growth rates. These and other challenges to submerged fermentation of filamentous microorganisms require innovative solutions to effectively harness the benefits of these organisms in application where resources are limited, e.g. aboard spacecraft or space stations or in challenging terrestrial environments.

More recently, some significant strides have been made in the development of systems and methods for culturing filamentous fungi that do not require active aeration or agitation of the liquid culture, particularly to produce biomats with significant tensile strength. However, the availability of oxygen to the fungus can still present a challenge even in these newer methods and systems. In addition, without wishing to be bound by any particular theory, it is believed that the surface area of the fungus/feedstock interface may also be a limiting factor in the growth of filamentous fungi in these applications.

There is thus a need in the art for systems and methods for culturing filamentous fungi in fermentation media that overcomes these and other drawbacks of submerged fermentation. It is further advantageous for such systems and methods to provide the fungus with greater oxygen availability and/or surface area in contact with a feedstock than previous systems and methods.

SUMMARY

It is one example of the present disclosure to provide a method for culturing a filamentous fungus in a fermentation medium, comprising (a) aerating the fermentation medium to provide an air-medium colloid (AMC); and (b) culturing the filamentous fungus in or on the AMC to form a biomass of the filamentous fungus. In one aspect, the present disclosure refers to a method for culturing a filamentous fungus in a fermentation medium, comprising: (a) aerating the fermentation medium to provide an air-medium colloid (AMC); and (b) culturing the filamentous fungus in or on the AMC to form a biomass of the filamentous fungus in the form of a coherent mat.

In examples, the AMC may be used as a self-contained bioreactor system that isolates the filamentous fungus from a surrounding environment, thus providing a fungal growth system that does not require environmental control.

In examples, the AMC may comprise a gelation agent, a viscosity modifier, and/or a humectant or other water activity-reducing component.

In examples, the AMC may comprise an inoculum of the filamentous fungus.

In examples, the method may further comprise inoculating the AMC with an inoculum of the filamentous fungus.

In examples, the AMC may comprise a stabilizer. The stabilizer may, but need not, comprise xanthan gum. A mass ratio of the fermentation medium to the stabilizer in the AMC may, but need not, be between about 100:1 and about 1,000:1. The stabilizer may, but need not, be selected from the group consisting of polysaccharide gums (e.g. xanthan gum, guar gum, locust bean gum, konjac root gum), anionic surfactants (e.g. carboxylates,

phosphate esters, sulfate esters, sulfonate esters), cationic surfactants (e.g. primary, secondary, or tertiary amines, quaternary ammonium salts), ceteareth 20, cellulose, diacetyl tartaric esters of mono- and diglycerides (DATEM), diglycerides, emulsifying wax, glycerol monostearate, lecithins, monoglycerides, mustards, non-ionic surfactants (e.g. polysorbate 20, polysorbate 80), soaps, sodium phosphates, sodium stearoyl lactylate, zwitterionic surfactants, saponins, starches, modified starches, plant protein surfactants (e.g. soy protein isolate, pea protein isolate), animal protein surfactants (e.g. casein, whey protein isolate), microparticulates, silica, and combinations and mixtures thereof.

In examples, a volume fraction of air in the AMC may be between about 0.05 and about 0.95, between about 0.1 and about 0.9, between about 0.2 and about 0.8, between about 0.3 and about 0.7, between about 0.4 and about 0.6, or about 0.5.

In examples, the AMC may be a foam that is stable over at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, or at least about 30 days.

In examples, the filamentous fungus may belong to an order selected from the group consisting of *Ustilaginales*, *Russulales*, *Polyporales*, *Agaricales*, *Pezizales*, and *Hypocreales*.

In examples, the filamentous fungus may belong to a family selected from the group consisting of *Ustilaginaceae*, *Hericiaceae*, *Polyporaceae*, *Grifolaceae*, *Lyophyllaceae*, *Strophariaceae*, *Lycoperdaceae*, *Agaricaceae*, *Pleurotaceae*, *Physalacriaceae*, *Omphalotaceae*, *Tuberaceae*, *Morchellaceae*, *Sparassidaceae*, *Nectriaceae*, and *Cordycipitaceae*.

In examples, the filamentous fungus may belong to a species selected from the group consisting of *Ustilago esculenta*, *Hericulum erinaceus*, *Polyporous squamosus*, *Grifola fondosa*, *Hypsizygus marmoreus*, *Hypsizygus ulmarius*, *Calocybe gambosa*, *Pholiota nameko*, *Calvatia gigantea*, *Agaricus bisporus*, *Stropharia rugosoannulata*, *Hypholoma lateritium*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Tuber borchii*, *Morchella*

esculenta, *Morchella conica*, *Morchella importuna*, *Sparassis crispa*, *Fusarium venenatum*, MK7 ATCC Accession Deposit No. PTA-10698, *Disciotis venosa*, and *Cordyceps militaris*.

In examples, the method may further comprise, prior to or during step (b), adding a food-grade or food-safe additive to the AMC.

It is another example of the present disclosure to provide a filamentous fungal biomat, produced by a method as described herein. In one aspect, the present disclosure refers to a filamentous fungal biomat having a dry density of at least about 0.20 g/cm^3 and a tensile strength of at least about 255 kPa, produced by a method comprising: (a) aerating a fermentation medium to provide an air-medium colloid (AMC); and (b) culturing a filamentous fungus in or on the AMC to form a biomass of the filamentous fungus in the form of a coherent mat. In another aspect, the present disclosure refers to a filamentous fungal biomat, having a dry density of at least about 0.20 g/cm^3 and a tensile strength of at least about 255 kPa.

In examples, the biomat may have at least one of the following properties: (a) a thickness of at least about 1.75 mm; (b) a mass of at least about 295 grams per square meter of a top surface area of the AMC; (c) a dry density of at least about 0.20 g/cm^3; (d) a tensile strength of at least about 255 kPa; and (e) a carbohydrate content of at least about 47 wt% when dry.

It is another example of the present disclosure to provide a filamentous fungal biomat, having at least one of the following properties: (a) a thickness of at least about 1.75 mm; (b) a mass of at least about 295 grams per square meter of a top surface area of the AMC; (c) a dry density of at least about 0.20 g/cm^3; (d) a tensile strength of at least about 255 kPa; and (e) a carbohydrate content of at least about 47 wt% when dry.

In examples, a biomat as described herein may comprise a stabilizer.

In examples, a biomat as described herein may comprise a food-grade or food-safe additive.

It is another example of the present disclosure to provide a foodstuff, comprising at least a portion of a biomat as described herein. In one aspect, the present disclosure refers to a foodstuff comprising any one of the biomats disclosed herein.

It is another example of the present disclosure to provide a structural material, comprising at least a portion of a biomat as described herein. In one aspect, the present disclosure refers to a structural material comprising any one of the biomats disclosed herein.

It is another example of the present disclosure to provide a textile material, comprising at least a portion of a biomat as described herein. In one aspect, the present disclosure refers to a textile material comprising any one of the biomats disclosed herein.

It is another example of the present disclosure to provide an air-medium colloid (AMC), comprising a fermentation medium; and air, colloidally dispersed throughout the fermentation medium.

In examples, the AMC may further comprise a stabilizer.

In one aspect, the present disclosure refers to a composition, comprising: an air-medium colloid (AMC), comprising: a fermentation medium; and air, colloidally dispersed throughout the fermentation medium; and a biomass, in the form of a coherent mat, of a filamentous fungus, cultured in or on the AMC.

For purposes of further disclosure and to comply with applicable written description and enablement requirements, the following references are incorporated herein by reference in their entireties:

Elka S. Basheva et al., "Unique properties of bubbles and foam films stabilized by HFBII hydrophobin," 27(6) *Langmuir* 2382 (Feb. 2011).

Graeme P. Boswell and Fordyce A. Davidson, "Modelling hyphal networks," 26(1) *Fungal Biology Reviews* 30 (Apr. 2012).

Andrew R. Cox et al., "Exceptional stability of food foams using class II hydrophobin HFBII," 23(2) *Food Hydrocolloids* 366 (Mar. 2009).

Monika S. Fischer and N. Louise Glass, "Communicate and fuse: how filamentous fungi establish and maintain an interconnected mycelial network," 10 *Frontiers in Microbiology* 619 (Mar. 2019).

Luke Heaton et al., "Analysis of fungal networks," 26(1) *Fungal Biology Reviews* 12 (Apr. 2012).

While specific examples and applications of the present disclosure have been illustrated and described, it is to be understood that the disclosure is not limited to the precise configuration and components described herein. Various modifications, changes, and variations which will be apparent to those skilled in the art may be made in the arrangement, operation, and details of the methods and systems of the present disclosure disclosed herein without departing from the spirit and scope of the disclosure. It is important, therefore, that the claims be regarded as including any such equivalent construction insofar as they do not depart from the spirit and scope of the present disclosure.

The advantages of the present disclosure will be apparent from the disclosure contained herein.

As used herein, "at least one," "one or more," and "and/or" are open-ended expressions that are both conjunctive and disjunctive in operation. For example, each of the expressions "at least one of A, B, and C," "at least one of A, B, or C," "one or more of A, B, and C," "one or more of A, B, or C," and "A, B, and/or C" means A alone, B alone, C alone, A and B together, A and C together, B and C together, or A, B, and C together.

It is to be noted that the term "a" or "an" entity refers to one or more of that entity. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising," "including," and "having" can be used interchangeably.

The examples and configurations described herein are neither complete nor exhaustive. As will be appreciated, other examples of the disclosure are possible utilizing, alone or in combination, one or more of the features set forth above or described in detail below.

CLAIMS

1. A method for culturing a filamentous fungus in a fermentation medium, comprising:

(a) aerating the fermentation medium to provide an air-medium colloid (AMC); and

(b) culturing the filamentous fungus in or on the AMC to form a biomass of the filamentous fungus in the form of a coherent mat.

2. The method of claim 1, wherein the AMC comprises an inoculum of the filamentous fungus.

3. The method of claim 1, further comprising inoculating the AMC with an inoculum of the filamentous fungus.

4. The method of claim 1, wherein the AMC comprises a stabilizer.

5. The method of claim 1, wherein a volume fraction of air in the AMC is between about 0.05 and about 0.95.

6. A filamentous fungal biomat having a dry density of at least about 0.20 g/cm^3 and a tensile strength of at least about 255 kPa, produced by a method comprising:

(a) aerating a fermentation medium to provide an air-medium colloid (AMC); and

(b) culturing a filamentous fungus in or on the AMC to form a biomass of the filamentous fungus in the form of a coherent mat.

7. The biomat of claim 6, further comprising a stabilizer.

8. The biomat of claim 6, further comprising a food-grade or food-safe additive.

9. The biomat of claim 6, having at least one of the following properties:

(a) a thickness of at least about 1.75 mm;

(b) a mass of at least about 295 grams per square meter of a top surface area of the AMC; and

(c) a carbohydrate content of at least about 47 wt% when dry.

10. The biomat of claim 6, wherein the AMC further comprises an inoculum of the filamentous fungus.

11. A filamentous fungal biomat, having a dry density of at least about 0.20 g/cm^3 and a tensile strength of at least about 255 kPa.

12. The biomat of claim 11, having a thickness of at least about 1.75 mm.

13. The biomat of claim 11, having a mass of at least about 295 grams per square meter of a top surface area of a growth medium on which the filamentous fungal biomat is formed.

14. The biomat of claim 11, having a carbohydrate content of at least about 47 wt% when dry.

15. The biomat of claim 11, further comprising a stabilizer.

16. A foodstuff comprising the biomat of any one of claims 6-15.

17. A structural material comprising the biomat of any one of claims 6-15.

18. A textile material comprising the biomat of any one of claims 6-15.

19. A composition, comprising:

an air-medium colloid (AMC), comprising:

a fermentation medium; and

air, colloidally dispersed throughout the fermentation medium; and

a biomass, in the form of a coherent mat, of a filamentous fungus, cultured in or on the AMC.

20. The composition of claim 19, wherein the AMC further comprises a stabilizer.

21. The composition of claim 19, wherein a volume fraction of air in the AMC is between about 0.05 and about 0.95.

22. The composition of claim 19, wherein the AMC is a foam that is stable over at least about 1 day.

23. The composition of claim 19, wherein the filamentous fungus belongs to an order selected from the group consisting of *Ustilaginales*, *Russulales*, *Polyporales*, *Agaricales*, *Pezizales*, and *Hypocreales*.