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Conversion of Biomass into Bioenergy for a Sustainable Circular Bioeconomy: Potential Solution to the Environmental Issues

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Abstract

The conversion of biomass into energy, also known as bioenergy, is the energy derived from biodegradable materials and is considered a potential solution to global energy problems. Concerning global sustainability, the reliance on fossil fuels and greenhouse gas emissions that contribute to global warming are particularly alarming. In order to address pressing issues and crises, an alternative and potentially viable solution is necessary. Currently, the most prevalent biomass feedstocks for energy conversion are plants, crops, and their wastes, which are potential resources and can be converted into biofuels, biopower, and a variety of bioproducts. The primary biomass sources in Malaysia are sewage treatment plant (STP) sludge managed by the Indah Water Konsortium (IWK), a national sewerage company, and oil palm industrial (OPI) wastes considered oil palm biomass (OPB) as well as abundant food waste generated daily. Current advanced research and development seeks an effective and efficient solution to biomass by converting waste to bioenergy, which could be a complete and viable solution with the generation of revenue and a circular economy approach at the point of generation for sustainable development. This keynote address provides an overview of the research on 'Turning waste into valuable bio-products,' with a focus on biofuels (bioethanol, biomethane and biogas) and bioenergy from the perspective of green technology. In Malaysia, various biofuels and bioenergy are currently being developed from a variety of domestic and industrial waste sources. The sources and characteristics of various biomass feedstocks in Malaysia are to be discussed. The topic discusses how data from research and development (R&D) could be scaled up to the commercial level for the recovery of renewable energy using green technology. For sustainable development, certain case studies on the bioconversion of food waste, sewage sludge, and oil palm industry waste into biofuels and bioenergy are to be shared.In addition, some results are shared on theinvestigation of the potential of food waste (FW) and activated sludge (AS) as co-substrates for improving biogas production through anaerobic digestion (AD). The research aims to determine the optimal FW-AS ratio and its application in an anaerobic sequencing batch reactor (SBR) while evaluating the impact of various organic loading rates (OLR): 10g, 25g, and 50g VS L^{-1} d^{-1} on biogas production and microbial composition. Dark fermentation at the AD experiments revealed a 25% increase in biogas and methane production. In AD-microbial electrolytic cell (MEC) system, the optimized H-MEC system demonstrated a yield of 92% of the biomethane obtained with more than 90% of CO_2 conversion from the AD system.

Keywords: *Biomethane; Bioethanol; Biomass; Anaerobic digestion; Microbial electrolysis cell*

1 Introduction

Anaerobic digestion is a versatile and promising technology that addresses key contemporary challenges: waste disposal and the generation of renewable energy (Wang et al., 2023). It yields biogas, primarily composed of methane (CH_4) and carbon dioxide (CO_2), suitable for conversion into compressed natural gas (CNG) as a clean fuel source. This process involves four distinct phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, orchestrated by diverse microbial consortia (Deb et al., 2023). Hydrolysis was the initial phase, breaking down complex macromolecules into simpler compounds, catalyzed by exoenzymes. Acidogenesis follows, with acidogenic bacteria converting these compounds into volatile organic compounds, including volatile fatty acids (VFAs) and other byproducts (Harirchi et al., 2022). Acetogenesis further transforms these acids into acetate and hydrogen, led by acetoclastic methanogens. The final step, methanogenesis, converts acetate into methane, carbon dioxide, and hydrogen, primarily mediated by acetotrophic and hydrogenotrophic methanogens (Kim et al., 2004; Castillo et al., 2015; Deb et al., 2023).

Biological methanation, synonymous with anaerobic digestion, is a microbiological process that decomposes organic matter in the absence of oxygen, yielding biogases and digestate residues (Deb et al., 2023). This process relies on a consortium of naturally occurring environmental microflora, mainly facultative or strictly anaerobic

microbes, with specific roles in the digestion process (Harirchi etl al., 2022; Deb et al., 2023). Organic matter serves as the primary nutrient source, supplemented by essential trace elements to ensure stable biogas production (Carrillo-Reyes et al., 2019; Deb et al., 2023). Biogas predominantly comprises methane and CO_2, with trace amounts of other gases like hydrogen sulfide, water vapor, and ammonia. An emerging trend was the utilization of biogas as a sustainable alternative fuel for heating, cooking, and electricity generation (Harirchi et al., 2022). While previous research has highlighted the benefits of co-processing food waste (FW) and activated sludge (AS) in biogas production, these findings were predominantly based on batch tests (Carrillo-Reyes et al., 2019).

Methane-enriched biogas, a valuable renewable energy source, is generated through the process of anaerobic digestion (AD). This intricate biological process relies on anaerobic bacteria to decompose organic materials such as manure, sewage, municipal waste, and food waste (Gujer & Zehnder 1983; Deb et al., 2023). As these organic materials undergo transformation within the AD system, they are converted into biogas. This biogas typically consists of approximately 55–75% methane (CH_4), along with 45–25% carbon dioxide (CO_2), and trace amounts of hydrogen sulfide (H_2S), hydrogen (H_2), and other gases (Ryckebosch et al., 20211). The AD process unfolds in two distinct phases: the acidogenic phase and the methanogenic phase (Gujer & Zehnder 1983). In the acidogenic phase, organic matter undergoes initial breakdown into simpler compounds, while in the subsequent methanogenic phase, methane is produced through specific microbial activities. The robust composition of biogas, coupled with its sustainable origins, underscores its importance as a clean and renewable energy source. To enhance hydrogen (H_2) production, it is crucial to reduce the growth of methanogenic bacteria since they utilize H_2 as a precursor for methane (CH_4) production. While lowering the pH to create acidic conditions can be effective in limiting methanogenesis, slightly acidified conditions within the pH range of 5 to 6 were found to be ineffective in controlling methanogenesis, especially in a setup focused solely on H_2 production, as seen in an MEC-only treatment (Hu et al., 2008).

In numerous studies involving microbial electrolysis cells (MECs), it has been observed that methanogens tend to utilize the generated H_2 for CH_4 production (Hu et al., 2008). Interestingly, CH_4 production persists even when the electrical voltage is no longer applied, underscoring the resilience of methanogens in MEC systems. A particular MEC study employed a low hydraulic retention time (5.3 hours) to remove methanogens from the reactor, yet CH_4 production was still detected (Deb et al., 2023). Recent experiments have reaffirmed the role of methanogens as impediments to H_2 production in wastewater treatment plants utilizing MEC technology. This is because CH_4 production reduces the concentration and purity of H_2. However, it's worth noting that only a limited number of MEC studies have been carried out using actual waste materials (Liu et al., 2012). Moreover, MEC treatment volumes are often kept relatively small, typically less than 0.3 liters, with a substantial electrode surface area within the MEC. This characteristic can make scaling up the technology an expensive endeavor. To address the challenge of methanogenic activity, Liu et al., (2012) explored a method involving the pre-treatment of waste activated sludge fermentation liquid using bi-frequency ultrasonics and alkaline addition before feeding it into an MEC. This approach effectively suppressed methanogenic activity, leading to increased H_2 production and the elimination of CH_4 production. However, it also introduced significant process complexity (Liu et al., 2012).

From a technical perspective, the process of producing bioethanol from lignocellulosic materials has been extensively studied and recently reviewed (Barampouti et al., 2019). This production process consists of several key stages, each serving a specific purpose such as Pretreatment, Enzymatic Hydrolysis, Fermentation and Ethanol Recovery. The initial phase is pretreatment, which focuses on altering the structural characteristics of the raw material. The goal is to make it easier for enzymes to access the lignocellulosic material and enhance the production of sugar monomers. This step is crucial for maximizing the efficiency of the subsequent processes. Enzymatic hydrolysis is a critical step that targets the breakdown of structural carbohydrates like starch, cellulose, and hemicellulose. During this stage, pentoses and hexoses, which are valuable sugar components, are liberated from the lignocellulosic material. These liberated sugars are essential for the fermentation process that follows. In the fermentation step, microorganisms are introduced to metabolize the readily available sugars obtained from enzymatic hydrolysis. This microbial metabolism results in the production of ethanol, a key objective of the entire bioethanol production process. The final step involves recovering the ethanol produced during fermentation. This typically involves distillation techniques to separate and purify the ethanol for use as a biofuel. In terms of production cost, one of the most financially demanding stages, which significantly contributes to the overall cost of bioethanol production, is the enzymatic hydrolysis step (Deb et al., 2019). This cost challenge poses a significant barrier to the widespread adoption of ethanol production from lignocellulosic materials. One potential solution to mitigate this cost challenge is to consider on-site production of the necessary enzymes instead of relying on commercially available enzyme preparations. Only a limited number of organisms have the capability to naturally produce the required enzymes for lignocellulosic biomass breakdown. Many of

these organisms belong to species such as *Aspergillus*, and *Trichoderma* (Barampouti et al., 2019; Deb et al., 2023).

The primary objective of this study is twofold. First, it aims to stimulate the metabolic system for the synthesis of specific enzymes by employing food waste. Second, it seeks to harness these enzymes for the purpose of breaking down food waste (FW) into ethanol, biogas and methane. To enhance ethanol, biogas and methane production, the investigation also includes the examination of three strategies: the introduction of cellulase and amylase, methanogenesis microbes and *Saccharomyces cerevisiae*.This study aims to bridge the gap between batch and continuous reactor performance. It seeks to determine the optimal FW-AS ratio for ethanol, biogas and methane production and investigate the impact of organic loading rates (OLR) in a continuous reactor over a 30-day period (biogas production). The OLR significantly influences ethanol, biogas and methane production, particularly through its effect on hydrolysis and the efficient production of ethanol, biogas and methane contains and volatile fatty acids (VFA). This research promises to optimize ethanol, biogas and methane production and enhance the understanding of the interplay between substrate ratios, OLR, and microbial composition in continuous bioreactors.

2 Experimental

2.1 Inoculum, Substrate and bioreactor

The inoculum used in this study was anaerobic activated sludge obtained from the Dasherkandi Sewerage Treatment Plant in Dhaka, Bangladesh. Anaerobic activated sludge was distinct from conventional aerobic activated sludge, as it operates in oxygen-deprived conditions, specifically for biogas production processes (Deb et al., 2023). Characterizing activated sludge involves assessing various parameters to understand its composition and quality in the context of biogas production or other bioprocesses. The pretreated inoculum had the following values per gram of inoculum: total solids (TS) 0.99g, volatile solids (VS) 0.79g, chemical oxygen demand (COD) 3.09g, carbohydrates 0.06g, and proteins 0.24g. Additionally, waste activated sludge (WAS) was sourced from a traditional municipal wastewater treatment plant, subjected to dewatering through a press, and stored at 4°C.Food waste (FW) was collected daily from a restaurant and supermarket over a span of 2 weeks, with only the fermentable components retained while non-fermentable materials such as bones and inert items like plastic or paper were discarded. The daily FW samples were combined, homogenized, and finely ground using a blender (1200W grinder, China) to achieve a particle size of <0.5 mm. Subsequently, this mixture was preserved at 4°C to prevent spontaneous fermentation.

2.2 Enzymatic hydrolysis of FW

Liquid-state cultivation (LSC) was employed to maximize enzyme production, with a focus on achieving the highest possible enzyme yield. During this process, the pretreated food waste (FW) was added to the cultivation in a specific ratio (100–10, 90–20, 80–30, 70–40, 60–50, and 50–60), indicating that for different ratio such as 10mL of the inoculum, 100mL was FW. Additionally, a carefully determined amount of a commercial cellulase and amylase enzyme (Spirizyme® Fuel) was introduced to attain either 10 to 50 Units of cellulase and amylase per gram of carboxymethyl cellulose (CMC)and starch. To prevent microbial contamination, sodium azide was included at a concentration of 0.02% (w/v). The enzymatic hydrolysis of the mixture was carried out at a controlled temperature of 30 ± 1°C using a rotary shaker set at 150 rpm. Periodically, samples were collected, subjected to centrifugation at 50ml for 10 minutes to separate solids from the liquid, and then analyzed for reducing sugar content.In simpler terms, this part of the experiment involved using solid-state cultivation to generate enzymes, and these enzymes were combined with pretreated food waste and commercial cellulase and amylase. The goal was to achieve specific enzyme activity levels and convert cellulase and starch in the FW into reducing sugar. Sodium azide was added to prevent unwanted microorganisms from contaminating the process. The hydrolysis reaction occurred at a consistent room temperature while monitoring the reducing sugar production over time.

2.3 Conversion of FW into bioethanol

Food waste was transformed into ethanol using a two-step process that involved enzyme production through liquid-state cultivation (LSC) and simultaneous saccharification and fermentation (LSF) carried out by the mesophilic fungus or a combination of fungus and the yeast *S. cerevisiae*. Initially, mesophilic fungus was cultivated using LSC, a method described previously for the generation of cellulolytic and amylolytic enzymes (Deb et al., 2019, Deb et al., 2023). In this step, the fungus was grown on a liquid substrate to produce these enzymes. The entire LSC culture, including the fungal mycelia and the enzymes it produced in situ, was then transferred to pretreated food waste (FW). The ratio of LSC to FW was maintained at 100–10, 90–20, 80–30, 70–40, 60–50, and 50–60(expressed as ratios based on volatile solids). The fermentation process was conducted in a rotary shaker operating at a controlled temperature of 30 ± 1°C and 150 rpm in Erlenmeyer flasks equipped with

specialized rubber stoppers. These stoppers served a dual purpose: they maintained anaerobic (oxygen-free) conditions required for fermentation and allowed for the release of carbon dioxide, a byproduct of ethanol production. When using a mixed culture, additional baker's yeast was introduced to enhance the fermentation process. This method allowed for the efficient conversion of food waste into ethanol with careful attention to maintaining the necessary environmental conditions for the fermentation process to succeed.

2.4 Biogas potential yield test

Various combinations of FW–AS (% VS) were examined to assess their impact on biogas potential yield (BPY) at a concentration of 10 g VS-L^{-1}. These combinations included (FW-AS ratio) 100–10, 90–20, 80–30, 70–40, 60–50, and 50–60 (expressed as ratios based on volatile solids). Additionally, individual treatments using FW and AS (100–0 and 0–100) a control strategy were employed as benchmarks for comparison. The BPY assessments were conducted using an Automatic Methane Potential Test System equipment, following a standardized protocol for biogas potential determination as outlined by Carrillo-Reyes et al., (2019). The batch reactors, with a total volume of 1000 mL and a working volume of 860 mL (along with a 140 mL headspace), were used for these tests. The incubation was maintained at a constant temperature of 30°C. All treatments were performed in triplicate, and the biogas production attributable to the inoculum alone was subtracted from the BPY calculation for all treatments.

2.5 Conversion of FW into biomethane based on AD-MEC

The conversion of food waste (FW) into biomethane without the use of Microbial Electrolysis Cells (MEC) primarily relies on the tried-and-true process of anaerobic digestion (AD). In this environmentally friendly approach, collected and prepared food waste is introduced into an anaerobic digester, where microorganisms work their magic in the absence of oxygen. The digestion process unfolds through several stages, starting with hydrolysis, followed by acidogenesis, acetogenesis, and ultimately methanogenesis, where biomethane is generated. The resulting biogas, comprising methane, carbon dioxide, and trace gases, is collected, purified, and made suitable for various applications, such as electricity generation, heating, or as a vehicle fuel. Unlike MEC-based approaches, which involve electrochemical reactions to boost hydrogen production, this traditional AD process relies solely on natural microbial processes to produce biomethane, contributing to sustainable energy generation and food waste management. The initial inoculum for this experiment was sourced from the effluent of a prior AD-MEC study, featuring a combinations included (FW-AS ratio) 100–10, 90–20, 80–30, 70–40, 60–50, and 50–60. Following a 30-day digestion period before commencing the microbial electrolysis cell (MEC) phase, the contents of both treatment reactors were combined and reintroduced into the digesters. Additional measurements revealed a pH level of 6.5, an oxidation-reduction potential (ORP) of -900 mV, and an electrical conductivity of 33.12 ms/cm. Each digester, encompassing both the AD-MEC and AD-only systems, possessed a total active volume of 1000mL.

2.6 Experimental set-up of anaerobic digestion

The FW–AS ratio that yielded the highest BPY was chosen for biogas production in an anaerobic digestion based sequencing batch reactor (SBR). The SBR, an acrylic cylinder with a 1000mL reaction volume, 860mL working volume, 120mL headspace, and a 50% exchange volume, was inoculated with 10mL of anaerobic sludge pretreated at 30°C for 1 hour to inhibit methanogenic microorganisms. The initial inoculum activation involved ten operation cycles, each lasting 1-2 hours, using a solution containing 15gL^{-1} of glucose and a mineral medium composed of 41.6 g NH_4Cl, 4g K_2HPO_4, 2g $MgCl_2.6H_2O$, 1.6g $FeSO_4.7H_2O$, 40mg $CoCl_2.6H_2O$, 40mg $MnCl_2.4H_2O$, 40mg KI, 8mg $NiCl_2.6H_2O$, and 8mg $ZnCl_2$.Following inoculum activation, experiments were conducted with a fixed feedstock of 10g VS L^{-1} (FW–AS ratio of 100–10). Reaction times of 12, 8, and 4 hours were employed, corresponding to the tested organic loading rates (OLR) of 10, 25, and 50g VS L^{-1} d^{-1}, respectively. The fill, settle, and draw phases lasted 10, 60, and 10 minutes, respectively. The reactor incorporated deflectors on the walls to ensure thorough mixing. A temperature controller using a recycling water pump maintained a constant temperature of 30°C inside the reactor. The pH was controlled at 5.5 using a pH controller with NaOH 1 mol L^{-1}. Stirring and pump operations were regulated by software programmed in Labutilizing a USB6008 data acquisition card connected to a personal computer. Gas flowmeter monitored the biogas flow rate. The cumulative biogas volume was calculated over time through numerical integration implemented in Lab. Daily biogas samples were collected to assess the percentage of methane, hydrogen and carbon dioxide. The cumulative CH_4, H_2, and CO_2 production (CH, H and CO) data was fitted to a modified Gompertz equation (Equation 1).

$$\text{CH(t)} = CH_{max} \exp\left\{-exp\left[\frac{R_{max}}{CH_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where CH(t), H(t), and CO(t) represents the total methane, hydrogen and carbon dioxide produced, CH_{max}, H_{max} and CO_{max} were the maximum methane, hydrogen and carbon dioxide production, R_{max} (mL CH_4 H_2 CO_2 d^{-1}) was

the maximum methane, hydrogen and carbon dioxide production rate, and λ (day)was the lag time before exponential biogas such as methane, hydrogen and carbon dioxide production.

2.7 Analytical analysis

The analytical analysis of all parameters such as total solids, dissolved solids, volatile solids, pH (Instruction's manual supplied by Hanna Instruments), fatty acids, chemical oxygen demand, protein and microbial growth (APHA, 2005; Barragán et al., 2017) involved in this research was conducted according to the standard methods. Furthermore, enzyme activities, including cellulase (CMC) and amylase (CMC), were assessed using carboxymethyl cellulose and starch substrates, respectively, following the methodology detailed in the study conducted by Deb et al.,(2023). The biodegradation rate was monitored in each step of the process to evaluate the effective bioconversion. The results were subjected to ANOVA analysis, followed by a post-hoc Tukey test to compare differences among the treatments.

3 Results and Discussion

3.1 Anaerobic digestion (FW and AS) characterization

Table 1 provides the characterization of both FW and AS for anaerobic digestion. Both residues exhibited high moisture content, exceeding 70%, indicating their suitability for a wet anaerobic digestion process. FW primarily consisted of 95% easily degradable material but had low alkalinity, which could lead to the rapid accumulation of volatile fatty acids (VFA), acidification, and potential inhibition of dark fermentation. In contrast, AS had four times higher alkalinity compared to FW. Co-processing FW with AS effectively enhanced the system's buffering capacity, mitigating VFA accumulation and process inhibition. Additionally, AS could be introduced during FW fermentation to control pH within the optimal range of 5.5 to 7.5, optimizing biogas production (Ramos et al., 2017) and obviating the need for expensive alkalis.

Table 1. Physicochemical characterization of the anaerobic digestion

Parameters	Food Waste	Activated Sludge
TS (g/g)	0.39	0.18
VS (g/g)	0.38	0.12
VS/TS	97.4	66.7
COD (mg g^{-1} TS)	1300	1288
Carbohydrates (mg g^{-1} TS)	208	52
Proteins (mg g^{-1} TS)	221	108
Alkalinity (mg $CaCO_3$ L^{-1})	150	610
pH	4.35	8.12
Iron (mg L^{-1})	0.56	66
Nickel (mg L^{-1})	0.02	0.8
Calcium (mg L^{-1})	6.98	193
Aluminum (mg L^{-1})	1.31	34

Furthermore, AS contained significantly higher levels of trace metals, some of which play crucial roles in metabolism, cellular growth, and the activation of enzymes and coenzymes during dark fermentation (Deb et al., 2019) for biogas production. Notably, metals like Fe and Ni were essential for the biosynthesis of methanogenesis, which facilitate proton reduction and biogas formation (Moreno et al., 2023). AS also exhibited higher concentrations of Ca and Al, which were essential for promoting microbial growth and cell retention, preventing cell washout in continuous bioreactors (Alam & Deb, 2020).

3.2 Hydrolysis of FW

The efficiency of a multienzyme system consisting of cellulose and amylase, which was produced reducing sugar during liquid-state cultivation (LSC), was assessed for its effectiveness in the hydrolysis of food waste (FW). However, it was observed that the initial cellulose and amylase production levels were relatively low. To overcome this limitation, the hydrolysis mixture was supplemented with different FW-Enzyme ratios, ranging from 100–10, 90–20, 80–30, 70–40, 60–50, to 50–60. Over time, the concentration of reducing sugar in the hydrolysis mixture gradually increased and stabilized after 36 hours, as depicted in Figure 1.

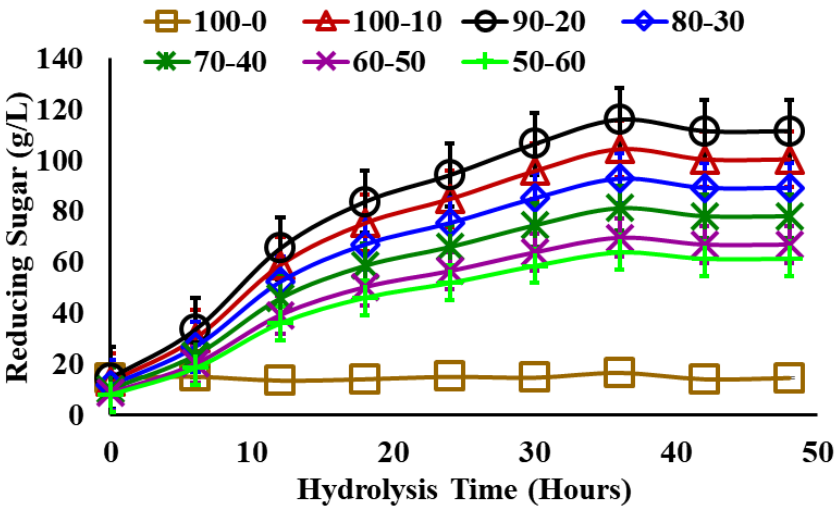


Figure 1. Hydrolysis course of reducing sugar release during FW hydrolysis by mixed enzyme system. Other factors were fixed pH 6.5, agitation time 150 rpm and room temperature (30°C±2).

Notably, the mixed enzyme system resulted in a release of 116g/L of reducing sugar, corresponding to a hydrolysis yield of 700.69%based on the cellulose and starch content present in the FW. The presented Figure 1 offers a comprehensive view of the enzymatic hydrolysis process applied to food waste under various FW-AS ratios and time intervals. The data elucidates several key trends and observations. Firstly, there is a consistent and time-dependent increase in the concentration of reducing sugar, signifying the progressive breakdown of complex carbohydrates within the food waste. Secondly, the choice of FW-AS ratio has a discernible impact on the rate and magnitude of sugar production, with higher ratios leading to more rapid sugar release. Additionally, a notable phenomenon is the plateauing effect observed around the 36-hour mark, hinting at a potential limit to sugar extraction, possibly due to factors like enzyme saturation or substrate availability (Padella et al., 2019). Lastly, variations in sugar yield at the 36-hour interval among different FW-AS ratios underline the complexity of the hydrolysis process and the influence of specific conditions on its outcome. These insights shed light on optimizing enzymatic hydrolysis for sugar production, with implications spanning industries such as bioenergy and biotechnology.

3.3 Bioethanol production by mixed enzyme with *S. Cerevisiae*

This study is focused on the production of bioethanol, an important renewable fuel, through the utilization of a process called simultaneous saccharification and fermentation (SSF). SSF is a particularly promising approach as it combines the steps of hydrolysis (the breakdown of complex carbohydrates) and fermentation (the conversion of sugars into ethanol) into a single integrated process. This integration offers several key advantages, most notably the avoidance of an issue called end-product inhibition of b-glucosidase, which can hinder the conversion of sugars to ethanol, and the elimination of the need for separate reactors, simplifying the overall production process. The primary objective of this research is to investigate the feasibility of converting food waste (FW) into valuable bioethanol using the SSF process in a batch mode setup. The results of this study are quite promising, indicating a significant achievement in terms of bioethanol production. Specifically, the study reports that after 72 hours of fermentation, the ethanol production reached an impressive concentration of 68.40 g/L (as shown in Figure 2).

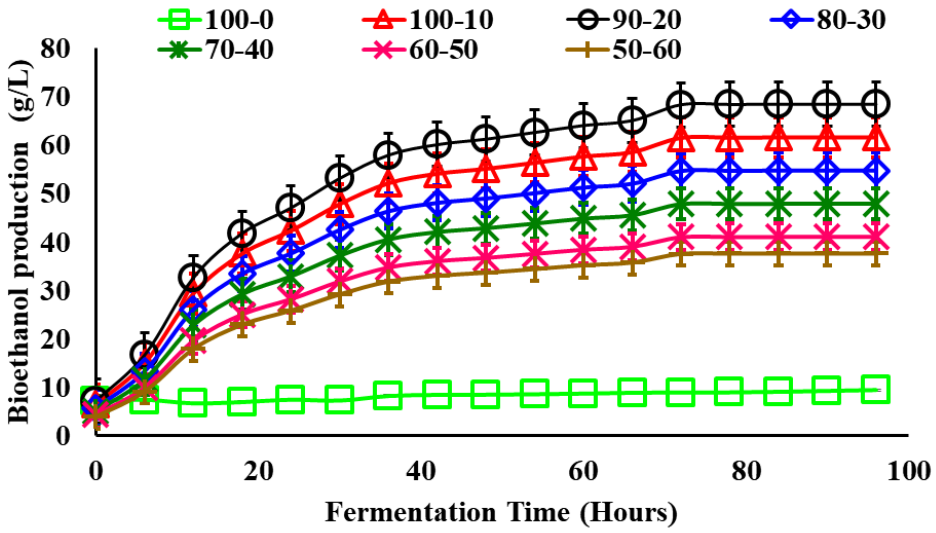
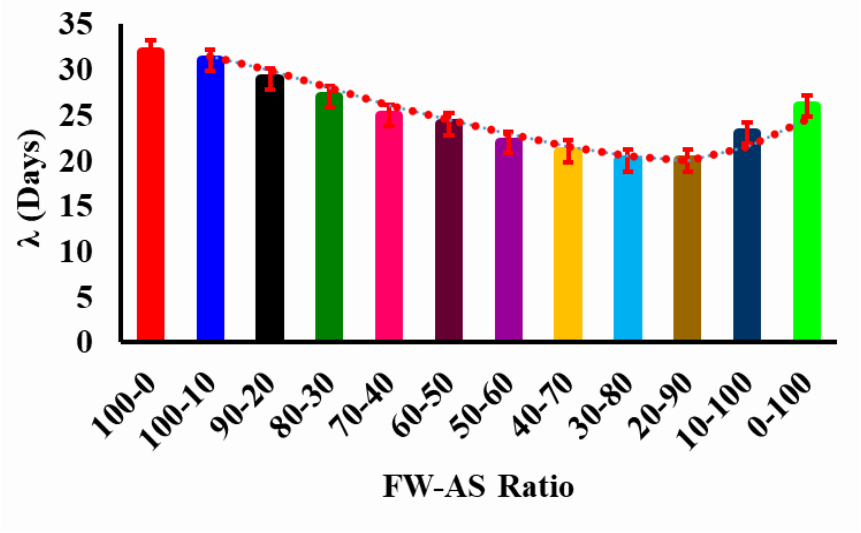


Figure 2. Bioethanol production from FW by mixed enzyme with *S. Cerevisiae*

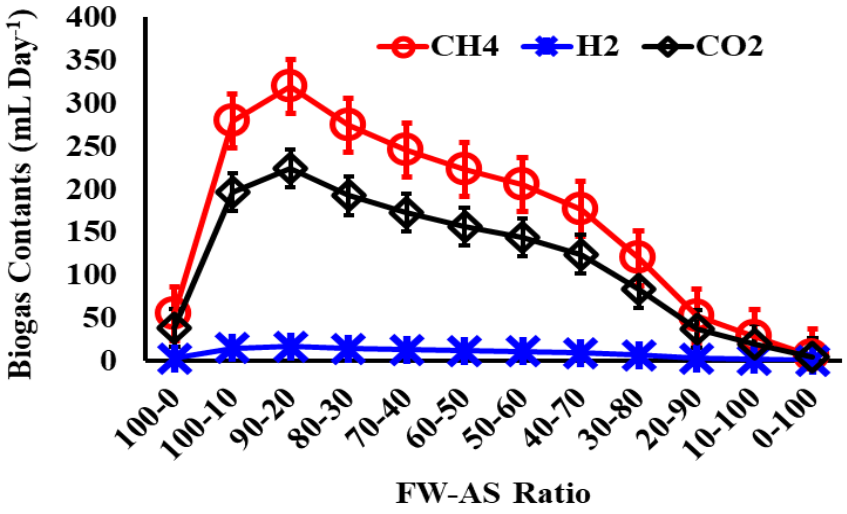
The significance of this outcome lies in its representation of approximately 31.8% of the maximum theoretical yield of bioethanol achievable from the soluble fraction of food waste (FW). This calculation serves as a pivotal benchmark, indicating the efficiency of the simultaneous saccharification and fermentation (SSF) process in harnessing the potential of FW for bioethanol production (Zhao et al., 2016). Notably, this level of efficiency implies that a substantial portion of the FW's soluble components can be effectively converted into bioethanol, making the SSF process a promising avenue for sustainable bioenergy production. By efficiently transforming FW into a valuable bioethanol resource, this research underscores the potential to simultaneously address waste management concerns and contribute to clean and renewable energy production, aligning with broader sustainability objectives (Rootzén et al., 2023). This outcome demonstrates the effectiveness of the SSF process in efficiently converting food waste into a valuable bioethanol resource. Such findings hold significant promise for sustainable bioenergy production, waste reduction, and environmental sustainability. This research underscores the potential for innovative approaches like SSF to contribute to a more sustainable and environmentally friendly energy landscape.

3.4 Biogas potential yield test

Figure 3 illustrates the results for (a) lag time (λ), (b) R_{max} (maximum biogas Such as CH_4, H_2 and CO_2 production rate), and CH_{max}, H_{max} and CO_{max} (total biogas production) across different waste compositions, including individual waste streams and various FW–AS ratios. Overall, the co-processing of FW with AS demonstrated improved fermentation performance. One notable benefit of adding AS was the immediate reduction in lag time (λ), which decreased from 32 days in the case of individual FW to 24 hours when FW–AS ratios of 80–30 and 30–80 were employed ($P < 0.001$). R_{max}, representing the maximum biogas production rate, exhibited distinct values for individual waste streams, with FW being nearly ten times higher than AS, and this value decreased as the proportion of AS increased in the FW–AS ratios. CH_{max}, H_{max} and CO_{max} indicating the total amount of biogas produced, followed a similar decreasing trend with increasing AS in the FW–AS ratios. However, the 100–10 ratio displayed a higher CH_{max} than both individual waste streams and other FW–AS ratios ($P < 0.01$). Notably, individual AS had a very low CH_{max} due to its limited availability of carbohydrates compared to FW. So, the percentage of CO2 in the biogas is 40%.



(a)



(b)

Figure 3. Kinetic parameters and biogas yield performance for each of the FW–AS ratios in batch test: (a) lag time; (b) R_{max} production at different FW–AS ratios.

However, WAS addition did reduce the amount of methane, possibly due to the activation of solventogenesis in methane producing bacteria, resulting from VFA accumulation in ratios with a higher FW content. These findings align with those reported by Kim et al.,(2004), who proposed an optimal co-digestion ratio of 87% FW and 13% sewage sludge for improved H_2 production compared to individual FW.

Figure 4 plays a pivotal role in elucidating the dynamics of biogas production and volatile solids (VS) removal within the context of different FW–AS ratios during batch testing. Notably, the data unveils a significant trend: as the proportion of AS increases in the FW–AS ratios, the efficiency of volatile solids (VS) removal experiences a noticeable decline. This observation implies that the presence of AS can influence the breakdown of organic matter, potentially slowing down the degradation of VS. Intriguingly, despite these fluctuations in VS removal efficiency, biogas production remains remarkably consistent. The study reports an average biogas production rate of approximately 57% across all treatment groups, regardless of the specific FW–WAS ratio ($P < 0.05$). This remarkable stability in biogas production underscores the robustness of the process, suggesting that AS, even in varying concentrations, has a limited impact on the overall biogas yield. Furthermore, the study's findings align with prior research, reinforcing the reproducibility and reliability of these results. In essence, Figure 4 provides invaluable insights into optimizing anaerobic digestion processes involving food waste and AS, offering potential improvements in both biogas generation and sustainable waste management practices.

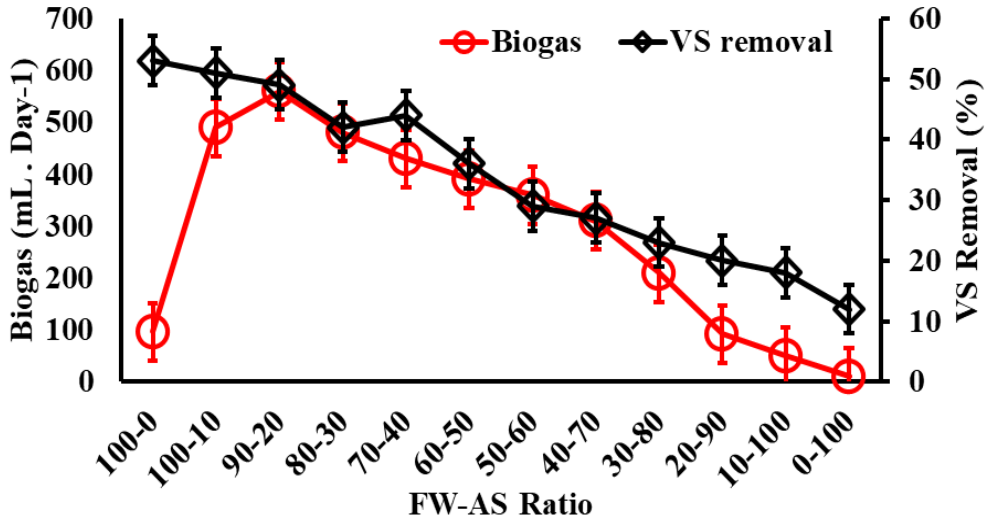


Figure 4. At different FW–AS ratios biogas production and removal of volatile solids (VS).

Even a small amount of AS contributed to enhanced biogas production, likely due to its alkalinity contribution, which stabilized the system's pH, and the presence of essential micronutrients like Ni and Fe, crucial for enzyme biosynthesis, including methanogenesis responsible for biogas production (Kim et al.,2004).

3.5 Biogas production from FW–AS co-digestion in the SBR

The inoculum underwent activation using glucose following the procedure outlined by Castillo-Hernández et al.,(2015). Subsequently, the SBR was supplied with a FW–AS ratio of 90–20 and operated for a total of 30daysat varying organic loading rates (OLRs). Specifically, the OLRs applied were 10g VS L^{-1} d^{-1}, 25g VS L^{-1} d^{-1}, and 50g VS L^{-1} d^{-1}. Figure 5 displays the biogas production throughout the SBR operation. During the glucose activation phase, the CH_4, H_2 and CO_2 percentage averaged 57, 3 and 40%. However, with the transition to the FW–AS substrate, the biogas percentage decreased. The variation in OLR had a notable impact on biogas percentages. Specifically, the OLR of 25g VS L^{-1} d^{-1} resulted in the highest biogas production which was around 500mL/g VS, reaching 57 ± 8% at 30 days.

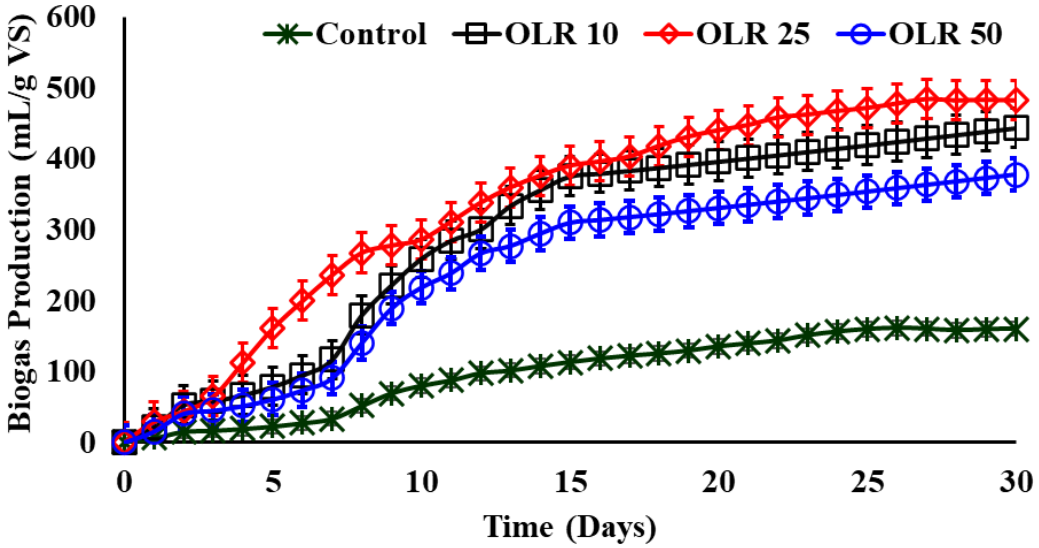


Figure 5. Biogas during SBR operation at different OLRs

In contrast, increasing the OLR led to the lowest biogas production, which averaged 18 ±3% ($P > 0.05$). As the OLR increased from 10 to 25 g VS L^{-1} d^{-1}, *Methanogenic spp.* became the second dominant species. *Methanogens* were well-known for their ability to produce methane from lactic and acetic acids (Ohnishi et al., 2012). This suggests that the co-processing of food waste (FW) and activated sludge (AS) did not significantly alter the most abundant members of the microbial community. However, with a further increase in OLR from 25 to 50 g VS L^{-1} d^{-1}, the abundance of *Mitsuokella spp.* reached 21%. *Mitsuokella spp.* were non-spore-forming, strictly anaerobic, fermentative bacteria that have previously been identified in biogas-producing reactors (Lan et al., 2002). Notably, *Mitsuokella*has the capability to hydrolyze phytate, the primary storage form of phosphorus in foods (Lan et al., 2002). This observation suggests that the higher availability of phytate in the SBR operated

at an OLR of 50 g VS L^{-1} d^{-1} might have stimulated the growth of *Mitsuokella*, although further research was needed to explore this phenomenon in depth. Conversely, *Enterococcus and Veillonella* exhibited a positive correlation, with substrate hydrolysis being more pronounced at an OLR of 10 g VS L^{-1} d^{-1}. Enterococcus was known for its ability to degrade complex polysaccharides such as xylan (Jumas et al., 2004), while *Veillonella* primarily produces acetic and propionic acids as major end products (Jumas et al., 2004).

3.6 Biomethane production by integrated AD and MEC with methanogenesis microbe

BioMethane production through the integration of Anaerobic Digestion (AD) with Microbial Electrolysis Cell (MEC) technology, involving methanogenic microbes, represents an innovative and promising avenue in the fields of renewable energy and waste management. In this process, Anaerobic Digestion serves as the initial phase, where microorganisms break down organic matter in the absence of oxygen, yielding methane and carbon dioxide. Methanogenic microbes play a pivotal role in this stage, converting organic acids and hydrogen into methane. The introduction of MEC technology enhances the overall efficiency of this process by providing an electrode interface for electroactive bacteria, facilitating electron transfer and, consequently, improving methane production. The collaboration between AD, MEC, and methanogenic microbes leads to a higher concentration of methane in the produced biogas, which can be further purified into valuable biomethane. This integrated approach not only boosts renewable energy production but also contributes significantly to sustainable waste management and greenhouse gas reduction, showcasing its potential in addressing both energy and environmental challenges. The results of the study reveal significant enhancements in biomethane production when the AD-MEC treatment was compared to the AD-only treatment, particularly during the MEC-inclusion period spanning days 20 to 30. The AD-MEC treatment exhibited a remarkable 92.0% increase in biomethane production over this timeframe. To put it in quantitative terms, the AD-MEC treatment yielded 560 mL of useful gases (a combination of 525.28mL Day^{-1} CH_4, 17.92 mL Day^{-1} of CO_2 remaining and32.25 mL day^{-1} of H_2) during an additional 36-hour digestion period, oxidation-reduction potential (ORP) of -900 mV, pH of 6.5 and room temperature 30°C (±2) whereas the AD-only treatment produced 319.2 mL of methane, 224.6 mL of $CO_2$and 16.8 mL of hydrogen. These findings are consistent with previous research (Zhang et al., 2015; Xiao et al., 2018), which similarly reported an 80-100% boost in biogas and methane production upon incorporating MEC into AD processes. So, the percentage of CO_2 in the biogas is 3.2% as shown in Figure 6.

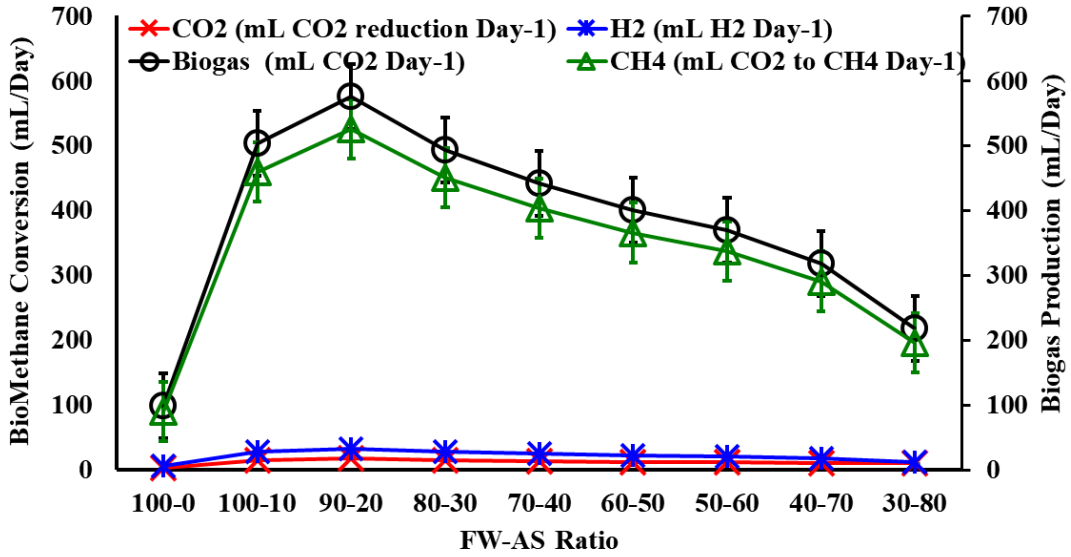


Figure 6. Biogas upgrading (CO_2 to CH_4 Conversion) AD-MEC for days 20–31 after MEC introduction (days 0–30 not shown).Other factor are36-hour of digestion period, oxidation-reduction potential (ORP) of -900 mV, pH of 6.5 and room temperature 30°C (±2).

Furthermore, the AD-MEC treatment also demonstrated a reduction in the concentration of carbon dioxide (CO_2) in the total gas composition, accounting for 3.2% compared to the AD-only treatment's 40.2%. This highlights the dual benefits of MEC integration: lowering CO_2 levels while simultaneously increasing the generation of valuable gases like methane and hydrogen within the biogas. Notably, the novel MEC design, which confined both the cathode and anode within a single chamber, played a pivotal role in this success. By reducing the distance between these electrodes, potential ohmic losses were minimized, thereby facilitating electron flow between the anode and cathode. Recent studies (An & Lee, 2013; Kadier et al., 2016) have corroborated the importance of limiting electrode distance in enhancing gas production, as extensive electrode separation can impede electron transfer, leading to increased ohmic losses.

Conclusion

This study demonstrates the considerable potential of harnessing food waste (FW) and other wastes to produce valuable biofuels/bioenergy, including bioethanol, biomethane, and biogas. Through the utilization of a multi-enzyme system combined with *S. cerevisiae* and methanogenesis microbes, the processes of biofuels are accelerated and enhanced its production. When integrated with *S. cerevisiae* in a mixed-microbial culture, bioethanol production exhibited a substantial increase in volumetric productivity, with a notable enhancement of approximately 23% when supplemented. In the AD system, SBR process indicated that a FW–AS ratio of 90–20 significantly improved bioethanol, biomethane, and biogas production, achieving approximately a 25% increase compared to FW alone. It is highlighted that the integration of AD with the MEC technology showed the significant increase of biomethane at high yield of 92%. This integration presents a promising avenue for significantly enhancing energy production from food waste anaerobic digestion, even when implemented as a post-digestion refinement step after 30 days. In essence, this research sheds light on the intricate microbial dynamics involved in the anaerobic digestion of FW and AS, uncovering key factors that influence biofuel production efficiency. It underscores the potential of this integrated approach to not only address waste management challenges but also contribute substantially to sustainable energy production and environmental protection.

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