Waste and Biomass Valorization
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ORIGINAL PAPER

Valorization of Oil Palm Frond as a Renewable Carbon Source for Microbial Bioflocculant Production: A Green Approach to Agricultural Waste Management

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Abstract

Despite Oil Palm Fronds (OPFs) being the most abundant harvestable lignocellulosic biomass residue in Asian region, they remain underutilized, contributing to greenhouse gas emissions when left to decompose in plantations. This study explores the feasibility of using enzymatically hydrolyzed OPF hydrolysate as a carbon source to partially substitute glucose in bioflocculant production. We hypothesize that OPF hydrolysate can serve as a viable carbon source for bioflocculant synthesis while minimizing environmental effect. Enzymatic hydrolysis with Cellic® Ctec3 without acid or alkaline pretreatment resulted in the highest reducing sugar yield (13.3 ± 0.2 g/L). A 40:60 OPF hydrolysate-to-glucose ratio yielded optimum bioflocculant yield (8.3 g/L) without compromising flocculating activity (87%). The produced bioflocculant remained stable across pH 3–7 and 4–30 °C. A preliminary life cycle assessment estimated a 58.8% carbon footprint reduction can be achieved compared to the glucose-only benchmark. These findings demonstrate OPF's potential as a sustainable feedstock for bioflocculant production.

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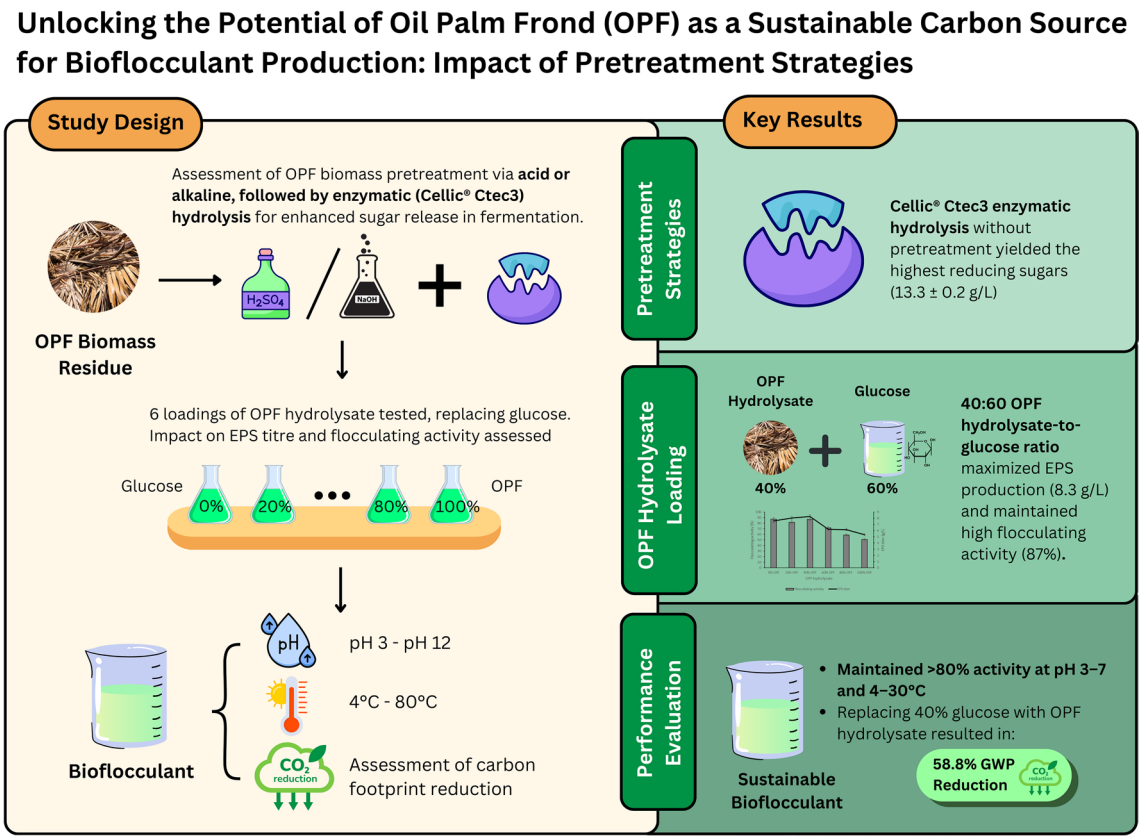
Graphical abstract



Keywords Agricultural waste management · Bioflocculant production · Biomass valorization · Oil palm frond · Sustainable bioprocess

Highlights

- Valorizes underutilized oil palm fronds (OPF) biomass from agricultural waste into a value-added bioproducts, addressing waste and emissions simultaneously.
- Mild enzymatic hydrolysis of untreated OPFs yielded 13.3 g/L reducing sugars, proving its viability as a carbon source.
- Optimized OPF-glucose supplementation (40: 60 ratio) maximized both bioflocculant yield (8.3 g/L) and performance (87% flocculating activity), demonstrating effective agro-waste utilization.
- The bioflocculant performed well across broad environmental conditions, suitable for diverse wastewater applications.
- Preliminary life cycle assessment shows a 58.8% reduction in carbon footprint compared to glucose-only production, aligning with circular economy goal.

Statement of novelty

This study introduces the first investigation into the use of oil palm frond (OPF) hydrolysate as an alternate carbon source for microbial bioflocculant production using *Bacillus licheniformis* CGMCC 2876. Unlike previous research that relies on conventional substrates such as glucose, this work explores an underutilized and abundant agricultural residue, addressing both valorization and sustainability challenges. Additionally, the process integrates pretreatment optimization and hydrolysate loading studies to enhance fermentable sugar recovery and bioflocculant yield. A preliminary life cycle assessment further quantifies substantial reductions in global warming potential (GWP), positioning OPF-based bioflocculant production as a sustainable, low-carbon alternative that aligns with circular bioeconomy principles.

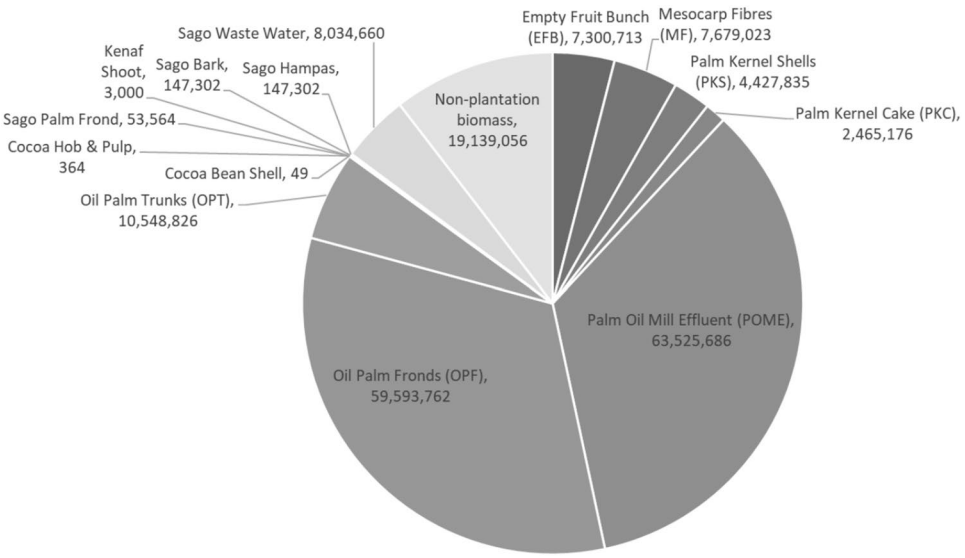


Fig. 1 Estimated quantities of biomass residue produced in Malaysia in 2022. Units are in tonnes. [23]

Introduction

Malaysia is one of the world's largest producers of Crude Palm Oil (CPO), accounting for 24% of global production in 2023/2024, second only to Indonesia (56%), with Thailand being the third-largest producer (5%). Given the scale of CPO production, large volumes of oil palm biomass residues are generated annually in Malaysia, both in palm oil mills and on plantations. Currently, attempts are being made to reduce Greenhouse Gas (GHG) emissions associated with the accumulation and subsequent decomposition of oil palm biomass by repurposing waste biomass into value-added products. Among the various residues generated by the palm oil industry, Oil Palm Fronds (OPF) are the most abundant, accounting for 65% of lignocellulosic biomass residues-produced by the palm oil industry [46]. According to Malaysia's Biomass Action Plan 2023–2030, plantation biomass residues accounted for 89.2% of all usable biomass in 2022, with OPF alone contributing 59.59 million tonnes, representing 33% of the total biomass residue, as shown in Fig. 1. This makes OPF the largest source of lignocellulosic biomass in the country. Due to its widespread availability, OPF has traditionally been treated as solid agricultural waste on oil palm plantations. However, its high lignocellulosic content presents significant potential for value-added applications, particularly as a feedstock for bioconversion processes [9, 30, 44].

OPF fibre contains a significant quantity of glucose and xylose, making it attractive as a potential carbon source for fermentation-based bioprocesses [32]. However, the OPF first needs to be treated to make accessible these fermentable sugars. Various techniques, such as steam explosion, acid hydrolysis, alkaline hydrolysis, and enzymatic hydrolysis, may be employed to break down the lignocellulosic components of the oil palm frond—cellulose, hemicellulose, and lignin—into fermentable sugars [33]. As with all lignocellulosic biomass, their unique structure necessitates a tailored pretreatment strategy to maximize the yield of the fermentable sugar extraction from OPF. Once released, these fermentable sugars can then be biochemically converted into value-added products, such as bioflocculants.

Bioflocculants have recently garnered much attention due to their non-toxic, harmless, and biodegradable properties, offering a sustainable alternative to conventional flocculants. Bioflocculants are polymers produced by microorganisms that may be utilized for a variety of industrial fields, including wastewater treatment, microalgae harvesting, activated sludge dewatering, heavy metal ion adsorption, and nanoparticle synthesis. They are particularly useful in the clarification and separation of fermentation products in industries such as food processing and pharmaceuticals, which typically have stringent health and safety requirements [24]. Bioflocculants are composed of exopolysaccharides (EPS) and proteins produced by microorganisms. Despite their advantages, however, their high manufacturing costs relative to standard inorganic and organic polymeric flocculants, is the primary barrier to the large-scale manufacture and deployment of bioflocculants. Various attempts have been made in recent years to reduce the cost of bioflocculant production, including the search for low-cost alternative feedstocks. Agro-industrial wastes, such as OPF, are not only abundant and geospatially concentrated, but also rich in organic substances, making them viable sources of carbon and nitrogen for economically competitive bioflocculants production [28].

Recent studies have increasingly explored the use of agro-industrial by-products as sustainable carbon sources for bioflocculant synthesis by various microorganisms. In this context, agro-industrial wastes, such as rice hull, rice

stover, potato by-products, peanut hull, corn cob, and wheat bran, were utilized to formulate a non-synthetic medium for bioflocculant production. For example, a 2025 study demonstrated that *Pseudomonas boreopolis* GO2 utilizes corn stover as the sole carbon source for bioflocculant synthesis [43]. Another research also highlighted the direct use of untreated agricultural residues such as corn stover, corn cob, potato residues, and peanut shell by *Cellulosimicrobium cellulans* L804, reporting over 80% flocculating activity and bioflocculant yields up to 4.75 g/L under optimized conditions [19]. These findings highlight the practical and economic benefit of using various agro-industrial wastes as effective substrates for cost-efficient, large scale bioflocculant manufacturing.

Based on current literature, no studies related to OPF as a carbon looking out for bioflocculant synthesis. Despite their abundance, several challenges must be solved before OPF can be consider for large scale application. The main challenges are the pretreatment process because of the high cost and energy consumed in the pretreatment stage of lignocellulosic, with its complex structure. Efficient pretreatment methods are required to break down this biomass into simpler sugars, enhancing its accessibility for microbial bioflocculants production. Furthermore, OPF may contain certain compounds that inhibit microbial growth during fermentation, posing an additional challenge for its utilization.

Another challenge lies in fact that bioflocculants derived from OPF may not exhibit the same level of effectiveness as those produced from other sources, such as molasses or starch. However, if these limitations can be overcome, OPF has the potential to emerge as a valuable and sustainable resource to produce sustainable, low-carbon footprint bioflocculant. This study investigated the possibility for bioflocculant generation from *Bacillus licheniformis* CGMCC 2876 utilizing OPF as an alternate carbon source. The effect of OPF pretreatment and the OPF hydrolysate loading was also evaluated to optimize the production process. Additionally, a preliminary life cycle assessment based on raw material input was also conducted to estimate the reduction in Global Warming Potential (GWP) achievable through OPF utilization in the process.

Material and Method

OPF Collection and Preparation

OPFs were collected from the Oil Palm Estate owned by University Kebangsaan Malaysia, located at Bangi, Selangor. To eliminate impurities and moisture, the OPF biomass was thoroughly washed and then heated in an oven at 50 °C for 24 h. Following this, it was ground and sieved to a particle size of 200 mesh. These OPFs were characterized using CHNS for elemental composition and BET for surface area. For comparability reasons, three pretreatment method were employed in this study to process the biomass. The pretreatment techniques utilized included acid-based pretreatment followed by enzymatic hydrolysis, alkaline-based pretreatment followed by enzymatic hydrolysis, and enzymatic hydrolysis using Cellic® Ctec3 alone.

Pretreatment and Hydrolysis of OPF

Acidic Pretreatment

The OPF was crushed and was subjected to an acidic pretreatment using 0.4 M dilute sulfuric acid solution (solid-to-liquid ratio 1:10). The mixture was treated under high-pressure conditions at 121 ^{o}C for 60 min. After acid hydrolysis, the pH was adjusted to 4.8 by addition of 2 M sodium hydroxide. The remaining material was then decanted from the OPF under vacuum filtration, and the residue washed with 150 mL distilled water. The dried material was then put into a desiccator for enzymatic hydrolysis, while the supernatant was analyzed for reducing sugar using dinitro salicylic acid (DNS) method [22].

Alkaline Pretreatment

Ground OPF (200 mesh) was treated with alkaline using 2% w/v sodium hydroxide solution at 50 ^{o}C for 60 min with 1: 10 solid-to-liquid loading. The mixture was then neutralized with 2 M hydrochloric acid after treatment. the supernatant was then removed from the OPF using a vacuum filter and the material was rinsed with 150 mL of distilled water. The material was then weighed. The dried solid was then placed in a desiccator for enzymatic hydrolysis.

Enzyme Hydrolysis

The study used enzyme hydrolysis in 100-mL Erlenmeyer flasks with biomass washed three times with sodium citrate buffer (pH 4.8, 0.05 M). The process was done for 72 h at 50 ^{o}C with 150 rpm shaking speed, with 10% solid loading. The experiment used Cellic® Ctec3 from Novozyme with 20 FPU/g enzyme loading which was chosen as optimum after loading up to 80 FPU/g was tested. After enzymatic hydrolysis, the samples were places in 100 ^{o}C water bath for 10 min to inactive the enzyme. The solid residue and supernatant were then separated by centrifugation at 12,000 rpm for 10 min. Total reducing sugars were quantified using DNS method.

Experimental Strain and Culture Media

Bacillus licheniformis CGMCC 2876 was used throughout this study and was sourced from the laboratory stock of our collaborator at the College of Chemistry and Chemical Engineering, Xiamen University, China. The strain was cultured on Luria Bertani (LB) agar in a sterile Petri dish and incubated at 37 °C for 24 h. Following this, it was inoculated into 50 mL of seed medium in a 250 mL Erlenmeyer flask and grown on a reciprocal shaker at 200 rpm and 37 °C. After 36 h of incubation, the seed culture was prepared for further experiments. The seed medium contained the following components (g/L): glucose, 10; yeast extract, 0.5; urea, 0.5; KH_2PO_4, 0.1; K_2HPO_4, 0.1; NaCl, 0.1; and $MgSO_4.7H_2O$, 0.2. For bioflocculant production, 4% of the seed culture (v/v) was inoculated into 50 mL of fermentation medium at 200 rpm for 48 h in a 250 mL Erlenmeyer flask at 37 °C. The fermentation medium contained the following components (g/L): glucose, 6.95 (varied according to experiment); yeast extract, 1.8; urea, 2.67; KH_2PO_4, 5.6; K_2HPO_4, 1.4; NaCl, 2; and $MgSO_4$, 0.048. The pH of all media was set to 7.2 at the beginning. All media were prepared using distilled water and sterilized at 121 °C for 15 min [42]. Batch fermentation was done in the same condition as pre-cultivation. Medium samples (5 mL) were taken at 3 h interval to measure cell growth and flocculating activity. The effect of different carbon source concentration (0- 11.12 g/L) was also tested for optimum flocculating activity. OPF hydrolysate was used to substitute glucose as carbon source with different concentration (0- 100% of optimum carbon source concentration).

Chemical Analysis of Bioflocculant

Sugar Content

The total sugar level was determined using the phenol–sulfuric acid method [29] with D-glucose as the standard curve. In brief, pure bioflocculant was first dissolved in 1 mL of distilled water. Then, 1 mL of 8% (v/v) phenol was added to the solution followed by 5 mL of 98% (v/v) sulfuric acid. The solution was kept in a water bath for 10 min, then agitated vigorously for 1 min. The absorbance was then measured at an optical density of 490 nm with a spectrophotometer (Mapada-V-1100D, Shanghai, China).

Protein Content

The quantity of protein of the produced bioflocculant was determined by the Bradford test [5] with bovine serum albumin (BSA) as the standard. Initially, 200 µL of was pipetted into a cuvette from each solution. Pour 1 mL of Bradford reagent into the dilutions. The mixture was allowed to stand at room temperature for 2 min. The solution was then measured at 595 nm with a spectrophotometer (Mapada-V-1100D, Shanghai, China).

Determination of Bioflocculant Activity

After 56 h of fermentation, the culture broths were centrifuged at 8000 rpm for 15 min to separate the cellular biomass. The cellular biomass was discarded, and three times the volume of ethanol was added to the supernatant to precipitate the crude products. The precipitated products were re-suspended in distilled water and lyophilized to produce the purified products. The cell-free culture supernatants were used to observe flocculating activity of the bioflocculant produced by *Bacillus licheniformis* CGMCC 2876. Various dosages of bioflocculant were evaluated, ranging from 0.25 to 1 ml, to determine the optimal dosage for further evaluation of the Flocculating Activity (FA). FA evaluation utilized a kaolin clay suspension. The evaluation for FA was prepared by adding 20 mL kaolin clay suspension (5 g/L) along with 0.5 g/L $CaCl_2$ and 1 mL bioflocculant sample; the mixture was swirled and then left to stand for 5 min at room temperature. After the 5-min standing time, the turbidity of the supernatant (OD550) was measured with ultraviolet spectrophotometer (Mapada-V-1100D, Shanghai, China). A control was performed under the same conditions with distilled water in place of bioflocculant sample.

The flocculating activity was quantified by measuring the reduction in turbidity of the supernatant, employing the equation establish by Lei et al. [16], as follows:

$$Flocculating\ activity(\%) = \frac{A-B}{A} x100\% \quad (1)$$

In Eq. (1), A and B represent the optical density of the control and sample at 550 nm. All measurements were conducted in triplicates to ensure accuracy and reproducibility.

Evaluation of OPF Hydrolysate as a Carbon Source for Bioflocculant Production

To determine whether oil palm frond (OPF) hydrolysate could serve as a viable carbon source for *Bacillus licheniformis* CGMCC 28756 bioflocculant production, shake-flask studies were performed using various OPF hydrolysate concentrations. Furthermore, to assess the impact of carbon source on EPS production, bacterial cultures were cultivated in media enriched with OPF hydrolysate or glucose, and different concentrations of carbon sources were evaluated. Four combinations of mixed carbon sources consisting of OPF hydrolysate and glucose were evaluated, with the total sugar concentration (in this scenario total sugar was either OPF hydrolysate or glucose) kept constant at 6.95 g/L (w/w). The ratios of OPF hydrolysate to glucose were: 0:100, 20:80,

40:60, 60:40, 80:20, and 100:0 (w/w). For control medium, glucose was used as sole carbon source.

Variables Influencing Flocculation Activity

To determine the flocculation behavior of the bioflocculant extracted from *Bacillus licheniformis* CGMCC 2876, studies were performed on the effect of temperature and pH towards the flocculating activity in suspensions of kaolin. The pH of kaolin suspension was modified by adding 0.5 M HCl and 1 M NaOH from the range pH 3 to pH 12, and to determine the effect of temperature on the flocculating activity, kaolin suspension samples were heated to various temperatures (from 4 to 80 °C). The activity of the bioflocculant was determined using methods previously described. Each experiment was carried out in triplicate to provide reliable results. The aim of using extreme levels of pH and temperature was to discover the conditions in which bioflocculant flocculation was optimally performed.

Assessment of Global Warming Potential Reduction

The GWP reduction was assessed by comparing two cases: Scenario 1, a benchmark fermentation using 100 % glucose as the sole carbon source, and Scenario 2, the best-performing OPF hydrolysate loading identified in this study (40 % OPF hydrolysate + 60 % glucose). A batch production of 1 tonne of bioflocculant was used as the functional unit for both scenarios. The GWP life cycle inventory (LCI) was constructed using a mass-balance approach combined with literature-derived emission factors. Each raw material input (e.g., glucose, OPF biomass, yeast extract, salts, and enzymes) was multiplied by its corresponding GWP factor obtained from published sources [4], Carbon [7, 8, 11].,[21, 31, 40, 41]).

The assessment was limited to the cradle-to-fermentation-medium preparation (gate) stage. Emissions from enzymatic hydrolysis and downstream processing were assumed negligible and were excluded from the LCI. This approach follows the general structure of life cycle assessment as outlined in ISO 14040/44 and represents a simplified, preliminary LCA, providing a comparative estimate of GWP impacts for the two scenarios while acknowledging our study's limitations.

Results and Discussions

Bioflocculant Synthesis and Flocculation Performance

As shown in Fig. 2, bioflocculant synthesis by *Bacillus licheniformis* CGMCC 2876 followed a typical bacterial growth pattern when cultivated using glucose as the conventional carbon source. The early exponential phase was observed at approximately 9 h, followed by stationary phase at around 12 h. Extracellular polymeric substances (EPS) was produced in the exponential growth phase, reaching maximum yields of 10.6 g/L and a flocculating activity of 87.76% at 48 h (late exponential phase). In the current study, both EPS yield and flocculating activity demonstrated a progressive decline after 48 h of fermentation. Remarkably, EPS production continued to increase even as the bacterial

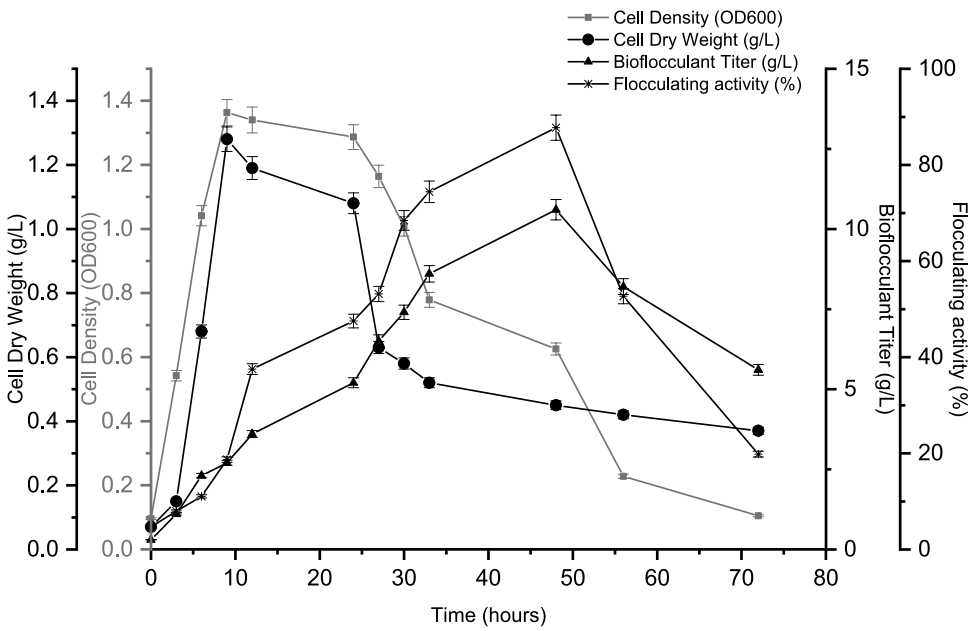


Fig. 2 Time courses of growth, bioflocculant production, and flocculating activity of *Bacillus licheniformis CGMCC* 2876 on incubator shaker at 200 rpm and 37 °C for 72 h

growth rate declined. This may be due to cellular stress induced by nutrient depletion and the accumulation of toxic metabolites by-products, which can stimulate EPS synthesis as a defensive mechanism [26]. This is consistent with previous studies reported by Sheng et al. [35], indicating an inverse correlation between EPS production and bacterial biomass. Since EPS synthesis and bacterial growth are correlated, it can be inferred that EPS production and flocculating activity is partially growth-dependent. In other words, while EPS generation is closely linked to microbial growth and biomass accumulation, additional factors such as nutrient depletion, and environmental stress can promote EPS production even when growth slows or declines, reflecting its dual role in balancing growth-related biosynthesis with stress-induced protective functions [10, 36].

Bioflocculant production and flocculating activity increased steadily during fermentation, peaking around 48 h before gradually decreased in declining phase due to nutrient depletion, microbial autolysis, and enzymatic degradation [25]. This trend aligns with findings in R. *erythropolis* fermentations using potato starch waste water, where activity peaked at 60 h and decreased by 90 h [12]. Additionally, microbial exoenzymes may accelerate deflocculation by depolymerizing bioflocculant macromolecules into smaller monomeric units. This degraded materials can acts as alternative nutrient and energy sources for the microorganisms once essential substances in the culture medium become depleted [3].

Carbon Source Optimization for Enhanced Bioflocculant Production

The carbon source concentration significantly influenced EPS production and flocculating activity in *Bacillus licheniformis* CGMCC 2876. As shown in Fig. 3, the highest flocculating activity (81.43%) was achieved at a glucose concentration of 6.95 g/L after 48 h of fermentation. Further increases in glucose concentration did not enhance flocculating activity, indicating that EPS synthesis had reached its optimal substrate utilization threshold. However, high excess glucose concentrations might have inhibited EPS production and reduced flocculating efficiency because of catabolite repression. Therefore, 6.95 g/L glucose concentration was selected to be the optimal concentration, balancing maximal bioflocculant yield with cost-effective substrate usage.

Dosage-Dependent Flocculation Efficiency

The bioflocculant dosage had a significant impact on flocculating activity. Dosages ranging from 0.25 to 1 mL per 20 mL of kaolin suspension (5 g/L) were evaluated, with 1 mL demonstrating the highest performance (89.33%), as shown in Fig. 4. In contrast, the lowest dosage (0.25 mL) yielded only 59.49% efficiency attributable to insufficient bioflocculant to neutralize the negative charges on kaolin particles. Flocculation requires a critical dosage balance: under-dosing limits bridging formation, while over-dosing increases medium viscosity that stabilizes kaolin particles and hindering particles aggregation [2]. In subsequent experiments, 1 ml of bioflocculant was selected as the standard dosage to assessing flocculating activity.

Physicochemical Characterization of Oil Palm Frond (OPF) Biomass

The OPF biomass had a carbon-rich (45.59%) content as characterized by CHNS analysis, with a low amount of hydrogen (5.98%), and trace of nitrogen and sulfur (0.004% and 0.015% respectively), which is similar with previous values reported by Zahari et al. [45] and Tan et al. [38]. The relatively low level of nitrogen and sulfur contents (< 0.02%), indicating absence of significant microbial growth inhibitors. After conducting knife-milling and passing the dried OPF through 200-mesh sieving, it was found from BET analysis that the average surface area was enhanced to 2.875 m^2/g,

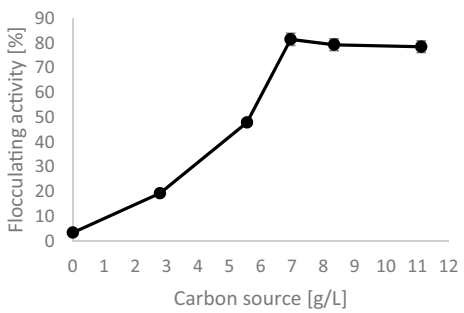


Fig. 3 Flocculating activity of the bioflocculant at various glucose concentrations in a chemically defined medium. A fixed bioflocculant dosage of 0.5 mL was used in all assay

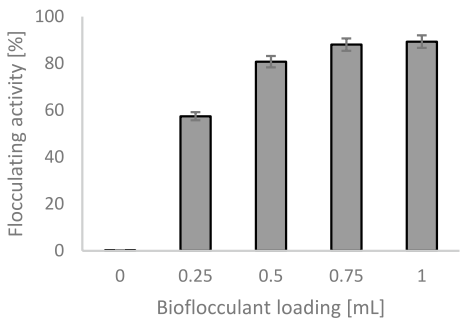


Fig. 4 Flocculation efficiency as a function of bioflocculant dosage. The bioflocculant loading dosage was varied from 0.25 to 1 mL to evaluate its influence on flocculation performance

demonstrating promising changes to improved hydrolysis efficiency, positioning OPF as a viable carbon source for microbial cultivation systems.

Enzymatic Hydrolysis: Role of Enzyme Loading

Enzymatic hydrolysis of OPF using Novozyme's commercial cellulase Cellic® Ctec3 demonstrated dose-dependent sugar release patterns. The negative control (0 FPU/g biomass) yielded minimal reducing sugars (1.97 g/L), whereas an enzyme loading of 20 FPU/g biomass yielded a sevenfold increase in hydrolysate sugar concentration (13.3 g/L). However, diminishing increases in sugar concentration were observed as the enzyme loading went over 20 FPU/g biomass. This study found that increasing enzyme loadings has limited positive impact on increases sugar concentration, most likely due to saturation of accessible substrate or limitation in biomass digestibility. These findings indicate that excessive enzyme supplementation does not proportionally improve polysaccharides conversion, likely due to competitive enzyme inhibition. Consequently, 20 FPU/g biomass in 0.05 M sodium citrate buffer (pH 4.8) was selected for further experiments as the optimal loading, balancing hydrolysis efficiency with economic feasibility for subsequent experiments.

Comparative Analysis of Pretreatment Strategies for OPF Valorization

Among the various pretreatment methods (including physical size reduction and chemical acid or alkaline treatments), enzymatic hydrolysis of mechanically ground OPF (200 mesh), without any chemical (acid/alkaline) pretreatments, produced the highest concentration of total reducing sugar (13.3 ± 0.2 g/L; Fig. 5). The efficiency of converting oil palm fronds (OPF) into fermentable sugars is significantly affected by its highly complex lignocellulosic structure [34]. This structure includes interconnected cellulose, hemicellulose, and lignin, which collectively present substantial challenges for efficient hydrolysis. Lignin forms covalent bonds with both cellulose and hemicellulose, resulting a rigid matrix that limits enzymatic accessibility and hinders efficient hydrolysis [27]. Oil palm frond (OPF), though structurally similar to hardwoods, contains a relatively high lignin content (29.5% (w/v))[15], that impacts pretreatment efficiency. This study investigates the efficacy of two chemical pretreatment techniques -dilute sulfuric acid and sodium hydroxide-for processing biomass. Alkaline pretreatment effectively degrades OPF's lignin matrix [15], but it also promotes the formation of fermentation-inhibiting phenolic compounds resulting from lignin decomposition and leaves significant residual lignin that promotes non-productive enzyme binding. This accumulation can hinder subsequent enzymatic hydrolysis and microbial fermentation process. In contrast, dilute sulfuric acid enhances the accessibility of cellulose and hemicellulose for enzymatic hydrolysis, yielding higher amount of reducing sugar than alkaline pretreatment but generated undesirable by-products like furfural, hydroxymethyl furfural (HMF), and acetic acid that resisted removal [37].

Mechanical size reduction of the raw material has been acknowledged to enhance OPF valorization by increasing biomass porosity and improving accessibility of the substrate during the pretreatment process. Indeed, particle size reduction has been known to disturb cellulose crystallinity, break interpolymeric bonds, and promote lignin breakdown, leading to higher hydrolysis efficiency, lower energy consumption, and increased fermentable sugar yields. In particular, smaller particles sizes result in better conversion [1]. This study shows that mechanical grinding alone is sufficient to disrupt OPF's structure and surpasses chemical pretreatments in terms of sugar production. In addition, grinding prevented inhibitor formation while maintaining hydrolysis

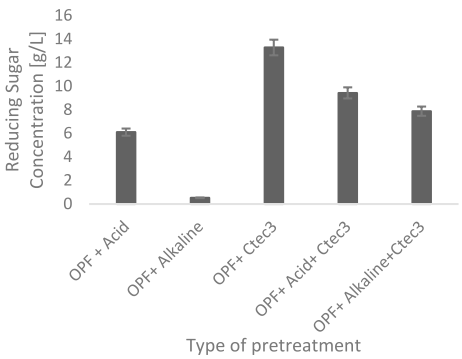


Fig. 5 Analysis of reducing sugar yields from oil palm frond using various pretreatment methods. The efficiency of each pretreatment was assessed according to the concentration of released reducing sugars

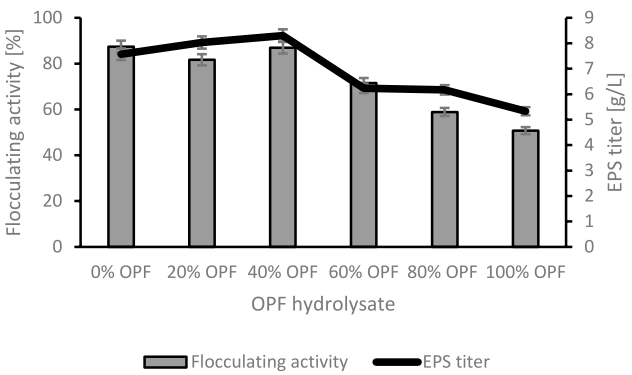


Fig. 6 Impact of oil palm frond (OPF) hydrolysate-to-glucose ratio on flocculating activity and EPS production after 48 h fermentation

efficiency, offering a sustainable valorization approach for OPF that combines operational ease with high conversion yields.

Impact of OPF Hydrolysate-to-Glucose Ratio on Bioflocculant Production

Figure 6 illustrates the effects of substrate on the rate of extracellular polymeric substance (EPS) production. Microorganisms react to various stimuli when generating EPS, including the amount of nutrients or the quantity of available carbon and nutrients. OPF hydrolysate generally consists of fermentable sugars such as glucose, xylose, various monosaccharides, and along with nutrients such as organic acids, amino acids, and minerals, which might stimulate microbial growth and promote EPS production.

The study indicates that mixing 40% oil palm frond (OPF) hydrolysate with 60% glucose resulted in the highest EPS production (8.3 g/L) and maximum flocculating activity (87%). The EPS production surpassed the yield obtained from using OPF hydrolysate alone (5.3 g/L) and the control treatment using glucose as the sole carbon source (7.6 g/L). The combination of OPF hydrolysate and glucose provides a more balanced nutrient environment meaning that sugar might be more efficiently utilized through microbial metabolism compared to using either glucose or OPF hydrolysate on their own.

By substituting 40% of the glucose with OPF hydrolysate, the flocculation activity reached 87%, which is comparable to the original flocculation activity of 87.4% achieved with glucose alone. Reduction in flocculation efficiency (0.48%) indicates that OPF hydrolysate may serve as a substitute for glucose as a carbon source, resulting in only a slight decline in flocculation activity, thereby reinforcing the potential of OPF as a sustainable alternative to glucose in bioflocculation production.

Higher concentrations of OPF hydrolysate suppress EPS titer and flocculation activity due to imbalanced carbon to nitrogen ratios. Additionally, the viscosity of the culture medium increased over time, which presented further inhibition to bacterial growth. At 40% OPF hydrolysate, microbial metabolism that favors EPS production is optimized, but higher OPF hydrolysate concentrations could bring cells into stress response mechanisms, limit oxygen and nutrient diffusion potential thus compromising microbial activity. Chemical analysis identified that the bioflocculant from *Bacillus licheniformis* CGMCC 2876 was producing EPS, using OPF as a carbon source, comprised of 84% carbohydrate and 16% protein (w/w).

Oil palm frond (OPF) shows a high potential as an agro-industrial substrate for bioflocculant production. As presented in Table 1, the bioflocculant synthesized using OPF achieved a flocculating activity of 87%, which is comparable to that of established substrates such as corn cob, corn stover and rice bran. Moreover, the bioflocculant yield from OPF was notably high, reaching 8.3 g/L, demonstrating its feasibility as an efficient and cost-effective substrate for bioflocculant synthesis. The effectiveness of OPF can be attributed to its rich content of fermentable sugars and essential minerals, which facilitate microbial proliferation and EPS production. These findings align with Sustainable Development Goal 12 by promoting responsible consumption and production, where OPF hydrolysates are valorized for use in waste water treatment, food processing, and industrial effluent recovery, thereby contributing to waste minimization and resource efficiency.

Flocculating Activity of the Bioflocculant

Performance Under Different pH

The bioflocculant produced by *Bacillus licheniformis* CGMCC 2876 exhibited high stability across a broad pH range (pH 3–7), maintaining over 80% flocculating activity, as shown in Fig. 7. Optimal performance (90.71%) was achieved at pH 6 using pure bioflocculant. However, under

Table 1 Agro-based materials utilized as substrates for microorganisms producing bioflocculants

Carbon source	Microorganism strain	Yield (g/L)	Flocculation activity [%]	Refs.
Palm oil mill effluent	*Bacillus marisflavi* NA8	9.72	80	[6]
Palm oil mill effluent	*Bacillus velezensis*	2.03	18.5	[14]
Rice bran	*Bacillus agaradhaerens* C9	12.94	87.2	[17]
Rice stover hydrolysate	*Rhodococcus erythropolis*	2.37	75.8	[13]
Peanut hull hydrolysate	*Pseudomonas veronii L918*	3.39	92.5	[19]
Corn steep liquor	*Corynebacterium glutamicum Cg1-P30*	0.52	91.92	[20]
Corn cob powder	*Cellulosimicrobium cellulans* L804	4.75	> 80	[19]
Oil palm frond hydrolysate	*Bacillus licheniformis* CGMCC 2876	8.3	87	This study

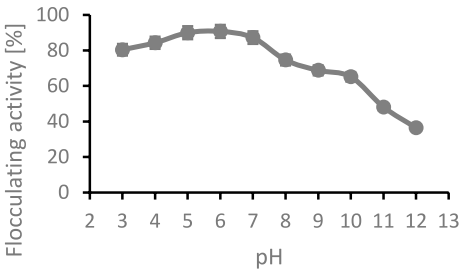


Fig. 7 The effect of acidic and alkaline conditions on flocculating activity of bioflocculant produced by *Bacillus licheniformis* CGMCC 2876

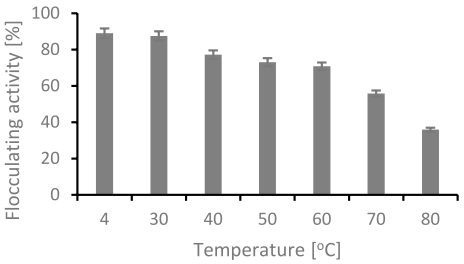


Fig. 8 Temperature optimization profile for bioflocculant activity from *Bacillus licheniformis* CGMCC 2876

alkaline conditions (pH > 7), its performance declined significantly, with activity dropping from 74.06% (pH 8) to 36.43% (pH 12) likely due to alkaline-induced polysaccharide degradation, potentially involving either molecular rearrangement or chain degradation.

Performance Under Different Temperature

Temperature has a significant effect on the thermostability of bioflocculants which affects their structure, activity, and efficiency in flocculation processes. Assessing thermostability can help to optimize their performance in various environmental or industrial applications. Thermostable bioflocculants are especially useful in industries where temperature fluctuation is common, like wastewater treatment. Figure 8. Illustrates the influence of temperature on the bioflocculant's flocculation effectiveness. The bioflocculant exhibited maximum flocculation efficiency (89%) between 4 and 30°C and then dropped to a low of 55.8% when the temperature increased to 70°C. This reduction likely results from structural instability, thermal decomposition, or lost interaction with the suspended particle at high temperature. The bioflocculant derived from *Bacillus licheniformis* CGMCC 2876 using OPF as a carbon source demonstrated limited thermostability, due to its ahigh carbohydrate content (84%), which is mainly polysaccharide-based nature, makes it sensitive to thermal degradation [39]. Additionally, the protein component (16%), which facilitates binding and charge-mediated flocculation, is also subjected to denaturation at high temperatures, which would lead to loss of functional groups involved in flocculation [18].

Environmental Consideration- Global Warming Potential (GWP) Reduction from OPF

The GWP assessment revealed a significant decrease in GHG emissions can be achieved upstream of the bioflocculant fermentation by substituting a portion of glucose with OPF hydrolysate in the fermentation medium. As can be seen in Table 2 and Fig. 9, Scenario 2 (40 % OPF + 60 % glucose) resulted in a net GWP reduction of 1067.06 kg CO2 eq./tonne of bioflocculant (58.8 % reduction) compared to the benchmark Scenario 1 (100 % glucose). This reduction was achieved through two mechanisms: substrate composition adjustment and an increase in bioflocculant yield. For the former, substrate substitution alone achieved a GWP reduction of 959.10 kg CO_2 eq./tonne, primarily due to a

Table 2 Mass balance and GWP for Scenario 1 (100% glucose) and Scenario 2 (40% OPF hydrolysate and 60% glucose). A batch production capacity of 1 tonne bioflocculant was used as basis for both scenarios

Component	Scenario 1 (100% Glucose)	Scenario 2 (40% OPF Hydrolysate + 60% Glucose)	GWP per unit [kg CO_2 eq.]*	GWP of Scenario 1 [kg CO_2 eq.]	GWP of Scenario 2 [kg CO_2 eq.]	Ref
Glucose (kg)	914.47	502.41	0.3987	364.57	200.3	[11]
OPF Biomass (kg)	N/A	2,518.07	-0.3156	N/A	-794.82	[4, 41]
Yeast Extract (kg)	236.84	216.87	3.204	758.84	694.84	[40]
Urea (kg)	351.32	321.69	0.714	250.84	229.68	[21]
KH_2PO_4 (kg)	736.84	674.7	0.287	211.47	193.64	[31]
K_2HPO_4 (kg)	184.21	168.67	1.15	211.84	193.98	[7]
NaCl (kg)	263.16	240.96	0.06	15.79	14.46	[8]
$MgSO_4 \cdot 7H_2O$ (kg)	6.32	5.78	0.1504	0.95	0.87	[31]
Cellic® Ctec3 (FPU)	N/A	503,614.50	2.84E-05	N/A	14.3	[31]
Net GWP (kg CO_2 eq.)	1,814.31	747.25				

* GWP values are in kg CO_2 eq. per kg, except for Cellic® Ctec3, which is in kg CO_2 eq. per FPU

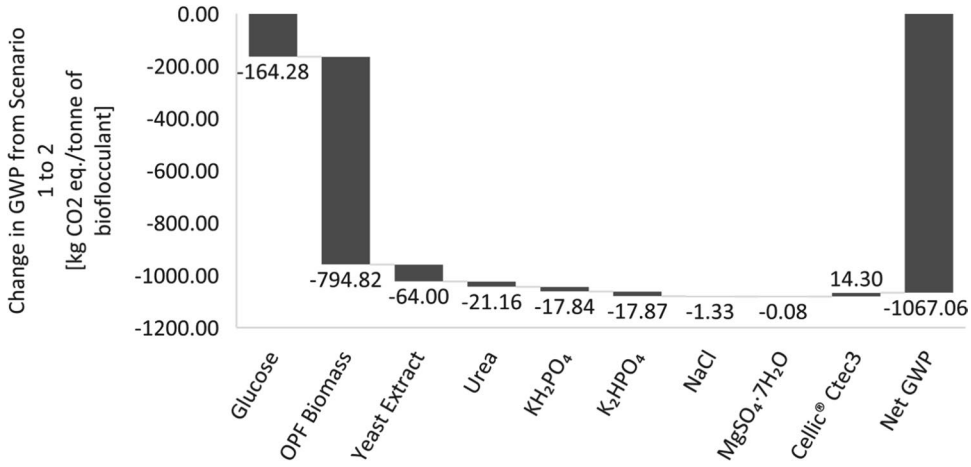


Fig. 9 Waterfall chart of change in GWP from scenario 1 to 2 by raw material inputs

9.05 % reduction from decreased glucose input and a 43.81 % reduction from OPF biomass utilization. The substantial contribution of OPF biomass is owed to the avoidance in emissions that would otherwise have resulted from decomposition in the field, which was considered in the calculations. These avoided emissions outweighed the estimated emissions resulting from the processing of the OPF including collection, transport, grinding, and drying. This effectively makes the utilization of OPF biomass residue a net carbon sink in the process. In addition, the process demonstrated a 9.2 % increase in bioflocculant yield, leading to a lower material input requirement per tonne of product. This reduction in the remaining material balance accounts for -107.96 kg CO2 eq./tonne of bioflocculant (5.95 % reduction). However, it is worth noting that the inclusion of Cellic® Ctec3 enzyme in Scenario 2 introduced a minor GWP increase of 14.30 kg CO2/tonne of bioflocculant, attributable to the embedded carbon footprint from enzyme production and purification. Despite this, the marginal increase is significantly outweighed by the savings from OPF utilization. In summary, this preliminary assessment demonstrates the potential of OPF hydrolysate as an avenue for carbon footprint reduction on the raw materials input front. By integrating OPF hydrolysate, bioflocculant, plants, and, indeed, the wider bio-based industry can significantly lower their carbon footprint while promoting circular bioeconomy principles. Future assessments should consider a more comprehensive life cycle approach, incorporating energy use during hydrolysis and downstream processing, to further validate the long-term environmental sustainability of this approach.

Conclusion

This study shows that oil palm frond (OPF) hydrolysate may be used as a low cost and with long-term sustainable carbon source for bioflocculant production. The high yield of reducing sugars was obtained by enzymatic hydrolysis of OPF without chemical pretreatment supported the growth of *Bacillus licheniformis* CGMCC 2876 and extracellular polymeric substances (EPS) synthesis. The optimal fermentation condition, with an OPF hydrolysate-to-glucose ratio of 40:60, produced a bioflocculant yield of 8.3 g/L with 87 % flocculating activity, indicating that OPF may partially replace glucose without losing performance. A preliminary assessment also revealed that a 58.8 % reduction in Global Warming Potential (GWP) can be achieved in comparison to the 100 % glucose benchmark, validating the environmental benefits of OPF valorization. The research provided valuable insights into the influence of pretreatment conditions on reducing sugar concentration released from OPF. These findings lay the foundation for sustainable and efficient bioflocculant production through microbial fermentation, with promising applications across various bioprocessing industries.

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Data availability The datasets analyzed during the study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tan Jian Ping reports financial support was provided by Institution of Chemical Engineers. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Abolore, R.S., Jaiswal, S., Jaiswal, A.K.: Green and sustainable pretreatment methods for cellulose extraction from lignocellulosic biomass and its applications: a review. Carbohyd. Polym. Technol. Appl. **7**, 100396 (2024)
2. Agunbiade, M.O., Van Heerden, E., Pohl, C.H., Ashafa, A.T.: Flocculating performance of a bioflocculant produced by *Arthrobacter humicola* in sewage waste water treatment. BMC Biotechnol. **17**(1), 1–9 (2017). https://doi.org/10.1186/s12896-017-0375-0
3. Aljuboori, A.H.R., Idris, A., Abdullah, N., Mohamad, R.: Production and characterization of a bioflocculant produced by *Aspergillus flavus*. Bioresour. Technol. **127**, 489–493 (2013). https://doi.org/10.1016/j.biortech.2012.09.016
4. Boateng, C.O., Lee, K.T.: An oil palm-based biorefinery concept for cellulosic ethanol and phytochemicals production: sustainability evaluation using exergetic life cycle assessment. Appl. Therm. Eng. **62**(1), 90–104 (2014). https://doi.org/10.1016/j.applthermaleng.2013.09.022
5. Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**(1), 248–254 (1976). https://doi.org/10.1016/0003-2697(76)90527-3
6. Bukhari, N.A., Loh, S.K., Nasrin, A.B., Jahim, J.M.: Enzymatic hydrolysate of Palm Oil Mill Effluent as potential substrate for bioflocculant BM-8 production. Waste Biomass Valoriz. **11**(1), 17–29 (2018). https://doi.org/10.1007/s12649-018-0421-8
7. Carbon Cloud. (2016). *Diammonium phosphate*. https://apps.carboncloud.com/climatehub/product-reports/id/188526940712
8. Carbon Cloud. (2025). *Salt (NaCl) · 0*. https://apps.carboncloud.com/climatehub/product-reports/id/5403842093
9. Chaturvedi, S.: Types of biomass burning in South East Asia and its impact on health. Eqa **50**, 55–79 (2022). https://doi.org/10.6092/issn.2281-4485/15539
10. Flemming, H., Wingender, J.: The biofilm matrix. Nat. Publ. Group **8**(9), 623–633 (2010). https://doi.org/10.1038/nrmicro2415
11. GFLI. (2025). *GFLI Fee Structure Membership*. 0–1. https://globalfeedlca.org/gfli-database/accessing-the-database/
12. Guo, J., Liu, J., Yang, Y., Zhou, Y., Jiang, S., Chen, C.: Fermentation and kinetics characteristics of a bioflocculant from potato starch wastewater and its application. Sci. Rep. **8**(1), 1–11 (2018). https://doi.org/10.1038/s41598-018-21796-x
13. Guo, J., Yu, J., Xin, X., Zou, C., Cheng, Q., Yang, H., Nengzi, L.: Bioresource technology characterization and flocculation mechanism of a bioflocculant from hydrolyzate of rice stover. Bioresour. Technol. **177**, 393–397 (2015). https://doi.org/10.1016/j.biortech.2014.11.066
14. Hassimi, A.H., Ezril Hafiz, R., Muhamad, M.H., Sheikh Abdullah, S.R.: Bioflocculant production using palm oil mill and sago mill effluent as a fermentation feedstock: characterization and mechanism of flocculation. J. Environ. Manage. **260**, 110046 (2020). https://doi.org/10.1016/j.jenvman.2019.110046
15. Kristiani, A., Abimanyu, H., Setiawan, A.H., Aulia, F.: Effect of pretreatment process by using diluted acid to characteristic of oil palm's frond. Energy Procedia **32**, 183–189 (2013). https://doi.org/10.1016/j.egypro.2013.05.024
16. Lei, X., Chen, Y., Shao, Z., Chen, Z., Li, Y., Zhu, H., Zhang, J., Zheng, W., Zheng, T.: Effective harvesting of the microalgae *Chlorella vulgaris* via flocculation-flotation with bioflocculant. Bioresour. Technol. **198**, 922–925 (2015). https://doi.org/10.1016/j.biortech.2015.08.095
17. Liu, C., Hao, Y., Jiang, J., Liu, W.: Valorization of untreated rice bran towards bioflocculant using a lignocellulose-degrading strain and its use in microalgal biomass harvest. Biotechnol. Biofuels **10**(1), 1–12 (2017). https://doi.org/10.1186/s13068-017-0780-6
18. Liu, W.J., Wang, K., Li, B.Z., Yuan, H.L., Yang, J.S.: Production and characterization of an intracellular bioflocculant by *Chryseobacterium daeguense* W6 cultured in low nutrition medium. Bioresour. Technol. **101**(3), 1044–1048 (2010). https://doi.org/10.1016/J.BIORTECH.2009.08.108
19. Liu, W., Zhao, C., Jiang, J., Lu, Q., Hao, Y., Wang, L., Liu, C.: Bioflocculant production from untreated corn stover using *Cellulosimicrobium cellulans* L804 isolate and its application to harvesting microalgae. Biotechnol. Biofuels **8**(1), 1–12 (2015). https://doi.org/10.1186/s13068-015-0354-4
20. Liu, Y., Zeng, Y., Yang, J., Chen, P., Sun, Y., Wang, M., & Ma, Y. (2023). *A bio fl occulant from Corynebacterium glutamicum and its application in acid mine wastewater treatment*. *February*, 1–12. https://doi.org/10.3389/fbioe.2023.1136473
21. Masjedi, S., Kazemi, A., Moeinaddini, M., Khaki, E., Olsen, S.: Urea production: an absolute environmental sustainability assessment. Sci. Total. Environ. **908**, 168225 (2024). https://doi.org/10.1016/j.scitotenv.2023.168225
22. Miller, G.L.: Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **31**(3), 426–428 (1959). https://doi.org/10.1021/ac60147a030
23. Ministry of Plantation and Commodities. (2023). National Biomass Action Plan 2023–2030. In *Ministry of Plantation and Commodities*. Ministry of Plantation and Commodities (KPK). https://www.kpk.gov.my/kpk/images/mpi_biomass/27122023-National_Biomass_Action_Plan.pdf
24. Mishra, B., Kallem, P., Yadavalli, R., Mandal, S.K., Reddy, C.N., Sumithra, B., Lakshmayya, N.S.V., Bana, F.: Industrial wastewater treatment using extracellular polymer substances/bioflocculants: a review. Appl Water Sci **15**(3), 1–20 (2025). https://doi.org/10.1007/s13201-025-02401-9
25. Mohammed, J.N., Majiya, H., Adisa, J.M., Kassim, Z.J.: Production of bioflocculant through fermentation of spoilt orange juice with *Bacillus* spp isolated from sediment of local clay pot. J. Appl. Sci. Environ. Manage. **27**(1), 169–175 (2023). https://doi.org/10.4314/jasem.v27i1.24

26. Mohammed, J.N., Dagang, W.R.Z.: Culture optimization for production and characterization of bioflocculant by Aspergillus flavus grown on chicken viscera hydrolysate. World J. Microbiol. Biotechnol. **35**(8), 121 (2019). https://doi.org/10.1007/s11274-019-2696-8
27. Mujtaba, M., Fraceto, F.L., Fazeli, M., Mukherjee, S., Savassa, S.M., de Meiros, A.G., do Espírito, A.S.P., Mancini, S.D., Lipponen, J., Vilaplana, F.: Lignocellulosic biomass from agricultural waste to the circular economy: a review with focus on biofuels, biocomposites and bioplastics. J. Clean. Prod. **402**, 136815 (2023). https://doi.org/10.1016/j.jclepro.2023.136815
28. Mussatto, S., Teixeira, J.: Lignocellulose as raw material in fermentation processes. Appl. Microbiol. Microb. Biotechnol. **2**, 897–907 (2010)
29. Nielsen, S. S. (2010). *Phenol-Sulfuric Acid Method for Total Carbohydrates*. 47–53. https://doi.org/10.1007/978-1-4419-1463-7_6
30. Noor, M. M. (2023). *Zero Burning Techniques in Oil Palm Cultivation: an Economic Perspective – OPIEJ*. Oil Palm Industry Economic Journal Vol. 3 (1). https://opiej.mpob.gov.my/zero-burning-techniques-in-oil-palm-cultivation-an-economic-perspective/
31. Olofsson, J., Barta, Z., Börjesson, P., Wallberg, O.: Integrating enzyme fermentation in lignocellulosic ethanol production: life-cycle assessment and techno-economic analysis. Biotechnol. Biofuels **10**(1), 1–14 (2017). https://doi.org/10.1186/s13068-017-0733-0
32. Ooi, Z.X., Teoh, Y.P., Kunasundari, B., Shuit, S.H.: Oil palm frond as a sustainable and promising biomass source in Malaysia: a review. Environ. Prog. Sustain. Energy **00**(00), 1–11 (2017)
33. Rahman, A.K.H., Nashir, N.M., Zainuddin, N.I.: Production of fermentable sugars by oil palm fronds (OPF) through fungal solid state bioconversion. IOP Conf Series: Mater. Sci. Eng. **932**(1), 012030 (2020). https://doi.org/10.1088/1757-899X/932/1/012030
34. Rizal, A.N.F.A., Ibrahim, M.F., Zakaria, M.R., Abd-Aziz, S., Yee, P.L., Hassan, M.A.: Pre-treatment of oil palm biomass for fermentable sugars production. Molecules **23**(6), 1–14 (2018). https://doi.org/10.3390/molecules23061381
35. Sheng, G.P., Yu, H.Q., Yue, Z.: Factors influencing the production of extracellular polymeric substances by *Rhodopseudomonas acidophila*. Int. Biodeterior. Biodegrad. **58**(2), 89–93 (2006). https://doi.org/10.1016/j.ibiod.2006.07.005
36. Sheng, G., Yu, H., Li, X.: Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol. Adv. **28**(6), 882–894 (2010). https://doi.org/10.1016/j.biotechadv.2010.08.001
37. Sukruansuwan, V., Napathorn, S.C.: Use of agro-industrial residue from the canned pineapple industry for polyhydroxybutyrate production by *Cupriavidus necator* strain A-04. Biotechnol. Biofuels **11**(1), 1–15 (2018). https://doi.org/10.1186/s13068-018-1207-8
38. Tan, J.P., Jahim, J.M., Harun, S., Wu, T.Y., Mumtaz, T.: Utilization of oil palm fronds as a sustainable carbon source in biorefineries. Int. J. Hydrogen Energy **41**(8), 4896–4906 (2016). https://doi.org/10.1016/j.ijhydene.2015.08.034
39. Tawila, Z.M.A., Ismail, S., Dadrasnia, A., Usman, M.M.: Production and characterization of a bioflocculant produced by *bacillus salmalaya* 139si-7 and its applications in wastewater treatment. Molecules (2018). https://doi.org/10.3390/molecules23102689
40. Union, E. (2023). *The EU yeast sector and its carbon footprint Summary and Presentation of the study*. *June*.
41. Wakhid, N., Hirano, T.: Carbon dioxide emissions from frond decomposition in oil palm plantations on tropical peat. J. Oil Palm Res. **35**(3), 538–542 (2023). https://doi.org/10.21894/jopr.2022.0067
42. Xiong, Y., Wang, Y., Yu, Y., Li, Q., Wang, H., Chen, R., He, N.: Production and characterization of a novel bioflocculant from *Bacillus Licheniformis*. Appl. Environ. Microbiol. **76**(9), 2778–2782 (2010). https://doi.org/10.1128/AEM.02558-09
43. Xu, Y., Feng, J., Hu, Y., Chen, L., Qin, W., Chen, C., Yan, M., Guo, H.: Hub metabolites promote the bio fl occulant production in a biomass-degrading bacterium *Pseudomonas boreopolis* GO2. Microb. Physiol. **35**, 1–12 (2025). https://doi.org/10.1159/000542892
44. Zahari, M.A.K.M., Abdullah, S.S.S., Roslan, A.M., Ariffin, H., Shirai, Y., Hassan, M.A.: Efficient utilization of oil palm frond for bio-based products and biorefinery. J. Clean. Prod. **65**, 252–260 (2014). https://doi.org/10.1016/j.jclepro.2013.10.007
45. Zahari, M. W., Alimon, A. R., & Wong, H. K. (2012). Utilization of oil palm co-products as feeds for livestock in Malaysia. *Biofuel Co-Products as Livestock Feed – Opportunities and Challenges*, 243–302.
46. Zakaria, M.R., Farid, M.A., Hafid, H.S., Andou, Y., Hassan, M.A.: Practical role of oil palm fronds in Malaysia's sustainable palm oil industry. Ind. Crops Prod. **222**, 119753 (2024). https://doi.org/10.1016/J.INDCROP.2024.119753

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