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Enhancement of biofuel production via microbial augmentation: The case of dark fermentative hydrogen

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ARSTRACT

This review portrays the status and perspectives of bioaugmented hydrogen fermentation, an emerging strategy of process intensification. Firstly, the paper introduces the potentials and limitations of dark fermentative hydrogen production and describes the technologies available for its enhancement including bioaugmentation. The theoretical background and practical features of augmentation methods for biohydrogen generation are subsequently assessed and surveyed in details. Furthermore, a throughout evaluation of the recent and novel achievements reported in the concept of "augmented hydrogen fermentation" is given in association with (i) bioreactor start-up, (ii) utilization of solid wastes, wastewaters, (iii) the feasibility in continuous systems as well as in complementary – integrated – applications. The article is intended to provide an insight to the advancements made for realizing more viable biohydrogen formation via bioaugmentation and hence it might be encouraging for further studies in the field.

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1. Introduction of strategies to improve fermentative hydrogen production

The continuous development of processes serving bio-based product formation is a way to promote the sustainability that these materials offer $[1]$. The production of biohydrogen, a potential biofuel and renewable energy carrier is governed by several biotic and abiotic factors. In the former group, hydrogen synthesizing bacteria, as microscopic bio-engines play a key role and power dark fermentative reactors. They act as the pumping heart of the technology and their performances are substantially affected by the fermentation conditions (abiotic parameters). The effectiveness of hydrogen forming bioreactors is primarily assessed by taking H_2 productivity and yield into account. Therefore, the ultimate aim of all the research efforts taken is to establish attractive systems in both terms, which rely heavily on the whole cell living biocatalysts. Nowadays the dark fermentative route is the most practically applicable to carry out biotechnological hydrogen production, however, this process has particular limits since a considerable part of the substrates used are converted into various soluble metabolic products rather than $H₂$. This means that the energy chemically stored in the raw material is not fully recoverable in the form of molecular hydrogen gas and a liquid residue with high chemical oxygen demand is obtained. This fraction is the major side-product of dark fermentative hydrogen technology and can be considered as a multi-compound mixture containing short-chain volatile fatty acids (e.g. acetic acid, butyric acid, propionic acid) and other constituents such as alcohols (e.g. ethanol, butanol), depending on the circumstances i.e. type of inoculum [\[2\].](#page--1-0) The notable formation of these substances (acids, alcohols) is one of the greatest challenges for fermentative hydrogen technology since it significantly cuts the achievable H_2 yields (mol $\rm H_2$ mol $^{-1}$ substrate) and dictates economy. Up to now, not a single organism (wild-type strains) has been discovered in the nature that could exceed the Thauer-limit, equaling to 4 mol \rm{H}_{2} mol $^{-1}$ glucose and being far behind the theoretical maximum of 12 mol $\rm H_2$ mol $^{-1}$ glucose [\[3,4\].](#page--1-0) This is definitely a key driver and many efforts have been put in the recent decades to facilitate the utilization of the organic matter rich spent media and push the upper bound performances. In particular, downstream-associated processes (Fig. 1) have been introduced and spotlighted [\[5\].](#page--1-0) In this repertoire, the most traditional complementary option is anaerobic digestion (methane production), where organic components are degraded to get methane $[6,7]$. In that way, a blend of hydrogen and methane, called biohythane can be obtained and considered as a clean vehicle fuel possessing beneficial combustion properties [\[8,9\]](#page--1-0). In such a sequential (two-step) design, biogas formation, as second stage after the hydrogen producing reactor, might be replaced by photo-fermentation when dark fermentation

effluent is biotransformed into extra hydrogen by the aid of (sun) light [\[10,11\]](#page--1-0). Besides, additional competing techniques referred as Bioelectrochemical Systems (BESs) are also among the possibilities [\[12\].](#page--1-0) Traditional BESs are built in two-chamber arrangement, where an anaerobic anode and an aerobic/anaerobic cathode are spatially detached – mostly by a thin proton-selective membrane – and at the same time connected by external wiring. For dark fermentation, two derivatives of BESs, namely microbial fuel cells (MFC) and microbial electrohydrogenesis cells (MEC) are the most potential systems [\[13](#page--1-0),[14\].](#page--1-0) In principles, both in MFC and MEC, special electrochemically-active microorganisms work under anaerobic environment as part of a biofilm formed on the anode surface and have the capability to transform a wide range of organic streams – e.g. wastewaters as well as the effluent of the hydrogen producing bioreactors – either into bioelectric power (in case of MFC) or hydrogen (in case of MEC) [[15](#page--1-0)–17]. Therefore, MFC and MEC offer solutions to assist the conversion of biohydrogen production residue and simultaneously fulfill the dual aim of waste (effluent) reduction and energy (carrier) generation (from the effluent) $[18]$. The further attractiveness of these methods is the reduced ecological impact since the theoretical cathode-side products of MFC and MEC are only water and $H₂$, respectively. Additionally, it has turned out that the introduction of MEC technology could lead to impressive, 9.6 mol H_2 mol⁻¹ glucose hydrogen yield, representing 80% of the theoretical maximum [\[19\].](#page--1-0) Hence, dark fermentative biohydrogen production integrated with MEC stands as a hot field for research. Another possibility to utilize the volatile fatty acid-rich fermentation effluent is the production of biologically synthesized polyesters e.g. polyhydroxyalkanoate [\[20,21\]](#page--1-0) which could have an industrial market [\[22\].](#page--1-0) Some alternative routes for the valorization of dark fermentative effluents in the frame of "Hydrogen biorefinery" have been introduced by Sarma et al. [\[23\]](#page--1-0).

Beyond these downstream-side methods exploiting the potential of high energy content fermentation spent media, various paths of upstream-related process improvements have been applied. These include, but are not limited to the optimization of reactor operation and fermentation circumstances, basically temperature, pH, substrate concentration, hydraulic retention time, organic loading intensity [\[24](#page--1-0)–[27\]](#page--1-0), hydrogen recovery by membrane separation to reduce partial pressure and purify the product [\[28](#page--1-0)-[32\]](#page--1-0), reactor design [\[33](#page--1-0)-[36\]](#page--1-0) and inoculum selection/pretreatment [\[37](#page--1-0)–[39\]](#page--1-0). All of these methods aim to ensure better surrounding to the microbial catalysts and thereby achieve better gas formation performance. Moreover, the employment of recombinant (metabolic engineered) strains appeared as a straight way forward to exceed the low to moderate actual gas production capacities [\[4,40\]](#page--1-0).

Fig. 1. Downstream-side possibilities of improving dark fermentative H_2 production efficiency.

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