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Fermentative hydrogen production by conventionally and unconventionally heat pretreated seed cultures: A comparative assessment

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ABSTRACT

In this study, the effects of pretreatment temperature and time during conventional and unconventional, microwave-assisted heat shock on the hydrogen producing capability of anaerobic seed sludge from soluble starch was focused. It was found that the different heat transfer techniques resulted in seed cultures with comparable hydrogen production potentials, with the highest obtainable values of approximately 0.9 L H_2/L-d. A comprehensive, statistical analysis revealed that both treatment temperature and time could be designated as significant process variables, however, in distinguishable extents for the two alternative methods. The results indicated that microwave-based sludge pretreatment needed remarkably shorter curing times (2 min) to eliminate H_2-consuming, methanogenic activity in comparison to the conventional heat shock method (30 min). It was also demonstrated that microwave irradiation increased the soluble organic matter content in the seed sludge.

1. Introduction

Biohydrogen is a prosperous energy carrier that receives significant attention from the scientific research community [1]. It has the potential to contribute to a more sustainable development but probably it will only be realized in relatively long terms [2]. This is because further advances have to be accomplished in order to make it a more competitive alternative both from technological and economical points of views. One particular issue to address is related to the production yields and rates, which are among the most important criteria for the evaluation of the overall biohydrogen process economy [3]. In fact, tremendous efforts have been put to improve them both on the upstream [4] and downstream sides [5,6]. As a result, dark fermentative biological hydrogen generation is nowadays the most promising way to go considering these practical aspects [7,8]. For example, hydrogen producing microorganisms have the ability of utilizing complex feedstock, even valueless and abundant waste materials [9–11]. However, it is also quite obvious that feasible hydrogen production needs the assistance of diverse microbial population (present in biogas sludge) rather than pure cultures. Beside the benefits of mixed consortia, when such inocula are used for

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the bioconversion of organic substances into biohydrogen, their sufficient pretreatment is usually required to suppress the activity of undesirable species such as methanogenes, homoacetogenes, etc. which are capable to inherently cut the obtainable hydrogen turnout. These pretreatments can be performed by exposing the community of strains to unfavorable conditions e.g. to elevated temperature, which refers to heat shock. This method is the most routinely applied one for the enrichment of the hydrogen-evolver bacteria in the population since it is relatively fast, easy to conduct and highly reliable [12].

Actually, there are different kinds of this technique altering in the mode of heat transfer and distinguished as conventional and unconventional ones. In general, the application of heat shock for pretreatment purposes relies on the conventional way meaning that the sources of inocula are usually kept in a hot (even boiling water) bath. Nevertheless, unconventional heat transfer takes place due to direct energy irradiation to the media, which can be ensured by microwave irradiation [13]. In summary, the application of conventional heating for energy input is a wide-spread opportunity, however, energy transfer and its rate are dependent on the heat conductivity of both the vessel containing the reaction mixture and the reaction mixture itself. On the contrary, unconventional, microwave-aided heating is independent of such parameters [14,15].

Consequently, the possible advantage of employing microwave irradiation is that a remarkably higher power density (amount of energy input to a certain volume of reaction media in a given timeframe) can be ensured so that it may lead to a reduction in the curing times needed for efficient sludge pretreatment.

It is to note that the usage of microwave energy is emerging to enhance the digestibility of complex (e.g. lignocellulosic) raw materials so as to achieve enhanced biohydrogen fermentation performance [16–18]. However, microwave applications for seed culture pretreatment are not well studied, especially not in microwave reactors under controlled conditions as intended herewith. Up to our knowledge, the current literature is lacking a comparative assessment of the conventional and unconventional heat treatments for seed enrichment purposes.

Therefore, in this work, it was aimed to investigate the feasibility and impacts of these different, conventional and microwave-assisted, unconventional pretreatment methods on the biohydrogen production potential of mixed microbial consortia.

2. Materials and methods

2.1. *Seed sludge and related analytical methods*

Seed sludge was collected from a domestic biogas fermenter processing mainly food waste. To calculate soluble COD, reducing sugar and protein contents, appropriate samples were centrifuged (5 min, 12,000 rpm) and then filtered through a 0.45 μm pore size membrane disc in order to get rid of the suspended solids. The supernatant obtained was used to determine soluble COD by following the procedure of APHA [19]. Reducing sugars in the supernatant solution were analyzed by o-toluidine method and expressed as mg glucose^{eqv}/L. The concentration of proteins (mg/L) in the liquor was estimated based on the Folin reaction.

2.2. *Sludge pretreatment conditions*

Seed inocula were pretreated either by means of conventional and unconventional methods. The exact treatment circumstances are presented in Tables 1 and 3. Conventional thermal curing was carried out in an ordinary water bath with occasional mechanical stirring (30 s in each 5 min). For unconventional, microwave-aided method a commercially available, lab-scale instrument was used (Discover series, BenchMate model, CEM Corp., USA). The device has 5 mL capacity sealable tubes to treat samples, in our case anaerobic biogas sludge. It is equipped with a magnetic stirrer, able to maintain a constant experimental temperature with the accuracy of ±1 °C through air cooling and by automatically varying the power output (amount of energy emitted to a certain volume of sample in a given time – J/mL-s). To attain the desired pretreatment temperatures output power of 100 W was set as an upper limit. For all the pretreatments (being either conventional or unconventional) the reaction times have started when the samples reached the desired temperature.

2.3. *Hydrogen fermentation experiments*

Biohydrogen production measurements were performed in 100 mL batch vials sealed by rubber stoppers. 25 mL working (liquid phase) volume was applied. Initial pHs were set to 6.5 by droplets of 0.5 M HCl. Prior to starting the fermentations, high purity N_2 sparging was employed for 5 min to provide a fully anaerobic environment in the bottles. The closed vessels were placed in a reciprocal air-bath shaker at 150 rpm under controlled temperature (37 °C) for 24 h. Potato starch (5 g/L) and yeast extract (2 g/L) was added to the pretreated inocula as substrate and external nitrogen/mineral source during the experimental runs, respectively.

Determination of biogas produced was checked by glass syringe method after 24 h when fermentations were over. Headspace gas composition was determined by gas chromatography (HP 5890 gas chromatograph, CarboPlot column – 90 °C, TCD – 115 °C, N_2 carrier gas).

Table 1 – The pretreatment circumstances applied during conventional heat shock.

Pretreatment temperature (°C)	Pretreatment time (min)	H_2 productivity (mL H_2/mL-d)
75	30	0.78
75	50	0.76
85	40	0.73
85	40	0.65
85	40	0.82
95	30	0.57
95	50	0.07