Biosafety of human environments can be supported by effective use of renewable biomass

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Edited by David Zilberman, Department of Agriculture and Resource Economics, University of California, Berkeley, CA; received July 8, 2021; accepted November 11, 2021

Preventing pathogenic viral and bacterial transmission in the human environment is critical, especially in potential outbreaks that may be caused by the release of ancient bacteria currently trapped in the permafrost. Existing commercial disinfectants present issues such as a high carbon footprint. This study proposes a sustainable alternative, a bioliquid derived from biomass prepared by hydrothermal liquefaction. Results indicate a high inactivation rate of pathogenic virus and bacteria by the as-prepared bioliquid, such as up to 99.99% for H1N1, H5N1, H7N9 influenza A virus, and *Bacillus subtilis* var. *niger* spores and 99.49% for *Bacillus anthracis*. Inactivation of *Escherichia coli* and *Staphylococcus epidermidis* confirmed that low-molecular-weight and low-polarity compounds in bioliquid are potential antibacterial components. High temperatures promoted the production of antibacterial substances via depolymerization and dehydration reactions. Moreover, bioliquid was innoxious as confirmed by the rabbit skin test, and the cost per kilogram of the bioliquid was $0.04427, which is notably lower than that of commercial disinfectants. This study demonstrates the potential of biomass to support our biosafety with greater environmental sustainability.

biomass | bioliquid | permafrost | human environments | biosafety

The importance of biosafety in the human environment has attracted significant attention due to global pandemic or endemic disease outbreaks that have threatened the global economy and life security. The main transmission of pathogenic microorganisms occurs via environmental media infected with bacteria or viruses (1, 2), such as the transmission of influenza A virus via the fecal–oral route (3). As permafrost gradually thaws due to global warming, ancient viruses and bacteria such as *Bacillus anthracis* can be released into the environment and cause severe health damage (4, 5). When global warming continues to intensify, such events may become more common on a global scale.

Disinfection is important to prevent the transmission of diseases. However, current commercial disinfectants have several disadvantages, including toxic byproducts and a high carbon footprint due to complex preparation processes (6). Permafrost exists in ecologically fragile areas, and a massive use of commercial disinfectants such as sodium hypochlorite can potentially lead to produce carcinogenic trihalomethanes (6). In addition, commercial disinfectants rely heavily on nonrenewable raw materials and thus are not conducive to sustainable development. Some commercial disinfectants are also not effective for spores (such as *B. anthracis*) of bacteria (7), due to its single component which also might spread a superbug (8). Therefore, renewable, environmentally friendly, and effective disinfectants are urgently needed to establish the biosafety of human environments.

Biomass is widely available and renewable, and has typical multiantibacterial structures, such as phenols and ketones (9). Although a known biomass pyrolysis-derived bioliquid (also named pyroligneous acid) has certain antibacterial components (10), its effective concentration is low (11). This challenge, however, can be overcome by the wet processing of biomass combined with subcritical hydrothermal liquefaction, which is a highly efficient technology for biomass cracking with the benefit of low temperature (12). In this study, a renewable disinfectant was developed from biomass and its effectiveness against highly pathogenic microorganisms was examined. The study demonstrates that effective use of biomass has the potential to support the biosafety of human environments with greater sustainability.

Results and Discussion

Low-Molecular-Weight and Low-Polarity Compounds Have Excellent Antibacterial Activity. *Escherichia coli* (gram-negative) and *Staphylococcus epidermidis* (gram-positive) are typical pathogenic bacteria (13). The inactivation rate of *E. coli* and *S. epidermidis* by bioliquid can reach to 100% (Fig. 1 *A* and *B*) due to damage to the cell wall (Fig. 1*C*). The half-maximal effective concentration (EC_{50}) was 2,015, 1,426, and 1,452 mg/L for *E. coli* and 782, 196, and 137 mg/L for *S. epidermidis* for the bioliquid produced at 210 °C, 270 °C, and 330 °C, respectively. These results suggest that the antibacterial activity of the bioliquid produced at high temperatures was better.

To identify the antibacterial components of bioliquid, the molecular structures of bioliquid were qualitatively investigated. The Van Krevelen diagrams of CHO chemicals suggest that the hydrothermal temperature promoted the transfer of compounds from lignin/carboxylic-rich alicyclic molecule–like structures to aromatic structures, as indicated by the downward-moving H/C values (Fig. 1*D*). Further, low-molecular-weight and low-polarity compounds were easily produced at high temperatures, which was demonstrated by the decrease of *m*/*z* and O/C ratios from 414.5 to 371.5 and from 0.423 to 0.375, respectively (Fig. 1*E*). Accordingly, the relatively abundant compounds with both $m/z > 400$ and oxygen number > 7 were clearly transformed to lower-molecular substances and lower-oxygen substances (Fig. 1*F*). This occurred because

Author contributions: F.Y., S.Z., and X.Z. designed research; F.Y., Wei Zhao, T.Q., Wang Zhao, Y.C., X.M., G.S., D.P., S.Z., and X.Z. contributed new reagents/analytic tools; F.Y., Wei Zhao, T.Q., Wang Zhao, Y.C., X.M., L.L., H.S., Y.Y., Y.Z., S.Z., and X.Z. analyzed data; and F.Y., Y.Y., Y.Z., S.Z., and X.Z. wrote the paper.

The authors declare no competing interest.

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This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2106843119/-/DCSupplemental.

Published January 10, 2022.

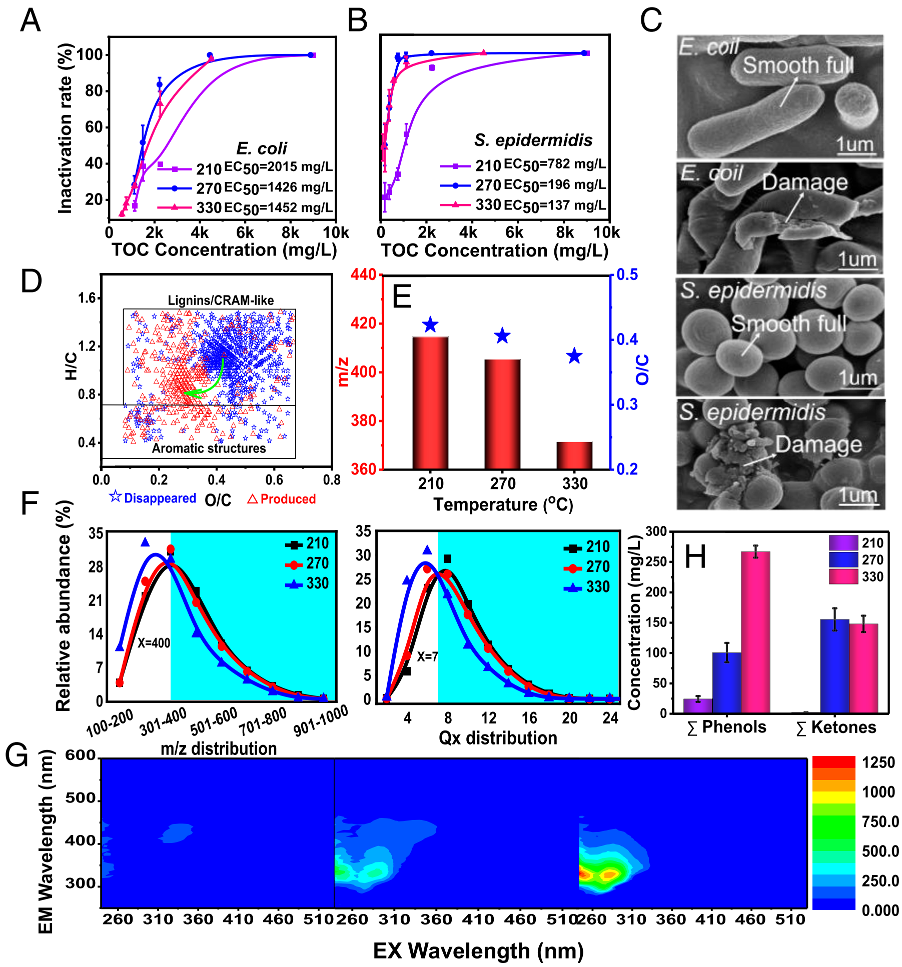

Fig. 1. The antibacterial components evaluation of bioliquid against *E. coli* and *S. epidermidis*. (*A* and *B*) Inactivation rate of *E. coli* and *S. epidermidis* treated by bioliquid. (*C*) SEM of *E. coli* (*Top*) and *S. epidermidis* (*Bottom*) before (first image) and after (second image) treatment by the bioliquid produced at 270 °C. (*D*) Van Krevelen diagram of CHO for the bioliquid produced at 330 °C compared to 210 °C. (*E*) The *m/z* and O/C ratio and (*F*) relative abundance of *m/z* and Ox distribution. (*G*) Fluorescence EEM spectra and (*H*) phenol and ketone concentrations of the bioliquid. The green arrow in *D* indicates the change direction of H/C and O/C mole ratio for the bioliquid produced at 330 °C, compared to 210 °C. TOC, total organic carbon.

lignocellulose was gradually depolymerized and dehydrated, leading to bond breaks and oxygen separation with an increasing hydrothermal temperature (12). The excitation–emission matrix (EEM) results further suggested that substances at 239/330 nm and 275/312 nm (excitation/emission) were related to phenol-like compounds produced by lignin depolymerization (12, 14). The fluorescence intensity increased notably with the increasing hydrothermal temperature, which demonstrated that its concentration increased (Fig. 1*G*). Similarly, gas chromatography mass spectrometry (GC-MS) (Fig. 1*H*) illustrated that the concentrations of low-boiling phenols and ketones (low-molecular-weight compounds) and those of the typical antibacterial components (15) were high for bioliquid yielded at 270 °C and 330 °C. Therefore, it can be concluded that the bioliquid produced at high hydrothermal temperatures (270 °C and 330 °C) presented better inactivation effects because of the high concentration of low-molecular-weight and low-polarity compounds.

Broad-Spectrum Potential of the Bioliquid for the Inactivation of Typical Pandemic-Causing Pathogens. To further investigate the broad-spectrum potential and response to pandemic outbreaks, the inactivation of the influenza A virus (H1N1, H5N1, and H7N9) and *B. anthracis* by bioliquid produced at 270 °C was tested in a biosafety level 3 laboratory. Quartz sand (the main component of soil) and masks were simulated as H1N1 and *B. anthracis* carriers. The cell viability was 100%, and was innoxious after bioliquid was diluted to less than 1.23% for Madin–Darby Canine Kidney (MDCK) cells (Fig. 2*A*). The inactivation rates of H1N1 (mask and quartz sand as carriers), H5N1, and H7N9 were all 99.99% (Fig. 2*B*), and that of *B. anthracis* (quartz sand as carrier) was 99.49%. The results strongly indicate that the carriers did not influence the inactivation of H1N1, and bioliquid can potentially prevent the transmission of pandemic-causing bacteria and viruses. In addition, the inactivation rate of *Bacillus subtilis* var. *niger* spores (a typical challenge bacterium used to evaluate the disinfection effect) was 99.99%, indicating that the disinfection function of bioliquid had a broad spectrum. Furthermore, the bioliquid was innoxious to rabbit skin (Fig. 2 *C* and *D*), which suggests that this bioliquid is safe in case people or animals unwittingly touch it. Moreover, the results of earthworm toxicity evaluation indicated that bioliquid was safer than phenolic disinfectant and hypochlorite disinfectant (Fig. 2*E*). The inactivation rates of *E. coli* and *S. epidermidis* by bioliquid derived from different types of biomass were all up to 100%, which indicated that bioliquid derived from all types of biomass has a great antimicrobial ability. The result of EC_{50} further indicated that biomass with high lignocellulose and low ash content may be preferable for the inactivation effects (Fig. 2 *F* and *G*). The potential production quantity and cost of this bioliquid was estimated to be 113.02 million tons and $0.04427/kg, respectively,

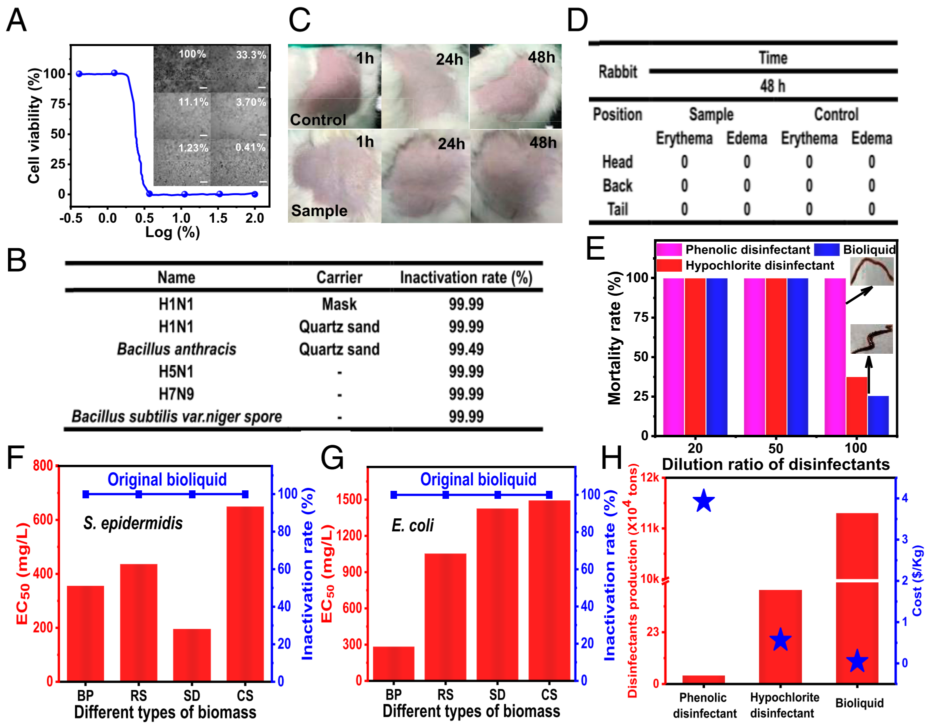

Fig. 2. Inactivation of epidemic-causing pathogens and disposable skin tests of the bioliquid produced at 270 °C. (*A*) Cytotoxicity of the bioliquid to MDCK cells. (Scale bars, 20 μm.) (*B*) Inactivation of H1N1, H5N1, H7N9, *B. anthracis*, and *Bacillus subtilis* var. *niger* spores. (*C* and *D*) Disposable skin tests for rabbits. (*E*) The earthworm toxicity evaluation. (*F* and *G*) Bioliquid derived from different types of biomass for *S. epidermidis* and *E. coli* inactivation. (*H*) The potential production of the bioliquid as a disinfectant, and the corresponding cost per kilogram in China.

which are beneficial for large-scale production, due to the abundance and low cost of renewable materials compared to those of the currently available commercial disinfectants (Fig. 2*H*).

Overall, the developed bioliquid is a good candidate for the prevention of an outbreak and for establishing better biosafety of human environments by inactivating H1N1, H5N1, H7N9, and *B. anthracis*. In addition, this bioliquid has a broad spectrum of action and a low cost, as it is obtained from renewable biomass, which is advantageous for large-scale applications. Given the potential demonstrated in this study, the separation method for the enrichment of low-molecular-weight and low-polarity compounds should be more broadly studied to promote the commercial application of bioliquid.

Materials and Methods

Bioliquid was acquired from biomass by hydrothermal liquefaction at different temperatures, and its effectiveness against highly pathogenic microorganisms such as H1N1, H5N1, H7N9, and *B. anthracis* was tested. In addition, the correlation between inactivation effects and the molecular structure of bioliquid was estimated by inactivating *E. coli* and *S. epidermidis*. For more detailed information, see *SI Appendix* for the extended materials and methods.

Data Availability. All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. This research was supported by National Natural Science Foundation of China (Grant 21876030) and the Natural Science Foundation of Shanghai (Grant 19ZR1403800).

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