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Effect of Localized Temperature Difference on Hydrogen Fermentation

Seongwon Im [1], Mo-Kwon Lee [1,2], Alsayed Mostafa [1], Om Prakash [1], Kyeong-Ho Lim [3] and Dong-Hoon Kim [1,*]

[1] Department of Smart-City Engineering, Inha University, 100 Inharo, Michuhol-gu, Incheon 22212, Korea; deback3838@naver.com (S.I.); mklee@hit.ac.kr (M.-K.L.); ama_mostafa@ymail.com (A.M.); opky72@yahoo.in (O.P.)

[2] Department of Environmental Health, Daejeon Health Institute of Technology, 21 Chungjeong-ro, Dong-gu, Daejeon 34504, Korea

[3] Department of Civil and Environmental Engineering, Kongju National University, Cheonan, Chungnam 31080, Korea; khlim@kongju.ac.kr

* Correspondence: dhkim77@inha.ac.kr; Tel.: +82-10-2639-9379; Fax: +82-32-873-7560

Abstract: In a lab-scale bioreactor system, (20 L of effective volume in our study) controlling a constant temperature inside bioreactor with a total volume 25 L is a simple process, whereas it is a complicated process in the actual full-scale system. There might exist a localized temperature difference inside the reactor, affecting bioenergy yield. In the present work, the temperature at the middle layer of bioreactor was controlled at 35 °C, while the temperature at top and bottom of bioreactor was controlled at 35 ± 0.1, ±1.5, ±3.0, and ±5.0 °C. The H_2 yield of 1.50 mol H_2/mol $hexose_{added}$ was achieved at ±0.1 and ±1.5 °C, while it dropped to 1.27 and 0.98 mol H_2/mol $hexose_{added}$ at ±3.0 and ±5.0 °C, respectively, with an increased lactate production. Then, the reactor with automatic agitation speed control was operated. The agitation speed was 10 rpm (for 22 h) under small temperature difference (<±1.5 °C), while it increased to 100 rpm (for 2 h) when the temperature difference between top and bottom of reactor became larger than ±1.5 °C. Such an operation strategy helped to save 28% of energy requirement for agitation while producing a similar amount of H_2. This work contributes to facilitating the upscaling of the dark fermentation process, where appropriate agitation speed can be controlled based on the temperature difference inside the reactor.

Keywords: temperature difference; H_2 fermentation; agitation speed; energy requirement

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1. Introduction

The development of hydrogen (H_2) production technology has gained significant attention due to its high energy content (142 kJ/g) and its cleaner nature on combustion [1–3]. At present, the commercial production of H_2 is achieved via coal gasification, gas reforming, etc., which are not sustainable and environmentally benign due to the depletion of the fossil fuels and the generation of greenhouse gaseous emissions [4,5]. On the other hand, water splitting technology and biologically mediated ways could provide a sustainable way of producing H_2 [6,7]. Among various bio-H_2 production routes, the production of green hydrogen by use of organic wastes as feedstock has a huge potential to become an important source of hydrogen in the future if operating under ambient temperature and pressure conditions. Dark fermentative H_2 production (in short "dark fermentation"), in particular, is considered an environmentally friendly and practically suitable process for commercial bio-H_2 production, due to its high production rate, simple operation, and handling of various organic wastes [8–12].

There are various factors, such as pH, temperature, substrate types and lactic acid contamination can affect the H_2 fermentation performance [13–16]. Among them, temperature is a critical operational factor, since it influences the microbial growth, enzymatic activity, and population dynamics [17,18]. H_2 production in the dark fermentation process

is conducted with various temperature regimes from mesophilic to hyper-thermophilic conditions. It has been reported that keeping a constant temperature in an appropriate range of 35–40 °C is important in mesophilic H_2 fermentation, whereas fluctuations in temperature beyond the optimal range negatively affected the performance [18]. The detailed heterogenous temperature profile was not reported, but it can reach up to 2–5 °C in the full-scale anaerobic digester [19].

The agitation intensity is reported to enhance the bioenergy yield by providing a sufficient environment for the nutrient transfer to the microorganisms, heat transfer, and release of the produced biogas from the digestate mixture [20]. However, the energy consumption for the agitation intensity is reported to be up to 50% of the overall energy input of the wastewater treatment process [21]. The application of intermittent and short mixing strategy was considered as an alternative option over continuous stirring to cut down the energy cost and even to improve the biogas yield [22]. Although several strategies were employed previously to study the impact of mixing in the wastewater treatment process, there is no rule of thumb regarding the agitation or recycling intensity required for stable reactor operation. Besides, the temperature variations inside the bioreactor (top, middle, and bottom) layer are a crucial factor to be considered for applying the agitation intensity for better mixing and reducing the energy input of the wastewater treatment process. Moreover, the information about the agitation intensity on dark fermentation was rarely reported [23]. To the author's knowledge, the effects of localized temperature differences inside the bioreactor on H_2 production have never been reported.

Based on the above, the aim of this study was to investigate the effect of localized temperature difference (±1.5 to ±5 °C) inside the reactor on dark fermentation. The temperature difference was generated by using water jackets to maintain a different temperature continuously, as shown in the schematic diagram (Figure 1). Furthermore, flux balance analysis (FBA) was employed to understand the mechanism for enhancement. The energy demand for agitation was also assessed using the operational strategy of stirring speed control responding to the temperature gap.

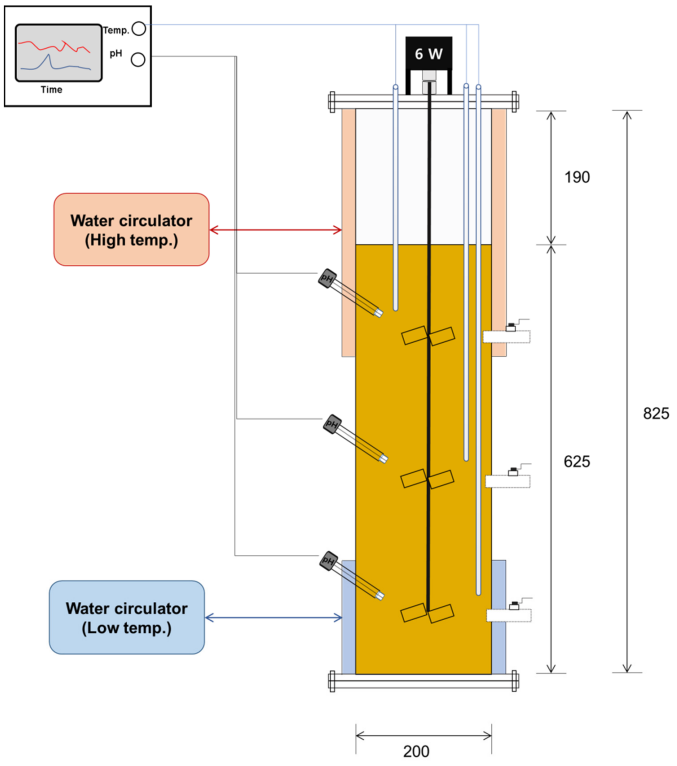

Figure 1. A schematic diagram of bioreactor system used in this study.

2. Materials and Methods

2.1. Inoculum and Substrate

The inoculum for H_2 fermentation was collected from an anaerobic digester in a local wastewater treatment plant in Korea. The pH, and volatile suspended solids (VSS) concentration of the sludge were 7.5, and 25.0 g/L, respectively. The sludge was filtered through a 2.0 mm sieve to remove large particles, and then heat-treated at 90 °C for 20 min to inactive methanogenic activity [24]. Glucose was used as a substrate and the concentration was adjusted to 10 g chemical oxygen demand (COD)/L. Nutrients were supplied according to COD:N:P:Fe ratio of 100:5:1:0.33 by using NH_4Cl, KH_2PO_4, $FeCl_2 \cdot 4H_2O$. To minimize the effects of trace metal deficiency, various trace metals were added as follows (in $mg \cdot L^{-1}$): Na_2MoO_4 $4H_2O$, 5; H_3BO_3, 50, $MnCl_2$ $4H_2O$, 50; $ZnCl_2$, 50; $CuCl_2$, 30; $NiCl_2$ $6H_2O$, 92; $CoCl_2$ $6H_2O$, 50; Na_2SeO_3, 50) [25].

2.2. Experiment

Batch experiments were conducted using a completely stirred tank reactor with a working volume of 20 L (200 mm ID), controlled at 35 ± 0.1 °C (Figure 1). The heat-treated sludge was inoculated in the reactor at a VSS concentration of 10.0 g/L. After seeding with media and substrate addition, the reactor was purged by N_2 gas (99.99%) for 10 min to establish an anaerobic condition. The pH inside the reactor was controlled at 6.0 ± 0.1 using a pH sensor (APH-200VD, South Korea), pH controller (samsnK.com-96pH(ORP)-L4, South Korea). The temperature was measured by temperature sensor (TC-V, range (−)50–300 °C, accuracy ±3%, South Korea) and maintained by a water bath circulator equipped with built-in water jacket. Experiments were conducted to investigate the effects of localized temperature difference in a stirred tank reactor on dark fermentation: control (35 ± 0.1 °C), E1 (35 ± 1.5 °C), E2 (35 ± 3.0 °C), E3 (35 ± 5.0 °C). The temperature variation was generated by using water jackets (CW10G) filled with cool and hot water at certain degrees to maintain a different temperature. For example, in the case of E3, the temperature of the bottom part of the reactor was adjusted to 30 °C, the middle part was 35 °C, and the top part was 40 °C, by circulating cool water (10 °C) at the bottom, and hot water (42 °C) at the top at the pumping rate of 10 L/min. In addition, the E4 experiment was performed to compare the energy required for agitation speed. The agitation speed was 10 rpm under small temperature difference (<±1.5 °C) (for 22 h), while it was increased to 100 rpm (for 2 h) when the temperature gap between top and bottom of reactor became larger than ±1.5 °C. When the temperature cross over was more than 36.5 °C, the sensor activates stirring to 100 rpm. To rotate the broth, a 6 W AC induction speed control motor (Brand: SPG Motor, Model: S6I06GB-V12) was used. The power consumption was measured using a digital power meter. At the end of the experiment, samples were taken from the top, middle, and bottom of the reactor to analyze carbohydrates and organic acids.

2.3. Analysis

The concentrations of VSS and COD were measured according to Standard Methods [26]. We settled the sludge for 1 day, and then after pretreatment, such as heating evaporates most of the water present in the sludge. The glucose concentration was measured by the colorimetric method, as previously described [27]. The amount of produced biogas from the reactor was determined by water displacement method and was adjusted to the standard conditions of temperature (0 °C) and pressure (760 mmHg) (STP). The H_2 and CO_2 content in the biogas was measured by gas chromatography (GC, Gow Mac series 580) equipped with a thermal conductivity detector (TCD) using mole-sieve 5A and porapack Q (80/100 mesh) as a separation column. N_2 gas (99.99%) was used as a carrier gas with a flow rate of 30 mL/min and the temperatures of injector, detector, and column were kept at 70, 50, and 80 °C, respectively.

Liquid samples obtained from the reactor were diluted 10 times with distilled water and filtered through 0.2 um pore size syringe filter to analyze soluble carbohydrate, and organic acids. Organic acids such as lactate, formate, acetate, propionate, and butyrate

were analyzed by a high-performance liquid chromatograph (HPLC) (LC-20A series, SHIMADZU Co.) with an ultraviolet (215 nm) detector (UV1000, SHIMADZU) and an Aminex fast acid analysis column (HPX-87H, Bio-Rad Lab.). The mobile phase was 0.005 M H_2SO_4 applied at a 0.6 mL/min flow rate and the temperatures of detector, oven, and column were 40, 35, and 90 °C, respectively. The flux balance analysis (FBA) model, previously developed by Chaganti et al. [28], was applied for experimental data analysis. The steps we followed for FBA, and the utilized abbreviations were the same as those stated in previous studies [28,29]. The goal of applying FBA was to investigate whether the reutilization of H_2 for acetate production, i.e., acetogenic H_2 consumption, varies among batches or not. FBA basically considers that acetate can be produced from two possible reactions, i.e., (i) acetyl coA, and (ii) H_2 reaction with CO_2. For FBA calculation, a (30 × 30) matrix was used. The numbers of intracellular, and extracellular reactions were 14, and 16, respectively. For solving the metabolic network linear equations, MetaFluxNet software (Version 1.8.6.2) was adopted. Further details regarding FBA can be found in previous studies [28,29]. The Pearson correlation coefficient was calculated to measure linear correlation between organic acid and H_2 production.

3. Results and Discussion

3.1. Fermentation Performance

The temperature variations at the different heights of the bioreactor are depicted in Figure 2. Within 5 h, the temperature at the middle layer became around 35 °C, while the temperature at the top and bottom reached 35 ± 1.5, ±3.0, and ±5 °C, depending on the operational conditions. This indicates that the control of temperature and pumping rate of cool and hot water circulating reactor surface was successful to make localized temperature difference as planned. In addition, pH difference in three parts (top, middle, and bottom) was negligible (data not shown), indicating that 100 rpm agitation speed was enough to provide sufficient mass transfer of soluble matters through the whole region of the bioreactor.

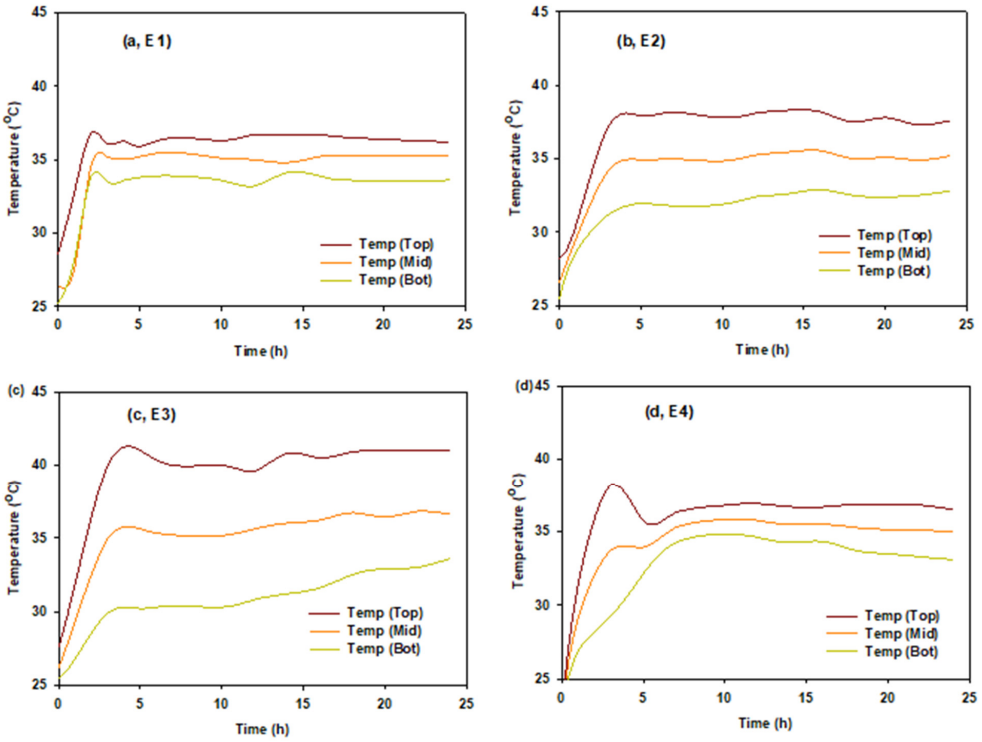

Figure 2. The change of temperature at top, middle, and bottom layer of reactor planned to deviate at (**a**) E1 (35 ± 1.5 °C), (**b**) E2 (35 ± 3.0 °C), (**c**) E3 (35 ± 5.0 °C), and (**d**) E4 (35 ± 1.5 °C) during H_2 fermentation.

The total amount of H_2 production declined as the temperature gap between top and bottom became larger (Figure 2). The H_2 yield of 1.50 mol H_2/mol $hexose_{added}$ was achieved at ±0.1 and ±1.5 °C, while it dropped to 1.27 and 0.98 mol H_2/mol $hexose_{added}$ at ±3.0 and ±5.0 °C, respectively (Figure 3). The temperature difference inside the bioreactor also affected the substrate removal efficiency and biomass growth. Glucose removal efficiency ranged 83 to 92% where the highest removal was observed in the control (±0.1 °C), and the lowest value was attained at ±5.0 °C. Ranges of removal efficiency and H_2 yield are near to the ranges previously stated in literature [30,31]. After fermentation, biomass concentration increased from (initial) 10 g VSS/L to 10.7 g VSS/L at the control (±0.1 °C), whereas at the temperature variations of ±1.5, ±3.0 and ±5.0 °C it increased to 10.4–10.6 g VSS/L. However, the difference in these two parameters did not seem great enough to tell the difference in H_2 production performance. This is because those two parameters can vary, while the H_2 production performance can be almost the same, and vice versa [32]. Further, the increase in removal efficiency did not always lead to increased H_2 productivity [33,34]. In addition, both the removal efficiency and biomass concentration were found to be microbially community dependent [35].

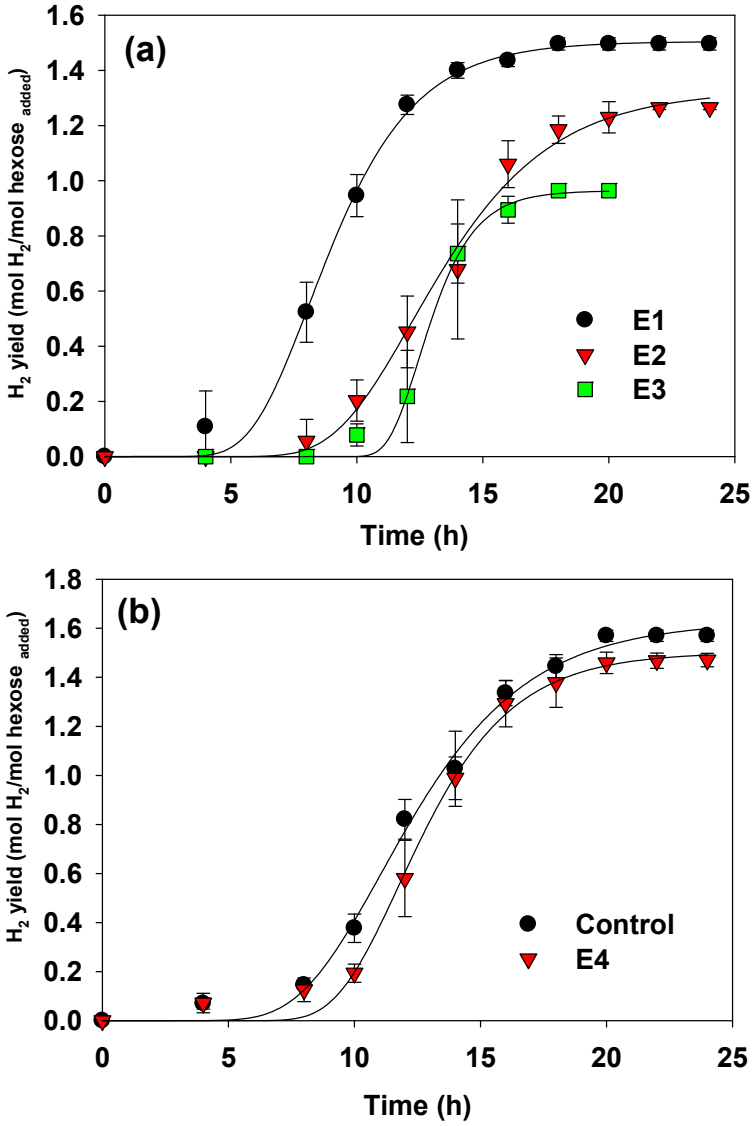

Figure 3. Cumulative H_2 yield for (**a**) E1 (35 ± 1.5 °C), E2 (35 ± 3.0 °C), E3 (35 ± 5.0 °C), (**b**) control (35 ± 0.1 °C), and E4 (35 ± 1.5 °C) batches.

Figure 4 shows the organic acids production profile under various temperature fluctuations. There was a slight difference in the carbohydrate and organic acid concentration in the samples taken from the reactor at the top, middle, and bottom. There was a slight difference (standard deviation <1%) in the carbohydrate and organic acid concentration in the samples taken from the reactor at the top, middle, and bottom. These results implied that the dissolved contents inside the reactor were mixed well during H_2 experiment.

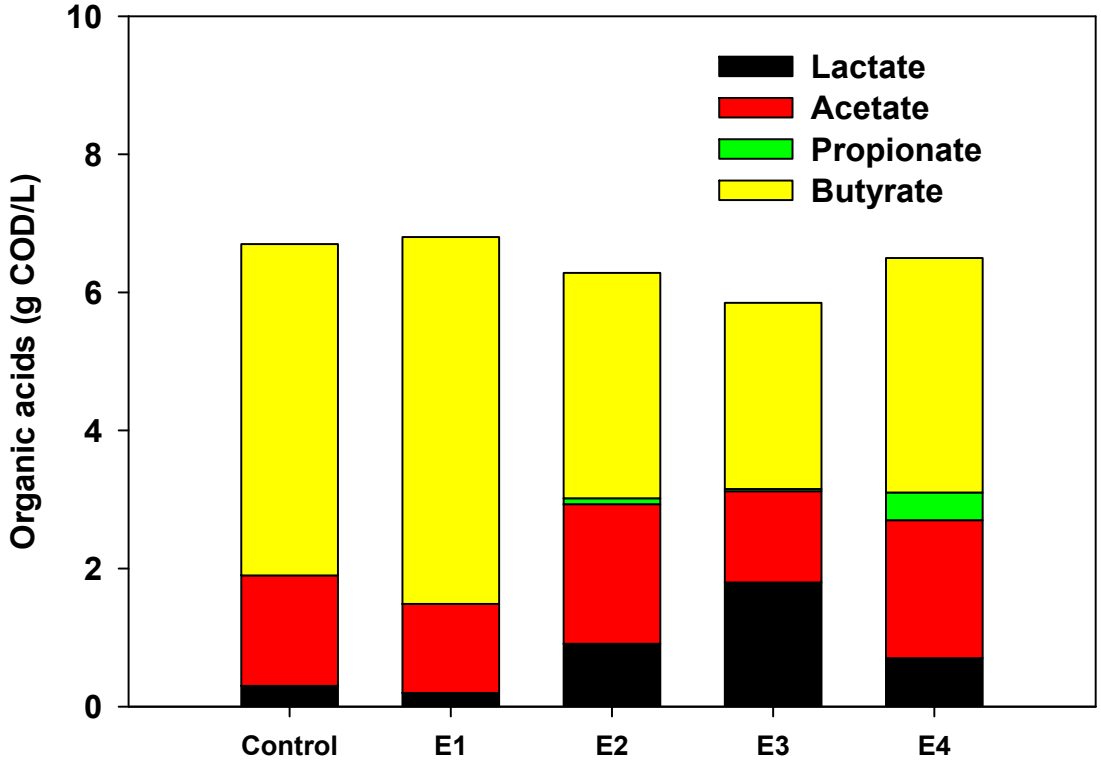

Figure 4. Organic acids profile for control (35 ± 0.1 °C), E1 (35 ± 1.5 °C), E2 (35 ± 3.0 °C), E3 (35 ± 5.0 °C), and E4 (35 ± 1.5 °C) batches.

The propionate concentration was negligible. The total organic acids production of 6.7 g COD/L was observed at the control experiment (±0.1 °C), and a similar trend of organic acids accumulation was noted at the experimental condition at ±1.5 °C. The organic acids production was dropped to 6.3 and 5.9 g COD/L at ±3.0 and ±5.0 °C. The major organic acids production during the experimental conditions was acetate, butyrate, and lactate, while formate was not detected.

Butyrate was the major dominant metabolic product for all conditions, and higher concentrations of 4.80 and 5.31 g COD/L were observed at control and ±1.5 °C, respectively. On the other hand, it dropped to 3.26 and 2.70 g COD/L at ±3.0 and ±5.0 °C, respectively. The accumulation of lactate was limited at the control 35 ± 0.1 °C and ±1.5 °C, whereas the concentration was higher at ±3.0 and ±5.0 °C. The localized temperature difference inside the reactor could affect organic acids distribution pattern (Figure 4). A maximum lactate accumulation of 1.8 g COD/L was observed at ±5.0 °C. As indicated earlier, the lactate production is not beneficial for H_2 production, which is mainly produced by lactic acid bacteria [36,37]. Further, previous study confirmed that the more lactate production, the less H_2 that would be generated [38]. The possible suggestion to overcome this problem has been mentioned by culturing lactate utilizing hydrogen producing bacteria (LU-HPB) such as *Megasphaera elsedenii* [16]. Experimental results were found to match with statistical analysis. In specific, a high positive correlation was observed between H_2 yield and HBu generation, (Pearson's r value of +0.920). On the other hand, H_2 yield inversely correlated with HLa production (Pearson's r value of −0.999).

Energy balance on a COD basis and molar conversion for batches under various localized temperature differences are provided in Table S1 (see Supplementary Material). The considered fractions were acetate, butyrate, propionate, lactate, H_2, biomass, and residual glucose content. The fraction of biomass was calculated by the change in VSS content, whereas the molecular formula was assumed to be ($C_5H_7O_2N$). For all batches, the total sum was higher than 89.6%, referring to the accuracy of analysis. In all batches, the highest portion of energy distribution was assigned to butyrate. On the other hand, residual glucose molar conversion varied among batches, whereas it reached its maximal value of 0.17 mol H_2/mol $hexose_{added}$ in E3. For further understanding of the H_2 production performance, we calculated acetogenic H_2 consumption by applying FBA on the obtained experimental data.

FBA is an informative tool for analyzing carbon/electron distribution and understanding the performance of various H_2 producing batches [29]. The list of the reactions, adopted for establishing FBA is given in a previous work [28]. Based on FBA results, we could calculate acetogenic H_2 consumption, H_2 production by hydrogenase activity, and net H_2 production. Figure 5 provides a thorough explanation for H_2 production from tested batches. Details regarding the calculations can be found in a previous work [29]. Apparently, a correlation between obtained H_2 yield values and H_2 production by hydrogenase activity can be noticed. Further, E2 had the highest acetogenic H_2 consumption of 0.51 mol H_2/mol $hexose_{added}$. This can further support H_2 yield results. Then, it can be concluded that both acetogenic H_2 consumption and lactate generation are enough for explaining the attained H_2 production results.

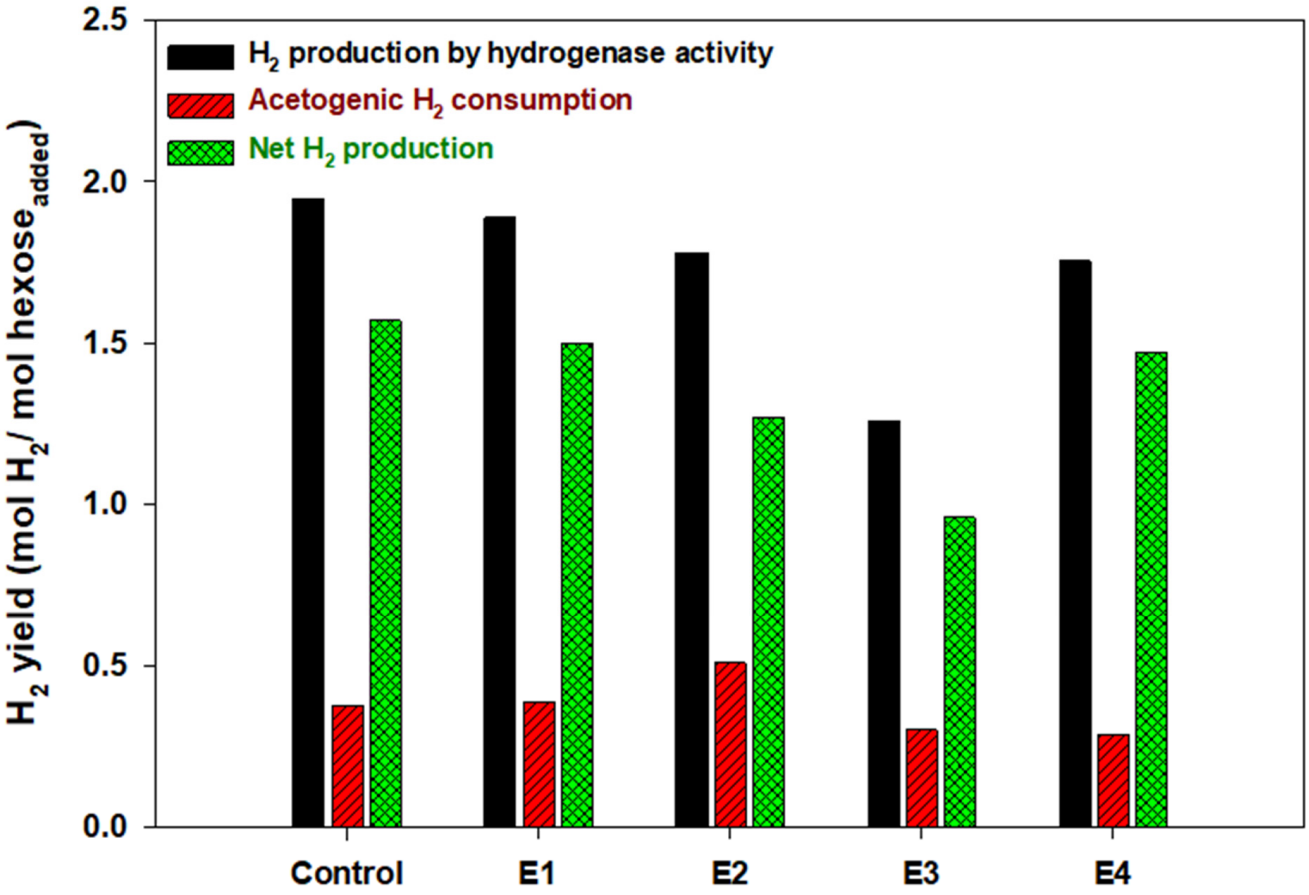

Figure 5. A thorough explanation for H_2 production from control (35 ± 0.1 °C), E1 (35 ± 1.5 °C), E2 (35 ± 3.0 °C), E3 (35 ± 5.0 °C), and E4 (35 ± 1.5 °C) batches, using flux balance analysis.

3.2. Energy Reduction by Agitation Speed Control

It was found that the temperature difference at ±1.5 °C had no significant effect on H_2 yield, compared to the control (35 ± 0.1 °C). Then, the experiment E4 was performed to compare the energy required for agitation speed during H_2 fermentation (Figure 6). The agitation speed was 10 rpm under small temperature difference (<±1.5 °C), while it increased to 100 rpm when the temperature difference between top and bottom of reactor became larger than ±1.5 °C (Figure 2). When the agitation speed increased to 100 rpm, the temperature gap became smaller less than ±1.5 °C after certain time.

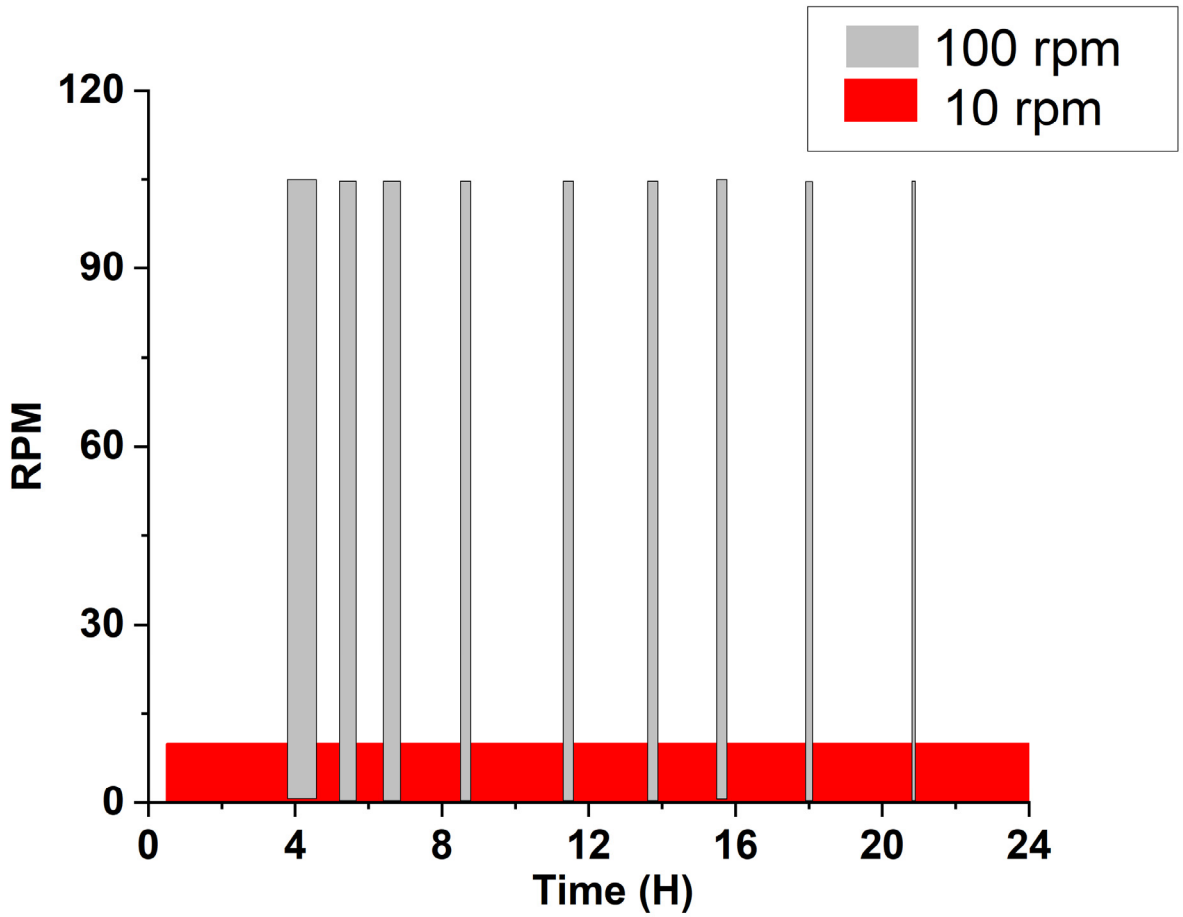

Figure 6. The change in agitation speed during dark fermentation (the agitation speed was 10 rpm under small temperature difference (<±1.5 °C), while it increased to 100 rpm when the temperature gap between top and bottom of reactor became larger than ±1.5 °C).

The H_2 yield of 1.47 mol H_2/mol $hexose_{added}$ was achieved at E4 condition, which was almost similar to the control condition (1.50 mol H_2/mol $hexose_{added}$) (Figure 3b). The economic benefit, in terms of the reduction in energy consumption for operating the agitation speed were compared. The control reactor at a fixed rotation of 100 rpm during the temperature difference needed an energy demand of 510 kJ (5.9 W × 24 h × 3.6), whereas the operational strategy E4 consumed a low energy input of 367 kJ as it was set up like "10 rpm for 22 h and 100 rpm for rest of the 2 h" = {(4.1 W × 22 h + 5.9 W × 2 h) ×3.6}. The net energy gain of 28% has been observed when the rotational speed was reduced from 100 rpm to 10 rpm. Figure 6 depicts the time interval for mixing at different rotations per minute for a day in the case of E4. Srirugsa et al. [39] has reported energy gain of11% when the rotational speed was reduced from "100 rpm for 24 h" mode to "100 rpm for 8 h + 10 rpm for rest 16 h" mode. Moreover, a reduction in mixing speed from 150 rpm to

25 rpm resulted in 83% reduction in equivalent energy consumption, while having same output [40].

In full-scale anaerobic digesters, mixing is not continuous, rather it is intermittent. However, such intermittent mixing was found to be random and irrational. For example, when Zhu et al. [41] reduced the intermittent period from 12 to 4 h, H_2 productivity increased by 4%. On the other hand, reducing the intermittent stirring period from 8 to 2 h, sharply lowered H_2 productivity by 30% [41]. Therefore, regulating intermittent mixing is a must for avoiding drops in H_2 production. For efficient mixing regulation, important parameters can be sensed and then used for deciding the intermittent mixing condition. Herein, we proposed sensing the temperature difference as a tool for regulating intermittent mixing.

The target of lowering agitation speed was reducing the energy demand, without affecting the performance. Our results confirmed that H_2 production, under low mixing speed or intermittent mixing, has not been significantly affected by such change in mixing. Energy reduction, acquired in this study, can have significant impact when it is upscaled to industrial level.

4. Conclusions

The total amount of H_2 production declined as the localized temperature gap became larger with the increased lactate production. H_2 yield decreased from 1.50 to 0.98 mol H_2/mol $hexose_{added}$ when the gap increased from $\pm$0.1 °C to $\pm$5.0 °C with the lactate concentration increase from 0.2 to 1.8 g COD/L. At $\pm$1.5 °C, a similar H_2 yield was attained with the control. Operating the bioreactor with differences in agitation speed responding to the localized temperature gap exhibited a similar H_2 yield of 1.47 mol H_2/mol $hexose_{added.}$ Through this strategy, it was possible to save 28% of energy required in agitation but acquiring same amount of H_2. This work emphasized the role of temperature in the fermentation process, and it shows temperature could have been a potential point to control energy consumption through agitation in the optimization process.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en14216885/s1, Table S1: Energy balance on COD basis and molar conversion for batches under various localized temperature differences.

Author Contributions: Conceptualization, D.-H.K.; methodology, M.-K.L.; software, A.M.; validation, S.I.; formal analysis, M.-K.L.; investigation, S.I., A.M. and O.P.; writing—original draft preparation, S.I., M.-K.L., A.M. and O.P.; writing—review and editing, D.-H.K. and K.-H.L.; visualization, M.-K.L. and A.M.; supervision, D.-H.K. and K.-H.L.; project administration, D.-H.K.; funding acquisition, D.-H.K. All authors have read and agreed to the published version of the manuscript.

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