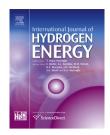
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## Enhanced mesophilic bio-hydrogen production of raw rice straw and activated sewage sludge by co-digestion

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#### ABSTRACT

In this study, batch biohydrogen production by co-digestion of raw rice straw and activated sewage sludge was investigated with different inoculum heat treatment, pH, S/X ratio (based on VS) and substrate sizes under mesophilic condition. In order to achieve a high bio-hydrogen yield and methanogens activity inhibition, heat treatment of inoculum was optimized at different exposure times (30, 45 & 60 min) and temperature ranges (80, 90 and 100 °C) prior to dark fermentation process. Collected data was analysed using response surface methodology (RSM). The heat treatment of inoculum at 100 °C for 60 min produced the highest bio-hydrogen yield of 14.22 NmL  $H_2/g$  VS at concentration of 70.97% and Production of 0.073 NmL CH<sub>4</sub>/g VS at 0.17% concentration in total produced biogas. The raw rice straw was also co-digested with heat-treated inoculum at different ratios of volatile solids (2:1, 4:1 and 6:1) and initial pH (4, 4.75 and 5.5) as numerical variables and 4 categories of substrate size ((250-500 µm], (500 µm-2mm], (2-20 mm), [20-30 mm]). The highest bio-hydrogen yield of 14.70 NmL/g VS was recognized at the optimum initial pH of 5.01 and S/X ratio of 4.54:1 using 2-20 mm rice straw.

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#### Introduction

Nowadays, many research works and pilot or industrial projects are being conducted all over the world revolving around biofuels. Bio-hydrogen as an energy career is becoming more attractive gradually and many studies cover wide range of issues in this field. Main subjects of these studies mostly include production, optimisation, method development and consumption of bio-hydrogen.

List of abbreviations: ASS, activated sewage sludge; S/X, substrata to inoculum ratio (based on VS); HPY, bio-hydrogen production yield; NmL, gas volume at NTP (0 °C, 1013 hPa); RS, rice straw.

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Bio-hydrogen inoculum and feedstock are the main components of bio-hydrogen production that can significantly affect bio-hydrogen production yield (HPY). Mixed culture is a mixture of different bacteria that contains a community of bio-hydrogen producer bacteria as well. Mixed culture can be obtained from aerobic or anaerobic sludge in wastewater treatment plants or compost piles or any other source of bacteria [1]. Since mixed culture contains different types of bacteria, it also contains methanogens or other hydrogen consuming bacteria. Methanogens, in one of their metabolism stages consume hydrogen to produce methane. Therefore, to produce bio-hydrogen from activated sewage sludge as a mixed culture, some treatment methods should be applied on it to inhibit methanogens activity or other hydrogen consuming bacteria [2]. There are few studies [3,4] that have used untreated mixed culture, but the yield is mostly lower compared to studies which have used treated mixed culture. There are many different methods to treat mixed microflora inoculum. Pretreatment methods reported in most research works mainly include heat-shock [2,5,6], acid [7,8], base [8], aeration, freezing and thawing, chloroform, sodium 2bromoethanesulfonate or 2-bromoethanesulfonic acid [2], photocatalytic pretreatment [8,9] and ultrasonic treatments [8,10]. Heat shock is the most commonly used methods for treatment of mixed culture. Wide ranges of time, mostly from 15 to 80 min and temperature mostly from 60 to 120 °C have been reported in different studies to treat different types of mixed cultures [11-15]. Published research works on heattreatment of inoculum introduce many different heat treatment conditions as the optimum heat-treatment condition. Choosing one of these conditions could be somewhat effective on inhibition of methanogens activity but there was no guarantee that the chosen heat treatment is the optimum heat treatment for our inoculum. Therefore, the plan for heat treatment in this study was to choose a range of time and temperature which was widely used in research works and try to find the optimum point for the ASS used.

Lignocellulosic waste materials are a huge group of biomasses that has bio-hydrogen production potential. Agricultural residues as a source of lignocellulosic waste materials are extensively used in bio-hydrogen production studies. Rice straw, wheat straw, sugar cane bagasse, beer lees, jackfruit peel and food waste are some of waste materials that are recently used in some research works for bio-hydrogen production [2,13,16-21]. Lignocellulosic materials have complex structure made from cellulose, hemicellulose, and lignin and consist of 50-80% carbohydrates in dry weight [22]. Pretreatment methods have been used to break down the complex structure of lignocellulose to make them a more biodegradable feedstock for biofuel production. These methods can be divided to 3 major groups, Physical pretreatment (size reduction, stream explosion and gamma rays usage), Chemical pretreatment (acidic and alkaline pretreatments) and biological pretreatment (enzymatic pretreatment). Mostly, a combination of these technologies produces better results [23]. The overall goal of all these methods are to change the structural and compositional properties of lignocellulosic biomass like presence of lignin and hemicellulose, decrease crystallinity of cellulose and to enhance accessible surface area; moreover it is expected from these technologies to make cellulose and

hemicellulose more accessible for enzymes and bacteria [22]. It should be considered that these pretreatment methods are always costly and sometimes not environment-friendly due to the usage of acid, base or the amount of energy used. As an example, biological pretreatment that seems more environment-friendly compared to the chemical pretreatment, is recognized as a time consuming process (10-14 days) and cannot be considered as a proper industrial method. On the other hand, the requirements of this bio-process, large space for conducting the process and the controlled environment, make it less attractive for large and industrial scales [23]. Size reduction is one of the most simple and widely used physical treatment methods of lignocellulosic waste materials in both lab and industrial scales [23,24]. In most researches, very fine lignocellulosic particles are used as raw lignocellulose [4,25], while some researches indicate that size reduction less than 0.4 mm has not any significant effect on yield of lignocellulosic waste material hydrolyses [4,23,26]. Grinding and milling are used to make lignocellulosic particles smaller than 2 mm.Since the Specific Energy Requirement (SER) to carry out milling or grinding is high, it is very important to do these mechanical treatments in a proper way [24].

Temperature is one the most affecting factors in biohydrogen production. Bio-hydrogen can be produced by mesophillic (25–40 °C), thermophilic (40–65 °C), extreme thermophilic (65-80 °C) and hyperthermophilic (>80 °C) microorganisms of the archaea and bacteria domains [27]. Under the same operating conditions a thermophilic bioreactor has a higher yield of bio-hydrogen production compared to a mesophilic bioreactor. On the other hand, a thermophilic experiment consumes more energy compared to a mesophilic experiment, and the difference between yields in thermophilic and mesophilic is not always highly significant considering the difference in the amount of consumed energy. Conducting a fermentation process in the mesophillic condition by optimization of other effecting factors on HPY will save lots of energy which is important for a successful large scale project [28].

pH is another important affecting factor for metabolism of microorganisms. The reason for that is pH directly affects hydrogenase activity or metabolic pathways [29]. Many studies are done to investigate pH effect on bio-hydrogen production using different inoculums and substrates [29–33]. Since most of the studies were conducted in batch mode without a pH controller, only the effect of initial cultivation pH, shortly called initial pH, on fermentative biohydrogen production was investigated. Many different pH rates are reported as the optimum pH. Davila-Vazquez et al. (2008) [34], reported pH 7.5 as the optimum pH, while Ramos et al. (2012) [29], or Fan et al. (2004) [35], have reported pH 5.5 as the optimum initial pH.

Volatile solid has a significant role in a successful fermentation process. S/X ratio (based on VS) could be an indicator of C/N ratio. Effect of C/N ratio is proven to be important for growth of microorganisms [36–38]. High initial VS impairs mass transfer between microorganisms and substrate while the very low VS restricts metabolisms in other ways. In different studies various ranges of initial total or volatile solids are elaborated [25,29,39]. There are few studies that deliberated S/X ratio for bio-hydrogen production [40].

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