

Wheat Straw Optimization via its Efficient Pretreatment for Improved Biogas Production

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Abstract

The complex indigenous configuration of non-bio-labile wheat straw necessitates its pretreatment to optimize the breakdown of its structural components for its ultimate conversion into biogas by means of anaerobic digestion. In this research work, wheat straw was pretreated with potassium hydroxide (KOH) to facilitate its improved biodegradability. The pretreatment of wheat straw was also obvious in terms of its crystallinity resulting in the improved amorphous regions compared to the control wheat straw. The results showed that pretreated wheat straw digestion transpired into comparatively higher removal of TS (86%), VS (89%) and total lignin, cellulose and hemicellulose (22%) than that obtained with control wheat straw. Maximum biogas production accrued was 1550 mLN per day with optimized dosing of KOH compared to 967 mLN per day obtained with control wheat straw, implying that the cumulative biogas production was improved by 45% using pretreated wheat straw than that using control wheat straw. These results suggested that pretreated wheat straw digestion led to a significant improvement in the biogas yield.

Keywords: Wheat Straw; Pretreatment; Hydrolysis; Lignocellulose; Anaerobic Digestion; Biogas.

1. Introduction

Harnessing biogas energy could be one of the most important options to undertake to realize one of the goals of clean energy as outlined under the banner of united nation's sustainable development goals [1]. Wheat straw is a by-product of wheat cropping process that is left behind after the wheat is mechanically separated from inside the straw. Wheat straw is one of the potential low-cost lignocellulosic biomass used as a key substrate in bio-refineries and for biogas production [2]. However, there is a technical hitch in the effective utilization of wheat straw as a feedstock for the purpose of making it a potential source of clean energy. The internal structural configuration of wheat straw is such that the innate constituents of wheat straw such as Lignin, Hemicellulose and Cellulose (LHC) are intertwined in a way that makes their overlapping presence in wheat straw as intricate.

The level of recalcitrance of the LHC needs to be compromised so as to overcome the limitations that are expected per se during the course of enzymatic and microbial degradation of wheat straw [3]. To address this complexity, wheat straw pretreatment is required beforehand to make the LHC compliant to an extent so that it results in its optimum biodegradability during the course of its digestion. Different techniques have already been adopted for the pretreatment of lignocellulosic biomass to improve upon the production of biogas [4]. Compared to physical and

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biological techniques, chemical method is reportedly low-cost yielding faster reaction rate as well as higher process efficiency during the course of biodegradation of otherwise recalcitrant wheat straw organics [5, 6]. Alkaline pretreatment of wheat straw is favoured due to its dual advantage of not only increasing the process pH, but also resulting in secure enough disintegration of the LHC to optimize their subsequent conversion into the biogas [7]. In addition, alkaline pretreatment leads to an increase in the accessible surface area and porosity of the straw, as well as decreases the cellulose crystallinity and induces disruption in the lignin structure [8].

In this research work, wheat straw pretreatment was carried out using lower proportional ratios of wheat straw, KOH and water in contrast to comparatively higher ratios used by other researchers [9]. For biogas production from optimised characteristics of wheat straw, anaerobic digestion of treated wheat straw was carried out in conformity to the standard parameters such as organic loading rate, temperature and pH [10, 11]. Temperature at which the anaerobic digestion occurs can significantly affect the conversion of substrate into final product and stability of the process [12]. Current research uses mesophilic temperature range (35-37 °C) for the effective conversion of the substrate, thus providing the inherent biota an enabling environmental condition to aid in the biodegradation process [13]. The structure of the current research work entails collection of wheat straw, preparation of wheat straw samples with proper size and ratios, pretreatment of wheat straw using different ratios of KOH followed by anaerobic digestion of both treated and control samples of wheat straw and their subsequent investigation. The structure of this article follows the basic criteria of IMRAD that is Introduction covering the background of the previous research and contribution of the current research followed by materials used and their methodology and finally mention of the experimental results obtained and their discussion in agreement with the work done by others before summarizing the value of this research in the form of conclusions.

2. Research Methodology

2.1. Experimental Set-up

Figure 1 shows the flow chart comprising of the components involved in the research plan. Wheat straw (Figure 2a), generated after wheat harvesting, was air-dried *in-situ* for two weeks. The dried straw was then transported to the lab and chopped by a paper chopper (PC 500, Stadia Co, Tianjin, China) to reduce the particle size up to around 3cm. After that it was grounded to a particle size of 5-10 mm in a hammer mill. After the physical treatment of straw (Figure 2b), the straw was stored in plastic bags at room temperature before being pretreated in glass serum bottles of 1000 mL with 1%, 3%, 6% and 9% of potassium hydroxide (KOH) denoted as K1, K3, K6 and K9 respectively with a constant organic loading rate of 65 g.L⁻¹. The prepared bottles were immediately wrapped with plastic film, tightly tied with plastic ring and were kept at ambient temperature for four days along with the sample bottle as control for further analysis. After four days of pretreatment, the samples were then analysed for pH value.



Figure 1. Flowchart of research plan



Figure 2. Wheat straw images before (a) and after (b) physical treatment

2.2. Anaerobic Digestion

The anaerobic digestion of treated and untreated or control wheat straw samples was carried out in batch mode at $35 \pm 1^\circ\text{C}$ with a working volume of 0.8 L. All the reaction bottles were also inoculated with 15 g/L of the activated sludge as reported in the literature [14]. The sludge characteristics were determined to be comprised of (in g/L) TS (49.2%), VS (29.5%) and the mixed liquor suspended solids (34.8%). The prepared sample bottles were then placed in incubator tank for anaerobic digestion of the bottle contents at mesophilic temperature range (Figure 3).

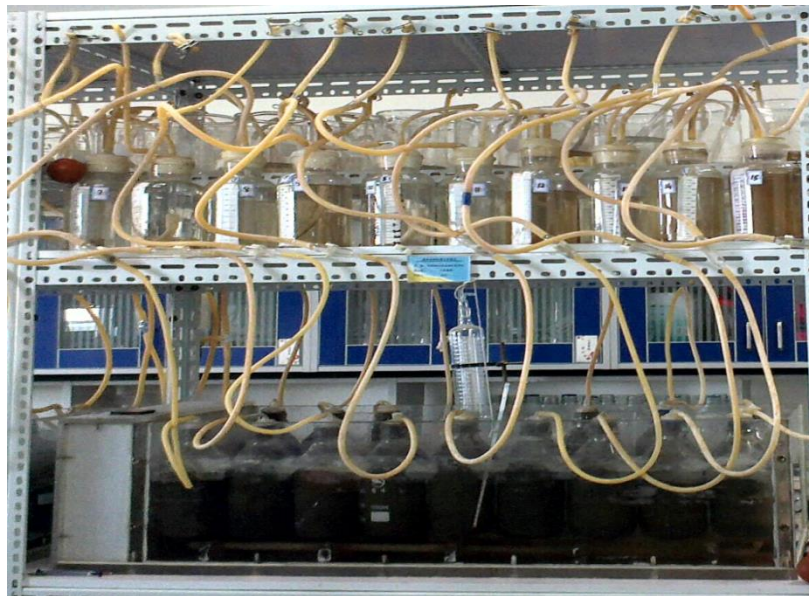


Figure 3. Anaerobic digestion of pretreated and control wheat straw samples in incubator tank

2.3. Analytical Procedure

The amount of biogas produced is a key factor in anaerobic digestion and the more the gas is produced, the more waste is degraded. The volume of biogas produced was analyzed daily via water displacement method. The biomethane yield was calculated using ideal gas equation at STP. The methane content of biogas and that of CO_2 produced during the course of anaerobic digestion were analysed daily via Gas Chromatograph (SP-2100, BeifenRuiLi Co, Beijing China) by withdrawing 0.25 mL of the gas samples from each glass bottle. The methane production was calculated by multiplying methane content with the amount of biogas production.

2.4. Chemical analysis

TS, VS and MLSS of wheat straw, sludge and their mixture were determined as per the standard methods [15]. The total carbon (TC) and Total Nitrogen (TN) were analysed by TC analyzer (Vario EL/micro cube elemental analyzer, Germany). The pH value was measured by pH meter (CHN868, Thermo Orion, USA). The lignin, cellulose and hemicellulose content were determined using a fiber analyzer (ANKOM, A20001, USA). The crystallinity was determined by using a D8 Advanced /Max 2500 X-ray diffractometer equipped with a sealed tube Cu Ka source. Scanning were obtained from $2\theta = 5$ to 60 degree with a wavelength of 1.5406 nm. The X-ray unit was operated at 40 KV and 40 mA. Crystallinity of cellulose was calculated corresponding to a peak deconvolution method to analyze the XRD data including the data needed to calculate the crystallinity index, as $\text{CrI}(\%) = [(I_{002} - I_{am}) / I_{002}] \times 100$, Where CrI is Crystallinity index, I_{002} is the maximum diffraction from the (002) plane at $2\theta = 22.3^\circ$ and I_{am} is intensity of background scatter measured at $2\theta = 18.3^\circ$ [16].

2.5. Data analyses

All the measurements were analysed on a mean result of three samples with standard deviation. The significant difference was determined by means of ANOVA using Metlab 7.10.0.

3. Results and Discussion

3.1. Effect of KOH Treatment on Wheat Straw Characteristics

Wheat straw pretreatment was carried out to cause internal disorientation in its structural properties so as to make it readily responsive during the course of its digestion. The pretreatment results suggested that alkali treatment of wheat straw induced breakdown of lignocelluloses comprising of three main polymeric constituents Lignin, cellulose and hemicellulose (LHC) in terms of their reduced concentration levels as reflected via the altered physical characteristics

of treated wheat straw [17]. The induced changes in wheat straw characteristics are directly proportional to its efficient biodegradability under the influence of its anaerobic digestion leading to enhanced biogas production [18]. The changes caused in the chemical composition of wheat straw under the impact of the alkali exposure are shown in Table 1. The data in the Table transpires that, on the whole, KOH-treatment of wheat straw with different proportions yielded in reduced values for TS, VS and LHC compared to the values observed in the control test. These results are in agreement with those reported by Xiaoying Liu et al. [19] and Sharma [20]. The breakdown pathway of wheat straw lignocelluloses under the influence of KOH treatment entails cracking up of the lignin seal as a first step, followed by disintegration of hemicellulose cover over cellulose before the cellulose crystalline structure is disrupted. The KOH-treated wheat straw resulted in hydrolysis of its main constituent, i.e. cellulose, the original concentration of which was compromised by a decrease of 2.2%, 5.3%, 9.9% and 11.5% with KOH dosing of 1%, 3%, 6% and 9% respectively. The combined LHC contents also showed a decrease by 10%, 14%, 22%, and 26% with K1, K3, K6 and K9 respectively, implying that higher dosage of KOH induced higher decrease in the overall concentration of LHC compared to the original concentration of untreated wheat straw.

Table 1. Effect of KOH-pretreatment on Wheat straw constituents

Wheat straw	TS	VS	Cellulose	Hemicellulose	Lignin	LHC
K0 (untreated)	92.7 ± 2.1	84.2 ± 1.8	41.6 ± 1.3	28.3 ± 1.2	12.5 ± 0.7	82.4 ± 7.3
K1 (treated)	12.4 ± 0.7	10.5 ± 0.8	40.7 ± 1.6	27.9 ± 1.7	10.7 ± 0.5	79.3 ± 5.8
K3	12.6 ± 0.6	9.8 ± 0.4	39.4 ± 1.3	22.2 ± 1.1	10.6 ± 0.6	72.2 ± 4.2
K6	12.2 ± 0.7	8.8 ± 1.0	37.5 ± 1.3	17.6 ± 0.9	9.1 ± 0.5	64.3 ± 3.9
K9	13.1 ± 0.8	11.2 ± 0.2	36.8 ± 1.6	15.7 ± 0.5	8.6 ± 0.4	61.1 ± 4.5

*Values are means ± SD (n = 3)

3.2. Effect of KOH-Treatment on Wheat Straw pH

Figure 4 depicts the pH profile of wheat straw during the course of its treatment with different proportion of KOH. The graph shows that the pretreatment of wheat straw has increased the pH value depending upon the alkali concentration applied. With the addition of hydroxyl ions into wheat straw via the mixing of KOH, the pH of treated wheat straw increased immediately by 18%, 35%, 57% and 100% with K1, K3, K6 and K9 respectively. However, the pretreatment duration was observed to be complete in four days when the desired level of decrease occurred in the LHC concentration. The induced degradation of wheat straw LHC was also directly proportional to the pH, as from the start of wheat straw pretreatment process until the end of day four, a gradual decrease was observed in the pH value in direct proportion to the KOH dosage applied. This meant that higher the KOH dosage was applied, the higher the decrease was observed during four days of wheat straw pretreatment [21, 22].

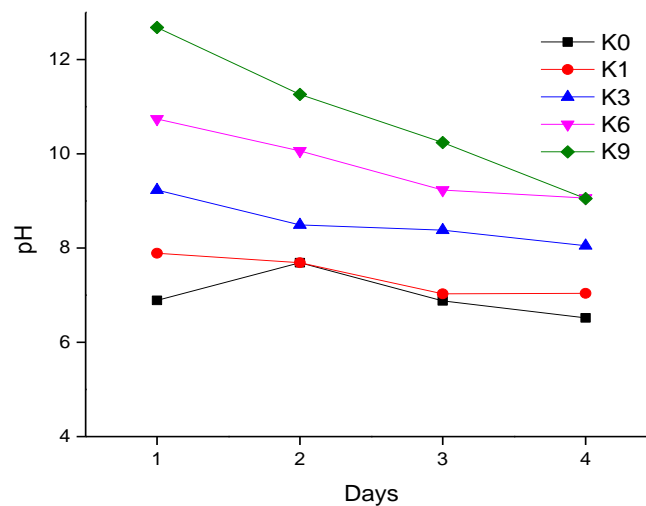


Figure 4. pH profile during 4-day wheat straw pretreatment with KOH

3.3. X-ray Diffraction

X-ray diffraction (XRD) of the samples was carried out to assess the innermost microstructure of cellulosic wheat straw in terms of their crystal features. Figure 5 shows the XRD pattern for both treated and control wheat straw samples, whereas crystallinity index values are given in Table 2. It is clearly shown in Figure 5 that almost similar XRD shapes were observed for both control and treated wheat straw samples producing the peaks on two theta degree at 22.3° and 18.3° respectively. The higher peak represents the existence of crystalline cellulose structure that is

normally protected under the stable coverings of both hemicellulose and lignin. Secondary peak being the minor one, represents the presence of highly controlled amorphous structure of cellulose [23]. KOH permeation down in the cellulose compartment generates swelling that is indicative of the fact that the alkali compound has actually penetrated the cellulose surface. Crystallinity is the ratio of crystalline cellulose amount to sum of the sample quantity and that of amorphous and crystalline cellulose [24]. This ratio was observed to be enhanced for crystalline cellulose from 48% to 52% compared to control wheat straw cellulose, with the highest ratio being obtained with higher alkali concentration used. The impact of alkali exposure up on wheat straw can be associated with the hydrolyzation activity being spurred on the amorphous region of wheat straw resulting in the pore surface ratio being increased, which in turn is beneficial for optimum biogas production from treated wheat straw during the course of its anaerobic digestion.

Table 2. Crystallinity (CrI) of untreated and treated wheat straw

KOH Loading	CrI (%)
Untreated straw	48
1%	48.5
3%	49
6%	50
9%	52

Values are means \pm SD (n = 3)

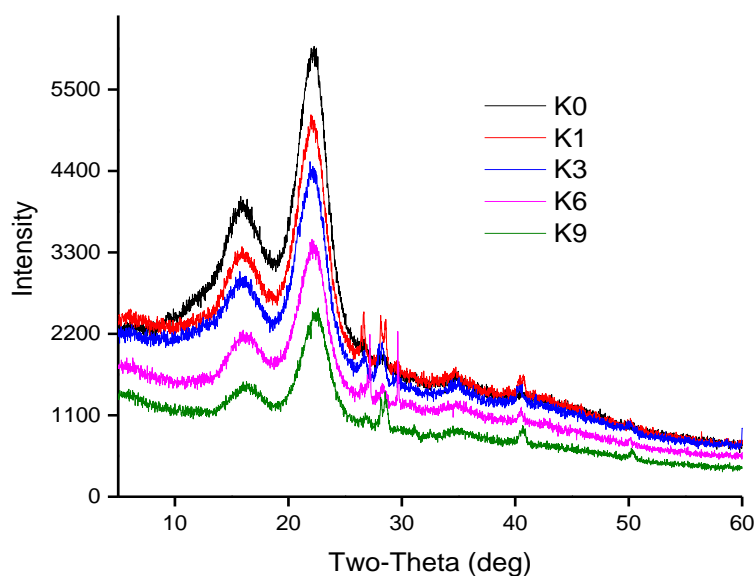


Figure 5. XRD pattern of untreated and treated-KOH wheat straw

3.4. Biogas Production

Figure 6 presents the daily account of biogas production. Anaerobic digestion of all the samples was carried out at mesophilic temperature of $35 \pm 1^\circ\text{C}$. The Figure shows a typical trend for all the treatment protocols applied on wheat straw as reflected in terms of biogas production, which continued until day six of the digestion, when the production rate was observed to be on the lower side. The decline in the production rate could be due to the decrease in pH value caused by the possible accumulation of unionized $\text{NH}_3\text{-N}$, excreted in the medium via wheat straw degradation during the course of digestion, resulting in the acidification of the medium [25]. Furthermore, the pH decline was also caused by the formation of HCO_3^- when the OH^- ions come into contact with the evolving CO_2 in the presence of volatile fatty acids that are germinated during the process and stabilized due to the buffering capacity established in the system [26-28]. The higher biogas production, as observed from day seven until day fifteen of the digestion, was restored after the pH was regulated via the addition of calcium hydroxide in the medium. Overall, the biogas production rate was observed to be 1103, 1207, 1515, 1330 and 967 mL_N per day for K1, K3, K6, K9 and K0 respectively. The decrease in the biogas production rate with K9 might be afflicted with the consumption of certain proportion of the organics present in wheat straw samples by the fermentative microbes, the unforced generation of which was caused by the excessive addition of the alkali compound during the course of wheat straw pretreatment.

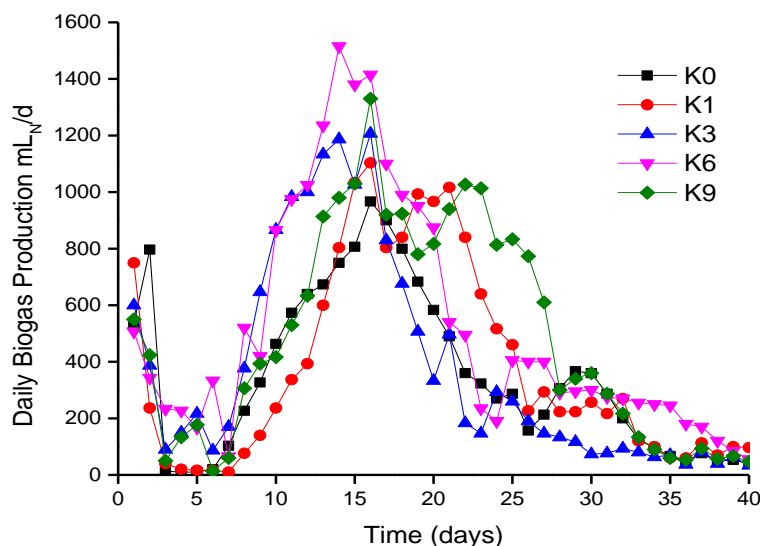


Figure 6. Biogas production rate from control and treated wheat straw

Figure 7 shows the total biogas production (TBP) accrued from wheat straw with and without pretreatment. Compared to TBP from control wheat straw run of 14001 mL_N, TBP was higher from wheat straw treated with K1, K3, K6 and K9 with TBP rate of 15003, 15002, 20006 and 19005 mL_N, respectively. These results are in harmony with those reported elsewhere in the literature [7, 29, 30]. The TBP values indicate that wheat straw pretreatment protocol of K6 was the most optimum option, which yielded into TBP of 45% higher ($P < 0.05$) than all the other runs.

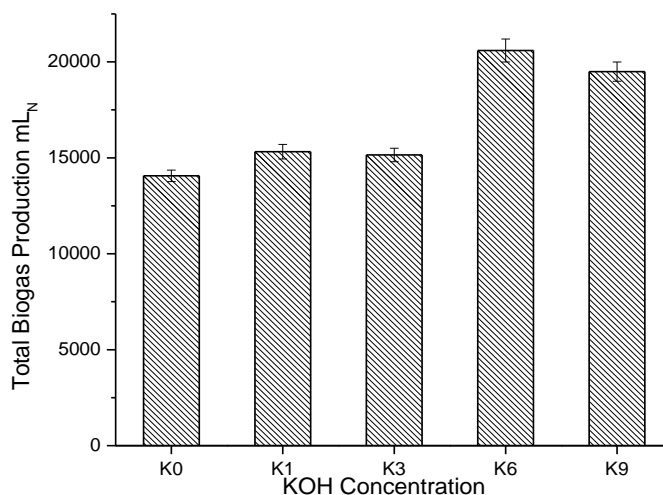


Figure 7. Total biogas production obtained from wheat straw with and without treatment (mean value, n=3)

4. Conclusion

For effective and optimized utilization of wheat straw, by product of wheat harvesting process, it needed to be pretreated with the given alkali compound (KOH) so as to bring changes in its complex internal structure to make it readily responsive for further processing. This research work suggested that wheat straw pretreatment with 6% dosage of KOH yielded, in overall terms, into viable results compared to other pretreatment protocols including proper disintegration of the tight-knit wheat straw constituents, enhanced cellulose crystallinity and optimum total biogas production. The optimized pretreatment option of K6 likely induced a probable makeshift in the characteristics and chemical composition of wheat straw so as to influence marked degradation of wheat straw constituents like TS, VS and LHC. To sum up, these research findings may prove to be a helpful baseline study in realizing the potential of this type of research at the large scale.

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6. Conflicts of Interest

The authors declare no conflict of interest.

7. References

- [1] Cakir, Ali Kemal. "Investigation and Evaluation of Potential Options to Determine the Methane Gas." *Civil Engineering Journal* 4, no. 8 (August 27, 2018): 1824. doi:10.28991/cej-03091117.
- [2] Griffin, Matt E., Katherine D. McMahon, Roderick I. Mackie, and Lutgarde Raskin. "Methanogenic population dynamics during start - up of anaerobic digesters treating municipal solid waste and biosolids." *Biotechnology and bioengineering* 57, no. 3 (1998): 342-355. doi:10.1002/(sici)1097-0290(19980205)57:3<3C342::aid-bit11%3E3.0.co;2-i.
- [3] Yu, Qiong, Ronghou Liu, Kun Li, and Ruijie Ma. "A Review of Crop Straw Pretreatment Methods for Biogas Production by Anaerobic Digestion in China." *Renewable and Sustainable Energy Reviews* 107 (June 2019): 51–58. doi:10.1016/j.rser.2019.02.020.
- [4] Jaffar, Muhammad, Hairong Yuan, Dexun Zou, Yanping Liu, Rashid Mustafa Korai, Yunzhi Pang, and Xiujin Li. "Wheat straw pretreatment with potassium hydroxide for enhanced biogasification and fertilizer value." *Fresenius Environmental Bulletin* 24, no. 10b (2015): 3491-3498.
- [5] Zheng, Yi, Jia Zhao, Fuqing Xu, and Yebo Li. "Pretreatment of Lignocellulosic Biomass for Enhanced Biogas Production." *Progress in Energy and Combustion Science* 42 (June 2014): 35–53. doi:10.1016/j.pecs.2014.01.001.
- [6] Mancini, Gabriele, Stefano Papirio, Piet N.L. Lens, and Giovanni Esposito. "Increased Biogas Production from Wheat Straw by Chemical Pretreatments." *Renewable Energy* 119 (April 2018): 608–614. doi:10.1016/j.renene.2017.12.045.
- [7] Zhong, Weizhang, Zhongzhi Zhang, Yijing Luo, Shanshan Sun, Wei Qiao, and Meng Xiao. "Effect of Biological Pretreatments in Enhancing Corn Straw Biogas Production." *Bioresource Technology* 102, no. 24 (December 2011): 11177–11182. doi:10.1016/j.biortech.2011.09.077.
- [8] Liew, Lo Nicee, Jian Shi, and Yebo Li. "Methane Production from Solid-State Anaerobic Digestion of Lignocellulosic Biomass." *Biomass and Bioenergy* 46 (November 2012): 125–132. doi:10.1016/j.biombioe.2012.09.014.
- [9] Kim, Moonkyung, Byung-Chul Kim, Kyoungphile Nam, and Yongju Choi. "Effect of Pretreatment Solutions and Conditions on Decomposition and Anaerobic Digestion of Lignocellulosic Biomass in Rice Straw." *Biochemical Engineering Journal* 140 (December 2018): 108–114. doi:10.1016/j.bej.2018.09.012.
- [10] Donoso-Bravo, A., C. Retamal, M. Carballa, G. Ruiz-Filippi, and R. Chamy. "Influence of Temperature on the Hydrolysis, Acidogenesis and Methanogenesis in Mesophilic Anaerobic Digestion: Parameter Identification and Modeling Application." *Water Science and Technology* 60, no. 1 (July 1, 2009): 9–17. doi:10.2166/wst.2009.316.
- [11] Banerjee, A., P. Elefsiniotis, and D. Tuhtar. "Effect of HRT and Temperature on the Acidogenesis of Municipal Primary Sludge and Industrial Wastewater." *Water Science and Technology* 38, no. 8–9 (October 1, 1998): 417–423. doi:10.2166/wst.1998.0833.
- [12] Mancini, Gabriele, Stefano Papirio, Gerardo Riccardelli, Piet N.L. Lens, and Giovanni Esposito. "Trace Elements Dosing and Alkaline Pretreatment in the Anaerobic Digestion of Rice Straw." *Bioresource Technology* 247 (January 2018): 897–903. doi:10.1016/j.biortech.2017.10.001.
- [13] Lettinga, G. "Challenge of Psychrophilic Anaerobic Wastewater Treatment." *Trends in Biotechnology* 19, no. 9 (September 1, 2001): 363–370. doi:10.1016/s0167-7799(01)01701-2.
- [14] Zhang, R. "Biogasification of Rice Straw with an Anaerobic-Phased Solids Digester System." *Bioresource Technology* 68, no. 3 (June 1999): 235–245. doi:10.1016/s0960-8524(98)00154-0.
- [15] APHA AWWA, W. E. F. "Standard methods for the examination of water and wastewater 20th edition." American Public Health Association, American Water Work Association, Water Environment Federation, Washington, DC (1998).
- [16] He, Yanfeng, Yunzhi Pang, Yanping Liu, Xiujin Li, and Kuisheng Wang. "Physicochemical Characterization of Rice Straw Pretreated with Sodium Hydroxide in the Solid State for Enhancing Biogas Production." *Energy & Fuels* 22, no. 4 (July 2008): 2775–2781. doi:10.1021/ef8000967.
- [17] Kaparaju, Prasad, María Serrano, Anne Belinda Thomsen, Prawit Kongjan, and Irimi Angelidaki. "Bioethanol, Biohydrogen and Biogas Production from Wheat Straw in a Biorefinery Concept." *Bioresource Technology* 100, no. 9 (May 2009): 2562–2568. doi:10.1016/j.biortech.2008.11.011.
- [18] Wałowski, Grzegorz. "Experimental Assessment of Porous Material Anisotropy and Its Effect on Gas Permeability." *Civil Engineering Journal* 4, no. 4 (May 3, 2018): 906. doi:10.28991/cej-0309143.

- [19] Liu, Xiaoying, Steven M. Zicari, Guangqing Liu, Yeqing Li, and Ruihong Zhang. "Pretreatment of Wheat Straw with Potassium Hydroxide for Increasing Enzymatic and Microbial Degradability." *Bioresource Technology* 185 (June 2015): 150–157. doi:10.1016/j.biortech.2015.02.047.
- [20] Sharma, Rajat, Vijaykumar Palled, Ratna R. Sharma-Shivappa, and Jason Osborne. "Potential of Potassium Hydroxide Pretreatment of Switchgrass for Fermentable Sugar Production." *Applied Biochemistry and Biotechnology* 169, no. 3 (December 29, 2012): 761–772. doi:10.1007/s12010-012-0009-x.
- [21] Gray, Kevin A, Lishan Zhao, and Mark Emptage. "Bioethanol." *Current Opinion in Chemical Biology* 10, no. 2 (April 2006): 141–146. doi:10.1016/j.cbpa.2006.02.035.
- [22] Sambusiti, C., E. Ficara, F. Malpei, J.P. Steyer, and H. Carrère. "Effect of Sodium Hydroxide Pretreatment on Physical, Chemical Characteristics and Methane Production of Five Varieties of Sorghum." *Energy* 55 (June 2013): 449–456. doi:10.1016/j.energy.2013.04.025.
- [23] Zheng, Mingxia, Xiujin Li, Laiqing Li, Xiaojin Yang, and Yanfeng He. "Enhancing Anaerobic Biogasification of Corn Stover through Wet State NaOH Pretreatment." *Bioresource Technology* 100, no. 21 (November 2009): 5140–5145. doi:10.1016/j.biortech.2009.05.045.
- [24] Goto, Masakazu, and Yasuhiko Yokoe. "Ammoniation of Barley Straw. Effect on Cellulose Crystallinity and Water-Holding Capacity." *Animal Feed Science and Technology* 58, no. 3–4 (May 1996): 239–247. doi:10.1016/0377-8401(95)00903-5.
- [25] Liu, C.F., F. Xu, J.X. Sun, J.L. Ren, S. Curling, R.C. Sun, P. Fowler, and M.S. Baird. "Physicochemical Characterization of Cellulose from Perennial Ryegrass Leaves (*Lolium Perenne*)." *Carbohydrate Research* 341, no. 16 (November 2006): 2677–2687. doi:10.1016/j.carres.2006.07.008.
- [26] Kim, Sehoon, and Mark T. Holtzapfel. "Effect of Structural Features on Enzyme Digestibility of Corn Stover." *Bioresource Technology* 97, no. 4 (March 2006): 583–591. doi:10.1016/j.biortech.2005.03.040.
- [27] Ward, A.J., and M.S. Kumar. "Bio-Conversion Rate and Optimum Harvest Intervals for *Moina Australiensis* Using Digested Piggery Effluent and *Chlorella Vulgaris* as a Food Source." *Bioresource Technology* 101, no. 7 (April 2010): 2210–2216. doi:10.1016/j.biortech.2009.11.053.
- [28] López González, Lisbet Mailin, Han Vervaeren, Ileana Pereda Reyes, Ann Dumoulin, Osvaldo Romero Romero, and Jo Dewulf. "Thermo-Chemical Pre-Treatment to Solubilize and Improve Anaerobic Biodegradability of Press Mud." *Bioresource Technology* 131 (March 2013): 250–257. doi:10.1016/j.biortech.2012.12.167.
- [29] Taherdanak, Mohsen, and Hamid Zilouei. "Improving Biogas Production from Wheat Plant Using Alkaline Pretreatment." *Fuel* 115 (January 2014): 714–719. doi:10.1016/j.fuel.2013.07.094.
- [30] Yang, Dongyan, Yunzhi PANG, Hairong Yuan, Shulin Chen, Jingwei MA, Liang Yu, and Xiujin Li. "Enhancing Biogas Production from Anaerobically Digested Wheat Straw Through Ammonia Pretreatment." *Chinese Journal of Chemical Engineering* 22, no. 5 (May 2014): 576–582. doi:10.1016/s1004-9541(14)60075-6.