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# Improving biogas production from wheat plant using alkaline pretreatment

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## HIGHLIGHTS

- A mixture of lignocellulosic and starchy biomass was used as a substrate.
- Effect of a wide range of temperature (0–100 °C) was studied on pretreatment.
- Crystallinity as well as surface layer of wheat plant were influenced.
- Yield of 404.4 ml CH<sub>4</sub>/gVS by 54.5% enhancement over raw substrate was obtained.

## ARTICLE INFO

### Article history:

Received 27 April 2013  
Received in revised form 19 July 2013  
Accepted 23 July 2013  
Available online xxx

### Keywords:

Wheat plant  
Alkaline pretreatment  
Anaerobic digestion  
Biogas

## ABSTRACT

Alkaline pretreatment of wheat plant (WP), including its grains and straw, was investigated under different conditions in order to enhance biomethane production at mesophilic temperature. Alkaline pretreatment was performed using 8% (w/v) NaOH solution at different temperatures (0, 25, 50, 75 and 100 °C). The best improvement in the yield of methane production was achieved by pretreatment at 75 °C for 60 min, giving a methane yield of 404 ml g<sup>-1</sup> VS. The highest glucose content was also obtained under this pretreatment. The cumulative methane yield for pretreated WP at 25, 50 and 75 °C increased the methane yield around 47.5%, 40.8% and 54.5% higher than that of the untreated WP, respectively, while pretreatment at 0 and 100 °C was not effective in improving the biogas production. Qualitative analysis of pretreated WP using Scanning Electron Microscopy and Fourier Transform Infrared showed the reduction of crystallinity as well as the removal of surface layers of lignin and hemicellulose.

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## 1. Introduction

Since the beginning of the industrial revolution, the required energy for the developed industries has been extremely increased all around the world [1]. However, ease of access to the fossil fuels during almost two centuries has decreased the available fossil fuel reservoirs, causing the rising prices. Therefore, the energy supply for the future has become one of the most important global problems [2]. On the other hand, combustion of fossil energy carriers like petrol, natural gas and coal has led to the release of CO<sub>2</sub>, NO<sub>x</sub> and SO<sub>x</sub>, which all cause huge environmental problems and adverse effects on human health and ecosystem [3]. In order to solve these problems, the European Commission has set the goal to increase the energy of renewable sources up to 20% by 2020, as compared to 8.5% in 2005. To reach this goal, the use of all existing renewable energy sources needs to be increased and improved [4].

Different kinds of biomasses like energy crops, agricultural wastes and various organic wastes such as organic fraction of the municipal solid waste have a great potential to be converted to

renewable energies such as biogas and bioethanol [5]. Biogas, which is known as a clean and renewable form of energy, could be augmented to the conventional energy sources [6]. The advantage of biogas over other existing renewable energies is that it can be easily applied by the energy consumers that use the existing technologies. In the past few years, biogas production by the anaerobic digestion of wastes has been developed [7].

Biological conversion of agricultural wastes such as wheat straw, rice straw and sugar beet plays an important role in the supply of the growing energy demand of the society in a sustainable manner [8]. The wheat plant (WP) is one of the most agricultural products in which the annual global consumption of wheat grain is in excess of 550 million tones. About 370 million tones are used for human consumption and 90 million tones are fed to ruminants and non-ruminants. The other parts are used as seed for industrial use or the lost post-harvest [9].

Sufficiently inexpensive sugar-rich streams from renewable agricultural biomass can become the basis for a wide variety of chemicals and fuels, replacing petroleum and other fossil feedstocks. However, conversion of these wastes by anaerobic digestion to biogas at a high yield requires effective and economical pretreatments. Plant biomass such as WP is composed of carbo-

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hydrates (starch, cellulose, hemicellulose and simple sugars), proteins, lignin, lipids, pectin, minerals and some other minor components. The major components of WP are cellulose and starch [10]. Pretreatment of the agricultural biomass by mechanical size reduction, heat treatment and/or chemical treatment usually improves its digestibility and therefore, the yield of biogas production [11]. Without an effective pretreatment, cellulosic whole plants would be much more difficult to hydrolyze completely than would starchy grains during anaerobic digestion. With increasing cellulose content in the whole plant biomass, much more recalcitrant cellulosic feedstock is expected. The combination of the lignocelluloses (cellulose, lignin and hemicellulose) and starch in the WP makes it difficult to process it as a single unit with conventional methods.

A wide variety of methods (e.g., concentrated or dilute acids or bases, high temperatures, radiation in various forms) have been investigated to pretreat the lignocellulosic biomasses to increase their yield of the gained sugars and digestibility [12]. Similarly, many treatment techniques have been studied to improve the rate and extent of conversion of starchy materials such as corn, wheat grain and other grains to fermentable sugars or more digestible starches [13]. Sometimes, a combination of steam, heat and/or pressure has been used to gelatinize the starch. In some other cases, they have been followed by digestion with starch hydrolyzing enzymes. It is necessary to mention that the application of an inappropriate pretreatment could degrade some of the sugars, e.g., acids or aldehydes, which reduce the yields and inhibit the subsequent biological conversion of the remaining sugars [14]. The previously applied successful technologies do not simultaneously increase the rate and extent of both starch and lignocellulose conversion to sugars. Instead, there has been more focus on either starch or lignocellulose conversion, but not both.

Alkaline pretreatment is known as an effective pretreatment method which can solubilize the lignin and also neutralize various acidic products released from the lignocellulosic complex [15,16]. Moreover, the presence of a small amount of residual alkali remaining in the treated solids may be helpful to prevent the pH reduction during subsequent acidogenesis process [17]. Therefore, alkaline pretreatment is more effective and compatible with subsequent anaerobic digestion when compared to other pretreatment methods such as thermochemical ones [18].

The objective of this study was to investigate the biomethane potential of WP and its improvement by means of alkaline pretreatment using NaOH. The effects of temperature in alkaline pretreatment on the yield of methane production were studied. Furthermore, changes in the composition and structure of the WP as a result of the pretreatments were also investigated.

## 2. Material and methods

### 2.1. Wheat plant (WP)

The wheat plant (WP) was collected from the Farm of Isfahan Agricultural Research and Development Center (IARDC). Then, it was air dried to the moisture content of less than 10% (w/w). The dried WP was milled to obtain particle sizes of less than 1 mm. Then, it was stored in air tight containers at room temperature prior to use. The ratio of total grain to total wheat plant was 47% (w/w) and 12% (w/w) of total grain was husk.

### 2.2. Microbial inoculum

The inoculum was obtained from a 3000 m<sup>3</sup> municipal solid waste anaerobic digester operating at 37 °C in wastewater treatment plant of Isfahan, Iran. Before use, the inoculum was sieved through a 1 mm screen to remove large particles and grit.

### 2.3. Pretreatments of WP

Alkaline pretreatments under different operational conditions were applied for WP biomass. 5 g of WP (dry basis) was mixed with 95 g NaOH solution (8% w/v). The mixture was then mixed for 10 min at room temperature [19]. Then, the mixture was incubated for 60 min at five different temperatures: 0, 25, 50, 75 and 100 °C, while it was being mixed every 10 min during the period of incubation. The incubated mixtures were then centrifuged at 4800 rpm (Werk NT.Baujhar Ekin, Universal 320 R, Hettich, Germany) for 5 min at room temperature. They were also neutralized to pH 7 by being washed with distilled water through vacuum filtration. The pretreated substrates were kept at 4 °C until use.

### 2.4. Anaerobic digestion

The methane potential of raw and pretreated WP was determined by anaerobic digestion in 118 ml serum glass bottles. Anaerobic digestions were carried out at 37 °C in batch mode [20]. Each bottle was supplemented with 20 ml inoculum and a certain amount of untreated or pretreated WP samples in order to have a volatile solids ratio of 1:2 for substrate: inoculum. Then, deionized water was added up to a total volume of 25 ml. Bottles were closed with butyl rubber seals and aluminum caps. The headspace of each bottle was flushed with 80% nitrogen and 20% carbon dioxide gas mixture to obtain anaerobic conditions. Furthermore, deionized water and inoculum were used as a blank so that the gas production of the inoculum alone could be determined.

In order to measure the production of CH<sub>4</sub> and CO<sub>2</sub> during the digestion, gas samples of 0.25 ml were withdrawn regularly from the headspace of each bottle with a pressure-tight syringe, making it possible to take gas samples at the actual pressure. The mass of methane and carbon dioxide was then determined in each sample by direct measurement using a gas chromatograph (GC). By assuming ideal gas mixtures, the methane and carbon dioxide contents were calculated in the flask headspace using the data from the GC measurements [20]. Immediately after sampling, excess gas was removed in order to avoid pressures higher than 2 bars. This was followed by a second gas sampling for analysis. The amount of CH<sub>4</sub> and CO<sub>2</sub> produced between the two subsequent samplings in each flask was calculated from the difference of mass of methane determined after releasing the overpressure and the mass of methane, which, in turn, was determined at next sampling time before the release. A gas with a known composition was used as the standard in each measuring occasion. The digestion experiments were continued until the gas production was ceased and the accumulated gas production was kept at stable levels. The accumulated methane gas volume is expressed as gas volume per gram of volatile solids at normal conditions with one standard deviation and at 95% confidence level. All experiments were performed in triplicates.

### 2.5. Analytical methods

#### 2.5.1. Biogas analysis

Methane and carbon dioxide were analyzed using a gas chromatograph (Sp-3420A, TCD detector, Beijing Beifen Ruili Analytical Instrument CO) equipped with a packed column (Porapack Q column, Chrompack) and a thermal conductivity detector with injection temperature of 140 °C. The carrier gas was helium operating with a flow rate of 20 ml min<sup>-1</sup> at 50 °C. A 250 µl pressure tight gas syringe (VICI, Precision Sampling Inc., USA) was used for the gas sampling. All results of methane volumes are presented at standard conditions.

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