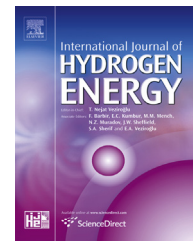




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Mesophilic biohydrogen production from calcium hydroxide treated wheat straw

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ABSTRACT

The use of $\text{Ca}(\text{OH})_2$ pre-treatment to improve fermentative biohydrogen yields, from wheat straw was investigated. Wheat straw was pre-treated with 7.4% (w/w) $\text{Ca}(\text{OH})_2$ at ambient temperature (20 °C) for 2, 5, 8, and 12 days, prior to 35 °C fermentation with sewage sludge inoculum. Biohydrogen yields were evaluated during dark fermentation and simultaneous saccharification fermentation (SSF) of total pre-treated straw material and compared to those from separated solid and hydrolysate fractions. $\text{Ca}(\text{OH})_2$ pre-treatment followed by SSF, exhibited a synergistic relationship. On average, 58.78 mL- H_2 g-VS⁻¹ was produced from SSF of pre-treated and filtered solids. This was accompanied by approximately a 10-fold increase in volatile fatty acid production, compared to the untreated control. By omitting pre-treatment hydrolysate liquors from SSF, H_2 production increased on average by 35.8%, per VS of harvested straw. Additional inhibition studies indicated that CaCO_3 , formed as a result of pre-treatment pH control, could promote homoacetogenesis and reduce biohydrogen yields.

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Introduction

Hydrogen can be produced from renewable resources and when used as a fuel can reduce emissions and improve air quality, compared to fossil fuel alternatives. Hydrogen's energetic content of 122 kJ g⁻¹ makes it an attractive energy carrier. However, one of the current obstacles, to wide-scale adoption of hydrogen fuel, is an economical and sustainable method for its large-scale production [1,2]. One method of obtaining renewable hydrogen gas, is from microbial dark fermentation [3,4]. Lignocellulosic material has been identified as an attractive dark fermentation feedstock due to its

low-cost, wide-geographic availability and large overall abundance. One such lignocellulosic material is wheat straw, of which it is estimated at least 500 Mt are produced annually as a by-product of food production [5–7]. It is widely accepted that lignocellulosic materials, firstly require effective pre-treatments, to create easily fermentable feedstocks [8–12].

A number of studies have found strong acid pre-treatments, such as HCl and H_2SO_4 , can effectively enhance biohydrogen and biomethane yields from lignocellulosic materials [8,13–17]. However the use of alkaline pre-treatment offers a number of advantages over acid pre-treatment. These include firstly, that alkaline reagents are more environmentally friendly than many strong acids and can be

Abbreviations: WSS, filtered wheat straw solids; WSH, wheat straw hydrolysate; TWS, total wheat straw.

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recovered for reuse [18,19]. Secondly, during dark fermentation a reduction in pH due to volatile fatty acid (VFA) accumulation can eventually inhibit biohydrogen production and a high pH feed (or pre-treatment by-product) could provide alkali to counteract this occurrence [20].

Alkaline pre-treatments have been reported to partially solubilise lignocellulosic materials, increasing their susceptibility to enzymatic breakdown. This occurs through the reactions of saponification, saponification and peeling of reducing end groups, as outlined by Hendriks and Zeeman [21]. A significant advantage of using alkali is that little degradation of cellulose occurs [18,22]. Generally alkali pre-treatments require reduced temperatures and pressure compared to other pre-treatment technologies leading to simpler reactor design and reduced capital cost. However, they require longer time periods, usually in the order of hours or days rather than minutes [23–27].

To date mainly NaOH has been used to investigate alkaline pre-treatment for dark fermentation [13,15,16,28–31]. A number of investigators have suggested using $\text{Ca}(\text{OH})_2$ as an alternative due to its ease of handling, reduced cost and recoverability as calcium carbonate, via lime kiln technology [9,12,19,21,32]. Additionally, Xu and Cheng suggested that the poor solubility of lime, could allow for a more stabilised higher pH during pre-treatment and that Ca^{2+} ions could provide linkages between biomass, reducing the loss of solids compared to NaOH treatment [33].

$\text{Ca}(\text{OH})_2$ has been reported to enhance biomethane yields from lignocellulosic containing grass and straw materials [34–36]. At present, little information on using $\text{Ca}(\text{OH})_2$, for dark fermentation processes, is available in literature. Cao et al. investigated $\text{Ca}(\text{OH})_2$ pre-treatment of cornstalk for thermophilic dark fermentation [20]. Cao et al. reported a H_2 yield of 155.4 mL g^{-1} volatile solid (VS) from cornstalk, following at 50 °C pre-treatment for 96 h and using a lime loading of 0.10 g/g biomass (a 38.1% increase compared to the non-treated control) [20].

Multiple investigators have demonstrated that the use of enzymes can increase yields and rates of H_2 production [37–39]. At present the application of enzyme treatment is usually restricted by costs, however enzyme prices are expected to continue to fall [40]. Two types of enzyme usage for dark fermentation processes have been described; separate hydrolysis and fermentation (SHF) and simultaneous saccharification fermentation (SSF). Quéméneur et al. compared SSF and SHF, of milled wheat straw [37]. After applying either enzyme treatment types; Quéméneur et al. achieved between 11.06 and 19.63 mL- H_2 g-VS $^{-1}$, in comparison to between 5.18 and 10.52 mL- H_2 g-VS $^{-1}$ from non-enzyme controls [37]. Following H_2SO_4 pre-treatment at 120 °C for 90 min, Nasirian et al. reported a 125.11 mL- H_2 g-VS $^{-1}$ from SSF of wheat straw solids compared to 47.89 mL- H_2 g-VS $^{-1}$ when using SHF. SSF of wheat straw was described as beneficial over SHF by Quéméneur et al. and Nasirian et al. [37,39]. SSF can reduce the occurrence of enzymatic product inhibition and the sterilization of feedstocks, to avoid losses of fermentable compounds, is unnecessary.

To date, the majority of previous $\text{Ca}(\text{OH})_2$ pre-treatment investigations have been enzyme hydrolysis studies for bioethanol production purposes [19,41,42]. Some authors have

reported inhibition of cellulose hydrolysing enzymes, by soluble compounds resulting from $\text{Ca}(\text{OH})_2$ pre-treatment. Hydrolysis enzymes may be inhibited by solubilised lignin [43,44], which can be released by lime pre-treatment [18]. Chang et al. observed calcium acetate inhibition of lignocellulose hydrolysing enzymes [32]. Therefore the formation of organic acids during dark fermentation means that resultant calcium based salts, could also reduce hydrolytic enzyme activity. S. Kim and Holtzapfel found no inhibitory impact of $\text{Ca}(\text{OH})_2$ pre-treatment liquors on fermentation of corn stover [42]. However; in an enzymatic hydrolysis study by Cheng et al. washing rice straw was found to significantly improve glucose conversion yields, following pre-treatment by $\text{Ca}(\text{OH})_2$ [9]. In addition some investigators have reported cations to cause inhibition of fermentative biohydrogen production [45]. Therefore, Ca^{2+} in pre-treatment liquors could directly contribute, to a potential reduction in biohydrogen yields.

Any inhibitory properties of pre-treatment hydrolysates, could limit their use for provision of alkali, during dark fermentation. Furthermore, if pre-treatment hydrolysates are completely removed from the process, any solubilised carbohydrates which they contain would remain unfermented. Hence, an optimisation scenario may arise between the amounts of soluble fermentable compounds present and the level of inhibition which the hydrolysate incurs. This is why it is important to understand the influences of pre-treatment hydrolysates, on subsequent biohydrogen yields, to ascertain their best use.

In this study interactions between biohydrogen yield, SSF, $\text{Ca}(\text{OH})_2$ pre-treatment and the division of resultant pre-treatment solids and hydrolysate were tested. This investigation had three aims:

- 1) Establish if room temperature $\text{Ca}(\text{OH})_2$ pre-treatment could improve biohydrogen production from wheat straw.
- 2) Test the influence on biohydrogen yields, by fermenting the pre-treated wheat straw solids and hydrolysate separately.
- 3) Examine the sequential combination, of $\text{Ca}(\text{OH})_2$ pre-treatment and SSF, on biohydrogen production.

To the best of the author's knowledge fermentative biohydrogen from alkali pre-treated wheat straw is reported here for the first time.

Materials and methods

Wheat straw pre-treatment

Raw wheat straw was supplied (in ~5 cm cuts) by Porters Farm (Dixon Brothers Ltd., UK) and sieved through a 0.5 mm sieve using a hammer mill. The wheat straw was stored in dry conditions at room temperature.

5 g-VS wheat straw was mixed with 62.5 mL of 80 mM $\text{Ca}(\text{OH})_2$ (using a 2 M stock solution) and sealed in a 150 mL plastic bottle. These bottles were magnetically stirred at 20 °C for periods of 2, 5, 8 and 12 days. Straw solids were collected in a Whatman® 4 filter (Sigma–Aldrich company Ltd., UK), through which a further 120 mL of deionised water (diH_2O)

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