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4Research Article

Enhancement of Pretreatment Feasibility of Lignocellulosic Substrates for Biogas Production with Alkaline Lake Water

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Abstract: To improve the biogas efficiency from lignocellulosic feedstock, an alkaline pretreatment method was investigated based on the sustainable use of a natural resource. A new aspect of this study is the use of water from Lake Van (Lake Van Water, LVW), the world's largest alkaline lake with a surface area of 3,755 km2, located in an agricultural area, instead of alkaline solutions made with chemicals in laboratories. Wheat straw is the main cultivated waste as lignocellulosic feedstock and was also selected for this experiment. The alkaline pretreatment was carried out on a pulverised straw (2 cm max) with the LVW and alkaline water (NaOH solution). The experimental pretreatments were carried out for 120 h at a temperature of 37 °C in a climate-controlled room. Batch tests for anaerobic digestion were conducted for 60 days under mesophilic conditions (37 °C) to assess biochemical methane potentials. The methane yield of both types of pretreated wheat straw gave higher values than untreated wheat straw. It was also indicated that the WS pretreated with the LVW had a higher methane yield than the WS pretreated with the NaOH solution. In this study, an economic feasibility evaluation was also conducted. The LVW was determined to be a better economic alternative to NaOH for the pretreatment of wheat straw, a model lignocellulosic biogas substrate.

Keywords: Alkaline pretreatment, Biomethane yield, Lake Van, Wheat straw

Lignoselülozik Substratlardan Biyogaz Üretiminde Ön Arıtma Fizibilitesinin Alkali Göl Suyuyla Artırılması

Öz: Lignoselülozik hammaddelerden biyogaz üretim verimliliğini artırmak için sürdürülebilir doğal bir kaynağın kullanımına dayanan bir alkali ön-arıtım yöntemi incelenmiştir. Dünyanın en büyük üçüncü gölü ve birinci sodalı gölü olan Van Gölü'nün alkali suyu bu çalışma için seçilmiştir. Lignoselülozik hammadde olarak başlıca tarım atığı olan buğday samanı kullanılmıştır. Azami 2 cm boyundaki parçalanmış buğday samanlarının alkali ön-artımı Van Gölünün bazik suyu ve laboratuvarda hazırlanan bazik su (NaOH çözeltisi) ile muamele edilerek sağlanmıştır. Deneysel ön arıtımlar, iklim kontrollü bir odada 37 °C sıcaklıkta gerçekleştirilmiştir. Biyokimyasal metan potansiyelinin tespiti için, kesikli anaerobik çürütme deneyleri, mezofilik koşullarda (37 °C) 60 gün süreyle yürütülmüştür. Her iki ön artımdan geçmiş buğday samanında da, ön arıtımdan geçmemiş samana kıyasla daha fazla metan eldesi gözlenmiştir. Aynı zamanda, sonuçlar Van Gölü suyuyla muamele edilen buğday samanının NaOH çözeltisiyle muamele edilenden daha yüksek metan eldesi olduğunu göstermektedir. Bu çalışmanda, bir de ekonomik fizibilite değerlendirmesi yapılmıştır. Sonuçlar göstermektedir ki, biyogaz üretimi için lignoselülozik bir substrat olarak buğday samanın ön-artımında, NaOH yerine Van Gölü suyu kullanımı ekonomik olarak avantajlıdır.

Anahtar Kelimeler: Alkali ön-arıtım, Biyometan eldesi, Buğday samanı, Van Gölü

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1. Introduction

Energy consumption has increased owing to industrial development. Recently, sustainable energy has gained more attention, including all renewable energy sources, such as wind, biomass, and tidal. Wheat straw (WS) is a common lignocellulosic substrate (730 million tons in 2014) (Zheng et al., 2018) predominantly comprising cellulose (35–45%), hemicellulose (20–30%), and lignin (8–11%) (Asghar et al., 2015). To improve biogas potential, lignin and hemicellulose in lignocellulosic materials should be pretreated to increase enzyme performance in the hydrolysis phase, which is the rate-limiting stage in biogas production (Kang et al., 2012; Sapci et al., 2013; Zheng et al., 2018). Numerous pretreatment approaches, such as mechanical, thermal, and chemical, have been applied to agricultural residues today (Horn et al., 2011; Estevez et al., 2012; Sapci et al., 2013; Mancini et al., 2018; Fjørtoft et al., 2019; Rani et al., 2022; Archana et al., 2024).

To increase enzymatic digestibility, the lignin in the lignocellulosic substrate should be removed from the raw materials. Pre-treating straw with caustic soda causes a large decrease in hemicellulose and lignin content (Fjørtoft et al., 2019; Rani et al., 2022). A recent study comparing four different alkali-based pretreatments indicated that alkali pretreatment was the most effective technique for removing noncellulosic polymers (Li et al., 2016). Fjørtoft et al. (2019) reported that methane production from straw was high for 20–50 days with NaOH pretreatment. Alkaline solutions efficiently remove lignin by breaking ester bonds, thereby increasing the porosity of the biomass. Structural analysis of WS pretreated with NaOH indicated that most of the amorphous cellulose and partially crystalline cellulose were hydrolyzed during enzymatic hydrolysis (Zheng et al., 2018). Based on these results, it can be concluded that alkali pretreatment aids delignification.

Alkaline (soda) lakes have been found worldwide, such as in the USA (Searles Lake) (Kempe & Kazmierczak, 2011), Africa (Lake Basaka, Lake Shala) (Lanzén et al., 2013), Austria (Lake Neusiedl) (Felföldi et al., 2009), India (Lonar Lake) (Surakasi et al., 2010), Hungary (Böddiszék) (Felföldi et al., 2009) and Turkey (Lake Van, Lake Salda) (Kempe & Kazmierczak, 2011). They appear to be related to active tectonic and volcanic zones. Soda lakes can reach pH levels greater than 10.5 and alkalinities greater than 150 mEq per L. Lake Van, which contains a high alkaline content, has a pH of 9.7 and an alkalinity of 151 mEq per L (Kempe & Kazmierczak, 2011).

One of the objectives of this study was to investigate the effect of alkaline pretreatment using water from an alkaline lake (Lake Van) on the digestibility of WS under mesophilic conditions. This was investigated by determining the biochemical methane potential of pretreated straw. The second objective of this study was to evaluate the economic viability for commercial applicability. For this reason, WS pretreated with water from Lake Van and WS pretreated with NaOH were investigated from a financial perspective.

2. Materials and Methods

2.1. Biomass collection

WS was used as a lignocellulosic substrate and was harvested in a farming area near Malatya, Turkey. After arriving at the laboratory, the WS was maintained at room temperature to reduce temperature changes. The WS was immediately placed in a desiccator to protect it from undesired humidity until the start of the trial.

2.2. Natural alkaline lake water collection

Lake Van is located in eastern Anatolia, Turkey, at 38° 38′ N, 42° 49′ E. The lake has a large surface area (3.522 km^2) and is at a high altitude (1648 m above sea level). After the Caspian Sea and Lake Issyk Kul, Lake Van is the third largest lake on Earth (576 km^3). It is also the deepest lake (maximum depth of 451 m) and largest alkaline lake in the world (Reimer et al., 2009).

The values of the anions and cations dissolved in Lake Van water are listed in Table 1 (Yiğit et al., 2017).

The lake water was collected from 38° 37' 55.7708" N, 42° 27' 10.5098" E. The water samples (5 L) were immediately transferred to the laboratory for analysis. The alkalinity of the lake was measured as 6.3 g NaOH-eq per L.

Table 1. Anions and cations dissolved in Lake Van water (Yiğit et al., 2017)

Components		Lake Van water (LVW) (mg·L⁻)
Main Cations	Ca^{2+}	335.3
	Mg^{2+}	117.1
	Na^{2+}	8612.6
	K^{2+}	473.6
	Li^{2+}	0.3
Main Anions	F^{-}	4.8
	Cl^{-}	10.5
	Br^{-}	20.5
	NO_3^{-}	3.7
	SO_4^{2-}	13.6
	PO_4^{3-}	2900.4

2.3. Drying and milling process

To obtain a suitable particle size, the WS was chopped using a tilted grinder (SM 108-132, Süper Mikser Endüstriyel Mutfak Makine ve Ekipmanları Sanayi ve Ticaret Limited, Şirketi, Turkey). The residues were sieved between 16- and 20-mm sieve trays to remove dust particles and make the sample homogeneous using laboratory test equipment (Matest Sieve Shaker, Treviolo, Italy). After sieving, the sample was kept in a desiccator. This was labelled as untreated WS.

Table 2 shows the proximate and physicochemical properties of untreated WS in the literature and in this study.

Table 2. Properties of wheat straw

	Constituent	(Chandra et al., 2012)	(Sapci, 2013)	(Cheng et al., 2019)	(Liu et al., 2019)	(Kumar et al., 2019)	(Zhang et al., 2020)	(Rahmani et al., 2022)	This Study
Proximate Analysis (wt%)[1]	Moisture	5.80	6.0	7.1	5.29 [3]	<10	2.74	-	5.5
	Volatile Matter	88.90	74.1	76.7	95.21	92.90	59.33	86.66	85.86
	Ash	11.10	3.3	7.0	-	-	-	13.3	8.15
	Fixed Carbon	-	16.5	9.2	-	-	28.66	-	-
Ultimate Analysis (TM%)[2]	C	45.80	48.4	45.5	43.92 (C/N)	41.53	41.82	58.01 (C/N)	-
	H	6.0	5.7	5.7	-	8.03	5.21	-	-
	N	0.42	0.6	1.0	-	0.35	0.80	0.19 (NH4-N dry g/kg)	-
	O	-	41.6	47.9	-	45.28	39.44		-
Physico-chemical Analysis	pH	4.49-8.10	7.4	-	-	-	-	7.1	7.5
Compositional Analysis	Cellulose	35.10	46.4	-	38.58	38.44	-	39.2	-
	Hemi-cellulose	25.60	26.4	-	31.05	29.80	-	28.6	-
	Lignin	7.5	8.6	-	9.11	17.46	-	14	-
	Rest	31.80	-	-	21.26	-	-	4.9	-

[1] Weight presence of mass, [2] Total matter, [3] Calculated from data in the reference.

2.4. Pretreatment processes

In this study, a chopped sample of WS (2.33 g) was chemically activated with 0.9% NaOH solution (the solution ratio was decided regarding the alkalinity of the LVW, which is 6.3 g NaOH-eq per L). The experiment was carried out for 120 h (43 rpm) at mesophilic (37 ± 0.5 °C) temperature (Chandra et al., 2012).

The chopped sample of WS (2.33 g) was chemically activated with LVW for 120 h (43 rpm) at mesophilic (37 ± 0.5 °C) temperatures.

2.5. Anaerobic digestion process

The same inoculum source was used for each batch test. Fresh manure from the Adabag farm in Bitlis, Turkey was used as the inoculum. The pH, total solids, and total volatile solids of the inoculum were measured as 7.5 ± 0.1, 3.32% (weight per weight), and 2.85% (weight per weight), respectively.

Twelve identical 1000 mL anaerobic batch vessels were operated in triplicate to observe the methane yield of pretreated WS using the same organic load (volatile solid and M/F was 2% (weight per weight) and 1/5, respectively), retention time (60 days), and incubation conditions (37 ± 0.5 °C). After feeding the inoculum (140.85 g) and water (up to 600 g of total wet weight) into these vessels, which already contained the pretreated WS, all vessels were immediately closed with GL45 caps (DWK Life Sciences GmbH, Mainz, Germany). Both the control group, containing untreated wheat straw and inoculum, and the blank group (containing only inoculum) were placed in 1000 mL vessels as pretreated reactors and filled with distilled water to fix the total volume. The vessels and gas lines were flushed with N_2 gas. The incubation was performed at 37 ± 0.5 °C with orbital agitation at 43 rpm in a dark room. The volumetric method was used to quantify the generated biogas (Filer et al., 2019). Before quantification, H_2S and CO_2 in the biogas were removed using gas traps with a concentrated NaOH solution (Bekmezci et al., 2021). The methane yield in the biogas was determined after passing the biogas through the gas trap equipment, as shown in Figure 1.

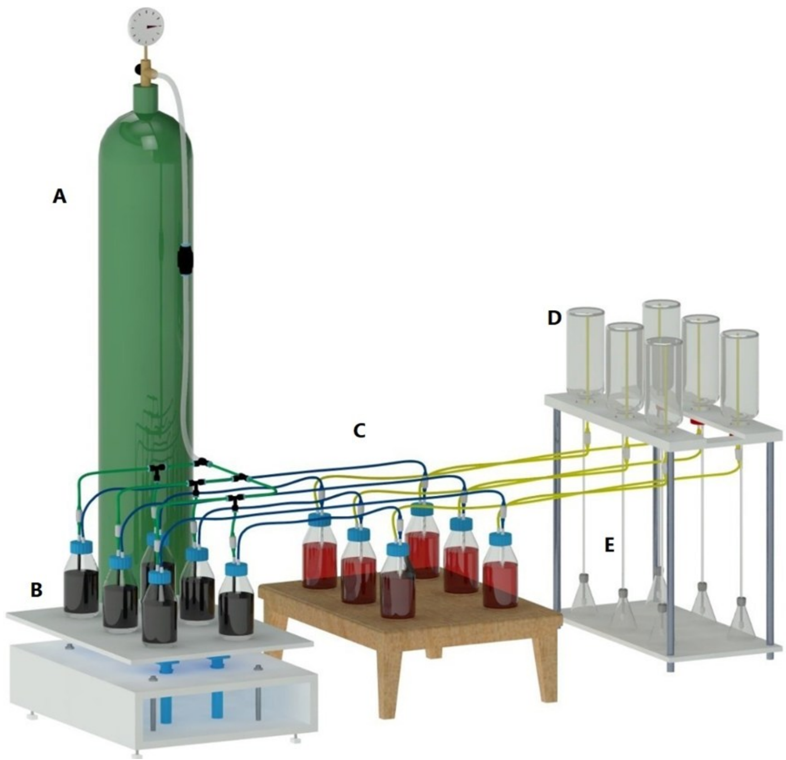

Figure 1. Experimental design of the biochemical methane potential test. A: Nitrogen gas for purging. B: Reactors on orbital shaker. C: Gas traps for removal of H_2S, CO_2, and moisture. D: Liquid displacement bottles. E: Collection vessels for displaced liquids.

2.6. Analytical methods

Total volatile solids and total solids were determined according to Standard Methods (APHA, 1999). The pH measurements were performed using a pH meter (Hach- HQ40d, Hach Company, USA). The alkalinity content was determined according to Standard Methods (APHA, 1999).

2.7. Economic feasibility

To evaluate the economic feasibility of pretreatment with LVW, investment and operational costs were calculated. The calculations were based on a possible small-scale biogas plant within a real farm (Adabag) near Lake Van (Figure 2). The hypothetical plant was fed manure and WS obtained from cow barns and agricultural activities of the farm.

The farm was assumed to have 200 beef cattle. The average beef cow weight was 600 kg. The manure generation rate is 8% of their weight per day, as reported by the Ministry of Agriculture and Forestry (Republic of Turkey) (2019). The total manure production rate was calculated using Equation 1.

$$Total\ manure\ production\ rate = NOA \times MGR \times AAW \tag{1}$$

where *NOA* represents the number of animals, *MGR* represents the manure generation rate, and *AAW* represents average animal weight.

C/N in the manure and WS were 14.35 to 1, calculated according to Palma's study (Font-Palma, 2019), and 127 to 1 according to Rynk et al. (1992), respectively. Other nutrients were assumed sufficient for the biomass to be fed to the reactor. The optimal C/N ratio is assumed to be 25 1 for methanogenic digestion (Yen & Brune, 2007). To satisfy the 25 to 1 C/N ratio, the appropriate WS-to-manure ratio was calculated according to Equation 2:

$$C/N\ ratio\ in\ the\ reactor = (d \times 14.35 + b \times 127) \div (d + b) \tag{2}$$

where *C* represents the mass of carbon in the substrate, *N* represents the mass of nitrogen in the substrate, *d* represents the amount of animal manure, and *b* represents the amount of wheat straw.

To satisfy the requirements for the pretreatment process of the WS, the NaOH amount and volume of the LVW per year were calculated according to Equations 3–7:

$$Wheat\ straw\ required\ per\ unit\ of\ manure = b \div d \tag{3}$$

where *b* represents the amount of wheat straw, and *d* represents the amount of animal manure.

$$Needed\ WS\ amount\ (daily) = WS\ required \times TMP \div 365\ days \tag{4}$$

where *WS required* represents the wheat straw required per unit of manure and *TMP* represents the total manure production in a year.

$$Needed\ WS\ amount\ (yearly) = Needed\ wheat\ amount\ (daily) \times 365\ days \tag{5}$$

$$Needed\ NaOH\ amount\ (yearly) = WS \times (0.0932\ g\ NaOH \div 2.33\ g\ WS) \tag{6}$$

where *WS* represents the needed wheat straw amount.

$$W\ (yearly) = Needed\ WS\ amount \times (14.71\ mL \div 2.33\ g\ WS) \tag{7}$$

where *W* represents the needed LVW.

The multipliers in Equations 6 and 7 are derived from the values used in this trial, as 0.0932 g of NaOH or 14.71 mL of the LVW for 2.33 g of WS.

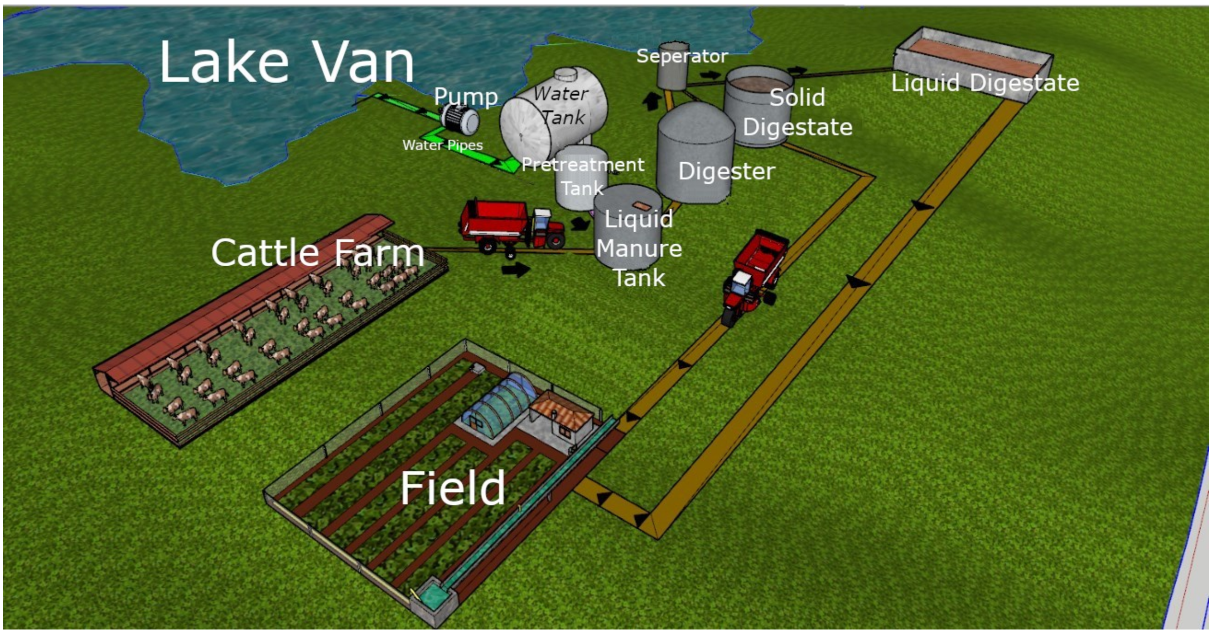

Figure 2. Overview of the hypothetical plant: The LVW is pumped to a water tank. WS is pretreated with LVW at the pretreatment tank. The generated manure collected in a liquid manure thank. The manure and pretreated WS are digested at the digester. The unitization of the generated biogas is not shown herein. The liquid and solid (sludge) portions of the digestate is separated and used at the agricultural fields as fertilizer.

3. Results and Discussion

3.1. Effects of two pretreatment processes on biochemical methane production from wheat straw

The batch pretreatment experimental results showed that the pH of the pretreated WS was acceptable for anaerobic digestion. After pretreatment, the pH values of the samples were measured as approximately 7.5 (Table 3). The operating pH range for the anaerobic digester is recommended as 5.5 to 8.5 (Archana et al., 2024). Therefore, pretreated WS samples with their liquid phases were directly used for the BMP test without pH adjustment.

Table 3. The pH level of wheat straw

Pretreatment Solution	Before Pretreatment	After Pretreatment
LVW	9.77 ± 0.03	7.59 ± 0.06
NaOH	12.08 ± 0.2	7.51 ± 0.04

The methane production from two control groups, the untreated WS with inoculum and only inoculum without substrate (blank), filled with distilled water until the total weight was 600 g, was found to be 87.30 mL CH_4 per g VS and 84.30 mL CH_4 per g VS, respectively. These test results are the lowest among the literature data (Chandra et al., 2012; Sapci, 2013; Mancini et al., 2018; Liu et al., 2019; Rahmani et al., 2023). The low and similar methane generation in both controls suggests that the inoculum was not acclimated to the substrates without pretreatment. Mancini et al. (2018) reported that biomass composition could be influenced by many factors, such as the variety of wheat, growth conditions, and harvesting methods. One of the key factors for anaerobic digestibility is the inoculum. Most studies in the literature used inocula from an anaerobic digester (Sapci, 2013; Mancini et al., 2018; Fjørtoft et al., 2019; Liu et al., 2019; Bertasini et al., 2024; Xu et al., 2024). When fresh manure is used as an inoculum, it should be pre-incubated under anaerobic conditions before use to obtain higher yields, as reported by Rani et al. (2022).

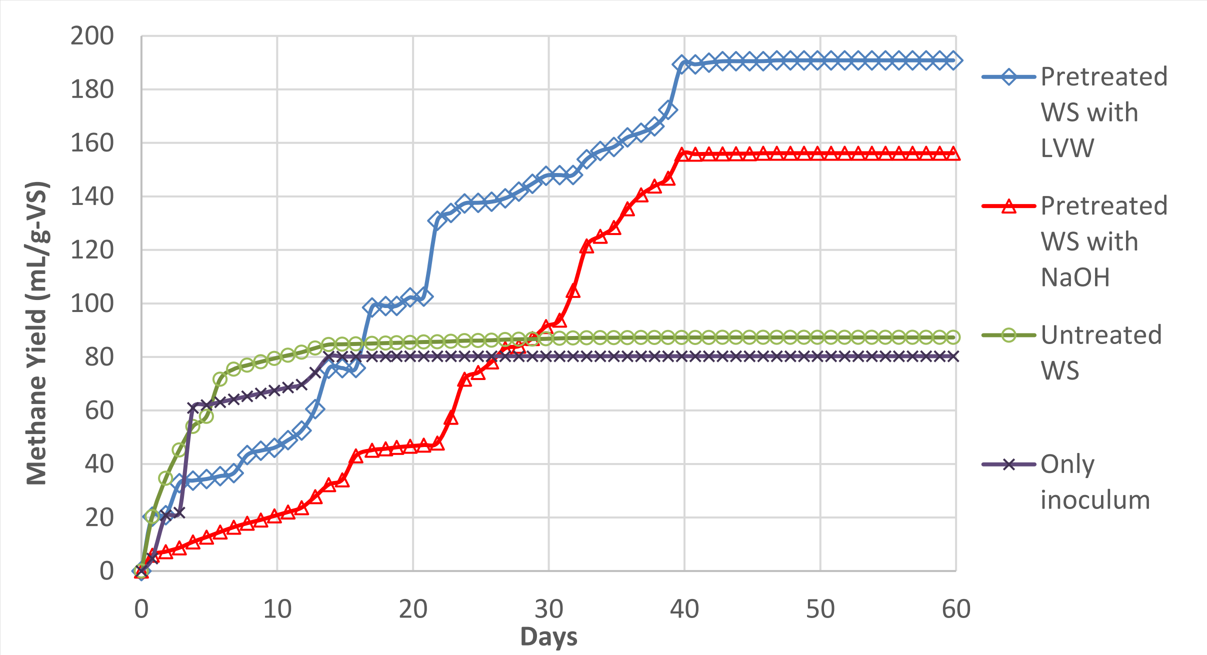

Figure 3. Cumulative biomethane production of inoculum, untreated and pretreated WS.

Figure 3 shows the methane yields during the 60-day trial period. Methane production from the untreated WS and blank reactors stopped completely after 15 days. It was also observed that the methane yields of both pretreated WS types were almost two times higher than those of the controls. Therefore, alkaline pretreatment using NaOH and LVW was associated with an increase in methane yield from lignocellulosic biomass.

Additionally, WS pretreated with LVW gave an approximately 22% higher methane yield than WS pretreated with the NaOH solution. Cations in water can affect lignocellulosic structures because of their cation exchange actions. It is thought that divalent ions, such as calcium and magnesium, have a positive effect on the dissolution of the lignin matrix by interacting with the phenolic groups in its structure. Thus, the microbial accessibility of the cellulose and hemicellulose fractions increases, which positively contributes to biogas production (Singh et al., 2014). Hence, not only the alkaline features of LVW, but also the ions it contains, may support anaerobic bacteria in breaking down the lignocellulosic structure.

Moreover, the dissolved ions contained in LVW are essential nutrients for microbial metabolism. The growth and enzymatic activity of anaerobic microorganisms depend on cations and anions. For example, the presence of low concentrations of sodium is essential for methanogenic bacteria, presumably because it is important for the formation of ATP or oxidation of NADH. When the concentration of potassium is below 400 mg/l, functioning in both the mesophilic and thermophilic temperature ranges is improved. However, if potassium is more than this amount, sodium, magnesium, ammonium, and calcium are effective in moderating the toxicity of potassium. However, the optimal concentrations should be considered to achieve mitigation effects. Calcium was maintained at 837 (Appels et al. 2008). Table 1 indicates that the amount of potassium was higher than 400 mg/l, and calcium did not have a high optimum concentration. Anaerobic bacteria should not be adversely affected, as it was observed that WS pretreated with LVW gave approximately 22% more methane yield than WS pretreated with NaOH solution.

Reimer et al. (2009) reported that the dissolved phosphate concentration was 3.5 µmol per kg in LVW, which was very high compared to the nitrogen species during the warm seasons. The dissolved inorganic carbon was determined to be 101 mmol/kg during the same season. Reimer et al. (2009) informed that the molar Mg to Ca ratio in the lake water increased to recent values of 40 to 1 in the surface water due to the persistent loss of calcium (Reimer et al., 2009). Therefore, the water quality was supportive of both physical and chemical properties (such as trace elements) of the methane yield from the biomass.

3.2. Economic feasibility evaluation

The costs of the delivery pipe and pump from the lake to the facility, as well as the reservoir water tank for the LVW, were evaluated as investment costs. Operating costs were calculated to include both the supply of NaOH and the pump energy. The resulting comparison with the local prices is shown in Table 4.

Regarding pretreatment with NaOH for WS, 15 tons of NaOH are consumed annually. Because 1 ton of NaOH is 840 € (Kimya Borsası, n.d.), 12 600 € is needed for the chemical supply of the process only. If LVW is chosen as the pretreatment solution, 2310 m^3 of lake water is required. To pump the LVW into the reactor, the annual energy cost was calculated as 175 € for the chosen pump model (Table 4). This model was selected based on the head losses and flow rates calculated for the hypothetical biogas plant location (Figure 2). The materials and prices for bringing water were obtained from a local construction company (MEM-KA İnşaat, Turkey) (Table 5). The fixed cost of water transportation was calculated as 1267.1 €.

Table 4. Cost comparison of pretreatment with NaOH and the LVW

	NaOH	Lake Van water	Comment
Investment Costs	Mixer Cost Pretreatment reactor Pump (fresh water) General equipment	Mixer Cost Pretreatment reactor Pump (fresh water) General equipment	Not compared, since both cost the same
	NaOH storage cost was not evaluated since the farm has excess storage space.	Transport of the LVW to the pretreatment unit: Pipeline (330 €) Pump (244 €) Reservoir for the LVW (Tank) (610 €) Others (38 €)	Cost calculations made and presented in Table 5
Operational Costs (per year)	NaOH supply (12600 €)	Electricity cost for the LVW (175 €)	Separate cost calculations were made
	Dosing Costs	Dosing Costs	Not compared, since both cost the same
	Fresh water pumping Electricity for mixing Supply of WS General costs	Fresh water pumping Electricity for mixing Supply of WS General costs	Not compared, since both cost the same

Table 5. Materials and prices for bringing the LVW to the pretreatment unit

Component	**Diameter**	**Each**	**Unit Price [1] (€)**	**Total Price (€)**
KS 12 / 1.5 Kw Double Stage Close Coupled Pump 220 V (Varan pompa, 2019)	Inlet: 1 in Outlet: 1 in	1	244	244
Tank	Inlet: 50 mm	1	610	610
Pipe (100 m)	32 mm	11	30	330
Dust holder	2 in	1	15.3	15.3
Connector	32 mm	15	0.8	11.5
Check valve	2 in	1	10.7	10.7
Reduction connector	50 by 32 mm	1	1.9	1.9
Nipple	2 in	2	1.5	3.1
Connector, inside treat	50 mm	2	2.3	4.6
Clack	2 in	1	5.8	5.8
Stainless pipe	2 in (6 m)	1	29.5	29.5
Steel connector	2 in	1	3.1	3.1
Total				1267.1

[1] Prices include taxes and are provided by MEM-KA İnşaat, Turkey.

When the calculations are examined and an economic assessment is made, the use of NaOH in biogas plants accounts for many of the costs. If WS pretreatment is performed with LVW, the extra investment cost compared to pretreatment with NaOH will be highly amortized in the first year (within the second month of operation). The cost difference between the two methods is so high that a possible negative decision to build a pretreatment unit with NaOH can be flipped to a positive one using alkaline LVW.

4. Conclusion

The alkaline pretreatment process using not only NaOH, but also LVW was associated with an increased methane yield from wheat straw.

WS pretreated with LVW yielded approximately 22% more methane than WS pretreated with NaOH solution. Therefore, the water quality was supportive of both physical and chemical properties (such as trace elements) of the methane yield from the biomass.

In this study, an economic feasibility evaluation was conducted. It was concluded that the use of lake water was significantly more cost-effective than the use of NaOH for pretreatment of wheat straw for biogas production.

Consequently, there are many publications in the literature on alkaline pretreatment methods. In this study, the effect of alkaline natural water sources on biogas production from agricultural waste was examined using an innovative approach compared to chemical solutions. As a result, extremely important information that can be applied by local people was obtained. If the information obtained is briefly summarized, it was determined that (1) more efficient biogas can be obtained when the lignocellulosic substrate is pretreated without the need for chemical additives, (2) it is economically sustainable because of the use of local resources, and (3) its application is practical and environmentally friendly.

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Author Contributions (CRediT)

Ozan K. Bekmezci: Lead author, conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing – original draft, writing – review & editing.

Zehra Sapci: Conceptualization, methodology, data curation, validation, writing – original draft, writing – review & editing.

Ezgi Piro: Investigation, visualization.

Hülya Durmaz: Funding acquisition, resources, supervision, writing – review & editing.

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