Impact of medium pH regulation on biohydrogen production in dark fermentation process using suspended and immobilized microbial cells

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ABSTRACT
The effect of pH regulation on biohydrogen production was studied using suspended and immobilized mixed cultures. Four sets of dark fermentation processes were carried out using suspended cells under regulated pH (Sus_R) and non-regulated pH conditions (Sus_N), the nalginate-immobilized cells under pH regulated (Imm_R) and non-pH regulated conditions (Imm_N). Sus_R showed a peak hydrogen fraction of 44% and complete glucose degradation, compared to Sus_N with a peak hydrogen fraction of 36% and a glucose degradation of 37%. Imm_R experiments showed a peak biohydrogen fraction of 35%, while the peak hydrogen fraction observed with Imm_N was 22%. The highest hydrogen fraction was observed using Sus_R conditions. A 100% glucose degradation was observed in both pH regulated and non-regulated processes using immobilized cells. The rate of pH change was slower for immobilized cells compared to suspended cells suggesting a better buffering capacity under non-pH regulated conditions. The study showed that biohydrogen production with suspended cells in a non-regulated pH environment resulted in early termination of the process and lower productivity.

KEYWORDS
pH regulation; biohydrogen production; suspended cells; immobilized cells; dark fermentation

Introduction
A large fraction of the global energy needs currently relies on fossil fuels and the constantly increasing human population has led to an upsurge in the global energy demand which may eventually result in the complete depletion of fossil fuel reserves in the near future [1]. In addition, fossil fuels are expensive as their combustion results in the release of various pollutants including greenhouse gases, and thereby, raising concerns for global climate change [2,3]. These challenges have fuelled the need to seek for alternative energy sources that are sustainable, renewable and non-polluting. Biohydrogen has been identified as a propitious alternative owing to its high energy content as well as renewable and environmentally friendly qualities [4–6]. Furthermore, fermentative biohydrogen production offers the advantage of being less energy intensive and can be operated at ambient temperature thus, making it a feasible option for large-scale hydrogen production [7]. Hydrogen production through the dark fermentation technology is the most commonly used production process [8]. It refers to the process of converting organic materials to biohydrogen through a cascade of biochemical reactions by fermentative microorganisms in the absence of oxygen [1,8] and has the dual advantage of being a non-polluting biofuel as well as an effective method for wastewater treatment [1]. Clostridium species have been found to play a key role in biohydrogen production. Hydrogen production by Clostridia typically employs the acetate/butyrate pathway, in which pyruvate is broken down to produce hydrogen, acetate, butyrate and ethanol as major end-products [9,10]. Clostridium species generally produce hydrogen gas during their exponential growth phase until the population reaches stationary phase. At this point, the metabolism shifts from hydrogen and acid production to solvent production. This shift in metabolism known as solventogenesis is induced by a drop in the pH of the fermentation broth below a certain threshold [11].

The hydrogen-producing efficiency of Clostridium has been reported in several studies [2,11]. Clostridium species have an optimum yield that varies between 1.1 and 2.6 mol H$_2$/mol glucose, depending on the substrate type and process conditions. Khanal et al. [11] reported a maximum hydrogen yield of 1.4 mol H$_2$/mol glucose using a mixed culture of Clostridium species on glucose-based medium. On the other hand, Liu et al. [2], using cultures of three Clostridium isolates, obtained hydrogen yields ranging from 1.72 to 1.83 mmol H$_2$/mmol of glucose with the highest yield being achieved by a pure culture of Clostridium butyricum.
Although fermentative biohydrogen is a promising energy source, in order for its production to become commercially viable, in-depth knowledge of the impact of key physico-chemical parameters such as pH and the limitation of flask experimental-based models of process development are required to overcome the challenges associated with biohydrogen production such as low yield and high production costs [1, 12]. The medium pH has been reported as one of the major parameters affecting biohydrogen production. Bacteria generally respond to changes in internal and external pH by shifting their activity associated with various metabolic processes such as proton translocation, substrate degradation [13, 14]. Variations in pH can disrupt molecular structures and affect intracellular and extracellular reactions. For example, at extreme pH, proteins are denatured and the DNA double-helix strand is destabilized. Since many microorganisms involved in anaerobic fermentation produce acids as metabolic by-products [15], there is a constant drift in the linearity of the pH within the production system. This drift leads to metabolic shift in hydrogen-producing bacteria, which could result in the inhibition of product formation. Reported flask-scale experimental studies of biohydrogen without pH control abound in the public domain. A study by Liu et al. [16] reported an initial pH of 7.0 without pH regulation for the production of biohydrogen using a combination of dark and photo-fermentative batch culture. In the same vein, various studies on biohydrogen production at flask scale only stated the initial pH value without indication of further regulation during the fermentation process [3, 11]. To a great extent, the sensitivity of metabolic shift under pH drift poses significant challenges on experimental models with the pH parameter among the input variables. This is evidenced in the assessment of the initial pH and glucose concentration interaction on biohydrogen production kinetics [17].

The use of immobilized cell systems in bioprocess development has several advantages as compared to suspended cells systems. These include better handling and the repeated use of cells as well as improved solid to liquid separation efficiency [18]. Immobilized cell culture systems for the continuous production of hydrogen using pure and mixed cultures have been reported [18–20]. However, there is a dearth of knowledge in the public domain on the potential of cell immobilization as a pH-buffering matrix for anaerobic biofuel fermentation processes.

Various studies for biohydrogen production have been carried out using shake flask bioreactors. These flasks are advantageous in the fact that they allow the operation of simultaneous experiments [21]. Inspite of their widespread acceptance, shake flasks have significant limitations. For example, conventional flask shakers shake according to linear reciprocating movement. This concept is well established and the associated mixing deficiencies are well understood [22]. Some of the challenges include the dissimilarities in oxygen transfer due to differences in the agitation systems of shake-flask and pilot-scale fermenters as well as inconsistencies in aeration, mixing efficiency, temperature and pH regulation systems [23]. The ramifications of these deficiencies impact on data validity for process scale-up. The non-linearity associated with unregulated pH poses many difficulties for bioprocessing and has been linked to reduced bioprocess yields. Ironically, most reported biohydrogen flask-scale data generated under non-pH-regulated culture systems form the basis of process scale-up in pH-regulated pilot-size bioreactors.

On account of the above, this study aims at investigating the effects of regulated and non-regulated pH on hydrogen production in shake-flask bioreactors using suspended and immobilized cell systems.

**Materials and methods**

**Inoculum development**

The hydrogen-producing mixed consortium used in this study was obtained from the anaerobic digested sludge collected from Darvill wastewater treatment plant, Pietermaritzburg, South Africa. The collected sludge was immediately transferred to the laboratory and stored at 4 °C. A combination of alkaline and heat treatment was applied as previously described by Faloye et al. [24]. This pre-treatment step was included to inactivate methanogens and promote the survival of endospore-forming hydrogen producers.

**Fermentation process in batch reactors using suspended cells**

Two sets of fermentation processes were carried out in duplicate in 250 mL conical Erlenmeyer flask bioreactors under both regulated and non-regulated pH conditions. The reactors were fed with glucose as a substrate at a concentration of 10 g/L and supplemented with inorganic salts at the following concentration (g/L): 1.5 KH2PO4, 1.5 K2HPO4, 2.0 (NH4)2SO4, 0.1 FeCl, 0.1 CaCl2·2H2O, 0.05 ZnSO4, 0.01 Na2MoO4 and 0.08 MnCl2·6H2O. Each reactor was inoculated with 25 mL of pre-treated sludge and made up to a total working volume of 250 mL with the inorganic salts media. Anaerobic conditions were induced by purging the reactors with nitrogen gas for 5 min. The initial pH was adjusted to 6.5 with 0.1 mol/L NaOH or 0.1 mol/L HCl. Fermentation
experiments were carried out in a duplicate under non-regulated pH conditions in a shaking water-bath with operational set-points of 37.5 °C and 180 rpm for temperature and agitation, respectively. Control experiments under regulated pH at 6.5 were run in parallel. The pH control systems consisted of an Alpha 190 automatic controller connected to a peristaltic dosage pump dispensing an amount of 1 mol/L NaOH whenever the pH drift below the set-point value. Thus, a closed feedback control loop was implemented as shown in Figure 1. All experiments were carried out in triplicates for a process time of 48 h.

**Fermentation process in batch reactors using immobilized cells**

Two sets of immobilized culture experiments were carried out under regulated and non-regulated pH conditions. The mixed microbial cells were immobilized by entrapment into the sodium alginate beads. Sodium alginate (Sigma) was dissolved in distilled water and autoclaved for 15 min to form a final alginate concentration of 3%. The sodium alginate solution when cooled was mixed with an equal volume of the pre-treated anaerobic sludge to a form a 50:50 alginate-to-sludge ratio. Granular activated carbon (GAC) (2%) was added to the mixture to increase bead porosity. The alginate/slugde mixture was then transferred through a peristaltic pump into a 0.2 mol/L CaCl solution to form beads of approximately 0.3 mm in diameter.

Each reactor was inoculated with a 20% v/v concentration of alginate-sludge beads and made up to a total working volume of 250 mL with the inorganic salts solution containing 10 g/L glucose. The initial pH was adjusted to pH 6.5. Regulated pH experiments were set up as described above in Figure 1. All experiments were carried out in a duplicate in a water-bath shaker at a constant temperature of 37.5 °C and an agitation of 180 rpm for a process time of 48 h.

**Process monitoring and analysis**

Changes in the biohydrogen fraction of the evolving gas were measured continuously using the F-Lab Biogas Software previously described by Gueguim Kana et al. [25] with a sampling frequency of 1 min and equipped with a BCP-H2 sensor (Blue-Sens, Germany). The sensor has a measuring range of 0%–100% hydrogen and employs the thermal conductivity measurement principle. Effluent samples were taken from the reactor at the lag and the exponential phase, at optimal hydrogen production as well as at the decline phase and the residual glucose concentration was measured using a glucose analyser (Model 200 Select, YSI, USA). For non-pH-regulated experiments, the pH was continuously monitored at 1-min interval using a pH probe (Crison, South Africa) connected to a programmed Arduino Microcontroller data-logger and the Parallax spreadsheet was used for database storage.

**Microbial analysis**

The morphology of the hydrogen-producing organisms was analysed using phase contrast and scanning electron microscopy (SEM) for the suspended and the immobilized cell culture systems, respectively. For phase contrast analysis, 50 μL of effluent was sampled at peak hydrogen production, mixed with 3% glutaraldehyde and viewed. For SEM analysis, alginate beads were sampled at peak hydrogen production and fixed with 3% glutaraldehyde for 3 h. The fixed samples were then dehydrated stepwise in a graded series of ethanol.
solutions, ranging from 0% to 100% ethanol, and critical-point dried using carbon dioxide. Finally, the dried beads were sputter-coated with gold prior to SEM observation.

**Statistical analysis**

One-way statistical analysis of variance was used to compare the biohydrogen peak fractions in the suspended cells with non-regulated pH, the suspended cells with regulated pH, the immobilized cells with non-regulated pH and the immobilized cells with regulated pH. The analysis was carried out using Microsoft Excel.

**Results and discussion**

**Impact of culture medium pH on hydrogen production in dark fermentation using suspended mixed cultures**

**Biohydrogen production under non-regulated pH conditions in suspended cell cultures**

Figure 2 illustrates the biohydrogen and pH evolution under non-regulated pH conditions using suspended cells. A slow decline in pH from an initial value of 6.5 was observed in the first 5 h of fermentation, followed by a rapid drop in pH with a simultaneous increase in the biohydrogen fraction. Thus, the lag phase lasted for 5 h. The short lag phase observed in this culture system may be accounted for by the high accessibility of the cells to glucose substrate as it is a monosaccharide that is easily broken down by microorganisms. Previous studies have indicated that the duration of the lag phase may be influenced by medium pH. For instance, O’Sullivan and Condon [26] observed that the lag phase was shorter under alkaline pH and mesophilic temperatures, compared to acidic and thermophilic temperature conditions. The hydrogen fraction increased exponentially to a peak value of 36% after 12 h, corresponding to a pH of 4.67 (Figure 2). Biohydrogen production predominantly occurs in the acidification stage where carbohydrates are rapidly converted to hydrogen by fermentative bacteria. This results in the accumulation of acid by-products such as volatile fatty acids, causing a drop in the pH [27] and ultimately leading to the termination of hydrogen production. This drop in pH can also lead to a complete microbial inactivation depending on the magnitude of the decrease [28].

It has been found that a high concentration of non-dissociated acids increases the ionic strength of the solution, switching the metabolic pathway from hydrogen production to solvent production. The extent to which pH is able to affect the metabolic pathway of the hydrogen-producing community may vary depending on the inoculum as well as the characteristics of the substrate used [29,30]. These acids diffuse across the cell membrane and rapidly dissociate because of a higher intracellular pH, resulting in the acidification of the cytoplasm by the release of H⁺ ions [31]. This causes an increase in the energy required to maintain cell pH homeostasis and thus the energy of the cell is redirected towards maintaining neutrality. The movement of non-dissociated acids across the cell membrane may also disrupt the proton motive force. Disruption of the proton motive force largely affects nutrient transport into the cell as well as crucial enzymatic reactions and may also lead to DNA alteration and microbial inactivation [32].

The decline in hydrogen production observed below pH 4.67 (Figure 2) might have been triggered by the transition of the fermentation system from acidogenesis to solventogenesis, a phenomenon which typically occurs at pH 4.5. This metabolic shift results in the production of more neutral by-products such as acetate or alcohols, as it can be noticed that the pH of the fermentation system continues to decrease throughout the fermentation process. However, after optimum hydrogen fraction (36%) was reached, the rate of pH change became slower. The duration of the peak hydrogen production was relatively short (3 min) with prevailing pH 4.67, which may be due to the rapid drift in medium pH to inhibitory levels under non-regulated pH conditions. Khanal et al. [11] reported that with an initial pH of 6.5, the fermentation pH dropped more rapidly and the hydrogen production phase was shorter. It was suggested that the rapid hydrogen production observed at this pH was accompanied by rapid acid-production to inhibitory levels and thus, reducing the buffering
capacity of the system. The onset of solventogenesis can account for the short production phase observed in the non-regulated pH study. This metabolic shift occurred as a result of volatile fatty acid and hydrogen accumulation within the batch system. Thus, the removal of excess hydrogen as well as the regulation of pH at an optimal set-point can be used to sustain hydrogen production [32]. A possible relationship between the change in fermentation pH as well as the evolution of the hydrogen fraction was also investigated. A strong correlation was observed between the hydrogen evolution trend and the pH at exponential production, the peak production and the decline phase with a correlation coefficient of 0.93, 1 and 0.93, respectively.

**Substrate degradation profile under non-regulated pH condition in suspended cells culture**

Substrate degradation analyses revealed that the glucose concentration decreased from an initial concentration of 10–9.9 g/L after 7 h of fermentation, corresponding to the early exponential phase. A residual glucose concentration of 7.3 g/L was observed at the peak production corresponding to hydrogen fraction of 36% with a prevailing pH value of 4.67; then, substrate degradation slowed down significantly to a final concentration of 6.3 g/L at the end of the fermentation process. The overall substrate consumption, therefore, was 37%, as shown in Table 1. This suggests that the decrease in hydrogen production may be due to acid inhibition rather than substrate depletion and that pH 4.67 may be the minimum threshold for hydrogen production and substrate degradation. These findings are similar to a study by Lay [33] which suggested that hydrogen production may proceed at an optimum rate when the fermentation pH is maintained at a set-point of 5.2 but decline if the pH is below 4.7. Due to accumulation of volatile fatty acids during glucose fermentation, the pH generally decreased and it has been reported that high levels of dissociated Volatile fatty acids (VFAs), present in the fermentation media, increased the ionic strength of the solution leading to cell lysis [34]. Butyrate is well known as one of the major VFA constituents in fermentative hydrogen production. In a study by Zheng and Yu [35] investigating the effects of butyrate on hydrogen production, glucose degradation was found to be high (98%) at butyrate concentrations ranging from 0 to 12.4 g/L of added butyrate. However, glucose degradation was reduced to only 36% at a concentration of 25.08 g/L of added butyrate.

**Biohydrogen production and substrate degradation profiles under pH regulated conditions in suspended cells culture**

The hydrogen evolution trend under pH-controlled conditions using suspended cells is shown in Figure 3. A lag phase of 5 h was observed, followed by an exponential increase in the biohydrogen fraction to a peak hydrogen fraction of 44% at 21 h. This peak hydrogen production phase lasted up to 45 min, compared to 3 min observed in non-regulated pH conditions. Peak production was followed by a gradual decline in hydrogen production as the carbon source gradually depleted. These results suggested that pH regulation with suspended cell cultures increased the biohydrogen fraction and the process productivity. Glucose analysis showed that the concentration of glucose decreased from an initial value of 10–7 g/L at the early exponential phase. A residual glucose concentration of 4.4 g/L was measured at optimum hydrogen production at 21 h which decreased to 0 g/L at 24 h. These results showed that pH regulation enhances substrate degradation. Fang and Liu [9] showed that continuous control of pH within a range of pH 5.5–7.0 resulted in complete substrate degradation, whereas the control of pH below a set-point of 5.5 resulted in the partial degradation of glucose.

### Table 1. Biohydrogen production under regulated and non-regulated pH conditions using suspended and immobilized cells.

| Temperature | pH | Regulation | System type | Max hydrogen fraction | Glucose degradation |
|-------------|----|------------|-------------|----------------------|--------------------|
| 37.5 °C     | 6.5| No         | Suspended   | 36%                  | 37%                |
| 37.5 °C     | 6.5| Yes        | Suspended   | 44%                  | 66%                |
| 37.5 °C     | 6.5| No         | Immobilized | 21%                  | 100%               |
| 37.5 °C     | 6.5| Yes        | Immobilized | 35%                  | 100%               |

**Figure 3.** A comparative evolution of hydrogen fraction and pH under regulated (pH 6.5) and non-regulated pH conditions using immobilized cells.
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Biohydrogen production under non-regulated pH conditions with immobilized cells culture

Figure 3 represents the hydrogen fraction and corresponding pH evolution using a mixed microbial population immobilized in sodium-alginate beads under non-regulated and regulated pH conditions. The lag phase lasted for 5 h, throughout which the pH remained relatively stable at 6.5. A steady drop in pH was observed after the fifth hour. This decrease in pH was accompanied by a concomitant increase in biohydrogen fraction. This pattern of sharp decrease in pH during the exponential phase of hydrogen production was observed in all four culture systems. The hydrogen fraction increased exponentially up to a peak fraction of 22% after 16 h, corresponding to a pH value of 4.54. This peak was maintained for 1 h during which the pH remained fairly stable at 4.5. Presumably, cell immobilization increased the buffering capacity of the system, therefore, sustaining a longer duration of hydrogen production at peak fraction.

Although the immobilized cell system resulted in a lower peak hydrogen fraction compared to suspended cells, the extended peak production phase implies that cell immobilization, even under non-regulated conditions, could enhance biohydrogen production. The lower hydrogen fraction obtained with alginate immobilized cells in flask culture may be attributed to vigorous gas production in the flask cultures which reduces the density of the beads, causing them to float and be clustered at the surface of the culture medium at the conical top of the flask (Figure 4) and thus, reducing the availability of nutrients [18]. These observations suggest that a novel reactor configuration may be appropriate for biohydrogen investigation with alginate immobilized cells at laboratory scale. The slopes of the pH evolution curves for both immobilized and suspended cells were 0.0003 and 0.0006, respectively. This is indicative that the rate of pH change was slower for immobilized cells compared to suspended cells.

Effect of non-regulated pH on substrate degradation using immobilized cells

The glucose concentration decreased from an initial concentration of 10–7.2 g/L at exponential phase and then to 4.9 g/L at peak hydrogen production. The final residual glucose concentration was 0 g/L revealing a complete utilization of carbon substrate of the medium. This suggested that the decline in hydrogen production may be anchored to the depletion of glucose or pH limit threshold as discussed above. Correlation studies showed a strong relationship between pH change and hydrogen production. Strong correlation values were observed between the hydrogen evolution and pH trends at lag phase, exponential phase as well as the decline phase with correlation coefficients of 0.94, 0.93 and 0.92, respectively.

Biohydrogen production and substrate degradation profiles under pH regulated conditions in immobilized cells culture

Figure 3 also shows the evolution of biohydrogen under regulated pH conditions using immobilized cells. A lag phase of 8 h could be observed. A rapid upsurge in the hydrogen production with a peak hydrogen fraction of 35% was observed at the 15th hour. The peak hydrogen production phase lasted up to 35 min. This was followed by a gradual reduction in the production of hydrogen, which may be attributed to nutrient depletion within the fermentation medium. To assess this possibility, glucose
degradation was monitored at regular intervals. Glucose was added to the initial culture medium at a concentration of 10 g/L under regulated pH conditions. During the exponential phase (5–15 h), the glucose concentration in the fermentation medium decreased from 10 to 8.1 g/L. At peak hydrogen production, the measured glucose concentration was 0 g/L. This suggested that cell immobilization enabled a complete utilization of the substrate and thus, hydrogen production ceased due to the depletion of substrate in the fermentation system. The lower peak hydrogen fraction observed in the study with immobilized cells culture systems, can be accounted for by bead flocculation and also by inefficient mixing [18]; however, these systems still presented the advantage of complete substrate degradation. Statistical analysis showed that the peak hydrogen fraction from the process run under regulated pH conditions was significantly different to the peak fraction obtained under non-regulated pH conditions (p-value < 0.05; F-value 3.7; F-critical 1.5). It was also revealed that the peak hydrogen fraction obtained using suspended cells was significantly different compared to that obtained using immobilized cells (p-value < 0.05; F-value 2.5; F-critical 1.0). These data substantiate the impact of pH regulation and cells immobilization on biohydrogen production.

**Microbial analysis**

Minton and Clarke [36] stipulated that hydrogen production occurred during the exponential growth phase of Clostridia. SEM (Figures 5 and 6) showed a microbial population consisting predominantly of rod-shaped cells, which suggested the presence of hydrogen-producing Clostridia. The microbial community structure significantly affects the hydrogen-production efficiency [37]. Our previous investigation of this sludge revealed the presence of Clostridium genus with species including C. aminovalericum, C. intestinale, C. tertium, C. sortagofome and C. beijerinckii as well as C. butyricum as the most abundant species [22]. C. butyricum along with C. beijerinckii have been reported to produce large amounts of acetic, butyric and formic acid during the fermentation process [38]. Bacillus species have also been found to add to biohydrogen production by contributing to the hydrolysis of the substrate resulting in higher substrate-degradation efficiency and thus, improving the biohydrogen yield [39]. Other microbial species reported in biohydrogen production also include Klebsiella and Enterobacter [37].

The alginate beads used as an entrapment matrix are represented in Figure 5(a–c). Figure 5(b) shows a dense, mixed microbial population which occurs on the surface of the bead run under regulated pH conditions. However, Figure 5(c) shows the surface of a bead run under non-regulated pH conditions. The surface of this bead appears to be smooth with no visible growth on the surface. This observation suggests that the change in pH of the media surrounding the outer surface may have resulted in the microbial population being washed-off the surface of the bead.

The cross-sectional area of the beads was observed by SEM (Figure 6). This analysis showed a mixed microbial population present with an irregular spatial distribution. More microbial cells were visible towards the outer edges of the bead (Figure 2(d)) compared to the centre of the bead (Figure 1(d)). This observation raises the possibilities of gradients (nutrients or pH) across the bead with a greater concentration of nutrients at the outer edges of the bead which gradually decreased towards the centre of the bead. The GAC was added to the alginate–sludge mixture to increase the bead porosity and enhance the transfer of nutrients across the beads. Mass

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Image: Figure 5. Scanning electron microscopy (SEM) images of unprocessed alginate bead (a), bead ran under regulated pH conditions (b) and bead ran under non-regulated pH conditions (c). Note: Magnification 3000 ×. Scale bar = 200 μm.

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and heat transfer across the beads could be potential limitations for the use of immobilized cell systems and thus, an investigation of the optimum GAC content could enhance the efficiency of the culture modes.

Although the findings showed that the use of suspended cells generated a higher hydrogen fraction as compared to immobilized cells, these systems showed premature process termination with an incomplete substrate utilization when the medium pH was not regulated. Regulation of pH in suspended cell systems increased the substrate degradation thus resulting in a higher hydrogen fraction. Immobilized cell systems, on the other hand, allowed for complete substrate degradation even under non-regulated pH conditions. Additionally, this system showed a better buffering capacity under non-regulated pH conditions as the rate of change in pH was slower as compared to the suspended cell system. However, the hydrogen fraction observed with immobilized cells was lower compared to hydrogen production with suspended cells due to cell flocculation that limited the release of gas out of the bead matrices. Thus, the reactor configuration may improve the hydrogen production for these culture systems with alginate immobilized cells.

Conclusions

The regulation of culture medium pH increased the hydrogen fraction and glucose degradation for both the suspended and the immobilized cells. Drift of pH was more pronounced with the suspended systems compared to the immobilized systems, which displayed relative stability, suggesting that cell immobilization may have improved the buffering capacity of the system. However, biohydrogen research in flask systems was limited by gas entrapment within the immobilization matrix, causing flocculation of the beads and reducing cell metabolism. These findings highlight the sensitivity of dark fermentation to pH oscillations in flask experimentation and the relative pH stability in alginate-immobilized culture systems, making them suitable for experimentation when conventional feedback pH control loops are difficult or expensive to implement.

Disclosure statement

No potential conflict of interest was reported by the authors.

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