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# Performance of microwave treatment for disintegration of cephalosporin mycelial dreg (CMD) and degradation of residual cephalosporin antibiotics

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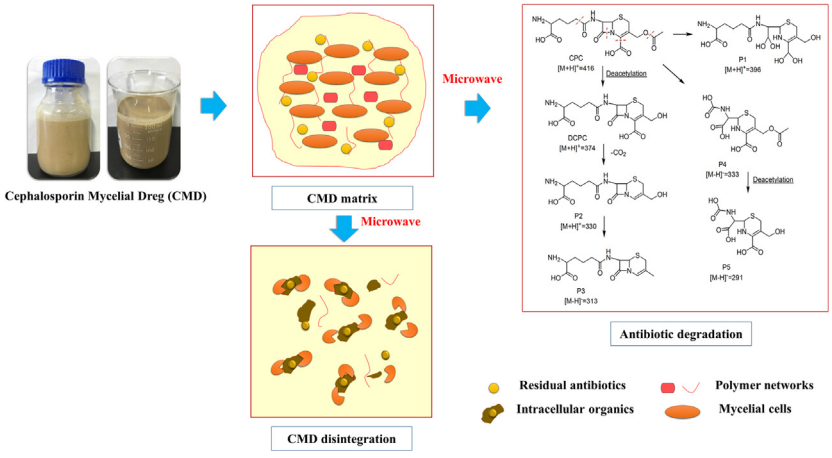
## HIGHLIGHTS

- Cephalosporin mycelial dreg treatment with microwave was first investigated.
- Temperature significantly influenced the cephalosporin mycelial dreg disintegration.
- The degradation ratio of residual antibiotics exceeded 99.9% under MW treatment.
- Most intermediate products of residual antibiotics lost the antibacterial activities.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Significant amounts of cephalosporin mycelial dreg (CMD) are still being generated from biopharmaceutical processes, representing both an economic and environmental burden for pharmaceutical factories. This study investigates the microwave (MW) treatment of CMD at a relatively mild temperature (100 °C) within 15 min. The results reveal that the MW treatment disintegrates the CMD efficiently and that the residual cephalosporin C (CPC) is almost degraded after sufficient irradiation. MW heating temperature strongly influences the polymer's release. SCOD (soluble chemical oxygen demand), soluble proteins and carbohydrates have significant positive correlations to the temperature ($r = 0.993$, 0.983 and 0.992, respectively; $p < 0.01$). 3D-EEM fluorescence spectra indicate that the key organic matters relate to temperature as well as microwave energies. Furthermore, more than 99.9% of the residual antibiotics in CMD are degraded by MW irradiation without antibacterial activities that are proven by the possible degradation pathway we elucidate. These results suggest that microwave irradiation treatment not only disintegrates CMD and destroys mycelial cells but also degrades the residual cephalosporin antibiotics, which implies the possibility for practical applications.



## 1. Introduction

Antibiotic mycelial residue is generated in the amount of 1.3 million tonnes each year in China [1]. Cephalosporin mycelial dreg

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(CMD) is one kind of antibiotic mycelial residue with approximately 50% of the total production. It is the precipitation of fermentation broth that is used for extracting cephalosporin C (CPC), a bulk drug for synthetic cephalosporins. The fact that antibiotic residue develops the microbial resistance unavoidably threatens the health of mankind [2]. Therefore, CMD has been added into the list of national hazardous waste category since 2008 [3]. It is identified for environmentally friendly treatment or safety disposal, such as an incineration process. However, due to the high water content of CMD, drying process followed by incineration is energy consuming and dramatically limited, which heavily perplexes the bio-pharmaceutical industry.

Hence, it is necessary to develop an efficient treatment/pretreatment method to solve the disposal problems. Based on the similarity of physicochemical properties of various antibiotic mycelial residues, organic matters (e.g., proteins, carbohydrates) can be utilized by microbial processes [4]. Wu et al. [5] found that anaerobic treatment of lincomycin manufacturing biowastes achieved with stably residual lincomycin in hydrolysate. Yang et al. [6] co-composted penicillin mycelial dregs and sewage with a significant increase of antibiotic resistance genes. Moreover, several other reports have had similar results [7–9]. All of the publications above indicate that a direct microbial biodegradation process may be not suitable for the treatment or disposal of these kinds of biowastes with residual antibiotics. It is obvious that the antibiotic mycelial residue has relatively low biodegradability, as it consists of a large amount of mycelial cells agglomerated together, resulting in compact flocs. Furthermore, the residual antibiotics can frequently suppress the microbiological process and even facilitate the development of bacterial antibiotic resistance [2,10,11]. Several physiochemical alternatives have thus been explored. The optimized conditions of hydrothermal treatment for CMD were 120–160 °C for 30–60 min [12] and the thermal-alkaline method was also developed for CMD treatment [13]. However, relatively high temperature and alkali addition were energy consuming, and the increase of electrolytes of treated CMD was not beneficial to the subsequent disposal process.

Microwave (MW) irradiation at a relatively mild temperature and without any addition of chemicals is sufficient for biomass or biowaste treatment, such as sludge and lignocellulosic biomass [14,15]. It can significantly disintegrate biomass matrix and lyse microbial cells to enhance biodegradability [16]. But there is limited information about the influence of microwave treatment on CMD disintegration as well as on destruction of mycelia that produce the antibiotics (CPC). Based on previous studies, the optimization of MW treatment process may be mainly based on the CMD solubilisation, releasing organic matters (e.g., proteins, carbohydrates) and intracellular components into a medium through CMD matrix disintegration and mycelial cell disruption. Regarding sludge conditioning with MW, solubilisation effectiveness is supposed to be caused by temperature rather than contact time [17]. Differences exist between CMD and municipal sludge; therefore, the influence of mild temperature (40–100 °C) and the contact time through further extending MW irradiation at the boiling point (100 °C) on CMD disintegration was investigated.

On the other hand, the removal of residual antibiotics retained in CMD is also a significant factor in evaluating the most appropriate conditions for CMD treatment. Particularly, microwave with high frequency electromagnetic radiation results in the movement of ions and vibrations of polar molecules, which may degrade CPC efficiently due to the strong polarity [14] and thermal instability [18]. Nevertheless, the degradation pattern and degradation products of residual antibiotics in CMD are lacking adequate information; therefore, CPC degradation needs to be studied before carrying out practical applications. Herein, this study was to evaluate the effectiveness of CMD disintegration through microwave irradiation conducted at a mild temperature range (40–100 °C). Additionally, the degradation pattern of residual antibiotics in CMDs and its possible byproducts via microwave irradiation were also investigated.

## 2. Materials and methods

### 2.1. Source materials, experimental equipment and CMD conditioning

The tested CMDs were obtained from Chinese biopharmaceutical factories, the Harbin Pharmaceutical Group Co. (Harbin, China). CMDs were collected using metal buckets. Afterwards, they were stored at 4 °C for use. The physiochemical properties of CMD are shown in Table S1. The standard of CPC (≥95%) was supplied from Zhongrun Pharmaceutical Co. (Shijiazhuang, China).

Fig. S1 presents the schematic diagram of MW apparatus. A laboratory-customized microwave oven (frequency 2.45 GHz, Preekem Scientific Instruments Co., Shanghai, China) with mechanical stirring (60 rpm) includes the power-constant mode. Inside the vessels, a thermocouple could record the temperature profiles of the CMDs. The reaction process could be observed by a camera at the top-right corner. The condensator could prevent moisture loss from evaporation.

CMD samples (100 g) were added to a 500 mL customized round-bottom flask. Before MW irradiation, a given amount of deionized water (100 mL) was added to the flask. Then, CMD samples were exposed to MW irradiation and heated to the target temperatures (40 °C, 60 °C, 80 °C, 100 °C). After cooling down to room temperature, the treated CMD samples were used for analysis. CPC-Na standard solution (200 mL 2.0 wt.%) was prepared to investigate the possible degradation pathways of CPC via MW irradiation.

### 2.2. Analytical methods

Total solids content (TS), volatile solids content (VS), chemical oxygen demand (COD), water content and pH were measured in accordance with standard methods [19]. The degree of CMD solubilisation was calculated based on a previous report [17]. Before analysing the released polymers, CMD samples were centrifuged at 4000 rpm for 10 min, followed by being filtered through 0.45 μm nylon syringe filters. Contents of soluble protein and carbohydrate were measured according to the Lowry method [20] and the phenol-sulfuric acid method [21], respectively. Fluorescence spectra were obtained by a JASCO FP-4500 fluorescence spectrophotometer (JASCO Co., Tokyo, Japan) according to Liu et al. [15].

Concentration of CPC was measured by the modified method developed by Sun et al. [22]. Total phase CMD samples (which include both soluble and sorbed fractions) were extracted by sonication with extract solution (50: 50 (*v*:*v*) MeCN: $H_2O$), followed by being centrifuged at 4000 rpm. The extraction procedure was repeated. Dichloromethane was added to the combined extracts of total phase samples to extract the acetonitrile. After cleaning up and concentration, the extract was then filtered through a 0.22 μm nylon syringe filter (Titan 2, USA) and transferred into 2 mL screw cap amber glass vials (Agilent, USA) for analysis. The extraction and clean-up steps are detailed in the Supporting Information (Fig. S2). An Agilent 6460 liquid chromatograph (Agilent, Palo Alto, CA, USA) interfaced with triple quad liquid chromatograph tandem mass spectrometer was used to analyse the CPC and scan for its degradation products. The flow rate was 1 mL/min. Gradient elution procedure was as followed: acetonitrile (A) and 0.1% formic acid in water (B): time 0 min, 90% B; 5 min, 50% B; 8 min, 70% B; 15 min, 90% B; 20 min, 90% B. The LC–MS/MS was performed in multiple reac-