutrophication of surface waters. In Japan, there are more than 100 million livestock farmers that have to observe the wastewater treatment standard in Japan.

Table 1 shows wastewater quality from milking parlor. COD concentrations were around 1000 to 2500 mg/L, SS were more than 600 mg/L. TN concentrations were also high, 163-1139 mg/L. TP concentrations were 65.7 -136.3 mg/L. Comparing wastewater from milking parlor with national effluent standard in Japan, all items should be removed. Not only chemicals, but also coliform bacteria in wastewater should be decreased under 3000 cells/ml.

Activated sludge method is popular system for animal wastewater treatment. To activate aerobic microorganisms, aeration was done. This aeration use energy about 56% of all energy required for wastewater treatment. Wastewater treatment using activated sludge from the farm, amount of wastewater was

Table 1. Wastewater quality from milking parlor

COD	1163 ~ 2586	mg/L
BOD	1383 ~ 2476	mg/L
SS	670 ~ 5833	mg/L
Coliform bacteria	134 ~ 6200	cells/mL
TN	163 ~ 1139	mg/L
TP	65.7 ~ 136.3	mg/L
pH	5.8 ~ 7.2	

Yoshio et al.(2004), Sato et al.(2005)

about 3 ton/day, cost of electricity for aeration was about 13000 yen/month, and CO_2 emission by aeration was 79 kgC/month. To achieve the Kyoto Protocol, we should reduce CO_2 emission from wastewater treatment. That reason, constructed wetland received attention.

Constructed wetland was wastewater treatment system using natural purification. It had several advantages, energy-saving, less maintenance, low cost, natural landscape and biodiverse habitats. The first constructed wetland; full scale of FWS (Free water surface CW) was started in Netherlands in the late 1960s. There are various constructed wetlands in the world. Constructed wetlands treat various wastewater, sewage, mining water, landfill leachate, industrial effluent, surface run-off, agricultural run-off, and road run-off.

There are some reports about animal wastewater treatment with CW. Hammer(1992) reported marsh-pond-meadow wetland. This system consists 3 steps. This system achieved to remove the 71% of ammonia. United states Department of Agriculture, Natural Resources Conservation Service(NRCS, 1991, 1992) recommended subsurface flow CW. Vegetation was commonly selected Giant reed in Europe, and NRCS.

Ammonia oxidation is critical to grobal nitrogen cycling and is ofen though to be driven only by ammonium-oxidizing bacteria. The recent finding of new ammonia-oxidizing organisms belonging to the archaeal domain challenges this perception. Two major microbial groups are now believed to be involved in ammonia oxidation: chemollithotrophic ammonia-oxidizing bacteria(AOB) and ammonia-oxidizing archaer(AOA).

Removal of coliform bacteria using constructed wetlands was the one of assignments for reuse of water. There were some reports that removal of indicator organisms with constructed wetlands. However the mechanisms for disappearance of coliform bacteria is not clear enough. This study, monitoring the number of coliform bacteria in the constructed wetland system, was intended to clarify the factors leading to the decrease of coliform bacteria.

In this study, constructed wetland Kawatabi was hybridized with vertical and horizontal flow systems to treat wastewater from a milking parlor. To reuse animal wastewater by treatment with constructed weteland, water quality of treated water using constructed wetland was investigated during treatment. Especially, we investigated about ammonia oxidizing microbes' presents, and the number of coliform bacteria.

2.Material and method

2.1 Study site

The systems were located in Miyagi prefecture, Japan. It snows in winter. They consisted of three types of five-stage CWs for dairy wastewater (Fig.1). The area was 111 m^3, and the depth was 70 cm. The beds were consists of sand and gravel. The first to fourth stages were operated vertical flow bed, the fifth stage was horizontal flow bed. Type A and C were planted with *Phragmites*, type B was not. Water levels of Type A and B were 0 cm, that of Type C was 35 cm (Fig. 2).

2.2 Wastewater and operating

Wastewater was flow from the dairy farming into the CWs twice a day. The volume of wastewater was about 2 m^3 per day. Wastewater quality was shown in Table 1.

The operated conditions adopted the rotation method that was cycled the inflow period and the stop period. The inflow period was for four days from three days, the stop period were for 10 days.

Wastewater and treated water were sampled from September to December every week. Then the sample was measured water qualities, COD, BOD, TN, TP, NH_4-N, NO_2-N, NO_3-N.

2.3 Soil sampling and DNA extraction

The sand and gravels were collected from below the surface to approximately 20 cm when the inflow period was started, in December 2009. Three samples were collected from each condition. From 2 g to 15

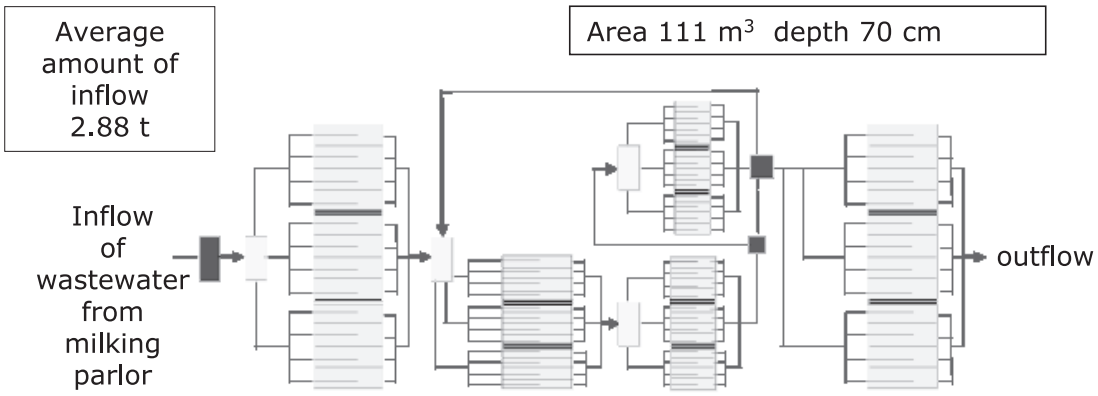

Fig. 1. Schematic of constructed wetland in Kawatabi. Hybridized with vertical and horizontal flow systems to treat wastewater from a milking parlor.

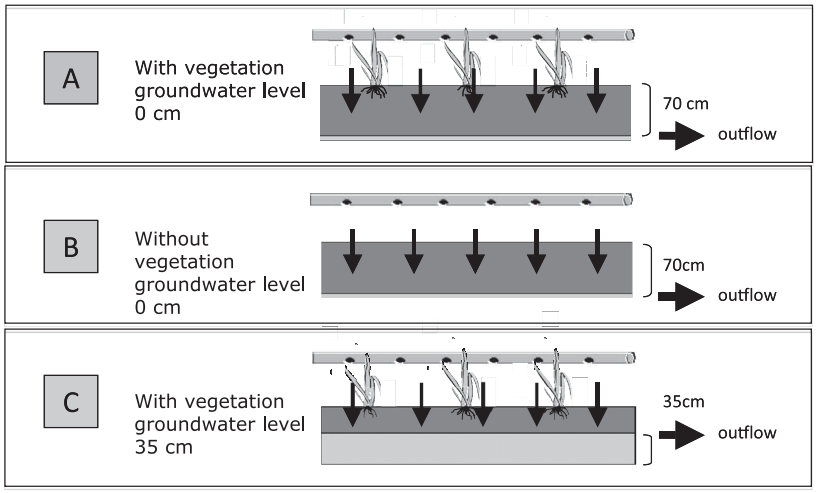

Fig. 2. 3 Types of the constructed wetland in Kawatabi. A has vegetation, and the groundwater level is 0 cm. B has no vegetation, and the ground water level is 0 cm. C has vegetation, and the groundwater level was 35 cm.

g of sand or gravels were shaken with PBS buffer for 10 mins using voltex mixure, after that suspending solution was transfer to a centrifuged tube. The solution was centrifuged by 15000 rpm for 5 mins, the precipitation was used for DNA extraction. DNA from the sand and gravels were extracted using Power Soil Extraction kit (MOBIO Laboratories, Inc, CA, USA), as described in the manufacturer's instructions. Extracted DNA was purified with PowerClean DNA Clean-Up Kit (MIOBIO Laboratories, Inc, CA, USA), and then the DNA sample was diluted 10 times with distilled water.

2.4 Real-time PCR

The *amoA* genes of AOB and AOA were amplified with previously described primers (Rotthauwe et al., 1997, Tourna et al., 2008). Copy numbers of 16S rRNA gene of ANAMMOX bacteria were quantified with the real-time PCR assay(Tsushima et al., 2007).

Reaction mixtures of 20 µl contained 10 µl of SYBR Premix Ex Taq II (Takara, Japan), 32 pmol of forward and reverse primers, and 5 µl of template. Amplification, detection, and data analysis were performed Chromo4 (Bio-Rad laboratories BV, The Netherlands). The amplification program used for AOA was as follows: 94˚C for 5 min; 50 cycles of 30s at 94˚C, 30 sec at 56˚C, and 1 min at 72˚C. That for AOB was as follws: 95˚C for 5 min; 50 cycles of 30s at 95˚C, 1 min at 55˚C, and 1 min at 72˚C. The amplification program used for ANAMMOX bacteria was as follows: 50˚C for 2 min, 94˚C for 10 min; 50 cycles of 15s at 94˚C, 1 min at 60˚C. The PCR cycle

after which the fluorescence signal of the amplified DNA is detected (threshold cycle[C_T]) was used to quantify the concentration of AOB and AOA *amoA* gene copies. Quantification was based on comparison of the sample C_T value with the C_T values of a calibration carve based on known copy numbers of the *amoA* gene of AOB or AOA. The AOB numbers were calculated by assuming two amoA gene copy numbers per cell (Chain et al., 2003) and the AOA numbers by assuming one amoA gene copy number per cell.(Mincer et al., 2007)

2.5 Phylogenetic analysis

Phylogenetic analysis of the amoA genes of AOA were performed on soil sampled at the third step in condition C. DNA was isolated from the soil samples, and the *amoA* gene of AOA were amplified as described above. DNA products were purified using the DNA clean-up kit (PowerClean, MO BIO) according to the supplier's protocol. ExoSAP-IT (Amersham Biosciences, Tokyo, Japan) was used to remove the excess primers and dNTPs, and the ABI PRISM 3130 ×l Genetic Analyzer (Applied Biosystems) was used for sequencing. Sequence accuracy was confirmed by 2-directional sequencing. Phylogenetic analysis was performed with MEGA version 3.1 using the neighbor-joining method following the alignment of the archaeal *amoA* sequences using Clustal W.

3. Results and Discussion

3.1 Ammonia oxidizing microbes in the CW

Fig. 3 shows the results of number of ammonium oxidizing microbes. Results of 3 types of wetlands were shown. These results showed that there were ammonia oxidizing microbes in all wetland. Ammonia oxidizing bacteria was dominant. AOB in the wetland C was more than the others. There were 10^2-10^3 cells/cm^3. Additionally, AOA was detected in the wetland C. Leininge et al. (2006) reported that AOA might be the most abundant ammonia-oxidizing organisms in soil ecosystems on Earth. Park et al. (2006) reported that AOA was detected from the wastewater treatment plants. The constructed wetland was consisted of sand, and wastewater was inflow. These conditions were enable to AOA could grow in the CW. In this result, AOA was detected only from the wetland C. The reason why was not obvious. However, water level of the wetland C was higher than the other. It is possibility that the difference of water level was affected on the amount of ammonia oxidizing microbes.

Number of ANAMMOX bacteria was around quantification limits. Number of ANAMMOX bacteria of B type constructed wetlands in October was more than 10^2 cells/cm^3. The optimal temperature of ANAMMOX growth is known from 20 to 43 degree C (Strous, 1997). In our results, the temperature in December was 6 degree C. That is why, the number of ANAMMOX in December were very low. Fig. 4 shows TN removal from October to December. TN removal in October is higher than that in December. Dong et al.(2007) reported that the depth of 80 cm conventional vertical flow beds into a 25 cm un-

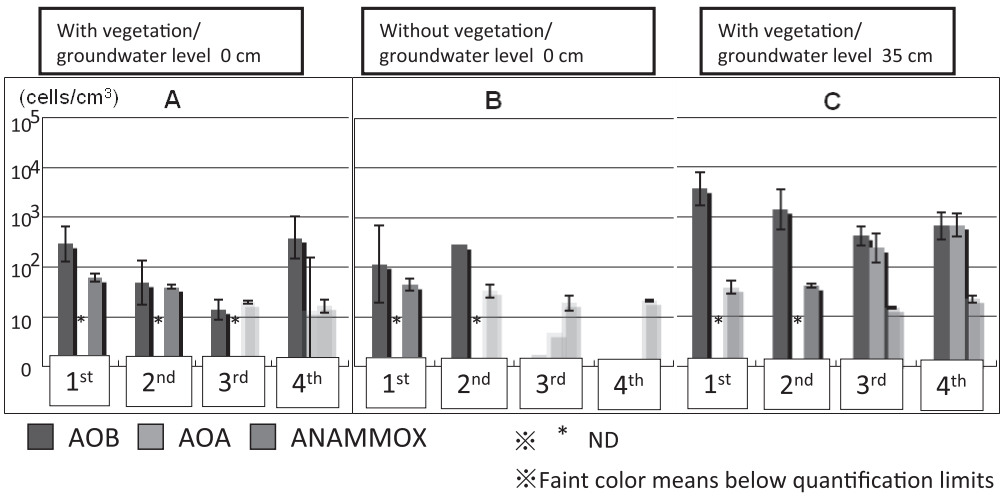

Fig. 3. Number of ammonia oxidizing microbes in December. Blue bars show the number of ammonia oxidizing bacteria. And orange bars show ammonia oxidizing archea.
Green bars show anammox bacteria.

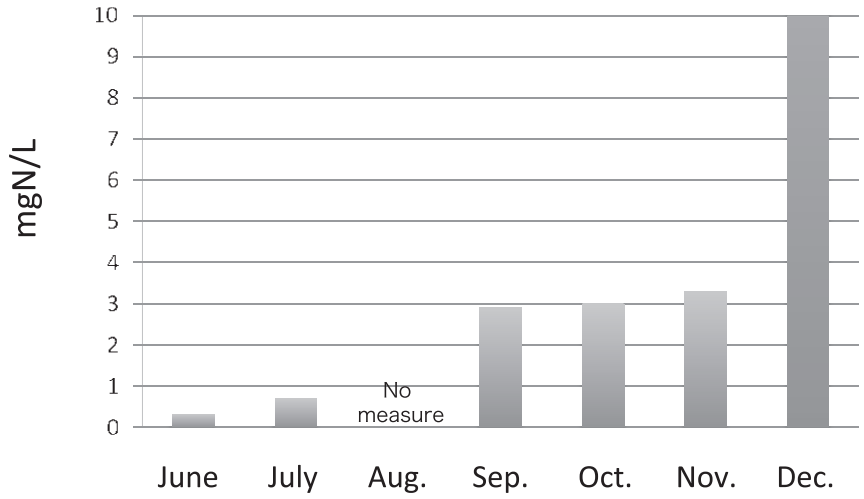

Fig. 4. TN concentrations of treated water thorough the 5th step of the CWs.

saturated layer and a 55 cm saturated layer, this new model cannot only get betted performance in removal efficiency of total nitrogen, but also achieved a promoting growth of ANAMMOX bacteria which will function on further removal the ammonium in horizontal flow bed. This report showed that the amount of ANAMMOX was related the removal of nitrogen. Comparing the TN removal and number of ANAMMOX, higher TN removal was observed when number of ANAMMOX is higher. This result suggested that ANAMMOX bacteria contributed to TN removal in the constructed wetlands.

3.2 Removal of coliform bacteria using CW

Fig.5 shows the number of coliform bacteria. After the start of operation, the coliform bacteria count at each stage 1-2log units decreased by about the fifth in the treated water is less 10 CFU/ml, showed high removal efficiency of coliform bacteria. After the rotation operation, a decrease in the number of coliform in each stage was about 0-1log. The numbers of coliform bacteria in treated water were from 10^3-10^4 CFU/ml. Coliform bacteria could be detected in soils of the constructed wetlands with average count 10^5 CFU/g. This may suggest that coliform bacteria

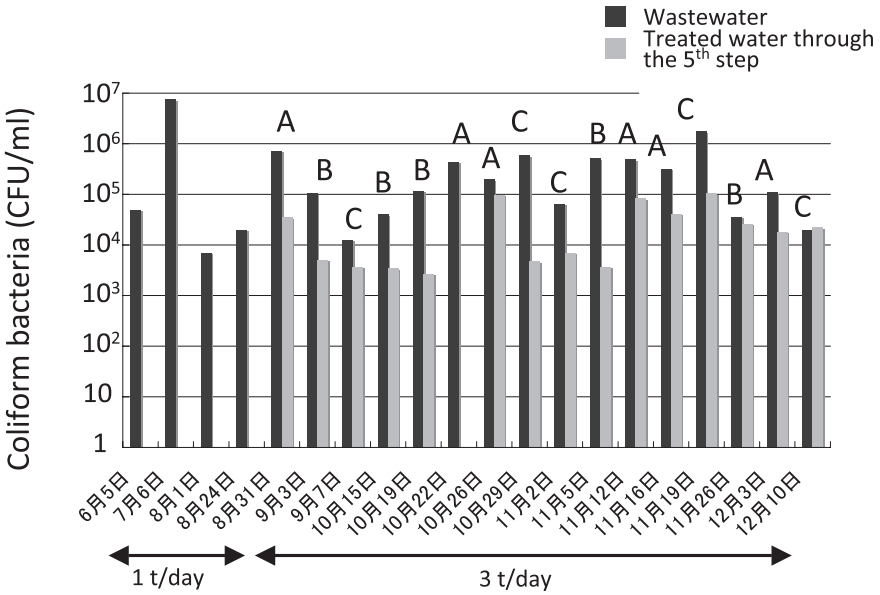

Fig. 5. Seasonal changes of number of coliform bacteria. A, B, C means type of constructed wetlands. From September, rotation running was started, so wastewater inflow increased up to 3 t/day. black bar show the number of coliform in wastewater, and gray bar show the number of colifrom in treated water through the 5th steps.

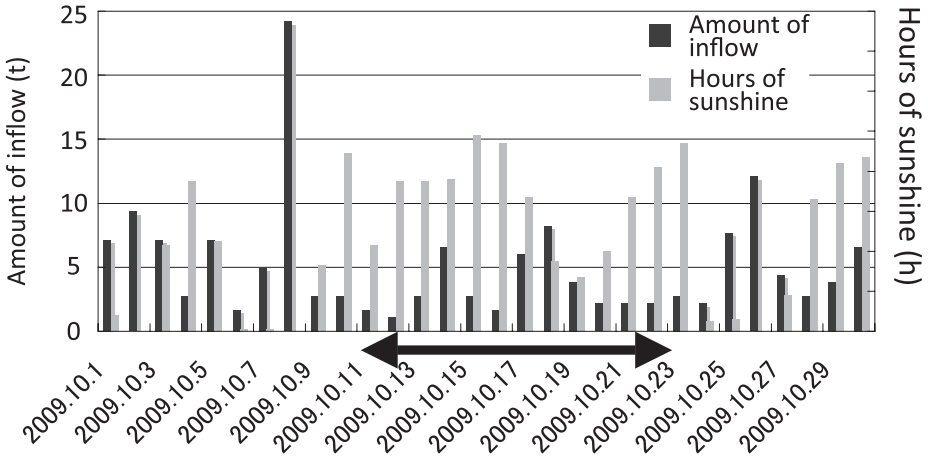

Fig. 6. Hours of sunshine and loading amount of wastewater and rain. Black bar show amount of inflow. Gray bar show hours of sunshine.

derived from the soil flow out when wastewater go through the wetlands. However only on October 22, showed a good reduction. Removal of coliform bacteria is 1-2 log units for one-stage systems and increases to 5.5 log units for multi-stage systems. From the analysis of the relationship between factors such as water quality and sunshine duration etc, coliform count, tended to decrease the number of coliform bacteria at times of low water inflow and longer daylight hours (Fig. 6). Thus, suggesting that contribute to reduction in the number of coliform bacteria in soil drying. Reed (*Phragmites* sp.) was known that high rate of transpiration (Larcher, 2004). Comparing between reed and surface of river, transpiration of reed was more than 10 times higher than quantity of evaporation from surface of river (Oshibe, 2004). Vegetation at the constructed wetland contributed to dry the soil in the wetland.

3.3 Recycle of wastewater

The agricultural water standard for rice cropping in Japan requires that TN concentation was below 1 mg/L. TN concentrations by the constructed wetland Kawatabi, were lower than 1 mg/L from June to August. When loading rate was 1 t/day, and it was warm seasons, treated water by the CW can meet the regulations for agricultural water.

The reclaimed to use for landscape water, the number of coliform bacteria have to lower than 1000 CFU/100 ml. When loading rate was 1 t/day, the number of coliform bacteria was lower than 100 CFU/100 ml. When loading amount was about 1 t/day and summer time, treated water can use for reclaimed water as landscapes water.

Conclusions

To reuse animal wastewater, nitrogen and coliform bacteria have to be removed. In this study, the number of ammonia oxidizing microbes and coliform bacteria in the constructed wetlands were investigated. Ammonia oxidizing bacteria was dominated in ammonia oxidizing microbes of the constructed wetland. ANAMMOX bacteria was detected in October from the constructed wetland, it is considered that ANAMMOX bacteria contributed to remove nitrogen in the constructed wetland. It is considered that soil-drying was one of the important factors for removing coliform bacteria. Treated water with the constructed wetland Kawatabi can use for reclaimed water as landscapes water, when the loading rate of wastewater was 1 t/day in warm season.

Reference

Chain, P., Lamerdin, J., F. Lamrimer, W. Regala, V. Lao, M. Land, L. Hauser, A.Hooper, M. Klotz, J. Norton, L. Sayavedra-Soto, D. Arciero, N. Hommes, M. Whittaker and D. Ar. (2003) Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph Nitrosomonas europaea. J. Bacteriol., 185: 2759-2773.

Dong, Z. and Sun, T. (2007) A potentioal new process for improving nitrogen removal in constructed wetlands- Promoting coexistence of Partial- nitrification and ANAMMOX. Ecological Engineering,

31: 69-78.

FAOSTAT Food Supply; http://faostat.fao.org/site/345/default.aspx

Hammer, D. A. (1993)Constructed wetlands for wastewater treatment: an overview of a low cost technology. Presented at the Constructed Wetlands for wastewater treatment conference, Middleton, Country Cork, Ireland.

Larcher, W. (2004) Plant physiological ecology, ver.2, Springer

Leininger, S., T. Urich, M. Schloter, L. Shwark, J. Qi, G. W. Nicol, J. L. Prosser, S. C. Schuster and C. Schleper (2006) Archaea predominate among ammonia-oxidizing prolaryotes in soils. Nature, 442(17): 806-809.

Mincer, T. J., M. J. Church, L. T. Taylor, C. Preston, D. M. Karl and E. F. DeLong (2007) Quantitative distribution of presumptive archearl and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. 9: 1162-1175.

NRCS (1991) Technical requirements for agricultural wastewater treatment. Natl. Bull., No.210, 1-17, Natural Resource Conservation Service, United States Department of Agriculture, Washington, DC.

NRCS (1992) Agricultural waste management field handbook, Part 651. National Engineering Handbool 210-. Natural Resource Conservation Service, United States Department of Agriculture, Washington, DC.

Oshibe, R. and K. Kan (2004) Effect of reed field on reduction of streamflow. Japan Society Civil Engineers, vol.59, Disk1, 523-524.

Park, H., G. F. Wells, H. Bae, C. S. Criddle and C. A. Francis (2006) Occurrence of Ammonia-Oxidizing Archaea in Watewater Treatment Plant Bioreactors. Applied Enviorn. Microbiol., 72(8): 5643-5647.

Rotthauwe, J., K. Witzel and W. Liesack (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol., 63: 4704-4712.

Strous, M., E. V. Gerven, P. Zheng, J. G. Kuenen and M. S. M. Jetten (1997) Ammonium Removal from Conecentrated Waste Streams with the Anaerobic Ammonium Oxidation (ANAMMOX) Process in Different Reactor Configuration. Water Research, 31(8): 1955-1962.

Tourna, M., T. E. Freitag, G. W. Nicol and J. L. Prosser (2008) Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environmental Microbiology, 10:1357-1364.

Tsushima, I., T, Kindaichi and S. Okabe (2007) Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR. Water Research, vol. 41:785-794.