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TOXIC IMPACT OF HEAVY METALS AND MICROBIAL COMMUNITY VARIETY DURING ANAEROBIC FERMENTATIVE HYDROGEN PRODUCTION

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Organic industrial and municipal waste is contaminated with various heavy metals. The studied samples of compost, solid and liquid digestate contain heavy metals such as Cd (0.16 mg/kg), Co (3.98 mg/kg), Cr (17.7 mg/kg), Cu (76.2 mg/kg), Ni (14.48 mg/kg), Pb (39 mg/kg), Zn (159.2 mg/kg). Attempts to produce hydrogen gas from contaminated organic waste have revealed that some heavy metals, such as Zn in low concentrations, can stimulate hydrogen gas production. However, the toxicity of heavy metals to fermentative hydrogen production has been thoroughly investigated. This study investigated the effect of heavy metals alone or in combination on biological hydrogen production. The results can be used as reference data for a comprehensive analysis of the effects of various heavy metal types at different concentrations. The microflora for seeding was collected from a sewage treatment plant and inoculated with synthetic medium.

The variable region of the 16S rDNA gene was amplified by PCR using the extracted DNA as a template. A clip 341f GC was added to the forward primer and the second step of the PCR procedure was repeated.

A semi-4-parameter logistic model was used to determine the chemical impact on the biological process and to indicate the concentration of heavy metals needed to inhibit hydrogen production by half.

The correlation between two heavy metals can be evaluated based on the value of the additive toxicity Index (AI). If the calculated AI value is greater than 0, it is a synergistic heavy metals interaction.

The modified Gompertz equation was used to estimate the production potential, maximum production rate, and lag time of hydrogen gas in the presence of single heavy metals. The results were similar to those of another study under the same conditions. The concentration of Zn had a significant influence on the hydrogen gas yield. The yield declined as the Zn concentration was increased to over 1.0 ppm. The results showed that Zn had a positive effect on the hydrogen yield in the relevant concentration range, but higher Zn concentrations inhibited the metabolism of hydrogen production. In addition, higher Zn concentrations had a negative effect on nitrogenase activity.

The Cd concentration at 0e20 ppm showed an inhibitory effect on the accumulation of hydrogen production. At a concentration above 1.0 ppm, the hydrogen gas production decreased to 597.82 mL/L. Cd is a biological inhibitor that can block the expression of genes related to iron and copper homeostasis, and can inhibit the methanogenetic process. However, at very small concentrations, Cd has a stimulating effect on the fermentative hydrogen production in mixed culture.

At all concentrations, the addition of Cu to the experimental batches showed an inhibitory effect on hydrogen production. The highest hydrogen gas yield was 952.55 mL/L at 0.5 mg/L Cd and the lowest hydrogen gas yield was 178.58 mL/L at 20 ppm Cd.

The Pb supplementation inhibited hydrogen production in the present study. The inhibition ranged from 41% to 85% for Pb concentrations from 0.50 to 20.0 mg/L, and the hydrogen yield increased by 3.5% at a low concentration of Pb (0.5 mg/L). This study investigated the effects of two heavy metals together on fermentative hydrogen production. The concentration of each heavy metal was the same as the concentration used in the single heavy metal experiment.

The present study found that the presence of more than 5.0 mg/L of Zn cd mixture had a stimulating effect on hydrogen production during sucrose fermentation, but the presence of 0.5 or 1.0 mg/L of Zn cd mixture had an inhibition effect on hydrogen production compared with the control experiment (sucrose only).

A 4-parameter logistic model was used to estimate the IC50 for hydrogen production in the presence of heavy metals. The lowest IC50 value indicated the highest inhibitory effect, and the relationship between the average cumulative hydrogen production and the IC50 value contributed to the predictive behavior of bio-hydrogen production. The hydrogen gas yield was stimulated at low concentrations by Pb, Cu, Cd, and Zn, but decreased at higher concentrations.

The additive index was calculated to understand how the two heavy metals interacted when mixed. The Zn cd interaction showed a less inhibitory effect by being antagonistic, and the cumulative hydrogen production was higher than that with a single Zn or Cd application. In this study, the ratio of n-butyrate/acetate (HBu/HAc) was used to indicate the performance of the hydrogen fermenter. The HBu/HAc ratio ranged from 0.2 to 2.9 depending on the heavy metal concentrations.

Using the 16S rDNA gene-directed PCR-DGGE method, 213 bands were identified in 16 different positions (or 16 species). Most of the bacterial taxa were related to the phylum Bacteria. Bordetella sp., Lactococcus raffinolactis, and non-cultured bacteria are not clearly recognized as hydrogen-producing strains, but a Bordetella avium strain can generate hydrogen and polyhydroxybutyrate.

The Shannon-Weaver index (H') was used to measure the diversity of the microbial community in the batch reactor. The highest index values were detected for Cu Cd at 20.0 mg/L and Cu Pb at 5.0 mg/L.

The Pielou index was used to indicate the richness of hydrogen-producing bacteria in microbial communities. A higher Pielou index indicated a relatively higher production of hydrogen. This study demonstrated that heavy metals have an effect on the rate of fermentative hydrogen production. The amount of hydrogen produced per HBu/HAc ratio differs between single heavy metals and mixed heavy metals, and the richness of the hydrogen-producing co-culture is an important factor in hydrogen production.

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