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An Economical Method for Simultaneously Improving Pretreatment and Anaerobic Fermentation Effects on Corn Straw Using Ultra-Low Concentration FeCl_2

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Received: 10 March 2020; Accepted: 1 April 2020; Published: 7 April 2020

Abstract: Low pollution, slight corrosion, and low cost are the main challenges in the conversion of biomass to biogas. In this work, based on the whole process optimization of biomass conversion, an effective method using an ultra-low concentration of FeCl_2 was proposed to simultaneously promote the biochemical reaction and improve the pretreatment effect. The concentration of FeCl_2 in the pretreatment was determined according to the requirements of the minimum amount which led to the optimal performance of the anaerobic system and had economical importance. The effects of FeCl_2 in the pretreatment and anaerobic fermentation were evaluated by comparing with that of distilled water in the production of hydrolysis products and organic acids. The optimal condition was obtained at 180 °C and 10 min with 1×10^{-5} mol/L FeCl_2 pretreatment. At that condition, hemicellulose achieved a high conversion of 97.6%, and the released xylose reached nearly 95%. At least 77.5% of the organic matter in the solution can be utilized for subsequent fermentation. FeCl_2 pretreatment also accelerated the anaerobic acidification fermentation process and promoted organic acid yields. The work provided a meaningful and economical choice for the conversion technology of biomass to biogas.

Keywords: FeCl_2 ; pretreatment; catalyst; ultra-low concentration; anaerobic fermentation; biogas; nutritional components; whole process optimization; corn straw

1. Introduction

Lignocellulose materials draw a lot of attention because they are abundant, low-priced, and renewable. Furthermore, they play a significant role in the utilization of alternative energy sources in the future which can be used to produce biofuels, such as biogas or bioethanol. Reasonable utilization of lignocellulose biomass can reduce CO_2 emissions [1,2]. To improve lignocellulose utilization efficiency, destroying the complex rigid structure of lignocellulose becomes the main challenge in the conversion from biomass to biofuel [3]. The conversion process is generally considered to have three steps: pretreatment, enzymatic hydrolysis, and fermentation to produce biofuel [4]. Pretreatment is considered the most important and efficient step for the degradation of lignocellulose [5]. It can break the lignin envelope and liberate its crystalline structure to be used more easily in the subsequent hydrolysis process [6].

At present, many kinds of pretreatment methods have been tested, including dilute acid [7,8], alkaline [9,10], liquid hot water [11,12], steam explosion [13,14], ionic liquid [15,16], and so on. Among these methods, dilute acid is considered low-cost for lignocellulose conversion, which can significantly enhance xylose yields from hemicellulose and expose cellulose to enzymes for high yields from subsequent hydrolysis [17–20]. However, the strong equipment-corrosion and harmful by-products can still not be completely resolved during the dilute acid pretreatment process. Besides that, the addition of reagents needs to be considered at different reaction stages for green technology [6]. Avoiding the repeated addition of reagents can simplify the reaction process, thereby saving human and financial resources. Therefore, to find suitable catalysts, which play a part in both pretreatment and fermentation, is vital for the overall process of lignocellulose conversion to biofuel.

Some researchers have found that inorganic salts, especially iron salts, can improve the hydrolysis effect of lignocellulose with the different pretreatment processes. FeCl_3 is an effective catalyst for the hydrolysis of peat with HCl [21]. In corn stalk, 0.1 mol/L FeCl_3 can release about 90% of xylose between 140 °C and 200 °C for 20 min [22]. $\text{Fe}(\text{NO}_3)_3$ as a catalyst, is found to be more efficient than other inorganic salts, such as FeSO_4 , FeCl_3 , $\text{Fe}_2(\text{SO}_4)_3$, for corn straw silages pretreatment [23]. FeSO_4 can enhance enzymatic hydrolysis and encourage the inversion of chemical composition and structure in corn stalk. This is because Fe^{2+} can adsorb at the positions of C_2 , C_3 , C_6 , O_5 of the cellulose pyran ring and make the pyran ring bond rupture, which result in the crystalline structure of cellulose breaking and the surface area improving [4]. FeCl_2 is found to improve the degradation of both xylose and furfural, and about 60% xylan is turned into non-saccharide products in sweet sorghum bagasse with liquid hot water [24].

In addition to serving as a catalyst in lignocellulose pretreatment, the iron element is found to play an important role as the nutrient additive in anaerobic fermentation [25,26]. Iron as a trace metal not only participates in the synthesis of anaerobes, but also often appears in the enzyme system of anaerobes as coenzymes, cofactors, and electron acceptors, and has a stimulatory effect on the methane production stage of anaerobes [27,28]. It can provide nutrition to the bacteria and promote microbiological activity. Organic matter is degraded to biogas and its methane content is increased by trace metals [29–31]. Adding iron also speeds up the degradation of volatile fatty acids accumulated [32]. Therefore, the effects of iron salts are reflected in a series of the biomass conversion process, as shown in Figure 1.

Figure 1. Role of iron salt in the whole process from lignocellulose to biogas.

As a trace nutritional supplement, the minimum concentration of iron salt is recommended to obtain economic importance for optimal performance of the anaerobic system, since the addition of trace elements may increase the operating costs of the process. A more thorough understanding of the minimum amount will allow a more economical and effective control of the process [33,34]. Some studies have explored the concentration of Fe^{2+} or FeCl_2 to the anaerobic system. Additives of

10 mg/L Fe^{2+} every 45 days at a hydraulic residence time (HRT) of 30 days can successfully perform the methane fermentation [31]. The minimum requirement of iron added in the mesophilic fermentation is 1.9 ± 0.1 mg/L [28]. Adding 1 mg/L $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ into the reactor volume of 1 L every day is suggested to reduce the concentration of volatile acids in the fermentation [35]. To improve the conversion of acetic acid to methane, the optimum dissolved iron concentration is recommended between 25.4 and 253.5 mg/L [36]. Since the biomass originally contains a certain concentration of Fe^{2+} , the additives of Fe^{2+} should be limited at 1.3–2.5 mg/L in the anaerobic system [37]. Based on the above research results, the minimum amount of FeCl_2 in the anaerobic system should be as low as 1 mg/L. The concentration of FeCl_2 in the pretreatment process should also be as low as possible so that Fe^{2+} in the hydrolysate can meet the minimum concentration requirements of the anaerobic system.

The main goal of the work was to investigate the influence of an ultra-low concentration FeCl_2 on both pretreatment and anaerobic fermentation in view of the whole process optimization. The catalytic property of FeCl_2 and its effect of pretreatment on corn straw conversion were evaluated. Further, the role of FeCl_2 in anaerobic fermentation was studied by acidogenic fermentation with the hydrolysate at the optimal condition of FeCl_2 pretreatment.

2. Materials and Methods

2.1. Raw Materials and Sample Preparation

All corn straw used in the study was taken from Wuqing, Tianjin, China. The air-dried crop samples were first cut into about 2–3 cm segments with a hay cutter, then ground several times until they all passed through a 20-mesh sieve, and finally stored in a polyethylene container at room temperature for use. They were dried at 60 °C before pretreatment to avoid the effect of moisture during storage.

The analytical procedure of National Renewable Energy Laboratory (NREL) [38] was performed to measure the components of corn straw, including cellulose, hemicellulose, lignin, and ash in biomass samples. The main components of corn straw raw material were cellulose 38 g, hemicellulose 21.8 g (containing 17.7 g xylan and 4.1 g arabinan), lignin 6.9 g, ash 4.3 g, and others 29 g in 100 g dry matter (DM).

2.2. Pretreatment Experimental Setup

Pretreatment was performed by the in-house-built laboratory-scale salt bath equipment (Figure 2). It included an electric controller, an electric mixer, and a salt bath. Hydrothermal reactions took place in the stainless steel cylindrical reactor tank with a total volume of 200 mL and were monitored by matching temperature sensors. The temperature of the salt bath was balanced through the mixer and the temperature of the reaction was regulated by the electric controller. The salt bath with a liquid mixture of KNO_3 and NaNO_2 in a proportion 1:1 was used to give the needed temperature. The highest temperature and the maximum pressure that the reactor could withstand were 300 °C and 40 MPa, respectively. The average heating rate was about 20–30 °C min^{-1} . When the designed temperature inside the reactor was reached, the pretreatment time started to be counted. After reaching the targeted time, the reactor was taken out from the salt bath and sunk into a cold water bath for cooling to below 50 °C. The mixture after the reaction was filtrated by a vacuum pump, obtaining a solid phase and a liquid phase. The solid phase was washed with deionized water, weighed and analyzed for lignin and ash according to the NREL analytical approach. The liquid phase was analyzed to use HPLC for the degradation products, such as glucose, xylose, arabinose, formic acid, acetic acid, furfural, 5-hydroxymethyl furfural (5-HMF), and levulinic acid.

2.3. Determination and Analysis of Samples

The concentrations of oligosaccharide, single-molecule, and volatile fatty acids in the hydrolysis products were all quantified by a High-Performance Liquid Chromatography (HPLC) system (Shimadzu,

LC-20A) equipped with Bio-Rad Aminex column (HPX-87H, 7.8 mm). Of all the products, saccharides including glucose, xylose, and arabinose, and organic acids including formic acid, acetic acid, propionic acid, butyric acid, and isovaleric acid, were measured by a refractive index detector. The hydrolysis byproducts such as furfural, 5-HMF, and levulinic acid were measured with an ultraviolet detector. The mobile phase was 5 mmol/L H_2SO_4 at a flow rate of 0.6 mL/min with a column-oven temperature set at 65 °C.

Figure 2. In-house-built salt bath reaction device and reactor tank.

Chemical Oxygen Demand (COD) in the hydrolysis products was determined according to the national standard (China, 2002). The sample digestion process was accelerated by a microwave oven (KDB-III, Qingdao, China). The measurement process was carried out according to the instructions for the microwave oven. A digital pH-meter (pHS-3C, China) was used to determine the pH in the work. A scanning electron microscope (SEM) (Zeiss Supra 55, Germany) was used to compare the pretreatment effect on straw micromorphology by distilled water and an ultra-low concentration of FeCl_2 .

Total sugar included glucose, xylose, and arabinose. Total by-products obtained included furfural, 5-HMF, formic acid, and levulinic acid. Available products were the sum of total sugar and total by-products at a certain pretreatment condition. The amount of cellulose conversion refers to the total yields of the main products such as glucose, 5-HMF, formic acid, and levulinic acid after cellulose hydrolysis. The amount of hemicellulose conversion refers to the total yields of the main products such as xylose, arabinose, and furfural after hemicellulose hydrolysis. Cellulose conversion and hemicellulose conversion are defined as follows:

$$\text{Cellulose conversion (\%)} = \frac{\text{Cellulose hydrolysates in solution (g)}}{\text{Cellulose content in corn straw (g)}} \times 100\% \quad (1)$$

$$\text{Hemicellulose conversion (\%)} = \frac{\text{Hemicellulose hydrolysates in solution (g)}}{\text{Hemicellulose content in corn straw (g)}} \times 100\% \quad (2)$$

2.4. Combined Severity Analysis

Combined severity (CS) is an effective parameter for analyzing the hydrolysis degree which correlates three important factors including temperature, time, and catalyst concentration [39]. It helps to evaluate and model the pretreatment process faster [40]. The severity factor is defined as follows [39,41,42]:

$$\text{CS} = \log(R_0) \text{ (Hydrothermal pretreatment)} \quad (3)$$

$$\text{CS} = \log(R_0') = \log(R_0) + \log[H^+] \text{ (Acid pretreatment)} \quad (4)$$

In Equation (4), the acid pretreatment is mainly aimed at the influence of dilute acid, so $[H^+]$ can be calculated directly by acid concentration. However, hydrogen ion concentration in the solution is

not equivalent to the concentration of catalyst with FeCl₂ pretreatment. Therefore, combined severity should be calculated by the following equation:

$$CS = \log(R_0') = \log(R_0) - \text{pH} \quad (5)$$

R_0 , respects the severity parameter, which analyzes and evaluates the influence of reaction temperature and reaction time on the pretreatment. R_0 , is defined as follows [40]:

$$R_0 = t \times \exp[(T_H - T_R)/14.75] \quad (6)$$

t , respects the reaction time (min). T_H is the reaction temperature (°C), and T_R is the reference temperature, which is generally regarded as 100 °C.

2.5. Pretreatment Schemes

In order to investigate the catalysis of an ultra-low concentration of FeCl₂, distilled water was used for pretreatment comparison. In view of the whole process optimization and the requirements of the minimum amount limited in the anaerobic system, the concentration of FeCl₂ was set to lower values, 5×10^{-4} mol/L, 1×10^{-4} mol/L, 5×10^{-5} mol/L, and 1×10^{-5} mol/L. The mass ratio of corn straw to FeCl₂ solution was set at 1:20. Pretreatment experiments were carried out at the temperature range of 160–220 °C and the time of 20 min. To evaluate the ultra-low concentration FeCl₂ in more detail, more pretreatment experiments were carried out at the temperature range of 160–220 °C and the time range of 10–40 min. Meanwhile, FeCl₂ solution was prepared when it was used in the experiment, to prevent Fe²⁺ from being oxidized.

The work schemes with about 1×10^{-5} mol/L FeCl₂ pretreatment were shown in Table 1. Different schemes had different pH, temperature, and time with 1×10^{-5} mol/L FeCl₂ pretreatment. In order to investigate the influence of 1×10^{-5} mol/L FeCl₂ more comprehensively, the temperature was set to a relatively wide range of 160–240 °C. The pH before the reaction fluctuated between 5.75 and 5.9 due to an error. To reduce experimental errors, each experimental result is the average of three repeated experiments under the same conditions.

Table 1. Pretreatment schemes with 1×10^{-5} mol/L FeCl₂, pH before and after reaction and CS.

Run	T/°C	t/min	pH (BR)	pH (AR)	lgR ₀ ' (CS)
1	160	10	5.85	4.72	−3.08
2	160	20	5.88	4.33	−2.81
3	160	30	5.87	4.16	−2.63
4	160	40	5.86	4.05	−2.49
5	180	10	5.76	3.95	−2.4
6	180	20	5.8	3.75	−2.14
7	180	30	5.79	3.48	−1.96
8	180	40	5.83	3.12	−1.87
9	200	10	5.77	3.44	−1.83
10	200	20	5.81	3.35	−1.56
11	200	30	5.8	3.21	−1.38
12	200	40	5.79	3.11	−1.24
13	220	10	5.86	3.1	−1.33
14	220	20	5.9	3.2	−1.07
15	220	30	5.81	3.08	−0.8
16	220	40	5.84	3.21	−0.7
17	240	10	5.82	3.12	−0.7
18	240	20	5.8	3.16	−0.38
19	240	30	5.78	3.13	−0.18
20	240	40	5.75	3.05	−0.03

Legend. BR and AR represent before the reaction and after the reaction, respectively.

2.6. Anaerobic Acidification Fermentation

The seed sludge came from an anaerobic fermentation tank at medium temperature (37 ± 1 °C) utilizing pig manure. Anaerobic acidification fermentation was performed in two reagent bottles (numbered 1[#] and 2[#]) with a total volume of 1 L and a working volume of 800 mL. The hydrolysate from 1×10^{-5} mol/L FeCl_2 pretreatment optimum condition was added in the bottle 1[#]. Distilled water was added into bottle 2[#] for comparison. The two reagent bottles were placed in the water bath shaker at 37 ± 1 °C. The mixed liquor volatile suspended solids (MLVSS) were determined according to the standard methods [43]. The sludge concentration in the bottle was 4000 mg MLVSS/L and the organic load was 1.0 g COD/g MLVSS [44,45] at the beginning of the acidification fermentation. Regulating the pH value was 7.0 ± 0.05 before fermentation.

3. Results and Discussion

3.1. Catalytic Role of FeCl_2 in Pretreatment

3.1.1. Comparison of Pretreatment Effects of FeCl_2 and Distilled Water

Biomass is subjected to autohydrolysis at certain temperature conditions, such as 150–240 °C [46]. Therefore, the catalytic action of an ultra-low concentration of FeCl_2 on pretreatment was evaluated by comparing with distilled water under the same pretreatment conditions. The pretreatment influences of FeCl_2 and distilled water on the yields of glucose, xylose, and arabinose in the reaction solution are shown in Figure 3.

Figure 3a shows the effects of both an ultra-low concentration of FeCl_2 and distilled water on the yield of glucose under different pretreatment conditions, including temperature and concentration. It can be seen that the yield of glucose resulted from various FeCl_2 solutions are higher than that caused by distilled water at the same temperature. This indicated that an ultra-low concentration of FeCl_2 can strengthen the destruction of the cellulose structure and produce more glucose. However, the yield of glucose declined with increasing temperature, this implied that the glucose degraded partly at high temperature.

In Figure 3b, the yield of xylose with FeCl_2 pretreatment was also notably higher than that of distilled water, and more xylose was obtained than glucose in a short time. It indicated that FeCl_2 exhibited high catalytic activity for hemicellulose degradation and xylose release. Additionally, Figure 3b showed different variations compared with Figure 3a. As the temperature went up, xylose production increased rapidly and reached the maximum value at 180 °C with different FeCl_2 pretreatments, and there was no obvious change from 180 °C to 200 °C. Different from FeCl_2 pretreatments, xylose yield rapidly decreased at 180–200 °C by distilled water pretreatment. The results showed that the increase in temperature had a great effect on straw self-hydrolysis. Xylose produced by an ultra-low concentration of FeCl_2 at 220 °C was not much different from that produced by distilled water, because hemicellulose had already been mostly degraded, and xylose had undergone secondary degradation the same as glucose. It can be seen that FeCl_2 played its catalytic role better at a temperature below 220 °C.

In Figure 3c, FeCl_2 had a significant catalytic effect on arabinose production, which was the same as that of glucose and xylose. Although the curve in Figure 3c had a similar downward trend as in Figure 3a, the slope of Figure 3c was larger, which indicated that the output of arabinose decreases faster with increasing temperature. When the temperature went up above 200 °C, the yield of arabinose dropped to below 1 g and changed little. It indicated that most of the arabinose in the solution was degraded.

Figure 3. Comparison of pretreatment effects of an ultra-low concentration of FeCl₂ and distilled water on corn straw with the reaction temperature of 160 °C, 180 °C, 200 °C, 220 °C, and the time of 20 min. The amount of sugar produced in the reaction solution are as follows: (a) glucose; (b) xylose; (c) arabinose; Legend. DW(♦), A(■), B(●), C(▲) and D(▼) represent distilled water, 1 × 10⁻⁵ mol/L, 5 × 10⁻⁵ mol/L, 1 × 10⁻⁴ mol/L, and 5 × 10⁻⁴ mol/L, respectively.

As can be seen from Figure 3a–c, FeCl₂ compared with distilled water played a more positive role to promote straw degradation because more monosaccharides were produced. This indicated that FeCl₂ had an excellent catalytic performance. In view of the optimization of the whole process from pretreatment to fermentation, the minimum amount of FeCl₂ should be considered for the optimal performance of the anaerobic system. As 1 × 10⁻⁵ mol/L FeCl₂ can give promising degradation results and has an economical advantage due to its low concentration, the minimum amount would be further studied to evaluate the effect of an ultra-low concentration of FeCl₂ on straw hydrolysis as well as fermentation.

3.1.2. Microstructure Comparison by the Pretreatment of FeCl₂ and Distilled Water

Figure 4 shows the microscopic morphology of straw in different pretreatment conditions. It can be seen that untreated corn straw was broken in a small part due to mechanical action during sample preparation in Figure 4a. Most of its surface was smooth, and the long chain of cellulose is clearly visible. However, there are different damage degrees for the straw surface after the pretreatment of distilled water and FeCl₂ in Figure 4b,c. In Figure 4b, the straw only had a little damage on the surface. Some of the fibers were detached and the fiber long chain can be recognized after the hydrolysis of distilled water. In Figure 4c, the straw was damaged seriously after the hydrolysis of FeCl₂. The cellulose long chain was obviously broken. The dense structure was widely opened and the surface was stratified and no longer smooth. The roughness of the straw surface increased greatly [47],

indicating a major degradation of hemicellulose [48]. It was quite clear that FeCl_2 showed a stronger influence on straw hydrolysis than distilled water from the damage degree of straw. It indicated an ultra-low concentration of FeCl_2 had an excellent catalytic performance to degrade straw. This was in agreement with the results shown in Figure 3.

Figure 4. SEM images (at a 500 magnification) of untreated straw and the pretreatment samples by distilled water and FeCl_2 at 180 °C and 10 min. (a) untreated corn straw; (b) pretreated with distilled water; (c) pretreated with 1×10^{-5} mol/L FeCl_2 .

3.2. Effect of an Ultra-Low Concentration of FeCl_2 on Cellulose Hydrolysis

Figure 5 exhibits the effect of 1×10^{-5} mol/L FeCl_2 pretreatment on cellulose degradation with four products, including glucose, 5-HMF, levulinic acid, and formic acid [49]. As seen in Figure 5, when CS was low between -3.08 and -2.14 , more glucose was obtained and the content of glucose reached the maximum value of 5.7 g at 180 °C and 10 min. Meanwhile, cellulose degradation also reached the highest value of 36.06%. By comparison, the degradation of cellulose was not more than 25% when corn stalk was pretreated with 0.1 mol/L FeSO_4 at 140–200 °C [4]. With the increase of CS, the glucose yield in the solution fluctuated up and down and overall, showed a decreasing trend. Unlike glucose, 5-HMF increased with temperature and time. It indicated that glucose is easier to be further decomposed to 5-HMF above 220 °C. In other words, the high temperature would promote glucose degradation and produce more 5-HMF.

5-HMF is thought to inhibit the fermentation process and thus affect methane production [50]. Under different pretreatment conditions, the yields of 5-HMF were very low, in the range of 0.09–1.9 g/100 g. Additionally, the high temperature may inhibit levulinic acid production. Because levulinic acid is a hydrolysate of 5-HMF [51], its amount reflects the hydrolysis degree of 5-HMF. It could be seen that the content of levulinic acid increased with increasing CS below 220 °C. When CS reached -1.56 , that is, the reaction condition was 200 °C and 20 min, the yield of levulinic acid reached its peak at 4.09 g and then decreased continuously between 200 °C and 220 °C. It dropped to a minimum of 0.66 g at 240 °C although there was a small rebound. Obviously, the amounts of formic acid and levulinic acid were higher than 5-HMF below 220 °C. It indicated that 5-HMF was degraded in a large amount.

Combined with cellulose degradation, it can be concluded that the pretreatment conditions with medium or low CS can not only improve the degradation of cellulose but also provide more available substrates for subsequent fermentation. More importantly, the yields of 5-HMF were extremely low with 1×10^{-5} mol/L FeCl_2 pretreatments, so that it would not have a significant inhibitory effect on subsequent anaerobic fermentation.

Figure 5. Effect of 1×10^{-5} mol/L FeCl_2 pretreatment on cellulose degradation.

3.3. Effect of an Ultra-Low Concentration of FeCl_2 on Hemicellulose Hydrolysis

Figure 6 shows the influence of different conditions on hemicellulose and its main products including xylose, arabinose, and furfural. It is shown that the yield of xylose fluctuated as CS increased. At 180 °C and 10 min, it reached a maximum of 16.3 g. Additionally, the arabinose yield and hemicellulose degradation all reach the maximum at the same condition, where the maximum glucose yield was also obtained. It indicated that this reaction condition was more favorable for monosaccharide production. When CS was higher than -1.83 (200 °C, 10 min), xylose yield decreased significantly. Nevertheless, furfural yield increased continuously and reached a maximum value of 5.9 g at 220 °C and 10 min. It showed that more xylose was degraded to furfural and other by-products at the harsher pretreatment condition. It is noted that furfural, like 5-HMF, is considered as an inhibitor of fermentation [51]. So, rather more xylose and arabinose than furfural are needed. It can be seen that the change trends of cellulose and hemicellulose were similar, which was consistent with the reported results [22]. The degradation of hemicellulose of 97.6% was achieved at 180 °C and 10 min. It was far higher than that of cellulose compared with Figure 5. This is related to the amorphous structure of hemicellulose.

Figure 6. Effect of 1×10^{-5} mol/L FeCl_2 pretreatment on hemicellulose degradation.

3.4. Effect of Ultra-Low Concentration of FeCl_2 on the pH Value

Figure 7 shows the relations between the pH after reaction and two main acid products, including formic acid and acetic acid, in the hydrolysate. Formic acid and acetic acid are two important organic

acids produced in the hydrolysates. As can be seen, the content of formic acid was always higher than that of acetic acid in the temperature range of 160–200 °C. It is a fact that formic acid mainly comes from the degradation of 5-HMF and acetic acid is from the acetyl in the lignocellulose [2,51]. This indicated that 5-HMF degrades faster than the acetyl group within this range. Formic acid can be converted to biogas directly in the anaerobic digestion [2], while acetic acid is considered as an inhibitor of fermentation activity [52]. When the temperature rose to 220 °C, the concentration of acetic acid increased rapidly, while formic acid drops sharply. Combined with Figures 5 and 7, it indicated that inhibitors such as acetic acid, furfural, and 5-HMF, were more likely to be produced at high temperatures above 220 °C. As a result, the reaction temperature should be controlled below 220 °C to limit the generation of inhibitors.

Figure 7. Effect of 1×10^{-5} mol/L FeCl_2 pretreatment on acid production.

Because of the higher yields of formic acid and acetic acid, they significantly affected the pH value of the solution after the reaction. It can be seen from Table 1 that the pH of the solution before the reaction was in the range of 5.75–5.9. It gradually decreased from 4.72 to 3.05 as CS rose. The pH is an important factor during biomass conversion, the change of that reflected the amount of acid produced after the pretreatment.

3.5. Effect of an Ultra-Low Concentration of FeCl_2 on Total Hydrolysates

Furfural and 5-HMF have been considered inhibitors to hinder anaerobic fermentation. However, recent research [53] found that pretreatment helps to dissolve or alter lignin structure and enlarge methane yield. Furthermore, furfural and 5-HMF as pretreatment by-products did not inhibit anaerobic digestion at a certain concentration and could be used as a carbon source to lead to biogas production. Furfural and 5-HMF can be converted into methane by methanogenic microorganisms at a concentration of 1 g/L and in the presence of xylose at 1 g/L. Some researchers have found that furfural and HMF will inhibit the anaerobic microbial growth or metabolic processes when their concentration is higher than 1.9 g/L and 2.5 g/L, respectively [2]. Additionally, it has been proved that the microbial community had a good ability to adapt the inhibitors, such as furfural, HMF, and phenol compounds, after 57 days of biogas production [54]. Therefore, according to these studies, pretreatment conditions including temperature, catalyst concentration, and reaction time, need to be investigated more comprehensively. Not only sugar yield and the inhibition of by-products but also the possible positive effects of by-products on fermentation should be considered.

As can be seen from Figure 8a, total sugar production increased sharply with low CS. When CS exceeded -2.4 (180 °C, 10 min), the yields of total sugar began to decline rapidly until it remained at a low level. The change in the trend of the total by-product was relatively gentle compared with total sugar. It illustrated that the effect of CS on total sugar yield was higher than that of the by-product.

The variation trend of total sugar and the total by-product was consistent with the theoretical correlation between the combined severity factor and the reaction degree [39].

Figure 8. Effect of 1×10^{-5} mol/L FeCl_2 pretreatment on total products and COD. (a) Total sugar and total by-product, (b) Available products and COD.

In order to increase biogas production from biomass, available products containing total sugar and total by-products should be as much as possible. In Figure 8b, available products reached a peak of 32.7 g at 180 °C and 10 min. However, to find out the optimum condition of FeCl_2 pretreatment, the favorable concentration of furfural and 5-HMF should be considered. According to the previous literature, the concentration of furfural and HMF is suggested to be lower than 15–20 mm which is no inhibition for the anaerobic fermentation. Therefore, combined with the yield of furfural and 5-HMF in Figures 5 and 6, a conclusion can be reached that the optimum condition of 1×10^{-5} mol/L FeCl_2 pretreatment was a reaction temperature of 180 °C and holding time of 10 min. Additionally, the value of COD in the solution did not fluctuate greatly above 200 °C, while the available products dropped sharply. It was speculated that many other small organic molecules were also formed at such high reaction intensity.

3.6. Mass Balance during Ultra-Low Concentration of FeCl_2 Pretreatment

In Figure 9, the organic products as important carbon sources for subsequent fermentation were not less than 77.5% of COD in the liquid fraction under the optimal condition. The content of the main by-products only accounted for 18% of all organic products. Among all the by-products, the production of 5-HMF, furfural, and acetic acid are low, 0.3 g, 1.5 g, and 2.2 g, respectively. Moreover, 30.7% of cellulose converted, and 69.6% of lignin was degraded. In particular, the release of xylose was as high as 95% and the conversion of hemicellulose reached 97.6%. It indicated that the ultra-low concentration of FeCl_2 had a significantly positive impact on hemicellulose removal and xylose release.

3.7. Effect of Pretreatment on Acid Fermentation

The effect of pretreatment on acid fermentation was evaluated with the hydrolyzate of the optimum pretreatment. The concentration of main volatile fatty acids (VFAs) and the variation of the pH during acidogenic fermentation were shown in Figure 10.

Figure 9. Mass balance of treated corn straw under the optimal condition of 1×10^{-5} mol/L FeCl_2 pretreatment.

Figure 10. VFAs distribution and the pH during acidogenic fermentation with the hydrolysate. (a) 1×10^{-5} mol/L FeCl_2 (bottle 1[#]); (b) distilled water (bottle 2[#]).

The production of acetic acid was the most in all products, which was the important organic compound to be utilized during the anaerobic fermentation process [34]. The sum of acetic acid and butyric acid accounts for more than 70% during 26 h to 54 h in bottle 1[#], exhibiting the feature of butyric acid fermentation type [55,56]. The pH of bottle 1[#] dropped quickly between 0 and 44 h and reached its lowest point at 44 h in Figure 10a. Compared with that in bottle 1[#], butyric acid-type fermentation took place between 44 h and 54 h in bottle 2[#], and the lowest pH appeared relatively late at 54 h in Figure 10b. It was obvious that the acidification process proceeded faster, and more VFAs were produced in bottle 1[#] than those in bottle 2[#]. It indicated that the FeCl_2 pretreatment accelerated the acidogenic fermentation process and promoted the production of organic acids.

In summary, the ultra-low concentration of FeCl_2 not only improved the effect of straw degradation as the pretreatment catalyst but also directly served as the nutritional supplement for subsequent fermentation, which avoided repeated addition of nutrients and greatly reduced the cost of raw material and labor. Low requirements for equipment saved the cost of the device due to slight corrosion by the ultra-low concentration of FeCl_2 . Moreover, the hydrolysate after FeCl_2 pretreatment can effectively improve the potential of straw to biogas.

4. Conclusions

This work proposed an economical and efficient method for the conversion of biomass to biogas. Based on the whole process optimization of biomass conversion, combined with the recommended minimum amount of FeCl_2 as a nutritional composition in the anaerobic system, the pretreatment effects of an ultra-low concentration of FeCl_2 on corn straw were investigated. The remarkable catalytic role

of FeCl_2 was confirmed by comparison with distilled water pretreatment. An ultra-low concentration of FeCl_2 as the catalyst showed significant effects on hemicellulose hydrolysis and xylose release in the pretreatment. The optimized condition of 1×10^{-5} mol/L FeCl_2 pretreatment that combined the influence of total sugars and total by-products on fermentation, was found to be the temperature of 180 °C and the holding time of 10 min. At this condition, FeCl_2 pretreatment accelerated the acidogenic fermentation process and increased organic acid yields. These results will help to enhance the potential of straw to biogas.

Author Contributions: Y.Q. performed pretreatment experiments, data analysis and paper writing; X.L. provided financial support and determination of experimental scheme; Z.Z. helped to collect the raw material and set up experimental equipment; S.Z. provided writing guidance and language modification of the full paper. All authors contributed to the editing and reviewing of the document. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation Project, China, 51876180.

Conflicts of Interest: The authors declare no conflict of interest.

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