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Revealing the factors influencing a fermentative biohydrogen production process using industrial wastewater as fermentation substrate

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Abstract

Background: Biohydrogen production through dark fermentation using organic waste as a substrate has gained increasing attention in recent years, mostly because of the economic advantages of coupling renewable, clean energy production with biological waste treatment. An ideal approach is the use of selected microbial inocula that are able to degrade complex organic substrates with simultaneous biohydrogen generation. Unfortunately, even with a specifically designed starting inoculum, there is still a number of parameters, mostly with regard to the fermentation conditions, that need to be improved in order to achieve a viable, large-scale, and technologically feasible solution. In this study, statistics-based factorial experimental design methods were applied to investigate the impact of various biological, physical, and chemical parameters, as well as the interactions between them on the biohydrogen production rates.

Results: By developing and applying a central composite experimental design strategy, the effects of the independent variables on biohydrogen production were determined. The initial pH value was shown to have the largest effect on the biohydrogen production process. High-throughput sequencing-based metagenomic assessments of microbial communities revealed a clear shift towards a *Clostridium* sp.-dominated environment, as the responses of the variables investigated were maximized towards the highest H_2-producing potential. Mass spectrometry analysis suggested that the microbial consortium largely followed hydrogen-generating metabolic pathways, with the simultaneous degradation of complex organic compounds, and thus also performed a biological treatment of the beer brewing industry wastewater used as a fermentation substrate.

Conclusions: Therefore, we have developed a complex optimization strategy for batch-mode biohydrogen production using a defined microbial consortium as the starting inoculum and beer brewery wastewater as the fermentation substrate. These results have the potential to bring us closer to an optimized, industrial-scale system which will serve the dual purpose of wastewater pre-treatment and concomitant biohydrogen production.

Keywords: Biohydrogen, Central composite experimental design, Microbial inocula, Metagenomics, Industrial wastewater

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