Advanced Powder Technology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Advanced Powder Technology

journal homepage: www.elsevier.com/locate/apt

Original Research Paper

Adhesion control of fungal spores on solid surfaces using hydrophilic nanoparticles

Toshiyuki Nomura [a,*], Mana Minamiura [a], Kazuto Fukamachi [a], Shohei Yumiyama [a], Akira Kondo [b], Makio Naito [b]

[a] Department of Chemical Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan
[b] Joining and Welding Research Institute, Osaka University, 11-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan

ARTICLE INFO

Article history:
Received 28 October 2017
Received in revised form 22 December 2017
Accepted 15 January 2018
Available online xxxx

Keywords:
Fungal spores
Adhesion
Adhesive force
Hydrophilic treatment
Silica nanoparticles

ABSTRACT

Mold growth can trigger a variety of serious problems such as allergies and asthma. Designing surfaces that are unfavorable for the adhesion of fungal spores is considered an effective method to prevent fungal growth. In this study, the effect of hydrophilic surface treatment on the adhesion of fungal spores onto substrates was investigated using *Aspergillus oryzae* as a model fungus. The fungal spores that strongly adhered on the hydrophilic substrates under atmospheric conditions were easily removed by lightly washing by hand in water. These experimental results agreed well with thermodynamic predictions based on contact angle measurements. In addition, the removal ratio of the fungal spores on substrates coated with silica nanoparticles was higher than that on plasma-treated glass. It is believed that the contact area between a spore and substrate depended on the substrate roughness. Atomic force microscopy revealed that there was almost no adhesive force between the spores and glass substrate coated with silica nanoparticles. These results suggest that hydrophilic treatment using hydrophilic silica nanoparticles is more effective than hydrophilic plasma treatment to prevent fungal spore adhesion on glass substrates.

1. Introduction

Molds are ubiquitous and their growth indoors can cause a variety of serious problems such as allergies and asthma [1–5]. Molds are a group of fungi that are characterized by having filamentous hyphae. Hydrophobins are a group of amphiphilic proteins expressed by fungal and are widely distributed throughout the fungal kingdom but not found in other organisms [6,7]. Hydrophobin monomers are secreted at the tip of the growing hypha. In the atmosphere, hydrophobin monomers secreted by emerging aerial hypha cannot diffuse into the air and assemble at the cell wall–air interface, changing the surface of fungal cells from hydrophilic to hydrophobic. In contrast, hydrophobin monomers diffuse to the medium–air interface in aqueous environments and the surface of the submerged fungal cells retain their hydrophilic nature [8,9]. Therefore, in the atmosphere, fungal spores that allow fungi to reproduce also have hydrophobic surfaces and readily adhere onto various kinds of solid interfaces. Once favorable growth conditions for fungal spores (temperature, humidity, and nutrition) are present, the spores start to grow and spread extensively and can cause a variety of serious problems. Designing surfaces that are unfavorable for the adhesion of fungal spores is considered an effective method to prevent fungal problems.

Fungal spores are microscopic biological particles. The surface physicochemical properties of substrates can affect the colloidal behavior (dispersion and adhesion) of spores. To control the adhesion of fungal spores, it is important to know the interaction between spores and substrates. However, it is difficult to estimate the biological interaction of spores because of their heterogeneous surfaces. Atomic force microscopy (AFM) is a powerful tool to measure biological surface forces because it can directly measure the interaction forces between surfaces [10,11]. Several AFM studies of spore surface force measurements using a spore probe have been reported [12–14].

In this study, the effect of hydrophilic surface treatment on the adhesion of fungal spores onto substrates is investigated. *Aspergillus oryzae* is used as a model fungus. Two kinds of surface hydrophilic treatment are carried out: plasma treatment and coating with hydrophilic nanoparticles. The contact angles for various probe liquids with different polarities are measured using the sessile drop technique. The surface tensions of substrates and the change of free energies when fungal spores adhere onto each substrate are calculated. The stability of fungal adhesion on the

* Corresponding author.
E-mail address: nomura@chemeng.osakafu-u.ac.jp (T. Nomura).

https://doi.org/10.1016/j.apt.2018.01.007
0921-8831/

substrates is estimated thermodynamically. Furthermore, the adhesive force between a single spore and each substrate is directly measured using a colloidal AFM probe technique.

2. Experimental

2.1. Fungal strain and growth conditions

Aspergillus oryzae niaD300 was used as a model fungal strain. The fungus was grown on an agar plate at 30 °C for 4 days in standard minimal medium containing appropriate supplements [15].

2.2. Preparation of glass substrates

Three kinds of glass substrate were prepared: glass slides (S2815, Matsunami Glass, Kishiwada, Japan) (denoted as untreated glass), hydrophilic glass slides treated with plasma (denoted as plasma-treated glass), and glass slides coated with hydrophilic fumed silica with a Brunauer–Emmett–Teller equivalent diameter of 7 nm (Aerosil 300, Nippon Aerosil, Tokyo, Japan) (denoted as nanosilica-coated glass). The plasma-treated glass was prepared using a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY, USA) operated at the middle level under reduced air pressure for 5 min. The nanosilica-coated glass was prepared using the attrition-type milling apparatus shown in Fig. 1 following a modified procedure described previously [16,17]. A rotor with oval-shaped heads was set in the chamber of the apparatus. Strong compressive force was induced at the powder bed between the rotor head and chamber wall. The inner diameter of the chamber was 150 mm. The rotor and chamber were made from stainless steel. Untreated glass (1 cm^2) was fixed on the chamber wall. The gap between the rotor head and substrate was 1 mm. Fumed silica (15 g) was put in the chamber. NH_4HCO_3 (0.4 g) was also added to form a powder bed during processing. The silica nanoparticles and substrate were then mechanically processed at 4000 rpm for 30 min. Finally, the treated substrate was detached from the chamber and heated at 120 °C for 12 h. Prior to use, each substrate was sonicated in ultrapure water for 5 min, rinsed with ethanol, and then dried in a clean box.

2.3. Atomic force microscopy imaging

The surfaces of the glass substrates and the fungal spore were imaged using an AFM (MFP-3D-BIO-J, Oxford Instruments Asylum Research, Santa Barbara, CA, USA) integrated with an inverted optical microscope (Eclipse TE2000, Nikon, Tokyo, Japan) in tapping mode in an air-conditioned laboratory (24 ± 2 °C). AFM imaging was performed using a silicon cantilever probe (OMCL-AC200TS, Olympus, Tokyo, Japan) with a nominal spring constant of 9 N/m at a scan speed of 0.2 Hz and 1024 pixels per line scan. The root mean square (RMS) roughness of the glass substrates was measured by the embedded software in the AFM system.

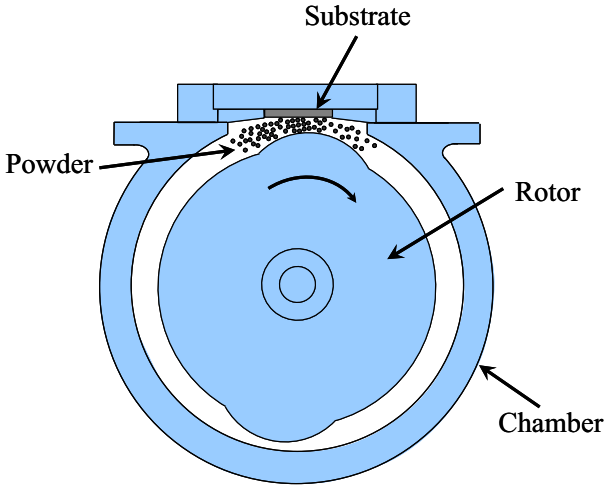

Fig. 1. Attrition-type milling apparatus used for powder coating on substrates.

2.4. Contact angle measurement

The contact angles between the glass substrates and specified probe fluids (water, formamide, and α-bromonaphthalene) were measured using a contact angle analyzer (FTA125, First Ten Ångstroms, Portsmouth, VA, USA) following a previously described procedure [18,19].

2.5. Surface energy estimation

The total surface tension γ^{Total}, Lifshitz–van der Waals component γ^{LW}, and acid–base component γ^{AB}, which can be expressed as $2\sqrt{\gamma^+\gamma^-}$, where γ^- and γ^+ indicate the electron donor and acceptor, respectively. The surface tensions were calculated from at least five independently measured contact angles using the van Oss approach [20,21].

2.6. Calculation of Gibbs free energy

The adhesion of fungal spores onto the substrate was estimated using the change in Gibbs free energy (ΔG^{Total}), defined as the sum of free energy changes in the Lifshitz–van der Waals (ΔG^{LW}) and acid-base (ΔG^{AB}) interactions [20,22]:

$$\Delta G^{LW} = -2\left(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}}\right)\left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}}\right) \quad (1)$$

$$\begin{aligned}\Delta G^{AB} = {} & 2\left(\sqrt{\gamma_B^+} - \sqrt{\gamma_S^+}\right)\left(\sqrt{\gamma_B^-} - \sqrt{\gamma_S^-}\right) \\ & - 2\left(\sqrt{\gamma_B^+} - \sqrt{\gamma_L^+}\right)\left(\sqrt{\gamma_B^-} - \sqrt{\gamma_L^-}\right) \\ & - 2\left(\sqrt{\gamma_S^+} - \sqrt{\gamma_L^+}\right)\left(\sqrt{\gamma_S^-} - \sqrt{\gamma_L^-}\right) \quad (2)\end{aligned}$$

where the subscripts B, L, and S refer to the fungal spores, dispersion liquid, and substrate, respectively.

2.7. Removal of adhered spores from substrates

The lawn of fungal spores formed on the agar plate was traced carefully with a glass substrate (1 cm^2) without touching the agar, causing the spores to be fully adhered on the substrate. First, the surface of the substrate was blown with an air duster for 20 s. Next, the edge of the substrate was grasped by tweezers and immersed in pure water. The substrate was then washed by shaking by hand for 20 s in the water. Finally, the substrate after washing by hand was immersed in fresh pure water and then washed in an ultrasonic cleaner for 20 s. The adhered spores on the surface of the substrate were observed using an optical microscope (BX60, Olympus) with a 20× objective lens.

2.8. Preparation of a single spore probe

A tipless cantilever (MLCT-O10, Bruker, nominal spring constant = 0.6 N/m) was used to prepare a single spore probe. The cantilever was sequentially rinsed with chloroform, ethanol, and deionized water for 15 min each. The cantilever was cleaned using the plasma cleaner operated at the middle level under reduced air pressure for 5 min. The cleaned cantilever was immersed in 5 mM NaOH solution for 15 min and then rinsed with deionized water.

Please cite this article in press as: T. Nomura et al., Adhesion control of fungal spores on solid surfaces using hydrophilic nanoparticles, Advanced Powder Technology (2018), https://doi.org/10.1016/j.apt.2018.01.007