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Review Article

Applications and Investigations of Ozone in Cereal Grain Storage and Processing: Benefits and Potential Drawbacks

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A B S T R A C T

Pest Control (insects and microorganisms including mould, fungi and bacteria) development in stored grains is essential at the time of storage after harvest. It is a great challenge since it currently leads to a grain yield loss around 3–10% in developed countries, and can reach 50% in certain countries if proper steps cannot be taken. Many studies have demonstrated that ozone treatment can be used to reduce levels of either biological or chemical contaminants in grains. Ozone use is more convenient, because it quickly decomposes into O_2 and hence does not leave residues. It is easily generated by either corona discharge (CD), ultraviolet (UV) or electrolysis of water on the site where it is required and so eliminates the need to store dangerous chemicals. It can be applied in the gaseous or aqueous state and has been demonstrated to be at least as efficient a disinfectant as chlorine. It has been approved as an antimicrobial agent by the US Food and Drug Administration (FDA 2001) for direct contact with all foods, and recently approved by the French Food Safety Agency for grain treatment. It has been found that Ozone react either directly or indirectly i.e. by the production of superoxide radical ion and its hydrogenated form, the hydro-peroxide radical with many endogenous compounds in the product. Ozone can react with sulfhydryl-containing proteins which play an important role in cereal product quality. It has been found that there is no effect of ozone treatment on corn grain germination, using 50 ppm of ozone. If ozone concentration was maintained under 0.98 mg/g of grains per minute, no effect on germination was observed even after 45 minutes of treatment. It affects the germination rate only when high dose is used for short exposure time. Ozonation efficiency varies depending on the nature and state of the target, as well as the ozonation conditions (temperature, time, water content). The benefits of ozonation must be compared against possible alternative techniques (as for example ionisation, pulsed light, etc.) in terms of cost, efficiency and potential drawbacks. Ozone treatment could also be used in combination with other type of process.So it is necessary to study the efficiency of ozone and UV light in combination to reduce the microbial mass.

Keywords

Pest control, ozonation, ozonator, seed germination, crona discharge

Introduction

Fumigation is widely used for disinfestation of durable foodstuffs. This technology is versatile and valuable from the point of view of stored products protection i.e. it gives low cost-effective solution to problems of insect attack in stored grain, oilseeds, pulses, coffee etc. but these have created problems with environmental contamination, health concern both for the public and workforce, particularly with respect to potential or suspected carcinogenicity of some fumigants along with the production of residues. Ozone has been used to control pest development in stored grains, to disinfect flours and to degrade potentially toxic molecules. It can be applied in the gaseous or aqueous state and has been demonstrated to be at least as efficient a disinfectant as chlorine, and more convenient, because it quickly decomposes into O_2 and hence does not leave residues (Graham 1997). It may easily generated onsite i.e. the place where it is required, by either corona discharge (CD), ultraviolet (UV) or electrolysis of water (Kim *et al.,* 1999), eliminating the need to store dangerous chemicals. It has been approved as an antimicrobial agent by the US Food and Drug Administration (FDA 2001) for direct contact with all foods, and recently approved by the French Food Safety Agency (AFSSA 2004; Pernot *et al.*, 2007a) for grain treatment. It has been found that ozone react either directly or indirectly with many endogenous compounds in the product by the production of superoxide radical ion and its hydrogenated form, the hydroperoxide radical (Khadre *et al.,* 2001). Ozone reacts with sulfhydryl-containing proteins (Cataldo 2003), which play an important role in cereal product quality also (Lindsay and Skerritt 1999).

Ozone application in grain storage

The control of insects and microorganisms including mould, fungi and bacteria development in stored grains is essential as it currently leads to a grain yield loss around 3–10% in developed countries, and can reach 50% in certain countries (Jayas 1999; Magan and Aldred 2007).

Insect control

Storage grains are very susceptible to a number of insects, such as *Tribolium*, *Sithophilus* and moths. These insects cause considerable damage and somewhere has potentially developed resistance to the currently used insecticides. Europe and USA has reduced the permitted amount of pesticide due to increasing concern towards green environment or even ban their uses. In such a scenario, ozone as a fumigant is an interesting alternative to applied chemicals for the control of insect development. Kells *et al.,* (2001) evaluated the efficiency of ozone fumigation in a corn grain mass (around 9 tons) against adult insects, such as the red flour beetle (*Tribolium castaneum*), maize weevil (*Sithophilus zeamais*) and larvae from the Indian meal moth (*Plodia interpunctella*). He has found that the lower dose was also significantly efficient but led to a lower insect mortality (77–99.9%) depending on the insect species. Ozonation conditions were also pointed out to be critical for ozone efficiency. Kells *et al.,* (2001) pointed out that an apparent air velocity of 0.03 m/s was necessary for ozone penetration through corn grain mass if a concentration of 50 ppm was used. No any correlation has been found between the resistance to phosphine and insect mortality following insect treatment, suggesting that the insect defence mechanisms developed for phosphine resistance are ineffective for ozone. Indeed, variability in the lethal time to kill 50% of the insect population only varied by 1.6-fold in each species evaluated, depending on the origin of the collected insect sample. Since, no cross resistance between the two gases was observed, as also reported before by Qin *et al.,* (2003), ozone could be considered as a potential viable alternative to overcome insect resistance to chemicals.

Microorganism control and Reduction of toxic chemical levels

Fungi development in grains depends on a number of factors, including cultural practices and field weather conditions, date of inoculation, resistance of the plant and storage conditions (Edwards 2004; Jouany 2007). The majority of infecting fungi types, such as *Aspergillus*, *Fusarium* and *Penicillium*, were able under certain conditions to produce mycotoxins, which have been shown to be harmful for humans and livestock. After harvest, the fungi growth and mycotoxin production can be reduced by drying grains or maintaining a moisture content below 14% and a low temperature of storage (Homdork *et al.*, 2000; Lugauskas *et al.,* 2007; Magan and Aldred 2007) but this will not ensure fungi removal, which can only be achieved by grain abrasion (Laca *et al.,* 2006; Rios *et al.*, 2009). The fungicidal efficacy of gaseous ozone for the reduction of *Aspergillus parasiticus* in corn was demonstrated by Kells *et al.,* (2001). 63% reduction of the fungi load was observed in a batch of around 9 tons of grains using 50 ppm ozone for 3 days.

In the case of stored wheat grain moisture content was enhanced to around 22%. Indeed, an increase in the moisture content and thus in the water activity of wheat grains was shown to enhance the fungicidal effect of ozone on wheat grains (Table 2.2); this was also further confirmed by Raila *et al.,* (2006). The presence of water potentially could accelerate ozone decomposition and thus the production of oxidant radicals able to react rapidly with organic compounds (Khadre *et al.,* 2001). Wu *et al.,* (2006) also observed an increase in ozone efficiency with a rise in temperature from 10 to 40 °C (Table 2.2) when controlling insect contamination.

n.d. (Not determined)

As wet and warm conditions are the most detrimental for stored grains, favouring microorganism development, the enhanced activity of ozone under the same conditions is advantageous. Therefore, interest in ozone use as a potential fungicide against the main grain contaminants has been demonstrated. Therefore, Ozone can also be considered as helping to reduce mycotoxin accumulation during grain storage due to its inactivating action on fungi. Furthermore, its oxidant properties could also be used for mycotoxin degradation and detoxification, as demonstrated by McKenzie *et al.,* (1997). McKenzie *et al.,* (1998), who reported a 95% decrease in the aflatoxin B1 level produced by *Aspergillus* strains in naturally contaminated corn grains using around 7 mg/kg of ozone per minute for a 92-hour exposure. These results were supported by feeding poultry – which are the most sensitive animal to aflatoxicosis – with either nontreated or ozone-treated ground contaminated grains mixed with soybean.

Effects on grain germination

It has been found by Mendez *et al.,* (2003), there is no effect of ozone treatment on corn grain using 50 ppm of ozone. These results were also later confirmed by dos Santos *et al.,* (2007) using 100 ppm of ozone at 4.6 L/min. Rozado *et al.,* (2008) did not observe any changes in physiological quality of corn grains following ozone treatment (50 mg/kg ozone injected at a flow of 0.8 L/min) also. Allen *et al.,* (2003) demonstrated that the effect of ozone on the germination capability of barley grains was dose dependent.

If ozone concentration was maintained under 0.98 mg/g of grains per minute, no effect on germination was observed even after 45 minutes of treatment. Violleau *et al.,* (2008) further demonstrated that short-term ozone exposure resulted in a higher germination rate of corn seeds compared to samples ozonated for longer periods, which resulted in lowering of germination levels.

Table.1 Fumigant Listed in Bond (1984) with current status and threats

Fumigant	Threat[a]	Status[b]
Acrylonitrile	Suspect carcinogen, residues	
Carbon disulphide	Lack of interest	*
Carbon tetrachloride	Ozone depletor, residues	
Chloropicrin	Almost forgotten	*
Dichlorvos	Residues, alleged carcinogen	*
Ethylene dibromide	Environmental contamination, fertility effects, alleged carcinogen	
Ethylene dichloride	Not very effective, alleged carcinogen	
Ethylene oxide	Suspect carcinogen, residues	
Ethyl formate	Almost forgotten	*
Hydrogen cyanide	Lapsed Codex Alimentarius registration	*
Methallyl chloride	No food registration	
Methyl bromide	Ozone depletor, alleged carcinogen	*
Methyl formate	Almost forgotten	*
Phosphine	(see text)	*
Sulphuryl fluoride	No food registration	
Trichloroethylene	Not very effective, residues	

[a]Suspect "carcinogens", [b]Materials are either still in common use or may no longer be registered

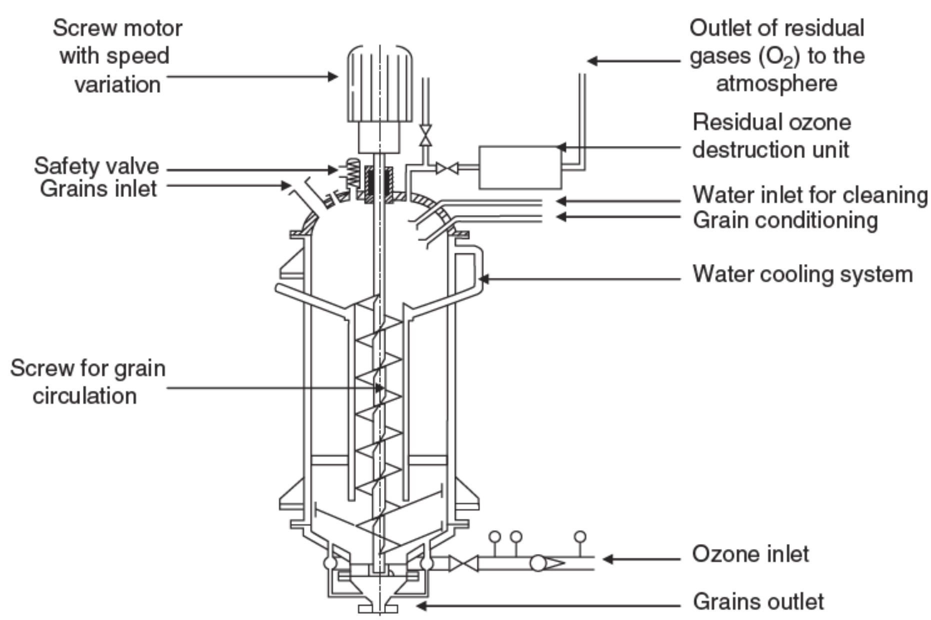
Table.2 Percentage of insect mortality reported by Isikberg *et al.,* (2009)

Ozonation conditions	Adults (n = 25)	Pupae (n = 25)	Larvae (n = 25)	Eggs (n = 100)	Insect species
Empty space	100	100	100	62.5	*Ephesia kuehniella*
Top of wheat grain mass	100	100	100	100	
Bottom of wheat grain mass	100	90	92	3	
Empty space	4	14	74	4	*Tribolium confusum*
Top of wheat grain mass	5	44	86	8	
Bottom of wheat grain mass	1	22	72	6	

Table.3 Percentage of spore survival reported by Wu *et al.,* (2006)

Ozonation conditions (T, grain moisture content)	% spore survival	
	T	T + 30
20 °C, 16.1%	30.1	26.5
20 °C, 19.3%	9.2	5.2
20 °C, 21.9%	3.1	0
10 °C, 16.1%	38.9	n.d.
40 °C, 16.1%	9.7	n.d.

Fig.1 Oxygreen Reactor (Dubois *et al.,* 2006)



Industrial applications

Ozone treatment of wheat grains (before processing) was reported to improve flour safety and potentially its technological properties (Yvin *et al.,* 2001; Pernot *et al.,* 2007a, b). Oxygreen (Figure 3.1), a specific process has been developed, which enables treatment of grain with 0.5–20 g of ozone per kg of grain (80–160 g/m3 TPN ozone in gas vector) for a treatment time of 5–70 minutes. Ozone treatment generally results in flours with increased dough toughness, which is in accordance with the observed effect on the wheat storage protein network (Figure 6.3; Desvignes *et al.,* 2008). It is interesting to note that ozone treatment before wheat grain fractionation leads to a reduction in the required energy for grinding and the resultant flours are slightly enriched with compounds from the aleurone layer (Desvignes *et al.,* 2008). The aleurone layer is a grain tissue which contains biochemicals that enhance flour nutritional properties. However, this observed effects on microbial mass or milling energy reduction could also be partly related to the debranning efficiency of the ozone treatment under the described conditions (Coste *et al.,* 2007).

Coste and Dubois (2005) reported another interesting potential application of ozone in wheat flour processing. Ozone use during dough formation was investigated, with the ozone added through the water added to flour, yeast and salt mixes, as a gas in the kneader, and through both methods. The total amount of ozone added varied between 4 and 60 mg/kg of produced dough per hour. The authors concluded that ozone addition allows a reduction in the mixing time or speed for a similar dough quality and thus could lead to an energy gain of between 15 and 23% during mixing. The resultant dough also appeared to develop faster and to be more resistant to overmixing.

Many studies have demonstrated that ozone treatment can be used to reduce levels of either biological or chemical contaminants in grains. Ozonation efficiency varies depending on the nature and state of the target, as well as the ozonation conditions (temperature, time, water content). In order to improve the control and prediction of ozone efficiency, an improved understanding of ozone diffusivity as influenced by equipment design for grain treatment, the grain tissue structure (porosity) and the ozonation parameters is needed. Similarly, the effect of ozone on grain biochemical compounds, which depend on the physiological state of the plant material, ozone penetration and treatment duration, also needs to be better characterised in order to control potential detrimental effects.

Furthermore, ozone treatment in some cases also appears to facilitate a reduction in the required processing energy. However, only a few economical evaluations of ozone treatment have been reported for grain decontamination (Pereira *et al.,* 2008a) or for flour production. The price of flour produced with the Oxygreen process is twice that of flour for untreated grains.

The benefits of ozonation must be compared against possible alternative techniques (as for example for grain or flour decontamination using ionisation, pulsed light, etc.) in terms of cost, efficiency and potential drawbacks. Finally, ozone treatment could also be used in combination with other type of process, as reported by Laszlo *et al.,* (2008), who studied the efficiency of ozone and UV light in combination to reduce the microbial mass in wheat flours.

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