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Bioflocculants from isolated strain or mixed culture: Role of phosphate salts and Ca^{2+} ions

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ABSTRACT

In literature, bioflocculants were produced by pure or mixed cultures with added phosphate salts as pH buffers. Flocculation and sedimentation tests of particulate suspensions with Ca^{2+} ions as co-flocculant were commonly conducted for verifying the efficiency of the produced bioflocculants. However, the validity of these literature works may be questioned since many of the associated experimental works were conducted without enough controls. This study applied a newly isolated strain, *Ochrobactium ciceri* W2, to produce a biopolymer from acid hydrolyzed corn stover with phosphate salts as pH buffer. Sufficient flocculation efficiency of the broth was noted on kaolin suspensions with added Ca^{2+}. Flocculation and sedimentation tests using individual ingredient of the fermenting broth revealed that the corn stover hydrolysate before W2 fermentation and the phosphate buffer alone could sufficiently flocculate the kaolin suspensions. Conversely, the purified biopolymer produced by W2 presented no flocculating activities. Further flocculation tests noted that phosphate buffers and Ca^{2+} could synergetically flocculate the kaolin suspensions. Since most bioflocculant studies in literature dosed high levels of phosphate salts in fermentation and Ca^{2+} in flocculation stages, the efficiency of so-produced bioflocculants may be over-estimated. Revisions on bioflocculant studies with individual ingredient in the applied medium tested are advised.

1. Introduction

Bioflocculants produced by microorganisms are regarded environmentally safe in numerous applications including wastewater treatment and downstream processing for food and fermentation industries [1]. Production of bioflocculants using mixed or pure cultures was studied (Table 1). The effective strains studied included *Bacillus* sp., *Halomonas* sp., *Scenedesmus* sp., *Chryseobacterium* sp., *Penicillium* sp., and *Pseudoalteromonas* sp., and the adopted organic substrates were mostly glucose and sucrose [2]. In these tests, high concentrations of phosphates salts (0.1–5 g K_2HPO_4 and 0.1–2 g KH_2PO_4) were added as pH buffer. The entire fermented broth was generally used as flocculants in the flocculation tests to reduce the production cost.

Efforts were made to isolated and characterize the so-yielded biopolymers in the fermentation tests [3–6]. More *et al.* [7] characterized the biopolymers produced by nine bacterial strains from sludge to flocculate kaolin suspensions. In general, the yielded biopolymer is a mixture of compounds of sugars, proteins and acids. To the authors' best knowledge, there is no comprehensive study on the control on whether the medium before fermentation or other added integrant contributing to the noted flocculation efficiency. Restated, the existing literature results have conducted no proper controls.

A few studies considered the use of hydrolysates from agricultural wastes for bioflocculant production [8–12]. This study aims at (1) using a newly isolated strain, *Ochrobactium ciceri* W2, to produce biopolymer from acid hydrolyzed corn stover and evaluate the flocculation efficiency of the so-yielded broth on kaolin suspensions; (2) examining the flocculation efficiencies of each ingredient in the fermenting broth.

2. Materials and methods

2.1. The hydrolysate and strain

The dried corn stover was collected from Harbin suburb, Heilongjiang province, China. The stover corn was hydrolyzed by

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Table 1
Bioflocculant production studies by isolated strains and mixed cultures.

Authors	Strain	Maximum flocculating activity (%)	Phosphate contents (l^{-1})	Ca^{2+} (l^{-1})	Substrate
Deng *et al.* [17]	*Aspergillus mucilaginosus*	99.6	5 g K_2HPO_4 + 2 g KH_2PO_4	0[e]	Glucose
Salehizadeh and Shojaosadati [20]	*Bacillus firmus*	>85	5 g KH_2PO_4	5550 mg $CaCl_2$	Glucose
Shih *et al.* [23]	*Bacillus licheniformis*	NA[a]	0.5 g K_2HPO_4	150 mg $CaCl_2$	Citric acid + glutamic acid + glucose
Deng *et al.* [24]	*Bacillus mucilaginosus*	98.1	1 g K_2HPO_4	0[d]	Glucose
Fujita *et al.* [25]	*Citrobacter* sp. TKF04	98.4	1 g K_2HPO_4	0[e]	Acetate + propionate
Jang *et al.* [26]	*Citrobacter* sp. TFK04	NA[c]	1 g K_2HPO_4	50 mg $CaCl_2$	Acetic acid
Kim *et al.* [27]	*Citrobacter* sp. BL-4	NA[c]	0	500 mg $CaCl_2$	Acetate
He *et al.* [28]	*Corynebacterium glutamicum*	<70	0.1 g KH_2PO_4	575 mg $CaCl_2$	Sucrose
Li *et al.* [29]	*Corynebacterium glutamicum*	NA[c]	0.1 g KH_2PO_4	No FA tests	Sucrose
He *et al.* [30]	*Corynebacterium glutamicum*	NA[a]	0.1 g KH_2PO_4	575 mg $CaCl_2$	Sucrose + corn steep liquor
Lu *et al.* [32]	*Enterobacter aerogenes*	NA[g]	5 g KH_2PO_4	0[e]	Carbohydrates
Prasertsan *et al.* [33]	*Enterobacter cloacae* WD7	91	5 g K_2HPO_4 + 2 g KH_2PO_4	4400 mg $CaCl_2$	Glucose
Zhang *et al.* [34]	*Nannocystis* sp. NU-2	90	2 g K_2HPO_4	0[h]	Starch
Oh *et al.* [35]	*Paenibacillus* sp. AM49	83	0.3 g K_2HPO_4 + 0.3 g KH_2PO_4	888 mg $CaCl_2$	Glucose
Gong *et al.* [36]	*Paenibacillus polymyxa* BY-28	NA	3 g KH_2PO_4	50 mg $CaCl_2$	Glucose
Zhang *et al.* [37]	*Sorangium cellulosum*	>99	2 g K_2HPO_4	10[m]	Starch
Elkady *et al.* [15]	*Bacillus mojavensis*	96.12[f]	5 g K_2HPO_4 + 2 g KH_2PO_4[f]	150 mg $CaCl_2$	Glutamic acid
Mabinya *et al.* [38]	*Halomonas* sp.	88	5 g K_2HPO_4 + 0.2 g KH_2PO_4	240 mg $CaCl_2$	Glucose
Cosa *et al.* [39]	*Virgibacillus* sp.	70.4	5 g K_2HPO_4 + 0.2 g KH_2PO_4	300 mg $CaCl_2$	Glucose
Kim *et al.* [40]	*Scenedesmus* sp.	95	0.3 g K_2HPO_4 + 0.3 g KH_2PO_4	944 mg $CaCl_2$	Glucose
He *et al.* [41]	*Halomonas* sp. V3a'	95	2 g KH_2PO_4 + 5 g K_2HPO_4	500 mg $CaCl_2$	Glucose
Zhang *et al.* [11]	*S. cerevisiae*	NA	2 g KH_2PO_4	48–648 mg Ca^{2+}[i]	Glucose
Ji *et al.* [42]	*Bacillus licheniformis*	99.2	5 g K_2HPO_4 + 2 g KH_2PO_4	999 mg $CaCl_2$	NA
Zhang *et al.* [43]	*Proteus mirabilis*	>90	5 g K_2HPO_4 + 2 g KH_2PO_4	10000 mg $CaCl_2$	Glucose
Liu and Cheng [44]	*Penicillium* sp.	96	5 g K_2HPO_4 + 2 g KH_2PO_4	450 mg $CaCl_2$	Glucose
Liu *et al.* [45]	*Chryseobacterium daeguense*	96.9	0.3 g K_2HPO_4	0[j]	Glucose
Wu *et al.* [46]	*Corynebacterium glutamicum*	NA[a]	0.1 g KH_2PO_4	582 mg $CaCl_2$	Glucose
Li *et al.* [47]	*Agrobacterium* sp.	NA	5 g K_2HPO_4	820 mg $CaCl_2$	Surcose
Yang *et al.* [1]	*Paenibacillus polymyxa*	>98[b]	5 g K_2HPO_4 + 2 g KH_2PO_4	27 mg $CaCl_2$	Surcose
Li *et al.* [14]	*Bacillus licheniformis*	97.9	5 g K_2HPO_4 + 2 g KH_2PO_4	500 mg $CaCl_2$	Glucose
Li *et al.* [48]	*Pseudoalteromonas* sp.	NA[a]	1gNa_2HPO_4 + 0.3 g KH_2PO_4	1110 mg $CaCl_2$	Flour
Xia *et al.* [49]	*Proteus mirabilis*	93.13	5 g K_2HPO_4 + 2 g KH_2PO_4	0[k]	Glucose
Gong *et al.* [50]	*Serratia ficaria*	95.4	5 g K_2HPO4 + 2 g KH2PO4	200 mg $CaCl_2$	Glucose
Lian *et al.* [51]	*Bacillus mucilaginosus*	>93[l]	2 g Na_2HPO_4	NA[l]	Sucrose
You *et al.* [52]	*Bacillus subtilis*	>97	5 g K_2HPO_4 + 2 g KH_2PO_4	150 mg $CaCl_2$	Glucose
Zhao *et al.* [2]	Mixed culture	97.35	0.5 g K_2HPO_4 + 5 g KH_2PO_4	150 mg $CaCl_2$	Synthetic + fermentation liquor
More *et al.* [7]	*Bacillus* sp. 7	>80%	Sludge supernatant	150 mg Ca^{2+}	Sludge as substrate
Wang *et al.* [13]	*Ochrobactrum ciceri*	94	5 g K_2HPO_4 + 2 g KH_2PO_4	150 mg $Ca(OH)_2$	Corn stover hydrolysate
Shu and Hsu [12]	*Schizophyllum commune*	NA	1.5 g KH_2PO_4	$Ca(OH)_2$ added to adjust pH	Detoxified rice hall hydrolysate
Elkady *et al.* [15]	*Bacillus mojavensis*	96.11	5 g K_2HPO_4	150 mg $CaCl_2$	Glutamic acid
Feng and Xu [16]	*Bacillus* sp. BF3-3	98.1	0.5 g KH_2PO_4	58.8 mg Ca^{2+}	Glucose
Nwodo *et al.* [53]	*Streptomyces* sp.	87	5 g K_2HPO_4 + 2 g KH_2PO_4	300 mg $CaCl_2$	Glucose

[a] Different definition in flocculating activity reported.
[b] Dual conditioning with 121 mg/l PACl and 99.75 mg/l biopolymer.
[c] No flocculating activity data were reported.
[d] 500 mg/l $MgSO_4 \cdot 7H_2O$ was added in fermentation broth.
[e] 200 mg/l $MgSO_4 \cdot 7H_2O$ was added in fermentation broth.
[f] Phosphate salts were removed from biopolymer, and flocculating activity was measured with purified biopolymer with no ion dosage was *ca.* 50%.
[g] Sedimentation rates were reported.
[h] 30 mg/l of $FeCl_3$ provided 90% flocculating activity.
[i] Presented in the recycled wastewater for flocculation.
[j] Adding ions reduced flocculating activities.
[k] Adding Mg^{2+} ions to increase flocculating activities.
[l] Depending on the type of wastewaters to be treated.
[m] Adding in fermentation broth.

diluted 1.7% (w/w) sulfuric acid with solid–liquid ratio 1:10 at 121 °C for 120 min. The lignocellulosic material was hydrolyzed and the produced reducing sugar measured by dinitrosalicylic acid (DNS) method (Miller, 1959). After hydrolysis the supernatant was collected at 9000 × *g* centrifugation and was mixed with sufficient $Ca(OH)_2$ to neutralize pH. The so-yielded precipitates were removed by centrifugation. The yielded hydrolysate was used in subsequent tests.

The strain was isolated in Wang *et al.* [13] which was from activated sludge sample collected at a wastewater treatment plant at Harbin, China. The strain was isolated by agar plate technique and was identified using PCR-DGGE technique as *O. ciceri* W2.

2.2. Bioflocculant production and flocculating activity tests

The isolates in Section 2.1 were individually cultivated in 250 ml flask containing cultivation medium of composition (per liter): 230 ml hydrolysate, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.1 g NaCl, 0.5 g urea and 0.5 g yeast extract at pH 7.5 and 30 °C.