Two kinds of behavior of fruit peel coagulant in treating low carbon source wastewaters

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ABSTRACT
Based on present serious phenomenon of insufficient carbon source in municipal sewage in China, the two kinds of effect of hybrid-fruit-peel (HFP) coagulant (coagulation and external carbon source (ECS)) on treating low carbon source wastewater was studied, mainly compared with that of sodium acetate (NaAc). The feasibility of HFP as an ECS was researched, and then its coagulation behavior, sludge properties, nitrogen removal were studied mainly with aeration mode and sequencing batch reactor (SBR), respectively. The results showed the ration of biochemical oxygen demand (BOD5) to chemical oxygen demand (COD) released from HFP was 0.67−0.76, within the scope of easily biodegradable. Within a short aeration time, HFP posed obvious coagulation behavior. HFP sludge gave larger extracellular polymer (EPS) and lower dehydrogenase activity (DHA) than NaAc. For HFP, much larger size sludge flocs appeared, the connection among the flocs became closer, and the biofacies and number of microorganisms (Opercularia Coaractata, Nematode, Vorticella, etc.) increased significantly. HFP can be used as an excellent ECS for low carbon source wastewater, and probably also have the ability of slow-releasing carbon, and can be applied to municipal sewage or other wastewaters with insufficient carbon source.

Keywords: Coagulation, External carbon source, Fruit peel, Sludge

Graphical Abstract

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1. Introduction

In recent years, nitrogen removal from water bodies is an urgent problem around the world with the rapid development of economy because nitrogen has adverse impact on environment (eutrophication phenomena) and human health (blue baby syndrome) [1-2], especially in China. Nitrogen removal is mainly conducted by biological denitrification process including two types of heterotrophic and autotrophic [2-4], in which heterotrophic denitrification is found the most common and effective process for nitrogen removal, because heterotrophic denitrification is characterized by having the advantages of high efficiency, low running cost, and more practical [3, 5]. However, organic carbon is indispensable substance for microbial growth for heterotrophic denitrification, directly impacting the effect of denitrification. Therefore, sufficient carbon sources are required to the biological denitrification process to complete successfully the denitrification heterotrophic denitrification [3, 6].

However, the phenomena of lower chemical oxygen demand (COD)/total nitrogen (TN) (C/TN or C/N) in municipal wastewater was very serious in China [7-9], in which the level of organic matter is often lower than 200 mg/L [1, 10, 11]. In addition, the discharge of saline wastewater (brine) from several industries (such as desalination, textile, oil and gas) degrades water quality and thus water cannot be directly used for potable water and industrial applications [12-15].

According to relevant reports from the Ministry of Housing and Urban-Rural Development of the People Republic of China, the biochemical oxygen demand (BOD5)/N of the inlet was often less than 4 in around 70% sewage treatment plants in China [11], resulting in higher nitrogen (N) level (> 15 mg/L) in tail waters in about 90% sewage treatment plants [16]. How to remove N efficiently from sewage is quite crucial for wastewater treatment because N discharge has many adverse effects on the environment and health, especially on eutrophication caused by over discharge of N and phosphorus (P) [1, 2]. Recently, with the increasing improvement of water environment quality requirements, some local sewage plants issued stricter effluent discharge standards, resulting in more and more serious issues in low carbon source for sewage plants. For instance, the TN of the effluent was required to be less than 10 mg/L [17, 18] in Beijing and Tianjin according to the local wastewater discharge standards. This puts forward more stringent requirements on the denitrification efficiency of sewage plants in some local district, thus resulting in the more prominent problem of low carbon sources. How to make the discharge of low carbon source sewage meet the discharging standard has become a study focus which needs to be urgently solved in wastewater treatment industries. Currently, the widely used methods include the optimization of influent modes [19, 20], the application of new nitrogen removal processes [21-25] and utilization of external carbon sources (ECS). New nitrogen removal processes mainly include shortcut nitrification-denitrification, Simultaneous nitrification denitrification (SND), Anaerobic ammonium oxidation (ANAMMOX), and Sharon-anammox combined process. Meanwhile, the utilization of ECS is still one of the methods extensively utilized until now [26-28].

Generally, there are two types of ECS for lower C/N ratio wastewaters: traditional type and new type. Traditional ECS is generally composed of low molecular organic substances with large production, including alcohols, acids, carbohydrates, and mixtures of them [3, 29, 30]. Traditional ECS is also easily utilized by microorganisms, but also give obvious disadvantages when inappropriate dose (e.g. Methanol [2, 31]) was conducted or others, such as existence of biological toxicity, higher cost for transportation, highly flammable, or exceeding the standard of organic carbon in the effluent [32-34], etc. New type of ECS, including solid, liquid and gas, has attracted more and more attention for many years. Solid type includes biodegradable polymers [35-42], natural materials [40-43] and mixtures of them [3, 34, 44-46]. Liquid type [2, 41, 42, 47-50] refers to hydrolyzate or leachate of wastes containing organic matters, and industrial wastewaters, probably having toxic substances due to very complex composition, so needing to be further studied in application [51, 52]. Presently, a large number of relevant studies on solid ECS have been carried out for agricultural and forestry wastes (AFWs) [3, 46, 53-57]. However, most research focused on direct utilization of ECS derived from AFWs or other types [3, 33, 47, 58, 59] in treating lower carbon wastewater, but multi-function of them was rarely studied. Fruit peel coagulant having a wide range of raw materials is one kind of natural polymer coagulants which was developed from one kind of AFWs, that is, fruit peel. Fruit peel coagulant prepared from agricultural wastes can be wildly used in the field of water and wastewater treatment due to lower cost. Recently, the peel of banana, orange, grapefruit, etc [60-63] have been used to prepare coagulants and absorbents. These fruit peels are basically composed of pectin, cellulose, hemicellulose and lignin, etc (saccharide content almost reaches more than 70.61% in them), and also carry many active functional groups (such as carboxyl and hydroxyl) which are conducive to adsorption and coagulation for pollutants. The peel accounts for 30%–50% of the total weight in the fruits. Most of the fruit peel has been discarded to the environment, in which organic matters have also been wasted. However, as a water treatment agent, fruit peel coagulants give a great ECS potential for low carbon wastewater due to the large amount of easily degradable organic matters in it. But rare studies were conducted in this aspect up to now.

In this work, a hybrid-fruit-peel coagulant (HFP) (derived from fruit peels of banana and orange containing large amount of organic carbon [60]) was used as an ECS for low C/N wastewater, in which its coagulation behavior and ECS function were studied simultaneously. In addition, sodium acetate (NaAc) was used as a compared ECS due the following reasons: (1) NaAc is a widely used and excellent ECS for lower carbon wastewater treatment; (2) whether HFP can be used as an effective ECS is needed to be compared with other efficient ECS due to HFP is a new type of ECS.

Firstly, HFP was prepared according the previous research [60], and the raw sludge was acclimated. Secondly, the feasibility of HFP used as an ECS was assessed. Thirdly, HFP coagulation behavior was studied, in which the sludge settling properties and the influence of HFP on activity and micro-characteristics of
sludge were researched with aeration method and sequencing batch reactor (SBR). Lastly, nitrogen removal using HFP as an ECS with SBR in treating a real wastewater with lower C/N was studied.

This work is expected to fully exert the both coagulation and ECS function of HFP in treating lower C/N wastewaters. In addition, the fertilizer made from the sludge can also be used in farmland, further promoting fruit growth, thus forming a closed loop including efficient agricultural, solid waste disposal and wastewater treatment. Therefore, this work significantly reduces cost, and also achieved a closed chain cycle of carbon source, which is an important development direction of ECS in the future.

2. Materials and Methods

The flowchart of the experimental process in this research is displayed in Supplementary materials (Figure 1).

2.1. Preparation of HFP [60]

2.1.1. The preparation of peel powders of banana and orange

Some dry peels of banana (planted in Hainan province, China) and orange (planted in Guangdong province, China) were obtained respectively after natural drying 2 days at room temperature and then further dried in oven at 70 °C for 24 h using GZX-9140MBE Electric Heat Forced Air Drying Oven (Shanghai Boxun, China). The dry peels of banana and orange were smashed with CS-700 High Speed Multi-function Pulverizer (COSUAL/Chaoshuai, Zhejiang, China) to make peel powders of banana and orange with different particle size almost within diameter of 40 mm.

2.1.2. The preparation of HFP

One part of banana peel powder was mixed with three parts of orange peel powder to obtain 125 g mixed powder, then the mixed powder was added to 5 L sodium hydroxide solution (0.04 mol/L, industrial grade, Tianjin, China) under medium stirring for 2–3 min at room temperature, and was followed by 60°C heating for 10 min to obtain a leaching solution. The leaching solution was filtrated with qualitative filter paper (Tianjin, China) to obtain HFP coagulant with pH 10.4, density 1.102×10^3 kg/m, α(Saccharides) 70.3%, and α(Mixed powder) 25 g/L, respectively. HFP was sealed with adhesive tape and stored at 5°C before use.

2.2. Water Samples

The following four water samples were used in the following sections: pure water (i.e. deionized water), simulated water, real wastewater and tap water. The simulated water (24 L) contains glucose (12, 9.6, 7.2 or 4.8 g for different influent containing different amount of carbon source), ammonium chloride (4.1 g), potassium dihydrogen phosphate (1.055 g), magnesium sulfate heptahydrate (1.44 g), calcium chloride (0.48 g) and soil leaching solution (30 mL). The soil leaching solution was prepared as follows: 5 kg soil derived from the campus at University of Jinan was mixed with 15 L tap water under stirring 2–5 min, followed by 10–15 min precipitation, and then was filtered with ordinary gauze to obtain the filtrate as the soil leaching solution which was stirred for 1–2 min before usage. The real wastewater was obtained from the hydrolytic tank of wastewater plant located on the campus at University of Jinan, which was thoroughly stirred before use. The qualities of the four water samples were as follows. The pure water: turbidity 0.1–0.2 NTU, temperature 26–26.4 °C, pH 6.7–7.2. The simulated water: turbidity 1.83–2.12 NTU, color 0.011–0.015 AU, temperature 26–26.6 °C, COD 198.2–208.7 mg/L, pH 7.4–8.1, NH3-N and TN 51–56.2 and 51.2–57.7 mg/L. The real wastewater: turbidity 44.3–58 NTU, color 0.205–0.28 AU, temperature 26.5–26.7 °C, pH 7.4–8.1, COD 266–306 mg/L, NH3-N and TN 57–66 and 67–72 mg/L. The tap water: turbidity 1.66–1.75 NTU, color 0.009–0.01 AU, temperature 26.2–26.5 °C and pH 7.2.

2.3. Raw Sludge and Its Acclimation

The raw sludge was obtained from the secondary sedimentation tank in the No.2 plant with Anaerobic-Anoxic-Oxic (A2O) of Everbright Water Co. Ltd. (Jinan, China) with the following qualities: temperature=18.8°C, pH=7.23, sludge settling ratio after settling 30 min (SV30)=28 mg/L, and mixed liquor suspended solids (MLSS)=5138 mg/L. The simulated water in Section 2.2 was used as the acclimation water sample for the sludge, with the following acclimation conditions of temperature 25+5°C and pH 7–8, and the following four stages. (1) Initial stage (1 d). 50 L raw sludge was added to 20 L tap water and was then aerated with SB-9488 aeration pump (Shanghai, China) for 24 h (Aeration rate 64 L/h). (2) Stage 2 (7 d). The stage included aerobic, anoxic and settling and drawing, in which fast filling (Influent qualities: C:N:P=50:5:1, COD=500 mg/L, ammonia-nitrogen (NH3-N)=50 mg/L, total phosphorus (TP)=10 mg/L) and drawing were conducted, with the amount of both inflow and outflow of 24 L. One running period was 12 h including aerobic time 8 h with initial aeration rate of 64 L/h, anoxic time 2 h with stirring rate of 90–100 r/min, and settling time of 2 h. The aeration rate was continuously adjusted by measuring the dissolved oxygen (DO) of the effluent to make DO stay stable to be 2.5–3.5 mg/L, and finally the aerator was changed to the rate of 17 L/h and the MLSS reached 4000–4500 mg/L, respectively. (3) Adjustment stage (22–25 d). The C/TN of the inflow was adjusted to reach 4/1 (i.e. COD reduced to 200 mg/L) by reducing COD at the rate of 100 (mg/L)/(2d), and then COD, NH3-N and MLSS of the influent and effluent were measured, respectively. The sludge after acclimation was called acclimation sludge with the properties: temperature=25.7°C, pH=7.5, NH3-N=19.5 mg/L, COD=25.6 mg/L, SVI=34 mg/L, and MLSS=9427 mg/L. COD and NH3-N were measured with Potassium dichromate method and Nessler’s reagent colorimetric method (UV-5800 UV spectrophotometer, Shanghai Yuanxi), respectively, and MLSS was measured with gravimetric method.
2.4. Feasibility Study of HFP as an ECS

The pure water in Section 2.2 was used as water sample and sodium acetate (NaAc) was used as the compared ECS. The total organic carbon (TOC) was used in HFP and its releasing carbon capacity was studied, respectively. The TOC of HFP was measured first, and then the ultraviolet absorbance at 254 nm (UV$_{254}$), COD, NH$_3$-N and BOD$_5$ released from HFP in the pure water were measured respectively, compared with that of NaAc, in which the dosage of HFP and NaAc was based on the TOC level of measured respectively, compared with that of NaAc, in which after aeration 0, 1, 2, 3 and 4 h were filtered with 0.45 μm membranes to obtain the filtrates for UV$_{254}$ analysis with UV-5800 UV spectrophotometer. COD and NH$_3$-N of some mixtures after aeration 0, 1, 2, 3 and 4 h were measured with the same methods as Section 2.3. The results referred to the mean of the measurement values of the top and bottom samples.

2.4.1. Determination of TOC in HFP

The TOC of HFP was measured with Total Organic Carbon Analyzer (MultiN/C3100, Germany analytikjena AG). Three runs were performed in this test, and the results represented the averages.

2.4.2. Determination of UV$_{254}$, COD, NH$_3$-N and BOD$_5$ released from HFP

NaAc was used as the compared ECS with working concentration of 5 g/L based on TOC level.

Four 1-L beakers having 1 L pure water were used as the reactors for this experiment. Based on the TOC level of HFP obtained in Section 2.4.1, dosages of 0.045 and 0.181 g/L HFP (as TOC) was added to the first two beakers, respectively, marked with No. 1 and No. 2, respectively. Correspondingly, dosages of 0.045 and 0.181 g/L NaAc (as TOC) were added to the other two beakers marked with No. 3 and No. 4, respectively. The four beakers were stirred for 2–5 min firstly and then were aerated with aeration rate 30 L/h to obtain the four mixtures. After different aeration time (from 0 to 4 h), the four mixtures were taken out from the top and bottom of the beakers for the following measurement, respectively.

(1) Measurement of UV$_{254}$, COD, and NH$_3$-N. Some mixtures after aeration 0, 1, 2, 3 and 4 h were filtered with 0.45 μm membranes to obtain the filtrates for UV$_{254}$ analysis with UV-5800 UV spectrophotometer. COD and NH$_3$-N of some mixtures after aeration 0, 1, 2, 3 and 4 h were measured with the same methods as Section 2.3. The results referred to the mean of the measurement values of the top and bottom samples.

(2) Measurement of BOD$_5$. Some acclimation sludge in Section 2.3 was settled for 15 min to obtain the supernatant which was taken out as the microbial solution. Some mixtures after aeration 1 and 4 h were mixed with the microbial solution, respectively, then followed by measurement with BODTrak®RII BOD analyzer (Hach, USA) according to its instructions.

Three runs were performed for UV$_{254}$, NH$_3$-N and BOD$_5$, and the results represented the averages of the tests, and the error bars for all the data points represented the standard error of the mean of the three experiments.

2.5. Coagulation Behavior of HFP as an ECS

Acclimation sludge of 9 L in Section 2.3 was allowed to settle 60 min, and the precipitate was obtained after removing the supernatant. The precipitate was stirred for 3 min as the test sludge, simply called “sludge” in the following Sections. 100 mL sludge was added to a 100-mL measuring cylinder and was allowed to settle 30 min and “initial MLSS of sludge” was measured.

The water sample was the simulated water, real wastewater and tap water in Section 2.2. According to the results in Section 2.4 and lots of previous results, the dosage of HFP was selected as 0.09 g/L, and the same dosage of NaAc was used as the compared ECS.

Four 1-L beakers were used as the reactors for this experiment. First, 900 mL water sample and 100 mL sludge were added to each beaker, and then HFP was added to No. 1 and No. 2 beakers, and NaAc was added to No. 3 and No. 4 beakers, respectively. No. 1 and No. 2 beakers were aerated for 5 min and No. 3 and No. 4 for 180 min. After aeration, 100 mL mixtures were taken out to be introduced to 100-mL measuring cylinders to be allowed to precipitate 10, 20 and 30 min continuously, and the turbidity of the supernatants were measured with 2100AN Turbidimeter (Hach, USA) and MLSS and sludge settling ratio (SV) were also measured, in which the sludge volume index (SVI) was finally calculated according to Eq. (1).

\[ SVI = \frac{SV}{MLSS} \]  

Three runs were performed for turbidity, and the results represented the averages of the tests, and the error bars for all the data points represented the standard error of the mean of the three experiments.

2.6. Influence of HFP on Activity and Micro-characteristics of Sludge

NaAc was used as the compared ECS, and the simulated water in Section 2.2 was used as the water sample. Three conventional SBR (diameter=15 cm and height=26 cm) [64, 65] with effective volume of each reactor 1.8 L was used as the bioreactors in this experiment, in which the filling, reaction, settling, and drawing phases occur sequentially. The total running time was 144 h, in which 4 h was one running period including four stages of filling, reaction, settling, and drawing with their time of 3, 175, 55 and 3 min, respectively. The inflowing and outflowing volume were all 0.6 L, namely the hydraulic retention time (HRT) was 12 h. The dosages of HFP and NaAc were all 0.181 g/L, and the “initial MLSS of sludge” was 3000 mg/L. The effluent and surplus sludge was taken out every 12 h.

2.6.1. Dehydrogenase activity (DHA) and extracellular polymer (EPS)

After the SBR runs, 100 mL surplus sludge was taken out and was then allowed to settle 30 min. After drawing out the supernatant, the precipitate was obtained, and the precipitate was rinsed three times with pure water to obtain the clean precipitate for the measurement of DHA, Protein (PN) and Polysaccharide (PS). The DHA was determined by TTC-reduction method (at wavelength 485 nm), and the PN and PS were determined by Coomash brilliant blue colometry (at wavelength 595 nm) and Anthrone-sulfuric acid method (at wavelength 620 nm) with
UV-5800 Ultraviolet spectrophotometer (Shanghai, China), respectively, in which the EPS was calculated from PN plus PS. Three runs were performed for DHA and EPS, and the results represented the averages of the tests, and the error bars for all the data points represented the standard error of the mean of the three experiments.

2.6.2. Micrographs and biofacies
A little precipitate in Section 2.6.1 was taken out and placed onto the surface of glass slides, covered with cover glasses slowly, and then was observed and photographed with OLYMPUS CX31 optical microscope (Beijing Cnrico Technology Co., LTD, China).

2.6.3. Surface morphology
The surface morphology of the clean precipitate in Section 2.6.1 was analyzed with Evols 15 Scanning Electron Microscope (Zeiss, Germany).

2.7. Application of HFP in a Real Wastewater
The experiment was the same as Section 2.6, in which NaAc was also used as the compared ECS, and the real wastewater in Section 2.2 was used as the water sample. A certain amount of SBR effluent was taken out every running 12 h for the measurement of NH₃-N and TN by Nessler's reagent colorimetric method.

Fig. 1. Influence of aeration time on releasing ability of (a) UV₂₅₄, (b) BOD₅, (c) NH₃-N and C/NH₃, (d) B/C and (e) C/T from HFP and NaAc. C/NH₃, B/C and C/T indicate COD/NH₃-N, BOD₅/COD and COD/TOC. HFP: hybrid-fruit-peel coagulant. NaAc: sodium acetate.
and Alkaline potassium persilage digestion-UV spectrophotometric method (UV-5800 UV spectrophotometer, Shanghai Yuanli), respectively. The dosage of HFP and NaAc was all selected as 0.181 g/L.

Three runs were performed in this test, and the results represented the averages of the tests, and the error bars for all the data points represented the standard error of the mean of the three experiments.

3. Results and Discussion

3.1. Feasibility Study of HFP as an ECS

NaAc was used as the compared ECS to study the carbon releasing capacity of HFP. The result showed the average TOC content in HFP was 4.5 g/L. Fig. 1(a)-Fig. 1(e) display the releasing ability of UV254, BOD5, NH3-N and COD/NH3-N (C/NH3), BOD5/COD (B/C), and COD/TOC (C/T) from HFP, respectively.

As seen in Fig. 1(a) and Fig. 1(b), the carbon releasing level of HFP was almost the same as that of NaAc, in which their carbon releasing processes were almost completed immediately after they were added to the water sample. As also seen from Fig. 1(a), UV254 released by HFP was much higher than that by NaAc. UV254 mainly consisting of natural and easily degradable aromatic compounds containing C=C bond and C=O bond, was a kind of dissolved organic matters that can be utilized by certain microorganisms. Therefore, as an ECS, HFP probably promoted the proliferation of diverse microorganisms, in comparison with NaAc. This will have a positive effect on the adaptability of HFP to quality changes in wastewaters, making its application range more flexible than other type of traditional ECS.

As shown in Fig. 1(b), BOD5 released from HFP at the initial aeration state (aeration 0 h) and 4 h aeration was all higher than that from NaAc at different dosages. When the dosages were 0.045 and 0.181 g/L, BOD5 from HFP at 0 h aeration was higher 8 and 33 mg/L than that from NaAc, respectively, and was higher 14 and 34 mg/L than those from NaAc at 4 h aeration, respectively, indicating that the releasing capacity of biodegradable organic carbon from HFP was higher than that from NaAc with the increasing of aeration time. HFP was derived from banana and orange peels which are composed of protein, cellulose, lipids, polysaccharides, etc. [60], in which the cellulose is a macromolecule polysaccharide composed of glucose. The short-chain glucose can be easily utilized rapidly by microorganisms, while other polysaccharide requires some conversion reactions and longer time to be utilized, so HFP had a slow-releasing ability of biodegradable carbon, which was easily used by diverse microorganisms. Of course, the possibility of sludge bulking should also be considered to some extent.

HFP contains nitrogen substances such as protein, so only nitrogen released from HFP was displayed in Fig. 1(c). As seen in Fig. 1(c), NH3-N releasing level increased slightly with the increasing of aeration time within aeration 3 h when dosage was 0.045 g/L. When dosage was up to 0.181 g/L, NH3-N releasing level decreased slightly with the increasing of aeration time, basically tending to be constant at aeration 3 h, in which the C/NH3 releasing level ranged from 39.8 to 66.5. According to some literatures [26, 66-68], C/NH3 is needed to be larger than 2.86 for N removal in theory and more than 6 is generally suggested to be used in practice, so a little higher C/NH3 released from HFP almost gave little adverse impact on wastewater treatment process. Therefore, as an ECS, HFP maybe have greater efficiency and good practical significance.

As inferred from Fig. 1(d), COD released from HFP was higher than that from NaAc. At different dosages, B/C from HFP at initial state (0 h aeration) and 4 h aeration were all slightly lower than that from NaAc: B/C from HFP was 0.67−0.76, compared with 0.71−0.93 from NaAc, all greater than 0.45; both were basically within easy biodegradation scope and gave excellent biodegradability. As also shown in Fig. 1(d), the biodegradability of both HFP and NaAc decreased slightly with the increasing of dosage, moreover, the decreasing trend increased significantly with the increasing of aeration time, but the decreasing trend of HFP was less than that of NaAc. For instance, when the aeration time was 0 h, the difference of B/C from HFP between dosages 0.045 and 0.181 g/L was 0.01, in comparison with 0.09 from NaAc, but when the aeration time was up to 4 h, the difference from HFP was 0.09, compared with 0.21 from NaAc. The above results indicated that HFP can probably adapt to a wider range of water qualities or treating conditions.

According to the literature result [69], the biochemical process was relatively easier to occur in wastewaters with C/T lower than 2.67, but there have more substances which were not easily degraded by microorganisms when C/T was larger than 2.67. As shown in Fig. 1(e), C/T releasing from HFP was slightly higher than that from NaAc under different aeration time at different dosages, but both were lower than 2.67, indicating that HFP was composed of more biodegradable organic matters [68].

In a word, HFP can be used as an excellent ECS, probably suitable to a wider range of water qualities by its characteristics of slow-releasing capacity of biodegradable carbon.

3.2. Coagulation Behavior of HFP as an ECS

According to the previous experiment results, HFP dosage was selected as 0.09 g/L, the aeration time was set to 5 and 180 min, respectively. The simulated water, tap water and real wastewater in Section 2.2 were used as the water sample. The influence of settling time on supernatant turbidity and aeration time on sludge sedimentation performance was studied firstly, and then some quantitative analysis of coagulation behavior of HFP was conducted.

3.2.1 Influence of settling time on supernatant turbidity

The settling time was selected as 5, 20 and 30 min, respectively. The turbidity in the following Sections all refers to the residual turbidity of the supernatant.

As seen in Fig. 2(a) and Fig. 2(b), the turbidity of HFP for the simulated water (Fig. 2(a)) was basically similar to that for the tap water (Fig. 2(b)), moreover, the longer the settling time was, the higher the similarity was. The simulated water and tap water were all transparent (their turbidity was lower than 2 NTU), so HFP only gave coagulation action for the sludge, thus leading to a similar turbidity in their supernatant.
When the aeration time was 5 min, the turbidity of HFP was slightly higher than that of NaAc at different settling times, indicating that HFP maybe exerted coagulation function and simultaneously produced some flocs that were not easily to be settled. As for the influence of the coagulation behavior on sludge activity, it will be further studied in the following sections.

When the aeration time was 180 min, the turbidity of HFP was slightly lower than that of NaAc or almost the same, also giving lower turbidity than that at 5 min, indicating HFP as an ECS generated a certain function for sludge proliferation with the increasing of aeration time (like NaAc). Meanwhile, the coagulation behavior of HFP promoted both agglomeration of suspended particles and settlement of flocs, thus enhancing the sedimentation performance of sludge and decreased the turbidity of the supernatant as well.

For the real wastewater (Fig. 2(c)) with slightly higher turbidity (46.2 NTU), when the aeration and settling time were all 5 min, the turbidity of HFP was similar to that of NaAc, both giving higher turbidity than that in the raw real wastewater. However, the turbidity of HFP was lower than that of NaAc with the increasing of settling time, further demonstrating that HFP did exert an obvious coagulation function and produced coagulation effect on both sewage and sludge simultaneously. Moreover, HFP coagulation behavior promoted the sedimentation of flocs, that is, promoted the decrease of SS and enhanced the sludge settling properties. When the aeration time was 180 min, the turbidity of NaAc at settling 5 min was slightly lower than that of HFP, but the turbidity of NaAc was significantly higher than that of HFP with the increasing of settling time, suggesting that NaAc probably gave some adverse effect on the removal of SS with the increasing of settling time, further increasing the difficulty in the subsequent sludge treatment and dispose. In addition, it can be further seen from the turbidity difference of HFP between aeration 5 and 180 min that all the coagulation process of HFP was basically completed within aeration 5 min. Therefore, it can be considered that HFP as an ECS can give obvious coagulation behavior in the initial stage of aeration.

3.2.2. Influence of aeration time on sludge settling properties

Fig. 3 shows the comparison of the impact of aeration time on sludge settling properties after adding HFP and NaAc to different water samples.

As seen in Fig. 3(a), for NaAc, when the aeration and settling time were all 5 min, NaAc basically gave the same "sludge settling performance" (SSP) for the three water samples, almost completing the settling
process within 5 min. NaAc had no coagulation behavior, therefore, the SSP of NaAc aerated for 5 min was basically the same as that of sludge. When the aeration time was 180 min, NaAc almost gave the same SSP for both simulated water and tap water, but different from that for the real wastewater (There appeared to be some flocs formed by NaAc in the real wastewater that gave different settling performance from the flocs formed in the sludge). Based on the above analysis, the following conclusions can be drawn. (1) When NaAc was used as an ECS, there was almost no self-proliferation of microorganisms at aeration 5 min. (2) NaAc had no coagulation function, so the proliferation of sludge formed by NaAc mainly derived from the self-proliferation of microorganisms. (3) When the aeration time was 180 min, there appeared to be some microbial proliferation using NaAc as the ECS, resulting in the increasing of flocs amount and the changes in SSP, but the increment of microbial biomass was not large. (4) Based on the conclusions from (1) to (3), it can be inferred that HFP can't generate self-proliferation of microorganisms at aeration 5 min, so it can be judged that HFP exerted coagulation function according to the volume increase of flocs.

As seen in Fig. 3(a), the SSP of HFP in the simulated water aerated for 5 min was also basically the same as that in the tap water but having greater SV in the three water samples than that of NaAc. Moreover, both HFP and NaAc all gave slighter difference of SSP between the simulated or tap water and the real wastewater at aeration 5 min. Based on the conclusions above, it can be inferred that HFP gave obvious coagulation behavior and produced much more sludge flocs (giving different settling performance from NaAc) at aeration 5 min. When the aeration time was 180 min, HFP also appeared to have self-proliferation of microorganisms in addition to the coagulation reaction, thus leading to some difference of its SSP from that at aeration 5 min to a certain degree.

The SSP of HFP in the real wastewater was some different from that of the simulated water and tap water with the increasing of settling time at different aeration time. When the aeration time was 5 min, the difference is small, moreover, the difference became less and less with the increasing of settling time, gradually tending to be the same. When the aeration time was 180 min, the difference became greater, because HFP not only gave coagulation behavior for both sewage and sludge in the real wastewater, but also produced some self-proliferation of microorganisms. As also seen from Fig. 3(a), the SV of HFP at settling 30 min was much less than that of 5 min, indicating that HFP produced a large amount of flocs giving larger sed-

![Image](image-url)

**Fig. 3.** Comparison of performance index of sludge expressed in (a) SV, (b) MLSS and (c) SVI between HFP and NaAc after addition to different water samples at different aeration time. Dosage=0.09 g/L. HFP: hybrid-fruit-peel coagulant. NaAc: sodium acetate. HFP-5 and NaAc-5, HFP-180 and NaAc-180 indicate aeration 5 and 180 min after addition of HFP and NaAc. "Simulated", "Tap" and "Real" indicate "Simulated water", "Tap water" and "Real wastewater", respectively.
The calculated results were listed in Supplementary materials (Table 1) (all calculation was based on MLSS). The known amount was as follows: initial sludge=1014 mg/L, NaAc (existing in the form of ions)=0 mg/L, and HFP=0 mg/L, respectively. Based on the above conclusions (i.e., HFP completed coagulation within aeration 5 min), the increments of 62 and 73 mg/L can be considered as the sludge increments generated by microbial proliferation at aeration 180 min. The increment of sludge generated by coagulation was basically the same for aeration 180 and 5 min (Supplementary materials (Table 1)), further demonstrating HFP almost completed the whole coagulation process within aeration 5 min, and then fully exerted ECS function with the increasing of aeration time.

The increment of the sludge generated by HFP's coagulation in the real wastewater was larger than that in the simulated water (Supplementary materials (Table 1)), further demonstrating HFP gave coagulation for both sewage and sludge in the real wastewater, which was conductive to the removal of SS.

For HFP (Fig. 3(c)), the following results were obtained. (1) SVI of other situations were all greater than 100 mL/g except for the real wastewater aerated for 180 min, suggesting the sludge generated by coagulation at the initial aeration stage led to a slight decrease in SSP. (2) The SVI of the real wastewater decreased to 91.8 mL/g after aeration 180 min, demonstrating the adverse effect of sludge generated by coagulation on SSP decreased rapidly with the increasing of aeration time. Moreover, compared with the flocs derived from the coagulation, the flocs produced by microbial proliferation probably had greater settling rate. (3) For both simulated and tap water (HFP only had coagulation behavior for sludge), the SVI difference between aeration 180 and aeration 5 min was very small, suggesting the settling performance of the sludge generated by HFP's coagulation for sludge was basically not affected by aeration time, however, as for the effect of longer aeration time, needing to be further studied. While for NaAc, SVI were all less than 80 mL/g. Since NaAc had no coagulation behavior, the sludge generated within aeration 5 min was almost the same as the acclimation sludge (the amount of microbial proliferation could be ignored), demonstrating the acclimation sludge had good settling performance. When the aeration time was 180 min, the SVI of NaAc increased slightly, but the increasing was lower, further proving the previous conclusion (3): the microbial proliferation was also not great at aeration 180 min.
As also shown in Fig. 3(c), the difference of settling performance of the sludge generated by HFP in the three water samples was much greater than that by NaAc, moreover, the difference between aeration 5 and 180 min was also greater than that by NaAc, indicating HFP posed different action from NaAc and the complexity of these actions was also different from different water samples, further proving HFP as an ECS posed obvious coagulation performance.

3.3. Influence of HFP on Activity and Micro-characteristics of Sludge

In the following Sections, “S”, “S+T”, “S+T+HFP” and “S+T+NaAc” refer to the following systems: “Sludge”, “Sludge+Test water”, “Sludge+Test water +HFP” and “Sludge+Test water +NaAc”, respectively.
Test water” (Sludge was added to the water samples), “Sludge+Test water +HFP” (Sludge and HFP were added to the water samples) and “Sludge+Test water +NaAc” (Sludge and NaAc were added to the water samples).

3.3.1. Influence of HFP on DHA and EPS

Fig. 4 shows the comparison of EPS and DHA between HFP and NaAc used as ECS in the above four test systems. EPS [70] mainly includes PN and PS. As shown in Fig. 4(a) and Fig. 4(b), the EPS of the sludge in “S+T+HFP” was significantly higher than that in “S+T+NaAc”, while the DHA was slightly lower than that in “S+T+NaAc”, but both were much higher than that in “S” and “S+T”. Compared with “S”, the increasing rate of EPS in “S+T+HFP” and “S+T+NaAc” was 101.2% and 60.8%, respectively, while the promoting rate of DHA was 252% and 283%, respectively. According to the above difference trend of EPS increasing rate and DHA promoting rate between HFP and NaAc, it could be concluded that the coagulation behavior of HFP produced more EPS, but slightly decreasing the activity of sludge (probably caused by some semi-wrapping or semi-coverance of coagulation flocs on some active microorganisms), consistent with the above conclusions and the results in Fig. 1. Fig. 4 also shows that the difference of DHA between “S+T+HFP” and “S+T+NaAc” was relatively smaller, so HFP can be used as a high-quality ECS, having a possibility of slow-releasing carbon source as well.

3.3.2. Micrographs and biofacies

Fig. 5 shows the comparison of the micrographs and biofacies between the sludge (Fig. 5(a)) and the sludge in “S+T”, “S+T+HFP” and “S+T+NaAc” at aeration 3 d (Fig. 5(b)) and 7 d (Fig. 5(c)), and biofacies (Fig. 5(d)) collected from the sludge in “S+T+HFP”, respectively.

As shown from Fig. 5(a), the flocs of sludge in scattered distribution have clear edge boundaries. After 3 and 7 d aeration, the color of the sludge flocs was slightly deepened and overlapped, but the number of the flocs was still small, the edge boundaries was still clearer, and the macroscopic size was relatively even and smaller.

As seen in Fig. 5(b) and Fig. 5(c), the size of the sludge flocs in “S+T+HFP” and “S+T+NaAc” increased significantly after aeration 3 d, and some semi-transparent feather-like structures appeared at the edge boundaries, which was inferred to be transparent mucous substances produced by microorganisms, in which the mucous substances in “S+T+HFP” were significantly more than those in “S+T+NaAc”. Moreover, the size of the sludge flocs in “S+T+HFP” was also larger than that in “S+T+NaAc”, further demonstrating that HFP coagulation process promoted the generation of some larger-size flocs. Compared with aeration 3 d, when the aeration time was up to 7 d, the size of the sludge in “S+T+HFP” and “S+T+NaAc” was significantly larger, the semi-transparent mucous substances increased significantly, the connection between the flocs became closer, and the biofacies type and number of microorganisms also increased significantly. By observation (Fig. 5(d)) under magnification 400 times, some very active organisms appeared obviously, such as Opercularia coaractata, Suctorida, Arcella, Nematode, Vorticella, Wheel animalcule, etc. In addition, as also seen in Fig. 5(d), the dispersion degree of the sludge flocs in “S+T+HFP” was greater than that in “S+T+NaAc” (A large number of flocs in “S+T+NaAc” appeared

![Fig. 6](image-url)
to be in clustered state), indicating that “S+T+HFP” gave larger surface area and amount of sludge flocs which will be conducive to contact directly to pollutants and to remove pollutants. However, the sludge flocs in “S+T+NaAc” posed better SSP than that in “S+T+HFP”.

In a word, HFP can be used an excellent ECS for low carbon source wastewaters.

3.3.3. Influence of HFP on surface morphology

To further testify the increment of microorganisms, and also to compare the microorganism proliferation and microstructure (micromorphology) between HFP and NaAc in different conditions, SEM images [71-73] were conducted in Fig. 6. Fig. 6 shows the comparison of the surface morphology of sludge when HFP and NaAc were used as ECS between the above four test systems in Section 3.3.

As seen in Fig. 6(a), the size and shape of the sludge in “S” were uniform and regular, basically appearing to be a kind of round or round-like structures, having clear boundaries and apparent independence with each other. Compared with “S”, the regularity of the size and shape of the sludge in “S+T” (Fig. 6(b)) was obviously decreased, appearing to be some larger-size strip structures (presumably a kind of filamentous bacteria), but the size of most round structures changed little. Moreover, there were lots of adhesions among various structures, leading to their closer connection. As stated above, this simulated water was transparent, so there had almost no turbidity in it except for some nutrients, so the changes in sludge structures was basically caused by the increment of microorganisms themselves.

Compared with “S” and “S+T”, for “S+T+HFP” (Fig. 6(c)) and “S+T+NaAc” (Fig. 6(d)), there were many diverse sludge structures and the regularity of the structures further reduced, in which the number of round structures decreased and other structures significantly increased. There appeared some larger round and some other structures with larger size (presumably to be some Clock-worms, Twig worms, and Metazoan rotifers, etc.) on the surface of the sludge in “S+T+HFP”. Moreover, there appeared to be many strip connection among the structures, similar to that in the literature [72]. Compared with “S”, “S+T” and “S+T+NaAc”, the sludge in “S+T+HFP” gave the most diverse surface structures. For the sludge in “S+T+NaAc”, the size of most round structures also changed little, but there were more round-cluster structures.

3.4. Application of HFP in the Real Wastewater

Fig. 8 displays the comparison of nitrogen removal using HFP and NaAc as the ECS in the real wastewater, in which SBR was also used. As shown in Fig. 8, compared with NaAc, HFP gave different NH3-N removal at different running time: NH3-N removal by HFP was higher than that by NaAc from 0 to 60 h and 108 to 144 h, and was almost the same to NaAc from 60 to 108 h.

Fig. 7. Schematic diagram of double function of coagulation and carbon source exerted by HFP after added to wastewater in comparison with one action of carbon source of NaAc. HFP: hybrid-fruit-peel coagulant. NaAc: sodium acetate. C: polymers having coagulation behavior. C: biodegradable carbon substances.
h. So it can be considered that the removal of NH$_3$-N by HFP was similar to that by NaAc. The removal of TN by HFP was slightly lower than that by NaAc, reducing to the lowest value of 16.5 and 11.1 mg/L respectively.

Fig. 8. Comparison of nitrogen removal from the real wastewater with SBR process between different test systems of “S+T”, “S+T+HFP” and “S+T+NaAc”. Dosage=0.181 g/L, running time =144 h and HRT =12 h. HFP: hybrid-fruit-peel coagulant. NaAc: sodium acetate. “S+T”, “S+T+HFP” and “S+T+NaAc” indicate the following systems: “Sludge+Test water”, “Sludge+Test water +HFP” and “Sludge+Test water +NaAc”, respectively.

The removal of NH$_3$-N and TN by both “S+T+HFP” and “S+T+NaAc” was all much higher than that in “S+T”.

4. Conclusions

HFP have both coagulation behavior and ECS function for lower C/N wastewaters. Compared with NaAc, HFP gave the following properties: HFP can release enough organic carbon in treating wastewaters by testing both total organic carbon (TOC) contained in it and the organics (such as UV$_{254}$, BOD$_5$, C/NH$_3$-N and B/C) released from it under aeration state; the released carbon was easily utilized by microorganisms; HFP may have strong adaptability to wastewater quality as an additional carbon source.

As an ECS, HFP exerted obvious coagulation function at aeration time of 5 min, thus reducing the effluent turbidity, further facilitating the removal of subsequent SS. HFP gave ECS action during the later aeration stage. Like NaAc, HFP prompted a certain amount of microorganism proliferation and increased the volume of flocs at aeration 180 min.

In the simulated water, the coagulation behavior of HFP produced more EPS but slightly decreased the activity of the sludge. With the increasing of aeration time, the size of the sludge flocs, the semi-transparent mucous produced by microorganisms, and the types and numbers of the microorganisms in both “S+T+HFP” and “S+T+NaAc” all increased significantly, and much active organisms appeared. Simultaneously, compared with “S+T+NaAc” (flocs almost was in a clustered state), the surface area, quantity and dispersion degree of sludge flocs were greater in “S+T+HFP”.

Like “S+T+NaAc”, “S+T+HFP” gave higher removal of NH$_3$-N and TN than “S+T”.

HFP derived from fruit peels can be used as an excellent, flexible and stable ECS for wastewaters, simultaneously, its "green sludge" which can be used as fertilizer in agriculture also provides a better idea for efficient agriculture, realizing closed cycle of carbon source between agriculture and wastewater treatment. As an ECS, HFP can be applied to municipal sewage with insufficient carbon source or low C/N, or to other situations of water quality decline due to other reasons (such as wastewater discharge from certain industries of textile, oil and gas). In the future, the following study will be conducted: the substantial impact of HFP coagulation behavior on both microbial types and subsequent wastewater treatment results; systematic experiments of HFP on both treatment effects and process conditions of the above-mentioned various types of wastewaters; further optimization and improvement of preparation scheme of HFP based on the qualities of various wastewaters.

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Author Contributions

Q.H. (Researcher) conducted the experiments. Y. (Professor) put forward the idea of the whole experiment, performed the analyses and wrote the manuscript. S.Y. assisted Q.H. to conduct some experiments. J.W., X. and Y.T. collated some data. All authors have read and agreed to the published version of the manuscript.

Conflict-of-interests

We declare that we have no conflict of interest. There's no any financial/personal interest or belief that could affect our objectivity. This work does not exist any potential competing interests.

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