Wetland Biomass

- Chemical Benefits and Problems with Biogas Usage

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Abstract: Constructed wetlands are largely used for water treatment both in agricultural land and for treating water from municipal and industrial waste. These wetlands need to be managed in order to work properly. How to deal with the large amount of vegetation harvested in the wetlands has withdrawn a great concern. The application of using wetland biomass as the co-substrates in anaerobic digestion was studied in this project. Plant materials, mostly *Phragmites australis* (common reed) from three different wetlands were used as raw material to produce biogas. The methane production using reed material harvested from municipal wastewater, industrial wastewater and an agricultural wetland are 66, 106, 144 ml/g VS respectively, which were lower than the suggested number 180ml/g VS. The gas potential remains a lot to be improved such as harvesting at summer to reduce the lignin content and changing the co-digestion mixing level to adjust to the optimal C/N ratio. Chemical analyses were performed concerning the gas yield and the residue quality. The digested residues showed a low concentration of cadmium, providing a non-toxic possibility to be spread on farm land as fertilizers, and closing the nutrient circle from land into water and back to land again. Pretreatments in the biogas process are usually focusing on the reduction of the lignocellulosic content in the raw material. Assessment of costs and benefits is needed for using wetland reed in the biogas production and applying any pretreatment methods.

Keywords: wetland biomass, anaerobic digestion, biogas, common reed, nutrient level, heavy metals, pretreatment

Introduction

Constructed wetlands

For long periods of time, constructed wetlands are being used to remove contaminants from water before it reaches natural ecosystem, especially for wastewater from human dwellings and activities. This subject was demonstrated by several studies considering such topic (Verhoeven et al., 2006). In Sweden, such constructed wetlands are usually in the form of treatment for water rich in nutrients from agricultural landscape and post-treatment for wastewater in treatment plants, extracting heavy metals, phophates and nitrates (tillvaxtverket.se, 2003). Wetland plants are an integral part of these systems (Reed et al., 1988), either naturally grown or intentionally planted. General requirements of plants suitable for constructed wetland in wastewater treatment systems include: ecological acceptability; tolerance of local climate and species conditions; tolerance of pollutants and hypertrophic waterlogged conditions; ready propagation and rapid establishment, spread and growth; and high pollutant removal capacity, either through direct uptake, or indirectly by microbial transformations (Tanner, 1996). To maximize the removal of pollutants, vegetations in constructed wetlands is suggested to be harvested frequently, particularly important with respect to the removal of phosphorus and metals (California Stormwater Quality Association, 2003). Generally in a constructed wetland, vegetation grows and emerges during spring and summer. When autumn comes, vegetation starts to decay and will be decomposed during the rest of the year, contributing organic matter and nutrients to the system and the effluent flow, which is opposite to the initial target of treatment. For all the above reasons, harvesting wetland vegetation in constructed wetlands has been a subject of intense debate (Wrigley and Toerien, 1988; Thullen et al., 2005; Álvarez and Bécares, 2008).

According to an oral report by the local municipality of Halmstad, Sweden, 250 hectares of constructed wetlands were built in Halmstad and Laholm municipalities during the last 10 years. There has also been 5 to 10 hectares of stormwater ponds constructed. It is assumed that every year around 10 tons of wetland biomass (dry matter) can be harvested per hectare, from the scale of a 25-hectare wetland. Volatile solids (VS), the amount of the organic matter that can be digested, are estimated to be about 85% of the dry matter, also know as total solids (TS). When the biomass is used as raw material for biogas production, methane yield, according to the literature data, is estimated at about 180 ml/g VS (Hansson and Fredriksson, 2004; Ohlsson, 2012). A bus using biogas has a consumption of 2.5 Nm^3/10 km. If the annual usage is 8000 km, the annual gas consumption of a biogas-bus is then 20 000 Nm^3 (Dahl, 2012). If the biomass in a total area of 100 hectares of wetlands can be harvested, providing 1 000 tons of TS with 850 tons of VS. The total biogas produced is then around 153 000 Nm^3, which can support about 8 buses using biogas.

Biogas production

Anaerobic digestion is a series of processes where microorganisms break down biodegradable material in the absence of oxygen. It is used for multiple purposes to reduce the organic content of waste and produce energy in the meantime. As a result, anaerobic digestion is widely used as an alternative energy source technique worldwide.

The main products of anaerobic digestion are biogas and digested residues. Biogas is usually composed of approximately 60% of methane, 30% of carbon dioxide, and additionally a small part of other unwanted gases. The biogas produced can be used directly for producing heat and electricity, or upgraded to vehicle fuel such as natural gas quality biomethane (Swedish Gas Association, 2011), being a green alternative for fossil fuels. The by-product, or nutrient-rich digestate, from the biogas process can be used as fertilizer for agricultural use (Fredriksson et al., 2006). Almost all organic biodegradable material can be suitable as substrates in the biogas production (Anaerobic-digestion.com., 2007), such as sludge from waste water treatment plants, food wastes from households and restaurants, manure, different plant materials and process waters from food industries. Among them manure is mostly used as it contains the anaerobic bacteria and enough biomass essential for anaerobic digestion. Co-digestion of various materials often gives a higher methane yield than if each substrate is digested separately. Therefore, based on natural degradation of the organic matter, anaerobic digestion may offer a promising approach to turn the wetland plants into a raw material for the production of fuel and chemical feed stocks. By spreading the digested residue onto agricultural land, this also can somehow close the nutrient circle from farm into wetland and back to farm again. Utilizing biogas has various benefits in different aspects. The major advantages are (1) replacement of fossil fuels, (2) saving energy footprint of waste treatment, (3) reducing methane emission to atmosphere and (4) displacing chemical fertilizers (Chen et al., 2008).

Common reed (*Phragmites australis*) is an aquatic plant with a distribution in regions different climatic and habitat conditions. The area in Sweden is about 100 000 ha (Hansson and Fredriksson, 2004). It is also one of the most common plants in constructed wetlands for water treatment (Maddison et al., 2009). Some studies concerning the use of common reed as biogas raw material have been made during recent years ((Hansson and Fredriksson, 2004; Komulainen et al., 2008; Berglund and Börjesson, 2006), which suggested a potential of converting reed biomass into energy.

Lignocellulosic biomass such as wood residues, straws of rice, wheat and corn, and paper residues usually contain a high level of lignin content that is hard to be decomposed by bacteria and enzymes. Consequently, the high lignin content in the raw materials results in a long hydraulic retention time (HRT) and slow volatile solids removal rate (Hjorth et al., 2011; Teghammar et al., 2010). Therefore pretreatments of the biogas substrates are needed so as to increase HRT, biogas yields and degradation of hard degradable substances.

A wide range of factors have been reported to be inhibitory to the anaerobic digestion processes at certain concentrations. Problems such as low gas yield and digester failure are often encountered (Chen et al., 2008). Inhibition factors commonly encountered in anaerobic digesters include sulfide, ammonia, light metals, heavy metals, and other anthropogenic organic compounds such as solvents and pesticides in the waste. Considerable research efforts have been made to identify the mechanism and the controlling factors of inhibition (Chen et al., 2008; Gerardi, 2003; Yerkes et al., 1997). When vegetation from wastewater-treatment wetlands is used as substrates for the biogas production, contaminants from the water system may have negative impacts on the digestion process. Heavy metal inhibition is likely to occur, as removal of such pollutant is often one of the targets of most municipal wastewater treatment. The non-degradable heavy metal (e.g., cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), and zinc (Zn)) may readily accumulate to potentially toxic concentrations, and many failures of sludge digestion has been ascribed to the heavy metals transferred from the substrates (Kroeker et al., 1979; Jarrell et al., 1987; Lin, 1992). Only metals in soluble, free form are toxic to the microorganisms. Heavy metal ions with high solubility such as copper, nickel, and zinc are very toxic to methanogenic bacteria at relatively low concentrations (Mosey and Hughes, 1975; Gerardi, 2003). The toxicity of heavy metals can be described as the disruption of enzyme function and structure, by binding with thiols and other groups on protein molecules, or replacing commonly occurring metals in enzyme prosthetic groups, thus make the enzyme inactivated (Vallee and Ulner, 1972; Gerardi, 2003). Other explanations suggested by recent researches are binding substitutive ligands, redox reactions with sulfurs, fentontype reactions, membrane-transport inhibitions, and electron siphoning (Harrison et al., 2007). Possibility also exists that digestate from such process contains high levels of toxic substances, thus making it not suitable for further use as agricultural fertilizers.

The aims of this research were:

1. Assessment of the biogas potential of plants from several constructed water treatment wetlands.
2. Preliminary analysis of the impact of heavy metal level on the anaerobic process.
3. Analysis of the toxicity level and nutrient content of the digestates.
4. Suggestion of pre-treatment if needed.

Methods

Material description

Fresh plant samples are collected from below wetlands: wetland that receives the effluent of the waste water treatment plant (WWTP) in the west coast of Halmstad, Sweden, wetland that collects waste water from the industrial area at the harbor of Halmstad (H), and the experimental wetland (EVA) in Harplinge. Samples from EVA are separated as emergent and sub-merged vegetations. Plant samples from both WWTP and the harbor are common reed (*Phragmites australis*). The emergent vegetation from EVA (EE) is mostly common reed with little amount of reed canary grass (*Phalaris arundinacea*). The sub-merged vegetation from EVA (ES) is a mixture of Canadian waterweed (*Elodea canadensis*) and watermilfoil (M*yriophyllum alterniflorum*). All plant samples were cut into pieces shorter than 1 cm in order to be better decomposed.

Fig.1 Experimental wetland (EVA) at Harplinge.

Digestates of cattle manure from the biogas plant in Plönninge, Sweden were used as provider of anaerobic bacteria.

Experiment Design

The experimental series consisted of 5 reaction bottles. A reference bottle was included with just digestates of 300 g. Detail properties of each reaction bottle are shown in Table. 1.

Bottle	Source	Manure digestate(g)	Plant(g)
Ref	N/A	300	0
1	WWTP	308	200
2	H	295	50
3	EE	316	110
4	ES	305	194

Table.1 Composition of experiment bottles. WWTP: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation.

Bottles were settled in the temperature controlled closet at 37 °C consistently for a 27-day reaction. Gas content was periodically detected by GC (VARIAN CP-3800) and pH values were measured by pH meter after the reaction was stopped. Fresh plant samples and digested residue samples of each bottle were taken to measure total solids (dried at 105 ℃) and volatile solids (dried at 550 ℃) composition.

*: Bottle 3 was broken on the 19th day of reaction due to a lab accident, so it was consequently impossible to get some of the experiment results from this bottle.

Fig.2 Digestion bottles and the thermostat container.

Chemical Analysis

Samples were taken both before and after digestion from each of the bottles and dried at 550 °C for 2 hours for chemical analysis.

For metal content analyses, the dried samples were transferred into an autoclave flask and added with 20ml of 7M nitric acid respectively. After the acid digestion in the autoclave at 120°C under 2.5 atm. overnight, the each sample was filtered down and diluted into a 100ml volumetric flask. The analyses of heavy metals (copper, cadmium and zinc) were performed by the atomic absorption spectrometer (Varian SpectrAA 100).

For measurement of phosphorus concentration, the dried samples were transferred in to a tube and added with 8ml distilled water, 1.6ml potassium persulfate, and 0.1ml 4M sulfuric acid. All the samples were under acid digestion in the autoclave at 120°C under 2.5 atm. overnight. The solutions were all diluted by 1000 times, thus the effect of dilution should also be considered when using the results. The determination of phosphorus was then performed by a spectrophotometer using molybdenum blue.

For measurement of nitrogen concentration separately, samples from the bottles were dried at 105°C for 24 hours and grinded into smaller pieces afterwards. The analysis of nitrogen and carbon content was performed by the NC soil analyzer (Flash.EA 1112).

Calculation of Methane Production

The methane production is expressed as the volume of CH_4 produced from unit weight of volatile solids (VS), which is calculated by below formula:

CH_4 (ml/g) / VS=$V_{\text{total gas production}}$*(Methane content)/$M_{\text{substrate}}$*TS*VS

$V_{\text{total gas production}}$ is the amount of total gas produced in the process.

Methane content is the average percentage of methane in the total gas produced.

$M_{\text{substrate}}$ is the amount of plant material added to each bottle.

Results

All bottles showed almost immediate gas production after the start of the reaction (Fig.3). The two bottles, with reed vegetation from the waste water treatment plant and the harbor respectively, had similar production curve throughout the whole process. The daily gas productions both peaked to the top within the first five days and decreased gradually after that. It can be seen from the figure that these two bottles would have had a few more days in producing the biogas, if not artificially stopped, particularly the H bottle. The bottle with reed vegetation from EE showed a fluctuation in gas production in the beginning, and reached the peak on day 11. Then the accident happened on day 19, and because of this, no more data was recorded. The bottle with submerged plants from ES had encountered a reaction failure from day 2 and recovered a little gas production during the last 8 days. The reference bottle with only biogas digestate produced very little biogas, indicating that there was not much digestible material left from previous production.

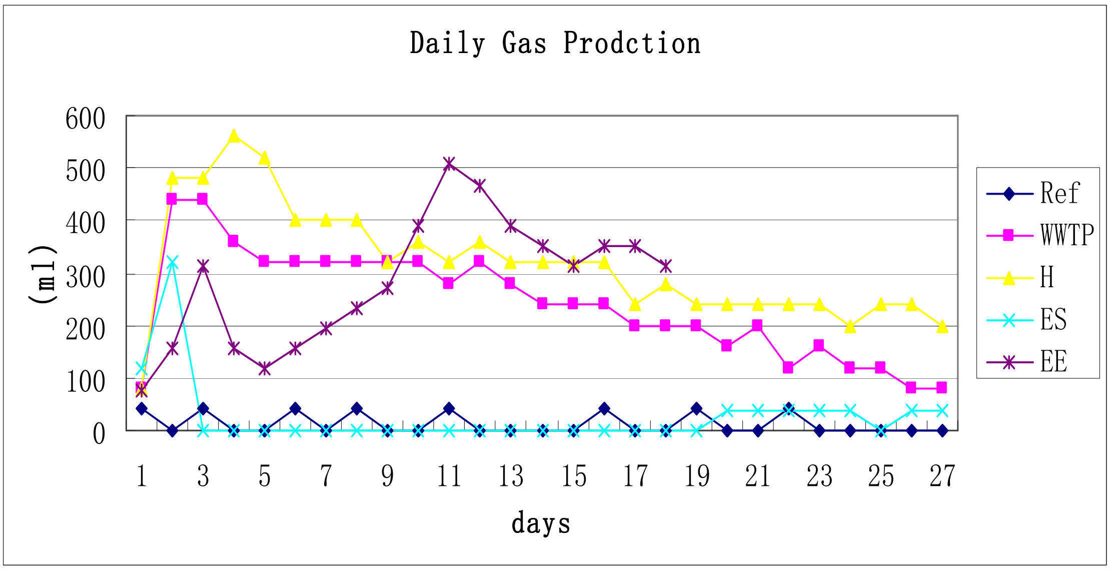

Fig.3 Gas production curve in each day with different substrates. WWTP: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation. Digestion was stopped by accident in bottle EE at day 19.

The cumulative gas production (Fig.4) in bottles WWTP, H, and EE showed a steady increase during the entire reaction. The reference bottle and ES, however, had little gas produced untill the end of the process.

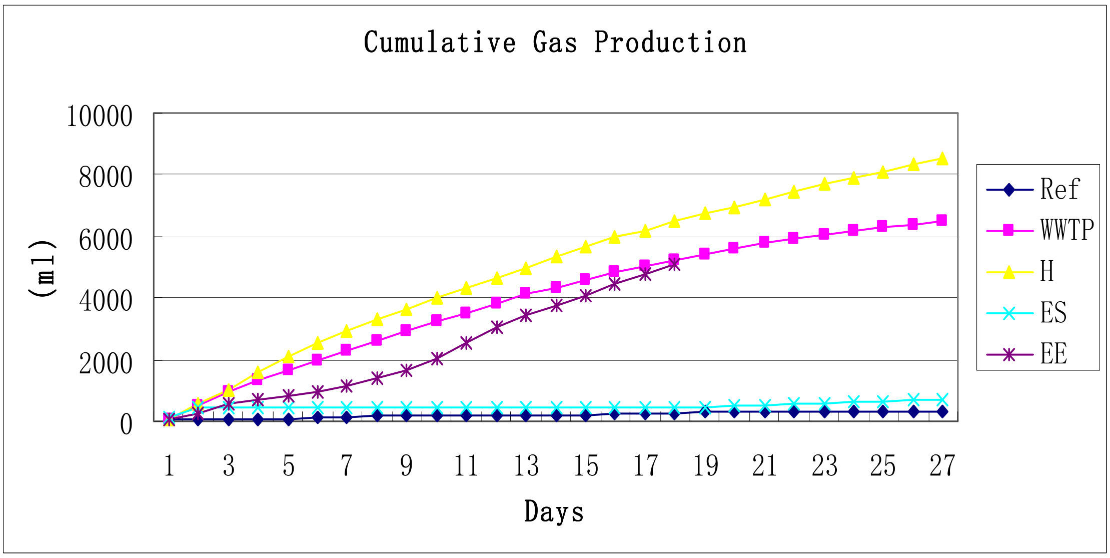

Fig.4 Cumulative gas production curve in each day with different substrates. WWTP: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation. Digestion was stopped by accident in bottle EE at day 19.

Methane content of the biogas yield in bottles WWTP, H and ES did not show much fluctuation during the whole 27 days, compared with bottle EE, which increased from day 5. The mean methane concentration is around 50~60% (Fig.5).

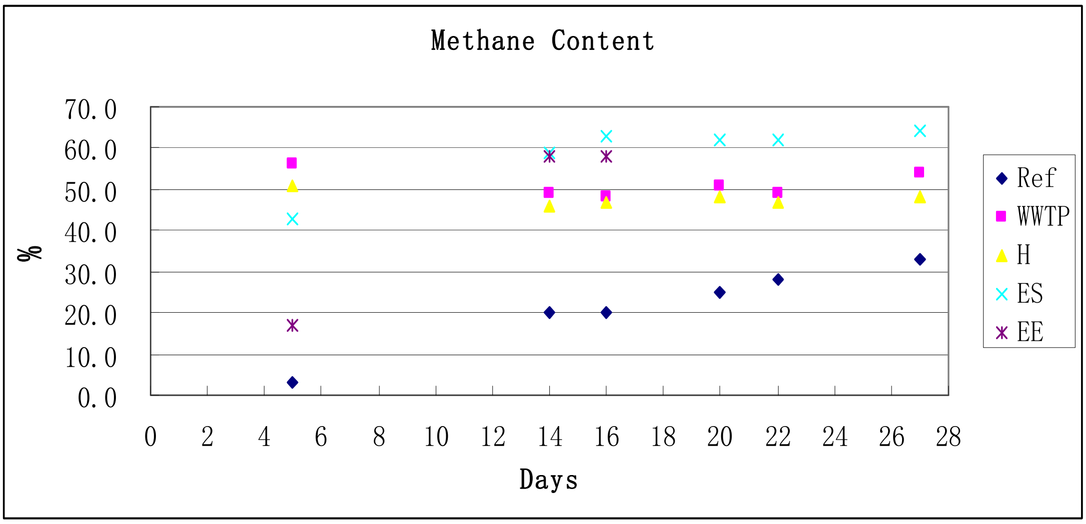

Fig.5 Methane content change through out the digestion. WWTP: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation.

Methane production of VS (Fig.6) showed rather big difference among different substrates sources. The bottle with reed from experimental wetland EE had the highest methane production of 143.7 ml/g VS, where as bottle ES had the lowest 24.5 ml/g VS. Considering the bottle EE was broken at day 18 of the total 27 days of reaction time, the real methane production can be much more. If extrapolated roughly from the existing results, total gas production from the bottle EE can reach 7000 ml with about 60% methane content. The methane production is then estimated to be about 190 ml/g VS.

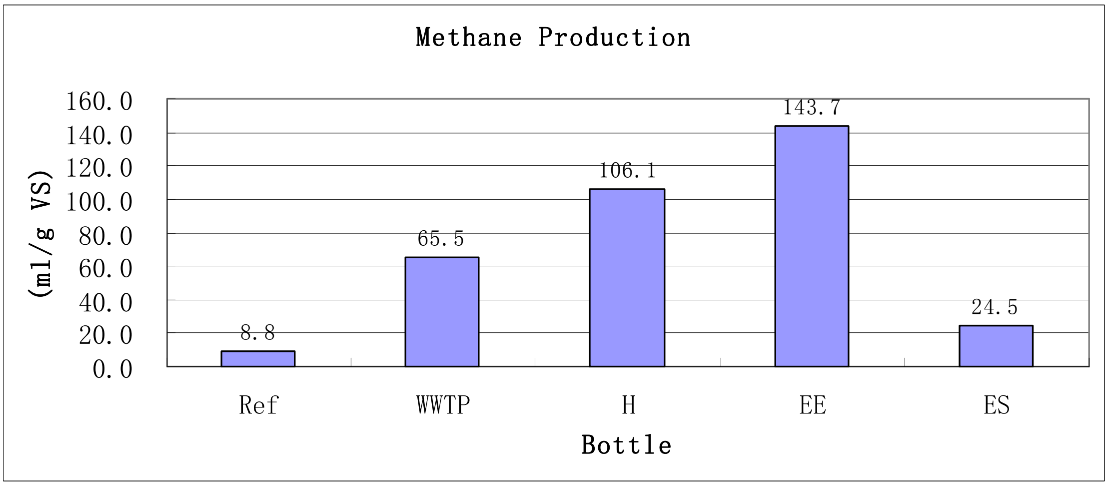

Fig.6 Methane production in different bottles. WWTP: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation.

Some characteristics of the materials used in this experiment are shown in Table 2. The TS content showed quite a big difference among the same reed species from the 3 different wetlands. The VS values of common reed from three different places are of the similar level, a bit higher than the suggested 85% of TS by Lars Ohlsson. C/N ratio varied in different results, where the reed sample from the wastewater treatment plant showed the lowest of 16:1, and the highest of 53 in reed sample from the experimental wetland with emergent vegetation.

Sample	Ref	WWTP	H	EE	ES
TS(%)	4.49	27.01	81.93	20.63	13.24
VS(% of TS)	73.17	92.55	93.54	90.90	71.93
C/N ratio	10	16	47	53	10
pH	8.0	7.6	8.0	*	8.0

Table.2 General characteristics of substrates of different sources. WWTP: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation. * The pH value was measured after the reaction was stopped, so the value for bottle EE was inaccessible due to the lab accident.

Table 3 stated the nutrient and metal levels of pre-and post samples including the post sample from the reference bottle. As the bottle EE was broken before the end of experiment, its analysis results of residue samples could not be reached.

The phosphorus level all showed an increase after the anaerobic digestion. The nitrogen level also increased after reaction in bottle WWTP and H, but a decrease in bottle ES.

Copper, zinc and cadmium levels were analyzed both before and after reaction. For Cu, both samples in bottle WWTP and H showed an increase in concentration after digestion, which can be attributed to the loss of organic contents that turned into biogas during the process. The Cu level in fresh EE and residue ES are below the detection limit of the equipment of 0.001 mg/g of TS. For Zn level, both samples in the bottle H and ES showed an increase after digestion, yet the sample in the bottle WWTP had an unexpected decrease, which is contrary to other bottles. Since the sampling and analysis was only conducted once, the reliability of the result remains to be doubted. For Cd, all samples are below the detection limit of 0.01 ug/g of TS.

Noted that the post-reaction samples were in fact the mixture of manure digestates and the digested vegetation, the nutrient and metal content in the digestates can also contribute to the increase in the residues.

Sample	W-fresh	H-fresh	EE-fresh	ES-fresh	W-post	H-post	ES-post	Ref-post
TN(mg/g)	25.5	8.9	7.3	29.3	26.3	19.9	27.4	31.7
TP(mg/g)	2.5	0.5	11.8	8.1	7.3	8	15.8	16.4
Cu(mg/g)	0.022	0.005	*	0.026	0.025	0.036	*	0.048
Zn(mg/g)	0.320	0.005	0.029	0.046	0.110	0.119	0.273	0.214

Table.3 Nutrient and metal levels of pre-and-post samples (of TS). W: wastewater treatment plant; H: harbor; EE: experimental wetland with emergent vegetation; ES: experimental wetland with submerged vegetation * Copper level of fresh material from EE and residue sample of bottle ES are below the detection line of the equipment.

Discussion

The targeted plant species for biogas production in this project is common reed, an aquatic plant with wide distribution in Sweden. For biogas production, the harvest time of the reed is important. When the reed is harvested too late in the growing season, it will contain an increased lignin content that affects the gas yield. Substrate with high level of lignin show recalcitrance against the hydrolysis process in the anaerobic digestion because of the high stability of the material to bacterial attacks (Taherzadeh and Karimi, 2008). It then extends the retention time and reduces the gas production. In the case of small-scale studies, summer harvested reed has shown good performance as a raw material for biogas production (Hansson and Fredriksson, 2004). The reed biomass used in this project is collected from wetlands in early spring of Sweden (Mar. 2012), which means it is in the stage of termination of growth after autumn and contains high level of lignin content. This factor may contribute to the result that the gas yield of substrates from WWTP and H, 65.5 and 106.1 ml/g VS respectively. This result was rather low compared to the number provided by the local authority, yet the estimated gas production of reed material from EVA, if the digestion hadn't been stopped by the accident, would have been about 190 ml/g VS, which corresponds with the suggested 180 ml/g VS (Ohlsson, 2012). The suggested carbon-to-nitrogen (C/N) ratio for biogas production is around 25~30, which is believed to be optimal for anaerobic bacteria growth (Yadvika et al., 2004; Yen and Brune, 2007; Zhong et al., 2012). The C/N ratio of plant samples from the wastewater treatment plant is extremely low compared with the other two sources, which may contribute to the low gas yield results. In this experiment, cattle manure was used for co-digestion and providing anaerobic bacteria. With its low value of C/N ratio, the manure can also be used to adjust the total C/N ratio by changing the mixing level with plant materials.

An advantage of the anaerobic digestion is that the plant nutrients in the digested material are not consumed and are stored in the digestates. Moreover, due to the decomposition of the organic material, the nutrients in the residues are made more easily available for the plants when used as fertilizers. General assumption by previous studies on nutrient levels in common reed is 25.73/3.39 mg/g dry matter for nitrogen (leaves/straw), and 1.69/0.63 mg/g dry matter for phosphorus (leaves/straw) (Hansson and Fredriksson, 2004). This number is relevant to the result of this project, suggesting that the biogas residues can be further utilized as a source of nutrient for agricultural use.

Heavy metals may play an important role in the inhibition of anaerobic digestion (Chen et al., 2008). From the experiment results reed substrates from the EE wetland showed the highest methane yield among the three reed substrates bottles, even though the production was stopped by accident at 2/3 of the whole reaction. As the EE wetland is an experimental wetland for agricultural wastewaters, the metal content may not be as high as that of industrial/municipal wastewaters. The copper level of the fresh material from the EE wetland was under the detection limit of the equipment (0.001 mg/g of TS), which suggests an explanation for the high gas yield.

The cadmium content in fertilizers has now been strictly under control, because Cd is a very toxic substance for human health and plant growth. The European Union stipulated that the Cd content of phosphate fertilizers not to exceed 20 mg Cd / kg P_2O_5 by 2015 (Oosterhuis, 2000), equivalent to approximately 10 mg Cd/ kg of common P fertilizers. The Cd levels in the biogas residues of this project were all under the detection line of 0.01 ug/g of TS, much lower than the EU regulation. This suggests that the residues are safe to be used as fertilizers with low toxicity. Copper content in the biogas residue is allowed in organic farming as fertilizers, with restrictions on its use to minimize copper accumulation. However, accumulation of Cu in the soil can also result in contamination. In order to prevent Cu accumulation, total Cu input to the soil need to be monitored (Tracy and Baker, 2005). Cu and Zinc both are favorable micronutrients for plant growing and human health, thus the low level of Cu and Zn content in the residue shows non-hazardous to be spread on agricultural land (Cakmak, 2009; Nziguheba and Smolders, 2008).

Pretreatment methods for the use of plant biomass in biogas production have been widely studied. Lignocelluloses (lignin, cellulose, etc.) are often major even sole components of many plant wastes. Treatment methods to reduce the lignocelluloses content or its negative impacts on biogas production have been largely discussed (Zhong et al., 2011). To achieve the purpose of sustainable development, an effective and economical pretreatment should meet the following requirements: (a) avoiding formation of other inhibitors for the biogas process, (b) minimizing the energy demand, (c) reducing the cost of material in constructing pretreatment reactors, (d) producing less waste, (e) using little/no chemical or a cheap chemical (Taherzadeh and Karimi, 2008). Chopping/milling is one of the most common and easy treatment methods. Chopping, by reducing the size of the materials, can improve the effectiveness in hydrolysis process, which improves the degradation of these materials and the biogas production (Zeng et al, 2007). It has been studied that materials of smaller particles were easier to be digested in biogas production, but it could also be more efficient if size reduction is combined with other pretreatments (Zhang et al, 1999). Possible drawbacks of chopping include the great energy costs during the process. Besides physical methods, chemical and biological methods have also been introduced for pretreatment lignocellulosic materials. Some common examples are

CO_2 explosion, alkaline hydrolysis, acid hydrolysis, wet oxidation, microorganism treatment, enzymatic treatment and etc (Taherzadeh and Karimi, 2008; Zieminski et al., 2012). Costs and benefits of each method should be carefully assessed before applying for industrial or commercial use.

Due to lack of time and lab conditions, the experiment conducted in this project is just a preliminary attempt in finding the potential of combining wetland biomass with biogas production. In future researches, parallel experiments for each source of biomass are needed as to further discuss the proportion of manure and plants to be used as co-substrates, the maximum gas production, and biogas potential of other wetland vegetations. Plant material such as common reed should be harvested in summer in order to achieve the optimal gas yield. Impacts of other heavy metals (e.g. zinc, chromium, lead) on the biogas process and residue should also be studied, especially on wetlands for municipal and industrial wastewaters in comparison of agricultural wetlands. Other factors that affect the production performance were not discussed in this thesis. By turning the wetland plant material into bio-energy, a more sustainable source of energy is discovered and the emission of CO_2 and other green house gases can be reduced.

Conclusion

- Common reed from wetlands for wastewater treatment showed a considerable potential for biogas production but did not reach the estimation from previous research.
- Influencing factors over the methane production include C/N ratio, lignin content, substrate composition, heavy metals, etc.
- Reed from the experimental wetlands for agriculture showed a lower Cu concentration compared with other two wetlands for wastewater treatment.
- Cd level in the biogas residues showed nonhazardous for use of fertilizers.
- Pretreatment of substrates needs the costs and benefits assessment.
- Further research is needed concerning different mixing level of manure residue and plant material, heavy metal and nutrient analysis, pretreatment method, and source of raw material.

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