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A novel method of rural sewage disinfection via root extracts of hydrophytes

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ABSTRACT

Based on the model organisms of coliphages T4 and f2, this study aimed to select hydrophytes that has a strong inhibition to viruses and can provide the scientific basis for using hydrophyte allelochemicals to reduce the virus concentration in constructed wetlands (CWs). Double agar overlay method was used to evaluate the inhibition of hydrophyte root extracts on coliphage growth through the plaque forming unit assay. The effects on coliphages T4 and f2 of the root extracts from five hydrophytes (i.e., *Phragmites communis*, *Typha latifolia*, *Arundo donax*, *Polygonum hydropiper*, and *Polygonum orientale*) were investigated. Results showed that the inhibition of root extracts from *P. hydropiper* was more effective on T4 and f2 than on other hydrophytes. Median effective concentration (EC_{50}) of *P. hydropiper* root on coliphage T4 was 0.6 mg L^{-1}, and the EC_{50} on coliphage f2 was 7.6 mg L^{-1}. Five solvents, namely, petroleum ether, chloroform, ethyl acetate, n-butanol, and distilled water, were used to extract and separate allelochemicals, which can inhibit coliphage growth, from *P. hydropiper* root. The inhibition rate (IR) of saturated n-butanol phase on T4 and f2 was found to be the strongest, followed by the ethyl acetate phase. The major components were identified via gas chromatography–mass spectrometry. According to the outcome, purchasing four alternative substances of allelochemicals verified the inhibitory effect on coliphage. Results showed that all the IRs on coliphage were below 40% when the concentration of four synthesized compounds were 10 mg L^{-1}. To provide a novel method of rural sewage disinfection, the specific allelochemicals of *P. hydropiper* root, which strongly inhibit coliphages, still require further extraction, isolation, and evaluation. Moreover, the inhibitory mechanism should be discussed in depth.

1. Introduction

The improvement of rural living standards and the prosperity of small- (population less than 200,000) to medium-size (population between 200,000 and 500,000) urban areas in China (Zhang et al., 2009) have led to the rapid increase of the amount of domestic sewage. The majority of the domestic sewage of rural, small-size, and medium-size urban areas are usually discharged to the environment, untreated, and have become important non-point pollution sources of water quality deterioration in China (Li et al., 2010). The sewage treatment system of constructed wetlands (CWs) has been widely used because of its low investment, low energy consumption, high efficiency, easy management, and aesthetic value (Locke et al., 2011; Saeed et al., 2012; García et al., 2013).

Domestic sewage carries a large number of pathogenic microorganisms of original infectious diseases, including gastrointestinal disease-causing bacteria, intestinal viruses, and bacteriophage. If these pathogenic micro-organisms (including bacteria and viruses) in the sewage are not removed in a timely manner, they can be a huge threat to human health and the environment (Zheng, 2005).

Common disinfection methods in sewage include ultraviolet (UV), chemical disinfection, constructed soil filter (CSF), and membrane bioreactor (MBR). The main mechanism of UV disinfection is to change the nitrogen-containing heterocyclic in the DNA and RNA of a pathogen cell, with the wavelength ranging from 200 nm to 275 nm. Thus, it will alter the biological activity of the nucleic acid and prevent microorganisms from replicating. However, the UV method has several disadvantages, including large power consumption, higher costs (Qiang et al., 2013), inability to sustain sterilization, and a disinfection effect largely determined by turbidity (Brahmi et al., 2010); studies also show that UV disinfection is powerless against viruses less than 2 μm in diameter (Templeton et al., 2005). In the chemical disinfectant method, sodium hypochlorite (Chamakura et al., 2011), chloramines (Lee and Westerhoff, 2009;

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Huang et al., 2013), chlorine dioxide (Shin and Sobsey, 2008), ozone (Thurston-Enriquez et al., 2005) and other agents are added to sewage, but this results in higher costs, more complex monitoring, and even secondary pollution. The CSF system involves the integration of soil organic matter, microorganisms, and plant sewage. The three aspects of pathogen removal in CSF are as follows: (i) property of media to retain pathogens during filtration, i.e., bacterial adhesion; (ii) unsuitable physicochemical environment for pathogen survival in CSF; and (iii) predation of these pathogens to regenerate bed for further adhesion treatment limits the survival of pathogenic microorganisms in the soil (Kadam et al., 2008). However, CSF can easily lead to soil pollution, which will take a long time to recover. MBR is the combination of a membrane-based filtration process like microfiltration or ultrafiltration system with a suspended growth biological reactor (Marti et al., 2011). Many microorganisms can be removed by the membrane, such as *Escherichia coli*, sulphite-reducing Clostridium spores, and somatic coliphages (Zanetti et al., 2010), but it may cause membrane fouling. The characteristics of the abovementioned methods indicated the difficult of using them in a CW sewage treatment system. Thus, a novel and safe wastewater disinfection method for rural areas is urgently needed.

Numerous studies have proved that plants can produce allelochemicals that can restrain the growth of other organisms. Pyrogallic acid and gallic acid, polyphenols whose proportions are identified in a *Myriophyllum spicatum* cultured solution, can significantly inhibit the activity of photosystem II, which is considered as a target site in *Microcystis aeruginosa* (Zhu et al., 2010). Endogenous jasmonic acid was exuded from barnyard grass roots that elicit the production of rice allelochemicals (You et al., 2011). Ethyl 2-methylacetoacetate (EMA), the anti-algal allelochemical isolated from *Phragmites communis* Tris, had a significant inhibitory effect on the growth of *Chlorella pyrenoidosa*, and *M. aeruginosa* (Hong et al., 2008). The whole fruit aqueous or hydroalcoholic extracts of *Punica granatum* L. (Punicaceae) showed high activity against the influenza virus (Sanchez-Lamar et al., 2008). Root exudates from bean plants create auto toxicity in a non-renewed culture solution, which leads to growth retardation and poor yield (Asaduzzaman and Asao, 2012). However, studies on hydrophytes for disinfection in CWs have rarely been reported recently. Especially the root of the plant is of great importance due to their active micro-environment (rhizosphere) (Huang et al., 2012).

Viruses are usually proposed as indicators in water treatment processes, such as somatic coliphages (Wu et al., 2010; Marti et al., 2011), coliphages (Kadam et al., 2008; Chiemchaisri et al., 2011), and F-RNA specific bacteriophages (Zanetti et al., 2010; Marti et al., 2011). Two viruses with bacterial hosts, coliphages T4 and f2, were chosen in this research as surrogates for human enteroviruses to assess the root aqueous extracts of five common hydrophytes, namely, *P. communis* (*Punicaceae*), *Typha latifolia*, *Arundo donax*, *Polygonum hydropiper*, and *Polygonum orientale*. Coliphage T4 is dsDNA, with 65 nm × 95 nm of the body and 25 nm × 110 nm of the tail, similar to those of *Adenovirus*, *Reovirus*, and *Rotavirus*. Coliphage f2 is line ssRNA, 24–26 nm of the body, similar to *Poliovirus*, *Coxsackievirus*, *Echovirus*, *Norwalk agent*, and *Hepatitis* A virus (Zheng, 2005). These two coliphages were chosen to test the modes mainly because they are non-pathogenic to humans and can be seeded with high concentrations in tracer experiments, which, in turn, results in simple and rapid assays (Molleda et al., 2008). The objectives of our study were to (1) choose hydrophytes whose roots have a strong inhibition to coliphages T4 and f2 and calculate the median effective concentration values; (2) examine the contribution of the five organic extract fractions in hydrophytes' roots to virus and (3) identify the allelochemicals in the inhibitoriest extracts. This work will provide the scientific basis for using hydrophyte allelochemicals to reduce the virus concentration in CWs.

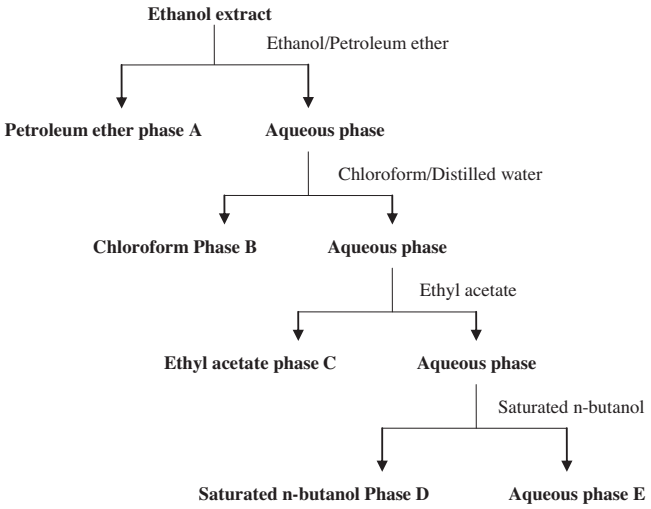

Fig. 1. Extraction procedures of allelochemicals from *P. hydropiper* root.

2. Materials and methods

2.1. Materials

Experiments were performed with the most widely available hydrophytes: *P. communis*, *T. latifolia*, *A. donax*, *P. hydropiper*, and *P. orientale*. The specimens were collected from Nansi Lake, Shan Dong province, China. Indicators of coliphages T4 and f2 and their host counterparts *E. coli* B and *E. coli* 285 were provided by Associate Professor Zheng Xiang from the School of Environment and Natural Resources, Renmin University of China.

2.2. Preparation of the hydrophyte root aqueous extracts

Specimens of the plant root were washed and air-dried under the shade (Noor Hashim et al., 2012), and then ground into powder. Next, 100 g powder was added into 1000 mL distilled water for 48 h in the dark, then the solution was centrifuged at 8000 rpm for 15 min. The supernatants were collected and filtered through a 0.45 μm membrane to obtain the aqueous extracts. Different volumes of extracts were added to distilled water to prepare the different concentrations (0, 0.05, 0.5, 5, and 50 g L^{-1}) of hydrophyte root aqueous extracts.

2.3. Extraction and separation of allelochemicals from P. hydropiper root

The powder specimens of *P. hydropiper* root were soaked in ethanol for 48 h in the dark (Li and Hu, 2005). The ethanol extract was then concentrated and dried at 40 °C under reduced pressure in an evaporator (EYELACCA-1110, Japan). The concentrated solution was separated by liquid–liquid extraction (Noor Hashim et al., 2012). The extraction procedure is shown in Fig. 1.

The ethanol extract was dissolved in 70 mL 90% ethanol and 30 mL petroleum ether, and then transferred to a separatory funnel. The mixture was shaken vigorously, then was settled to make the mixture separate into layers. After evaporation at 30 °C, fraction A