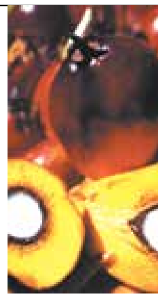

Novel Bioflocculant from Palm Oil Mill Effluent (POME) and its Potential Application

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INTRODUCTION

High valued biochemicals can be harnessed from palm oil mill effluent (POME) via microbial process apart from bioenergy *e.g.* methane or hydrogen. The potential pollution source of POME can be transformed into business opportunities by recovering and utilising the readily available nutrients for microbial fermentation into various bio-products. This bioconversion pathway makes use of the nutrient rich organic residues in POME as a substrate for specific microorganisms to consume and grow while concurrently produce biomass and some of the targeted bio-products.

Among the various potential biochemicals to be realised, microbial-derived flocculants (bioflocculants) are at the top of the list and received great attention for scientific and biotechnological consideration. Flocculating agents including bioflocculants have been widely used in industrial processes, including water and waste water treatment, heavy metals, toxic and colour removal, synthesis of nanoparticles as well as cell removal and biomass recovery *i.e.* microalgae harvesting.

MICROBIAL-DERIVED FLOCCULANTS

Bioflocculants from microbial sources have great potential to replace the synthetic or chemical flocculants. Although chemical flocculants have high flocculation efficiency and are low in cost, they pose severe drawback especially on human health as the monomers in used are neurogenic and carcinogenic. Bioflocculants have been well received globally because they are biologically active, environmental-friendly and safe for the ecosystem. However, the cultivation cost and low production yield of bioflocculants have hindered their practical applications.

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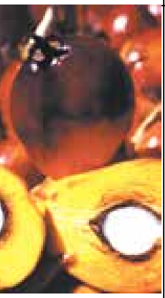

Low-cost renewable raw materials should be explored as an alternative to the commonly used substrates such as glucose, fructose, sucrose and L-glutamate. The use of nutrient rich waste material as a substrate for culturing the bioflocculant-producing microorganisms not only can reduce the production cost but also improve the feasibility of commercial production of bioflocculant. Various types of organic wastewater sourced from soybean juice, fishmeal, dairy, brewery, and starch have been attempted for bioflocculant production to lower their production cost. The utilisation of the by-products from the palm oil milling process *e.g.* POME is seen to be promising in reducing the production cost and improve the feasibility of commercial bioflocculant production.

BIOFLOCCULANT-PRODUCING BACTERIA FROM POME

Several strains of bioflocculant-producing bacteria were isolated from POME samples. They were screened for their capabilities to produce bioflocculant using kaolin clay as indicator. One such isolate showing the highest flocculating rate was selected and identified using 16S rRNA sequencing. The strain was designated as *Bacillus marisflavi* NA8 (Nurul Adela *et al.*, 2015). It is a gram-positive and rod-shaped bacteria (bacillus) capable of utilising the hydrolysed POME *i.e.* POME hydrolysate as a carbon source for bioflocculant production.

BIOCONVERSION OF POME INTO BIOFLOCCULANT

Generally, conversion of POME into bioflocculant involves three main processes (*Figure 1*): pre-treatment or substrate hydrolysis, fermentation and downstream processing *i.e.* separation/extraction of the targeted product.

- **Hydrolysis** – POME was first hydrolysed to simple sugars to facilitate the bacterial fermentation using a recombinant cellulase enzyme. The optimisation of POME hydrolysis to produce the fermentable sugars was carried out and a suitable fermentation medium *i.e.* POME hydrolysate was developed.

- **Fermentation** – The optimum culture conditions for bioflocculant production from POME hydrolysate was carried out by manipulating several process parameters - pH, temperature, inoculum size and nitrogen source in a shake flask. The highest flocculating activity was reached at 37ºC in a neutral medium (pH 7.0). 5 to 10% (v/v) of *B. marisflavi* NA8 inoculated in the POME medium gave the highest flocculating activity. The strain could efficiently produce bioflocculant using POME hydrolysate without having to add nitrogen as nutrient supplement. This implied that POME alone was a protein medium enriched sufficient for microbial growth. The suitability of

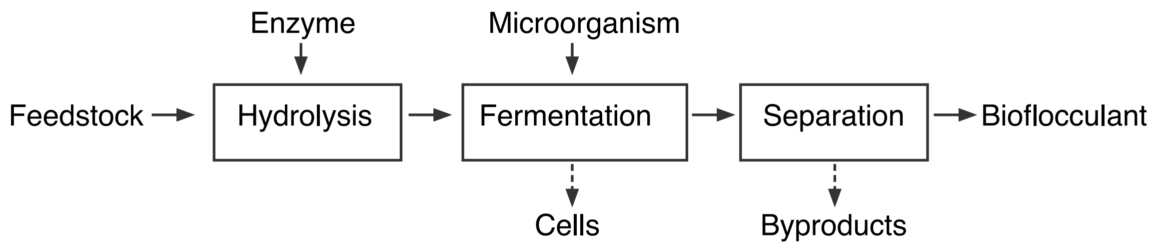

Figure 1. Typical steps for microbial conversion.

POME for *B. marisflavi* NA8 to survive in and the adaptability performed by this strain could largely reduce the production cost of bioflocculant (Nurul Adela *et al.*, 2016).

The bioflocculant production from POME was up-scaled using a 5 litre^{-1} bioreactor (Minifors™, Infors AG) with a 3 litre^{-1} working volume. The bioreactor was equipped with control modules to monitor the agitation, air flow, dissolved oxygen, pH and temperature. Temperature was maintained at 37°C, and the pH controlled at 7.0 by automatically adding 30% (w/w) NH_4OH and 5 M H_2SO_4 during the entire course of fermentation. Agitation was cascaded between 200 and 600 rpm. Rate of aeration was held at 1.0 vvm. IRIS (Infors AG) software was employed to monitor and record all available fermentation parameters in real time.

- **Extraction** – The fermentation broth was extracted using a centrifuge to remove bacterial cells followed by cold ethanol precipitation to separate the bioflocculant from the water.

  From 1 litre of culture broth, an estimated 9.72 g of bioflocculant could be obtained. Using this finding, a total of 49 kg bioflocculant can be obtained from 1 t of POME. The estimated potential production from a 60 t hr^{-1} palm oil mill is about 11 800 t of bioflocculant in a year.

CHARACTERISTICS OF BIOFLOCCULANT

The main characteristics of the produced bioflocculant are:

- Comprises 74% polysaccharide and 25% protein with 1% nucleic acid;
- Its elemental weight fractions are: C (29.6 ± 2.9%), H (6.4 ± 0.6%), N (4.5 ± 0.2%) and S (0.7± 0.04%) (relative weight percentage);
- The major functional groups identified by FTIR analysis are hydroxyl (-OH), amino (NH_2), carbonyl (C=O) and carboxyl (COOH) groups;
- It degrades mainly at 125°C by the thermogravimetric analysis (TGA); and
- It is thermostable and tolerant of extreme pH.

APPLICATION OF BIOFLOCCULANT

It is anticipated that the produced bioflocculant would precipitate suspended solids; in this case, its ability to aggregate microalgae cells during harvesting could be possible. When comparing the effectiveness of the produced bioflocculant with the chemical flocculant *i.e.* polyaluminium chloride (PAC), it was found that the produced bioflocculant was more efficient than PAC. At a lower dose (100 mg litre^{-1}), it was able to precipitate ~60% of *Chlorella vulgaris* UMACC283 (Loh *et al.*, 2017); while the PAC required >500 mg litre^{-1} to achieve the same result. This finding suggested that the produced bioflocculant could potentially be an alternative to many inorganic and synthetic flocculants. *Table 1* shows the summary of various microbial-derived bioflocculants and their functions.

CONCLUSION

POME has been demonstrated as a suitable medium for bioflocculant production. The production of bioflocculant from POME provides both the environmental and economic benefits. This value-addition hopefully can change the negative perception of POME as being polluting. The produced bioflocculant showed higher efficiency in precipitating microalgae than the conventionally-employed flocculants. With its high stability across a broad temperature and pH range, as well as its biodegradability, this POME-derived bioflocculant could be an attractive low-cost candidate for further exploitation in other industrial processes.

TABLE 1. APPLICATION OF MICROBIAL-DERIVED BIOFLOCCULANTS

Application	Remarks/examples	Reference
Water treatment	Bioflocculant from *Bacillus licheniformis* used for drinking water treatment	Li *et al.* (2009)
	Bacillus subtilis, Exiguobacterium acetylicum, Kebsiella terrigena, Staphylococcus aureus, Pseudomonas pseudoalcaligenes and *Pseudomonas plecoglossicida* to treat river water turbidity	Buthelezi *et al.* (2009)
	Pseudomonas aeruginosa and *Pseudomonas putida* able to remove natural organic matters in river water	Wang *et al.* (2012)
Wastewater treatment	Bioflocculant from *Serratia ficaria* applicable to treat different industrial wastewater *i.e.* brewery, soy a sauce brewing, meat processing, pulp and paper	Gong *et al.* (2008)
	Bacillus mucilaginosus able to remove 85% of COD and 68.5% of SS from starch wastewater	Deng *et al.* (2003)
	Bacillus mucilaginosus used to treat municipal, brewage, and pharmaceutical wastewater	Lian *et al.* (2008)
	Paenibacillus elgii B69 removed 68% of COD and 83% of turbidity	Li *et al.* (2013)
Colour removal from wastewater	Bioflocculant from *Bacillus, Exiguobacterium, Klebsiella, Pseudomonas* and *Staphylococcus* to decolorize dye (basic fushine) and Chromium	Buthelezi (2008)
	Klebsiella mobilis were efficient in flocculating some disperse dyes	Wang *et al.* (2007)
	Proteus mirabilis were effective in hazardous dye (basic blue 54) removal	Zhang *et al.* (2009)
Metal removal	A significant removal of Pb^{2+}, Zn^{2+}, and Hg^{2+} by bioflocculant from *Bacillus, Halomonas, Psedomonas* and *Paenibacillus*	Lin and Harichund (2011)
	Removal of Pb^{2+}, Cu^{2+}, and Zn^{2+} by *Bacillus subtilis*	Mikutta *et al.* (2012)
Toxic organic compound removal	Polycyclic aromatic hydrocarbon (PAHs) degradation removal	Zhang *et al.* (2011)
	Zooglea sp. and *Aspergillus niger* degraded 30% of pyrene	Jia *et al.* (2011)
Synthesis of nanoparticles	Nano-scale silver can be synthesised in reverse micelles using bioflocculant *Bacillus subtilis* MSBN17 as stabiliser	Sathiyanarayanan *et al.* (2013)
Microalgae harvesting	Microalgae *Nannochloropsis oceanica* DUT01 and *Chlorella minutissima* UTEX2341 harvested using bioflocculant from *Solibacillus silvestris* W01	Wan *et al.* (2013)
	Microalgae C. minutissima UTEX2341 harvested using bioflocculant from *Bacillus agaradhaerens* C9	Liu *et al.* (2015)
	Microalgae *Chlorella vulgaris* UMACC283 harvested using bioflocculant from *Bacillus marisflavi* NA8	This study

Note: COD = chemical oxygen demand, SS = suspended solids.

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