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A novel flocculant prepared by lignin nanoparticles-gelatin complex from switchgrass for the capture of *Staphylococcus aureus* and *Escherichia coli*

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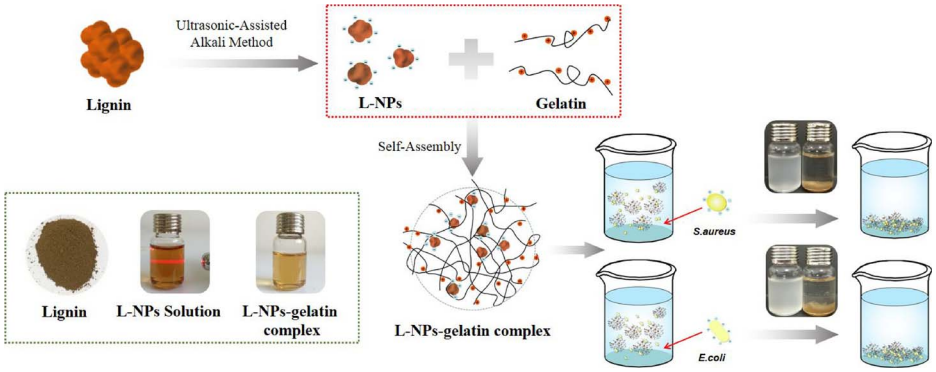
GRAPHICAL ABSTRACT



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ABSTRACT

Lignin nanoparticles (L-NPs) were firstly prepared from switchgrass lignin by using ultrasonic-assisted alkali method, after which L-NPs were assembled with gelatin to constitute L-NPs-gelatin complex as a novel flocculant. Both Gram-positive (*Staphylococcus aureus*, *S.aureus*) and Gram-negative (*Escherichia coli*, *E.coli*) strains were used to investigate the flocculation property of L-NPs and L-NPs-gelatin complex. It was found that L-NPs-gelatin complex exhibited relatively better flocculation capacity than L-NPs, in which the flocculation efficiency was greatly affected by pH and dosage. For both indicator strains, flocculation efficiency (> 95%) was achieved within 30 min at pH 4.5, while the flocculation efficiency of 90% costed 60 min at pH 5. The L-NPs-gelatin complex was promising to be applied for flocculating bacteria in wastewater treatment, enrichment and detection of microorganism, etc.

1. Introduction

Switchgrass (*Panicum virgatum* L.) with good tolerance to heat, cold and drought is a perennial C4 grass and considered to be a valuable feedstock for value-added products in term of its low agricultural input requirements and positive environmental impacts [1]. It is estimated that the productivity of switchgrass can reach about 14 tons per acre [2], which enable stable and sustainable supply of raw lignocellulosic material for bioethanol or other high-value biorefinery products. The main components of switchgrass are cellulose (32%–38%),

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Scheme 1. Schematic illustration of synthesizing the L-NPs by the ultrasonic-assisted alkali method.

hemicellulose (26%–32%) and lignin (17%-18%) [3,4], among which cellulose and hemicellulose can be hydrolyzed into fermentable sugars via various pretreatment methods [5]. However, the lignin is hard to depolymerize because of its aromatic polymer characterization, which hinders its further utilization for value-added products. It was estimated that only 5% lignin is developed for high-value products, while the rest is burned as fuel [6]. Waste of resources and environmental negative effects cannot be ignored. Hence, some researchers try to modify lignin by physical, chemical and biological methods [7,8], after which some modified lignin-derived products have been utilized in a few industries [9,10].

Nanotechnology provides new solutions for lignin utilization [6,11,12]. When lignin or lignin based materials are converted into nanostructure, both the specific surface area and surface active sites will increase, leading to the improvement of some properties, such as solubility, antioxidant activity and UV protection activity [13,14]. Recently, various methods were used to prepare lignin nanoparticle dispersions including self-assembly [11,15], ultrasonication [8,16], dialysis [6], precipitation [14,17], enzymatic degradation [12], hydroxymethylation [18,19]. Among these approaches, ultrasonication is usually considered as a prior choice, due to its ease of operation, low requirements for equipment and harmlessness to environment. Recently L-NPs have been used for some applications, such as biocide, drug delivery vehicles and UV protectants [14,20,21]. However, there was no report on the studies of L-NPs as microbes flocculants so far.

Most of the wastewaters, especially food processing wastewater contain microbes. These microbes usually include various pathogens that give rotten water which is harmful to environment and human safety. Hence, it is necessary to remove these bacteria. Bacterial cells are usually stable in wastewater because of electrostatic repulsion result from negative charges they carried [22]. Flocculation is always considered to be a convenient method to aggregate, settle and finally remove bacteria [23,24]. However, only few studies were done in which the acceptable bacteria removal was achieved with flocculants [25,26]. Flocculants mainly include inorganic coagulants, synthetic organic polymer flocculants and natural polymeric flocculants. Compared with other flocculants, natural organic flocculants are inexpensive and biodegradable [24]. As a natural material, L-NPs also have these advantages when it is used as flocculant. Moreover, L-NPs can be assembled with other materials to constitute complex to further improve the flocculation capacity. Gelatin was chose in the study. There are two main reasons: (1) Gelatin is a water-soluble protein derived from collagen. It carried positive charges when pH was lower than its isoelectric point, which endows the potential application in bacteria removing. (2) As a natural material, gelatin is usually obtained from animal skin, bones, and other tissues [27]. The resource is abundant and biodegradable. In addition, the degraded floc is benign to the environment. It will not produce secondary pollution. L-NPs was assembled with gelatin. L-NPs was lyophobic, when gelatin was added into L-NPs suspension, it will form complex with larger size. The complex flocculated with bacterial cells will be easily removed from the water.

In this work, lignin of switchgrass was fabricated into nanoparticles by ultrasonic-assisted alkali method, following with the gelatin combination to form L-NPs-gelatin complex. The structure and physiochemical properties of L-NPs and the L-NPs-gelatin complex were investigated by TEM, SEM, DLS zeta potential and FTIR spectroscopy. The flocculation properties of these materials were also investigated with different dosage, pH value, initial cell concentration and settling time, in which the *Escherichia coil* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) were used as indicator strains.

2. Materials and methods

2.1. Materials

Switchgrass (SG) was kindly donated by Professor Yongqing Ma at the Institute of Soil and Water Conservation, Northwest A&F University. The raw material was comminuted and sieved (100 mesh pass), after which it was dewaxed with benzene-ethanol (2:1 v/v) in a Soxhlet apparatus for 8 h. Lignin used in this study was isolated according to the method of National Renewable Energy Laboratory (NREL) [28]. Gelatin was purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Preparation of L-NPs and L-NPs-gelatin complex

The ultrasonic-assisted alkali method was used in preparation of lignin nanopartciles (L-NPs), which was similar with Glica's method [8] with some modifications. 0.21 g lignin was suspended in 30 mL deionized water, after which 900 μL 0.1 M NaOH was added. The lignin suspension was sonicated for 60 min at 400 W (Scheme 1). Thereafter, the suspension was centrifuged and the supernatant was reserved for dialysis. Finally, a stable nanodispersion was obtained. Samples were freeze dried for subsequent FTIR studies.

The dispersions of L-NPs-gelatin complexes were prepared by direct mixing the gelatin solution (1.0 mg mL^{-1}) with the L-NPs suspension (1.0 mg mL^{-1}) [29]. Solutions of gelatin were injected into L-NPs