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Review on the start-up experiences of continuous fermentative hydrogen producing bioreactors

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ABSTRACT

The start-up of continuous biohydrogen fermentations is a complex procedure and a key to acceptable hydrogen production performance and successful long-term operation. In this review article, the experiences gained and lessons learned from relevant literature studies dealing with various aspects of H_2 producing bioreactor start-up are comprehensively surveyed. Firstly, the importance of H_2-forming biosystem start-up including its main steps is outlined. Afterwards, the role of main influencing factors and methods (e.g. strain selection, seed pretreatment and inocula stimulation, switch-over time, bioreactor design, operating conditions) in avoiding the deterioration of starting a reactor is analyzed and presented in detail. Finally, the so far suggested applicable start-up strategies and the corresponding findings are critically discussed pointing out the advantages and disadvantages of each strategy.

Contents

1. Introduction

Hydrogen is an emerging candidate among the various alternative energy carriers. H_2 is believed to help the transition of current fossil-based economy to a renewable-based one [1], however, only if it is derived by sustainable processes. Though H_2 can be prepared by many conventional and mature methods (e.g. steam reformation of hydrocarbons), environmental-friendly methods such as with biological routes are required and still subjects to extensive research [2].

Nowadays, microbiologically produced hydrogen is recognized as an emerging way ahead, especially when formed via dark fermentation because of its inherent advantages such as relatively low energy demand (attributed to the gentle reaction conditions), the usability of wide range of feedstocks e.g. derivates of biomass, waste streams and agricultural residues [3–5], and the possibility to integrate with other e.g. membrane-based processes in order to accomplish the sufficient reuse of hydrogen producing cells [6] or to upgrade bioH_2 [7–9] so that it could be a viable feedstock in energy efficient fuel cells.

Nevertheless, additional efforts are still essential to make biohydrogen generation more attractive. From practical aspect, the two major criteria to be considered are H_2 production yields and rates. As a result of the investigations in the past decades, several factors were identified that significantly affect the main

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technological indicators mentioned above. Among them, bioreactor configuration and operation are apparent ones [10]. Regardless the type of the fermenter, it can be concluded that feasible biohydrogen fermentation should be conducted in continuous rather than batch systems [11], e.g. due to higher expectable process efficiencies.

The establishment of continuous flow bioreactors usually starts in batch mode, and it is to note that successful transition and reliable, long-term operation is highly influenced by the start-up strategy applied [12–14]. However, up to the authors' best knowledge, there is no recent review paper comprehensively surveying start-up experiences in continuous hydrogen producing bioreactors. Hence, in this work, the experiences gained and lessons learned from batch to continuous shifts are reviewed and suggestions are given to achieve proper continuous operation.

2. The role of start-up in the efficacy of continuous hydrogen production

Process instability is a frequently observed drawback in fermentative H_2 production [15] that could be attributed to multiple reasons as specified later in this paper. In fact, beyond steady-state operational parameters and medium composition, stable and continuous bioreactor operation to obtain acceptable hydrogen production performance is strongly dependent on the start-up phase [16]. It could involve the following steps:

- Selection of the hydrogen producing biocatalysts.
- Enhancement and acclimatization of H_2-forming strains to fermentation circumstances.
- System transition until steady-state is reached.

These steps in a line require great attention and comprehensive control in terms of environmental and operational circumstances to develop robustful H_2 fermenting culture [17]. Otherwise, starting a reactor may easily be deteriorated e.g. due to the insufficient growth and H_2 production capacity of microorganisms. Such bottlenecks can be avoided or at least mitigated by properly designed start-up strategy.

In the next sections of the paper, the aforementioned parts of the continuous dark fermentative bioreactor establishment are outlined and discussed in details.

3. Factors affecting the initiation of continuous H_2 fermenters

3.1. H_2 producing strains

Fermentative biohydrogen generation can be realized either by pure cultures [18] such as *Escherichia coli* [19,20] or mixed bacterial consortia [21,22] and both have their own benefits. For example, cultures of pure isolates may be easier to control but need constantly sterile environment to prevent contamination that is difficult and costly to maintain out of laboratories. Considering their application in a non-sterile environment, pure cultures may be used in the bioaugmentation of diverse H_2 producing population to attain better gas turnouts [23]. The restrictions of sterility criteria are the main reasons why mixed bacterial communities are preferred to their pure counterparts in real-case, scaled-up applications.

3.2. Pretreatment and stimulation

Conceptually, anaerobic, mixed H_2-producing consortia (e.g. in sewage sludge, biogas plants, etc.) are built up by co-existing and synergic species [24]. However, in most cases, they naturally occur together with H_2-consumer microorganism such as methanogenic archaea, homoacetogenic (producing acetate from CO_2 and H_2)-, lactic- and propionic acid bacteria which must certainly be suppressed or more preferentially totally eliminated [25]. As a consequence, regardless the source of mixed inocula, it should undergo initial pretreatment in order to select the desired whole cell biocatalysts. For such purposes, a lot of tools have been developed based on heat shock, addition of chemicals, swinging the oxidation–reduction potential (ORP) e.g. by aeration, high energy irradiation, alteration of pH, freezing and thawing [26–28]. These pretreatment techniques exploit the distinct sensitivity of strains present in the mixture and in general could provide a satisfactory starter culture to be used as seed inocula for subsequent biohydrogen fermentation. In other words, these procedures aim to eliminate hydrogen-consuming vegetative cells and on the other hand, are devoted to enhance acidogenic- and often sporulative H_2-forming cells [29].

Although culture pretreatments can effectively suppress undesired microbiological activity, they may also reduce the number of indigenous H_2-former bacteria, especially the ones with low stress tolerance. For these reasons, as a next step after culture pretreatment, treated inocula should be submitted to stimulative environment (e.g. to a batch reactor) to let the microbes proliferate so that a reasonable amount of active cellmass can be accumulated, harvested and further applied. Also, batch cultivation can play a role to help biofilm development on carrier materials (e.g. powdered- and granulated activated carbon) if an immobilized, continuous H_2 production system is to be implemented [30,31].

According to literature reports, pretreated inocula are more often than not dominated by spore-forming and robust H_2-producer species such as *Clostridium* sp. [13]; however some organisms of no utility (e.g. propionic acid and homoacetogenic bacteria) may also survive and reclaim their niche over time [21,25]. Changes in the microbial background can be revealed by the modern technical apparatus of molecular biology [32,33].

Furthermore, it is presumable that the age of the seed source – most commonly sewage or biogas (anerobic fermenter) sludge as suggested by Tables 1 and 2 – may also be a factor to take into account. It is assumable that the microbial community structure of anaerobic mixed cultures varies constantly during storage due to changes (e.g. concentration differences) within micro-environments. Consequently, aging of an anaerobic seed culture over time can result in the variation of the obtainable bacterial populations and their activity. Thus, it might lead to alterations in the attainable biohydrogen performances even though standardized, identical pretreatment conditions are ensured time after time to prepare H_2 producing inocula.

Moreover, beyond the goal of activating H_2-producer organisms [16,34,35], preliminary cultivation – mostly in batch – may also serve as a tool to acclimatize the microflora to certain substrates and their loadings e.g. to overcome inhibitory effect [36], which will induce a dynamic competition between the various groups of bacteria. Although batch-continuous start-up strategy was proposed by various authors to follow (for examples, please refer to Tables 1 and 2), some researchers reported adequate start-up directly in continuous operation [37–41].

The advantage of this strategy lacking initial discontinuous cell growth might be that in batch operation the nutrient concentrations as the time passes, especially at the end hours of the cycle, are insufficiently low and consequently a shift in the dominant strains could occur, depressing H_2 production [12]. During careful continuous adaptation, broth is constantly supplemented and such disadvantageous phenomenon may be avoided. Moreover, continuous (hydraulic detention time influenced) acclimatization strategy encompasses the so-called biokinetic control which causes the