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Prevention of Mold contamination
Ozone treatment

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Mold is a common pest that can cause diseases and decay property. Moreover, certain mold can produce toxic chemicals which leads directly or indirectly to additional health impacts and economic losses. Therefore, prevention of mold growth is a major concern, and disinfection of mold has become a center of attention.

The purpose of this thesis was to study about the effect of ozone in the disinfection of mold and the method of producing ozone. The usage of ozone for disinfection in indoor sector and food sector was studied by reading previous research work to understand the effect of ozone on mold. Similarly, available literature was examined to understand methods of ozone production treatment.

Ozone can be produced by electrical or photochemical method and it can be used in gaseous form or aqueous form to disinfect mold. Molds of all kinds are killed by ozone. However, concentration of ozone and treatment time vary depending on mold species and form of ozone used. The usage of ozone treatment was more beneficial in the food sector than in the indoor sector.

Keywords	mold, disinfection, ozone production, ozone treatment

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List of abbreviations

ct	Concentration and time
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FBD	Fine bubble Diffusion
GRAS	Generally Recognized as Safe
HEPA	High-Efficient Particulate Arresting
HV	High Voltage
MAP	Modified Atmospheric Packaging
NIOSH	National Institute for Occupational Safety and Health of USA
NOAA	National Oceanic and Atmospheric Administration of USA
NTP	Normal Temperature and Pressure
OSHA	Occupational Safety and Health Administration
RH	Relative Humidity
UV	Ultra Violet
CFU	Colony Forming Unit
WHO	World Health Organization

Contents

1 Introduction

Mold is ubiquitous and an important part of nature. Certain types of mold have also been used in our society for fermentation technology, food processing and production of antibiotics. For example, penicillin, an antibacterial compound, is derived from mold *penicillium* (Christensen, 1965). However, all kinds of mold are not useful and uncontrolled growth of mold can have a negative impact. The major concerns on mold problems are centered around health effect and economic impacts. Growth of mold decreases aesthetic values of properties and produces a distinct smell of rot. Therefore, growth of mold on our properties is not favorable. Also, mold spores cause allergies, respiratory problems, skin irritation and loss of concentration. Furthermore, unwanted growth of mold causes decaying of properties and which leads to generation of waste and economic loss. Lastly, certain types of mold can produce toxins which cause serious health impacts, ranging from mild allergies to death (EPA, 2010).

Different strategies can be adopted to control and prevent mold growth such as controlling physical features (such as temperature, pH and humidity), using chemical sterilizers and fungicides and removal of mold contaminated goods (Money, 2004; EPA, 2010). As a saying goes “prevention is better than a cure”, best way to avoid mold related problems is to prevent its growth. Therefore, sterilization of mold spores has been an area of interest. There are different methods of sterilization; however, this thesis focuses on chemical sterilization, mainly ozone treatment. Ozone has been used as an anti-microbial agent for more than a century. It has been basically used for water treatment purpose (Gottschalk, et al., 2009). Nevertheless, ozone has disinfection usage in other sectors as well. Furthermore, ozone is proven to be effective against mold and fungi (Rice, et al., 2002; O'Donnell, et al., 2012; Korzun, et al., 2008; Misra, et al., 2014).

This thesis is a result of theoretical study on ozone production and its effect on mold. Two sectors were chosen to study ozone disinfection: indoor sector and food sector. The indoor sector was chosen because indoor sectors are constantly having problems with mold and deal with sprawling companies claiming to achieve complete mold remediation with ozone (Money, 2004). Similarly, food industry constantly struggles to keep food safe from mold. Mostly, in developing countries, mold causes 5 to 10 percent food losses in post-harvest products (Royte, 2014).

This thesis explains the working principles of ozone generation, quantitative analyses and method of treatment. Previous researches in two different methods: fumigation and aqueous ozone treatment, are studied and are analyzed.

2 Theoretical Background

2.1 General characteristic of mold

Mold is a diverse group that consists of thousands of mold species and each species have their unique physiology. However, all types of molds exhibit some general characteristics. They are microscopic, multicellular organism that form tubular network of cells known as mycelium over its substrate and reproduce by means of spores.

2.2 Mold growth

The growth condition for mold is defined by following factors: availability of nutrition, temperature, relative humidity, water activity, pH and air affect the growth of mold. Even though, a general growth condition for mold growth can be set, due to the diversity in the group of species, different environmental conditions support different types of mold growth.

2.2.1 Temperature

Heat is one of the basic requirement to support all life. Generally, favorable temperature for growth of mold is 21 to 32ºC. However, temperature for growth of mold, as a group, can range from 0 to 50ºC (Christensen, 1965). Depending on the growth temperature range, mold is classified into 3 sub-groups: thermophiles, mesophiles, psychrotrophs and psychrophiles (Ryan & Ray, 2004). The classification is illustrated with table 2.1.

Table 2.1 Nomenclature of Mold type based on Favorable Growth Temperatures

Mold type	Psychrophiles	Psychrotrophs	Mesophiles	Thermophiles
Temperature range(ºC)	-10 to 20	0-30	25 to 45	45 to 54

Psychrophiles and thermophiles are known as extreme organisms as they grow in temperatures that normally do not support our life. Mesophiles and psychrotrophs grow in moderate temperatures. Most mold can survive freezing temperatures by going into dormant phase where their biological functions are inactive. However, high temperature slows mold growth, and temperature above 70ºC can kill mold in few seconds (Christensen, 1965).

2.2.2 Moisture

Moisture is a basic requirement for mold growth. The presence of moisture helps mold spores to germinate and mold colonies to grow. However, dependence of mold on moisture for growth varies amongst mold species. Some mold is aquatic, whereas some can tolerate drought conditions (Christensen, 1965).

2.2.3 Relative humidity

Humidity is a measure of amount of moisture contained in air and humid air means moisture containing air. Humidity is another important factor that influence mold growth. Humidity directly does not contribute to mold growth, however, humidity influences evaporation of water. Increase in humidity slows down evaporation process, due to which water stays in liquid form for longer which in turn favors mold growth. Also, increase in humidity would slow down dehydration of mold.

Similar to distribution of mold along temperature scale, different types of mold grow at varying humidity. Generally, humid atmosphere encourages mold growth. However, some mold can survive in atmosphere with low humidity.

Relative humidity is a percentage of amount of moisture contained in air to the maximum moisture carrying capacity of air at given temperature. In a closed system and at a given temperature, increasing moisture contained in air increases relative humidity. If the moisture content in air is equal to the maximum moisture holding capacity of air or saturation point, then the air is called saturated. Saturation point is directly related to temperature and increase in temperature would result in rise of saturation point. Slow molding occurs at regular presence of 70 percent relative humidity. The growth rate is hastened when relative humidity reaches 75 percent, and mold growth is observed within few weeks (Christensen, 1965).

2.2.4 Nutrition

Food is a basic requirement of to sustain life, and the process by which an organism obtains its food is called mode of nutrition. There are two types of organism based on the mode of nutrition: autotrophs and heterotrophs. Autotrophs are organisms that can produce their own food by processes such as photosynthesis or chemosynthesis. Green plants are the prevalent example of autotrophs. Heterotrophs are organisms that rely on other organisms for nourishment. Molds are heterotrophs and further classified into parasites and saprophytes. Molds that derive nutrient from dead organic matter are called saprophytes and the mold that derive nutrition from other living organisms or hosts are called parasites (Christensen, 1965; Money, 2004). This thesis is focuses on the saprophytic mold.

2.2.5 pH

Mold can survive in pH range between 3-7. However, some mold can survive alkaline environment of pH up 9 where as others survive in an acidic environment with a pH below 2.

2.2.6 Oxygen availability

All molds require oxygen for survival. However, some molds can survive in a low concentration of oxygen.

2.3 Reproduction in mold

Mold reproduces asexually by spore formation and fragmentation. Sexual reproduction is rare in mold. Figure 2.1 shows a general asexual lifecycle pattern for mold through spore formation.

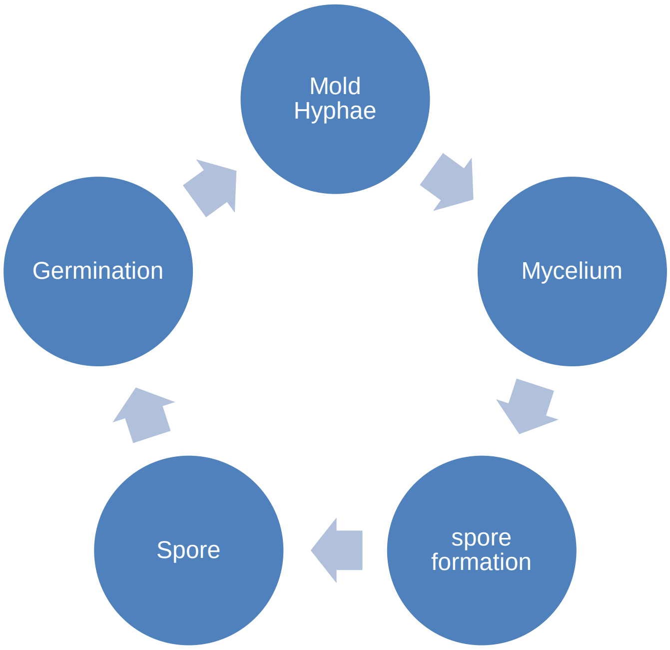

Figure 2.1 General life cycle pattern for mold

During its life cycle, mold reproduces by means of asexually produced spores. Germination of mold start with landing of spore over a food source. During favorable conditions (cool and moist), germination starts out from spore as formation of a tubule from the spore. Overtime the tubule grows into elongated and branched structure consisting of cells joined end to end. This structure is known as hyphae which might not be visible to naked eye. The growth of hyphae always continues along tips and it spreads over the surface of the substrate. The hyphae release enzymes over its substrate that digest any available organic matter. The digested organic matter is then absorbed into hyphae which sustain its growth. As the hyphae grows and becomes branched, it would appear as a network of thread like structures. This stage is visible and the thread like network is known as mycelium. Over time, mycelium matures and aerial stalks grows on them these stalks are known as conidiophores, and they produce spores, also known as conidia. Conidia are minute and can remain air borne. Upon finding a suitable location, the spore can germinate, restarting its life cycle. Even though most molds follow a similar reproduction pattern, the physiology of spores and mold colony differ according to species. Furthermore, the time period from germination to formation of visible colony varies amongst mold species. Usually, time period is about 24 to 48 hours under favorable conditions. Change in environmental condition would slow down the growth of mold; moreover, a completely different type of mold can replace the existing mold colonies which is better suited for the new environment.

Another type of asexual reproduction in mold is fragmentation. When mold colonies are fragmented or mycelial mass of mold is separated, each fragmented part of the colony can give rise to new mold colony. The fragmentation can occur by physiological action of the colony or through external action such as environment or other living organisms.

2.4 Mold related problems

Mold is commonly witnessed; they grow as discolored green/grey patches on food leftovers which are not disposed or stored properly. After a few days, the patches grow and start producing mold spores, but usually at this time, food is long discarded. This is the natural process known as decaying and by this time mold is actively decomposing the food components and absorbing it to sustain itself and reproduce. The food that has mold on it is called spoiled food. Spoiled food is unfit to eat, as it lacks nutrients and can cause stomach problems. The only option is to throw the spoiled food, which leads to food waste.

This is a small example of problem related to mold which is commonly faced. However, mold related problems are not only limited to spoiled food. Mold growth causes economic loss and health impacts in a wide range of sectors. The reason is that mold has a ubiquitous presence, grow on any surfaces, deteriorate any material, has potential to produce toxic substances and is able to survive in extreme environmental conditions (Christensen, 1965; Money, 2004). Due to the scope of this thesis mold related problems in the indoor and the food sector are studied.

2.4.1 Indoor sector

The indoor sector includes all indoor spaces in apartments, homes, offices, hospitals, industries and so on. Indoor areas provide adequate temperature and are filled with material for mold to grow on. The determining factor for mold growth is moisture. In clean and well ventilated areas, mold do not cause problem, whereas high humidity and low ventilation initiates the growth of mold. Apart from humidity, wet areas or hidden water damaged areas become breeding grounds for mold and other microbes alike. If the moisture source is not removed or fixed, mold can proliferate into large colonies as shown in Figure 2.2.

(a) (b)

Figure 2.2 Figure (a) and (b) showing growth of mold in damp places (Paragon, ei pvm) , (Stephen, 2015)

The photos in Figure 2.2 are examples of mold colonies in wet areas. In the first figure, seepage of waste water from drain pipe over the wall surface provided growth condition for microorganisms and mold. The second figure shows formation of mold colony on wet bathroom tile caused by lack of proper cleaning. Mold damage is always not visible as it can be seen from photo in Figure 2.3. The moisture contained behind wall papers were colonized by black colored mold. Another cause for mold infestation is water damages from hidden water pipe leakage and natural disasters like flooding and hurricanes.

Figure 2.3 Mold behind wall papers (Decker, ei pvm)

Growth of large mold colonies on walls and furniture reduces their visual appeal. Moreover, growth of mold can produce decaying smell which causes discomfort to occupants. Normally mold growth in indoor areas do not cause serious health impacts. However, for sensitive individuals, mold spores can induce health effects such as, allergies, nasal stiffness, sneezing, rashes, itchy skin and, redness of eye (EPA, 2010). Also, mold spores can worsen symptoms for asthma patients.

In some cases, people can suffer from mold related diseases when disease causing mold grows indoors (CDC, 2014). For example, in developing countries, farmers working with mold infested grains can suffer from pulmonary aspergillosis which is type of lung disease caused by *Aspergillius fumigatus,* a common mold in grains.

Apart from health effects mold growth in indoor areas have a significant economic impact. Economic impacts are caused when mold damages properties such as furniture, fabrics, pipework and the ventilation system. Certain objects might be treated and reused after mold growth; however, untreatable objects need to be discarded causing economic loss.

The best way to keep mold from growing again is to clean and dry the mold contaminated area and then control moisture. However, if the growth of mold is over 10 sq. ft. professionals should be contacted for a mold remediation process (EPA, 2010). The usage of ozone on the mold remediation would be discussed later. Some common indoor species of mold are Cladosporium spp., Penicillium spp., Alternaria spp. and Aspergillus spp.

2.4.2 Food sector

Mold can grow in all kinds of food products as they provide all the necessities for mold growth. The determining factor for mold growth in food would be temperature and method of storage. The growth of mold on food leading to its spoilage during processing till packaging stage is one of the reason for food loss. An example of food loss due to mold growing on common food item, tomato, is depicted in Figure 2.4. Consumption of mold-contaminated food can have impact on health as the food loses their nutritional value.

Figure 2.4 Growth of mold on tomatoes (Orange Mold, 2015)

Food loss is a major issue in developing countries. In tropical regions, half of the total fruits and vegetable produced is lost due to mold; in addition, total loss of harvested grains due to mold contamination in countries with low infrastructure is about 5 to 10 percent (Pitt & Hocking, 2009). Similarly, the total food loss in sub-Sahara in Africa would be enough to feed 3 billion people for a year. The main reason for food loss in these region is the lack of proper infrastructure or cooling facilities, disinfection and transport, storage and market. Food products are prone to spoilage naturally by senescence; therefore, adequate transport and efficient storage can significantly decrease food loss. Furthermore, usage of proper disinfectant can be employed to protect crops or produce from mold.

Common mold on food products are *Aspergillius* spp*., Mucor* spp*., Fusarium* spp*., Pennicilium* spp*. and Rhizopus* spp. (Condalab)*.*

2.4.3 Mycotoxins

According to FAO, mycotoxins are toxic compounds that are produced by certain type of fungi (FAO, 2016). They are either formed as a result of metabolic activities such as digestion or produced to sustain certain metabolic activities. The mold producing these toxins are called toxigenic mold. On the basis of their origin, mycotoxins are divided into following major groups:

1. Aflatoxins
2. Citrinin
3. Ochratoxin

4. Ergot
5. Patulin
6. Fusarium toxin

The origin of mycotoxin is complicated; a single species of toxigenic mold can produce different types of mycotoxins. Furthermore, a similar mycotoxin can be produced by wide range of toxicogenic mold.

The effect of mycotoxins varies widely and depend on the mycotoxins' chemistry, victim's physiology and defense mechanism against the mycotoxin. Some mycotoxins might not be lethal; however, others can be deadly. They are capable of causing wide range of health impacts ranging from mild discomforts to severe health effects such as organ failure, cancer and death (EPA, 2010; CDC, 2014). Also, an increase in dosage increases the risks involved with the toxin.

A case with mycotoxin was observed in Cleveland, Ohio. Growth of *Stachyborys chartarum,* was related to producing trichothecene (a type of fusarium mycotoxin) in indoor spaces caused wide spread panic. Exposure to this chemical can increase risk of lungs bleeding or pulmonary hemorrhage. However, growth of these mold became a fortune for some people as its growth was followed by multi-million lawsuits against building contractors and insurance companies (Money, 2004). This is an example of economic impact where contractors and insurance companies were at economic loss due to impact of mold. Similarly, according to FAO's reports and publications, mycotoxins cause worldwide food loss.

2.5 Ozone

Ozone is an allotrope of oxygen which consists of 3 atoms of oxygen. It is prevalently known to occur naturally between troposphere and stratosphere, at an altitude of 20 – 30 km from earth surface. Since, ozone concentration in this altitude range is higher than any other part of atmosphere this region called ozone layer. Due to the photochemical activity of ozone at this height, harmful UV rays are shielded off from earth surface (EPA, 2016). In the ozone layer, oxygen molecules are dissociated by UV radiation into mono atomic oxygen ions which combine with other oxygen molecules to form ozone. This reaction is a continuous and reversible process due to which ozone concentration remains more or less constant in the layer. In troposphere and over earth's surface ozone is formed during lightning storms (EPA, 2016).

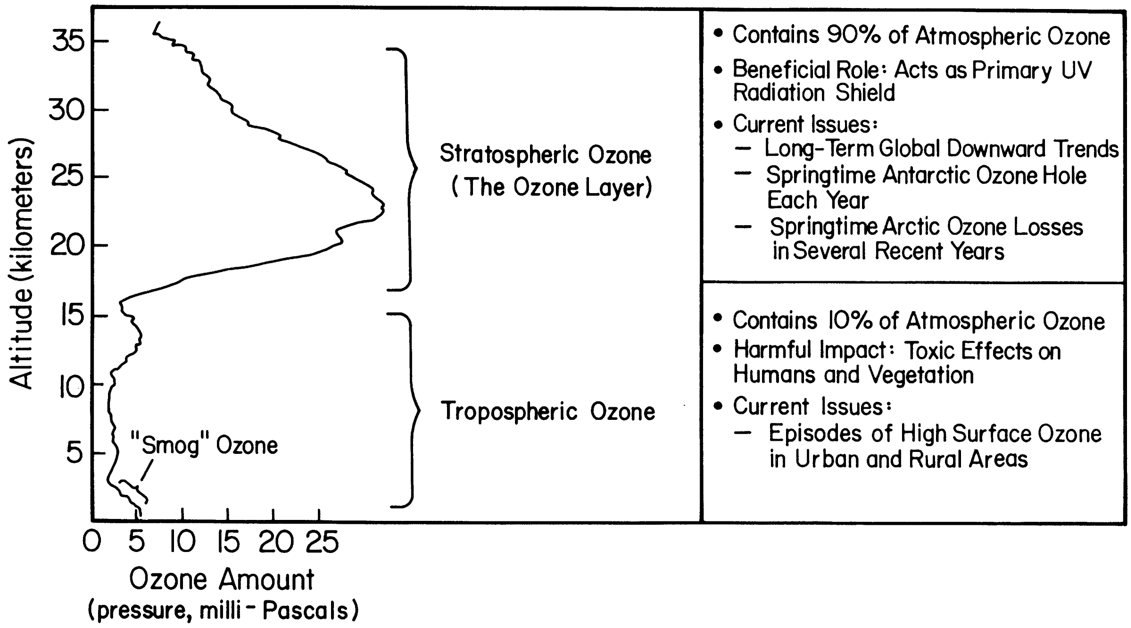

Figure 2.5 Ozone layer in stratosphere (NOAA, 2008)

Figure 2.5 shows distribution of ozone in atmosphere partial pressure of ozone gives an estimate on abundance on ozone. The partial pressure of ozone rises steadily from 15 km and upwards to a maximum value of 25 *mPa* at 20- 25 *km*. Beyond that height partial pressure of ozone start to drop. The concentration of ozone in the ozone layer is about 10 ppm.

At ground level the average ozone concentration is 0.01-0.04 ppm. However, the concentration of ozone largely varies depending on time of day and location. Heavy traffic areas and industrial areas have higher ozone concentration. Also, the ozone concentration in the day time (particularly during noon) is higher than any other time. The mechanism of ozone formation is chemical reaction between nitrogen oxides and volatile organic compounds which contribute towards ozone production. The peak ozone concentration can be as high as 0.4 ppm (World Bank Group, 1998).

Ozone is also produced naturally during electric discharges or simply lightning storms. During these storms, oxygen is ionized into monoatomic oxygen which combines with oxygen molecules to form ozone. Artificially ozone is produced by photochemical smog (World Bank Group, 1998). Other than that ozone is confined to ozone layer.

2.5.1 Properties of ozone

Physical properties

a) In normal atmospheric conditions, ozone is in gaseous state with strong odor which can be detected by humans at concentration as low as 0.003 to 0.01ppm (0.006-0.02 *mg/m³*) in clean air.
b) It is more soluble in water than oxygen, which increases with increasing pressure, decreasing temperature and decreasing pH.
c) It condenses in to deep blue colored liquid at temperature lower than 161 K and solidifies into violet black crystals below 80.6 K. (Wikipedia, n.d.; Lenntech, n.d.)

Chemical properties

a) Ozone is a self-decomposing gas and over time it decomposes back into oxygen and has half-life of a day in air at room temperature.
b) It is one the most powerful oxidants.
c) It oxidizes metal to metal oxides.
d) It decomposes organic double bonds (Wikipedia, n.d.; Lenntech, n.d.)

2.6 History of ozone

Ozone was first produced unintentionally by a Dutch chemist Martinus van Marum in 17th century during his experiment with electrifiers (Lenntech, ei pvm). During the experiment, a pungent smell was noted. However, it was in 1840 that a German researcher Schönbien identified similar smell during his experiment on electrolysis of water which was compared to smell during lightning storms. He later isolated the gas and gave name to it as “ozone” which was derived from a Greek work “*ozein*” meaning scent (Lenntech, ei pvm; Wikipedia, n.d.). Furthermore, he was the first person to research reaction between ozone and organic compounds. Therefore, discovery of ozone is attributed to Schönbien (Lenntech, ei pvm; Kogelschatz, 2003; Gottschalk, et al., 2009). After its discovery in 1840, many experiments for studying disinfection mechanism were conducted. Moreover, it was in 1857 that another German scientist, Siemens devised a first ozone generator (Kogelschatz, 2003). His model for ozone generation became the most popular and is still used with little modifications.

2.6.1 Ozone in disinfection

In 1891, tests were performed in Germany which proved that the ozone was effective against bacteria (O'Donnell, et al., 2012). The use of ozone, for drinking water treatment was extensively studied in France and Russia, which was followed by establishment of first ozone treatment plant for water disinfection in Netherlands in 1893 (Gottschalk, et al., 2009). This led to a development of trend on treating water with ozone for drinking purpose in Europe, until 1914. After 1914, however, the ozone treatment declined due to development of chlorine (O'Donnell, et al., 2012). The chlorine became a potent competitor for ozone in disinfection sector as usage of chlorine overcame the technical and economic difficulties of ozone generator at that time. Chlorine could be cheaply produced offsite and in large scale. Furthermore, it could be stored and dosed in adequate amount to continue its disinfecting action in water distribution network. On the other hand, ozone required to be produced onsite and efficiency of ozone generators were low at that time. Furthermore, ozone generator required high maintenance and needed replacement due to factors such as dielectric failure caused by high voltage. Due to this, ozone treatment was much more expensive than chlorine. This led to usage of chlorine as a primary disinfectant (Gottschalk, et al., 2009).

The interest in ozone resurged after 1970, with discovery of disinfection by products from chlorine treatment. Since chlorine is a very reactive gas, it reacts with wide range of inorganic and organic compound. In case of water, chlorine reacts with natural organics forming chlorinated byproducts which are harmful for human beings. From this discovery, it was only matter of time to discover any halogen based disinfectant (such as fluorine and bromine) could for form halogenated organic compounds (Gottschalk, et al., 2009).

However, the reemergence of ozone in disinfection purpose is not because it does not produce by-products. Ozone is a reactive gas and has astounding oxidation potential due to which it could oxidize wide range of compounds along with natural organics. The reason for ozone to gain interest was because in field of sanitation it can provide excellent antimicrobial result with relatively less contact time and low concentration, due to which it would produce far less by product. Moreover, with its low residual activity and decomposition to oxygen in atmospheric condition, ozone has been considered a safer choice (chemically) than Chlorine or chlorine based disinfectants.

3 Ozone production

Ozone has been used for more than a century for disinfection purpose and it has been proven efficient for removal of mold. The following chapter would discuss about how ozone is produced. There are two prevalent methods for production of ozone:

1) High voltage electric discharge (HV method)
2) UV radiation (Photochemical method)
   Other method involves usage of electrolytes which have been excluded from the study due to its complexity, and lesser known usage.

3.1.1 High voltage electric discharge

As the name suggest this method of ozone production uses electricity and its phenomenon is similar to ozone production during lightning storms. During lightning storms a huge amount of charge flow from cloud to the ground. During charge flow the air around the lightning are ionized. The ionized air contains oxygen ions/radicals which freely react with oxygen molecules forming ozone.

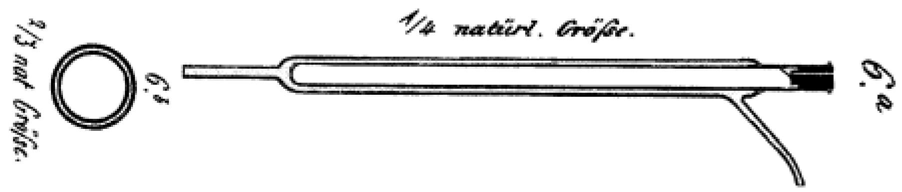

Figure 3.1 Siemens Ozone generator (Kogelschatz, 2003)

The phenomenon of production of ozone is similar to lightning storm, however, the electric discharges are confined between electrodes and ozone is produced in smaller scale. The first recorded experiment for generation of ozone was conducted by Siemens in 1857. Figure 3.1 shows experimental arrangement used by Siemens for ozone production. The experiment included a coaxially placed cylindrical electrodes separated a narrow gap of air and dielectric material. The electrodes were connected to a high alternating voltage which produced alternating electric field of sufficient amplitude in the space between the electrode and dielectric material. Due to alternating electric field, displacement current flows producing discharge in the air gap between electrode and dielectric material (Elaison, et al., 1994). The discharge is a form of ionization which occurs at a specific voltage depending on the nature of gas. Also, magnitude of voltage the gas can withstand without breaking down or ionizing is called its dielectric strength. Air has dielectric strength of 3MV/m under normal condition, i.e. 3kV/mm (Elert, 2000). So, the discharge can only occur if voltage per length between electrodes should be higher than dielectric strength of air.

During production of ozone, air is passed through the discharge zone which would in turn ionize oxygen molecules present in the inlet air. Upon application of high voltage, oxygen discharges earlier than nitrogen. The radicals formed would readily bond with oxygen molecules to form ozone (Kogelschatz, 2003; Ozone Solutions, n.d.; Lenntech, n.d.).

The formation mechanism can be understood from a simplified reaction as follows:

$$O_2 \rightarrow 2O + 2O_2 \rightarrow 2O_3 \qquad (1)$$

Due to wide varieties of ions formed in this technique, the chemistry for formation of ozone is a complex process. As it is mentioned above, ozone discharges earlier than nitrogen; however, as the applied voltage is increases the nitrogen oxides are formed. At higher voltages nitrogen and oxygen ions are formed and dominant product is NO, N_2O, NO_2, N_2O_5 and NO_3. Also, if nitrogen dioxide is initially present ozone formation can be interrupted.

A limitation of this method of ozone production is that hazardous oxides of nitrogen are formed. Furthermore, in case of air as infeed gas, humidity played an important role in functioning of ozone generation and longevity of equipment. This is because, nitrogen dioxide would dissolve in moisture to form nitric acid which corrode the equipment. This limitation is however removed by using pure oxygen or dry air as infeed gas. Furthermore, controlling voltage to reduce nitrogen oxide formation has been an area of ongoing research (Elaison, et al., 1994).

In case of pure oxygen as feed gas, the ozone production has higher yield. The limiting effect of ozone production is caused by the discharge itself. As the concentration of ozone increases, the high voltage causes discharge in ozone as well which would cause decomposition of ozone into oxygen. Industrial level ozone generators can produce ozone output from 5% *by wt*. (air) to 18% *by wt*. (pure oxygen) (Kogelschatz, 2003).

3.1.2 UV radiation

The production of ozone from UV radiations is another natural phenomenon that can be observed in ozone layer. This method utilizes the same phenomenon. In this method, input air is exposed to UV radiation of about 188*nm* wavelength, which is generated by a UV lamp. At this wavelength, oxygen molecule ionizes to monoatomic state by absorbing the energy from the radiation. The monoatomic oxygen then reacts with oxygen molecules to form ozone. In contrast to HV method, in UV method nitrogen gas is not ionized as UV radiation of this wavelength is only capable of dissociating oxygen molecules.

The feature of this method is that even though low concentration of ozone is produced hazardous oxides of nitrogen aren't formed. Also, the air didn't need desiccation prior to treatment. The production of low concentration of ozone is due to low efficiency of UV lamps and also the UV radiation has low penetration capacity so only the gas close enough to UV lamp is ionized. In case of pure oxygen as a feed gas output ozone can be about 20 *gm/kwh* (2% *by wt.*) and for air as infeed gas, ozone production is about 1-2 *gm/kwh* of UV (0.1-0.2% *by wt.*) (Smith, ei pvm).

4 Treatment methods

Disinfection with ozone is carried out mostly in two ways:

4.1 Fumigation

In this technique, the object or product to be treated is fumigated with ozone for required duration of time. The object is placed in an enclosed chamber or space which is supplied with ozone gas. This result in formation of sterile environment within the chamber that kill mold. This type of treatment has been carried out mostly in mold remediation of buildings.

4.2 Ozonized water

This method uses ozonized water to disinfect surfaces. Ozonized water is prepared by either Venture Injection method or Fine Bubble Diffusion (FBD) method (O'Donnell, et al., 2012).

4.2.1 Fine bubble diffusion method

This method works by dissolution of ozone in water by forming fine or micro bubbles. A conventional model for fine bubble diffusion method is depicted in Figure 4.1. Ozone is passed through a diffuser to bottom of a diffuser tank at a pressure higher than that of water column in the tank. The diffuser can be porous stone, frits or steel sieves which serves to disperse fine bubbles of ozone into the water volume. The bubbles drift upwards and expand while dissolving in the water. Undissolved ozone can be collected and converted back to oxygen from top of the tank.

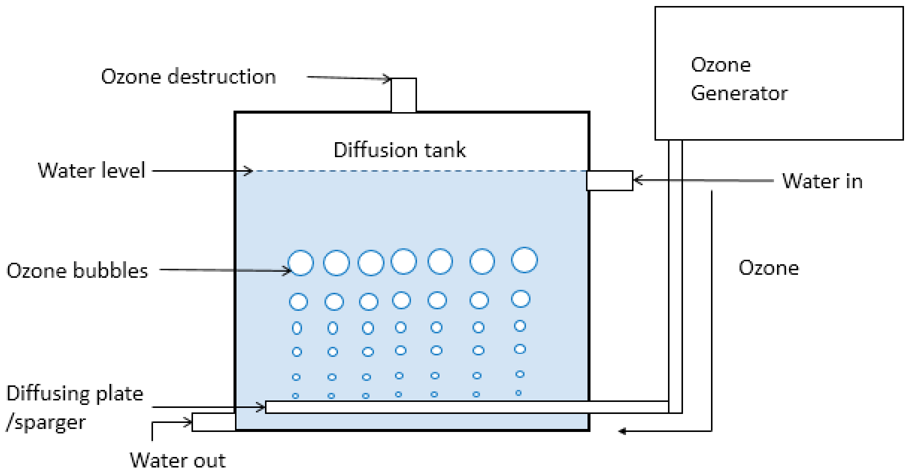

Figure 4.1 Schematic diagram of the Fine Bubble Diffusion(FBD) method

The dissolution of ozone into the water is affected by dispersion of ozone, size of bubble and contact time between ozone and water. For maximizing the dissolution of ozone, sinters with pore diameter of 5-micron, and countercurrent flow of water can be applied. Countercurrent flow of water increases contact time between ozone and water by lowering the drifting velocity of bubbles. Furthermore, sinters produce fine bubbles which increases contact area between ozone and water. As a precautionary step, pressure of ozone should not be very high as the high pressure causes heating which in turn decomposes ozone. Also, the ozone generator should be placed above the water column to prevent backflow of water into the ozone generator (O'Donnell, et al., 2012).

4.2.2 Venturi injection method

The working principle for this method is the Venturi effect. It is observed as a drop in pressure and increase in velocity of fluid flowing across a pipe when its movement is choked or constricted. A schematic of Venturi injector is presented in Figure 4.2. From the figure, when pressurized water is passed to the motive fluid nozzle it is constricted by the nozzle, which causes the water to eject from the nozzle in form of high velocity jet stream. The high velocity of jet stream creates a pressure drop in injection chamber. This loss in pressure acts as a suction which draws ozone towards the jet stream. The mixture of ozone and water then flows towards the diffuser throat where the velocity of water decreases and pressure increases again. The pressure of fluid in diffuser outlet should be lesser than that of the inlet pressure to prevent flow of water through the gas inlet (O'Donnell, et al., 2012).

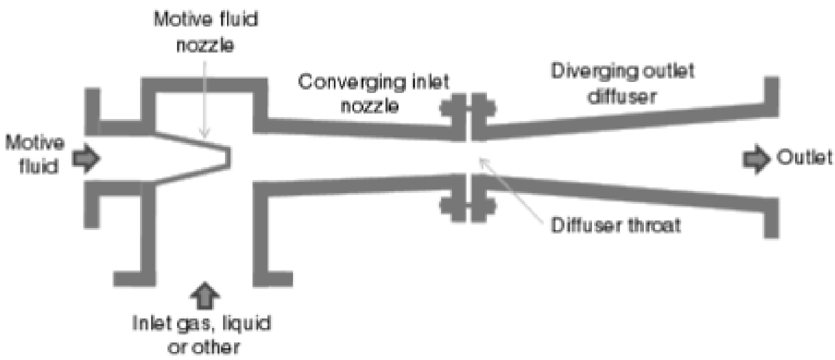

Figure 4.2 Schematic representation of a venture injection method (O'Donnell, et al., 2012)

In the Venturi injection method, there is always a slight negative pressure inside the system due to flow of water. Therefore, in case of leakage the air would flow inside the system rather than outflow of ozone. Furthermore, using hydraulic monitors/controllers can help detect a leakage so that process could be halted beforehand. In case of FBD method, the ozone is applied in pressurized condition; therefore, the leakage in ozone carrying pipes would cause outflow of ozone. Due to these reasons, the Venturi injection method is preferred over FBD for practical application (O'Donnell, et al., 2012).

Aqueous ozone treatment is considered superior to fumigation for disinfection because aqueous use provides better disinfection in lesser treatment time (Öztekin, et al., 2006). Some researchers report that the formation of more reactive ions such as hydroxyl ions in water is the cause for increased efficiency (Roushdy, et al., 2011; Gottschalk, et al., 2009). Also, water current and flows could remove mold from surfaces to be treated.

5 Analysis of ozone and mold

5.1 Ozone concentration measurement

The concentration of ozone is measured to gain an estimate of treatment time, disinfection efficiency and for process control.

Practically, there are two major ways for measuring ozone in air which are UV Absorption and Electronic sensing. In UV absorption technique, differential UV light absorption is utilized as a factor for measuring ozone. UV light of 254nm wavelength is passed through the reference medium and the sample medium containing ozone. Difference in the intensity of the UV light gives the absorbance value of the sample medium. The absorbance value would then be used to calculate the ozone concentration using the Beer-Lambert’s equation:

$$A = \in * l * c \ ,$$

where

A = absorbance

∈ = proportionality constant

l = path length of UV light in the medium

c =concentration

The concentration can be measured in different units based on the proportionality constant used. This method can accurately measure ozone concentration from 0.1 ppm to few percent by wt. (O'Donnell, et al., 2012). The absorbance value is measured by a UV spectrometer.

The next method utilizes electronic sensors which are quick and easy to use. However, this method is not as accurate as the UV method and is usually used in field where precise measurement of ozone is not required.

In addition to above methods, iodometric/ redox titration can be used to measure ozone concentration in laboratories.

5.2 Mold viability analysis

Mold viability analysis is the test to access the success of ozone treatment. The viability test can be done by enumeration of mold colonies which is grown from the ozone treated samples. The enumeration is prevalently done by direct plating and dilution plating technique (Pitt & Hocking, 2009).

5.2.1 Direct plating technique

In this technique, the samples treated with ozone are placed directly on a solid agar plate. Depending on the size of the sample 5 to 20 samples are placed. As a precautionary step the samples are placed into the plate using a disinfected forceps to prevent contamination with foreign microbes. Then, the plates are stored in an incubator for 5 days at favorable growth temperature for mold. After the incubation period, the number of samples infected are visually observed and counted. The result is expressed as a ratio of number of infected sample to the total number of sample. The ratio can also be expressed as percentage which is called moldiness percent. Furthermore, stereomicroscope can be used to identify individual mold species (Pitt & Hocking, 2009).

5.2.2 Dilution plating technique

First of all, a mother solution with an unknown concentration is prepared from a sample. Then a first fold dilution is performed by extracting 1ml of the mother solution into a test tube which is diluted to 10ml by adding 9ml of sterile buffer medium or diluent. The buffer medium is neutral and has low mortality rate. A typical buffer is 0.1% peptone (Pitt & Hocking, 2009). The diluted sample is serially diluted to obtain six consecutive diluted samples. In the end, six solutions with concentration 10^{-1} to 10^{-6} of mother solution is obtained. After that, a culture medium is prepared by spreading fixed amount of sample and each dilution (example: 0.1 *ml*) over separate Petri dishes containing appropriate growth medium. At least 3 set of culture medium is prepared to test repeatability of the test. The inoculated dishes are then stored for 5 days at 25^oC (Pitt & Hocking, 2009).

After the incubation period, the number of mold colonies are counted on the dishes. The number of colonies for each dilution and the original solution are expressed as an average of the triplicate set in unit *CFU/ml* or *CFU/g*. Then using the result, a relationship between dilution and concentration of mold is established which gives an estimation on concentration in original solution.

Furthermore, using these techniques, mold concentration in samples that are not treated with ozone could also be estimated. By, comparing the difference in mold concentrations in untreated and treated samples, the efficiency of ozone treatment can be calculated.

6 Effect of ozone on mold

The exposure time and concentration of disinfectant are directly proportional to disinfection rate. The product of these factor gives 'ct' values and is expressed in $mg\ l^{-1}\ min^{-1}$. Each 'ct' value corresponds to specific log reduction of viable spores or conidiophores for specific type of mold. The two studies below are the results showing effects of varying exposure time and concentration separately. The first study is an example of aqueous use of ozone and second study uses fumigation technique.

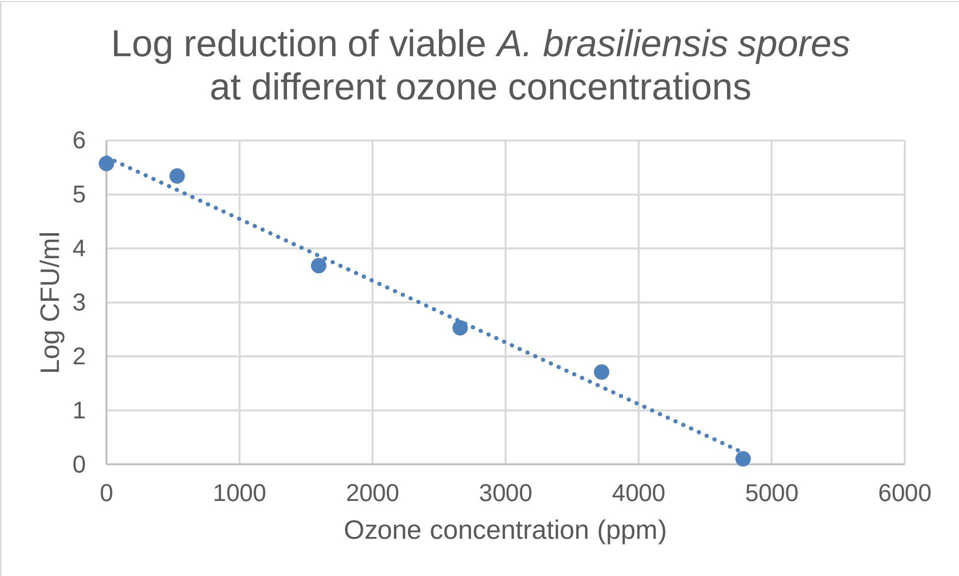

Figure 6.1 Reduction of A. Brasiliensis at different ozone concentration with 1 minute of exposure time (Roushdy, et al., 2011)

The Figure 6.1 is obtained from a study on sporicidal effects of ozone performed by Roushdy, M.M; Abdel-Shakour, E.H.; Abdel-Ghany, T.M., 2011. The aqueous samples contain spores of *Aspergillius Brasiliensis* were treated with varying concentration of ozone for 1 minute. The results show the decrease of total viable spores in an aqueous solution at all level of the ozone concentrations. Also, for 1 minute of treatment time, increasing the ozone concentration had increasing sporicidal effect.

The next study by Raquel R. Santos et al (2016) reported that the sporicidal effect increased by increasing the treatment time for a constant ozone concentration. The photos in Figure 6.2 shows decrease in moldiness with increase in treatment time.

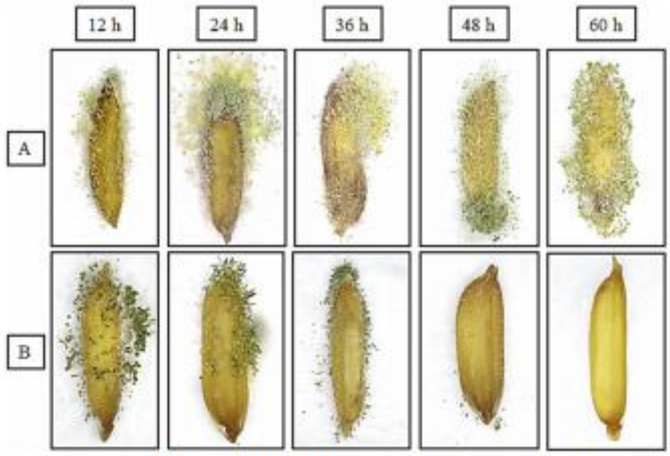

Figure 5. Rice grains observed with a stereoscopic microscope subjected to atmospheric air (A) and ozone gas (B) in different periods of exposure

Figure 6.2 Study on exposure time and mold growth inhibition (Santos, et al., 2016)

The rice samples were inoculated with *Aspergillius* spp. and *Penicillium* spp. and then fumigated with ozone. The concentration of ozone was maintained at 10.13mg/l or about 4800 ppm_v by continuous input of ozone gas. The rice samples treated for 60 hours with ozone showed no visible mold colonies. The study also revealed that *Aspergillius* was more resistant to ozone treatment than *Penicillium*, as moldiness decreased for *Aspergillius* at slower rate than for *Penicillium* under similar treatment conditions (Santos, et al., 2016). So, the ozone treatment has different disinfection rate for different mold species.

6.1 Ozone in food processing

It was in year 2001 that ozone was accepted for direct contact with food products for antimicrobial purposes and granted a GRAS (Generally recognized as safe) status by United States Food and Drug Administration. Wide range of food sectors can use ozone as a disinfectant such as fresh food packaging sector, grain storage facilities, meat processing and storage, sea food processing, soft drinks and juice processing, beer and other beverages. (Guzel-Seydim, et al., 2004; O'Donnell, et al., 2012).

Ozonized water is used in post-harvest treatment to wash post-harvest products, such as fresh fruits and vegetables, for sanitation and for maintenance of freshness, they are used in meat industry for sanitation of meat and equipment. Furthermore, fumigation technique can be used to disinfect dry foods where aqueous ozone treatment is not viable. In addition to these methods, novel techniques such as modified atmosphere packaging (MAP) can be implemented to prolong shelf life of food products (O'Donnell, et al., 2012). In this technique, ozone is synthesized inside the packages containing food through High Voltage ozone generation method. An example of MAP is a research conducted by Dublin Institute of Technology for treatment of strawberries. Ozone was generated by applying high voltage inside the strawberry package. The discharge was allowed away from the mass of strawberries for 5 mins. After which ozone concentration was about 1000 ppm. After 24 hours, there was reduction to 2 $\log_{10}$ of the microflora inside the packages. Apart from production of ozone, UV radiation was produced inside the strawberry package, which is known to have a synergistic effect on removal of microflora (Misra, et al., 2014).

6.2 Ozone in indoor mold

There has been extensive research in laboratory and in real life situation on fumigation treatment for treating mold (Rice, et al., 2013; Öztekin, et al., 2006; Korzun, et al., 2008). The total viable spores of indoor mold *stachyborys, aspergillius* and *cladosporium* was significantly reduced with fumigation by 5-12.8 ppm of ozone across 4-hours of exposure time (Korzun, et al., 2008). The experiment also showed that even with exposure to 11 - 12 ppm of ozone for 4 hours, some conidia of each mold species survived with lowered biosynthetic abilities. Therefore, further treatment or removal via physical methods was suggested.

Similar to this experimental study, practical application of ozone fumigation was studied and reported by Rice et. al. (2013). Their study compiled 3 different case studies of ozone treatment on mold contaminated home.

The ozone generation was carried out by their own commercially available ozone generator which used 14 inch UV lamp with capacity to produce 5-20ppm ozone within few hours in an average sized room. Depending on size of mold contaminated area, number of generator and their placement were decided by professional workers. Since the concentration of ozone was above health risk standard, professionals used proper protective gears when moving inside the rooms to for measurement purpose. The treatment was proceeded in six steps:

1. Inspection of mold damage.
2. Recommend steps to be carried out after treatment.
3. Clear out the spaces (remove pets, objects susceptible to damage by ozone, open all drawers and cabinets, remove ventilation filters).
4. Treat the spaces ozone for up to 4 hours. Since ozone concentration is higher than the daily allowed exposure limit the treatment area is vacated for this period. The periodic ozone concentration measurement was done by professionals in protective gear.
5. Drive ozone gas out of the building by using fans and ventilation.
6. Vacuum the ceiling walls floors and furnishing with HEPA filter to remove debris and available mold.

These studies concluded that fumigating with 5-20ppm of ozone over a time period of 4 hours was effective to reduce mold below detection and hazardous limit in indoor applications. Also, some mold colonies survive even after treatment. Therefore, vacuuming with a HEPA filter to remove debris and dead mold was recommended. Furthermore, removal of source of mold growth, for example moisture source, food source and mold contaminated filter, was carried out to prevent regrowth of mold (Rice, et al., 2013; Korzun, et al., 2008).

7 Ozone related risks

Ozone is a very reactive element and one of the most powerful oxidizers which contributes to its disinfection ability. however, the same reason for its disinfection efficiency also makes it a hazard. The risks of ozone can be categorized as follows:

1) Health risks
2) Physical risks
3) Environmental risks

Health risk: The ozone is a toxic gas has is known to cause numerous health problems in humans. At low concentration ozone causes irritation of mucous membrane and respiratory passages. Increase in concentration can lead to desensitization of nose and damages in lungs. High ozone concentration, up to 10*ppm*, can cause headache, coma and damage to respiratory system (O'Donnell, et al., 2012). Air quality guidelines published by WHO in 2006 has suggested that the permissible amount of ozone exposure should be is 100 *µg/m*^{3}(0.1 *mg/m*^{3}) at NTP averaged over the exposure duration of 8 hours (WHO, 2006). However, the Occupational health and Safety Administration(OSHA) of USA has defined permissible exposure limit of ozone to be 0.1 ppm (0.2 *mg/m*^{3} at NTP) averaged over the exposure time of 8hours (Gottschalk, et al., 2009). For short term exposure NIOSH has set 5ppm as exposure limit (McConnel, 2012). At concentration level of 50ppm and exposure duration of 30 minutes' ozone is fatal (Gottschalk, et al., 2009).

Physical risk: Ozone is highly oxidative and flammable gas. Substances such as natural rubber and plastics are vulnerable to ozone damage (McConnel, 2012). Risk of detonation under high concentration.

Environmental Risk: Ozone is recognized as toxic to aquatic ecosystem, as it kills microbial organism (McConnel, 2012). Ozone toxicity is higher in water than in air. Also, increase in RH of air increases toxicity of ozone in air (Santos, et al., 2016).

8 Conclusion

Ozone treatment has been studied in food industry and indoor sectors and depending on the degree of disinfection required and type of sector, a method of ozone generation can be chosen. Ozone generation with UV radiation is safer than with high voltage. However, former has far less yield for ozone production. Therefore, it is more suited for laboratorial, and small scale purposes. The high voltage can be utilized for large scale production of ozone which is suited for industrial usage.

Ozone is lethal on mold species in both indoor and food sectors. Two major factors that affect ozone treatment are treatment time and concentration of ozone. Both of the factors directly affect the efficiency of ozone treatment; as, increasing concentration and treatment time would increase mortality of mold. Also, for similar concentration of ozone, disinfection is faster with ozone in aqueous medium than in gaseous state.

Fast disinfection can be achieved with a higher ozone concentration but increasing the ozone concentration also increases health and material related risks. For example, in food sector high ozone concentration causes damaging of food product prior to disinfection. Therefore, from the health and material safety perspective, a safe level of ozone concentration should be selected for the required disinfection level, which would give an estimation on required treatment time with help of ct values.

In case of indoor sector, the usage of ozone can effectively reduce viable spores of mold in air. However, its use is limited and mostly focused on mold remediation because high level of ozone in indoor areas is not safe from the health perspective. Ozone is used in mold remediation process as a gaseous disinfectant to kill mold spores in air and surfaces. However, even after the ozone treatment, some mold colonies survive as ozone would not affect hidden mold inside wall papers or mold beneath surfaces of most material. Additional drawback of the ozone treatment is that the areas treated with ozone needs to have proper ventilation post treatment to reduce ozone concentration to safe level. Moreover, efficient ventilation system, replacement of air filters and proper cleaning can alleviate common mold related problems. Also, considering the fact that ozone does not kill mold beneath the surfaces, mold contaminated objects need to be discarded; therefore, it can be concluded that the ozone treatment for mold remediation in homes is a redundant step and only true benefit might be quick odor elimination. The use of ozonized water is not applicable in the mold remediation process, because ozonized water would dampen surfaces and mold would regrow.

In the food sector, however, ozone treatment can have many benefits. Depending on type of food, fumigation or ozonized water treatment can be used to disinfect large volumes of food. Ozone disinfection can be used prior to food storage or as a step in food processing. Also, ozone is a more environmentally friendly option in disinfecting food than other compounds such as chlorine and its derivatives. Furthermore, MAP techniques can reduce the risk to mold contamination and increase shelf life of food products. Collectively, food industries can benefit from ozone treatment by adopting environmentally safe disinfectant, reducing food loss and saving the environment.

Lastly, in both the sectors, disinfection with ozone is a step in preventing growth of mold; however, post treatment procedures, such as regulating temperature and humidity, must be adopted to prevent mold growth in future.

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