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OZONE AS AN ALTERNATIVE DISINFECTANT – A REVIEW

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It is important for consumers that the disinfectants used in the food processing industry should ensure the quality and microbiological safety of food. It is also important that they should not endanger their health through toxic intermediate products. Consequently, a growth of interest has been observed in alternative disinfectants, including ozone. An ozone molecule, owing to split of the third atom of oxygen, is a strong oxidant and an effective agent in destroying microorganisms. It is used for disinfecting waste and drinking water as well as throughout the food processing industry. Ozone is used for disinfecting equipment, production areas and in the food industry.

INTRODUCTION

It is beyond doubt that food processing plants must assure customers about the safety of their products. To this end, a number of disinfection methods and agents are used which are aimed at inhibiting the growth of pathogenic microflora, restricting food decay and prolonging its storage time. It is also important that they should not leave any harmful residues, but this condition is not always met. It is therefore important that alternative and effective disinfectants should be sought [Gűzel-Seydim *et al.*, 2004 a, Khadre *et al.*, 2001]. Such expectations can be met by ozone which has recently proved attractive not only for the food processing industry.

CHEMICAL AND PHYSICAL PROPERTIES OF OZONE

Ozone (O_3) is an allotropic form of oxygen. It is characterised by a high redox potential of -2.07 V, as compared to that of chloric (I) (a.k.a. hypochlorous) acid (-1.49 V), chlorine (-1.36 V) or oxygen (-0.40 V) [Kim *et al.*, 1999].

Ozone is a gas with a pungent, characteristic smell. At high concentrations in the air it becomes blue, whereas at low concentrations it is a colourless gas, lighter than the air. The half-life of ozone molecules in the air is relatively long and spans for *ca.* 12 hours; in aquatic solutions it depends on the content of organic matter. In other words: the lower the concentration of organic matter, the longer the ozone half-life [Graham, 1997]. Ozone dissolves in water ten times better than oxygen and its solubility decreases with an increase in water temperature [Holcman & Domoradzki, 2003; Gordon, 1995], which is shown in Table 1.

TABLE 1. Temperature and solubility relationship of ozone in water [Rice *et al.*, 1981].

Temperature (°C)	Solubility (L ozone/L water)
0	0.640
15	0.456
27	0.270
40	0.112
60	0.000

Ozone dissolves in water at pH below 7.0; at this level it does not react with water and it is present in the form of molecules. However, an increase in the pH value leads to a spontaneous decomposition of ozone, which results in producing highly reactive free radicals, such as hydroxyl · OH. At pH value of 8, nearly half of the introduced ozone is decomposed to various intermediate forms and to oxygen within 10 minutes [Gordon, 1995].

THE MECHANISM OF OZONE ACTION

An ozone molecule, owing to split of the third atom of oxygen, is a strong oxidant. It is the property which makes it very effective in destroying microorganisms. It has been proven that ozone destroys viruses inducing hepatitis A, influenza A, vesicular stomatitis, and infectious bovine rhinotracheitis. The inactivation takes place mainly as a result of damaging the protein and peptidoglycan molecules in a virus capsid. It is equally effective in destroying several strains of bacteriophages [Gűzel-Seydim *et al.*, 2004 b]. The bacteriocidal properties of ozone have also been

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demonstrated in the case of Gram-positive (*Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative microorganisms (*Yersinia enterocolitica*, *Pseudmonas aeruqinosa*, *Salmonella typhimurium*); in both spores and vegetative cells [Gűzel-Seydim *et al.*, 2004 b; Restaino *et al.*, 1995]. At first ozone attacks a cell's surface. Two major mechanisms have been identified through which the bactericidal effect of ozone is exerted. One of them is oxidation of sulfhydryl groups and amino acids of enzymes, peptides and proteins. The other mechanism is based on oxidising PUFA [Victorin, 1992]. In Gram-negative bacteria, the lipoprotein and lipopolysaccharide layers are the main sites of the destructive effect of ozone, which contributes to increased microorganism cell permeability and results in its lysis. Ozone acts as a general intracellular oxidant, whereas chlorine destroys selectively certain enzymes [Kim *et al.*, 1999; Khadre *et al.*, 2001].

Oocysts of the primate called *Cryptosporidium parvum* are also vulnerable to the cidal effect of ozone [Peeters, 1989], whereas chlorine has hardly any effect on them. It has also been found that, due to the increased permeability of oocyst membranes, a preceding exposure to ozone enables a better penetration by chlorine of the microorganism cell interior, which results in significant inactivation of oocysts [Gyurek *et al.*, 1996].

OZONATION OF WATER

The physicochemical properties of ozone, *i.e.* its relatively high solubility in water and a high redox potential (which destroys the structure of microorganisms), have enabled its commercial application in the 1880s for deodorisation of industrial waste and disinfection of drinking water [Koppenol, 1982; Kubiak, 2003].

Waste treatment is crucial for the environment's protection and for prolonging the availability of water resources. The past few years have brought new different methods of water purification. Safe water purification systems require reliable waste disinfection, which is the most important process in ensuring the protection of public health [Xu *et al.*, 2002]. Chlorination is still the most common method for deactivating the pathogens present in water and waste; however, alternative techniques have been appreciated because of an increasing amount of undesirable intermediate products of chlorination and its ineffectiveness in eliminating some epidemic microorganisms with low concentrations of chlorine [Tyrrel *et al.*, 1995; Lazarova, 2002]. It has been proven in the past that ozone is one of the more effective disinfectants and can be widely applied for deactivation of pathogens in drinking water [Langlais *et al.*, 1991; Facile *et al.*, 2000]. Its cidal features have been demonstrated in *Escherichia coli* and similar bacteria such as typical faecal indicators, because their presence is usually checked in waste treatment. As a result, deactivation of these bacteria was observed by 1-3 orders of magnitude and the effect was achieved without measurable concentrations of ozone in the solution. Other microorganisms were also taken into account, *e.g. Clostridium* sp. as a representative of more resistant bacteria, *Salmonella* sp., eneteroviruses or bacteriophages. Besides *Clostridium*, other microorganisms are relatively sensitive to ozone. The higher resistance of *Clostridium* bacteria indicates that they can be used as a significant indicator of microorganism resistance [Xu *et al.*, 2002].

Ozonation also causes a reduction in UV absorbance and colour, which may be regarded as an advantage in some later applications of purified waste.

THE USE OF OZONE IN THE FOOD PROCESSING INDUSTRY

Disinfecting production areas and equipment

A high reactivity and leaving no harmful residues make ozone an effective disinfectant in assuring the quality and microbiological safety of food [Kim *et al.*, 1999].

It is important in food processing plants that the areas which are in contact with food should be as microbiologically pure as possible. Therefore, it should be remembered that surfaces that visually seem clean may still be contaminated with a large number of microorganisms which can further contaminate food [More *et al.*, 2000]. To prevent this, after proper washing various cleaning agents are used. They include chlorine compounds, acids, iodine and four-valent ammonium compounds. Some food processing plants use thermal sanitization and/or radiation. Thermal sanitization is an effective method for destroying microorganisms contaminating food, however, steam and hot water are expensive to generate and excessive heat may damage equipment. Radiation methods are practically not used in food processing plants due to inherent risks related to the presence of radioactive materials. The application of chemical sanitising agents is the most common method used in the food processing industry. Chlorine-containing agents are widely used to disinfect water, waste and food processing plant equipment. However, these chlorine compounds have some drawbacks: harmfulness and irritating action at high concentrations, a tendency to form carcinogenic compounds and a toxic effect on the environment. Despite these disadvantages, chlorine has been used as a disinfectant for water and food for many years as it deactivates all types of vegetative cells. Although chlorination effectively reduces the frequency of food poisoning, chlorine reacts with many organic compounds and forms toxic intermediate products which adversely affect the public health and environment [Gűzel-Seydim *et al.*, 2004 a]. As a result, the interest in additional or alternative disinfectants has been observed to increase [Moore *et al.*, 2000].

Ozone dissolved in water was employed in a newly developed line for washing plastic containers used for storing and transporting meat. Water enriched with ozone is used instead of traditional washing agents. Ozone obtained from the atmospheric oxygen is introduced under controlled conditions to the circulating water under lowered pressure, allowing colder water – between 15 and 30°C – to be used for washing. Although colder water can be used as a result of applying ozone, this method stands out among other such methods as highly effective and having numerous advantages. It is not necessary to use aggressive chemicals or to heat water to 80°C at the final stage of the process. As a result, the pollution of the environment is not as high as in other methods.

Ozone causes also the coagulation of proteins and fats. As a result, fat "catchers" work more effectively and the degree of waste contamination is lower. Being the strongest oxidiser and disinfectant, ozone is able to oxidise remnants of fat and protein and remove them from the surface of containers. The reaction between ozone and the contaminated surfaces lasts several seconds when the concentration of contaminations is about 10%. Compared to traditional chemicals, this time is considerably shorter. Detailed microbiological tests have proven that at all stages of washing ozone caused the total inactivation of bacteria [Steigert & Franke, 2000].

Using gaseous ozone is a method which ensures the full sterility and can be applied in slaughterhouse to sterilize slaughtering tools, particularly knives, which can become a source of contamination during the slaughter, post-slaughter processing and partition of carcasses. Bacteriocidal properties of ozone have been confirmed in the study by Uradziński *et al.* [2005] with knives contaminated earlier with *Escherichia coli*, *Salmonella Enteritidis* and *Staphyloccocus* aureus. The knives were exposed to ozone in 20-minute intervals until they had become fully sterile. In the mentioned study, the total time needed to eliminate the bacteria was found to be 120 minutes, as this is how much it took to deactivate all the *Salmonella Enteritidis* and *Staphylococcus aureus*, although *Escherichia coli* was inactivated after 80 minutes of ozonation. This results from the stronger oxidising ability towards Gram-negative bacteria. In the current study, small tools were exposed to ozone in a device equipped with a UV lamp, emitting 185 nm of radiation, that was enclosed within a leak-proof ozone generator which produced ozone at a dose 0.03 mg/m^3 air. This device was made of stainless steel to enable its long-term use.

The use of ozone in food production

Apart from sterilising equipment and production area, ozone is used in the food processing industry to eliminate microorganism from the surface of meat from slaughtered animals and poultry carcasses as well as in the preservation of food and extension of its shelf life. Reduction of microflora, destruction of pathogens, disinfection and extension of the shelf life of a product can all be achieved by using ultraviolet radiation [Smith & Pillai, 2004]. It has been found that the inactivation of microorganisms by UV radiation results primarily from the damage to their DNA structure. However, some authors see the use of UV radiation in disinfection as problematic since some microorganisms are able to repair the damaged DNA [Morita *et al.*, 2002].

Contemporary studies have shown the effective bactericidal action of ozone towards microorganisms inducing food decay (*P. aeruginosa* and *Z. bailii*), faecal contaminants (*E. coli* and *E. faecalis*) and pathogens causing food poisoning, *e.g. L. monocytogenes*, *B. cereus*, *S. typhimurium* [Restaino *et al.*, 1995]. Despite its effectiveness against spores and vegetative forms of bacterial cells, it is hardly possible that ozone could be used directly to treat food, the reason being the presence of organic matter. Under these conditions, ozone is less effective in reducing the number of bacteria, as it first oxidises the components of the substrate and then the bacterial cells, which results in decreasing the concentration of free ozone with its bactericidal action. In order to achieve the desired reduction in the bacteria number, a higher concentration of ozone would be needed, which in turn might decrease the sensory and health-promoting properties of food [Unal *et al.*, 2001; Moore *et al.*, 2000].

Although the direct application of ozone in food preservation seems hardly probable, at present the food processing industry commonly uses a technique of food preservation which consists in the simultaneous use of methods with additional or synergistic action against pathogens. For example, exposing beef surface to ozone at a dose of 5 mg/L water increases the effectiveness of subsequent treatment at a temperature of 45–75°C against strains of *Clostridium perfringens* which produce enterotoxins. The resistance of both spores and vegetative cells is reduced as a result of exposure to ozone before thermal treatment in which the temperatures might prove ineffective, and applying higher temperatures might reduce the quality of meat, *i.e.* meat surface browning. This, in turn, is another argument for the synergistic application of ozone and moderate temperature in order to reduce the number of *Cl. perfringens* [Novak & Yuan, 2004]. However, the technique of food preservation needs to be carefully evaluated to ensure that the cells which retain their vital functions do not pose a threat of food poisoning or infection. The pathogens which survive the exposure to ozone are less dangerous during the consumption of food than those which survive sublethal thermal treatment [Novak & Yuan, 2003].

Williams *et al.* [2004] argues that applying ozone is a potential alternative method for thermal pasteurisation in controlling the population of *E. coli* O157:H7 and *Salmonella* sp. in apple and orange juice. Considerable reduction of this bacteria is caused by heat and ozone.

Similar results are caused by the simultaneous application of ozone and electric fields (pulsed electric field). Due to the simultaneous use of these two methods, microorganisms which cause damage to food, *i.e. L. leichmanii*, pathogens which cause food poisoning, *i.e. E. coli* O157:H7 and *L. monocytogenes*, are deactivated more effectively because the lethal effect of the electrical field is synergistically enhanced by the prior application of ozone [Unal *et al.*, 2001].

Using ozone during food storage

The success of most methods of food preservation depends on how the processed food is protected during storage from adverse environmental conditions. Food can be protected mainly by packaging. However it is essential that during storage the exposure to different conditions does not cause changes of physical and/or chemical properties of packing materials. On the other hand, efforts should be made to avoid any modification of containers which might affect the quality of food contained in them [Ozen & Floros, 2001]. It has been observed that treating plastic containers with ozone-enriched water reduces the number of bacteria stuck to their surface by 5 orders of magnitude [Khadre & Yousef, 1989]. Ozone is used in food storage for yet another purpose – for controlling odour. The presence of ozone at low concentrations of 0.01–0.04 mg/m^3 air in cold store and store rooms increases the feeling of air freshness.

Moreover, ozone enhances the flavour of fresh unstable products, such as fruit and vegetables, by oxidising pesticides and neutralising ammonia and ethylene produced during the ripening process. Reducing the ethylene level prolongs the permissible storage period and reduces the shrinking of fruit and vegetables [Jaksch *et al.*, 2004]. Some research has been conducted to examine the effect of ozone released continuously to a cold store room at doses of 0.3 and 1.0 mg/m^3 air, on the development of major diseases of grapes and citrus fruit. Ozone had an inhibiting effect on mycelium and considerably reduced the sporulation of *Penicillium digitatum* and *Penicillium italicum*. It should be emphasized that the ozonated room was closed throughout the storage period [Palou *et al.*, 2003].

Other uses of ozone

Apart from the above applications, ozone is widely used to reduce the number of microorganisms in spices. Spices improve the taste and flavour of food, however they usually contain a certain amount of microorganisms. A large amount of microorganisms can negatively affect the food produced with the use of such spices. It may decay prematurely, but it may also be harmful to health (*e.g.* aflatoxins). Spices and vegetables should be free from characteristics which might pose a threat to human health or reduce the usability and shelf life of products containing such spices. Naturally, no spice is free from microorganisms, but their number can be maintained at a low level. This can be achieved by proper harvesting, processing and storage of plants, and by physical methods, such as radiation, microwaves, dry and humid heating, or chemical methods, such as the use of hydrogen peroxide, acids and bases. But the use of gaseous ozone also reduces the amount of microorganisms [Anon., 1992].

Ozone is an effective pesticide used in grain storage. Experts estimate that 5–10% of the global food production is lost every year due to infestation by insects [Anon., 2003]. The gas is an alternative to currently applied agents, some of which can no longer be used for the purpose due to not only their negative effect on the environment, but also the deterioration of the quality of grain. The half-life of ozone is very short and insects are killed by very low doses, while many of the insecticides which are currently in use can be toxic to all organisms present in the storage site and vicinity, including humans. Consequently, on the 29 November 2002, the Ministry of Labour and Social Policy issued a regulation on the highest acceptable doses and concentrations of harmful agents in the workplace. In the regulation, the concentration of 0.15 mg/m^3 of ozone was established as the limit to which a worker can be exposed to during an 8-hour working day and an average working week without serious harm to his/her health or to the health of his/her future offspring.

The use of ozone for therapeutic purposes is possible because of its oxidising and bacteriocidal properties, among others. It is used for disinfection of wounds of various origin; in gynaecology it is used in the treatment of sexual organ ailments of bacterial, fungal and viral origins.

Positive preliminary results obtained in treating cancer in humans and animals suggest the possibility of its therapeutic use in oncotherapy. New therapeutic methods include both increasing oxygen molecular pressure and sensitisation of neoplastic cells hypoxia to the applied radiotherapy. Ozone has such properties, and due to this it enhances the sensitivity of neoplastic cells to the action of ionising radiation [Zieliński, 1997; Madej & Madej, 1993; Ożarowski, 2000].

Ozonotherapy is an effective way of stimulating the immune system in patients with a reduced immunity and/or its absence [Ożarowski, 2000]. In response to the stimulation of an organism with ozone, the immune system produces cytokine. This substance triggers a process of positive changes in the immune system and controls inflammatory processes as well as cell functioning and maturing.

SUMMARY

Ozone is one of the more effective disinfectants; it does not leave any harmful residues in food or on the surfaces which are in contact with it. In addition, compared to chlorine and other disinfectants, it is more effective against resistant viruses and spores. Exposing some products (*e.g.* fruit or vegetables) to ozone during their storage period extends their shelf life without affecting their sensory value.

The use of ozone does not require high temperature, hence it offers the possibility of energy saving. Ozone must be produced on the spot, which leads to savings on transport and storage costs of disinfectants. The cost of an ozone generator may raise concerns in a small businesses; however, such fears are unfounded because the purchase of such a generator may prove economical in the long run [Moore *et al.*, 2000].

Many people are critical of the use of ozone as it can produce an irritating substance in the air; it can be felt at low concentrations and can even be poisonous at higher concentrations. Despite such reservations, it must be said that when used under controlled conditions, it is an effective and totally safe disinfectant [Steigert & Franke, 2000].

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OZON JAKO ALTERNATYWNY ŚRODEK DEZYNFEKCYJNY

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