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Fusion of Vermicompost and Sewage Sludge as Dark Fermentative Biocatalyst for Biohydrogen Production: A Kinetic Study

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Abstract: The present study explores the synergy between vermicompost and the anaerobic sewage sludge as inoculum for biohydrogen production using food waste as a substrate. Experiments were designed and performed in two phases of operation. In the first phase, the vermicompost (VC) was used as inoculum and food waste as substrate at three different organic loading rates of 10 gVS/L (VC1), 20 gVS/L (VC2), and 30 gVS/L (VC3). In the second phase of operation, the inoculums were combined with a proportion of 50% (VC+AS). The study showed an effective biohydrogen production of 20 gVS/L when the mixing ratio of vermicompost and anaerobic sludge was 50:50. The results inferred that effective synergy was observed between the combined consortia of the inoculum, which induces a more effective metabolic pathway for enhanced hydrogen production. H_2 production was 33 mL/gVS (VC1), 48 mL/gVS (VC2), 35 mL/gVS (VC3), 46 mL/gVS (AS), and 50 mL/gVS (VC+AS). Heat pretreatment (100–120 °C) of the inoculum suppresses the methane-producing microorganisms and increases the hydrogen-producing microbes. In addition to hydrogen production, different metabolites are formed in the liquid phase, such as acetic acid, butyric acid, and propionic acid of 2.957 g/L, 4.286 g/L, and 2.123 g/L, respectively, with an energy content of 257 J/day with VC+AS. In addition, a kinetic model was studied for the cumulative hydrogen production curves using the modified Gompertz model, and the fit infers that the experimental data fitted well, with high coefficients of determination for VC+AS (R^2 (G) > 0.99).

Keywords: biofuel; dark fermentation; vermicompost; anaerobic sludge; food waste; bioenergy

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1. Introduction

Hydrogen is a low-carbon fuel with a high energy carrier because it has the opportunity to decrease greenhouse gas emissions by 57–73%, and it is a promising alternative source for the realm of fossil fuel depletion and global warming potential. It has attracted great attention since it does not emit CO_2, and combustion yields water. Therefore, hydrogen is a suitable clean alternative biofuel. At present, most of the hydrogen production is from nonrenewable sources such as natural gas (50%), petroleum-derived naphthenes and distillates (30%), coal (18%), and electricity (2%). However, the renewable source of hydrogen is one of the best biofuels, and the calorific value is highest at 122 kJ/g, which is 2.75 times more than other regular hydrocarbon fuels [1,2]. According to the life-cycle assessment, hydrogen generation from renewable source with carbon capture and storage is a net-negative CO_2 emission technology, with carbon dioxide removing potentials ranging from 8.84 to 11.60 kg CO_2 per kilogram H_2 [3]. The use of fossil fuel generates 5981 Mtons of CO_2 per year, however, biohydrogen production could capture 133 Mtons of CO_2 per year with a hydrogen potential of 12.10 Mtons/yr [4]. Generally, hydrogen is also produced from renewable sources such as thermochemical, electrolytic, biological, and photolytic processes [5]. Compared to all processes, biological methods are sustainable and eco-friendly, viz., bio-photolysis, photofermentation, and heterotrophic dark

fermentation processes, or the integration of these methods [6]. Dark fermentation converts various organic substrates to biofuels and other value-added products through a diverse heterotrophic group of anaerobic bacteria without light. Generally, the 1st generation biofuels are made from cereals, maize, and sugarcane, and the current scenario is moving towards the 2nd generation of biofuels obtained from organic waste. Dark fermentation can convert 2nd generation sugars such as lignocellulosic biomass to value-added chemicals [7]. The efficiency and stability of dark fermentation are driven by a complex community of microorganisms of different functional guilds and rely on the syntrophic activity of the microbial community driven by complicated biochemical reactions [8].

The biochemical pathways involved in dark fermentation for the production of hydrogen are similar to anaerobic digestion, such as hydrolysis, acidogenesis, and acetogenesis, using hydrolytic and fermentative bacteria. The main process is to inhibit methanogenesis by pretreating the anaerobic sludge and redirecting the pathway for hydrogen production [6]. The two main mechanisms for biohydrogen production are a catabolic formic acid transformation and the re-oxidation of nicotinamide adenine dinucleotide hydride (NADH), catalyzed by the hydrogenase pathway [9]. Hydrogenase promotes the reaction of protons and electrons for biohydrogen production. Hydrogen-producing bacteria change glucose to pyruvate by glycolytic routes that forms adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and by the reduced form of NADH in the dark fermentation process using glucose as the substrate [10]. Pyruvate ferredoxin oxidoreductase and hydrogenase further convert pyruvate to acetyl coenzyme A (acetyl-CoA), hydrogen (H_2), and carbon dioxide (CO_2). Depending on the microorganism and ambient circumstances, pyruvate could be metabolized to acetyl-CoA and formate and further converted to H_2 and CO_2 (Figure 1). Acetate, butyrate, and ethanol are all feasible conversions of acetyl-CoA. The theoretical conversion of glucose yields nearly 4 moles of H_2 [10].

$$C_6H_{12}O_6 + 6H_2O = 2CH_3COOH + 4H_2 + 2CO_2$$

Microbial hydrogen (H_2) was synthesized using organic waste as a substrate by a mixed microbial culture during the acidogenic stages of dark fermentation [11]. Dark fermentation is advantageous compared to photofermentation because it does not require light, fast cell growth, and has a higher evolution rate. The only concern is that the concentration of hydrogen produced is only 40–60%; hence, it could not be used as fuel directly, and it requires down-streaming processes [12]. To overcome this issue, enzymolysis and acid pretreatment could enhance the percentage concentration of hydrogen [13]. In the DF processes, selecting obligatory microflora such as mixed, pure, and co-culture is essential for efficient hydrogen production. Diverse anaerobic culture is well-suited inoculum for dark fermentation processes since it contains hydrogen producers and has dominant methanogenesis, such as acetotrophic, hydrogenotrophic methanogens, and sulfate-reducing bacteria, which might consume the produced hydrogen [14]. Hence, it is essential to follow strategies to terminate hydrogen consumers and allow hydrogen as the end product in its metabolic pathway [6]. Pre-treatment of parent inoculum is an effective technique for speeding up the hydrolysis phase and augmenting anaerobic digestion to increase H_2 production by decreasing the influence of rate-limiting steps [15]. Among the other pretreatment processes, thermal pretreatment is more effective. Hence, it suppresses the non-spore bacteria and allows spore-forming bacteria to grow, which enriches the H_2-producing bacteria. The selection of effective biodegradable organic fraction for the anaerobic process is essential for enhanced biohydrogen production. Research has been carried out using mixed culture to produce higher biohydrogen than pure culture because of its diverse organisms, ease of operation, wide substrate choice etc. [6].

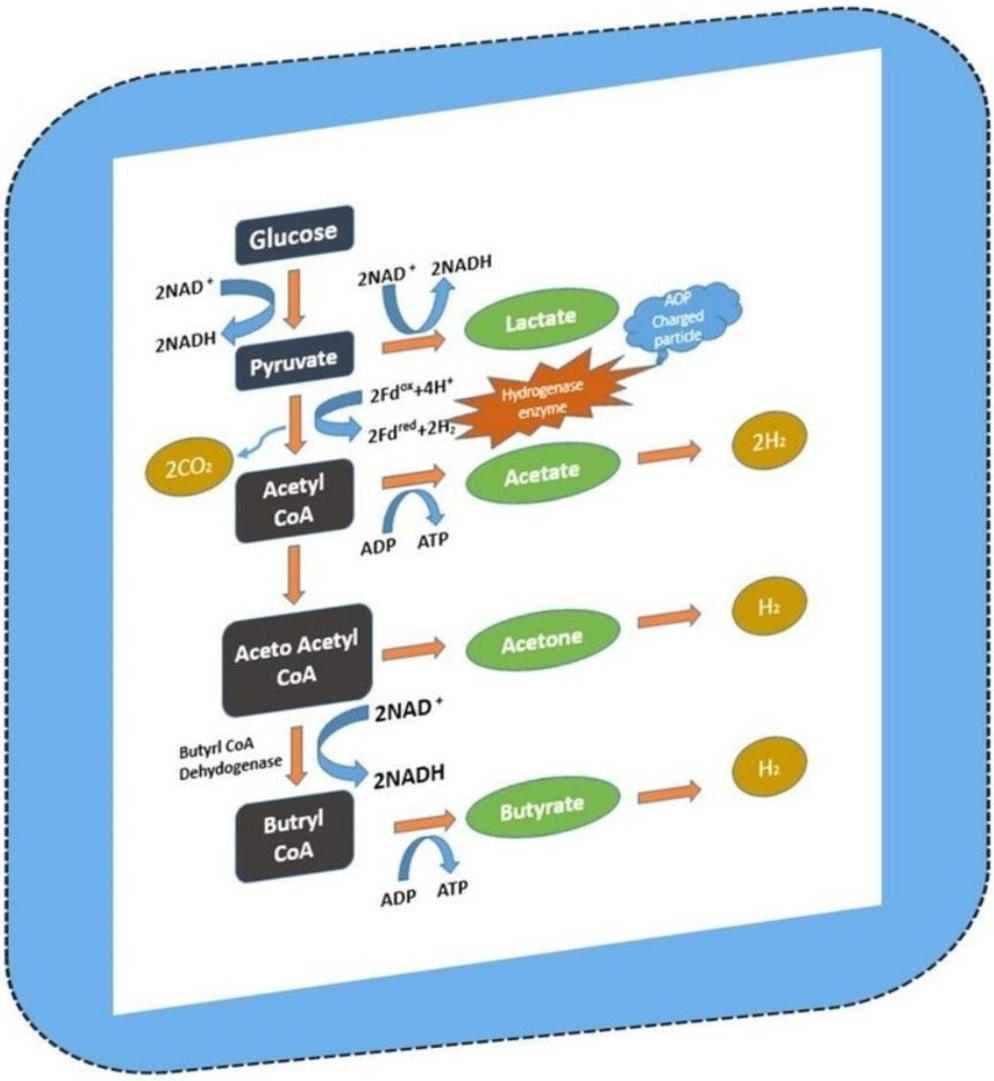

Figure 1. Biohydrogen production pathway in Dark Fermentation process.

In addition, combining several mixed consortia cleaves the complex substrates and yields the desired products at an augmented rate. Vermicomposting decomposes the organic wastes by different kinds of earthworms to improve waste conversion and product quality. The most common earthworms used in composting are Eisenia fetida, Eudrilus eugeniae, Perionyx excavates, Metaphire Californica, and Eisenia Andrei. Oceguera et al. (2019) identified vermicompost as a new inoculum and performed tests using E.fetida earthworms and agricultural wastes [16]. Pascualone et al. (2019) identified a mixture of vermicompost as inoculum and mild heat-treated fruit and vegetable waste as substrate [17]. The transit of organic matter via the earthworm encourages the development of an active microbial community, with a profusion of bacteria from the genus Clostridium, in the ingested material. The substrate uptake will be high in the self-selecting mixed consortia when enriched with a group of microorganisms, hence, combining different microorganisms that proliferate rapidly to gain maximum energy productivity with the high organic content as substrate.

In the dark fermentation process, the microbial metabolism in Clostridium species converts organics into hydrogen and other by-products. Combining the consortia of anaerobic sludge and vermicompost allows syntrophic interactions and enables the metabolic diversity of the organisms to produce hydrogen. Hence, using several mixed consortia enhances the substrate conversion efficiency, resulting in higher hydrogen production rates. Generally, hydrogen production depends upon several factors such as microbes, substrate,

pH, HRT, partial pressure, etc. Hence, optimizing the factors mentioned above is vital for effective hydrogen production. Food waste is an adequate feedstock for hydrogen production because its physicochemical and biological characteristics suit effective anaerobic degradation. Food waste has properties of moisture content (75–90%), COD (20–345 g/L), a carbohydrate level of 25.5–143 g/L, and C/N of 14–37 [18]. In addition to the above properties, particle size, nutrient content, volatile solid composition, and biodegradability of food waste should be considered for effective biohydrogen production [19]. To understand the larger-scale applicability, modeling studies were performed. The modeling allows determining optimal working conditions, which are theoretically possible, to analyze and estimate various processes.

The possibilities of biohydrogen production helps to analyze kinetic models that can be used to design and scale up laboratory experiments into industrial-size applications [20]. Several primary models were used for biogas production, such as Logistic, Gompertz, Baranyi-Roberts Von Schnute, Bertalanffy, Richards, Buchanan three-phase, and Huang models, etc. [21]. Studies reported that cumulative hydrogen production was correlated with the Gompertz equation and the regression coefficient was determined to identify the effective fit with experimental data [22]. Research has been carried out to utilize the Gompertz model to predict biohydrogen generation for dark fermentation using different substrates to predict several biological systems' behavior [23,24]. Gompertz's model is analysed based on the shape of the curve and the location parameter that shifts the curve horizontally without changing its shape. The parameter value is kept constant relative to the *x*-axis or the *y*-axis by characterizing type I and type II of Gompertz models. For energy analysis, thermodynamics could be an absolute control since the proton reduction is strongly energy-consuming (+79.4 kJ/mol of H_2). Most electron equivalents do not accumulate in H_2 due to the range of organic acids and alcohols [25].

In this context, the present research explores the fusion of vermicompost and sewage sludge as a biocatalyst for biohydrogen production using food waste as a substrate. The study has been carried out with two different phases of operation; the first phase evaluates the potential of vermicompost as inoculum using food waste as substrate with different organic loading rates. In the second phase of operation, both the inoculums were combined with a proportion of (50%VC+50%AS) for evaluation of biohydrogen production and other metabolites production. The experimental performance was assessed using biochemical analysis, gas analysis, and VFA production (Figure 2). Energy calculations were performed to calculate the hydrogen conversion efficiency and energy analysis to understand the potential of food waste and its conversion efficiency for biohydrogen production. A kinetic model was studied using the modified Gompertz model to validate the fit with the experimental data. The current research has a good potential for industrial applications.

Hydrogen has many industrial applications such as chemical, refining, metallurgical, glass, electronics, construction, and pharmaceutical, etc. It has broader applications mainly due to its reactivity rather than its physical properties.In fuel applications, it could be used for aerospace, automobile, and electrochemical cells for electricity production. Different kinds of feedstock are feasible for H_2 production on an industrial scale. However, natural gas is the most suitable fuel as a main raw material due to its abundance and low cost [26]. The produced hydrogen is used as feedstock in several chemical and petrochemical industries for producing syngas, ethanol, etc. [27]. Around 25% of feedstock is used as hydrogen in petroleum refining, 55% of hydrogen is used in ammonia production, and 10% in the methanol production industry. The amount of hydrogen produced from natural sources is 236,239 kg H_2/day. Similarly, hydrogen generation from biomass is 194,141 kg H_2/day [28]. According to Franchi et al. (2020), the cost of generating hydrogen using steam reforming varies from 1.33 to 2.30 USD/kg of H_2, compared to 2.50 to 5.30 USD/kg of H_2 via electrolysis and around 3.5 USD/kg H_2 via biomass gasification [29]. However, this application could apply to biohydrogen production.

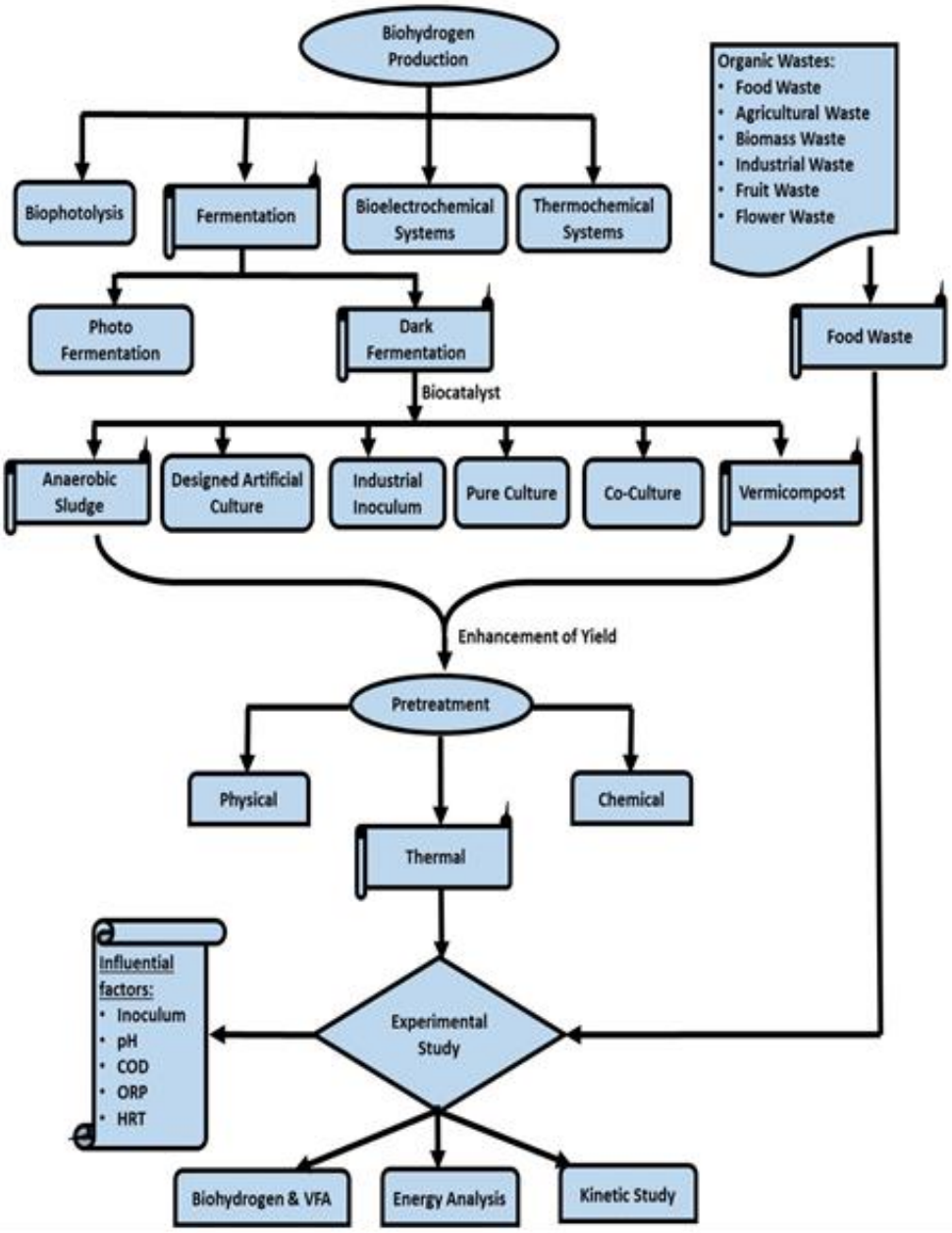

Figure 2. Overview of the biohydrogen production processes and the experiment performed in the study.

2. Materials and Methods

2.1. Substrate and Inoculum

The food waste was collected from the institute canteen and was prepared with the below-mentioned composition, such as rice (60%), potatoes (5%), eggs (5%), and cereals (5%), etc. The wastes were masticated using a food processor and filtered through a stainless steel mesh. The chemical compositions of food wastes were pH 7.2, VS 36.7 g/L, C/N ratio of 26.3, TS 39.3 g/L, and COD 51.38 g/L with a biodegradable fraction (BOD/COD) of 0.74. The mashed food waste was prepared as a slurry using distilled water and the organic content was adjusted to 10–30 gVS/L. The processed vermicompost was purchased from a local farm, milled, screened to 0.5 mm, and stored at 4 °C before the experiment. The characteristics of vermicompost were pH 6.9, COD 26.8 g/L, and VS 36.68 g/L. Anaerobic sludge was collected from the local effluent treatment, and the mother culture was well washed and filtered in a nylon filter before being used as an inoculum. The characteristics of the anaerobic sludge were pH 7.3, TS 15.5 g/L, VS 12.21 g/L, C/N 19.8, and COD 17.1 g/L.

Anaerobic sludge and vermicompost were dissolved separately with distilled water and heated by immersing in a hot water bath at 100 °C for 10–15 min. Heat treatment helps to select specific spore-forming bacteria for enhanced hydrogen production and suppression of methanogenesis [30].

2.2. Experimental Reactor Setup and Analysis

A dark fermentation study was conducted in a batch mode at ambient temperature and pressure using 500 mL glass reactors with 333 mL of working volume equipped with mechanical stirring of 120 rpm [31]. The reactors were completely air-tightened and connected to a gas syringe. Each 500 mL reactor consists of 300 mL of food waste suspension (1:1 dilution) and 10% of enriched culture. The anaerobic condition maintained in the reactors were sealed with a rubber stopper with provisions for sample collection to prevent oxygen access. The pH was adjusted to 6 ± 0.05 using 1N HCl or 1N NaOH [30]. The first phase of the experiments was performed with VS concentrations of 10 g/L (VC1), 20 g/L (VC2), and 30 g/L (VC3) at room temperature. All the experiments were performed in duplicate with a retention time of 48 h. The second phase of the experiment was performed by combining the inoculums with a proportion of 50% (VC+AS) (Figure 3).

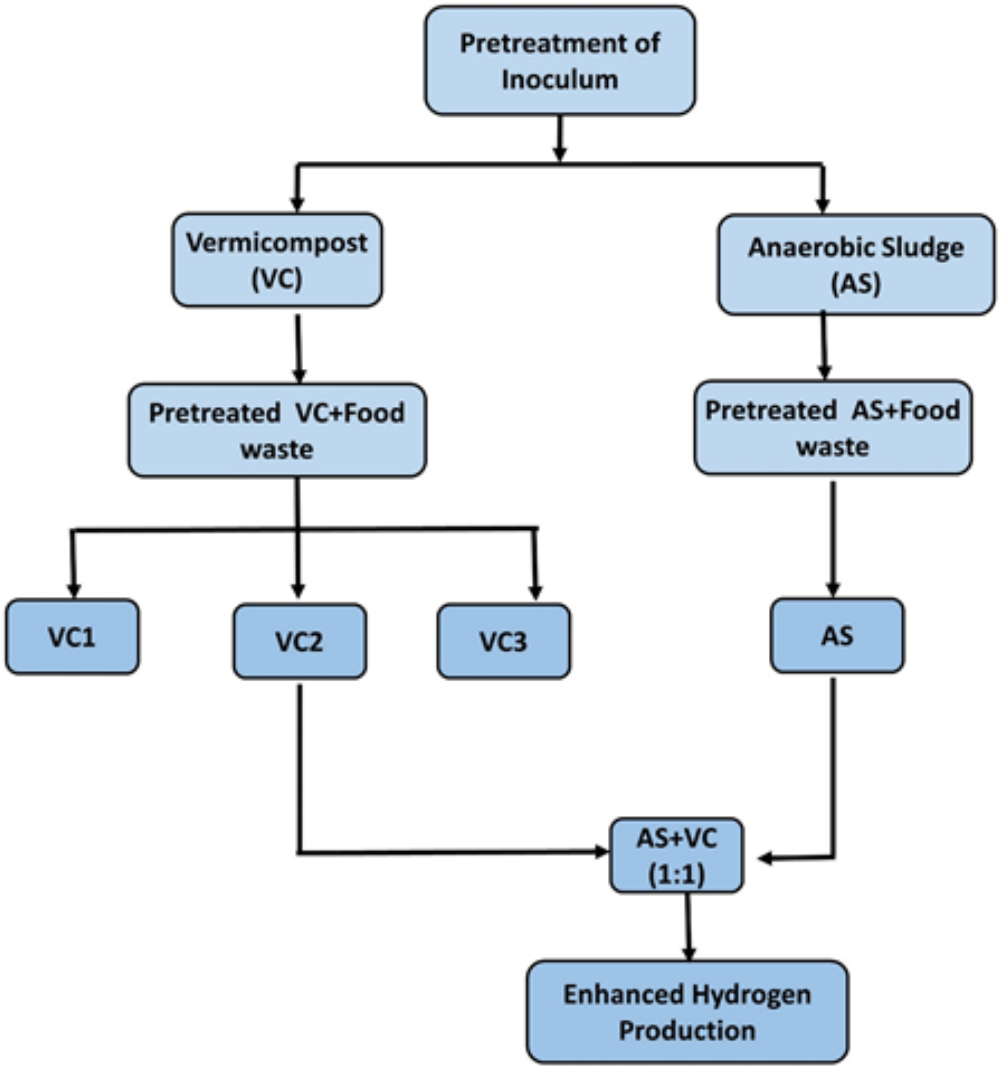

Figure 3. Biohydrogen production from food waste using pretreated anaerobic sludge (AS-20 mg/L), vermicompost as inoculum (VC1-10 mg/L, VC2-20 mg/L, VC3-30 mg/L) and (AS+VC 1:1).

2.3. Analysis

The total solids (TS), volatile solids (VS), and COD were measured according to the Standard Methods for the examination of wastewater samples [APHA, 2002]. pH and ORP were measured using a digital pH meter. The individual VFA composition was measured using high-performance liquid chromatography (HPLC). Thermofisher 3000 Series HPLC analyzed VFA in the medium with components such as an autosampler, vacuum degasser, thermostated column compartment, quaternary pump, and RID detector. The column used was a size of 4.6 mm × 150 mm × 5 µm. The mobile phase used in the HPLC was 20 mM aqueous phosphate buffer and acetonitrile, and the flow rate was 1.0 mL/min with an injection volume of 5 µL. The samples were prepared and diluted with 0.45 µm filter paper and 200 mg/L of standards for different organic acids such as acetic acid, succinic acid, butyric acid, and propionic acid. A gas chromatograph (Agilent 7890A) linked to a thermal conductivity detector (TCD) was used to classify the gas content, and the column used was a stainless steel column with a carrier gas (Nitrogen). The flow rate was fixed at 30 mL/min. The temperature was set at 40 °C, 80 °C, and 40 °C for the inlet, the detector, and the analytical column, and the gas injection volume was 0.2 mL.

2.4. Calculation of Hydrogen Conversion Efficiency and Energy Analysis

Hydrogen conversion efficiency (HCE) is the proportion of substrate converted to H_2. Probably, 1 kg of COD (food waste) can yield 468.83 L of H_2 (based on the acetate pathway of the dark fermentation process) [32]. The HCE of the substrate in the described reactor was determined using the formula:

$$\text{Hydrogen Conversion Efficiency (HCE,\%)} = (C \times 10{,}000)/(O \times Th \times Vs \times CODr) \quad (1)$$

where *C* is "cumulative hydrogen production (L)," *O* is "the organic loading (gCOD/L)," "the theoretical hydrogen yield" (*Th*; 0.468 L/gCOD), and Vs is "the substrate feeding volume to the reactor (L)," and *CODr* is "the substrate removal efficiency (%)".

Similarly, the calculation of hydrogen energy from dark fermentation is found using the formula [33]:

$$E_h = V_h \times \rho H_2 \times \alpha H_2 \quad (2)$$

where E_h is "the generated energy of hydrogen from dark fermentation (kJ/d)"; V_h is "the hydrogen yield from dark fermentation (m^3/d)"; ρH_2 is "the density of hydrogen (0.0899 kg/m^3)", and αH_2 is "the calorific value of hydrogen (1.43 × 10^5 kJ/kg)".

2.5. Mathematical Model and Simulation Analysis

A modified Gompertz model was performed using the Gompertz Equation (3) for all the batch experiments to characterize the progression of cumulative hydrogen production using simulation software OriginPro.

$$H = Hm \times \exp\{-\exp[(R \times 2.7183/Hm) \times (\lambda - t) + 1]\} \quad (3)$$

where H (mL) is the total biohydrogen production in the period t (h), Hm (mL) is the highest amount of hydrogen produced, R (mL/h) is the optimum hydrogen generation rate, and λ (h) is the lag period. Hm, R, and L values for each experiment were calculated by changing the biohydrogen production values in Equation (3) with a nonlinear regression analysis using Origin Software 2022.

3. Results and Discussion

3.1. Biohydrogen Production

In the first phase of the study, the biohydrogen production was evaluated using heat-shock pretreated vermicompost as inoculum and food waste as substrate at a varying organic load of 10 gVS/L (VC1), 20 gVS/L (VC2), and 30 gVS/L (VC3). The highest biohydrogen production rate was observed with VC2 (48 mL/gVS), indicating that an

adequate substrate availability improves the substrate utilization towards biohydrogen production. After VC2, the second highest biohydrogen production was recorded with VC3, with a rate of 35 mL/g VS. A lower biohydrogen production with VC3 can be attributed to the presence of inhibitors [34]. The lowest production was reported with VC1 (33 mL/gVS). Overall, the study infers that food waste is considered a potential substrate since it consists of 95% VS and 85% moisture; however, the performance varies based on the potential of the inoculum.

Lower biohydrogen production with VC3 compared to VC2 indicates substrate inhibition. Previously, it was reported that under the mesophilic condition, when the F/M was 6, the volumetric hydrogen production was higher; however, in the present study, it was observed to be less than 6, which might have influenced the biohydrogen production efficiency. Controlling F/M appropriately can enhance biohydrogen production [35]. VC2 reported higher performance due to the vermicompost's properties, such as structure, porosity, and moisture-holding capacity, which improves the organic waste's degradation rate [36]. The earthworm extract fragments the substrate, increases the solid fraction of digestate, and releases high-quality humus due to the effective enzyme activity [37]. Among all the experimental conditions, the combined action of the extract of earthworms and microorganisms degrades the waste and biochemically stabilizes the organic substrate through the bio-oxidation process.

In the second phase of the operation, using 20 gVS/L of food waste as substrate and pretreated anaerobic sludge (AS) as inoculum, the biohydrogen production was 46 mL/gVS. A diverse group of microorganisms, in which a series of biochemical reactions play a significant role in fermentation, manifests anaerobic mixed consortia. Mixed consortia are applicable for large-scale operations and should suit the substrate's non-sterile, and degraded complex nature [6]. Employing pretreated consortia possibly inhibits hydrogen-consuming microbes, subsequently enhancing hydrogen production. Generally, anaerobic culture cannot stimulate hydrogen production unless methanogens are suppressed, since it is an intermediate for methane formation. As mixed culture contains hydrogen-consuming microorganisms, pre-treatment paves its path toward acidogenesis, terminating methanogenic processes, allowing H_2 as an end product [38,39]. It also prevents other hydrogen-consuming bacteria from competitive growth and co-existence [6].

Studies reported a significant difference between untreated and pretreated inoculum [40]. An increase in hydrogen production (nearly 18 times) was observed with pretreated inoculum because of the enrichment of H_2-producing bacteria in the mixed culture [41,42]. In this experiment, the highest hydrogen production was observed with experiment VC+AS (50 mL/gVS), which infers the benefits of coupling anaerobic sludge and vermicompost (Figure 4). In addition, the pretreatment of vermicompost extracts facilitates mass reduction, waste stabilization, and pathogenic reduction, which helps to enhance hydrogen production [43].

3.2. Redox Conditions

pH is one of the vital factors in bacterial metabolism for biohydrogen production. Initially, pH was adjusted to 6.0 for all the experiments, and for every 6 h, a change in pH was observed. For all the experimental conditions, pH was gradually decreased with respect to time resulting for the production of fatty acids. Pretreated biocatalyst enhances the acidogenic-producing bacteria, which lowers the reactor' pH. For VC1 at an organic load of 10 g/L, the pH changed from 6 to 5.2 for the first 6 h of fermentation, with gas production of 5 mL/gVS. With the increase in operation time, the pH decreased to 4.52 and was found stable at 42 h. The maximum hydrogen production was achieved at 18 h (8 mL/gVS). The bio-oxidative process of organic matter is facilitated by microorganisms assisting in biohydrogen production, particularly from vermicompost inoculum sources [16]. VC2 with an organic load of 20 g/L reported pH variation from 6 to 5.0 for the first 6 h, and the amount of gas produced was observed to be 12 mL/g VS. With continuous operation at 36 h, the pH was dropped to 4.67. Change in pH was more with VC1 than VC2, indicating a good

buffering capacity. VC3 with an organic load of 30 g/L was reported with a pH variation from 6 to 5.3 for the first 6 h, and the amount of gas produced was observed to be 8 mL/gVS. With continuous operation, the pH was monitored at 6 h, and it decreased drastically at a particular stage at 36 h; later, the process reached stabled condition. Generally, a very drastic drop in pH produces rapid acid and might achieve the inhibitory levels impacting the buffering capacity of the system. Hence, the removal of excess hydrogen regulates the pH to sustain hydrogen production [44]. The maximum hydrogen production was achieved at 18 h (10 mL/gVS). Among all the experiments, VC3 reported lower performance than VC1 and VC2. The hydrogen production was also less, inferring that the availability of substrate and inoculum proportion was less (Figure 5). However, at 20 gVS/L, the performance of VC2 was higher; hence, further study was evaluated at 20 gVS/L.

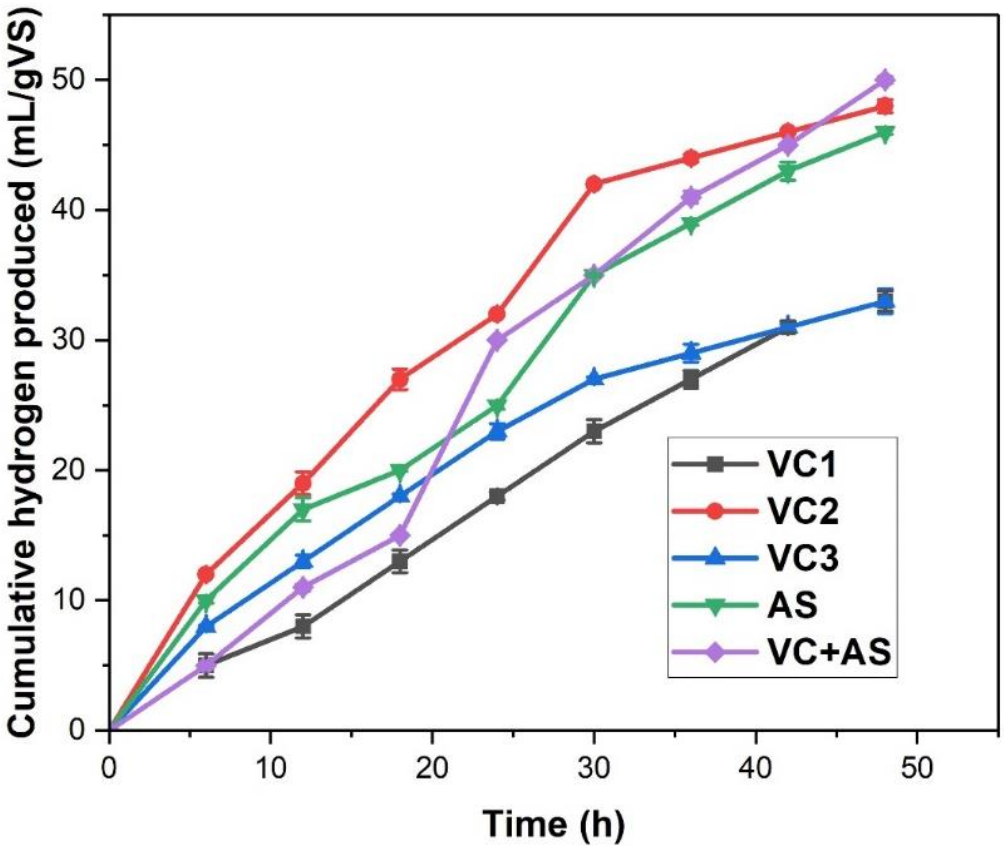

Figure 4. Cumulative hydrogen produced in different reactors (VC1, VC2, VC3, AS, and VC+AS).

Redox conditions were also measured for all the experimental conditions. Redox potential reflects the microbial metabolic activity, and controlling it at the appropriate time makes the fermentation processes more efficient. Since hydrogen production is the reduction process, the standard redox potential of H_2/H^+ is formed in the anaerobic fermentation processes. For VC1, the ORP was observed to vary from −40 mV to −170 mV. Similarly, for VC2, the ORP was observed to vary from −35 mV to −151 mV, whereas VC3 was reported as −21 mV to −130 mV. The conversion of organics by microbes led to acidifying the medium. Compared to VC1, VC2 reported higher hydrogen production, inferring that the earthworm extract favors an active microbial population with the passage of organic material. Previous studies described an abundance of the Clostridium genus, which supports the production of hydrogen production [45]. Reduction in pH disrupts the molecular structures, which affect intracellular and extracellular reactions, which causes drift in the pH's linearity, leading to a metabolic shift in the hydrogen-producing bacteria [46].

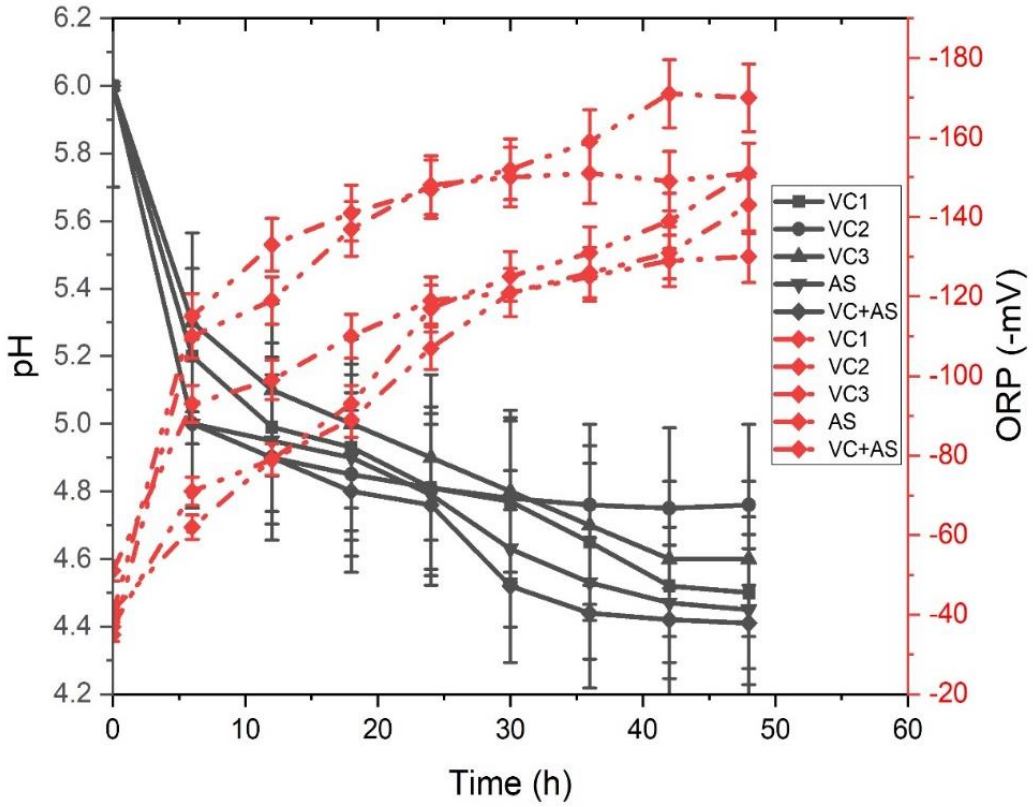

Figure 5. pH and ORP changes concerning different experimental conditions.

The experiment for AS was carried out using pretreated anaerobic sludge with 20 gVS/L. The pH was observed to vary from 6 to 5.0 for the first 6 h, and the amount of gas production during this phase was observed to be 10 mL/gVS. The ORP was observed to range from −37 mV to −143 mV. The prolonged operation reduced the pH at 6 h and stabilized at 42 h. Based on the optimum results from experiments for VC2 and AS, experiment VC+AS was designed using 50% vermicompost extract and 50% anaerobic inoculum. The pH variation was observed from 6 to 5.0 for the first 6 h. The ORP was observed to vary from −41 mV to −151 mV. With the prolonged operation, the pH was monitored to drop at the 6th hour and stabilize at the 36th hour. The maximum amount of hydrogen was displaced at 12 h (20 mL/gVS). The combined effect of both anaerobic inoculum and vermicompost reported enhanced performance with less retention time. The cornerstone of a cell's metabolic network is a conjugate combination that creates a comprehensive redox reaction [47]. Biohydrogen generation occurs mainly in the acidification phase, where carbohydrates are rapidly converted to hydrogen by fermenting bacteria and lead to acid by-products [48]. pH plays an essential role in the production of VFAs. However, pH regulation could induce microorganisms to reach optimum hydrogen production capacity, as pH variations in fermentation block hydrogenase's action [49], which plays an essential role in overall fermentation processes.

3.3. Volatile Fatty Acids

The acidogenic metabolism produces both H_2 and VFAs simultaneously. The acid intermediates produced in the process cause changes in the pH and the system's buffering ability [50]. The most significant level of VFA generated was 7.71 g/L at pH 4.5 in VC1, in the range of pH 6 to 4.5. The metabolites produced in terms of acetic acid, butyric acid, and propionic acid were composed of 2.81 g/L, 3.208 g/L, and 1.492 g/L, respectively. The initial alkaline condition favored the system posing as a pre-treatment step enhancing the hydrolysis rate. Enzymatic and microbiological activity results in high-quality nutrient-dense products, humus, and hormones converted into VFAs. Acetic acid pro-

duction was high, implying that hydrogen-producing bacteria were abundant. Propionic acid production was low, indicating that the overall retention period was short, limiting the proliferation of hydrogen-consuming bacteria and lowering the conversion rate [51]. Similarly, in experiment VC2, the pH was stated to have changed from 6 to 5.0 for the first 6 h, and the total VFA attained 7.9 g/L at pH 4.76 (Figure 6).

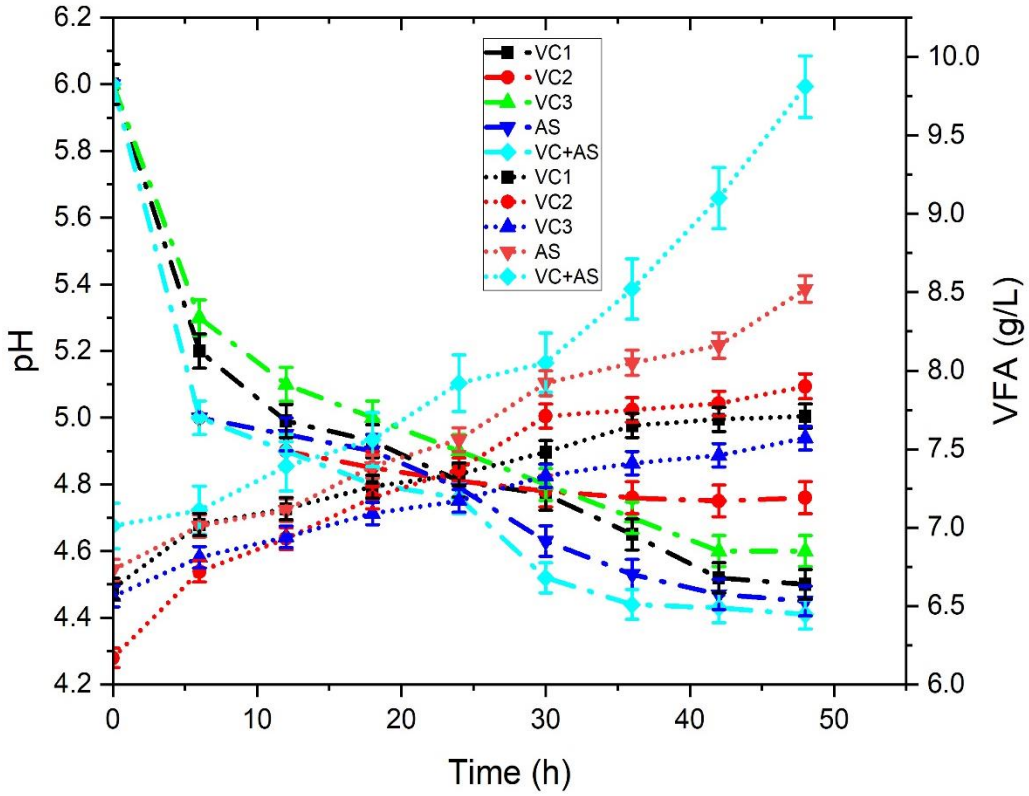

Figure 6. pH and VFA changes concerning different experimental reactors.

The complete degradation rate of processes with effective oxidation is improved when substrate availability is increased. Thermal pretreatment enhances the solubilization of the compost, allowing for more effective treatment [52]. At pH 4.76, VC2 obtained 2.21 g/L acetic acid, 3.62 g/L butyric acid, and 1.769 g/L propionic acids, respectively. According to Han et al. (2016), the proportion of butyric acid to acetic acid (B/A ratio) was more significant than the acetic acid/propionic acid concentration, which indicates substantial hydrogen production [53]. Experiment VC3 showed a pH shift from 6 to 4.6 and a maximum VFA of 7.57 g/L, with 2.426 g/L of acetic acid created, 3.282 g/L of butyric acid formed, and 1.562 g/L of propionic acid formed, which values were considered to be less than other experiments. Enhanced acetic acid generation can be related to the dominancy of the alkaline environment. Since the F/M ratio was lower, the degradation rate was lower, and hence the VFA production rate was minimal. Change in pH with AS changed from 6 to 4.5, with a total VFA production of 8.52 g/L, 2.414 g/L of acetic acid, 3.86 g/L of butyric acid, and 1.796 g/L of propionic acid, respectively. According to research, the optimal pH for active acidogenesis in the anaerobic micro-environment is 6 to 4.5 and 6 to 5.5, which prevents both methanogenesis and solventogenesis [53–55]. As a result, the pH range in this investigation was 6 to 4.5, implying acid generation was successful. Experiment VC+AS showed a pH change from 6 to 4.41, with a total VFA of 9.81 g/L, 2.957 g/L of acetic acid, 4.286 g/L of butyric acid, and 2.123 g/L of propionic acid (Figure 7).

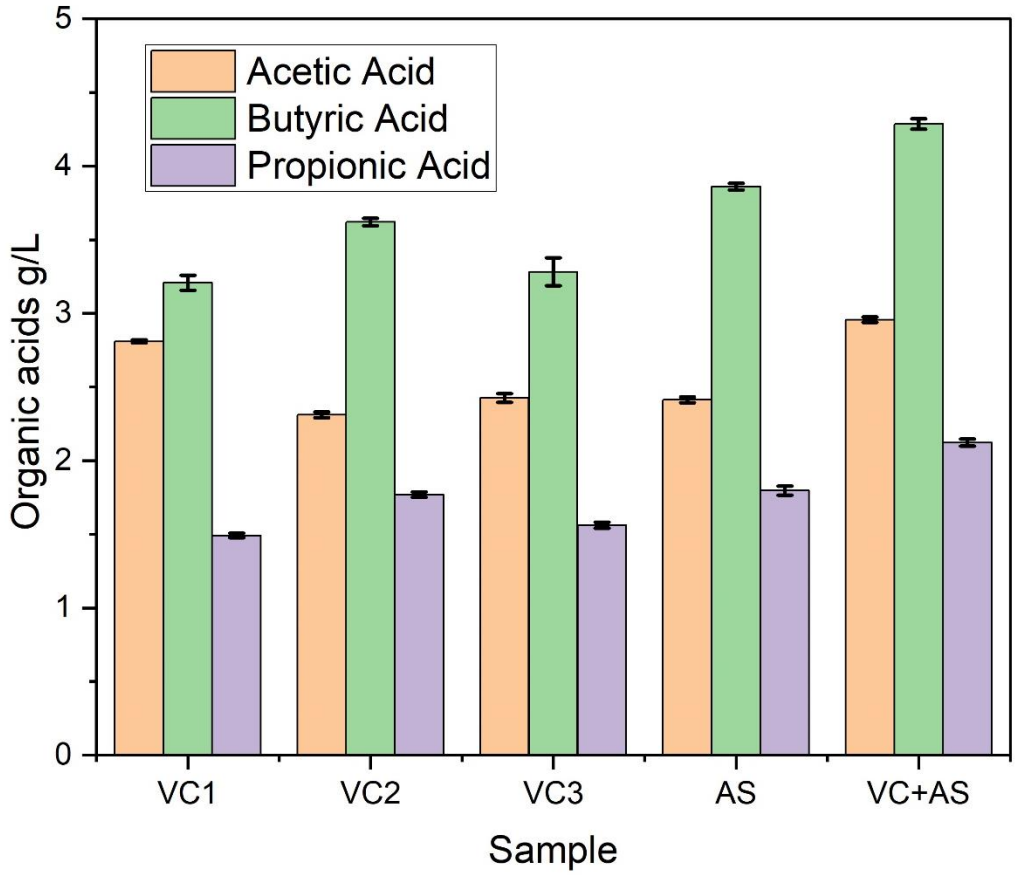

Figure 7. Acetic acid, butyric acid and propionic acid production in different reactors.

The reduction of methanogenic activity is responsible for increased fatty acid production [56]. Acetic and butyric acids were the most frequent VFAs in all the reactors, accounting for more than 70% of the total VFA output. Biohydrogen formation was enhanced considerably as the butyric acid concentration increased, and these findings were confirmed by Bansal et al. (2013) that anaerobic bacteria primarily produced hydrogen through butyrate-type fermentation [57]. Clostridium species are likely to be the dominant microbes in batch reactors [45,58,59]. They will be responsible for butyrate-type fermentation. The existence of food waste at optimal conditions allows the microbes to balance the system because the system buffering capacity may diminish when large amounts of acid are produced. The generation of VFAs in the fermenter involves a shift in the metabolic process and provides information on how to increase and boost H_2 production by altering or modifying the circumstances. As a result, adjusting the operational pH may be considered as a technique to maintain high VFA production efficiency while also aiming for a specific acid type, especially in long-term and continuous operations.

3.4. Substrate Degradation Efficiency

Food waste is an effective carbon source for hydrogen production in the dark fermentation processes. Experimental variations of VC1, VC2, and VC3 showed effective degradation; VC1 with an inlet concentration of 10 g/L of VS was observed to decrease to 5 g/L with a removal efficiency of 50%, where the total gas displaced was 33 mL/gVS. The organic sources have been reduced to soluble metabolites and H_2 production, inferring that the vermicompost effectively degraded the organic carbon source. Experiment VC2 was observed to decrease from 20 g/L to 12 g/L of VS with a removal efficiency of 40%, and the amount of gas displaced was observed to be 48 mL/gVS. The increase in the concentration of VS produced higher H_2 production, and the conversion rate of soluble metabolites was higher. However, the degradation rate decreased since the retention time was 48 h.

An increase in retention time might further reduce the concentration of VS without any inhibition in the system. Experiment VC3 reported a VS removal efficiency of 31% with a decrease of VS from 30 g/L to 20.3 g/L for 48 h, and the volume of gas displaced was 33 mL/gVS.

Higher concentrations of VS reveal substrate inhibition, in which a drastic drop in pH is observed that affects its buffering capacity and leads to a change in the metabolic pathway, resulting in less hydrogen production. Studies have reported that hydrogen production is more sensitive to retention time than substrate concentration [60]. Maintaining a suitable range of organic loading for effective microbial growth and stable hydrogen production is imperative, favoring the present study results [61]. Reactor AS shows the VS removal efficiency of 50% with VS reduction from 20 g/L to 10 g/L in 48 h, and the total gas displaced was found to be 46 mL/gVS. A higher degradation rate was observed in the anaerobic system because of the mixed bacterial growth population, which degrades the complex substrate. In particular, the pretreated acidogenic culture degrades the organic substrate into several metabolites and increases the reaction rate. Reactor VC+AS shows a VS removal efficiency of 60% with a reduction of VS from 20 to 8 g/L in 48 h, and the total gas displaced was found to be 50 mL/gVS (Figure 8).

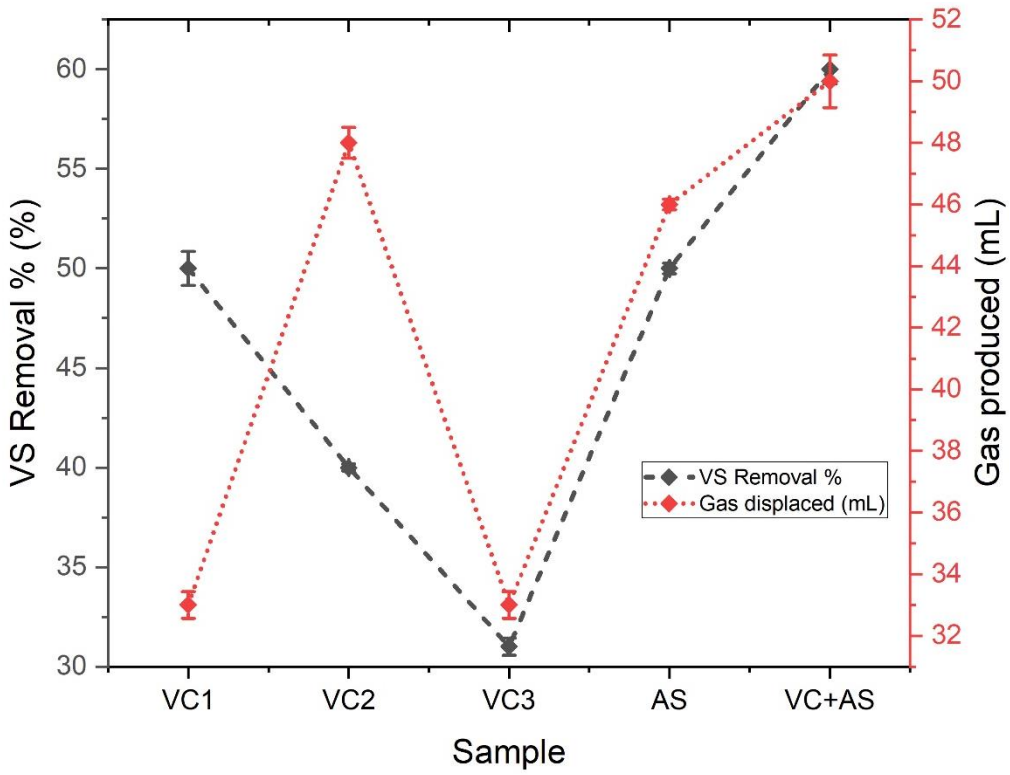

Figure 8. VS removal efficiency and gas produced in different reactors.

The vermicompost extract indirectly simulates the microorganisms and enhances the organic substrate degradation [62]. The compost characterizes the active phase by ingestion, and the microorganisms degrade the ingestion process quickly; hence, the degradation rate increases with both the inoculums. Table 1 describes the overall findings of other research on biohydrogen production from several biodegradable wastes.

Table 1. Biohydrogen Production from various biodegradable wastes.

S.No	Substrate Used	Inoculum Used	Cumulative Hydrogen Produced	VS Degradation Efficiency	References
1	Food waste	Anaerobic Sludge and Vermicompost	50 mL/g VS	60%	Present Study
2	Vegetable waste	Acid-treated anaerobic sludge	89 mL/g COD	65%	[63]
3	Food waste	Anaerobic sludge	57 mL/g VS	39%	[35]
4	Potato, pumpkin waste, other agro-industrial wastes	Anaerobic sludge	46 mL/g VS	-	[64]
5	Autoclaved FW	Anaerobic sludge	27.91 mL/g VS	-	[65]
6	Cheese whey and wheat straw hydrolysate	Anaerobic sludge	4554.5 H_2/L	-	[66]
7	Mild heat-pretreated FVW	Vermicompost	63.0 mL/g VS	46%	[17]
8	Waste wheat	Anaerobic mixed culture	654.7 L/kg	45%	[67]

3.5. Hydrogen Conversion Efficiency and Energy Analysis

Hydrogen gas produced by bacterial fermentation of glucose was limited to a yield of 4 mol/mol glucose [68]. Theoretically, 1 kg of COD (food waste) can produce 468.83 L of H_2 (based on the acetate pathway of the dark fermentation process) [32]. The hydrogen conversion efficiency of the food waste in the specified reactor was calculated using Equation (1). The increase in organic loading rate (10 to 30 g/L of VS) shows hydrogen conversion efficiency (13.51–27.16%). The highest hydrogen conversion efficiency was achieved with reactor VC+AS (27.16%), which might be due to lower methanogenic activity and higher acidogenic activity, whereas, with reactor VC3, 13.51% of HCE was described as the minimum (13.51%). It is apparent from the experimental data that, despite the load, the pretreated vermicompost can inhibit methanogens, which aids in the evolution of good H_2, resulting in higher conversion efficiency. The HCE noted from reactor VC3 was comparatively lower than all other reactors.

The ranking of HCE with different organic loading is 27.16% (VC+AS) > 20.14% (VC2) > 18.39% (AS) > 15.33% (VC1) > 13.51% (VC3). The reactor VC+AS reported higher conversion efficiency due to the potential of compost extract and the microorganisms. The mixed bacteria significantly impact vermicompost and simulate the conversion efficiency. However, pretreatment increases the hydrolysis rate in the system and eliminates unwanted pathogens for hydrogen production. Dark fermentation is more feasible without light limitations and can achieve a high production rate [69]. In food waste, the carbon balance of biohydrogen production is 32.5% of undigested food and 67.5% of CO_2. The study infers that 1 g of food waste produces 0.304 g of glucose, which can be converted into 245.7 mL and 205.8 mL of hydrogen in the batch and continuous reactor. The batch process is confirmed higher because of the washout of glucose in the effluent [70]. Higher VS removal and conversion efficiency were observed, inferring that the degraded substrate has converted to biohydrogen. The energy content of hydrogen is 120 MJ/kg–142 MJ/kg, which can be the only combustion product [71]. Studies have reported that the net energy ratio (NER) is equal to 1.9 using sweet potato residues, sugarcane bagasse, and sugarcane juice substrate. NER is the ratio of energy produced and total energy input, inferring that the production rate is more than the input energy [72]. In the present study, energy recovery is calculated as mentioned in Equation (2); the hydrogen energy from reactors VC1, VC2, VC3, AS, and VC+AS is 212.1 (J/day), 308.5 (J/day), 212.1 (J/day), 295.6 (J/day), and 321.3 (J/day), respectively, assuming the reactor's average performance is 80%. Therefore, the results of the hydrogen recovery from the reactors of VC1, VC2, VC3, AS, and VC+AS are 169.68 (J/day), 246.8 (J/day), 169.68 (J/day), 236.48 (J/day), and 257.04 (J/day), respectively (Figure 9).

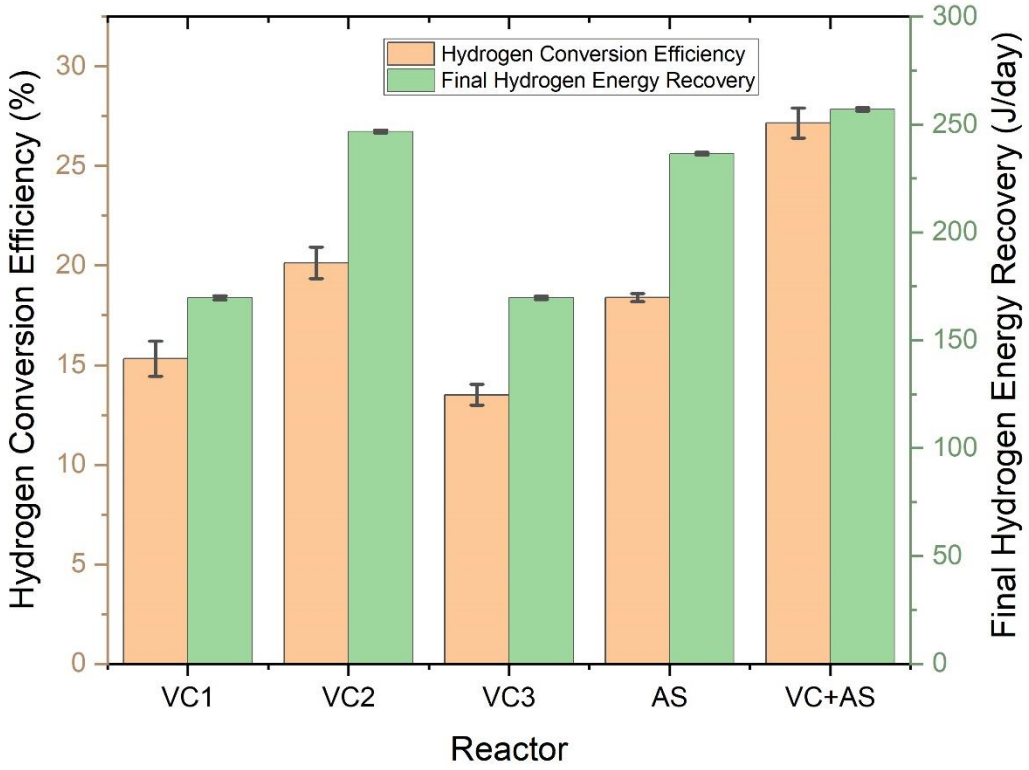

Figure 9. Hydrogen conversion efficiency and Energy recovery in different reactors.

The study inferred that the organic fraction present in the food waste could be an effective substrate converted to hydrogen. In the graph above, hydrogen energy and final hydrogen energy recovery are plotted for various reactors, inferring that the pretreated biocatalyst can suppress the other microorganisms that could consume hydrogen and enrich the hydrogen-producing microorganisms. This favors the production rate in the dark fermentation processes.

3.6. Substrate Mapping Based on Hydrogen Yields

The cumulative hydrogen production was correlated with the Gompertz equation, and the regression coefficient was determined to identify the effective fit with experimental data. Gompertz's model is divided based on the shape of the curve and the location parameter that shifts the curve horizontally without changing its shape. The parameter value is kept constant relative to the *x*-axis or the *y*-axis by characterizing type I and type II of Gompertz models. The model parameters used in the study were lag time, maximum hydrogen potential, and H_2 production rate. The regression factor R2 was generated by fitting the Gompertz model to experimental data of hydrogen accumulation. This model was highly suitable for describing the kinetics of VC1 (0.997), VC2 (0.997), VC3 (0.995), AS (0.982), and VC+AS (0.996), suggesting that the model fit the experimental data well.

The overall goodness of fit between the measured hydrogen production data and those fitted with the Gompertz equation was high in VC+AS, inferring the efficiency of the hybrid inoculum. Maximum cumulated hydrogen production yields (Hmax) were higher for VC+AS (50 mL/gVS). The cumulative hydrogen output of VC1, VC2, VC3, AS, and VC+AS is shown in Figure 10.

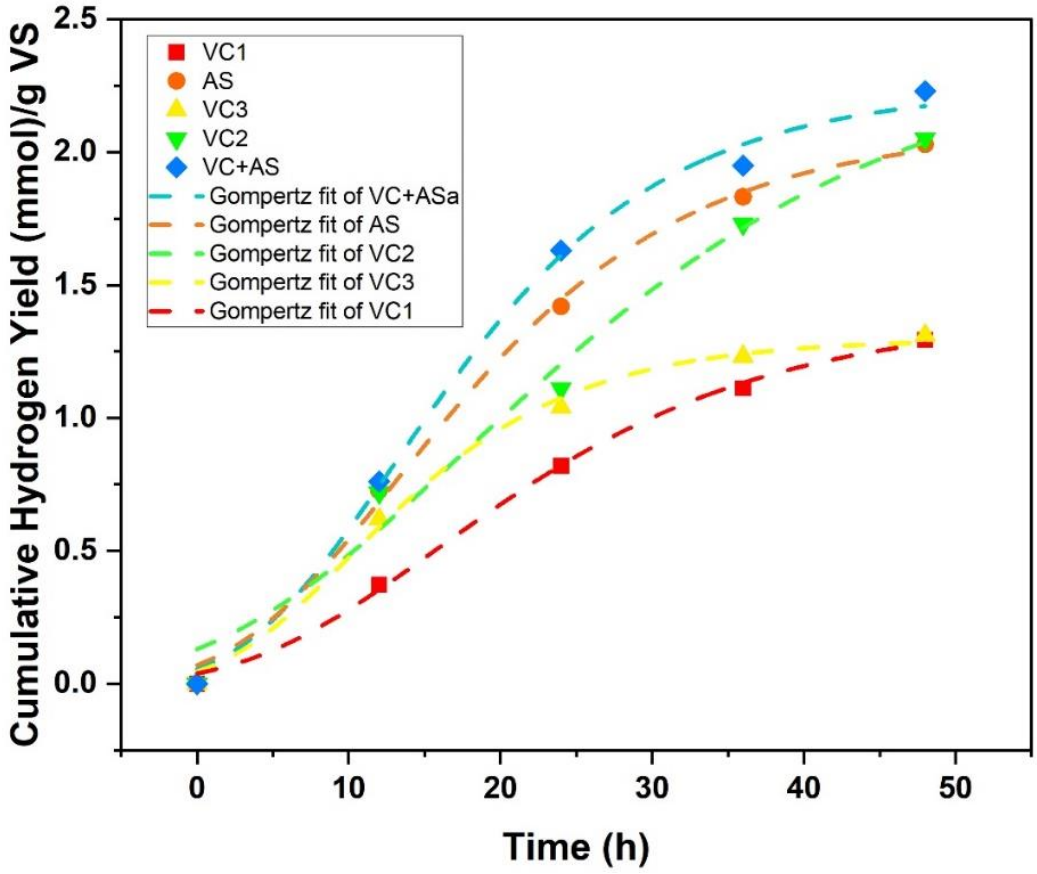

Figure 10. Gompertz model for biohydrogen production.

Table 2 discusses the difference in cumulative hydrogen output among fermentation techniques due to the various hydrogen-producing methods and routes in VC1, VC2, VC3, AS, and VC+AS. In other words, these data showed that the thermal pretreatment of the inoculum resulted in a more efficient microbial population.

Table 2. Estimated parameters of Gompertz equation for Biohydrogen Production.

Substrate	Reactor	Hm (mL)	R (mL/h)	λ (hr)	Production H_2 (mmol/g VS)	R^2
Vermicompost	VC1	33	0.04	3.41	1.224	0.997
	VC2	48	0.071	2.4	2.010	0.997
	VC3	33	0.05	1.69	1.267	0.995
Anaerobic Sludge	AS	46	0.05	1.16	1.927	0.982
Vermicompost + Anaerobic Sludge	VC+AS	50	0.083	3.05	2.107	0.996

The study infers that the pretreatment method suppresses the methane, and only CO_2 and H_2 were deducted. Studies conclude that the inner structure of lignocellulosic material should be preferable to the use of carbohydrates [73]. The findings presented were consistent with those of previous studies, demonstrating a link between biogas and biohydrogen generation and the carbohydrate content of the substrate employed [74].

4. Conclusions

The study concludes that the syntrophic fusion between the vermicompost and sewage sludge enhanced biohydrogen production and other metabolites. The combined inoculum acts as an effective biocatalyst, which increases the bio-oxidation property and balances the excellent redox conditions resulting in increased biohydrogen yield production. However,

regulating the fermentation condition using the pretreated biocatalyst simulates the acidogensis for suppressing the methane formation and leading to biohydrogen production. The modified Gompertz model results infers that the experimental data fit well with model data for the feasibility of industrial scale. The study concludes that the biohydrogen production from food waste is an environmentally sustainable process that paves a path for production of renewable bioenergy and managing the waste effectively. Hydrogen is being utilized in a wide variety of industries. However, biohydrogen also has the scope to be used in many industries. Further investigations could overcome the current industrial limitations such as poor yields and rates of hydrogen generation when converting organic waste to biohydrogen, large working reactor volumes, modern technology, and various storage and transportation facilities are necessary. Recent scientific breakthroughs in the biotechnology field involving metagenomics methods and genetic changes can also help make microbial-aided hydrogen production economically feasible and practicable in the coming years. In this regard, the hydrogen economy is booming towards green technologies, which will be able to replace non-renewable energy sources to sustain the world's energy supplies.

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Abbreviations

The following abbreviations are used in this manuscript:

Btu	British thermal units
GHG	Greenhouse gases
FW	Food waste
VC	Vermicompost
AS	Anaerobic Sludge
FAO	Food and Agriculture Organization
VFA	Volatile Fatty Acids
VIT	Vellore Institute of Technology
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
GC	Gas Chromatography
HPLC	High-Performance Liquid Chromatography
ORP	Oxidation Reduction Potential
APHA	American Public Health Association
HCE	Hydrogen Conversion Efficiency
THY	Theoretical Hydrogen Yield
F/M	Food to Microorganism ratio
NER	Net Energy Ration
VS	Volatile Solids

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