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돈분뇨 처리수 유래 질소와 인 제거를 위한 식물정화법 활용과 바이오매스의 바이오메탄 잠재성 연구

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Application of Phytoremediation for Total Nitrogen and Total Phosphorus Removal from Treated Swine Wastewater and Bio-methane Potential of the Biomass

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ABSTRACT

The aim of this study is to determine the removal efficiency of total nitrogen and phosphorus from treated swine wastewater by *Phragmites australis* and *Miscanthus sacchariflorus* var Geode Uksae−1, and to determine its biomass total energy value and biomethane potential. Plants were grown with a bedding mixture either soil and sand or soil, sand, and bioceramic. Treeated swine wastewater with Total nitrogen (TN) and Total phosphorus (TP) of 222.78 mg/L and 66.11 mg/L, respectively, was utilized. The TN and TP removal is higher in the bio−ceramic−soil−sand bedding media treatment. The highest TN removal of 96.14% was performed by *Miscanthus sacchariflorus* var Geode Uksae−1, but the elemental analysis shows that *Phragmites australis* contains more nitrogen than *Miscanthus sacchariflorus* var Geode Uksae−1, indicating higher nitrogen uptake. The highest TP removal of 98.12% was performed by Phragmites australis. The cellulose content of the plant grown with the bioceramic−soil−sand bedding was approximately 3−6% higher than that of the plant grown in the soil−sand bedding. Different growing substrates may have an effect on the fiber content of plants. The biomethane potential of the produced biomass of the plants was between 57.01 and 99.25 L−CH_4/kg VS. The lignin content is believed to inhibit the breakdown of plant biomass, resulting in the lowest methane production in the *Phragmites australis* grown in the soil−sand bedding media.

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Keywords : Total nitrogen, total phosphorus, biomass, biomethane

초 록

본 연구는 거대억새(*Miscanthus sacchariflorus* var Geode Uksae-1)와 갈대(*Phragmites australis*)를 활용하여 돈분뇨 처리수 유래 영양염류(질소 및 인) 제거를 정량적으로 분석하고 생산된 바이오매스의 총 에너지가와 바이오메탄 잠재성 분석을 목적으로 수행되었다. 식물들은 일반토양과 사질토 또는 일반토양, 사질토 및 바이오세라믹의 혼합 여재로 채워진 용기에서 다루어졌다. 사용된 돈분뇨 처리수의 총질소와 총인함량은 각각 222.78 mg/L 과 66.11 mg/L에 해당하였다. 총질소와 총인 모두 바이오세라믹 첨가구에서 높은 제거율을 보였다. 거대억새에서 총질소 제거율이 가장 높게 나타났다(96.14%). 하지만 식물체의 원소분석 결과 갈대의 질소함량이 거대억새보다 더 높게 나타나 갈대의 질소흡착력이 더 뛰어난 것으로 판단된다. 반면 가장 높은 총 인 제거율을 보인 처리구는 갈대로 98.12%의 값을 보였다. 식물체 셀룰로스 함량은 일반토양 처리구보다 바이오세라믹 처리구에서 약 3~6% 더 높게 나타나 바이오세라믹은 식물섬유 형성에 영향을 미치는 것으로 사료된다. 본 연구에서 생산된 바이오매스의 바이오메탄 잠재성 분석결과 약 57.01~99.25 L-CH_4/kg VS의 값을 보였다. 리그닌은 식물의 바이오매스 분해를 방해하는 요소로 일반토양-사질토 여재를 사용한 갈대 처리구에서 가장 높게 나타나 메탄 생산력이 떨어지는 것으로 판단된다.

주제어 : 총 질소, 총 인, 바이오매스, 바이오메탄

1. Introduction

Livestock wastewater contains a high amount of nitrogen and phosphorus. Total nitrogen in swine wastewater can be as high as 4,546 mg/L, and the total phosphorus can be as high as 2,765 mg/L[1]. Nitrogen and phosphorus are among the major pollutants of aquatic environment. A high amount of nitrogen and phosphorus may lead to eutrophication and groundwater contamination. Eutrophication will result in the depletion of oxygen, leads to the death of organisms in the water.

Technologies have been developed to reduce the total nitrogen and phosphorus in the wastewater, such as biological wastewater treatment systems utilizing microorganisms. Some examples of microorganism-based nutrient removal are the activated sludge system, enhanced biological phosphorus removal (EBPR). and anammox. Those systems are usually used as the primary wastewater treatment. However, in some cases, after the primary treatment, the nitrogen and phosphorus concentration is still high and does not achieve the discharge limit standard, especially if the wastewater naturally has high nitrogen and phosphorus compounds , such as livestock wastewater. In this case, further wastewater treatment is needed. One of the alternatives is to use plants to remove nutrients from the wastewater, which is often known as phytoremediation. However, the study of phytoremediation is mostly conducted with non-treated wastewater. Therefore, the study of nitrogen and phosphorus removal from the treated wastewater is needed.

Phragmites australis, or common reed, is a wetland grass that forms reed beds that occur in freshwater, brackish water and in some instances, marine littoral communities[2]. It has an extensive system of rhizomes that can penetrate into the soil up to a depth of 1 m. Reed often considered an invasive species. However, it is frequently studied for its potential for nutrient removal from wastewater in constructed wetland systems. It is often used as a plant for nutrient removal in constructed wetlants with horizontal or vertical flow. It is used to treat sewage wastewater[3], polluted agricultural wastewater[4], and post-treated domestic wastewater[5]. Reed is also used for water purification treatment. Furthermore, reed biomass can be utilized as a feedstock for bioenergy by combustion or anaerobic digestion and is also a potential source of lignocellulosic derived biofuel. The young reed plants are useful for livestock feed, especially ruminants. and reed biomass can also be utilized as a soil conditioner[6].

In addition to reed, another plant might also be a candidate for a nutrient removal system. There is a new *Miscanthus sacchariflorus* genotype that was recently found in South Korea named Geodae-Uksae 1. It was found in damp land in the southern part of the Korean Republic, which indicates it has the ability to survive under waterlogged conditions[7] and so might be suitable for wastewater treatment. *Miscanthus* sp. (silver grass) have been known as ideal lignocellulosic bioenergy crops that can substitute the first generation of bioenergy crops. It is a perennial grass that can be found easily in East Asia and the Pacific Islands and has a strong resistance to pests and diseases. Research regarding the utilization of *Miscanthus sacchariflorus* var Geode Uksae-1 for the removal of TN and TP from wastewater is lacking. Therefore, a study regarding the TN and TP removal with *Miscanthus sacchariflorus* is important to provide alternative plants that can be utilized for nutrient removal.

The sustainability of phytoremediation is also determined by how the biomass is utilized after it is used in the nutrient removal system. Most studies of phytoremediation mainly focus on the nutrient removal itself with regard to the production of biomass without more on the potential value of biomass. The integration of a nutrient removal study with the potential value of the biomass will provide an idea of the added value of the plant biomass that can be obtained from the nutrient removal system.

Finally, the aim of this study is to determine the removal efficiency of total nitrogen and phosphorus from treated swine wastewater using *Phragmites australis* and *Miscanthus sacchariflorus* var Geode Uksae-1. This study also wants to address the potential value of biomass in terms of its total energy value and biomethane potential.

2. Materials and methods

2.1 Experimental plant preparation and treated swine wastewater

The plants that are used in this experiment were reed (*Phragmites australis*) and silver grass (*Miscanthus sacchariflorus* var Goede Uksae 1). *Miscanthus sacchariflorus* var Geodae-Uksae 1 was obtained from the

Bioenergy Crop Research Center, National Institute of Crop Science (NICS), RDA which located in Muan, Chonnam, South Korea. Healthy rhizomes were transplanted into a pot with a soil : sand (1:1) mixture media and watered every 3 days to induce shoot growth. Treated swine wastewater used in this study has gone through anaerobic digestion and an aerobic treatment system.

2.2 Experimental design

The plants were grown in a container with a height of 50 cm and a diameter of 30 cm. The surface area of the container is 0.076 m^2. The bottom of the container was filled with gravel up to 5 cm in height. The upper bedding was either a mixture of soil and sand (1:1) or soil, sand, and bio-ceramic (2:2:1). The bio-ceramic utilized in this experiment was in form of pellets with a 2 mm diameter. The experiment was conducted in a batch system with these two plants. The 10% treated swine wastewater was used in this experiment. The treatment group was the system with the plants while the control is the system without plants. The experimental unit was replicated three times for a total number of 18 experimental units. Water sampling was conducted every week for 105 days.

2.3 Physicochemical characteristics of water

The total nitrogen, ammonia, nitrate, nitrite, and total phosphorus were analyzed by using Hach chemical reagents according to Hach protocols manual (DR5000, Hach USA).The total nitrogen and phosphorus removal was calculated using following formula:

$$TNremoval\,(\%) = \frac{(TN_{swine\,wastewater\,(initial)} - TN_{final})}{TN_{swine\,wastewater\,(initial)}} \times 100 \quad \cdots\cdots (1)$$

$$TPremoval\,(\%) = \frac{(TN_{swine\,wastewater\,(initial)} - TN_{final})}{TN_{swine\,wastewater\,(initial)}} \times 100 \quad \cdots\cdots (2)$$

2.4 Plant biomass analysis

Phragmites australis and *Miscanthus sacchariflorus* shoots were cut above the ground and weighed directly after harvesting. Before weighing, the plants were cleaned from the dirt to remove any contamination. The harvested plant biomass was air dried at the ambient temperature for 1-2 days and continued with drying in an oven at 60°C overnight. The dried plant biomass was ground and then passed through a 1 mm sieve. The moisture content was determined by oven drying at 103-105°C for 5 hours following AOAC standard method No. 935.29. The organic (volatile solid) and ash contents were determined by ignition at 550°C for 2 hours.

The Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analyses were performed using filter bag method with an ANKOM F57 filter bag. Approximately 0.5 g sample was placed inside the bag and reflux with the ADF or NDF reagent at 200°C for 1.5 hours. Then, the sample with filter bag was washed with warm water until the color was clear before washing with acetone. After that, the filter bag with the sample was dried at 105°C for 2 hours, and the weight was measured. The NDF and ADF were determined by the difference in weight were converted to a dry matter basis. The air-dried sample (0.5-1.0 g) was hydrolyzed with 70% H_2SO_4 at ambient temperature for 2 hours, then boiled and reflux for 4 hours. The mixtures were vacuum filtrated and dried at 105°C for 2 hours, followed by ignition at 550°C for 3 hours. The Klason lignin content was determined by the difference in weight before and after ignition. The carbon, nitrogen, hydrogen, oxygen, and sulfur contents were determined by an elemental analyzer (Thermo Fisher).

The higher heating value was determined by oxygen combustion of a sample using a bomb calorimeter (1341 Oxygen Bomb Calorimeter, Parr Instrument Company). The sample weight of approximately 0.5 - 0.75 g was pelletized and combusted with oxygen at 25 atm. The combustion temperature was recorded until the end of combustion. The combustion ended when the temperature reached a constant temperature. The HHV value was determined as MJ/kg of the dried sample.

2.5 Biomethane Potential (BMP) Assay

Biomass samples of the plants were put into 250 ml serum bottles. The weight was adjusted so that each serum bottle contained 0.5 g volatile solids of plant biomass. A BMP test media of 90 ml containing phosphate buffer, macronutrients, and micronutrients was added, and an inoculum of anaerobic microorganisms was added to bring the total volume to 100 ml. Blanks were prepared using the anaerobic medium and inoculum to correct for biogas production. The mixture was then purged with N_2 gas to remove oxygen for 5 minutes. The serum bottle was closed with a rubber cap and then sealed with aluminum crimps. All bottles were put into the shaker incubator, which maintained a temperature of 35°C and 120 rpm. At regular intervals the biogas production was measured with a glass syringe using the pressure displacement method. The gas composition (CO_2, CH_4, and N_2) was analyzed using Gas Chromatography (Agilent) equipped with a column HP-PLOT/Q and a Thermal Conductivity Detector (TCD). The methane gas production was calculated by using the following formula:

$$V_{CH_4t} = V_b \times C_{CH_4} \quad \cdots\cdots\cdots\cdots (3)$$

where,

V_{CH_4t} : Volume of methane at time t

V_b : Volume of biogas at time t

C_{CH_4} : Concentration of methane at time t

Meanwhile, the cumulative methane production was calculated by following formula,

$$V_{CH_4Cum} = V_{CH_4t_i} + V_{CH_4t_j} + \dots + V_{CH_4t_n} \quad \cdots\cdots\cdots\cdots (4)$$

where,

V_{CH_4cum} : Cumulative methane production

3. Result and Discussion

3.1 Nitrogen and phosphorus removal by Phragmites australis and Miscanthus sacchariflorus var Geode Uksae-1

The characteristics of the treated swine wastewater that was used in the experiment is shown in **[Table 1]**. The main component of the total nitrogen in the treated swine wastewater is nitrate, (NO_3^-) because the wastewater had gone through aerobic wastewater treatment. In the aerobic condition, nitrification may occur. The nitrification process converts ammonium into

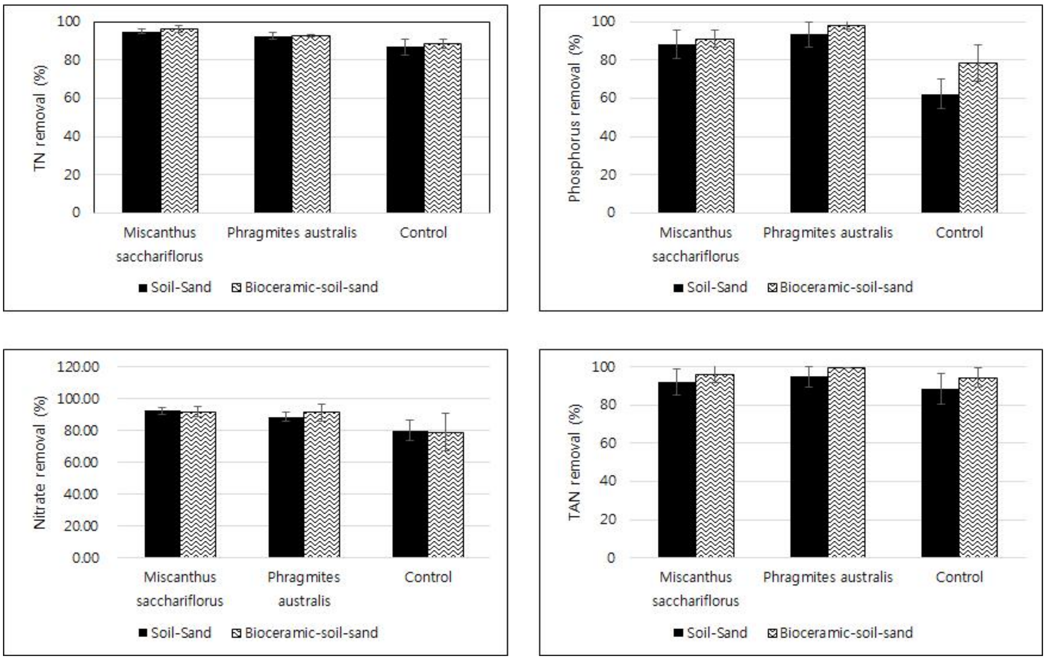
[Table 1] Chemical Characteristics of Treated Swine Wastewater

Chemical parameters	Concentration (mg/L)
Total Nitrogen (TN)	222.78
Nitrate (NO_3^-)	137.39
Nitrite (NO_2^-)	15.49
Total Ammonia Nitrogen (TAN)	33.50
Total Phosphorus	66.11

nitrate in aerobic conditions by nitrifying microorganisms. This also explains the low amount of total ammonia nitrogen in the treated swine wastewater.

In the bio–ceramic treatment, both *Miscanthus* and *Phragmites* exhibited a higher total nitrogen removal compared with the control, and as the plants absorb nitrogen for their metabolisms, the nitrogen concentration in the water was reduced. However, the removal of nitrogen in the control (without plants) is also high (more than 80%) **[Fig. 1]**. The adsorption of nitrogen by the substrate contributed to the high nitrogen removal. In addition, microbial activity, especially that which involves in nitrification and denitrification might also have contributed to the nitrogen removal, which was not observed during the study.

Between the two plants, the highest total



[Fig. 1] Total Total nitrogen, phosphorus, TAN and nitrate removal from the treated wastewater.

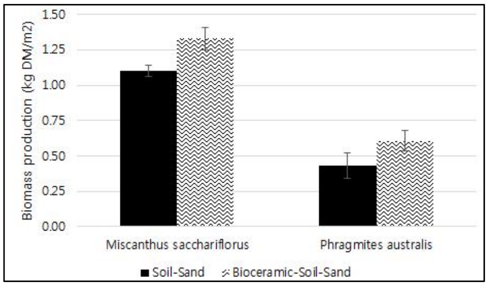
nitrogen removal of 96.14% with final TN effluent concentration of 7.33 mg/l was performed by *Miscanthus sacchariflorus* var Geode Uksae-1 and was not much different from the TN removal by Phragmites australis, which was 92.56% with final TN effluent concentration of 18.33 mg/l. However, the results of the elemental analysis **[Table 2]** show that *Phragmites australis* contains more nitrogen than *Miscanthus sacchariflorus* var Geode Uksae-1, indicating a higher nitrogen uptake.

Generally, the treatment with the bio-ceramic media removed most of total phosphorus from the water. The possible mechanism of phosphate removal in the bio-ceramic treatment is the absorption of the phosphorus into the bio-ceramics. In the bio-ceramic treatment, the system with *Phragmites* gives the highest percentage, 98.12%, of total phosphate removal for a final total phosphorus concentration under 5 mg/L. The plants also have a role in the reduction of phosphorus because plants take up phosphorus, and the phosphorus removal is generally higher in the systems with plants. Thus, this result shows that plants play an important part in the phosphorus removal process. In this experiment, the system with *Phragmites* has higher phosphorus removal in both mixtures of bedding media.

From the elemental analysis shown in **[Table 2]**, the composition of elemental nitrogen in *Phragmites australis* is higher than in *Miscanthus sacchariflorus*. This indicates that *Phragmites* takes up more nitrogen than *Miscanthus*. The other differences in element composition is that *Phragmites* has a sulfur content that is four times higher than Miscanthus. This indicates that *Phragmites* may take up more sulfur compounds. This result may also be related with the higher ash content of *Phragmites australis* **[Table 3]**.

3.2 Biomass production, characteristics, and methane potential

3.2.1 Biomass production



[Fig. 2] Biomass production of *Miscanthus sacchariflorus* and *Phragmites australis* in different bedding media

[Table 2] Elemental Analysis of *Phragmites Australis* and *Miscanthus Sacchariflorus* var Geode Uksae-1 with Different Bedding Media

	Bedding	C*	H*	O*	N*
Phragmites australis	Soil- Sand	40.8	5.5	36.0	1.7
	Bioceramic-Soil-Sand	40.4	6.0	35.5	1.2
Miscanthus sacchariflorus	Soil- Sand	43.1	5.6	38.6	1.5
	Bioceramic-Soil-Sand	41.6	5.9	39.9	0.9

* Unit in % dry matter

Phragmites and *Miscanthus* grown in the media with bio-ceramic material produce more fresh and dry weight biomass**[Fig. 2]**. The roots and rhizomes of the plants grown in the bedding media with bio-ceramic are also more abundant. The higher biomass and more extensive roots might be related to the higher nutrient removal in the system with bio-ceramics. More extensive roots might take up more nutrients from the water, resulting in a lower nutrient concentration in the water.

The reason why the bio-ceramic substrate produces greater biomass is not clear in this study. The assumption is that it might be because of the porosity created in the bedding mixture by the bio-ceramic material. The bio-ceramic material has a diameter of 2 mm and creates a more porous media compared with the soil and sand as a bedding media, which might result in higher gas diffusivity. Higher gas diffusivity provides higher gas exchange between the roots and the bedding media, which may result in improved plant growth. The gas diffusivity is highest when the media mixture contains 2-4 mm fragments[8].

3.2.2 Biomass characteristics

Based on the data shown in **[Table 3]**, the

[Table 3] Characteristics of *Phragmites australis* and *Miscanthus Sacchariflorus* Grown in Different Bedding Media

Characteristics	Bedding	*Phragmites australis*	*Miscanthus sacchariflorus*
Water content (%)	SS	53.64	51.87
	BSS	55.91	56.75
Dry matter (%)	SS	46.36	48.13
	BSS	44.09	43.25
Organic matter (% DM)	SS	86.88	91.19
	BSS	89.36	90.43
Ash (% DM)	SS	13.12	8.81
	BSS	10.64	9.57
Cellulose (% DM)	SS	20.53	24.63
	BSS	25.25	26.38
Hemicellulose (% DM)	SS	19.36	22.07
	BSS	23.93	23.64
Klason lignin (% DM)	SS	28.46	26.23
	BSS	24.4	22.73
Neutral Detergent Fiber (% DM)	SS	68.35	72.93
	BSS	73.61	72.75
Acid Detergent Fiber (% DM)	SS	48.98	48.88
	BSS	49.68	49.11
Higher Heating Value (MJ/Kg)	SS	16.82	17.00
	BSS	15.03	16.58

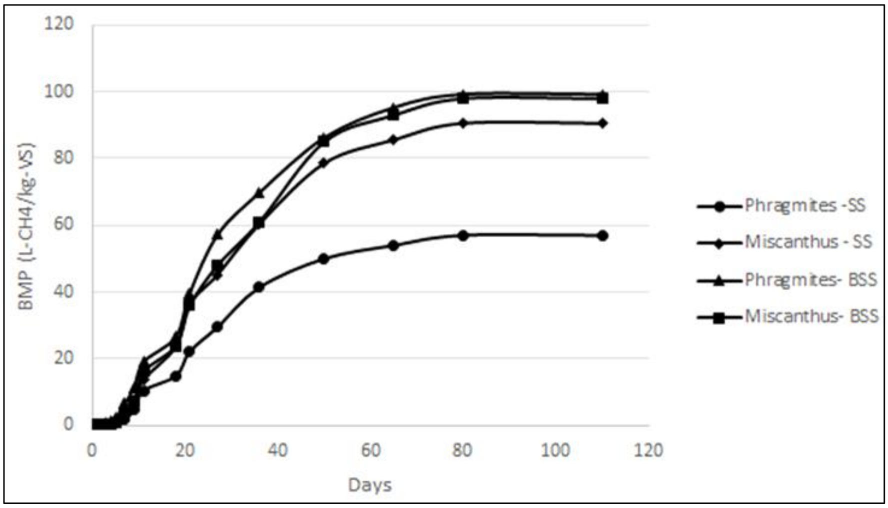
SS: Soil-Sand
BSS : Bioceramic-Soil-Sand

characteristics of the biomass of *Phragmites* and *Miscanthus* grown in different bedding media did not really differ. However, Miscanthus has a higher organic matter content than does Phragmites. This result might be because *Phragmites* has a greater ability to take up minerals than *Miscanthus*. Both *Phragmites* and *Miscanthus* have a high lignin content. The lignin content of Phragmites and Miscanthus was in the range of 22–26% dry matter. A higher lignin content might influence the degradation process in composting, and/or the pre–treatment for bioethanol production. Generally, there are no large differences in the fiber content between *Miscanthus* and *Phragmites*.

Cellulose is major cell wall component that accounts for 28–30% dry matter of forage grasses[9]. In this study, the cellulose content of *Phragmites* and *Miscanthus* ranges from 20–26% dry matter, which is in the range of reported studies. The cellulose content of the plants with the bio–ceramic addition have higher values compared with the plants with only soil and sand as a substrate. However, the difference is only approximately 3–6% higher with the bio–ceramic substrate, which is opposite to the lignin content, which is higher in the soil and sand substrate. From this result, a different substrate may have an effect on the fiber content of the plants. In the case of hemicellulose, different substrates did not give a consistent result. Therefore, it is assumed that the bedding media mixture in this study slightly affected the cellulose and lignin contents, while the effect on the hemicellulose content was unclear.

3.2.3 Biomass energy content and methane potential

The higher heating values (HHV) of Phragmites australis and Miscanthus sacchariflorus var Geode Uksae–1 are shown in **[Table 3]**. The heating values of the biomass were in the range of 15 – 17 MJ/kg



[Fig. 3] Cumulative methane production of *Phragmites australis* and *Miscanthus sacchariflorus* (SS: Soil–Sand; BSS: Bioceramic–Soil–Sand) dry matter. This result is lower than the energy content of *Miscanthus* and energy grass from reported studies, which is approximately 19.29 MJ/kg[10] dry matter However, it is comparable to other biomass fuel resources, such as rice straw and Sudan grass which is 15.61 MJ/kg and 17.39 MJ/kg, respectively[10].

The biomethane potential of the produced biomass was between 57.01 and 99.25 L-CH_4/kgVS**[Fig. 3]**. The biomethane potential observed in this study was in the range of the biomethane potential observed in lignocellulosic biomass. Previous studies showed that the biomethane potential of lignocellulosic biomass ranged between 49 and 417L-CH_4/kgVS[11,12]. Interestingly, the lowest and highest biomethane potentials belong to the *Phragmites australis* biomass from different bedding material, with the soil and sand mixture resulting in the lowest biomethane potential. This result might be mainly caused by biomass characteristics. *Phragmites australis* biomass with soil and sand as the bedding material has the highest lignin content at 28.46% DM, while the treatment with bioceramics has the lowest lignin content of 24.4% DM. Lignin is known to have negative correlation with biomethane potential because it can affect the digestibility of the cellulose and hemicellulose in the lignocellulosic biomass[10]. Therefore, the high lignin content of the *Phragmites australis* biomass with the sand and soil mixture is the main cause of the low biomethane potential. The relatively high lignin content of all the plant biomass observed in this study ranges between 24.4 and 28.46% DM **[Table 3]** and is also the main cause of the moderately low biomethane potential compared with the data from existing literature. Nevertheless, the biomass produced from this experiment can still be utilized as a substrate for anaerobic digestion, although pre-treatments might be required to increase the methane yield.

4. Conclusion

The removal of total nitrogen and phosphorus is higher when the bio-ceramic media is added to the bedding. The TN removal among the 2 plants was not significantly different, but the elemental analysis result shows that *Phragmites australis* contains more nitrogen than *Miscanthus sacchariflorus* var Geode Uksae-1, indicating higher nitrogen uptake. In the bio-ceramic treatment, the system with *Phragmites* gives the highest percentage, 98.12%, of total phosphate removal. The bio-ceramic media also promotes plant growth, resulting in higher plant production. The bedding media may also influence the fiber composition of the plant biomass, which then affects the biomethane potential. The biomethane potential of the produced biomass was between 57.01 and 99.25 L-CH_4/kgVS. The lignin content is believed to inhibit the breakdown of the plant biomass, resulting in the lowest methane production in the *Phragmites australis* grown in the soil-sand bedding media.

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