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TOPICAL REVIEW

Agro-industrial waste materials and wastewater as growth media for microbial bioflocculants production: a review

Saifeldin M Siddeeg1,2, Mohamed A Tahoon1 and Faouzi Ben Rebah1,2

1 Department of Chemistry, College of Science, King Khalid University, PO Box 9004, Abha 61413, Saudi Arabia
2 Chemistry and Nuclear Physics Institute, Atomic Energy Commission, PO Box 3001, Khartoum 11111, Sudan
E-mail: benrebah@yahoo.fr

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Abstract

Various microbial strains (bacteria, fungi, and microalgae) produced polymers variable in composition (protein, cellulose, polysaccharide, etc) with interesting flocculation properties such as the ability to remove large spectrum of pollutants (organic and inorganic materials, etc) from wastewater and the stability over a wide range of temperature, pHs and salt concentrations. These bioflocculants have been characterized and successfully tested in wastewater treatment and sludge dewatering. The production of microbial bioflocculants involves the culture step of the bioflocculant-producing microorganism in an appropriate medium, followed by polymer extraction. The production processing is mostly controlled by the microbial growth medium cost. Agro-industrial wastes including agricultural by-products (rice hull, rice stover potato by-products, peanut hull, corn cob, wheat bran, etc), sugar processing wastes and fermentation liquors contain nutrients such as nitrogen and carbon, which can sustain the microbial growth and bioflocculant production. Recently, the potential use of wastewater and sludge as growth media for various bioflocculant-producing microorganisms has been demonstrated. Interestingly, waste pre-treatments may be essential to enhance the microbial growth and the bioflocculant production. Bioflocculant properties (polymer yield, polymer composition, flocculating activity, etc) are controlled by the growth conditions. Moreover, the produced materials showed acceptable results for wastewater treatment and sludge dewatering. This new strategy reported in this review can decrease to some extent the environmental problems related to the disposal of agro-industrial wastes and wastewater sludges. At the same time, this could reduce the cost of microbial bioflocculant production.

1. Introduction

During growth, various microbial strains are able to synthesize bioflocculants with interesting characteristics (biodegradable, eco-friendly, with efficient flocculation properties, etc) (Ben Rebah et al 2018). Under specific conditions, microorganisms produce extracellular polymeric substances (EPSs) having various ecological and physiological properties (Imperiali 2019). They can be secreted outside the cell (known as slime EPS) or remain bonded to cell (Known as capsular EPS) (Costa et al 2018). These biopolymers were investigated in different practices including wastewater treatment, sludge dewatering, etc (Ben Rebah et al 2018, Wangad et al 2019, Salek and Euston 2019, Pessoa et al 2019, McCarthy et al 2019). Generally, the production of bioflocculant by growing microbial strains in growth media is a biological process consisting of three principal steps (Ben Rebah et al 2018). The first step is related to the preparation of the microbial strain useful for the production of the bioflocculant. Various factors, such as the morphology, the presence of extracellular polysaccharides and the evaluation of the flocculating activity were used to select the useful microorganisms. Moreover, the resulted polymer should be qualitatively analysed. The second step of the bioprocess, is the strain culture and in order to determine the time of the highest polymer production, the adequate growth medium composition and
operating parameters (pH, temperature, aeration, inoculation rate, etc.) should be optimized. Finally, actions of separation and extraction are needed to produce the polymer (Ben Rebah et al. 2018). Depending on various factors (strain, medium composition, operating parameters, etc.), the produced microbial flocculants varied in composition (polysaccharides, proteins, nucleic acid, etc.) and in the flocculating activity (FA) (Ben Rebah et al. 2018). The bioflocculant production can be enhanced through process optimization of various control parameters such as culture medium composition (carbon and nitrogen sources, the ratio carbon/nitrogen (C/N), growth factors, mineral salts, etc.) and growth conditions (food/microorganism (F/M), pH, temperature, aeration, inoculum rate, etc.) (Fang et al. 2020, Arias et al. 2020, Wang et al. 2019, Lv et al. 2019, Zhou and Xu (2019), Hu et al. 2019, Zhang et al. 2019). During the investigation of bioflocculant-producing microorganisms, researches started by using synthetic media containing simple sugar (glucose, sucrose, lactose, fructose, maltose, etc.), alcohols and organic acid as carbon sources, and yeast extract, peptone, NH₄Cl, etc., as nitrogen sources. Generally, each microbial strain has its preferred carbon and nitrogen sources to grow well and produce efficient bioflocculant. Many strains (Bacillus velezensis 40B, Proteus mirabilis, Rhodococcus opacus, Penicillium sp. HHE-P7, Proteus mirabilis, etc.) use effectively glucose to produce polymer with an interesting FA (>90%) (Ben Rebah et al. 2018, Czemierska et al. 2017, Zaki et al. 2013, Liu and Cheng 2010). Other strains such as Solibacillus silvestris W01 and Aspergillus flavus yield higher flocculant rate in the presence of maltose and sucrose, respectively (Ben Rebah et al. 2018, Aljuboori et al. 2013, Wan et al. 2013). Moreover, nitrogen sources have an effective function for each microbial strain. Because of its composition, yeast extract was frequently used as a preferred nitrogen source for large number of microbial strains belonging to various classes (Bacillus, Rhodococcus, etc.) (Ben Rebah et al. 2018, Czemierska et al. 2017, Zaki et al. 2013). Peptone was suitable for the culture of Proteus mirabilis and Paenibacillus elignii B69 (Li et al. 2013, Zhang et al. 2010). Sodium nitrate, ammonium chloride and urea allowed the production of glycoprotein by Klebsiella sp. ZZ-3 with a FA superior to 90% (Yin et al. 2014). In contrast, pH, temperature, inoculation and growth time course have critical effect on microbial growth, polymer production and flocculating activity. As illustrated by many authors, each microorganism grows and produces bioflocculant at an optimal pH and temperature. Also, both pH and temperature affect the flocculating activity (Aljuboori et al. 2013, Wan et al. 2013, Zhang et al. 2010, Liu et al. 2010). Similar effects were reported for both inoculum rate and the growth time. Depending on the inoculation, some strains produce bioflocculants during the logarithmic growth phase (Wan et al. 2013, Nwodo et al. 2014). Other strains produce polymers throughout the stationary phase as reported by Aljuboori et al. (2013). Therefore, it seems that every microbial strain has its definite operating parameters to yield bioflocculant with efficient flocculating activity. However, the used substrate for the growth remains a major factor for large scale microbial bioflocculant production. Generally, experiments conducted with synthetic media have been reported for laboratory level and its use is inappropriate for large scale, because of the expensive price (Elkady et al. 2011, Li et al. 2010, Liu and Cheng 2010). The investigation of low cost substrates easily available became a necessity opening new opportunity for an effective production and utilization of microbial flocculants. Interestingly, different materials such as agro-materials, agro-industrial by-products, wastewater and sludge, which are locally available in various regions, have been evaluated as media for bioflocculant-producing microorganism growth. In this overview we will discuss the prospective use of various materials and wastes as culture substrates for large number of microbial strains. Highlighting will be provided for wastewater and sludges, which have been confirmed to be useful for the growth of various microorganisms.

2. Agro-industrial materials for microbial bioflocculant production

New strategies of microbial bioflocculants production, using low cost materials such as agricultural and industrial by-products, were reported by various researches. A variety of plant materials and agricultural wastes are considered as a source of carbon, nitrogen and oligoelements offering the ability to sustain the growth of large number of microbial strains. This growth can be associated to the production of efficient bioflocculant materials. As summarized in table 1, various agricultural materials were prepared and used to formulate growth media for bioflocculant-producing microorganism. Generally, the synthetic medium, containing simple carbon source, nitrogen sources and mineral salts, has been modified by introducing new preparations as new carbon sources.

Several agricultural wastes (peanut, potato, corn, wheat and rice by-products, etc.) were employed to formulate a non-synthetic medium. Mostly, the growth medium contained mineral salts (MgSO₄, K₂HPO₄, MgSO₄), yeast extract supplemented with agricultural waste hydrolysate. The hydrolysate was prepared by acting sulfuric acid followed by centrifugation. Then, the resulted supernatant was neutralized with Ca(OH)₂, followed by centrifugation (to remove the precipitates). As reported in table 1, hydrolysates of potato-by-products, corn stover, corn cob, wheat bran, rice stover and rice hull were tested as sole source of carbon for Pseudomonas veronii L918. Based on the conducted experiments, peanut hull hydrolysate offered the best carbon
| Carbon source/preparation | Growth medium composition | Strains | Bioflocculant properties (Y, FA, BC) | References |
|---------------------------|---------------------------|---------|------------------------------------|------------|
| Rice straw                | 0.5% rice straw (w/v) in mineral salt medium | *Pseudomonas* sp. HP2 | Y: 1.75 g l\(^{-1}\) FA: 92.5% (kaolin) BC: 38.5% P and 47.8% PS | Qi et al 2019 |
| Untreated agave           | untreated biomass 1% (w/v) in mineral salt medium | *Pseudomonas Borealis* G 22 | FA: 91.2% Guo et al 2018a |
| Untreated corn stover     | Untreated miscanthus       | Pine powder | Untreated Wheat bran | Untreated Wood dust |
| Wheat straw pretreated with xylanase | 1% wheat straw (w/v) in mineral salt medium | *Pseudomonas Boreolis* G 22 | Y: 2.08 g l\(^{-1}\) (15 days) Guo et al 2018b |
| Crude sugar cane molasses | 12% molasses (w/v) supplemented with NaH\(_2\)PO\(_4\) solution and 6 g l\(^{-1}\) yeast extract | *Bacillus velezensis* KY471306 | FA: 90.9% (kaolin) Y: 7.6 g l\(^{-1}\); BC: 100% P Moghannem et al 2018 |
| Peanut hull: dried        | 20 g l\(^{-1}\) carbon source, 3 g l\(^{-1}\) Yeast extract, 1.3 g l\(^{-1}\) K\(_2\)HPO\(_4\), 0.2 g l\(^{-1}\) gSO\(_4\)\(_7\)H\(_2\)O, 10 g l\(^{-1}\) Na\(_2\)CO\(_3\) | *Bacillus agaradhaerens* C9 | FA: 64% (kaolin) Liu et al 2017 |
| Corn cob: dried           | Wheat straw: dried        | Corn stover: dried | Rice bran: dried | Rice bran: dried |
| Rice stover: hydrolysized (1.7% v/v H\(_2\)SO\(_4\)), supernatant collection, centrifugation (6000 rpm, 30 min) and neutralization (Ca(OH)\(_2\)) | 200 ml l\(^{-1}\) hydrolyzate, 4 g/l K\(_2\)HPO\(_4\), 2 g/l KH\(_2\)PO\(_4\), 0.2 g/l MgSO\(_4\), 0.1 g l\(^{-1}\) NaCl, 0.5 g l\(^{-1}\) urea, 0.5 g l\(^{-1}\) yeast extract | *Rhodococcus erythropolis* | Y:2.37 g l\(^{-1}\) (60 h); FA > 85% (kaolin); BC: 95.6% PS Guo et al 2017 |
| Corn cob, potato residues, and peanut shell: powdered and sieved (40 mesh sieve) | 20 g l\(^{-1}\) carbon source, 3 g l\(^{-1}\) yeast extract, 1.2 g l\(^{-1}\) K\(_2\)HPO\(_4\), 0.2 g l\(^{-1}\) MgSO\(_4\)\(_7\)H\(_2\)O, 0.4 g l\(^{-1}\) Na\(_2\)CO\(_3\) | *Cellulosimicrobium cellulans* L804 | FA > 80% (kaolin) Liu et al 2015 |
| Corn stover: powdered and sieved (40 mesh sieve) | 20 g l\(^{-1}\) carbon source, 3 g l\(^{-1}\) yeast extract, 1.2 g l\(^{-1}\) K\(_2\)HPO\(_4\), 0.2 g l\(^{-1}\) MgSO\(_4\)\(_7\)H\(_2\)O, 0.4 g l\(^{-1}\) Na\(_2\)CO\(_3\) | *Cellulosimicrobium cellulans* L804 | Y: 4.75 g l\(^{-1}\) (48 h) FA > 90% (Kaolin) Liu et al 2015 |
|                           |                           |                           | FA: 99.04% (C. Reinhardtii), FA: 93.83% (C. Minutissima) |           |
| Carbon source/preparation | Growth medium composition | Strains | Bioflocculant properties (Y, FA, BC) | References |
|---------------------------|---------------------------|---------|-------------------------------------|------------|
| Peanut hull: hydrolyzation (1.7% w/w H₂SO₄ at 121 °C for 2 h), supernatant collection, centrifugation (10,000 rpm, 10 min) and neutralization (Ca(OH)₂) | 300 ml l⁻¹ hydrolyzate, 0.6 g l⁻¹ K₂HPO₄, 0.1 g l⁻¹ MgSO₄·7H₂O, 3 g l⁻¹ yeast extract | *Pseudomonas veronii* L918 | BC: 68.6% PS and 28% P; Y: 3.39 g l⁻¹ (24 h), FA: 92.51% (ash-flushing wastewater) | Liu et al 2016 |
| Rice stover hydrolyzate: hydrolyzation (1.7% w/w H₂SO₄ at 121 °C for 2 h), supernatant collection, centrifugation (6000 rpm, 30 min) and neutralization (Ca(OH)₂) | 200 ml l⁻¹ hydrolyzate, 5 g/lK₂HPO₄, 2 g l⁻¹ KH₂PO₄, 0.2 g MgSO₄·7H₂O, 0.1 g l⁻¹ NaCl, 0.5 g l⁻¹ urea, 0.5 g l⁻¹ yeast extract | *Rhodococcus erythropolis* | BC: 77.14% PS and 4.84% P; Y: 2.4 g l⁻¹ (60 h), FA: 89.5% (kaolin) | Guo et al 2015 |
| Palm jaggery | 20 g l⁻¹ palm jaggery, 2.5 g l⁻¹ yeast extract, 1 g/l NH₄NO₃, 0.2 g l⁻¹ MgCl₂, 5 g/l K₂HPO₄, 0.1 g l⁻¹ NaCl | *Bacillus subtilis* MSBN17 | BC: 95.6% PS and 3.3% P; Y: 13.42 g l⁻¹ (48 h), FA: 94.26% (kaolin); Sathiyarayanana et al 2013 |
| Corn stover: hydrolyzation (1.7% v/v H₂SO₄), supernatant collection, centrifugation (5000 × g) and neutralization (Ca(OH)₂) | 230 ml l⁻¹ hydrolyzate, 5 g/lK₂HPO₄, 2 g/lKH₂PO₄, 0.2 g l⁻¹ MgSO₄·7H₂O, 0.1 g l⁻¹ NaCl, 0.5 g l⁻¹ urea, 0.5 g l⁻¹ yeast extract | *Ochrobactrum ciceri* W2 | BC: 58.6% PS and 17.8% P; Y: 3.8 g l⁻¹ (16 h); Wang et al 2013 |
| Detoxified rice hull hydrolysate (with activated charcoal 2% for 60 min) | 2 l of hydrolyzate supplemented with 3 g l⁻¹ yeast extract, 0.5 g l⁻¹ KH₂PO₄, 0.25 g l⁻¹ MgSO₄·7H₂O | *Schizopyllum commune* ATCC 38548 | FA: 92% (kaolin); BC: P and PS | Shu and Hsu 2011 |
| Sugar beet molasses/ starch molasses: various pretreatments (clarification, pH pretreatment, sulphuric acid, activated carbon, tricalcium phosphate) | 2 l of hydrolyzate supplemented with 3 g l⁻¹ yeast extract, 0.5 g l⁻¹ KH₂PO₄, 0.25 g l⁻¹ MgSO₄·7H₂O, pretreated molasses (30 g l⁻¹ carbohydrate) supplemented with 7 g/l K₂HPO₄, 2 g/lKH₂PO₄, 0.1 g l⁻¹ MgSO₄·7H₂O, 1 g/l(NH₄)₂SO₄, 0.5 g l⁻¹ peptone, 157.2 g l⁻¹ NaCl | *Halomonas sp.* AAD6 | BC: 90% PS, 4%–5% NA and 0.5% P | Sam et al 2011 |
| Flowers of forest tree, Madhuca latifolia L.: dry flower soaked in hot water for 2 h under agitation (220 rpm) at 29 °C | 20 g l⁻¹ flower extract, 0.5 g l⁻¹ yeast extract | *Azotobacter indicus* ATCC 9540 | Y: 6.10 g l⁻¹ (144 h), FA: 72% (kaolin); BC: PS; Sathiyanarayanana et al 2013 |

Y: Yield, FA: Flocculating activity, BC: Bioflocculant composition, PS: Polysaccharide, P: Protein, NA: Nucleic acid.
source yielding bioflocculant with the highest FA (91.93%) obtained with kaolin clay suspension (Liu et al. 2016). Therefore, peanut hull hydrolysate offered the required amino acids and carbon sources suitable for the growth of Pseudomonas veronii L918, since the raw material contains proteins (6%–7%), cellulose (35%–45%), lignin (27%–33%), lipids (1%) and ash (2%–4%) (Tanyildiz 2011). Interestingly, a bioflocculant yield of 3.39 g l\(^{-1}\) was obtained with medium containing 300 ml l\(^{-1}\) of peanut hull hydrolysate and after 24 h of Pseudomonas veronii L918 growth. The produced bioflocculant, was a 24.77 kDa polymer (77.14% polysaccharide and 4.84% proteins) with the ability to flocculate ash flushing wastewater (FA: 92.51%) (Liu et al. 2016). Similar experiments were reported while growing Ochrobactrum ciceri W2 in corn stover hydrolysate supplemented with salt solution (K\(_2\)HPO\(_4\), KH\(_2\)PO\(_4\), MgSO\(_4\), NaCl), urea and yeast extract. The maximum bioflocculant yield (3.8 g l\(^{-1}\)) was obtained with 230 ml l\(^{-1}\) of hydrolysate and after 16 h of growth. However, the highest FA (92%) was obtained after 30 h of culture. The tested strain produced proteoglycan polymer with efficient FA at large range of pH (1–10) at 30 °C and temperature (30 °C–100 °C at pH 7.0). For this strain, is very important to point out that both growth rate and polymer productivity were significantly increased in the presence of corn stover hydrolysate-based medium free of phosphate salt, which could reduce the cost related to phosphate salts (Wang et al. 2013). It is however worthy to mention that corn stover hydrolysate contains mainly xylose, which is easily used by the microorganism as reported by Ren et al. (2008). Similarly, a rice stover hydrolysate was also tested as carbon source for Rhodococcus erythropolis showing the production of 2.4 g l\(^{-1}\) polymer (MW = 3.93 \(\times\) 105 Da) consisting of protein (95.6%) and polysaccharide (3.3%) (Guo et al. 2015). This biopolymer efficiently enhanced the dry solids (DS = 18.4%) and specific resistance to filtration (SRF = 4.8 \(\times\) 1012 m kg\(^{-1}\)) of secondary municipal sludge. Moreover, good performances of swine wastewater pre-treatment were observed with an interesting levels of COD (48.3%), ammonium (43.6%) and turbidity (75.8%) removals obtained at pH 8 and with flocculant dose of 20 mg l\(^{-1}\) (Guo et al. 2015).

For reducing the inhibitory effects on microbial growth of various by-products (phenolic compounds, hydroxymethylfurfural, furfural, acetic acid, etc), resulted after acid hydrolysis of lignocellulosic materials, detoxification and enzymatic hydrolyses were employed (Hodge et al. 2009, Hu et al. 2009, Tian et al. 2009, Shu and Hsu 2011). For example, rice hull hydrolysate was detoxified with activated charcoal increasing the glycan produced by Schizochytrium commune from 8.3 mg l\(^{-1}\) (hydrolysate without detoxification) to 81.3 mg l\(^{-1}\) (under optimal condition of detoxified hydrolysate) (Shu and Hsu 2011). In the same context, Pseudomonas boreopolis G22 was able to transform various untreated biomass (corn stover, pine powder, wheat bran, wood dust, xylan) using the enzyme cellulase-free xylanase and produced polymer. While using untreated wood dust, the strain produced a 3.982 \(\times\) 105 Da polysaccharidic polymer (63.3%). This polymer flocculates kaolin suspension (FA: 97.1% obtained at a concentration of 3.5 mg l\(^{-1}\)) and microalgal biomass (FA: 95.7% obtained at 80 mg l\(^{-1}\)) (Guo et al. 2018a). Likewise, Pseudomonas veronii L918, with its enzymatic system produced in the presence of peanut hull hydrolysate a bioflocculant useful for ash-flushing wastewater treatment (Liu et al. 2016). The enzymes enhance efficiently the production of available carbon source for both growth and biopolymer production. The enhancement of the biopolymer production could be related to the transformation of the raw material into accessible nutrients. However, the biomass variability affects considerably the C/N ratio which may control the microbial performances as reported by Liu et al. (2016) and Chaisorn et al. (2016).

Molasses, the cane sugar processing wastes, were also investigated as carbon source for microbial bioflocculant production. For example, Bacillus velezensis KY471306 was cultivated in formulated medium containing diluted solution of crude sugar cane molasses supplemented with NaH\(_2\)PO\(_4\) solution and yeast extract. The optimal conditions obtained by statistical experimental design (molasses 12% (w/v), yeast extract 6 g l\(^{-1}\), 30 °C) allowed the production of 7.6 g l\(^{-1}\) of exopolysaccharide (glucose, galactose and mannose) with a molecular weight 1.14 \(\times\) 10\(^5\) Da (Moghanem et al. 2018). As reported above, various chemical pretreatments (sulphuric acid, tricalcium phosphate, etc) and detoxification with activated carbon were applied for the crude molasses before been used (Sam et al. 2011). Growth and bioflocculant production depend on the used material and the applied pretreatment. Consequently, pretreated molasses allowed the strain Halomonas sp. AAD6 to produce polymer composed mainly by carbohydrates (90%) with an interesting FA comparable to that obtained by conventional flocculants employed for wastewater coagulation-flocculation.

Plant materials were also investigated for the formulation of microbial growth media. However, as far as we know, the only experiment describing this technology was the use of flowers of forest tree, Madhuca latifolia L., to produce an exopolysaccharide bioflocculant of Azotobacter indicus ATCC 9540 (Patil et al. 2010). Water extract of dry flower material (dry flower soaked in hot water for 2 h under agitation (220 rpm) at 29 °C) was used for the strain culture yielding 6.10 g l\(^{-1}\) of polymer, higher to that obtained with sucrose and mannitol. This yield was obtained with 20 g l\(^{-1}\) of flower extract and 0.5 g l\(^{-1}\) of yeast extract and after 144 h of growth under controlled conditions (180 rpm, 30 °C and pH 7.0). A bioflocculant (FA = 72%) consisting of uronic acids, O-acetyl groups, and Orcinol was produced at an optimal concentration of 500 mg l\(^{-1}\) (Patil et al. 2010). The beneficial use of the formulated medium could be related to its high sugar content (30–36 g l\(^{-1}\)) as reported by He et al. (2004).
Fermentation liquor, resulted from biological processing of various biomasses in order to produce valuable bioproducts, was also used as a culture medium for bioflocculant-producing microorganisms (table 2). For example, biohydrogen fermentation liquor was used for culture of various Bacillus strains (e.g.: *B. fusiformis*, *B. subtilis* and *B. flexus*) isolated from biohydrogen fermenter. These strains were able to produce polysaccharides with the capability to flocculate kaolin suspension (FA > 72%) (You et al 2008). This liquor (with COD: 4490 mg/l and pH 4) contains ethanol (689 mg l⁻¹), acetate (613 mg l⁻¹), propionate (340 mg l⁻¹), butyrate (102 mg/l) and valerate (47 mg l⁻¹), which are suitable as carbon sources for the microbial growth. Likewise, an interesting bioflocculant was also produced using another liquor from hydrogen-producing reactor with the ability to treat domestic (turbidity removal > 98.7%) and black ink (Color removal > 93.0%) wastewater and sludge conditioning (Dong et al 2008). An effective bioflocculant (FA: 92.5%) was also yielded by a consortium of two strains (*Rhizobium radiobacter* and *B. sphaericus*) cultivated in a mixture of 100 day-fermentation liquor and a synthetic medium (1/1 v/v) (Zhao et al 2012). Remarkably, 100 day-fermentation generates various reducing sugar beneficial for an efficient microbial bioflocculant production (Xiong et al 2010).

The conducted experiments using plant material and agro-industrial wastes as potential growth media for various bioflocculant-producing microorganisms were expected to offer an attractive and processing strategy for economic large scale production of bioflocculants. It is slightly difficult to compare data from various experiments using different microbial strains and different formulated media. As seen in tables 1 and 2, no defined medium has been established for different microbial strains and the produced bioflocculant varied in composition and in FA. Despite of these variations, the produced polymer seems very attractive for various fields such as the wastewater treatment. Hence, it could replace chemical agents (Fe₂(SO₄)₃, AlCl₃, etc) for wastewater treatment and sludge conditioning (Ferretti et al 2003, Ben Rebah et al 2018). Generally, the preparation of waste-based medium should be done taken into consideration the waste material composition and the balance between the various nutrients required for the growth. Moreover, researches should be conducted to clarify the comportment of each strain in the presence of the waste materials. This will help to detect inducers and inhibitors of both growth and polymer production in waste-based medium.

### 3. Wastewater and sludge for microbial bioflocculants production

#### 3.1. Wastewater

Wastewater from various origins, such as formaldehyde wastewater (Zhao et al 2016) methanol wastewater (Cao et al 2015), dairy wastewater (Wang et al 2007), potato starch wastewater (Pu et al 2014), H-acid wastewater (Zhong et al 2014a), starch processing wastewater (Joshi et al 2017) have been used as a carbon source to produce microbial bioflocculants (table 3). For example, using methanol wastewater as nutrient resource for an isolated strain (*Turicibacter sanguinis*) allowed the production of bioflocculant (4.61 g l⁻¹) composed of polysaccharide (74.1%) and protein (24.2%). Interestingly, the produced polymer has the ability of arsenite removal from aqueous solution (Cao et al 2015). Although the variability of wastewater depending on the origin and the applied treatment process, they contain enough carbon, nitrogen, phosphorus and micronutrients able to sustain microbial growth for bioflocculant production. This could largely reduce the production cost, improve the feasibility of commercial bioflocculant production and ultimately minimize the disposal/pretreatment cost of wastewaters. Interestingly, the effects of additional nutrients on the growth and bioflocculants production by various microbial strains cultivated in wastewaters were studied. For example, the addition of glycerine as a carbon source (v/v: 1/100) and (NH₄)₂SO₄ as nitrogen source (0.5 g l⁻¹) to potato starch wastewater during the growth of *Candida anglica* allowed the production of bioflocculant with FA of 94.6%. Generally, the yeast extract increased the growth and the FA when added to formaldehyde wastewater, K-acid wastewater (Zhong et al 2016), H-acid wastewater (Zhong et al 2014a) and Chromotropic acid (Zhong et al 2014b). This effect could be explained by the presence in yeast extract of amino acids, inorganic nitrogen and growth factors (iron, calcium, magnesium, strontium, sodium, potassium, barium, manganese, copper, lead, aluminum and vanadium) at concentrations satisfying the nutritional requirements of microorganisms. Similarly, dairy wastewater supplemented with 2% ethanol (v/v), was used as a substrate for the production of a novel bioflocculant by *Klebsiella mobilis* (Wang et al 2007). Under optimized conditions, 2.58 g l⁻¹ of bioflocculant (100% polysaccharide) was produced with an interesting FA (95.4%) superior to that produced using conventional substrate. This bioflocculant was effective in flocculating some disperse dyes in aqueous solutions, in particular, Violet HFRL with a decolorization efficiency of 91% (Wang et al 2007).

The characterization of wastewater indicated the presence of complex organic material, which cannot be easily assimilated by a large number of microorganisms. In this context, an enzymatic hydrolysis was applied for palm oil mill effluent and the obtained hydrolysate was used to formulate growth medium (hydrolysate:
Table 2. Fermentation liquor as growth media for bioflocculant-producing microorganisms.

| Origin of the liquor/ preparation/Characteristics | Growth medium composition | Strains | Bioflocculant production (FA, BC) | References |
|--------------------------------------------------|----------------------------|---------|----------------------------------|------------|
| Rice straw 100 -day fermentation liquor: centrifuged (10 min at 9000 x g) | Fermentation liquor mixed with synthetic medium (Ratio 5/1) | *Rhizobium radiobacter* and *Bacillus sphaericus* | FA: 97.35% (kaolin) | Zhao *et al* 2012 |
| acetate : 5000 mg l⁻¹; propionate 1333 mg l⁻¹; butyrate: 2500 mg l⁻¹; valerate 833 mg l⁻¹ | Synthetic medium (10 g l⁻¹ glucose, 0.5 g l⁻¹ yeast extract, 0.5 g l⁻¹ urea, 5 g/1 K₂HPO₄, 2 g/1 K₂HPO₄, 0.2 g l⁻¹ MgSO₄, 0.1 g l⁻¹ NaCl) | *Bacillus fusiformis* | FA: 71% (Koalin) | You *et al* 2008 |
| Biohydrogen fermentation liquor: centrifugation (20 min at 12000 x g) and filtration (0.22 μm) | Fermentation liquor/inoculum prepared in liquor (ratio >1/0.1) | *Bacillus subtilis* | FA: 70.5% (Koalin) | |
| COD : 4490 mg l⁻¹; pH: 4.02; Ethanol :689 mg l⁻¹; acetate : 613 mg l⁻¹; propionate 340 mg l⁻¹; butyrate: 102 mg/l; valerate 47 mg l⁻¹ | | *Bacillus flexus* | FA: 69.1% (Koalin) | |
| Biohydrogen fermentation liquor | | | | |
| COD > 3000 mg l⁻¹ | Fermentation liquor mixed with synthetic medium (Ratio 9/1) | Strains F₂-F₆ | FA: 11.4%–50.3% (kaolin) | Dong *et al* 2008 |
| | Synthetic medium (10 g l⁻¹ glucose, 0.4 g l⁻¹ K₂HPO₄, 0.2 g l⁻¹ MgSO₄, 0.5 g l⁻¹ carbamide, 0.5 g l⁻¹ KH₂PO₄, 0.4 g l⁻¹ NaCl, 0.5 g l⁻¹ yeast extract) | | BC: 6.12% PS and 1.14% P | |

FA: Flocculating activity, BC: Bioflocculant composition, PS: Polysaccharide, P: Protein.
Table 3. Wastewater as growth media for bioflocculant-producing microorganisms.

| Industrial wastewater                        | Microbial strains                  | Growth conditions                                      | Bioflocculant properties (Y, BC, FA)                  | References          |
|-----------------------------------------------|-------------------------------------|--------------------------------------------------------|-------------------------------------------------------|---------------------|
| Starch wastewater                              | Klebsiella variicola BF1            | pH 7, T: 30 °C, 200 rpm, 48 h                          | Y: 7.5 g l⁻¹                                           | Nguyen et al 2019a  |
|                                               |                                     |                                                        | FA: 97.6% (Kaolin)                                      |                     |
|                                               |                                     |                                                        | BC: 83.1% PS and 10.6% P                               |                     |
| Seafood wastewater                             | Chlorella vulgaris SAG 211–19       | pH 10, T: 27 °C, 14 d                                  | Y: 92% (algal harvesting)                              | Nguyen et al 2019b  |
| Domistic wastewater supplemented with molasses (5%) | Paracoccus denitrificans ISTOD1     | IN: 2%, T: 30 °C, 168 h                                | Y: 2.9 g l⁻¹                                           | Medhi and Thakur 2018|
|                                               |                                     |                                                        | FA: 68% (Kaolin)                                       |                     |
|                                               |                                     |                                                        | BC: 100% PS                                            |                     |
| Potato starch wastewater                       | Aspergillus niger A18               | pH 6.3, T: 28 °C, 2 d                                  | Y: 1.89 g l⁻¹                                          | Pu et al 2018       |
| Corn ethanol wastewater (50 g l⁻¹)             | Klebsiella variicola B16            | pH 4–10, IN: 1%, T: 15 °C–40 °C, 48 h                  | Y: 3.08 g L⁻¹                                          | Xia et al 2018      |
|                                               |                                     |                                                        | FA: 90.6% (Kaolin)                                      |                     |
|                                               |                                     |                                                        | BC: 100% PS                                            |                     |
| Palm oil mill effluent (treated with lignocellulase) | B. marisflavi NA8                  | pH 7, IN: 14%, T: 37 °C, 200–600 rpm,                 | Y: 9.72 g l⁻¹ (24 h)                                    | Bukhari et al 2018  |
|                                               |                                     |                                                        | FA: 80% (Kaolin)                                       |                     |
|                                               |                                     |                                                        | BC: 74% PS and 24% P                                   |                     |
| Potato starch wastewater                       | Rhodococcus erythropolis            | IN: 2%, T: 30 °C, 150 rpm, 60 h                        | Y: 1.18 g l⁻¹ (pH: 7.5)                                 | Guo et al 2018c     |
|                                               |                                     |                                                        | FA: 95.6% (Kaolin)                                      |                     |
|                                               |                                     |                                                        | BC: 92.3% PS and 7.6% P                                |                     |
| Food processing wastewater                     | Paenibacillus sp. A9                | pH 8.5, T: 30 °C, 150 rpm, 120 rpm, 10 d               | Y: 6.26 g l⁻¹ (pH: 7.5)                                 | Jiang et al 2019    |
|                                               |                                     |                                                        | FA: 87.64% (Kaolin)                                     |                     |
| Formaldehyde wastewater containing (NH₄)₂SO₄, yeast extract | Paenibacillus polymyxa | pH 6, IN: 7%, T: 30 °C, 120 rpm, 10 d                  | Y: 8.97 g l⁻¹ (pH: 7.5)                                 | Zhao et al 2016     |
|                                               |                                     |                                                        | FA: 94.7% (Kaolin)                                      |                     |
|                                               |                                     |                                                        | BC: 71.2% PS and 27.9% P                               |                     |
| K-acid wastewater containing urea and yeast extract. | Haloplanus vesicu | pH 7, IN: 12.5% T: 35 °C, 200 rpm, 96 h                | Y: 1.86–8.04 g l⁻¹                                     | Zhong et al 2016    |
|                                               |                                     |                                                        | FA: 81.86%–95.07% (dye wastewater)                      |                     |
|                                               |                                     |                                                        | BC: 78.6% PS and 21.1% P                               |                     |
| H-acid wastewater containing urea and yeast extract | Klebsiella pneumonia ZCY-7. | pH 7, IN: 9.65%, T: 98,60 °F, 200 rpm, 60 h            | Y: 8.9 g l⁻¹ (kaolin)                                   | Zhong et al 2014a   |
|                                               |                                     |                                                        | FA: 95.1%                                               |                     |
|                                               |                                     |                                                        | BC: 82.4% PS and 14.2% P                               |                     |
| Wastewater supernatant from anaerobic co-digestion of corn straw and molasses wastewater | Rhizobium radiobacter and Bacillus sphaericus | pH 7, IN: 8%, T: 30 °C, 140 rpm, 24 h                  | Y: 2.32 g l⁻¹                                               | Zhao et al 2017     |
|                                               |                                     |                                                        | FA: 91.3% (Kaolin)                                      |                     |
|                                               |                                     |                                                        | Y: 9.71 g l⁻¹                                           |                     |
|                                              |                                     |                                                        | FA: 85%–92.5% (Kaolin)                                  |                     |
| Chromotropic acid wastewater + urea and yeast extract | Sphingomonas yahuschieae | pH 6.9, IN: 7.74%, T: 37 °C, 200 rpm, 96 h             | Y: 9.71 g l⁻¹                                                                                   | Zhong et al 2014b   |
| Industrial wastewater                              | Microbial strains                  | Growth conditions                                                                 | Bioflocculant properties (Y, BC, FA)                                      | References                  |
|---------------------------------------------------|------------------------------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------|
| Alcoholic wastewater with urea                    | *Bacillus cereus*                  | pH 7, IN: $10^8$ cell l$^{-1}$, T: 30 °C, 120 rpm, 24 h                          | BC: 91% PS and 9% P                                                       | *Zhang et al* 2015          |
| Alcoholic wastewater with urea                    | *Pichia membranifaciens*           | pH 7, IN: $10^8$ cell l$^{-1}$, T: 30 °C, 120 rpm, 24 h                          | FA: 89.3% (Kaolin)                                                        | *Zhang et al* 2015          |
| Alcoholic wastewater with urea                    | *Bacillus cereus and Pichia membranifaciens* | pH 7, IN: $10^8$ cell l$^{-1}$, T: 30 °C, 120 rpm, 24 h | FA: 85% (at pH 3.6), 80% (at pH 7)                                       | *Zhang et al* 2015          |
| Palm oil mill effluent hydrolysate (20% v/v): enzymatic hydrolysis | *Bacillus marisflavi NA8*            | pH 7, IN: 10%, T: 37 °C, 150 rpm, 24 h                                           | Y: 32 g L$^{-1}$                                                          | *Nurul-Adela et al* 2016   |
| Diary wastewater with 2% ethanol (v/v)            | *Klebsiella Mobilis*                | IN: 5%, pH 6, T: 30 °C, 120 rpm                                                 | Y: 2.58 g L$^{-1}$                                                        | *Wang et al* 2007           |
| Methanol wastewater + yeast extract               | *Turicibacter sanguinis*            | IN: 8.5%, pH 7.5, T: 30 °C, 120 rpm                                             | Y: 4.61 g L$^{-1}$                                                        | *Cao et al* 2015            |

Y: Yield, FA: Flocculating activity, BC: Bioflocculant composition, PS: Polysaccharide, P: Protein.
20% v/v) for Bacillus marisflavi NA8. This medium allowed the production of 32 g l⁻¹ of bioflocculant with an interesting FA (80%) (Nurul-Adela et al. 2016).

According to table 3, the behaviors of microorganisms and specifically bioflocculant production varied much among the tested samples depending mainly on the microbial species and the origin of the wastewater used as growth medium. Wastewaters, which are abundant raw material, remains challenging and may reduce the microbial bioflocculant production cost. They can be used without any treatment process because of its organic composition (C, N, P, solids) and its nutrient value. In some cases, pre-treatment may enhance the bioflocculant productivity and reduce fermentation time. As reported by many studies, the technology of growing microbial strains in wastewater allowed the reduction of various pollutants (López-Pacheco et al. 2019a, 2019b). Therefore, wastewater as growth media can be exploited as an approach to considerably reduce pollutant load and simultaneously produce value added products such as microbial flocculants.

3.2. Wastewater sludge

Wastewater treatment processing produces huge quantity of sludge. Depending on their origin (municipal and/or industrial) and on the treatment process (physical, chemical and/or biological processing), the generated sludges varied in composition and are classified into primary, secondary, mixture or digested sludge. Generally, sludges contain water, organic and mineral substances, and various microbial strains. Interestingly, sludges can be used in various fields (as fertilizer in agriculture, to produce bricks for buildings, as growth media for industrial microbial growth, etc) (Ben Rebah et al. 2001, Zhang et al. 2018). In sludge, microorganisms produce extracellular polymeric substances and as reported above, these polymers consist mainly of polysaccharides, proteins and nucleic acids. They interact with sludge solids developing bioflocs allowing sludge dewatering (Flemming and Wingender 2001, Garnier et al. 2005, Houghton and Stephenson 2002, Sobeck and Higgins 2002). Therefore, various types of EPSs were extracted directly from sludge and their flocculating activity was evaluated (table 4). As indicated in table 4, the characteristics of extracted polymers are controlled by various factors such as the sludge origin and the used extraction methods (Nguyen et al. 2016, Liu et al. 2009). In some cases, polysaccharides or proteins represent the main compounds of the EPS as reported by many researchers (Liu and Fang 2002, Peng et al. 2012, Sponza 2003). For example, the mainly content of the EPSs extracted from acidogenic (sucrose-rich wastewater treatment) and methanogenic (phenolic wastewater treatment) sludges using formaldehyde and NaOH was respectively polysaccharide (62%) and protein (41%) (Liu and Fang 2002). Similarly, the highest protein content was reported for EPS extracted (using sonication-thermal method) from municipal activated sludges while compared to that from sludges sampled from industrial wastewater plants (petrochemical, printing, dyeing, pulp and paper, fruit processing, etc) (Peng et al. 2012).

However, is very important to point out that EPS content is controlled by other factors such as the F/M ratio, the wastewater type, the chemical composition, the treatment process, the C/N ratio, the time of sludge sampling, etc (Sponza 2003, Cetin and Erdincler 2004, Durmaz and Sanin 2001). Low F/M enhanced the EPS-protein percentage as reported by Sponza (2003). More effect was reported for C/N, but higher ratio (C/N = 40) reduced the EPS protein content (Cetin and Erdincler 2004, Durmaz and Sanin 2001).

As reported in table 4, the used extraction methodology affected the EPS yield, composition and flocculating activity. Therefore, is very important to optimize the EPS processing take into consideration the sludge characteristics and the operating parameters related to the extraction methods, which may help to generate an EPS with the required flocculating properties.

Because municipal and industrial sludges contained the needed elements (carbon, nitrogen, phosphorus and micronutrients) for microbial growth, they can be used for microbial bioflocculants production. In this context, as reported for wastewater different strains can be cultivated in most sludge types, allowing the production of efficient bioflocculants with specific properties (table 5). For example, bioflocculant materials were produced by several strains (isolated from wastewater, sludge, etc) while growing in sterilized sludge (20 g l⁻¹ SS) as sole carbon source. Depending on the strain origin and on the extraction method, the FA of the EPS varied from 72% to 80.2% (Nguyen et al. 2017). Likewise, it was reported by Nouha et al. (2015), the possibility of growing Cloacibacterium normanense in sterilized sludge (25 g l⁻¹ SS), which produced bioflocculant (FA 94.2%) with high efficiency of dewatering for both Kaolin suspension and municipal wastewater sludge. However, the sludge (25 g l⁻¹ SS) formed the same origin supplemented with crude glycerol (0.5%–2% w/v) was more suitable for the production EPS by the same strain. The highest EPS concentration reached 21 g l⁻¹ in the presence of 2% (w/v) of crude glycerol. Interestingly, FA reached 95.3% allowing the removal of various metals from wastewater such as Ni (85%), Fe (71%), Zn (65%), Al (73%); and Cu (36%) (Nouha et al. 2016).

Because sludges contain complex organic substances, which may limit the microbial growth, the transformation of these substances into available nutrient sources constitutes an essential action improving the microbial uptake of sludge. Depending on sludge origin and composition, various methods were applied including thermal, acid, alkaline, ultrasonic and microwave pretreatments. Pre-treatments may affect the
Table 4. Extracellular polymeric substance extracted from crude sludges.

| Wastewater sludge                                           | Extraction method                   | Yield          | Biofloculant composition                        | FA (%) | References        |
|-------------------------------------------------------------|-------------------------------------|----------------|-----------------------------------------------|--------|-------------------|
| Industrial sludge (membrane bioreactors)                    | Cation exchange resin               | 300–600 mg l⁻¹ | 100% PS                                       | 74–89  | Ajao et al 2018   |
| Municipal sludge (biofiltration)                            | Centrifugation                      | 430 mg l⁻¹     | 25% P, 62.5% PS and 12.5% NA                 | 45     | Nguyen et al 2016 |
|                                                             | Sonication                          | 3548 mg l⁻¹    | 45% P, 21% PS and 34% N                      | 47     |                   |
|                                                             | EDTA                                | 6244 mg l⁻¹    | 45% P, 21% PS and 34% N                      | 83     |                   |
|                                                             | Formaldehyde- EDTA                  | 4214 mg l⁻¹    | 40% P, 45% PS and 65% NA                     | 61     |                   |
|                                                             | Formaldehyde- sonication-EDTA       | 6816 mg l⁻¹    | 18.5% P, 12.5% PS and 69% NA                 | 73     |                   |
| Methanogenic sludge from phenolic wastewater reactor        | Formaldehyde–NaOH                   | 102 mg/g VS    | 41.3% P + 18.7% PS + 22.8% HS               | ND     | Liu and Fang 2002 |
| Acidogenic sludge from sucrose-rich wastewater fermentor   | Formaldehyde–NaOH                   | 179 mg/g VS    | 41.6% P + 62% PS + 8.4% HS                  | ND     | Liu and Fang 2002 |
| Municipal aerobic activated sludge                          | Formaldehyde–NaOH                   | 165 mg/g VS    | 33.2% P + 24.6> % PS + 30.6% HS             | ND     | Liu and Fang 2002 |
| Municipal sludge (biological aerated flier)                | Ultrasonication                     | 130–195 mg l⁻¹ | P and PS                                     | 92.33  | Liu et al 2009    |
| Municipal activated sludge                                  | Hydrochloric acid disintegration     | 1000 mg l⁻¹    | P and Ps                                     | > 96   | Zhang et al 2013  |
| Conventional activated sludge (70% domestic + 30% leachate) | Sonication-thermal                  | 358.8 mg l⁻¹   | Ratio P/PS: 6.8                               | ND     | Peng et al 2012   |
| Sludge from Printing and dyeing wastewater (anaerobic–oxic) | Sonication-thermal                  | 27 mg l⁻¹      | Ratio P/PS: 4.5                               | ND     | Peng et al 2012   |
| Sludge from paper and pulp wastewater (anaerobic–oxic–oxic) | Sonication-thermal                  | 1.3 mg/ml      | Ratio P/PS: 4.9                               | ND     | Peng et al 2012   |
| Municipal sludge (mechanical dewatered)                     | Hydrochloric acid disintegration     | ND             | PS                                           | 87–99.5| Sun et al 2012    |

FA: flocculating activity, ND: not determined, P: protein, PS: polysaccharide, NA: Nucleic acid HS: humic substance, versus: volatile solids.
Table 5. Wastewater sludge as growth media for bioflocculant-producing microorganisms.

| Wastewater sludge (type/pre-treatment) | Microbial strain/growth conditions | Bioflocculant characteristics (Y, BC, MW, FA)/applications | References |
|----------------------------------------|-----------------------------------|-------------------------------------------------------------|------------|
| Sterilized municipal sludge (SS:25 g/l) supplemented with crude glycerol (0.5%–2% w/v) | Cloacibacterium Normannense | Harvesting oloaginous yeast biomass: FA: 79% (combined with alum) | Yellapu et al 2018 |
| Municipal sludge (SS:25 g/l) treated with NaOH (pH 10 at 25 °C) and sterilized (121 °C, 30 min) | Rhodococcus erythropolis | MW: 4.21 × 10^3 D | Guo and Ma 2015 |
| pH 7, IN: 2% (v/v), 35 °C, 150 rpm and | | | |
| Sterilized municipal sludge (SS:20 g l⁻¹) | EPS-producing strains isolated from different sources (sludge, wastewater, etc), pH7, 30 °C, 220 rpm | BC: P | |
| Sterilized municipal sludge (SS:25 g/l) supplemented with crude glycerol (0.5%–2% w/v) | Cloacibacterium normannense | Y (B-EPS): 21.3 g l⁻¹ (72 h) Kaolin FA (B-EPS): 9.6%–95.3% (depending on the method of extraction for sludge) | Nouha et al 2016 |
| pH 7, IN: 2% (v/v), 30 °C, 180 rpm | | Heavy metal removal: | |
| Pretreated secondary sludge (SS = 25 g/l) using steam sterilization | Mixture of bacterial strains isolated from secondary municipal sludge | FA: 93% (depending on cation addition) COD: 51.4%–70.2% (depending on cations addition) Municipal wastewater treatment: FA:87.2%–94.3% (depending on cations addition) COD: 74.5%–80.4% (depending on cation addition) Brewery wastewater treatment FA: 76.4% (depending on cation addition and EPS dose) COD: 85.7%–88.4% (depending on cation addition and polymer dose) | More et al 2016 |
| | | FA: >93% (depending on cation addition) COD: 51.4%–70.2% (depending on cations addition) Municipal wastewater treatment: FA:87.2%–94.3% (depending on cations addition) COD: 74.5%–80.4% (depending on cation addition) Brewery wastewater treatment FA: 76.4% (depending on cation addition and EPS dose) COD: 85.7%–88.4% (depending on cation addition and polymer dose) | |
| Municipal sludge (SS:25 g/l): sterilized (121 °C, 30 min) | Cloacibacterium normannense | Kaolin FA: 94.2% | Nouha et al 2015 |
| | | FA: >93% (depending on cation addition) COD: 51.4%–70.2% (depending on cations addition) Municipal wastewater treatment: FA:87.2%–94.3% (depending on cations addition) COD: 74.5%–80.4% (depending on cation addition) Brewery wastewater treatment FA: 76.4% (depending on cation addition and EPS dose) COD: 85.7%–88.4% (depending on cation addition and polymer dose) | |
| | | Kaolin dewatering: | |
| | | CST: 59.9% Municipal wastewater sludge dewatering: CST: 37.6% SVI: 20 ml g⁻¹ | |
| Supernatant of pre-treated municipal sludge mixed with sterilized livestock wastewater (from anaerobic digestion reactor) | Rhodococcus erythropolis | BC: 91.2% PS, 7.6% P and 1.2% NA | Peng et al 2014 |
| | | Dye wastewater decolorization | |
| | | Color removal: 16.7%–85.7% | |

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produced EPS (yield, composition, FA, etc) by the cultivated strain. In the findings of Guo and Ma (2015), growing *Rhodococcus erythropolis* in sterilized and alkaline pre-treated sludge produced $4.21 \times 10^5$ Da bioflocculant composed mainly by protein. This polymer was useful for both wastewater treatment and sludge dewatering. The same strain produced bioflocculant (mainly protein) while growing in pre-treated (sterilization, alkaline-thermal and acid-thermal pre-treatments) swine wastewater sludge (SS ranged from 15 to 65 g/l). The bioflocculant concentrations were ranged from 2.9 to 4.1 g l$^{-1}$ depending on SS concentrations. However, FA exceeded 90% reported for broth and slim polymer (Guo et al 2014). In the same perspective, various combination of pre-treatment processes (thermal, chemical, ultrasonic and microwave pre-treatments) were applied on sludge before being used as growth media for *Rhodococcus erythropolis* (Peng et al 2014).

Interestingly, the thermal-alkaline pre-treated sludge combined with livestock wastewater (ratio 1:7 v/v) allowed the production of an effective bioflocculant (FA > 98% for pH 2–12) with the ability to remove color (the highest rate reached 85.7%). This polymer is composed of 91.2% of polysaccharides (Peng et al 2014). However, in the presence of treated activated sludge, the same strain produced a bioflocculant composed mainly of protein (84.6%) as reported by Junyuan et al (2013). Based on the collected information, bioflocculant production varied depending on the microbial species and on sludge composition, which affect the polymer yield and properties (composition and FA). First, high solids concentrations may reduce oxygen transfer and the dilution of the crude sludge, to obtain an adequate SS, may permit an optimal oxygen transfer during the culture.

**Table 5. (Continued.)**

| Wastewater sludge (type/pre-treatment) | Microbial strain/growth conditions | Bioflocculant characteristics (Y, BC, MW, FA)/applications | References |
|---------------------------------------|-----------------------------------|-----------------------------------------------------------|------------|
| Pre-treatments: Thermal (120 °C, 70 °C), alkaline, acid, ultrasonic, microwave | Ratio sludge/wastewater: 7:1 (v/v) | BC: 99.2% P; Y: 1.1–4.1 g l$^{-1}$ | Guo et al 2014 |
| Swine sludge from biofiltration plant (SS: 15–65 g/l) | *Rhodococcus erythropolis* | Kaolin FA (B-EPS): 71.5%–94.5% | |
| Pre-treatments: sterilization, alkaline-thermal and acid-thermal treatments | IN: 2% (v/v), pH 7, 150 rpm and 35 °C | Kaolin FA (S-EPS): 67.5%–92.8% | |
| Secondary municipal sludge (autoclaved at 121 °C, 15 min) | Bacterial strain isolated from sludge (Bacillus, Serratia and Yersinia strains) | Kaolin FA (C-EPS): 7.8%–94.5% | More et al 2012a |
| Municipal sludge from biofiltration unit (SS: 17–44.8 g l$^{-1}$) | Kaolin FA (B-EPS): 75% (B-EPS) | Y (B-EPS): 3.4 g l$^{-1}$ (alkaline thermal treatment for SS 17 g l$^{-1}$) | More et al 2012b |
| Pretreatments: Steam sterilization (121 °C, 15 min), alkaline (1 M NaOH)-thermal (121 °C, 15 min) treatment, acid (1 M HCl)-thermal (121 °C, 15 min) treatment | Kaolin FA (C-EPS): 26.4%–72.3% | Wastewater sludge dewatering | |
| Activated municipal sludge: sterilization, alkaline-thermal and acid-thermal pre-treatments | *Rhodococcus erythropolis* | Kaolin FA: 92.1% | Junyuan et al 2013 |

IN: inoculum size, DS: Dry solid, SRF: specific resistance to filtration, FA: flocculating activity, MW: molecular weight, Y: yield, BC: bioflocculant composition, P: protein, PS: polysaccharide, Y: bioflocculant yield, EPS: extracellular polymeric substance, B-EPS: Broth, C-EPS: Capsular EPS, S-EPS: Slim EPS, SS: suspended solids, CST: capillary suction time, SVI: sludge volume index.
Second, sludge composition such as, carbon, nitrogen, heavy metals, Mg, Ca, etc may have an inhibitory effect on bacterial growth, and pre-treatments may enhance nutrient availability and improve the bacterial multiplication and, consequently, bioflocculant production (Ben Rebah et al 2001).

4. Conclusions

This review summarized the use of various wastes in the production of microbial flocculants. Many Agro-industrial wastes including agricultural by-products, sugar processing wastes and fermentation liquors showed good potential as growth media for bioflocculant-producing microorganisms. Although accessibility, cost and pretreatments of these agricultural byproducts may limit bioflocculant production, their use remains a valuable option for recycling wastes fitting well with environmental sustainability. Interestingly, the use of wastewaters and sludge have attracted considerable attention as an alternative energy source, since they are abundantly available and containing nutrients beneficial in microbial bioflocculant production process. In some cases, waste pre-treatments may be necessary to maximize microbial bioflocculant production and flocculating activity. Nevertheless, each microbial strain has its own individual conditions for polymer production. Consequently, the medium composition should be optimized taking into account the microbial strain nutrient needs, the waste composition and the growth operating conditions (temperature, pH, aeration, etc). Moreover, the progress in waste technology (characterization, pre-treatments, etc) is still needed before large-scale application. Finally, in order to evaluate the competitive applicability of this new strategy of microbial bioflocculant production, economical and environmental study should be addressed taking in consideration the bioflocculants efficiency for pollutants removal at large scale and the disposal of the generated wastes including the removed pollutants and chemical related to polymer extraction process.

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ORCID iDs

Saifeldin M Siddeeg © https://orcid.org/0000-0002-3040-3834
Mohamed A Tahoon © https://orcid.org/0000-0002-3092-5296
Faouzi Ben Rebah © https://orcid.org/0000-0003-0574-4490

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