Microbial-Based Flocculants to Enhance Wastewater Sludge Dewaterability

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Various microorganisms (fungi, bacteria, and microalgae) are able to produce flocculating materials, such as polysaccharides, proteins, and glycoproteins. The ability of microorganisms to produce these molecules is identified based on many parameters, including the morphology and the existence of slimy extracellular polysaccharides. For this purpose, various methods (colorimetric, 16S rRNA gene sequence, etc.) and reagents (chelating agents, $CuSO_4$ solution crystal violet, etc.) are applied to isolate suitable microorganisms from soil, rivers, seawater, sludge, etc.

Keywords: bioflocculants ; sludge dewatering ; microbial-based flocculants

1. Introduction

Because of the intensification of industrial activities and the improvement in people's living standards, an increasing quantity of wastewater is generated, causing a serious health problem, mainly when it is discharged in the environment without treatment [1]. To manage wastewater from various origins (industrial and urban, etc.), wastewater treatment facilities are designed to remove pollutants using various methods, including physical, chemical, and biological processes. Generally, wastewater treatment processes generate large amounts of sludge, creating a potential threat to the environment and human health [2][3]. The obtained sludge with a lower solid content (under 8%) should be treated for final safe disposal [4][5]. Sludge handling and disposal is a significant step of the whole system, which costs as much as 50% of the total wastewater treatment cost [6][7]. However, sludge management cost is governed mainly by the efficiency of the methods used to separate liquids and solids in sludge [8], allowing the decrease in its volume and enhancing the post-treatment efficiency [9][10]. Generally, after mechanical dehydration, the sludge water content remains more than 70% [11], which should be reduced to meet the subsequent sludge reuse. The performance of sludge dewatering is controlled by various factors related to its composition, the particle size, the surface charge, the presence of extracellular polymers, etc. [12][13]. Because of their composition (mainly hydrophilic proteins and polysaccharides), extracellular polymers bind water molecules, allowing for high water content in sludge and making the sludge dewatering process difficult [14][15][16][17]. In order to enhance the efficiency of sludge dewatering, various methods are applied. These methods are classified into biological, chemical, and physical methods [18][19][20][21][22][23]. The biological methods are based on the use of enzymes that degrade proteins, allowing sludge floc fragmentation [18].

2. Microbial-Based Flocculants

Various microorganisms (fungi, bacteria, and microalgae) are able to produce flocculating materials, such as polysaccharides, proteins, and glycoproteins. The ability of microorganisms to produce these molecules is identified based on many parameters, including the morphology and the existence of slimy extracellular polysaccharides. For this purpose, various methods (colorimetric, 16S rRNA gene sequence, etc.) and reagents (chelating agents, $CuSO_4$ solution crystal violet, etc.) are applied to isolate suitable microorganisms from soil, rivers, seawater, sludge, etc. [24]. The general process of the preparation of microbial-based flocculants is illustrated in **Figure 1**.

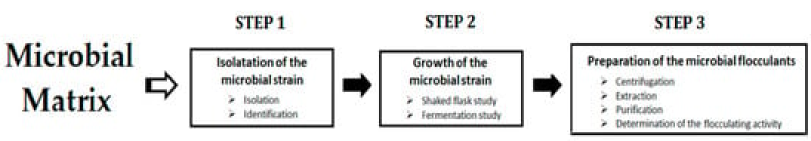

Figure 1. General process of the preparation of the microbial-based flocculants.

Generally, the microbial bioflocculants have been successfully applied for the removal of various pollutants (suspended solids, chemical oxygen demand, heavy metals, dyes, etc.) with high efficiency levels (>90%), allowing a significant flocculating activity (>70%) [24][25]. Interestingly they have the potential to improve sludge dewaterability, as indicated in **Table 1**.

Table 1. Applications of microbial-based flocculants for sludge dewatering.

		Crude Sludge Characteristics					Sludge Characteristics after Bioflocculation				References
Type of Sludge	**pH**	**SRF (m/kg)**	**CST (s)**	**MC (%)**	**DS (%)**	**Flocculation Conditions**	**SRF (m/kg)**	**CST (s)**	**MC (%)**	**DS (%)**	
Municipal anaerobically digested sludge	6.79	3.29×10^{13}	38.70			*Acidithiobacillus ferrooxidans* (10^8 cells/mL, 30 min, 180 rpm)	0.36×10^{13}	10.10	70.30		[26]
						Commercial cationic polymer (0.2%)	1.08×10^{13}	16.25	71.20		
Municipal secondary sludge		11.30×10^{12}			13.20	Pre-treated sludge flocculant (1.5 g/L), pH 7.5	3.40×10^{12}			22.50	[27]
						$Al_2(SO_4)_3$ (8 g/L, pH 6.5)	4.70×10^{12}			15.90	
						PAM (0.15 g/L, pH 7.5)	3.20×10^{12}			24.20	
						PAC (4 g/L, pH 7.5)	3.80×10^{12}			20.60	
						$FeCl_3$ (8 g/L, pH 6.5)	4.50×10^{12}			16.40	
Municipal secondary sludge	6.50	11.30×10^{12}			13.20	*Paenibacillus polymyxa* flocculant (1.5 g/L, pH 7.5)	3.60×10^{12}			21.70	[28]
Secondary sludge		11.30×10^{12}			13.20	*Paenibacillus polymyxa* flocculant (1.5 g/L, pH 7.5)	3.90×10^{12}			20.80	[29]
Secondary sludge		11.64×10^{12}				*Klebsiella pneumoniae* (0.1%/*wt*/*v*)	4.66×10^{12}			59.97	[30]
						$Al_2(SO_4)_3$	6.26×10^{12}				
						PAC	5.00×10^{12}				
Secondary sludge	6.23	29.00×10^5			3.19	*Proteus mirabilis* TJ-1 (7 mg) + $CaCl_2$ (12.5 mg/g Dw), (pH 7.5)	9.00×10^5				[31]
Chemically treated primary sludge	6.20	71.90×10^{12}	122.70		2.71	*Acidithiobacillus ferrooxidans* + Fe^{2+} (10% *v*/*v*)	5.00×10^{12}	20.00			[32]
Activated sludge	6.70	10.00×10^{12}	12.60		2.08	*Acidithiobacillus ferrooxidans* + Fe^{2+} (10% *v*/*v*)	$<5.00 \times 10^{12}$	7.90			[32]
Anaerobically digested sludge	7.70	8.30×10^{12}	19.50		2.10	*Acidithiobacillus ferrooxidans* + Fe^{2+} (10% *v*/*v*)	$<3.00 \times 10^{12}$	7.50			[33]
Anaerobically digested sludge	7.45	16.10×10^{12}	30.40		2.05	*Acidithiobacillus ferrooxidans* + Fe^{2+} (10% *v*/*v*)	$<1.00 \times 10^{12}$	<20			[34]

		Crude Sludge Characteristics					Sludge Characteristics after Bioflocculation				References
Type of Sludge	pH	SRF (m/kg)	CST (s)	MC (%)	DS (%)	Flocculation Conditions	SRF (m/kg)	CST (s)	MC (%)	DS (%)	
Chemically treated primary sludge	6.74	111.00 × 10^{12}	121.00		2.59	*Acidithiobacillus ferrooxidans* + Fe^{2+} (10% *v*/*v*)	11.10 × 10^{12}	10.00		31.40	[35]
Chemically treated primary sludge	7.03		86.90		2.00	Filamentous fungal strains (5% *w*/*v*), pH 6.85–7.15		35.50			[36]
Secondary sludge	8.04	10.87 × 10^{12}			13.10	*Klebsiella* sp. (6 mg/g Dw), pH 8	3.36 × 10^{12}			17.50	[37]
Municipal digested sludge	7.70		339.10	82.4		*Acidithiobacillus ferrooxidans* ILS-2 + Fe^{2+} (15% *v*/*v*)		31.30	60.10		[38]
						Acidithiobacillus ferrooxidans ILS-2 + Fe^{2+} (21% *v*/*v*)		26.20	48.60		
Secondary activated sludge	6.40	11.30 × 10^{12}			12.10	MBF10 *Rhodococcus erythropolis* (12 g/kg dry sludge)	4.80 × 10^{12}			19.30	[39]
						MBF10 *Rhodococcus erythropolis* (10.5 g/kg + PAC (19.4 g/kg))	3.20 × 10^{12}			23.60	
Municipal activated sludge	7.43	2.76 × 10^{12}	21.00			*Talaromyces flavus* S1	0.83 × 10^{12}	12.40			[40]

The bioflocculant produced by *Rhodococcus erythropolis* in alkaline thermal pre-treated sludge allowed a significant increase in both SRF and DS, reaching 3.4 × 10^{12} m/kg and 22.5%, respectively [27]. In the same research, the use of *R. erythropolis* supplemented with synthetic polymers (PAC and $Al_2(SO_4)_3$) increased the charge neutralization and bridging effect, allowing the enlargement of the flocs and, consequently, improving the sludge dewaterability [27]. However, for specific microbial strains there is a need for an energy substance (Fe^{2+}) for efficient production of biogenic flocculants [32][33][34][35]. For example, *Acidithiobacillus ferrooxidans* in the presence of Fe^{2+} (10% *v*/*v*) significantly improved the dewaterability of anaerobically digested sludge, and the values of SRF and CST passed from 16.1 × 10^{12} m/kg to less than 1 × 10^{12} m/kg and from 30.4 s to less than 20 s [34]. The same strain improved the dewaterability of various sludges (chemically treated primary sludge, activated sludge, and anaerobically digested sludge) and the highest reduction was observed for chemically treated primary sludge, with final values for SRF and CST of 5 × 10^{12} m/kg and 20 s, respectively [33]. Moreover, the biopolymer produced by the same strain (*Acidithiobacillus ferrooxidans*) reduced the SRF and the CST of municipal anaerobically digested sludge with an interesting reduction rate of MC (70.3%), SRF, and CST. The SRF and CST values passed from 3.29 × 10^{13} m/kg to 0.36 × 10^{13} m/kg and from 38.7 s to 10.1 s, respectively. The obtained reduction rates are higher than those reported for polyacrylamide (PAM) [26]. Similarly, the use of filamentous fungal strains for the dewatering of chemically treated primary sludge allowed the decrease in CST from 86.9 to 35.5 s in the presence of metal cations [36]. More recently, the strain *A. ferrooxidans* ILS-2 was added to municipal digested sludge in the presence of ferrous iron (10–21%), allowing a significant reduction in CST and MC values. However, this reduction increased when increasing Fe^{2+} loading, and the highest reduction was obtained with ferrous iron at 21%. Fe^{2+} loading at 21% reduced CST from 339.1 s (without strain and ferrous addition) to 26 s, and MC from 82.4% (without strain and ferrous addition) to 84.6% [38]. Therefore, higher loading of ferrous iron could improve the growth of *A. ferrooxidans* in sludge, and this strain transforms ferrous iron to biogenic ferric iron that acts as bioflocculant, allowing the enhancement of sludge dewaterability by the release of bound/stagnant water in extracellular polymeric substances in sludge.

A bioflocculant TJ-F1 obtained by growing *P. mirabilis* was tested for the dewaterability of a secondary sludge showing a higher reduction in SRF compared to a synthetic polymer P(AM-DMC). In the presence of 7 mg of the bioflocculant supplemented with 12.5 mg/g dw (dry weight) of the synthetic polymer and at pH 7.5, the SRT of the sludge reduced by 69% which is significantly higher than that obtained by P(AM-DMC) [31]. In the same context, the exopolysaccharide

Klebsiella sp. at a dosage of 6 mg/g dw and at pH 8 allowed a reduction in the secondary sludge SRF by 69%, giving a final DS of about 17.5% [37]. In the same research, the use of the bioflocculant supplemented with alum reduced the SRF by 84.2% and achieved a DS of 21.3% [37]. In this context, Serratia flocculant used for sludge dewatering allowed for a sludge volume index of 54 mg/L, obtained at a dosage of 0.3 g/L of the bioflocculant. However, with a synthetic flocculant, such as cationic polymers, a sludge volume index of 56 mg/L at a dosage of 0.3 g/L was achieved [41]. Similarly, the polysaccharidic bioflocculant produced by *Rhodococcus erythropolis* cultivated in rice stover hydrolysate showed better sludge dewaterability performances than synthetic polymer in terms of DS and SRF [39]. More recently, the spores of the filamentous fungus *Talaromyces flavus* S1 were used to inoculate activated sludge. This inoculation improved the dewaterability by 48% [40]. This improvement may be related to the polysaccharides produced by the fungal mycelium [42]. It was reported in the literature that extracellular polymeric substances have the ability to enhance the formation of biofloc, allowing higher settleability of sludge [43]. The content of the extracellular polymeric substances significantly affects their role in sludge dewaterability. Thus, higher carbohydrate content and lower protein content may increase sludge dewatering [44][45]. Likewise, it is very important to point out that sludge characteristics (sludge origin, pH, organic content, cationic content, etc.) affect the facility of extracellular polymeric substances to act in sludge conditioning [46]. Indeed, the use of microbial flocculant could increase the sludge calorific value, as reported by Kurade et al. [26][35]. Moreover, microbial flocculants act at lower dosages when compared to synthetic polymers, such as $FeCl_3$ and $Al_2(SO_4)_3$ [27].

According to the literature, sludge dewatering can be achieved by adding the microbial strain into sludge and the bioflocculant will be produced during the growth or by the application of a pure bioflocculant purified after its production by a selected microbial strain growing in an appropriate growth medium [47]. However, the microbial bioflocculant production is controlled by various factors including the culture medium and the operating conditions (C/N ratio, oligoelements, pH, temperature, aeration etc.) [24][48]. For large-scale production, optimization studies should be carried out in order to maximize the bioflocculant production. Moreover, the purification process and the preservation method should be taken into consideration in bioflocculant recovery. The optimization of the growth media and the purification process are considered as the main factors that control the product commercialization. For economical production, a low-cost medium should be developed and/or high-yield strains should be selected. In this context, various agricultural and industrial wastes (molasses, poultry processing waste, corn, rice, peanut, potato, corn, etc.) [49][50] and wastewaters generated by many industries (potato starch, brewery, corn ethanol, swine, palm oil mill, livestock, ramie biodegumming, etc.) have demonstrated their ability to replace standard microbial growth media for bioflocculant production [25]. This may considerably reduce the microbial flocculant production cost, as reported by Siddeeg et al. (2019) [25]. In the same way, another strategy was developed based on the screening of new microbial strains able to grow and produce flocculant in a culture medium low in nutrients [51]. Is also important to promote the selection of strains with the ability to produce bioflocculants that act without metal activation [51][52][53]. Furthermore, the microbial bioflocculant yield could be improved using genetic engineering [54]. The microbial diversity and the variability of the carbon sources may affect the nature and the characteristics of the produced bioflocculant (structure, composition, flocculating activity, etc.) [25]. Although these variations may limit the universal use of the produced microbial bioflocculant, these biopolymers seem suitable to replace synthetic polymers in the coagulation/flocculation process in wastewater treatment and sludge dewatering [24]. Generally, the research activities reported for sludge conditioning are limited and more investigations are needed to evaluate the flocculating activity at a large scale for sludge from various origins. A techno-economic feasibility should be conducted, taking into consideration the various parameters, such as the growth conditions (culture medium composition, operating parameters, extraction and purification of bioflocculants, etc.).

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