Modified Alginate Beads with Ethanol Extraction of *Cratoxylum formosum* and *Polygonum odoratum* for Antibacterial Activities

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**ABSTRACT:** Bacteria contaminations in water are concerned as environmental effects including human health, so water treatment is required before use. Although using extracted plant is interesting because of their good chemical compounds for bacterial inhibitions, no study has applied the extracted plant in bead materials for disinfection in wastewater. The current research attempted to extract *Cratoxylum formosum* and *Polygonum odoratum* for the synthesis of CFB and POB, and their antibacterial efficiencies were investigated by agar diffusion tests, antibacterial batch tests, adsorption isotherm and kinetics, and material reusability. C. formosum and P. odoratum leaves were ethanol-extracted, and their bead materials (CFB and POB) were synthesized. Furthermore, their characterizations of surface area, chemical compositions, and chemical functional groups were investigated. For field emission scanning electron microscopy and focused ion beam (FESEM-FIB) analysis, CFB and POB had spherical shapes with coarse surfaces. Energy-dispersive X-ray spectrometry (EDX) analysis of CFB and POB illustrated five main chemical compositions, which were carbon (C), oxygen (O), calcium (Ca), chlorine (Cl), and sodium (Na), whereas Fourier transform infrared (FTIR) spectroscopy analysis identified seven main chemical functional groups, which were O\(\cdot\)H, C\(\cdot\)H, C\(\equiv\)O, C\(\equiv\)C, N\(\equiv\)H, C\(\cdot\)O, and C\(\cdot\)Cl. Agar diffusion tests confirmed the abilities of CFB and POB to inhibit both *Staphylococcus aureus* and *Escherichia coli*, and batch experiments examined high antibacterial efficiencies of CFB of almost 100% on both bacterial types. The adsorption isotherm of CFB corresponded to the Freundlich model, which is related to the physiochemical adsorption process with multilayer or heterogeneous adsorption, and the adsorption kinetics of CFB was correlated to the pseudo-second-order kinetic model, which involved chemisorption relating to physiochemical interaction. Moreover, the desorption experiment confirmed the reusability of CFB. Therefore, CFB is a potential material to possibly apply for disinfection of wastewater.

1. **INTRODUCTION**

Because of increasing water consumption through many human activities such as household, agriculture, industry, and transportation may create water pollutions including bacterial contaminations in water,\(^{1,2}\) so access to clean water is necessary for safe life. Especially, *Staphylococcus aureus* and *Escherichia coli* are commonly found in contaminated water\(^{3-5}\) as Gram-positive and Gram-negative bacteria, and *E. coli* is also a member of fecal coliform bacteria.\(^6\) As a result, they are concerned as a cause of diseases such as diarrhea, boils, dysentery, typhoid fever, and septicemia.\(^7,8\) Therefore, water treatment is required before use or discharging for safety.

Several methods are generally used for disinfection of wastewater, such as chlorine, ozone, and ultraviolet light (UV); however, these methods have disadvantages. Chlorine leaves have unwanted odor, high contact time, and residual toxicity of effluent. Ozone and UV are characterized by expensive operating costs, high energy demand, and complicated maintenance.\(^9-11\) As a result, alternative methods were studied to overcome the drawbacks of the above traditional methods. Previous studies reported many materials of silver and metal oxides of ZnO, TiO\(_2\), and CuO that have been used for disinfection of wastewater.\(^12-19\) Silver-modified clinoptilolite, natural silver zeolite clinoptilolite, and silver on polydopamine-coated composite membrane as representative silver materials, and ZnO nanorods, ZnO nanodisks, and ZnO-nanostructured surfaces as representative ZnO materials have been applied for disinfection in wastewater.\(^14,15,17,18\) For other modified materials of Fe/TiO\(_2\) and CuO, porous ceramic disk filter coated with Fe/TiO\(_2\) nanocomposites has been reported...
against *Bacillus subtilis*, *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* in wastewater, and copper oxide (II) nanoparticles have been studied against *S. aureus* and *E. coli*. Although silver nanoparticles (AgNPs) are used as antibacterial agents, they might release hazardous substance of silver after treatment, which may damage nontarget organisms or create environmental pollution. For metal oxides such as ZnO, TiO$_2$, and CuO, many studies take advantage of metal oxides for improving raw materials for high antibacterial efficiencies; however, these metal ions might release treated effluents, which cause metal accumulations in the environment. Since plant extracts are naturally derived and do not contain any harmful substances, they are appropriate to be selected for disinfection in wastewater.

For plant extracts, *Piper betle*, *Mentha cordifolia*, *Garcinia cowa*, *Sechium edule*, *Piper sarmentosum*, *Limnophila aromatica*, *Polygonum odoratum*, and *Cratoxylum formosum* are interesting plants since they have appropriate chemical compounds such as rutin, catechin, and hydroxylchavicol. Therefore, it is possible to use them for bacterial inhibitions such as *S. aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Klebsiella pneumonia*, *P. aeruginosa*, and *E. coli*. Since *C. formosum* and *P. odoratum* have good chemical compounds such as phenols, alkaloids, flavonoids, xanthones, tannin, and terpenoids, previous studies have reported that these plants have high potential against bacteria. As a result, they were chosen to explore their antibacterial efficiencies on *S. aureus* and *E. coli*. In previous studies, the extracted *C. formosum* leaves have been used to inhibit many bacteria types such as *S. aureus*, *E. coli*, *Campylobacter jejuni*, *P. aeruginosa*, *B. cereus*, and *B. subtilis* by the agar diffusion method, and the results confirmed their high antibacterial efficiencies. Some studies have reported that the extracted *P. odoratum* leaves could inhibit many bacteria types of Gram-positive and Gram-negative bacteria including *S. aureus* and *E. coli* by the disk diffusion method. However, no study has been applied extraction of *C. formosum* and *P. odoratum* from bead materials for investigating disinfection in wastewater. Therefore, this study has attempted to extract *C. formosum* and *P. odoratum* from a powder form and then modify it to a bead form as novel.

**Figure 1.** Physical characteristics of *C. formosum* and *P. odoratum* in (a, d) leaf, (b, e) powder, and (c, f) bead forms, respectively.
antibacterial materials. Moreover, their antibacterial efficiencies on *S. aureus* and *E. coli* were investigated through batch experiments for probably disinfectant applications in waste-water treatment.

This study aimed to extract *C. formosum* and *P. odoratum* leaves with ethanol for synthesizing bead materials (CFB and POB) against *S. aureus* and *E. coli*, to characterize the surface morphologies, chemical compositions, and chemical functional groups by field emission scanning electron microscopy and focused ion beam (FESEM-FIB), energy-dispersive X-ray spectrometry (EDX), and Fourier transform infrared (FTIR) spectroscopy, to observe antibacterial properties by agar diffusion tests, to investigate antibacterial efficiencies by batch experiments, to examine adsorption isotherm and kinetics for understanding adsorption equilibrium and mechanism of materials, and to explore material reusability by desorption experiments.

2. RESULTS AND DISCUSSION

2.1. Physical Characteristics of Raw Materials. Figure 1a–f illustrates the physical characteristics of *C. formosum* and *P. odoratum* in leaf, powder, and beads. For *C. formosum*, the leaf is simple, opposite, ovate-shaped, blunt, or shortly pointed-apex-shaped, acute-base-shaped, and entire-leaf margin. Leaves are normally green in color, but young leaves are reddish-pink, as shown in Figure 1a. *C. formosum* powder (CFP) was dark brown in color, whereas *C. formosum* beads (CFB) were black in color, which are displayed in Figure 1b,c, respectively. For *P. odoratum*, the leaf is simple, alternate, ovate- or lanceolate-leaf-shaped, cuspidate-apex shaped, acute-base-shaped, and entire-leaf margin. Leaves have mixed colors of light green and dark green, as shown in Figure 1d. *P. odoratum* powder (POP) was light green in color, whereas *P. odoratum* beads (POB) were black in color, as displayed in Figure 1e,f, respectively.

2.2. Material Characterizations. 2.2.1. FESEM-FIB. The morphologies of *C. formosum* and *P. odoratum* in powder and bead forms by FESEM-FIB are demonstrated in Figure 2a–f. For the powder form, the *C. formosum* powder (CFP) and the *P. odoratum* powder (POP) were uneven structures with heterogeneous cracking or flake surfaces at 10 000× magnification with 5 μm, as shown in Figure 2a,d. For the bead form, Figure 2b,e presents *C. formosum* beads (CFB) and *P. odoratum* beads (POB), which had spherical shapes at 65×
magnification with 1 mm. Figure 2c,f presents the morphologies of CFB and POB at 10 000× magnification with 5 μm, which showed coarse surfaces; however, CFB had a smoother surface than POB, which might depend on the plant species. It was similarly found in other studies, which formed bead material from different extracted plant leaves (A. vasica).50

2.2.2. EDX Analysis. For EDX analysis, the chemical compositions of powder and bead materials (CFP, CFB, POP, and POB) are presented in Table 1. Three main chemical compositions, carbon (C), oxygen (O), and calcium (Ca), were found in all materials, whereas chloride (Cl) was observed in three materials except CFP. Potassium (K) and copper (Cu) were only detected in CFP and POP, whereas sodium (Na) was only realized in CFB and POB because of using sodium alginate in bead formation. Silicon (Si) was only found in CFP, and magnesium (Mg), rubidium (Rb), and manganese (Mn) were only noticed in POP.

The mass chemical compositions in percentage by weight of CFP, CFB, POP, and POB are also demonstrated in Table 1, with their three main chemical elements in order from high to low was C > O > Ca, similar to other studies.51–53

Table 1. Chemical Compositions of Antibacterial Materials in Percentage by Weight

| plant name   | materials | C    | O    | Ca   | Cl   | K    | Cu   | Na   | Mg   | Rb   | Mn   | Si   |
|--------------|-----------|------|------|------|------|------|------|------|------|------|------|------|
| C. formosum  | CFP       | 66.4 | 23.8 | 1.6  | 2.2  | 4.1  | 1.8  | 0.8  | 1.6  | 1.4  | 0.8  |
|              | CFB       | 52.7 | 32   | 9    | 4.6  | 1.7  | 0.9  | 1.7  | 1.6  | 1.4  | 0.8  |
| P. odoratum  | POP       | 65.8 | 24.8 | 1.2  | 1.2  | 2.4  | 0.9  | 1.6  | 1.4  | 0.8  | 1.6  | 0.8  |
|              | POB       | 49.5 | 26.3 | 13.8 | 9.9  | 0.5  | 0.8  | 1.6  | 1.4  | 0.8  | 1.6  | 0.8  |

Figure 3. FTIR spectra of (a) CFP, (b) CFB, (c) POP, and (d) POB.
results reported that the quantity of C was higher than O and Ca, although they used different plants (Hevea brasiliensis, Gmelina aborea, cinnamomum camphora, Thevetia peruviana). In addition, other chemical elements were also found, such as Si, K, Cl, Mg, Cu, and P, depending upon the plant species, some of which are found in this study. For K and Cu, CFP had 2.2% K and 4.1% Cu, whereas POP had 2.4% K and 0.9% Cu. For Na, CFB and POB were 1.7% and 0.5%, respectively. For Si, CFP was 1.8%. Finally, POP had 1.6% Mg, 1.4% Rb, and 0.8% Mn. As a result, the change of form from powder to beads affected the change of mass percentage in chemical compositions of materials by the increase in O, Ca, and Cl, whereas C was decreased. The increase in Ca and Cl might be from CaCl₂, which was used in a bead formation.

2.2.3. FTIR Analysis. The chemical functional groups of CFP, CFB, POP, and POB were investigated by FTIR spectroscopy in a board peak range of 4000–600 cm⁻¹ are represented in Figure 3a–d. All materials had similarly seven main functional groups, which were O–H, C–H, C=O, C≡C, N–H, C–O, and C–Cl. O–H (stretching of phenol compounds or hydroxyl or carboxylic groups) is normally detected in many plant-based extracts, and it relates with the organic molecules of carbohydrates and proteins in plants. In addition, phenol compounds in O–H also play an important role in bacterial inhibition. C–H (stretching of aliphatic hydrocarbon chains (alkanes) or bending of methyl groups (CH₃) or OOP bending of phenyl rings (aromatics)) could probably arise from phenyl ring. C=O is due to stretching of aldehydic and ketolic compounds, and C≡C (stretching of alkenes) represents heterocyclic compounds such as flavonoids, alkaloids, and phenol compounds (polyphenol) in plant leaves. N–H (bending of amide I or II) generally presents in protein of plants. C–O (stretching of ethers or ester) could probably arise from the carboxylic acid in plant leaves, and C–Cl is the chloride compound. Therefore, the change of form from powder to beads did not affect the change of main absorption bands of all materials.
The prominent bands of CFP in Figure 3a were observed at around 3272.60 (O−H), 2922.75 (C−H), 1718.69 (C=O), 1619.77 (C=C), 1515.25 (N−H), 1442.58 (C−H), 1195.40 and 1025.43 (C−O), 815.44 (C−H), and 784.99, 744.49, and 721.35 (C−Cl) cm⁻¹, and other studies also found these main functional groups in C. formosum leaves extracted in forms of O−H, C−O, and N−H. For CFB, the main peaks were detected at around 3318.15 (O−H), 2926.05 (C−H), 1716.60 (C=O), 1598.17 (C=C), 1515.99 (N−H), 1430.54 (C−H), 1249.82, 1190.03, and 1023.63 (C−O), 816.23 (C−H), and 753.00 and 615.67 (C−Cl) cm⁻¹ in Figure 3b. For POP, the prominent bands were observed at around 3335.10 (O−H), 2912.71 and 2852.61 (C−H), 1735.72 (C=O), 1596.59 (C=C or N−H), 1436.86 (C−H), 1195.80 and 1024.39 (C−O), 817.72 (C−H), and 744.62 (C−Cl) cm⁻¹ in Figure 3c, and this observation was similar in organic acid phenol and aliphatic amines in forms of O−H, C−H, C=C or N−H, and C−O in P. odoratum leaves to another study. Finally, the main peaks of POB were detected at around 3216.49 (O−H), 2912.71 and 2852.61 (C−H), 1735.72 (C=O), 1596.59 (C=C or N−H), 1436.86 (C−H), 1195.80 and 1024.39 (C−O), 817.72 (C−H), and 744.62 (C−Cl) cm⁻¹ in Figure 3d.

2.3. Results of Agar Diffusion Tests. Agar diffusion test is a method to examine the efficiencies of bead materials of C. formosum (CFB) and P. odoratum (POB) on S. aureus and E. coli, and their results are reported in Table 2 and Figure 4a–d. For S. aureus, the diameters of the inhibition zone at different concentrations of CFB and POB from 100 to 400 mg/mL on S. aureus were 6.0 ± 0.4, 6.2 ± 0.4, 7.4 ± 0.8, and 8.3 ± 0.9 mm for CFB, and 3.0 ± 0.0, 3.3 ± 0.1, 3.8 ± 0.4, and 4.8 ± 0.3 mm for POB, and the averages of four concentrations of CFB and POB were 7.0 ± 1.1 and 3.8 ± 0.7 mm, respectively. As a result, the average diameters of the inhibition zones of CFB and POB also showed the same tendency of
increasing antibacterial efficiencies with increasing concentrations of CFB and POB, and CFB presented stronger antibacterial activity on S. aureus than POB.

For E. coli, the diameters of the inhibition zone at different concentrations of CFB and POB from 100 to 400 mg/mL on E. coli were 6.1 ± 0.4, 7.5 ± 0.5, 8.6 ± 0.8, and 8.8 ± 0.3 mm for CFB, and 3.1 ± 0.1, 3.3 ± 0.1, 4.5 ± 0.3, and 5.0 ± 0.1 mm for POB, and the average of four concentrations of CFB and POB were 7.8 ± 1.2 and 4.0 ± 0.9 mm, respectively. As a result, the average diameters of the inhibition zones of CFB and POB also showed the same tendency of increasing antibacterial efficiencies with increasing concentrations of CFB and POB, and CFB presented stronger antibacterial activity on E. coli than POB.

For comparison, the results of the agar diffusion tests of CFB and POB on S. aureus and E. coli represented that CFB and POB had the same tendency of increasing antibacterial efficiency with increasing concentration. Since the average of four concentrations of CFB and POB was close to 300 mg/mL, this concentration was the appropriate one for inhibition on S. aureus and E. coli. However, CFB demonstrated higher antibacterial efficiencies on both S. aureus and E. coli than POB at all concentrations, where CFB represented a larger inhibition zone than POB, as shown in Figure 4a–d. Furthermore, both materials better inhibit E. coli than S. aureus because the cell wall of E. coli is thinner than that of S. aureus. Therefore, CFB demonstrated higher inhibition than POB on both bacterial types.

Possible mechanisms for S. aureus or E. coli inhibition by bead materials are demonstrated in Figure 5. The cell wall of bacteria contacted with materials and then extracted substances in materials such as phenolic compounds, alkaloids, flavonoids penetrated into cell to damage membrane, DNA, and main functions of bacteria. As a result, it caused cell death.

For the wastewater treatment application, the batch experiments were designed to confirm antibacterial efficiencies after preliminary microbiological tests. Since CFB represented higher inhibition than POB on S. aureus and E. coli, it was chosen for batch experiments.

2.4. Batch Experiments. 2.4.1. Effect of Dose. The dose effects of CFB on antibacterial efficiency against S. aureus and E. coli are presented in Figure 6a. For S. aureus, the antibacterial efficiencies of CFB increased from 96.50 to 99.99% with increasing dosage from 0.1 to 0.4 g, and they illustrated a value of almost 100% after 0.3 g. For E. coli, the antibacterial efficiencies of CFB increased from 96.50 to 100% with increasing dosage from 0.1 to 0.4 g, and they illustrated a constant value of 100% after 0.3 g. Since 0.3 g was the lowest dosage of CFB for almost 100% antibacterial efficiency on S. aureus and E. coli, this value was the optimum dose of CFB against both bacterial types. Therefore, 0.3 g was used for the next experiment for the effect of contact time.

2.4.2. Effect of Contact Time. The effect of contact time on the antibacterial efficiency of CFB against S. aureus and E. coli was investigated by varying times from 1 to 8 h, as shown in Figure 6b. For S. aureus, all values of contact time presented high antibacterial efficiencies of CFB in a range of 98.33–99.93%, and their average was 99.48%, which was close to a value of 3 h. Therefore, 3 h was an appropriate contact time against S. aureus. For E. coli, all values of contact time presented high antibacterial efficiencies of CFB more than 99.99%, and the contact times of 2–8 h were constant at 100%. Therefore, 2 h was an appropriate contact time against E. coli. The conditions of CFB were 0.3 g, 3 h for S. aureus and 0.3 g, 2 h for E. coli. These conditions were used to study the effect of pH.

2.4.3. Effect of pH. pH is another important factor to investigate the antibacterial efficiencies of CFB on S. aureus and E. coli because the acid, neutral, and base pH conditions may affect the inhibition of bacteria. In this study, pHs 5, 7, and 9 were selected for acid, neutral, and base conditions, and the result is presented in Figure 6c. All pH conditions demonstrated high antibacterial efficiencies of CFB of almost
100% on *S. aureus* and *E. coli*; however, a change of pH from 5 to 9 illustrated little different values of antibacterial efficiencies from 99.36 to 99.87% for *S. aureus* and from 99.95 to 99.98% for *E. coli*, respectively. As a result, the acid, neutral, and base pH conditions did not affect the CFB efficiency, and pH 7 was suitable chosen as the optimum pH of CFB against *S. aureus* and *E. coli* to achieve safe water quality. Therefore, the conditions of CFB were 0.3 g, 3 h, pH 7 for *S. aureus* and 0.3 g, 2 h, pH 7 for *E. coli*. They were used to study the effect of concentration.

### 2.4.4. Effect of Concentration

The result of the effect of concentration of CFB by varying the concentrations of *S. aureus* and *E. coli* from 10⁵ to 10⁷ CFU/mL is examined in Figure 6d. All concentrations represented a high antibacterial efficiency of approximately 100%, so the concentration factor did not affect its efficiency. Therefore, CFB had a high potential material against both *S. aureus* and *E. coli*.

In conclusion, only dose and contact time of CFB affected the antibacterial efficiency on *S. aureus*, whereas only the dosage of CFB affected the antibacterial efficiency on *E. coli*. Finally, water contaminated with *S. aureus* and *E. coli* of 10⁶ CFU/mL in the sample volume of 100 mL could be treated almost 100% by CFB under the optimum condition of 0.3 g, 3 h, and pH 7 for *S. aureus* and 0.3 g, 2 h, and pH 7 for *E. coli*. Since the inhibition of CFB on *E. coli* spent time less than *S. aureus*, CFB was a higher-potential material on *E. coli* than on *S. aureus*.

### 2.5. Isotherm Study

The adsorption isotherm studies of CFB on *S. aureus* and *E. coli* with fitting of Langmuir and Freundlich linear and nonlinear models are demonstrated in Figure 7a–f. Langmuir linear model is plotting of *C*ₐ/qₑ versus *C*ₐ, whereas Freundlich linear model is plotting of log qₑ versus log *C*ₐ. Langmuir and Freundlich nonlinear models are plotting of qₑ versus *C*ₑ. In addition, the equilibrium isotherm parameters are reported in Table 3.

For the Langmuir linear model, the maximum adsorption capacities (qₑₐ) of CFB on *S. aureus* and *E. coli* were 2.50 × 10⁹ and 3.33 × 10⁹ CFU/g, which indicated that CFB had possibly higher inhibition of *E. coli* than *S. aureus*. Langmuir linear adsorption constants (Kₑ) were 0.00013 and 0.00500 L/CFU. For the Langmuir nonlinear model, the maximum adsorption capacities (qₑₐ) of CFB on *S. aureus* and *E. coli* were 2.52 × 10⁹ and 3.36 × 10⁹ CFU/g and Langmuir nonlinear adsorption constants (Kₑ) were 0.00010 and 0.00486 L/CFU, respectively. The *R*² values of linear and nonlinear Langmuir models were 0.09 and 0.38 for *S. aureus* and 0.04 and 0.34 for *E. coli*. In the Freundlich model, 1/n is the constant depicting adsorption intensity, 1/n < 1 means favorable adsorption with different concentrations, and the adsorption capacity will decrease with increasing concentration.⁷³ For the Freundlich linear model, the 1/n values of CFB on *S. aureus* and *E. coli* were 0.54 and 0.88, and Freundlich linear adsorption constants (Kₑ) were 8.46 × 10⁶ and 2.30 × 10⁷ (CFU/g)(CFU/L)¹/n, respectively. For the Freundlich nonlinear model, the 1/n values of CFB on *S. aureus* and *E. coli* were 0.50 and 0.81, and the Freundlich linear adsorption constants (Kₑ) were 9.45 × 10⁶ and 2.57 × 10⁷ (CFU/g)(CFU/L)¹/n, respectively. The *R*² values of linear and nonlinear Freundlich models were 0.92 and 0.97 for *S. aureus* and 0.93 and 0.98 for *E. coli*, respectively.

For comparing the results of linear and nonlinear adsorption isotherms, the equilibrium isotherm parameters of CFB on *S. aureus* and *E. coli* in both Langmuir and Freundlich linear and nonlinear models had close values, which indicated that the results of the Langmuir and Freundlich linear models agreed to those of the Langmuir and Freundlich nonlinear models. Moreover, the actual experimental data of CFB on both *S. aureus* and *E. coli* were best fitted to Freundlich linear and nonlinear models shown in Figure 7a–f, which corresponded to the higher *R*² values of the Freundlich model than the Langmuir model shown in Table 3. Literature review shows that other studies also found the same results as this study, that is, the results of the adsorption linear model agreed to those of the nonlinear model, and the equilibrium isotherm parameters of both linear and nonlinear models had close values.⁷⁴−⁷⁸ As a result, both linear and nonlinear adsorption models are necessary to plot and calculate all isotherm parameters for confirming which adsorption model is the best-fit model to the actual experiment values to avoid errors.⁷⁹,⁸⁰

Finally, since the *R*² value is generally used to decide which adsorption isotherm model well explains an adsorption pattern of adsorbent, a higher *R*² close to 1 is suitably chosen. Therefore, the adsorption isotherm of CFB on *S. aureus* and *E. coli* corresponded to the Freundlich model in both linear model (*R*² = 0.92 and 0.93) and nonlinear model (*R*² = 0.97 and 0.98), which indicated that the adsorption pattern could be explained by a physiochemical adsorption process with multilayer or heterogeneous adsorption.⁸¹

### 2.6. Kinetic Study

Adsorption kinetics is an important study to explain an adsorption mechanism including rate of adsorption to time by adsorbent.⁸² Pseudo-first-order kinetic model and pseudo-second-order kinetic model reactions were used for the kinetic study of CFB by plotting log (qₑ − qₑ) versus time (t) for pseudo-first-order linear kinetic model and t/qₑ versus time (t) for pseudo-second-order linear kinetic model, respectively. Pseudo-first-order and pseudo-second-order nonlinear kinetic models are plotting of qₑ versus time (t). Moreover, the adsorption equilibrium of CFB on *S. aureus* and *E. coli* is represented in Figure 9 and the adsorption kinetic parameters are reported in Table 4.

| Table 3. Equilibrium Isotherm Parameters of CFB on *S. aureus* and *E. coli* |
|----------------------------------|---------|---------|---------|---------|---------|
|                                  | *S. aureus* | **nonlinear** | *E. coli* | **nonlinear** |
|                                  | **linear** | **nonlinear** | **linear** | **nonlinear** |
| qₑₐ (CFU/g)                      | 2.50 × 10⁹ | 2.52 × 10⁹ | 3.33 × 10⁹ | 3.36 × 10⁹ |
| Kₑ (L/CFU)                       | 0.00013    | 0.00010   | 0.00500   | 0.00486   |
| R²                               | 0.09       | 0.38      | 0.04      | 0.34      |
|                                | **Freundlich Model** |
| Kₑ (CFU/g)/(CFU/L)¹/n           | 8.46 × 10⁶ | 9.45 × 10⁶ | 2.30 × 10⁷ | 2.57 × 10⁷ |
| 1/n                             | 0.54       | 0.50      | 0.88      | 0.81      |
| R²                               | 0.92       | 0.97      | 0.93      | 0.98      |
For comparing the results of linear and nonlinear kinetic models, the adsorption kinetics of CFB on *S. aureus* and *E. coli* corresponded to pseudo-second-order linear and nonlinear models shown in Figure 8a–f. In addition, the adsorption kinetic parameters of CFB on *S. aureus* and *E. coli* in both pseudo-first-order and pseudo-second-order linear and nonlinear models had close values, which indicated that the results of linear kinetic models agreed with those of linear kinetic models. These results were similar in many studies that have been plotted in linear and nonlinear kinetic models to explain their results, and their results are normally in agreement with best-fit linear model coupling of the nonlinear model. Therefore, the plotting of nonlinear kinetic models helped to confirm the result of linear plotting to protect mistake processing data.

### 2.7. Desorption Experiment

Desorption experiment was studied for confirming the material reusability through several adsorption–desorption cycles because of probable industrial applications with high efficiency, long-time reuse, and reasonable budget. In this study, three cycles of adsorption–desorption were studied to confirm material reusability and 0.01 M HNO₃ solution was used for bacterial desorption on CFB. Table 5 confirms the high bacterial adsorption and desorption of CFB on *S. aureus* and *E. coli*, and their results were in the ranges of 90 ± 0.3–100 ± 0.0% and 87 ± 0.2–100 ± 0.0%, respectively. For probable reuse consideration, the bacterial adsorption and desorption efficiencies of CFB in three cycles on *S. aureus* decreased by 9 and 12%, respectively, and on *E. coli*, the values decreased by 10 and 12%, respectively. Therefore, CFB had a possible material for...

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**Table 4. Adsorption Kinetic Parameters of CFB on *S. aureus* and *E. coli***

|       | *S. aureus* | *E. coli* |      |      |      |
|-------|-------------|-----------|------|------|------|
|       | linear      | nonlinear | linear | nonlinear |      |
|       |             |           |       |       |      |
| *k₁* (min⁻¹) | 0.0144 | 0.0141 | 0.0311 | 0.0400 |      |
| *qₑ* (×10⁶ CFU/g) | 42.81 | 46.19 | 0.0001 | 0.0001 |      |
| *R²* | 0.92 | 0.95 | 0.34 | 0.35 |      |

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**Figure 8.** (a, b, d, e) Linear and (c, f) nonlinear adsorption kinetic models of CFB on *S. aureus* and *E. coli*. 

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Finally, CFB has a potential material for reuse and can possibly be applied for disinfection in wastewater treatment.

3. CONCLUSIONS

This study was the first to report the antibacterial activity of extracted *C. formosum* and *P. odoratum* leaves in bead materials (*C. formosum* beads (CFB) and *P. odoratum* beads (POB)) against *S. aureus* and *E. coli* in wastewater. CFB and POB had spherical shapes with coarse surfaces, and C, O, Ca, Na, Cl, O=H, C––H, C=O, C=O, C–O, and C–Cl were detected. Agar diffusion tests confirmed higher antibacterial activities of CFB than POB on both bacterial types. The optimum conditions of CFB were 0.3 g, 3 h, pH 7, 10^6 CFU/mL for *S. aureus* and 0.3 g, 2 h, pH 7, 10^6 CFU/mL for *E. coli* with almost 100% bacteria removal. Since the inhibition of CFB on *E. coli* spent time less than *S. aureus*, CFB was higher potential materials on *E. coli* than *S. aureus*. The adsorption isotherm and kinetics of CFB on *S. aureus* and *E. coli* corresponded to the Freundlich model and the pseudo-second-order kinetic model. Moreover, the desorption experiment confirmed material reusability. Therefore, CFB is a high potential material for inhibitions on *S. aureus* and *E. coli* and can possibly be applied for disinfection of wastewater treatment in the future.

For future works, other bacterial types of Gram-positive and Gram-negative bacteria should be investigated by CFB as bacterial competitions in the actual wastewater. In addition, the continuous flow study or column experiment is recommended to explore whether CFB may possibly apply for disinfection of real wastewater system.

4. MATERIAL AND METHODS

4.1. Materials and Chemicals. Raw materials were *C. formosum* in Hypericaceae and *P. odoratum* in Polygonaceae, which were collected from Sakon Nakhon province, Thailand, and only leaves were used in this study. All chemical reagents were of analytical grade without purification. Nutrient broth (NB) and nutrient agar (NA) (HiMedia, India) were used for the preparation of culture media, and the solvent of plant extraction was 99.9% ethanol (C₂H₅OH) (RCI Labscan, Thailand). Sodium alginate (Merck, Germany) and calcium chloride (CaCl₂) (KEMAUS, New Zealand) were used for the

| bacterial | cycle | adsorption (%) | desorption (%) |
|-----------|-------|----------------|----------------|
| *S. aureus* | 1 | 99 ± 0.4 | 99 ± 0.4 |
|           | 2 | 96 ± 0.3 | 92 ± 0.6 |
|           | 3 | 90 ± 0.3 | 87 ± 0.2 |
| *E. coli*  | 1 | 100 ± 0.0 | 100 ± 0.0 |
|           | 2 | 97 ± 0.2 | 94 ± 0.4 |
|           | 3 | 90 ± 0.4 | 88 ± 0.2 |
bead formation. For pH adjustments, 1% sodium hydroxide (NaOH) (RCI Labscan, Thailand) and 1% nitric acid (HNO₃) (Merck, Germany) were used.

4.2. Instruments. A hot-air oven (Binder, FED 53, Germany), an orbital shaker (GFL, 3020, Germany), a rotary evaporator (BUCHI, RE-111 Rotavapor, Switzerland), a freeze dryer and vacuum concentrator (LaboGene, Scanvac, Denmark), and a hot plate stirrer (Ingenieurbüro CAT, M. Zipperer GmbH, M 6, Germany) were used for material synthesis. For all aseptic experiments, an autoclave (TOMY, SX-700, Japan) to obtain initial concentrations from 10⁴ to 10⁷ CFU/mL. Next, ECF or EPO was added into sodium alginate solution and the samples were homogeneously mixed by a hot plate at 60 °C with a constant stirring speed of 250 rpm for 30 min. Then, 0.1 M CaCl₂ solutions were prepared for setting beads. After that, the mixed samples were added into a 50 mL glass syringe of 3 mm diameter and were added dropwise into a 0.1 M CaCl₂ solution. Then, the bead samples were soaked into 0.1 M CaCl₂ for 24 h. Finally, they were filtered, rinsed with deionized water (DI), air-dried for 24 h, and kept in desiccators before use. The extracted plants were called extracted C. formosum (ECF) and extracted P. odoratum (EPO).

For the raw material preparation, an autoclave (Termaks, KBP 6087, Norway) was used to incubate sample tests in agar diffusion tests, antibacterial batch experiments, adsorption isotherm, kinetics, and desorption experiments. The amount of ECF or EPO added into 2% w/v sodium alginate depended upon the concentration of plant extract to the volume of solvent solution prepared. For example, 0.1 g of ECF or EPO was added into 1 mL of 2% w/v sodium alginate for the concentration of 100 mg/mL.

4.3. Bacterial Culture and the Preparation of Bacterial Water Sample. S. aureus (DMST 562) and E. coli (DMST 4212) (DMST stands for Department of Medical Sciences Culture Collection, Thailand) were used in this study. The water samples were prepared by diluting S. aureus and E. coli of 10⁸ CFU/mL in sterile deionized water (autoclave: TOMY, SX-700, Japan) to obtain initial S. aureus and E. coli concentrations from 10⁴ to 10⁷ CFU/mL. Note: CFU stands for colony-forming unit, which is widely used to estimate the number of visible microorganisms in test samples.

4.4. Synthesis of Materials. A diagram of synthesis methods of bead materials (CFB and POB) is demonstrated in Figure 10, including three procedures of raw material preparation, plant extraction, and bead formation. The details are clearly explained below:

For the raw material preparation, C. formosum and P. odoratum leaves were washed with tap water, cut into small pieces, and dried in a hot-air oven (Binder, FED 53, Germany) at 50 °C for 12 h. Then, the dried samples were ground to powder by a blender to a sieve size of 125 μm. The powder materials were called C. formosum powder (CFP) and P. odoratum powder (POP) and kept in desiccators until use. For plant extraction, about 10 g of CFP or POP was added to 100 mL of ethanol into a 250 mL Erlenmeyer flask and was mixed by an orbital shaker (GFL, 3020, Germany) for 24 h with a constant stirring speed of 200 rpm at room temperature. Then, the mixed samples were filtered using a vacuum pump, evaporated using a rotary evaporator (BUCHI, RE-111 Rotavapor, Switzerland) at 50 °C, freeze-dried (LaboGene, Scanvac, Denmark), and kept at 4 °C until use. The extracted plants were called extracted C. formosum (ECF) and extracted P. odoratum (EPO).

For bead formation, a 2% w/v sodium alginate solution was prepared by a hot plate (Ingenieurbüro CAT, M. Zipperer GmbH, M 6, Germany) at 60 °C with a constant stirring speed of 200 rpm for 30 min. Next, ECF or EPO was added into sodium alginate solution and the samples were homogeneously mixed by a hot plate at 60 °C with a constant stirring speed of 250 rpm for 30 min. Then, 0.1 M CaCl₂ solutions were prepared for setting beads. After that, the mixed samples were added into a 50 mL glass syringe of 3 mm diameter and were added dropwise into a 0.1 M CaCl₂ solution. Then, the bead samples were soaked into 0.1 M CaCl₂ for 24 h. Finally, they were filtered, rinsed with deionized water (DI), air-dried for 24 h, and kept in desiccators before use. The bead materials were called C. formosum beads (CFB) and P. odoratum beads (POB).

Note: The amount of ECF or EPO added into 2% w/v sodium alginate depended upon the concentration of plant extract to the volume of solvent solution prepared. For example, 0.1 g of ECF or EPO was added into 1 mL of 2% w/v sodium alginate for the concentration of 100 mg/mL.

4.5. Material Characterizations. The surface morphology and chemical compositions of powder and bead materials (CFP, POP, CFB, and POB) were analyzed by field emission scanning electron microscopy and focused ion beam (FESEM-FIB) with energy-dispersive X-ray spectrometry (EDX) (FEI, Helios NanoLab G3 CX). The samples were mounted on aluminum stubs by double-sided carbon tape and coated by a gold-coater for 4 min using a 108 Auto Sputter Coater with thickness controller MTM-20 model (Cressington, Ted Pella, Inc.), and then they were analyzed by FESEM-FIB with a 10 kV accelerating voltage. The chemical functional groups of all materials were explored by Fourier transform infrared (FTIR)
spectroscopy (Bruker. TENSOR27, Hong Kong). The spectral range of 4000−600 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) was used for the scanning of FTIR spectra of samples, which was analyzed with an average of 16 scans over the entire covered range.

4.6. Agar Diffusion Test. Antibacterial efficiencies of bead materials (CFB and POB) were investigated by agar diffusion tests, and Figure 11 shows a diagram of two steps by agar diffusion tests. The details are explained below.

**Step 1:** Preparation of bacteria concentration: *S. aureus* and *E. coli* were prepared at a 10^8 CFU/mL concentration using a 0.5 McFarland standard.

**Step 2:** Antibacterial tests: CFB or POB were applied to nutrient agar using the three-dimensional swab technique, and then four pieces of CFB or POB were put in a plate test. Then, a plate test was carried out in an incubator (Termaks, KBP 6087, Norway) at 37 °C for 24 h and the result was reported by the average inhibition zone diameters. Triplicate experiments were applied to confirm the results.

4.7. Antibacterial Batch Experiments. A series of batch experiments were performed to investigate the effects of dose, contact time, pH, and initial concentration of CFB on *S. aureus* and *E. coli*. Four different doses of 0.1, 0.2, 0.3, and 0.4 g were examined with the highest antibacterial efficiency of CFB. The optimum dose of CFB was used to study the effect of contact time from 1−8 h, and then the optimum dose and contact time of CFB were applied to investigate the effect of pH. Three different pH values of 5, 7, and 9 were examined to determine the optimum pH for the antibacterial efficiency of CFB. Finally, the optimum dose, contact time, and pH of CFB were used for the effect of concentration, and four different bacterial concentrations of 10^4, 10^5, 10^6, and 10^7 CFU/mL were explored with the optimum concentration for the antibacterial efficiency of CFB.

For the control condition, a sample volume of 100 mL, a bacterial concentration of 10^6 CFU/mL, a shaking speed of 200 rpm, and a temperature of 25 °C were applied. Triplicate experiments of each factor were used to confirm the results, and the average was reported. All samples were analyzed using the plate count technique, and the antibacterial efficiency in percentage is calculated using eq (1)\(^{73}\)

\[
\text{antibacterial efficiency(\%)} = \left( \frac{(C_{0} - C_{e})}{C_{0}} \right) \times 100
\]

where \(C_{e}\) is the bacterial equilibrium in the solution (CFU/mL) and \(C_{0}\) is the initial bacteria concentration (CFU/mL).

4.8. Adsorption Isotherm. Adsorption isotherms can be used to describe the interaction of bacterial in the solution with CFB, which was analyzed using linear and nonlinear models of Langmuir isotherm equations (2)\(^{93,94}\) and (3)\(^{95}\) and Freundlich isotherm equations (4)\(^{96,97}\) and (5)\(^{98}\) respectively.

**Langmuir isotherm**

linear: \(\frac{C_{e}}{q_{e}} = \frac{1}{q_{m} K_{L}} + \frac{C_{e}}{q_{m}}\) (2)

nonlinear: \(q_{e} = q_{m} K_{L} C_{e} / (1 + K_{L} C_{e})\) (3)

**Freundlich isotherm**

linear: \(\log q_{e} = \log K_{F} + 1/n \log C_{e}\) (4)

nonlinear: \(q_{e} = K_{F} C_{e}^{1/n}\) (5)

where \(q_{e}\) is the capacity of bacteria adsorption on bead material at equilibrium (CFU/g), \(q_{m}\) is the maximum capacity of bacteria adsorption on bead material (CFU/g), \(C_{e}\) is the equilibrium of bacterial concentration (CFU/mL), \(K_{L}\) is the Langmuir adsorption constant (L/CFU), \(K_{F}\) is the Freundlich constant of adsorption capacity (CFU/g), and \(n\) is the constant depicting the adsorption intensity. Graphs of Langmuir and Freundlich isotherm models were plotted by linear plot features of \(C_{e}/q_{e}\) versus \(C_{e}\) and log \(q_{e}\) versus log\(C_{e}\), respectively. For nonlinear plot features of both isotherm models, they were plotted by the capacity of bacteria adsorption on bead material.

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Figure 11. Diagram of two steps by agar diffusion tests.
at equilibrium ($q_e$) versus the equilibrium of bacterial concentration ($C_e$).

For adsorption isotherm experiment, 0.3 g of CFB was added to a 250 mL Erlenmeyer flasks with variable bacterial concentrations from $10^4$ to $10^7$ CFU/mL. The control condition was a sample volume of 100 mL, a contact time of 6 h, pH 7, a temperature of 25 °C, and a shaking speed of 200 rpm.

4.9. Adsorption Kinetics. Adsorption kinetics can be used to explain the mechanism of antibacterial efficiency of CFB. Characteristic constants of sorption were investigated using linear and nonlinear models of a pseudo-first-order kinetic model following eqs $6^{99}$ and $7^{100}$ and a pseudo-second-order kinetic model following eqs $8^{90}$ and $9^{101}$ respectively. Pseudo-first-order kinetic model

linear: $\ln(q_e - q_t) = \ln(q_e) - kt$  

nonlinear: $q_t = q_e (1 - e^{-kt})$  

Pseudo-second-order kinetic model

linear: $t/q_t = 1/k_1q_e^2 + (1/q_e)t$  

nonlinear: $q_t = k_2q_e^2t/(1 + q_kt)$  

where $q_e$ (CFU/g) and $q_t$ (CFU/g) are the capacities of bacteria adsorbed by bead material at equilibrium and at time $t$, respectively, and $k_1$ (min$^{-1}$) and $k_2$ (g/CFU-min) are the reaction rate constants of pseudo-first-order and pseudo-second-order kinetic models, respectively. Graphs of pseudo-first-order and pseudo-second-order kinetic models were plotted by linear plot features of $\ln(q_e - q_t)$ versus time ($t$) and $t/q_t$ versus time ($t$), respectively. For nonlinear plot features of both kinetic models, they were plotted by the capacity of bacteria adsorbed by bead material at the time ($q_t$) versus time ($t$).

For adsorption kinetic experiment, 3 g of CFB was added to a 1000 mL beaker with a bacterial concentration of $10^6$ CFU/mL. The control condition was a sample volume of 1000 mL, a contact time of 8 h, pH 7, a temperature of 25 °C, and a shaking speed of 200 rpm.

4.10. Desorption Experiment. The possible reusability of CFB was investigated by the desorption experiment with three adsorption—desorption cycles. After an adsorption process, CFB was desorbed using 100 mL of 0.01 M HNO$_3$ solution with a shaking speed of 200 rpm for 2 h and a temperature of 25 °C. After that, the CFB was washed by deionization water and dried at room temperature. Then, the CFB was ready for the next adsorption cycle. The desorption efficiency in percentage is calculated following eq 10

$\text{desorption(\%)} = (q_d/q_e) \times 100$  

where $q_d$ is the amount of bacteria desorbed (CFU/mL) and $q_e$ is the amount of bacteria adsorbed (CFU/mL).

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P.N. contributed to investigation, writing—original draft, and visualization. P.P. contributed to supervision, conceptualization, funding acquisition, validation, investigation, methodology, writing—original draft, and visualization.

Notes

The authors declare no competing financial interest. The raw/processed data required to reproduce these findings cannot be shared at this time due to legal or ethical reasons. The raw/processed data required to reproduce these findings cannot be shared at this time as the data also form part of an ongoing study.

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