Abstract

The dwindling fossil reserves coupled with environmental pollution necessitate the search for clean and sustainable energy resources. Biohydrogen is emerging as a suitable alternative to fossil fuels and has received considerable attention in recent years due to its economic, social, and environmental benefits. However, the industrial application of biohydrogen has been hindered by low yield. Therefore, development of novel techniques to enhance the yield is of immense importance towards large-scale production of biohydrogen.

Thus, this research effort explored various options to enhance the yield of biohydrogen during dark fermentation process. Some options explored included (i) the utilization of feedstocks from the agricultural, industrial and municipal sectors, (ii) parametric optimization of biohydrogen production, (iii) investigation of biohydrogen production using metal ions and nitrogen gas sparging, and (iv) assessing the feasibility of biohydrogen scale-up study to pave the way for pilot-scale development. Solid biowaste feedstocks consisting of apple, bread, brewery residue, cabbage, corn-cob, mango, mealie-pap, pear, potato, and sugarcane were investigated for dark fermentative biohydrogen production using anaerobic mixed sludge. The experimental results showed that substrates which are rich in carbohydrates are suitable for dark fermentative biohydrogen-producing bacteria. Consequently, a maximum biohydrogen fraction of 43.98, 40.32 and 38.12% with a corresponding cumulative biohydrogen yield of 278.36, 238.32 and 215.69 mL H_2/g total volatile solids (TVS) was obtained using potato, cabbage, and brewery wastes, respectively. Based on these results, potato waste was chosen as a suitable substrate for subsequent biohydrogen production studies.

Parametric optimization was carried out on biohydrogen production via dark fermentation using potato waste as the substrate. Effects of operating variables such as pH, temperature,

fermentation time, and substrate concentration were investigated via response surface methodology (RSM) approach using a two-level-four factor (2^4) central composite design (CCD). The obtained predictive model (statistical model) was used to explain the main and interaction effects of the considered variables on biohydrogen production. In addition, the model was employed in the optimization of the operating conditions. Consequently, a second-order polynomial regression with a coefficient of determination (R^2) of 0.99 was obtained and used in the explanation and optimization of operating variables. The optimum operating conditions for biohydrogen production were 39.56 g/L, 5.56, 37.87 ^oC and 82.58 h for potato waste concentration, pH, temperature and fermentation time, respectively, with a corresponding biohydrogen yield of 68.54 mL H_2/g TVS. These results were then validated experimentally and a high biohydrogen yield of 79.43 mL H_2/g TVS indicating a 15.9% increase was obtained. Furthermore, the optimized fermentation conditions were applied in the scale-up study of biohydrogen production that employed anaerobic mixed bacteria (sludge) which was immobilized in calcium alginate beads. A biohydrogen fraction of 56.38% with a concomitant yield of 298.11 mL H_2/g TVS was achieved from the scale-up study.

The research also investigated the influence of metal ions (Fe^{2+}, Ca^{2+}, Mg^{2+} and Ni^{2+}) on biohydrogen production from suspended and immobilized cells of anaerobic mixed sludge using the established optimal operating conditions. A maximum biohydrogen fraction of 45.21% and a corresponding yield of 292.8 mL H_2/g TVS was achieved in fermentation using Fe^{2+} (1000 mg/L) and immobilized cells. The yield was 1.3 times higher than that of suspended cultures. The effect of nitrogen gas sparging on biohydrogen conversion efficiency (via suspended and immobilized cells) was studied as well. Cell immobilization and nitrogen gas sparging were effective for biohydrogen production enhancement. A maximum biohydrogen fraction of 56.98% corresponding to a biohydrogen yield of 294.83 mL H_2/g

TVS was obtained in a batch process using nitrogen gas sparging with immobilized cultures. The yield was 1.8 and 2.5 times higher than that of nitrogen gas sparged and non-sparged suspended cell system, respectively.

Understanding the functional role of microorganisms that actively participate in dark fermentation process could provide in-depth information for the metabolic enhancement of biohydrogen-producing pathways. Therefore, the microbial composition in the fermentation medium of the optimal substrate (potato waste) was examined using PCR-based 16S rRNA approach. Microbial inventory analysis confirmed the presence of *Clostridium* species which are the dominant biohydrogen-producing bacteria.

The results obtained from this research demonstrated the potential of producing biohydrogen using South African solid biowaste effluents. These feedstocks are advantageous in biohydrogen production because they are highly accessible, rich in nutritional content, and cause huge environmental concerns. Furthermore, optimization techniques using these feedstocks will play a pivotal role towards large-scale production of biohydrogen by increasing throughput and reducing the substrate costs which accounts for approximately 60% of the overall costs. The findings from this research also provide a solid basis for further scale-up and techno-economic studies. Such studies are necessary to evaluate the competitiveness of this technology with the traditional processes of hydrogen production. In summary, the findings from this research effort have been communicated to researchers in the area of biohydrogen process development in the form of peer-reviewed international scientific publications and conference proceedings, and could provide a platform for developing an economic biohydrogen scaled-up process.