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Research progress in bioflocculants from bacteria

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Abstract

Although one of the major users of flocculants are water and wastewater treatment industries, flocculants are also used in various food industries. The chemical flocculants are preferred widely in these industries due to low production cost and fast production ability. However, the negative effects of the chemical flocculants should not be neglected to gain the economic benefits only. Therefore, the researchers are working to discover efficient and economical flocculants from biological sources. Several attempts have been made and are still being made to extract or produce bioflocculants from natural sources such as plants, bacteria, fungi, yeast, algae, etc. The review revealed that significant amount of work have been done in the past, in search of bioflocculant. However, commercially viable bioflocculants are yet to be marketed widely. With the advent of new biotechnologies and advances in genetic engineering, the researchers are hopeful to discover or develop commercially viable, safe and environment-friendly bioflocculants.

Introduction

Food and water security are the main issues in any developing country. Agriculture and food sectors are the largest consumers of water in most of the countries in the world (Hoekstra *et al*., 2012; Chowdhury and Al-Zahrani, 2015; Proskuryakova *et al*., 2017; Vergine *et al*., 2017). River water is the main global source of fresh water for domestic, irrigation and industrial usages. However, high turbidity caused by fine colloidal particles in water is a common issue that reduces the aesthetic value of water making it unappealing for human consumption and other usage (Robert *et al.*, 2016).

At the same time, the cost of water treatment increases with high turbidity and other pollutants in the river water. One of the main sources of high turbidity is storm runoff from developing areas. As such, the countries in the tropical region are more susceptible to high turbidity in the rivers due to high rainfall inducing soil erosion (Ali *et al*., 2007).

Bioflocculant can be produced from plant parts (Rani and Jadhav, 2012; Alfred and Sangodoyin, 2013). It also can be derived from microbes, either from single culture (Lian *et al.*, 2008; Gong *et al*., 2008; Nontembiso *et al*., 2011; Okaiyeto *et al*., 2015) or mixed culture of two microorganisms (Molla *et al*., 2001; Wang *et al*., 2011; Luvuyo *et al*., 2013). In fact, compound produced by mixed culture possesses higher flocculating activity than the compound from single culture (Zhu *et al.*, 2008).

Natural bioflocculant is more important for food industries as the product is related to the health and safety of the people. Among the physicochemical methods most widely to treat wastewater produced from food industry is coagulation (Shahidi *et al*., 1999; Lui *et al.*, 2013). Particles, suspended solids and also organic matters have been effectively removed using coagulation and flocculation process (Agunbiade, 2016). For example, chitosan, a partially deacetylated polymer obtained from the alkaline deacetylation of chitin is applied to treat the wastewater from milk processing plant. Unlike river water, wastewater of the food industry generally contains leavenings, carbohydrates inorganic and organic salts, oil, sugar, starch, detergents, cleaning products and high concentration of proteins. This paper, however, reports the progress on the discovery and production of flocculants from various biological sources.

Coagulation and Flocculation Process

According to the International Union of Pure and Applied Chemistry (IUPAC) definition, coagulation or flocculation is a process of contact and adhesion

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whereby the dispersed particles form clusters. Flocculant causes aggregation between particles and cells by bridging and charge neutralization whereby force of attraction is formed between the flocculant and the oppositely charged particles (Lian *et al.*, 2008). In terms of the mechanism, coagulation is the initial process in particle sedimentation which involves high speed mixing such as 200 rpm. At this stage, flocculant or coagulant is added to coagulate particles. According to Tzoupanos and Zouboulis (2008), the mixing is done in short time (less than 1 minute) to allow the flocculant interacts with the particles.

In wastewater treatment, small tank is used for this process. Meanwhile, flocculation is the later stage after the particles coagulate is formed. Low speed mixing is used to allow the flocs settle down at the centre and bottom of the tank (Figure 1). Besides, low speed is preferable to avoid the flocs scramble. Flocculant might be added at this stage to increase the floc size, floc strength and settling rate although it is not as important as in the coagulation stage. Since settling rate also depends on the gravitational force, flocculation requires longer time than coagulation between 20 to 45 minutes to maximize the amount of flocs collected.

Large tank is used for flocculation and mulit-stages are being practiced in water treatment plant. The crucial part in turbid water treatment is in coagulation part since force of attraction is initiated at this stage (Okuda *et al.*, 2001). It is vital to understand the mechanism of coagulation and flocculation to establish the real effect of bioflocculant during treatment.

Flocculants

The most widely used method of reducing turbidity is by adding chemical flocculants in the treatment process, which is not so environmentally friendly. Chemical flocculants such as Polyaluminium chloride, ferric chloride and polyacrylamide have high flocculating activity and low cost (Zheng *et al*. 2008). However, the main concern of chemical flocculant is the long-term side effect towards human health and ecosystem. Alzheimer (Banks *et al*., 2006), neurotoxicity (Polizzi *et al.*, 2001) and cancer (Ruden *et al*., 2004) are among the diseases associated with their application in drinking water treatment. Besides, chemical flocculants are also unsuitable in food industry. Therefore, various researches are conducted to find natural bioflocculant for food industries (Shahidi *et al*., 1999; Roseiro *et al*., 2003; Gupta and Aku, 2005; Mudgil *et al*., 2014).

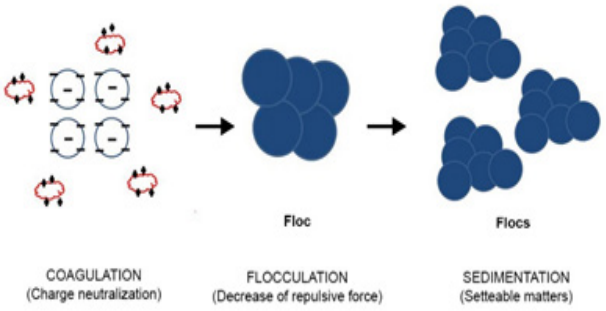

Figure 1. Charge Neutralization in Coagulation-Flocculation Process

Monomer of acrylamide is a strong carcinogen and remains in water after the treatment because it is highly water soluble and not readily adsorbed by sediments (Brown *et al*., 1988). Hence, safe alternative is required for the same purpose in water treatment.

Flocculants from Bacteria

Bioflocculant was also produced by *Paenibacillus elgii* B69 which was an exopolysaccharide composed of glucose, glucuronic acid, mannose and xylose (Li *et al*, 2003). The maximum bioflocculant production was about 25.63 g/L achieved with sucrose at 51.35 g/L, peptone at 6.78 g/L and yeast extract at 0.47 g/L. Bioflocculants are generally produced by microorganism including bacteria along the growth phase (Xia *et al*., 2008) whereby its characteristics influence the flocculating activity (Salehizadeh and Shojaosadati, 2001; Buthelezi *et al*., 2010).

The synthesis of extracellular polymer by living cells of microorganisms produces bioflocculant Produced bioflocculant are mainly composed of macromoecular substances such as glycoprotein, polysaccharide, protein, cellulose and nucleic acid (Al-Shahwani and Al-Rawi, 1989). A few latest research carried out on microbial flocculant and its application are given in Table 1. It can be observed that most of the works are done using fungi and bacteria, mainly due to easy isolation and culture process of the microbes.

Bacterial Cultivation

Single or mixed culture

Bioflocculant can be produced by cultivating single strain of bacteria. Lian *et al.* (2008) successfully isolated *Bacillus mucilaginosus* from the maize-farming fields in China. The activated strain then cultured for 6 days to obtain the supernatant. Abd-El-Hameem *et al.* (2008) has isolated three strains from *Bacillus* species whereby each can produce

Table 1. Research on Bioflocculants from Various Sources

Source	Flocculating Activity (%)	Major Findings	Application	Reference
Fungi: *Aspergillus niger*	86.2	Composition: Sugar (69.7%) and protein (28.5%)	Kaolin. 2 g/L concentration	Aljuboori *et al.* (2014)
Fungi: *Aspergillus parasiticus*	92.4	Composition: Sugar (76.3%) and protein (21.6%)	Reactive Blue 4 dye removal	Deng *et al.* (2005)
Bacteria: *Cellulomonas* sp., *Streptomyces* sp.	91	Source: Sucrose, peptone, MgCl2 Flocculant yield: 4.45 g/L	Kaolin. 4 g/L concentration	Nwodo *et al.* (2014)
Bacteria: *Methylobacterium* sp. Obi, *Actinobacterium* sp. Mayor	95	Flocculant yield: 8.20 g/L Dose: 1 g/L in 100 ml Kaolin	Kaolin. 4 g/L concentration	Luvuyo *et al.* (2013)
Bacteria: *Cobetia* sp., *Bacillus* sp.	90	Dose: 0.8 g/L pH: 8 Flocculant aid: Ca2+	Wastewater (brewery, dairy) and river water	Ugbenyen and Okoh (2015)
Bacteria: *Virgibacillus* sp.	70.4	Composition: Polysaccharide	Kaolin. 4 g/L concentration	Cosa *et al.* (2011)
Bacteria: *Bacillus* sp. Gilbert	91	Composition: Polysaccharide	Kaolin. 4 g/L concentration	Nontembiso *et al.* (2011)
Bacteria: *Klebsiella* sp. TG-1	84	Composition: Polysaccharide	Waste residue from the food industry	Liu *et al.* (2013)

bioflocculant to reduce Kaolin solution turbidity. Mix culture also being used to produce the single type of bioflocculant. In fact, mix culture may give bioflocculant with higher flocculating activity than those produced through single strain cultivation.

Nutrient

Different type of medium may lead support growth of different bacteria. Abd-El-Hameem *et al.* (2008) has successfully used yeast extract-peptone-glycerol (YPG) medium to isolate the *Bacillus* sp. whereby the main chemicals are yeast extract, peptone, and glucose dissolve in deionized water pH of 6.5 as according to Li *et al*. (2003).

Another isolation medium has been used was supplemented nutrient broth (SNB). Zaki (2011) has isolated *Bacillus subtilis* and *Pseudomonas* sp. from petroleum oil samples. The bioflocculant produced by those bacteria exhibited flocculating activity more than 90% when applied on kaolin suspension. Other medium used in previous studies were Luria Bertani (LB) and nutrient agar or broth. As such, various nutrients and supplements are being tested to get high yield of good quality bioflocculants.

According to Xia *et al.* (2008), peptone was the most cost-effective nitrogen source to support flocculant production. On the other hand, Li *et al.* (2009) mentioned that it was more economical to use 10 g/L of starch and 0.5 g/L ammonium chloride in production medium than 20 g/L of glucose, 0.5 g/L urea, 0.5 g/L yeast extract and 0.2 g/L ammonium sulphate in the screening medium.

Critical nutrition required are sucrose, peptone and magnesium chloride as mentioned by Nwodo *et al.* (2014). Li *et al*. (2009) found that the most preferred sources of carbon and nitrogen by *Bacillus licheniformis* were starch and ammonium chloride whereby the C/N ratio (w/w) was 30. Other nitrogen sources that can be used are beef extract, yeast extract and urea. Glucose, sucrose, lactose and ethanol are among the carbon sources that can be utilized to produce the bacterial flocculant (Xia *et al.*, 2008).

Temperature

Temperature in the growth process can be divided into three stages which are bacterial isolation, broth cultivation and preservation of the product. Gao *et al.* (2009) used 30°C to culture the bacteria in rotary shaker, 20°C for Jar test and 4°C to preserve the product in short term prior testing. Pure culture is preserved in glycerol stock at -80°C for long term preservation. He successfully isolated *Rhotia* sp. that gave 86% flocculating efficiency in Kaolin clay suspension. Most of the studies are conducted within temperature range of 20-35°C.

Other factors

Flocculating activity and bioflocculant yield can be affected by few factors such as metal cation presence, temperature, pH and inoculum size. (Luvuyo *et al*., 2013) Some of the previous studies mentioned Ca^{2+} presence as flocculant aid (Kurane *et al*., 1986; Ugbenyen and Okoh, 2013; Okaiyeto *et al*., 2015). Since coagulation process require bridge formation between the particles and the flocculant, initial adsorption is assisted by the cation presence. However, studies by Zhao *et al*. (2013) and Aljuboori *et al.* 2014 showed that some bioflocculants are cation-

independent which gave significant flocculating activity without metal cation presence in the kaolin suspension. Some of the bacteria species that have been successfully tested to produce flocculant are *Klebsiella* sp. (Liu *et al*., 2013), *Bacillus cereus* (Yang *et al*., 2007), *Rhizobium radiobacter* and *Bacillus sphaeicus* (Wang *et al*., 2011).

For lab scale fermentation, Erlenmayer flask was used to hold the broth culture on incubator shaker. Nwodo *et al.* (2013) incubate 200 ml Murashige and Skoog (MSM) fermentation medium in 500 ml flask while Xiong *et al*. (2010) produce the flocculant by inoculating the strain in 250 ml flask containing 100 ml medium. Larger flask can hold more volume of broth hence allow more biomass production. However, too high volume will cause incomplete agitation of the broth itself. Jang *et al.* (2001) has successfully cultured flocculant from *Citrobacter* sp. using fed-batch bioreactor.

In a study by Lian *et al.* (2008), 2 ml inoculum was cultured in 250-ml conical flask containing 100 ml culture medium for 6 days at 28°C. The lower portion of the culture was concentrated as the floccules and used as the flocculation material. The supernatant was decanted, estimated to be 85% of the total volume. In contrast, Okaiyeto *et al*. (2016) applied the cell-free supernatant as flocculating material and discard the biomass collected. By comparing these two studies, determining flocculating activity using floccules (74.6%) gave a higher turbidity removal percentage than using the supernatant (60%). However, to produce flocculant in form of floccules or biomass needs more source and time. The biomass can be extracted by concentrating the supernatant at 40°C. After cold ethanol is added, the precipitate is recovered, vacuum-dried and re-dissolved in distilled water (Deng *et al.*, 2005).

Properties of Bioflocculant

Besides the coagulation activity of the bioflocculant, the following parameters are also important for the flocculant to be easily stored, preserved, carried and applied to the industry.

Thermostability

High temperature may denature protein or peptide chains in the microbial flocculant resulting in reduced the flocculating activity. Luvuyo *et al.* (2013) cultivate the bacterial strains at 28°C to obtain the flocculant purify. The flocculant gave the highest flocculating activity at 86% when the optimum was 80°C. In fact, it showed thermostability over 70% at 80°C and 100°C. Meanwhile, Ugbenyen and Al-Okoh (2013) also successfully isolated another bacteria species with slightly higher flocculating activity which was 89.3% at 80°C proving that the bioflocculant is thermally stable between 50°C and 100°C.

Optimizing the temperature is important since bioflocculant application in the real industry will involve temperature changes due to seasonal climate and operating lines. Moreover, the collected supernatant which is used as the flocculating material is preserved at 4°C prior testing. The review on the temperature sensitivity of bioflocculant revealed that it is one of the parameters important for the long-term storage and preservation of the bioflocculant.

Characterization

FTIR spectrometry of the bioflocculant produced by a consortium of Streptomyces sp. and Cellulomonas sp. in Nwodo *et al.* (2013) showed the presence of carboxyl, hydroxyl and amino groups, indicating heteropolysaacharide compounds. The flocculant has woven clump-like structure as shown by SEM image and has heterogeneity characteristic. Luvuyo *et al.* (2013) also observed the presence of carboxyl and hydroxyl groups in the flocculant particle produced by a mixed culture of *Methylobacterium* sp. Obi and *Actinobacterium* sp. Mayor. Gong *et al.* (2008) showed that the major component of the flocculant produced by *Klebsiella mobilis* was neutral sugar without any protein. Meanwhile, Energy Dispersive X-ray spectroscopy (EDX) analysis performed towards flocculant produced by *Bacillus* sp. Maya indicated the presence of polysaccharide and protein in the compound (Ugbenyen and Okoh, 2013). Generally, the bioflocculants are some sort of polymers and proteins in nature.

Application

Microbial flocculant are, generally, potential for wastewater treatment (Mabinya *et al.*, 2011), drinking water purification (Nakata and Kurane, 1999), downstream process in food production and fermentation process (Salehizadeh and Shojaosadati, 2001). In wastewater treatment, dyes solution (Zhang *et al*., 2002; Deng *et al*., 2005), inorganic solid suspensions (Levy *et al*., 1992; Shih *et al*., 2001; Yim *et al*., 2007), and humic acids (Zouboulis *et al*., 2004) have been successfully treated with bioflocculants. Meanwhile, Gong *et al.* (2008) successfully applied the isolated bioflocculant to brewery, meat and soy-based processing wastewater from the industries.

Initial turbidity

Initial turbidity is an important factor to decide the dose of biocoagulant. During rapid test sedimentation, initial turbidity of the Kaolin is set between 500 and 550 NTU based on 4 g/L concentration. The clay powder is suspended in deionized water to obtain the desired turbidity as according to Kurane *et al*. (1986) and modified by Gao *et al.* (2006). It was observed that most of the turbidity reduction work was done for high range of turbid water, whereas, the river water turbidity is usually lower than 200 NTU most of the time, except the rainy days.

Dosage

In Cosa *et al*. (2011), 2 ml of cell-free supernatant and 3 ml of 1% $CaCl_2$ is added into 100 ml Kaolin suspension (4 g/L). The optimum dosage is varied depending on the type of raw water and the bacterial flocculant. However, it was observed that high dose of raw bioflocculant is required compared to the chemical flocculants.

pH

There are bioflocculant, which is able to show high flocculating activity in wide range of pH condition. *Bacillus toyonensis* is tolerant to pH 3-11 (Okaiyeto *et al*., 2015) giving more than 85% flocculating activity whereby the highest recorded at pH 3 (94%). The growth medium also supports the flocculant production at its best in acidic condition in which pH 5 give flocculating activity of 65% (Okaiyeto *et al*., 2015). The similar conditions were reported for flocculant production from Chryseobacteria daeguense W6 (Liu *et al*., 2010) and Bacillus spp. (Zufarzaana *et al.*, 2012). As such, the ability of bioflocculants in wide range of pH is an advantage over the chemical coagulants, which are usually efficient within narrow range of pH variation.

Real-field Application

The challenge in bioflocculant production is its real-field application. The bioflocculant production totally depends on the natural mechanism of the microorganism to produce the flocculant in conducive environment. The slow process actually requires high cost due to skilled worker, special equipment and the substrate. At the end, the flocculating activity exhibited by the bioflocculant is yet relatively low compared to synthetic flocculant thus require high dosage to ensure the reliable efficacy (Li *et al.*, 2003).

Lian *et al.* (2008) applied bioflocculant produced by *Bacillus mucilaginosus* into three types of wastewater namely domestic, brewage and pharmaceutical wastewater in lab scale setting. The removal rate of suspended solids was 93.9%, 93.6% and 88.4%, respectively. Ugbenyen (2015) applied glycoprotein bioflocculant produced by a mixed culture of *Cobetia* sp. and *Bacillus* sp. into river water, brewery wastewater and dairy waste water with resultant flocculating activities of 96.4%, 93.7%, and 82.2%, respectively.

Costing

Low cost medium in bioflocculant production will directly reduce the production cost hence make it competitive in real field application. Pretreated molasses has been used as fermentation substrate in a study by Sam *et al.* (2011). Activated charcoal detoxified rice hull hydrolysate was used to produce bioflocculant known as Schizophyllan glucan from Schizophyllum commune (Shu and Hsu, 2011). Solid residue of tofu production also has been used as the main medium component to produce bioflocculant from Klebsiella sp. TG-1 (Liu *et al.*, 2013). However, no exact amount of cost was mentioned to compare between synthetic and bioflocculant costs.

Concluding Remarks

Despite various studies conducted on the development and discovery of bioflocculant from natural materials, large-scale, economical and marketable production of bioflocculant is not reported yet. The main challenge is to produce high quality of large amount bioflocculant in short period of time. Until then, the industry has to rely on the chemical coagulants due to their efficiency and low cost, despite the known and unknown risks posed by the chemical coagulants.

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