Tan DT, Poh PE, Chin SK.

[Microorganism preservation by convective air-drying—A review.](#)

Drying Technology 2018

DOI: <https://doi.org/10.1080/07373937.2017.1354876>

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This is an Accepted Manuscript of an article published by Taylor & Francis in *Drying Technology* on 19/07/2017, available online: <https://doi.org/10.1080/07373937.2017.1354876>

Date deposited:

30/03/2018

Embargo release date:

19 July 2018

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Journal:	<i>Drying Technology</i>
Manuscript ID	LDRT-2016-0548.R1
Manuscript Type:	General Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Tan, David; Monash University - Malaysia Campus, Chemical Engineering Poh, Phaik Eong; Monash University - Malaysia Campus, Chemical Engineering Chin, Siew Kian; Newcastle University International Singapore
Keywords:	Cell survival, Dehydration inactivation, Heat pump drying, Hot air circulation oven drying, Spray drying, Thermal inactivation

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Microorganism Preservation by Convective Air Drying – A Review

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Abstract

At present, microorganisms are mainly preserved by freeze drying. There is, however, lack of studies conducted on various cheaper yet promising convective air drying alternatives. Convective air drying has been proven to produce dried culture with comparable cell survival and final moisture content to that of freeze drying. This paper aims to draw an understanding to application and suitability of convective air drying which includes spray-, oven, heat pump, fluidised bed, conveyor, and rotary drying to preserve microorganisms. The paper concludes that drying near ambient temperature and the addition of dehydration protectants are important to obtain satisfactory drying quality.

Keyword: cell survival, dehydration inactivation, heat pump, hot air circulation oven, spray drying, thermal inactivation

1 Introduction

Almost all of the natural-occurring products in our universe are readily degradable, or perishable, and will continue to grow, change, adapt and eventually be broken down back into nature [1]. Preservation comes into play as a mean to protect and suspend the object in its current desired state so that it can be of use at a later time and as part of maintaining the continuity of certain biological identity [2].

Preservation is widely practiced in the food industry with the aim to prevent food spoilage through the inactivation of bacteria, viruses, and yeasts [3]. Preservation of starter cultures is highly important in industries such as food and pharmaceutical [4].

In recent years, this concept has been aggressively extended to the area of microbiology as while the bulk of biodiversity on earth is dominated by microorganisms, only 10% of it has been characterised [5]. In addition, microorganisms play an essential role in recycling earth's naturally-occurring matters through biogeochemical cycle which mainly includes the hydrologic (water) cycle, carbon cycle, nitrogen cycle, sulphur cycle, and metal cycle [6]. All in all, having the preserved form of microorganism offers a wider range of applications for a substantially longer period of time as compared to its unpreserved liquid or slurry form [7]. In the area of scientific and industrial development, preservation makes it possible for various applications including observation of cells preserved on microscope slides, starter cultures for direct inoculation to fermenter or biological tank (e.g. yeasts, mesophilic mixed culture), bio-control agents such as bio-pesticides and bio-preservatives (e.g. *Lactobacillus plantarum*, *Beauveria brongniartii*) [8–10]. Moreover, preservation has also fueled the improvement of health-related products such as tablets containing beneficial probiotics, functional supplements in food products, and in fermented food products [8].

One of the areas that this paper focuses on is the development of preservation in industries that require a ready-to-use microbial starter cultures. Although it is traditionally possible to culture strains of bacteria from scratch, it is much more preferable to culture microorganism from a preserved starter cultures. This is particularly useful to obtain **rehydratable cell-culture with desired properties** which

has been well-developed prior to preservation process [11]. In contrast, newly cultured colonies often take after a relatively generic property from which the seed culture is derived, and thus require time to adapt [11]. Although adaptation period could be as short as 25 days in circumstances where the type of applications are highly similar [12], most applications are fairly distinctive and requires 100 or even up to 400 days of adaptation period [13,14]. For instance, utilising certain mixed culture from a one wastewater treatment plant to treat other type of wastewater requires a period of acclimatisation [15]. Starter culture was also proven to be useful in wastewater treatment applications where it has been reported to reduce operational problems at the early stage of the start-up process [16].

In general, preservation of microorganisms is based on suspending the microorganisms in an anabiosis state, where metabolism activity is much lower [17]. In this state, the bacteria can be stored away for longer time without the need of food [18]. Perhaps the oldest known method of preservation is drying, which was first applied to lengthen the shelf-life of food products [19]. Drying works on the concept of lowering moisture content of bioproducts, usually to approximately 5-8%, where biodegradation that might be caused by activity of microorganisms, enzymes, or non-enzymic chemical reactions is inhibited [20–22]. Similar concept can be applied to preserve microorganisms by removing water in the culture medium and thus suspending microbial activity [8]. Moreover, putting microbiological cultures in dry state enables easier transportation, storage, reduced odour issue and even quality control of certain microbial cultures [8,23].

At the moment, the most popular drying method for microorganism is freeze-drying due to its reliability in term of cell viability and relatively long storage time [24]. Freeze drying, however, is also one of the most expensive drying options as it operates at extremely low temperature and vacuum condition over a considerably long drying time [25,26]. Besides, there had been reports of intermittent instability and reduced shelf life due to hygroscopic and amorphous nature of freeze-dried products [27]. More economical drying methods to preserve microbial cultures are therefore highly desirable. Studies have shown that convective air drying is a promising technique as it often operates at near

1
2
3 ambient temperature and mild processing conditions [28]. Such condition is hypothesised to be
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5 favourable towards high cell survival of microorganisms [29].
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8 Majority of studies and reviews, however, largely revolve around freeze-drying and cryogenic
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10 preservation which are both costly [8,15,30,31]. Although convective air drying has been used to
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12 preserve many foodstuffs, research on its applicability for bacteria preservation is still limited [32–
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14 36]. This paper is aimed to critically review work on preservation of microbes; looking into the
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16 factors that will affect the cell viability in convective air drying and to suggest future directions on the
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18 use of convective air drying for microbial preservation.
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20 21 **2 Methods for microorganism preservation**

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23 While there are many means to preserve a desired culture of microorganisms, some methods might be
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25 more suitable than the other depending on the purpose of preservation. Based on applicability of the
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27 techniques, preservation methods can be distinguished into those that are limited to just laboratory
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29 scale and those that can be applied in industry, as some pilot techniques could be simple and reliable
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31 but are not scalable [15]. The key difference between laboratory and industrial scale is the amount of
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33 culture that needs to be salvaged [26]. For laboratory purposes, low cell survival is sufficient whereas
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35 large quantity (and therefore high cell survival) is required for industrial usage[15]. Typical laboratory
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37 preservation techniques include smearing and storing on agar or gelatine, cell immersion in paraffin
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39 oil, and the adsorption-desiccation of bacteria culture on filter-paper or on pre-dried plugs of starch or
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41 silica gel[15,37,38].
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44 High number of successfully preserved viable cells is particularly important to ensure possible direct
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46 inoculation to the process fluid where the bacterial culture is to be utilised [11]. The laboratory
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48 preservation methods mentioned above are unsuitable due to the complexity of the process,
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50 requirement of additives, and low recovery rate [15]. Preservation methods which are recognised to be
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52 practical for industrial use are subcultivation, freezing, and drying [39,40].
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Of the methods mentioned above, preservation by drying is deemed to be most desirable from economic perspective as it produces compact, easily stored, and relatively lightweight final product [41,42]. On top of that, obtaining a mixed culture by rehydrating dried cultures often leads to better purity, relative to subcultivation where culture is obtained through culturing a small amount of stock culture, which both consumes time and risks contamination [15]. Generally, the main objective for drying process is to produce a dried product of desired quality at a minimum cost. However, drying is one of the most energy-intensive unit operations that could easily take up to 15% of all industrial energy utilisations [43]. For instance, drying process accounts for 50%, 60%, and 70% of total energy in manufacturing of textile fabrics, farm corn, and wood respectively [43]. This occurrence is indifferent in residential applications, where drying consumes 9-25% of national energy in developed countries [43]. Thus, reduction in energy requirement per unit of moisture removal for drying technologies is essential [43].

One of the most popular drying techniques for microorganism is freeze-drying: a process which combines freezing and drying or certain form of moisture removal [44,45]. Freeze drying has been widely used for preservation of microbes and combines the advantages of both freezing and drying, i.e. highly stable, low mass, and long shelf life final product as outlined in Table 1 [15,20]. By applying optimised process, compatible protective agents, and favourable storage conditions, up to 90% cell recovery has been achieved [4]. It also, however, incurs the disadvantage of high operating cost shared by all preservation methods involving freezing [46,47]. On top of that, freeze-drying often involves the need to operate in vacuum environment which adds up to the cost and complexity when compared to other drying methods operated at near-atmospheric pressure [46,48,49]. In light of these issues, scientists have been trying to develop a cheaper option to obtain a dried culture for long-term preservation [50].

Convective air drying has been viewed as a promising alternative as it is far less costly to operate while capable of achieving higher volumetric reduction of about 5 to 10 times more than freeze drying

[30]. While high volumetric reduction might not be desirable for products such as food and fruits for retention of original shapes, aesthetic is hardly a concern for preservation of microorganisms. In freeze-drying, much higher energy needs to be supplied to induce sublimation in order to vaporise the frozen water molecules whereas convective air drying only relies on evaporation [52]. Based on study conducted by Rudy (2009), freeze drying consumes approximately 3.3 MJ/h while convective air drying consumes 3.05 MJ/h for every kg of wet starting material [53,54]. Although the energy consumptions might not seem to differ greatly, it should be noted that the analysis has not taken into account the pre-freezing process of freeze drying which could also significantly adds to the energy consumption [53]. In addition, the installment cost of freeze-drying unit is about twice of that for convective air drying such as spray drying [11]. The major cost of freeze drying comes from the requirement of refrigerant to achieve the extremely low operating temperature while convective air drying only requires heated air [55]. Similar to air-conditioning systems, refrigerants depletes over time and needs to be recharged after certain period. On top of that, mass reduction through convective air drying is about three times as high as that of freeze drying, which in turn correlates with cost of storage and transportation [30,56].

3 Factors affecting cell survival in drying

Cell survival is the most important parameter to compare the effectiveness of different drying techniques. After all, a drying technique will only be useful if the microorganisms retain its function and reproducibility afterwards [57]. Therefore, understanding the measure of microorganism survival and factors that could affect cell survival is essential before making a comparison of different drying methods that can be utilised for the preservation of bacteria culture. In other words, a reliable drying method is one that maintains cell viability and activity [58]. Cell viability refers to the ability to reproduce, while activity refers to retention of its original cell function [58]. In this paper, the term survival refers to both cell viability and activity.

In general, there are two ways by which microbial cells are inactivated in drying process, namely thermal inactivation or dehydration inactivation [29]. Thermal inactivation is the dominant factor when drying is conducted at high temperatures whereby microbial cells are killed by heat stress [26]. The mechanism of thermal inactivation is understood to be the denaturation of key cellular components which disrupts cellular activity and reproduction [59,60]. Some of the heat-sensitive cellular components include DNA/RNA, ribosomes, protein/enzymes, and cell membrane which are interdependent for cell survival [61]. In spray drying of Lactic Acid Bacteria (LAB), it was found that the most critical cellular component which could lead to irreversible cell damage is ribosome, thermally inactivated at temperature of 62-69°C based on study conducted on *Lactobacillus bulgaricus* and *Thermophilic campylobacters* [26]. The most critical cellular component, however, could differ at various drying temperatures for different species and strains as shown by Table 2.

Dehydration inactivation occurs when cells are deprived of water, which is the primary constituent in cell cytoplasm and responsible in maintaining the structure and biochemical cellular activities [65]. Dehydration mainly attacks cell membranes, which in turn causes destruction of phospholipid membrane and proteins in the membrane [66]. As a result cellular membrane loses its function to regulate and contain intracellular substance from leaking out as well as extracellular substances from entering the cell, eventually leading to cell death [67]. Dehydration inactivation often occurs when cellular water concentration falls below certain level, defined as the critical water concentration [15]. The inactivation, however, occur within a range of water concentration rather than at exact concentration due to biological variation of microorganisms in the culture [15]. The variation could arise from as little as difference in stages of cell growth or as much as the presence of different strains or species such as in mixed cultures [57,68]. The two mechanisms may, nevertheless, occur simultaneously during thermal drying process [29]. Factors influencing the degree of inactivation by the two pathways may come from the cell's resistance to inactivation (intrinsic) or from drying process conditions (extrinsic).

3.1 Intrinsic factors influencing cell survival

Intrinsic survival refers to cell's own ability to resist inactivation. The basic requirement of maintaining cell survival is the ability to prevent drying from damaging vital cellular structures, which are important to achieve successful rehydration [40]. Intrinsic survival varies greatly across different microorganisms. For instance, Gram-positive bacteria are more resilient to heat and osmotic stress as compared to Gram-negative bacteria [69]. This is due to the fact that Gram-negative bacteria possess thinner cell wall membrane and are therefore susceptible to changes in conditions surrounding the cells [69,70]. The intrinsic tolerance might even differ within the same strain for cultures extracted from different growth phase or cultured in different growth medium as found by Smelt and Brul (2014) [71]. Various literature indicates that drying of cultures extracted from stationary phase could result in better survival than those extracted from log phase or other phase in the growth curve [72]. In addition, better survival during drying and storage can be obtained by subjecting the cells to heat or osmotic shock beforehand to momentarily increase the stress tolerance [73]. Incorporating dehydration-protection into drying medium could also greatly increase survival ratio by minimising cell injury during drying process [74]. Reconstituted skim milk, sugar groups, and other similar solutes generally serve as effective protectants [74–76]. This allows improvement of intrinsic stress resistance through optimisation of growth conditions. On the other hand, this could bring about challenges in standardising microbial cultures for ease of comparison when different strains/species needs to be extracted from different growth phases [8].

Regardless of varying tolerances to stress among microbial cultures, the injury mechanisms to cells which occur during thermal drying process are theorised to be the same [8]. During dehydration process, the two main disturbances to cellular components are heat and osmotic stresses [77]. Both stresses at certain extent could cause irreversible loss of cell activity and reproducibility [77].

Fundamental structures of biopolymers such as protein and nucleic acid as well as any bioactive compounds could unwind due to heat treatment, followed by link breakages among monomeric units, which results in damages to monomeric units eventually [8,78]. In comparison, high osmotic stress

could lead to disruption in structural integrity of cellular components which eventually results in partial or complete loss of cellular function [79]. Subsequently, the loss of function, particularly those concerning antioxidant generations, may give rise to lipid peroxidation [80]. Lipid peroxidation refers to the breakdown of lipid fraction in the cell membrane through a chain-reaction of radical-fuelled oxidation [81]. As a result, vital cellular contents are lost through the weakened membrane and inevitably cause cell death.

3.2 Extrinsic factors influencing cell survival

External to the cell structures, factors that influence survival of microorganism are not only observable during drying itself, but could be extended to before and after the drying process [8]. Before drying commences, the growth stage, pH, and medium composition of the culture in which the microorganisms grow play an important factor in determining survival [82]. Similarly, conditions of storage and mechanisms of rehydration post-drying significantly affect the survival of the dried microorganisms [83]. Of particular interest in this paper, factors that affect the inactivation of microorganisms during the drying process are drying temperature, drying duration, drying rate, humidity of drying medium, and initial water content of the microbial sample [8].

Microorganism inactivation is found to be in direct proportion with drying temperature and drying duration [84,85]. However, inactivation is found to be inversely proportional with drying rate, humidity of drying medium and initial water content of the microbial sample [8,86]. In other words, drying with less amount of moisture in both the drying medium and sample under drying encourages higher survival. Beyond such simplification, there are cross-interactions among the individual factors in real practice, resulting in rather complex combined effect on survival [8,29]. Thus, it is worth to note that the trend of the factors described above might differ at certain drying condition. Table 2 illustrates some of these cases where contradiction is found for different temperature range due to the difference in the main driving force for cell inactivation and injury.

4 Convective Air Drying Technologies

The practice of preserving microorganisms by convective air drying began since 1970s and has quite recently been expanded towards the possibility of substituting freeze-drying process with comparable viability and stability during storage [87]. Studies have shown promising results of convective air drying in achieving greater than 80% cell survival [88]. Spray drying of *Lactobacillus paracasei* in a 300-litre pilot run attained up to 84.5% survival, in which the obtained dry product could be directly used as starter culture for cheese manufacture [4]. Three of the highly promising convective air drying methods: spray drying, hot air circulation oven drying, and heat pump drying are discussed in more detail below.

4.1 Spray Drying

Spray drying is a technique of converting liquid or slurry into dry powder by rapidly injecting the atomised slurry into a current of hot air [54,87]. Spray drying employs quick heat-mass transfer and generates high-quality powder that could closely resembles the original product after rehydration [89]. Millions of atomised droplets in the order of micrometre (10-200 μm) create extensively large surface area, which makes it possible for drying in a very short time when exposed to hot air in the drying chamber [87,90]. Atomisation, however, involves subjecting the materials under intense shear stress in order to produce the required fine droplets which will subsequently be in direct contact with hot air [26,91]. The main advantages of spray drying over other drying methods are short drying process (less than 30 s), flexibility in choice of particle size, and high quality product with no adverse effects as well as diversity and availability of machinery [92].

Incidentally, concern over high cell inactivation during the atomisation process has hindered the commercialisation for spray-dried cultures [40]. Atomisation process induces shear stress which disrupts cell and could eventually causes cell damage by irreversible protein denaturation [93]. In addition, atomisation involves converting the liquid microbial cultures into small droplets, which subjects the shear-injured cells to oxygen damage [93]. The severity of oxygen damage is further

increased when drying is conducted on anaerobic microorganisms [94]. Consequently, increased risk of cell deaths during drying and storage contributes to the challenges in commercial spray drying of probiotic products [40].

Furthermore, several other factors add on to concerns regarding low survival during spray drying and low stability during storage for application for microorganisms [95]. Factors that play a big role in survival and stability include drying temperature & duration, particle size, type of species/strain to be dried, culture medium, and whether or not pre-adaptation and/or protectants are added [54,96]. Under the right condition, spray drying of lactic acid bacteria can be conducted to achieve sufficiently high retention of cell activity and viability with even comparable quality to those produced through freeze-drying [87].

In relation to thermal inactivation, spray drying enables rapid drying owing to high surface area of the atomised cell cultures and thus short residence time (as low as 20-40s) of the cells in the heated drying dryer [97,98]. Although shorter residence time typically reduces the effect of thermal inactivation, in spray drying this might be outweighed by the need to supply high specific heat of evaporation within the short period of time [99]. Therefore, high inlet temperature of spray dryer is often required and might lead to substantial thermal inactivation [99,100].

It is, however, found that inlet temperature of spray dryer does not directly correlate to thermal inactivation [8]. This is due to cooling effect by evaporation of water-rich microbial sludge at the start of the drying process [101]. In fact, the survival of the bacterial cells is more strongly correlated to the outlet temperature of spray dryer [97]. As confirmed by several literature, survival rates generally increases with decreasing outlet spray dryer temperatures [102,103]. Nevertheless, the desired low thermal inactivation has to be weighed against the high residual water content in the dried product resulting from applying low outlet temperature [97]. Significant thermal inactivation can theoretically be avoided by ensuring that a suitable mass ratio of liquid-to-air is set in such a way that the amount of heat transferred from hot air to the liquid is the equivalent amount that is required for the moisture

to evaporate [15]. This in turn results in low outlet temperature as the hot air cools down through the heat transfer to liquid [99].

The next influencing factor for bacterial survival during spray-drying is particle size. Particle size greatly affects temperature and moisture gradient within the particle itself and the system as a whole [104]. In a spray dryer, the main factors affecting particle size are nozzle type and atomisation pressure, where large nozzle opening and low pressure results in large particle size and vice versa [105]. The particle size is also influenced by the physical properties of the drying material: viscosity, density, and surface tension [15,106]. While density and surface tension are intrinsic properties which do not change much with regard to the amount of liquid, viscosity can be significantly changed by adjustment of the cell concentration [15]. High solid concentrations results in larger particles and hence require longer drying time, which in turn causes higher thermal inactivation due to longer exposure to hot drying air [15]. Thus, attempt in reducing operating cost through having high solid concentration in the feed should be weighed against the resultant lower survival.

Spray drying has been adopted to preserve microorganisms, such as probiotics used in dairy industry [107]. Although currently preservation of starter cultures involving freezing is more commonly chosen, spray dried cultures are increasingly viewed as promising approach [107]. Spray dryer has been shown to produce dried probiotics with high processing rates and low operating costs [101]. However, sufficiently high number of viable bacterial cells over an adequate period of time is required for commercial application to provide buffer due to storage and transportation of the culture as viable cell counts could drop by 75% and 90% following drying process in 50 days and 200 days respectively [108]. Therefore, studies on spray drying have been carried with cheese cultures, yogurt cultures, and lactic acid bacteria to illustrate the suitability of spray drying to substitute the conventional freezing or freeze-drying [109].

In recent years, there has been technological advancement developed on spray dryers, such as tube spray dryer and flame spray drying [107]. A tube spray dryer is developed by reducing the drying

chamber into the shape of narrow tubes [110]. Tube spray drying allows the potential to precisely construct the drying profile along the length of the drying tube and has been proven to successfully dry sucrose well into its crystallised form [110]. The other emerging technique, namely flame spray drying focuses into the improvement of product quality and reduction of energy consumption [111]. In the drying of maltodextrin, flame spray dryer was found to be able to produce final product with less fractured particles while consuming up to 30% less energy when compared to conventional spray dryer [111]. Moreover, flame spray dryer offers saving in initial capital cost as it does not require auxiliary unit to preheat incoming air [111].

4.2 Hot Air Circulation Oven Drying

Hot air circulation oven drying, or in short oven drying is a rather simple process which works by flow of hot air through forced convection [112]. Oven drying is a traditional drying technique for food & bioproducts that is fairly cheap to operate [113]. The solid or liquid to be dried is exposed to flow of hot air where the solvent (normally water) slowly evaporates, leaving behind the dried residues as the dried product [87].

The mechanism of oven drying is evaporation induced by thermal perturbation to substrate, both externally by forced air convection and internally by heat conduction [87]. Due to simplistic mechanism, oven drying generally has low processing costs and fairly short drying time of about 5-11 hours [87]. The combined capital and operational costs of oven drying is only about 25.2% when compared to freeze-drying [73].

Convective hot air circulation oven drying is developed as an improvement from conventional thermal drying by introduction of air circulation [20]. Study conducted between convective hot air circulation oven drying and conventional thermal drying on kefir biomass reveals that the convective method gives higher drying rate, as moisture content is removed more rapidly by circulated air stream [54]. In addition, the final dry weight of kefir is the same for both methods, and consistent results were obtained at drying temperatures of 28, 33, and 38°C [31]. Moreover, the cell viability obtained from

convective method was higher than conventional thermal drying, largely due to the shortened drying time during which the biological cells are put under thermal and osmotic stress [31].

Literature study has found that the survival of microorganisms in oven drying depends more on the drying air temperature rather than humidity [114]. For example, the survival of bacterial culture decreased from 88.1% to 55.3% when drying conditions were changed from 15 min at 45°C to 9 min at 55°C [114]. Moreover, cell survival dropped significantly to less than 10% when drying was carried out at 95°C for 6 min [114].

Similar results were obtained by Katechaki et al (2010), where LAB were found to give significantly high cell viability (82-87%) with moisture content as low as 3% when dried at lower temperature range of 35 – 55°C [115]. Additionally, analogous behaviour is observed after storage at 4°C where survival rates were reduced to 70% for cells dried at 35°C and about 60% for cells dried at 45 - 55°C [115].

Drying by hot air circulation oven drying can be concluded to work best when conducted at low temperature and high relative humidity as air inlet temperature above 40°C and low RH levels were reported to decrease the cell viability after drying process [116,117].

4.3 Heat Pump Drying

Heat pumps are basically the reverse of refrigeration units, where both systems consist of the same main components, namely compressor, condenser, expansion valve, and evaporator [118]. Thus, heat pump dryers can be easily put together through slight modification of refrigeration units [2]. The main advantages of using heat pump technology are the energy saving potential and the ability to control drying temperature and air humidity [99]. Heat pump dryer works by transferring heat from condensing working fluid in heat pump cycle to the drying medium such as air, rather than through electrical heating, resulting in significantly high overall thermal efficiency [2]. Heat pump dryers have

been found to conserve up to 20% and 70% of energy when compared to freeze-dryer and electrical resistance dryer respectively [43,58,119].

In heat pump, almost all of the drying parameters can be adjusted to desired value [120]. Drying temperature is set by regulating the condenser capacity while drying medium velocity and humidity are adjusted by fan speed and frequency regulation of compressor capacity respectively, all of which are independent of ambient air conditions [121]. Heat pump normally operates at temperature range between -20°C and 50°C, with relative humidity ranging from 20% to 90%, and flow of drying medium adjustable between 0 to 3 m/s [122]. By having this capability, temperature or moisture sensitive product can be dried at selected desirable conditions [123].

Living cells and biological active molecules are generally sensitive to temperature [124]. Drying often comes in as final steps before commercialisation of such biotechnological products and it is important not to lose biological activity [124]. Conventional water removal methods, like evaporation, spray drying and freeze drying operates at temperatures either above 50°C or below -20°C [40]. The extreme low and high temperature could result in denaturing the biomolecules unless expensive cryo-protectant is added for the case of freeze drying [125]. Heat pump comes into play as the technique can operate at milder temperature, closer to the atmospheric condition which the microorganisms naturally inhabit and thus reduces loss in biological activity caused by abrupt temperature change [126,127].

Heat pump dryer has been used to successfully preserve *Lactococcus lactis* ssp *lactis* at optimum temperature of 25°C to achieve maximum cell survival of 92.8% when 15% dried yeast and 10% trehalose are added as protectants [58]. The suitable drying time was determined to be 6 h 40 min to achieve the maximum survival for drying with said protectants [58]. Alves-Filho and Stranmen found that heat pump drying of *Rhodococcus* sp for 1 hour at 20°C resulted in survival rate of 100% while achieving water content of 3.5% (w.b.) [128]. When the drying time of *Rhodococcus* was

increased above 1 hour, decline in survival was observed, signifying overdrying as shown by decrease of cell survival from 100% to 60% when drying duration was increased from 1 hour to 24 hours [128].

The key findings of the above convective air drying methods for the preservation of microorganisms are summarised in Table 3

4.4 Application of convective air drying in various studies

The real applications of convective air drying methods often incorporate the use of protective agents as it is found to greatly improve survival of bacterial cultures. In studies conducted by Gandhi et al [129], only 15.9% of initial bacterial cells survived the drying process when no dehydration-protectant is used. Subsequently, addition of sodium caseinate and lactose was shown to improve the survival *Lactococcus lactis* to 64.8% and 68.6%, respectively [130].

Overview of the studies conducted for the convective air drying methods discussed above together with the achievable survival are presented in Table 4 **Error! Reference source not found.**, note that each of the drying applications presented incorporates certain type of protectant. In each drying approach, the entries are sorted in descending order of survival. Several trends could be observed from Table 4, such as increase of survival values are achieved by incorporation of protective agents. In addition, survival is generally improved with decrease in drying temperature when type of protectant is kept constant.

Species presented in Table 4 are largely gram-positive as gram-negative do not seem to survive well throughout drying. Fortunately, majority of microorganisms which has beneficial industrial uses are of gram-positive bacteria, while pathogens such as *Escherichia coli* and *Salmonella sp.* are gram-negative [69,135,136]. Thus, drying has an added advantage of getting rid of pathogens while at the same time preserving the desired microbial cultures.

4.5 Other convective air drying techniques

Development of drying techniques as alternatives to freeze-drying is not limited to the only three above. In recent years, studies have been conducted to some other drying methods which include, but not limited to fluidised bed drying, conveyor drying, and rotary drying [26,137]. All of these dryers typically utilise hot air as drying medium which is heated by heat exchange with either steam or combustion gas from fuel burner [138]. Temperature of the steam is controllable by adjusting the flow rate of the steam itself while temperature of the combustion gas is controlled by adjusting flow rate of fuel into the burner [138].

Fluidised bed dryings is a process where heated air is passed through wet solid at a controlled speed so that the solid is suspended in the drying chamber [75]. Capability of operating at gentle drying condition while keeping drying duration short means fluidised bed dryer enjoys advantages of both spray dryer and heat pump dryer [139]. On top of that, particulates of drying materials are normally well dispersed by the vertical stream of hot air which promotes good mixing, high rate of heat transfers, and consistent heat distribution within the drying unit [138]. However, its applicability in drying of microorganisms still requires extensive work as the desired microbial cells are small and often get carried along with the drying air [75]. Insufficient studies conducted on fluidised bed drying largely accounts its current limitation and difficulty to be considered as a viable option for microbial preservation.

In conveyor drying, the materials being dried are loaded onto a perforated conveyer which then passes through a single drying chamber or several drying chambers in series [137,138]. Conveyor dryers may also consist of multilevel conveyor belts which are parallel to each other [137]. Inside the drying chambers, moisture is removed by passing stream of hot air which are normally heated by fuel combustion [138]. Individual drying chamber is typically fitted with a fan as a mean to control air velocity and ensure that hot air is uniformly circulated [138]. While conveyor drying is commonly used for drying of agricultural products, it is a promising drying technique for microorganisms as it

presents little physical stress and is one of the best continuous dryers with highly precise and consistent control on exposure of drying materials to the hot air [137]. Conveyor drying can also be practically adopted to convert most batch dryers into continuous dryers, for instance in the work conducted by Erbay and Hepbasili with single-stage heat pump-assisted conveyor dryer [140]. Such combination plays a big role in opening up possible applications of many batch dryers for industry.

Rotary drying are notably suitable to dry loose or powdery material such as soil and sludge [137].

Drying materials are tumble-dried in a drum-like chamber while hot air is passed concurrently through the cylindrical section which is often inclined at an angle with horizontal surface [141]. The operation of rotary drying can be a rather complex process due to the adjustable parameters such as temperature, humidity, rotational speed, flow rate of air, etc [141]. The tossing and turning motions of the drying material, however, could result in greater physical stress and strain when living cells is dried using rotary drying.

5 Drying of mixed culture

It is widely accepted that survival of microorganisms differs under different drying techniques and conditions. Cell survival, however, could differ even under the same drying techniques and conditions. This is especially true for drying samples consisting different species of microorganisms [142]. In certain cases, significant variation in survival was even found when different strains of the same species underwent the same drying process [59].

This is a prominent issue when drying is conducted for mixed cultures. Mixed cultures, as opposed to pure cultures, contain not only single species but at least two or more species of microorganisms [143,144]. Often, each of the various species contains strain variation. It is not uncommon that each strain and species of the microorganisms has different optimum drying conditions due the difference in cellular structures and contents which in turn determine resistance towards heat and dehydration

inactivation. On top of that, mixed cultures quite frequently contain unknown species which adds to the complexity and difficulty in defining the overall characteristics of the mixed culture [143–145].

While there cannot be one drying technique or drying condition that satisfies all microorganisms within a mixed culture, there are some ways to achieve the most optimum condition for a maximum collective survival of the mixed culture as a whole. For instance in drying of anaerobic sludge, drying conditions are chosen to suit methanogenic portion within the mixed culture as it is considered as the most vulnerable. Such choice is based on the viability and practicality of utilising the mixed culture following drying process, as methanogens have long doubling time relative to other groups of microorganisms within the anaerobic sludge: hydrolytic, fermentative, and syntrophic-acetogenic bacteria [146]. Drawing from this example, we can conclude that the most suitable drying condition in drying of mixed culture should not be judged by the value of cell survival alone, but consideration should be extended to ease in repopulation of each species or strain within the mixed microbial culture. An exception can only be made where nearly perfect (~100%) survival is achievable. A nearly 100% survival implies that essentially all microorganisms are viable and thus present in optimum proportion following drying process, deeming repopulation process unnecessary. In such case, choice of drying condition could be based on either the strain or strain that is most sensitive to heat or osmotic stress to enhance process stability and consistency, or cost and practicality of drying.

A considerably thorough search on published literature reveals that research into preservation of mixed culture is unexpectedly scarce and outdated. A study conducted in 2004 looked into the preservation of mixed culture containing four species: two species of LAB (*Streptococcus thermophilus* and *Lactobacillus acidophilus*) and two species of bifidobacteria (*Bifidobacterium longum* and *Bifidobacterium infantis*) [147]. It was found that LAB and bifidobacteria could be suitably preserved through freeze-drying with cell survival of 46.2-75.1% and 43.2-51.9% respectively 1.5-16.2% [147]. Meanwhile, comparatively lower cell survival of LAB and bifidobacteria of 1.5-16.2% respectively was obtained for spray drying [147]. In similar way,

researchers dated back to 1975 investigated a mixed starter culture containing two species: *Streptococcus cremoris* and *Streptococcus diacetylactis* for cheese manufacture [148]. While Efstathiou et al. (1975) managed to recover 96% of the initial cell count, the employed preservation method was freezing at -30°C which also requires storage in frozen condition at an ideal temperature of -30°C [148].

5.1 Current limitations and future research direction

As illustrated above, study in the area of drying for mixed culture is still extremely limited and significantly outdated. The majority of research work covers drying application for food and agricultural products, while the minority that does work on microbes mainly looks at pure cultures. This is largely due to the difficulty in standardisation of mixed culture between runs and replicates. It is well understood that the population distribution of each species within a mixed culture constantly changes depending on the feed and surrounding conditions. While identification and quantification of the microbial community distribution is possible, fairly laborious experimental work is needed for the numerous microorganisms inside a mixed culture consortium. Moreover even with knowledge of the microbial distribution, there is currently no effective and practical way to readily adjust the microbial count of each species within the mixed culture.

The main application of mixed cultures is in anaerobic bioreactor for the purpose of wastewater treatment [149]. However, start-up of anaerobic treatment system requires at least three months and sometimes up to seven months to reach steady state [150–152]. Furthermore, anaerobic digestion systems which treat high strength wastewater are especially prone to reactor upset which could lead to foaming and eventually major loss of mixed culture [153,154]. As an integral part of manufacturing plant, it is critical for wastewater treatment units to operate continuously and with as little disruption as possible. Thus, future direction on the technological advancement should include the preservation of mixed culture to hasten start-up and safeguard against process upset. This could be achieved by having readily usable mixed culture in dried form, which not only eases storage but also deters

biodegradation and halts the formation of flammable biogas as well as toxic hydrogen sulphide gas [155,156]. In addition, mass reduction of mixed culture due to drying also translates into lower storage space and transportation. This, however, is only feasible when the adopted drying method is economical. It is therefore vital to look into convective air drying as low-cost preservation alternatives for mixed cultures.

Subsequently, investigation on the type of protectants which best protects mixed culture throughout drying is worth looking into. As demonstrated by Table 4, certain protective agents seem have varying degree of effectiveness when incorporated with different species of microorganism. Several promising protectants which should be studied include reconstituted skim milk (RSM) and sugars such as trehalose, sucrose, maltodextrin [9,26]. The selection is mostly based on inexpensive and non-toxic nature of these substances [9].

Additionally, study into the the relation of drying preference between individual species or strains and the mixed culture as whole should also yield interesting and valuable findings in understanding the co-existence relations among microorganisms in a microbial consortium. The study should start with isolation of pure cultures from the mixed culture through spread-plate and/or streak-plate method, followed by polymerase chain reaction (PCR) [157]. Genomic DNA extraction can then be performed, where 16S rRNA sequence is amplified and then compared against databases [158][159].

6 Conclusion

Suitability of each method of drying is highly dependent on the type of microorganism to be preserved and thus heavily relies on empirical experimentation. In similar way, each type of drying methods discussed has its own benefit. Spray dryer is able to produce a product within a remarkably short period of time in the form of high quality powder that is easily reconstituted but requires careful adjustment to balance between desired low final moisture content and low outlet temperature which in turns encourage good survival. Heat pump dryer is by far the most energy-efficient drying technique

as it recaptures the heat utilised in the drying process and at the same time facilitates drying at mild to moderate temperature ranges, making it suitable for temperature-sensitive cultures. Lastly, convective air drying such as hot air circulation oven comes in as intermediate between the earlier two, with relatively short drying time, fairly low operational cost, simplistic mechanisms, and is able to produce dried product with comparable survival to spray-drying and heat pump drying. Overall, the common key to achieve high microbial survival in convective air drying seems to be drying at near ambient temperature and incorporation of dehydration protective agents.

7 Acknowledgement

The authors would like to acknowledge Ministry of Science, Technology and Innovation (MOSTI) e-Science Fund (03-02-10-SF0122) for the financial support to this project.

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Table 1 Common microbial preservation techniques for industrial use

Table 2 Contrast of responses on cell survivability between high and low temperature [8]

Table 3 Summary of drying techniques for microorganisms as alternatives to freeze-drying

Table 4 Parameters for drying of microorganisms using convective air drying technology

For Peer Review Only

Table 1 Common microbial preservation techniques for industrial use

Technique	General overview	Advantage	Disadvantage
Subculturing (subcultivation)	Retaining cultures of bacteria through periodic transfer cells from large number of colonies, e.g. old batch to new batch or by taping bacteria-rich downstream fluid and feed it to fresh process fluid upstream [15]	<ul style="list-style-type: none">• Prevents selection of spontaneous mutants [5]• Cheap, applicable universally• Requires no reactivation procedure [37]	<ul style="list-style-type: none">• Risk of genetic instability and contamination• Impractical for large number of cultures• Time consuming• Requires periodic transfer to fresh medium• [15,37]
Freezing (cryopreservation)	Preserving culture and storing it in frozen form in freezer or liquid nitrogen [37,43]	<ul style="list-style-type: none">• Long-term (almost infinite) storage• High genetic stability [51]	<ul style="list-style-type: none">• High cost and energy requirement of storage and transport of frozen culture [15]
Drying (desiccation)	Removal of moisture to halt microorganisms activity and thus extend shelf life [4,20]	<ul style="list-style-type: none">• Low operating cost• Reduced mass of dried product enables easier handling and transportation [4]	<ul style="list-style-type: none">• Dehydration inactivation is a major factor in low survivability [20]

Table 1 Contrast of responses on cell survivability between high and low temperature [8]

	Lower temperature (<37°C)	Higher temperature (>60°C)	Microorganism	Source
Effect of drying rate	Fast drying improves cell survival	Slow drying improves cell survival	Lactobacillus delbrueckii subsp. bulgaricus	[62]
Effect of initial water content	High water content improves cell survival	Low water content improves cell survival	Salmonella serotype Typhimurium	[63]
Cause of cell inactivation	Dehydration	Thermal	Lactobacillus plantarum	[64]
Characteristic cellular injury	Membrane damage	Degradation of ribosome and other cellular structures	Lactobacillus plantarum, Lactobacillus bulgaricus	[63,64]

Table 1 Summary of drying techniques for microorganisms as alternatives to freeze-drying

Technique	Overview	Drying Conditions	Main Advantages	Microbial Characteristics Suited
Spray Drying	rapidly turns wet materials into powder which can be easily reconstituted	High temperature (40-220°C) and atomisation pressure, very short drying time (20-40 seconds)	costs about 6.5 times less than freeze-drying, simple, and continuous operation	high heat and shear stress resistance
Convective Hot Air Circulation Oven Drying	evaporates water from wet materials by exposure of continuous flow of hot air	Moderate temperature (28-95°C), moderate drying time (5-11 hours)	costs about 8 times less than freeze drying rehydration is poor low survivability great reduction in volume	Moderate heat and osmotic stress resistance
Heat Pump Drying	evaporates water from wet materials by combination of heat and low humidity	Mild temperature (10-30°C), moderate to long drying time (7-24 hours)	most energy efficient, consumes about 10-20 times less than freeze drying	Low heat and osmotic stress resistance

Table 1 Parameters for drying of microorganisms using convective air drying technology

Drying technique	Microorganisms	Survivability (%)	Temperature (°C)	Protective agent	References
Spray drying	<i>Lactobacillus plantarum</i>	~100			[103]
	<i>Lactobacillus salivarius</i>	~100	140(in)/40(out)	10% (w/w) maltodextrin or 10% non-fat skim milk	
	<i>Pediococcus acidilactici</i>	~97			
	<i>Streptococcus lactis</i>	72	220(in)/77(out)	25% (w/w) condensed skim milk	[102]
	<i>Rhodococcus</i> sp.	13	107(in)/66(out)	5% K ₂ SO ₄	[129]
	<i>Lactobacillus plantarum</i>	~10			[130]
	<i>Lactobacillus kefir</i>	~2.0	180(in)/70-85(out)	11% (w/v) reconstituted skim milk powder	
Hot air circulation oven drying	<i>Saccharomyces lipolytica</i>	~0.52			
	<i>Lactobacillus plantarum</i>	63	30	Mixture of 1 g of potassium phosphate buffer solution and 1 g cell pellet	[131]
	<i>Penicillium bilaiae</i> Conidia	75	30	Carbohydrate mix of starch and wheat	[114]
	Kefir	90	28	Casein	[132]
Heat pump drying	<i>Lactococcus lactis</i>	93	30	10% (w/w) sorbitol	[58]
	<i>Lactococcus lactis</i>	4	30	None	
	<i>Rhodococcus</i>	35	10	None	[124]

Changes made to the manuscript ‘Microorganism Preservation by Convective Air Drying – A Review’, based on

*page numbers are of revised manuscript

Comments by Associate Editor

Comment #	Page, Line	Comments	Changes
1	22, 30	Please check the accuracy and completeness of all references.	Reference has been checked for completeness and correctness
2	15, 42 19, 45-48	Scientific names must be written in a correct format throughout the whole manuscript (main text and References section).	Scientific names (Latin names) have been checked for proper format
3	22, 21	Reference format should also be checked and revised as per the instructions on the journal's website.	Reference format has been revised as per format required by Drying Technology

Comments by Referee #1

Comment #	Page, Line	Comments	Changes
1	6, 3-4 6, 13-21 12, 12-16 15, 42-53 16, 3-5 17, 3-11 17, 20-24 19, 30-55 21, 7-13 21, 20-26	Authors have provided a good comprehensive survey of relevant literature. However, review needs to be more in depth and critical. Some parts involve only statements with one or more references without a quantitative discussion.	Statements and ideas throughout the text have been elaborated and supported by references. These statements have been highlighted in yellow in the manuscript. Some of these additions are also part of amendments made from other comments.
2	15, 48-53 16, 3-5	The section on heat pump drying has quantitative discussion whereas section 3.3 is a key part but does not critically discuss the results. It is useful but not sufficient to present tabular results. Prior works need to be critically discussed and not only listed.	Based on the referee's comments, the authors believe that the comment is referring to Section 4.3 instead of 3.3. We have taken the suggestion from the referee to include more details of prior work with in-depth discussion in Section 4.3 which can be found on page 15, line 20-40.
3	19, 30-55	The part on mixed cultures appears to be of significant interest. Please discuss in depth with examples.	In-depth examples on the preservation of mixed cultures have been included on page 19, line 15-41.
4	1, 38-42	The Abstract is too general. Please rewrite by giving more detail on what the review contains.	The authors agree with the referee's comment and have added more details into the abstract. However, in view of the word limit set by the journal (100 words), only slight modification was possible as currently the word count of the abstract is 99.

5	21, 7-13 21, 20-26	Can the section on future R&D needs be made more specific?	Under Section 5.1, the following were added: <ul style="list-style-type: none"> • Elaboration on the type of promising protectants starting from the sentence "Several promising protectants ..." • The basis of selecting types of protectants starting from the sentence "The selection is mostly based ..." • Elaboration on recommended techniques for microbiological identification starting from the sentence "The study should start with isolation ..."
6	22, 21	Please check all references. At least some are incomplete e.g. missing names of publishers.	The references were checked for completeness and amended where necessary.
7	n/a	Please check the manuscript for minor grammatical errors.	Manuscript has been proofread and run through grammar-checking tool to correct errors.
8	8, 22 11, 44	Note literature is singular; the word 'literatures' is used several times in the text.	The -s ending has been removed for the word Literatures throughout the text

Comments by Reviewer #2

Comment #	Page, Line	Comments	Changes
1	12, 52-55 13, 3-18	<p>Preservation of microorganisms by drying, especially spray drying, has been reviewed by other authors, for example:</p> <ul style="list-style-type: none"> - Santivarangkna, C., Kulozik, U., & Foerst, P. (2007). Alternative drying processes for the industrial preservation of lactic acid starter cultures. <i>Biotechnology progress</i>, 23(2), 302-315. - Peighambardoust, S. H., Tafti, A. G., & Hesari, J. (2011). Application of spray drying for preservation of lactic acid starter cultures: a review. <i>Trends in Food Science & Technology</i>, 22(5), 215-224. - N. Fu and X.D. Chen (2011). Towards a maximal cell survival in convective thermal drying processes, <i>Food Research International</i> 44, 1127–1149. - Broeckx, G., Vandenheuvel, D., Claes, I. J., Lebeer, S., & Kiekens, F. (2016). Drying techniques of probiotic bacteria as an important step towards the development of novel pharmabiotics. <i>International journal of pharmaceutics</i>, 505(1), 303-318. 	<p>The authors have included literature review the following references in Section 4.1:</p> <ul style="list-style-type: none"> • Schuck, P.; Jeantet, R.; Bhandari, B.; Chen, X.D.; Perrone, Í.T.; de Carvalho, A.F.; Fenelon, M. and Kelly, P.; Recent advances in spray drying relevant to the dairy industry: A comprehensive critical review. <i>Drying Technology</i> 2016, 34 (15), 1773–1790. • Mansouri, S.; Suriya Hena, V. and Woo, M.W.; Narrow tube spray drying. <i>Drying Technology</i> 2016, 34 (9), 1043–1051. • Piatkowski, M.; Taradaichenko, M. and Zbicinski, I.; Energy Consumption and Product Quality Interactions in Flame Spray Drying. <i>Drying Technology</i> 2015, 33 (9), 1022–1028.

		The authors should provide more recent developments or breakthroughs in the field of spray drying, instead of general discussing the well-known information, or focus more on the other convective drying techniques mentioned in this work.	Discussion on recent advances in spray drying, narrow tube spray drying, and flame spray drying can be found starting at 52 nd line of page 12.
2	16, 13 16, 51	The outline of the manuscript must be re-organized to make the structure more logical. For example, the applications of techniques (4.4) should be introduced after all the techniques (4.5). The item 5 is actually also discussing the application of convective drying in preservation of microorganisms.	Several sections in the text have been rearranged and reorganised improve the flow of manuscript <ul style="list-style-type: none">The second paragraph of Section 4.1 in the original submission has been made last paragraph in revision (page 12, line 31-50)Statement on page 9, line 46 on the original submission has been moved to page 8, line 21-34 However, the authors decided to retain the order of Section 4.4 and 4.5 as the Section 4.4 only discusses convection air drying techniques from Section 4.1-4.3. Section 4.5 then comes in as additional emerging techniques for microorganisms.
3	n/a	Please use continual line number.	Authors are unable to change the line numbering format as it is generated by ScholarOne Manuscript submission system.
4	1, 42	Page 1, Line 42: Missing a period.	Missing period has been inserted before the sentence "The paper concludes ..."
5	Table 2	Item 2: I suggest to use subtitle to introduce the three methods (i.e. sub-culturing, freeze-drying, convective drying) separately.	The three methods are included as part of general introduction into microorganism preservation which will then be narrowed down to a more focused discussion about convective air drying in Section 4. Tabular form was chosen for subculturing, freezing, and drying so that the methods can be concisely described and easily compared to one another. Therefore, the authors have decided to retain the section in its current form.
6	7, 15-24	Page 6, Line 55: Although there are a few reports supporting the importance of ribosomes in bacterial thermal death, it is still controversial to conclude ribosomes as the "most critical" cellular component responsible for bacterial thermal death. The "most critical" cellular component firstly driving cell death is probably	The following sentence "It was, however, found that the most" has been reworded and the statement "The most critical cellular component could differ at various drying temperatures for different species and strains" has been added to present the information more accurately and avoid misleading the readers.

		species/strain-dependent.	
7	13, 55 14, 12 14, 14 14, 16 14, 23 (23 changes)	Page 7, Line 5: Please check and revise the fonts of °C throughout the text.	Font for °C and µm has been replaced throughout the text and tables for consistency.
8	7, 49	Page 8, Line 14: "During thermal drying process" is more suitable.	The sentence has been amended according to the suggestion.
9	9, 16	Item 3.2 : "Extrinsic factors"	Subtitle correction has been made for Section 3.2.
10	Table 2	Table 2: Suggest to add a column of the microorganism species/strains used in those references.	A column specifying the investigated microorganism has been added to Table 2.
11	9, 37-38	Page 9, Line 5: Please provide the appropriate reference to support that the inactivation is direct proportion with drying rate. It is controversial to conclude the relation between cell inactivation and drying rate. The fast drying rate is possible to be helpful in protecting cells (the authors actually also showed it in Table 2).	The authors found that the contradictory ideas were due to the misplacement of the phrase 'drying rate'. This has been addressed by placing the phrase in the succeeding sentence. A supporting reference has also been added to strengthen the statement.
12	n/a	Page 9, Line 22-29: Suggest to remove this paragraph.	The referred paragraph with relatively low relevance has been removed as suggested by the referee.
13	10, 31	Page 10, Line 20: There should be a space between number and units. Please check throughout the text.	Space has been added between number and units, with the exception to °C and %.
14	12, 47	Page 10, Line 52: Remove the repeated comma.	Repeated comma has been deleted between the word 'cultures' and 'and'
15	11, 29	Page 11, Line 48: Please clarify the reason why the high inlet temperature is often required.	The preceding sentence has been revised to emphasise more clearly that high specific heat of evaporation needs to be supplied within a short period of time in spray drying, and thus high inlet temperature is required.
16	15, 42	Page 15, Line 10: Revise the name of bacteria.	Missing space in bacteria name has been added. It is now written as <i>Lactococcus lactis</i>
17	17, 35-54 18, 3-20	Item 4.5: Is solar drying a convective air drying method? Is there any report of using solar drying to preserve microorganisms? Is vacuum drying a convective air drying method?	The authors agree that solar drying and vacuum drying do not fit well in this Section 4.5. Thus, they have been removed and replaced with conveyor drying and rotary drying. The added methods can be found between page 17 line 35 and page 18 line 20.
18	22, 20 to 31, 38	Please revise the format of references according to the requirement of Drying Technology.	Format of references were updated to follow the requirement of Drying Technology

Comments by Reviewer #3

Comment #	Page, Line	Comments	Changes
1	2, 54 3, 3	Page 3 Line 3: The cited Statements are not found in source 11.	The ideas which were taken from the source have been highlighted in green. The referred source is the first and second paragraph of Section 11.1.
2	3, 37	Page 3 Line 37: Why does this statement only hold for mixed cultures?	As pointed out by referee, the statement holds for microbiological cultures in general and thus the word 'mixed' has been replaced with 'microbiological' in the text to reflect the right idea.
3	3, 54 4, 3-5	Page 4 Line 5: Were microorganisms investigated in the drying of lemon slices?	The idea that the authors are trying to express with the aid of reference [28] is the capability of convective air drying to operate near ambient temperature under mild processing condition. The sentence has now been split into two parts to improve clarity of the cited statement.
4	6, 11-13	Page 5 Line 54: According to other sources energy per removal of 1 kg water is 1.6 kWh for spray drying and 2 kWh for freeze drying. The number you mentioned sounds very high.	In the initial submission, values from different sources were taken as average and compared. In this revision, a more reliable source which provides both values from the same study has been used.
5	8, 21-34	Page 9 Line 46: Are these statements only true for convective air drying?	The statement on page 9 line 46 applies to general conditions as well. Thus, it has been moved to page 8, line 21 under Section 3.1
6	10, 31-33	Page 10 Line 20: Surface area is in the range of km2 as the droplet size is in the range of microns.	The authors meant to place the bracketed value after 'droplet size'. The error in placement of the bracketed value has been corrected to state the intended meaning.
7	10, 40-42	Page 11, Line 17 & 18: Decisive temperature is the wet bulb temperature and this is low even at high inlet temperatures. More important is a short residence time.	Weakly elaborated and relatively redundant sentence has been removed as per suggestion to place more emphasis on the suggested idea on page 10 line 40-42 which is in line with referee's comment.
8	12, 12-16	Page 12, Line 26: Droplet size mainly depends on the type of nozzle in the spray drier.	Following referee's comment, nozzle type and atomisation pressure have been included as factors affecting particle size to increase depth of the literature review.
9	13, 51-55 14, 3-5	Page 13, Line 26: This is contradictory to Table 2.	The statement is consistent with Table 2 when compared against the column for drying temperature of 37°C (~38°C), fast/shortened drying time improves viability
10	15, 14-16	Section 4.3: How is the drying	The authors agree that it is

		principle of heat pump drying different to oven drying regarding process engineering aspects. It only is a different way of reutilization of air which also could be used in other drying technologies. How does this affect survivability? In general it would be better to link survivability with process conditions and not construction of drier.	important to make comparison based on process conditions. The process conditions, however, have been included in the text. For instance on page 15 line 14-16. By categorisation based on the type of dryer is thought, the authors expect the paper could more easily relate to industry as well as other researchers.
11	15, 23-39	Page 14, Line 45: This section does not fit to the heading heat pump drying.	The paragraph aims to lead to the idea in the last sentence "Heat pump comes into play as", that heat pump is capable of drying at temperatures close to room temperature. Therefore, the authors have decided to keep the paragraph in Section 4.3.
12	15, 46	Page 15, Line 14: Contradictory to Table 2.	The authors found that the contradictory sentence was part of a truncated paragraph. It has been taken out to display only those statements backed up by reference.
13	17, 35-54 18, 3-20	Section 4.5: Most of the mentioned drying techniques are not convective but conductive or radiation drying techniques.	The authors agree with referee's view. Hence, vacuum and solar drying have been removed and replaced with conveyor drying and rotary drying. They can be found between page 17 line 35 and of page 18 line 20
14	Table 1	Table 1 last row: What is meant by half microorganisms?	There was a typo in the word 'halt' has been corrected
15	Table 2	Table 2: Can these findings be generalized? In my opinion not (see comments above).	Authors have added a column specifying the species of microorganisms investigated in Table 2. These findings, however, do still apply in general cases, for example in Comment #9 above.