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PRODUCTION OF MEDICATED BEDDING STRAW: CHALLENGES AND PERSPECTIVES

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Abstract. *Previous bacteriological findings have reported that animal disease outbreaks are associated with the quality of the animal environment. Animal bedding straw is a good source of bacteria and fungi, typically contaminated with (mycelia) yeasts and filamentous fungi species such as Aspergillus, Fusarium, Alternaria, Cladosporium, Epicoccum, Penicillium, Verticillium and Enterobacteria. The objective of this work was to assess the efficacy of different technologies on the production of medicated bedding straw. Four critical control points of an industrial straw disinfection processing line were identified. The levels of fungi and bacteria present in the straw during an industrially applied mechanical – chemical process were quantified. The plate counting revealed that propionic acid and formaldehyde chemicals reduced the microbial levels from the raw material and that they were more efficient on moulds than on bacteria. The potential use of ozone gas as an alternative greener technology to the current liquid chemical treatments was also evaluated. Trials conducted on ozone treatments (flow rates: 0.031, 0.125, 0.5 L/min, concentrations: 36, 99, 150 µg/mL, treatment time: 0, 5, 15, 30 mins, and residual times of 0 to 18 hrs) indicated that ozone successfully reduces the microbial counts and the fungi levels by more than 1.5 logs (cfu/g).*

Keywords. Ozone, antimicrobial, medicated straw, bedding, moulds, molds, propionic acid, formaldehyde, disinfection

Introduction

Straw is a popular bedding material for a large variety of livestock (e.g. cattle, horses, poultry) because it is quite absorbent, cheap, warm and easy to maintain. Straw has been shown to support the growth of moulds and its spores. These spores can then be released into the air as dust, which can affect livestock and farmers. Previous reports have shown that animals whose well-being is compromised are often physiologically and immunologically abnormal (Poole, 1997). Animal husbandry has become more important in agriculture with the introduction of increased legislation to protect animal welfare and the realisation that proper husbandry can improve not only animal welfare but productivity.

In a study by Wechsler et al. (2000) to compare different types of soft lying mats with straw bedding, regarding cow behaviour and leg injuries; no significant differences between cows kept in cubicle systems with soft lying mats and straw bedding were found, except in terms of leg injuries. Consequently, straw bedding was shown to be the most favourable option with respect to leg injuries located in the tarsal joints (Wechsler et al., 2000). However cows bedded on sawdust had greatest teat end populations of total coliforms and *Klebsiella,* while *Streptococci* were the most numerous on straw-bedded cows (Rendos et al., 1975). Blokhuisa et al. (2008) believed that bedding material such as straw may act as vehicle of diseases such as Foot and Mouth Disease (FMD) or Aujeszky's disease. These reports support the application of disinfection treatments to straw or other bedding materials before their use in a housing system.

The microflora in the straw will depend primarily upon the initial levels of contamination in the grain crop. In a study to examine toxin contamination of cereals crops (wheat and barley), the main fungal contaminant species identified were *Aspergillus flavus, Aspergillus fumigatus, Fusarium graminearum*, and *F. culmorum* (Tabuc et al., 2009). This supports the findings of Gutwiller (2006), who showed that bedding straw was contaminated by *Fusarium* toxins. Other studies have shown that chopped and baled straw had much greater populations of storage fungi, particularly *Aspergillus*, *Penicillium* spp., and *Wallemia sebi* (Magan, 1988). *Alternaria alternate, Aspergillus ochraceus, A. flavus* (Hassan, 1999*), F. avenaceum, F. sporotrichioides, F. oxysporum, Penicillium* have also been found in grains which make them potential straw contaminants (Mačkinaitė et al., 2006, Askun, 2007; D'Mello, 2004). Concerning bacteria, *Pasteurella haemolytica* was isolated from straw bedding samples (Burriel, 1997). Additionally, *Escherichia coli* and species of *Pseudomonadaceae, Micrococcaceae, Bacillaceae, Lactobacillaceae, Salmonella* spp (Laca et al., 2006) also stand as potential contaminants as they are present in cereal grains.

The use of sulphur dioxide and ammonia as fumigation agents have been investigated for controlling mould spoilage in storage grain (Magan and Aldred, 2007). Another preservative method is based on use of aliphatic acids, which have been employed to prevent spoilage and mycotoxin contamination of stored commodities, especially animal feed. Attempts have been made to use alternatives such as essential oils and anti-oxidants to prevent growth and mycotoxin accumulation in partially dried grain (Magan and Aldred, 2007). A number of commercial products predominantly based on propionic and sorbic acids are available. However, these require thorough coverage of the material to be completely effective (Magan and Aldred, 2007). There are several methods of application of such chemicals, whether it is dip coating, a spray-on application, or the creation of a gaseous environment by the evaporation of liquid in a sealed chamber. Marín et al. (2000) found that different mixtures of propionic and sorbic acids failed to inhibit the growth of *Fusarium* section *Liseola* species in trials. Other industrial practices include the treatment of straw with the combination of food grade bactericides and mould inhibitors. Chemicals such as propionic acid and formaldehyde are used

effectively for these decontamination treatments. Even though some of the previously discussed disinfectants are efficient, alternative environmental friendly treatments should also be considered. Ozonation is a well-established method of disinfecting water whether it is for drinking supplies, bottled water, swimming pools or wastewater treatment plants (Guzel-Seydim et al., 2004; Tiwari et al., 2010). Ozone disinfection is a green alternative to chemical disinfection. Its capacity to react with numerous chemical groups, offers many advantages, such as wide antimicrobial spectrum which combined with a high oxidation potential makes it an attractive processing option for the food industry (Tiwari et al., 2010). Ozone decomposes quickly, and leaves no residues while fruits and vegetables treated with ozone were found to have a considerably longer shelf-life (Guzel-Seydim et al., 2004; Tiwari et al., 2010).

Restaino et al. (1995) studied the antimicrobial properties of ozonated water on food related microorganisms and established that ozone was very effective in destroying gram positive bacteria (*Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis*), and gram negative bacteria (*Pseudomonas aeruginosa*, and *Yersinia enterocolitica*). Ozone destroys bacteria by attacking the bacterial membrane glycoproteins and/or glycolipids (Guzel-Seydim et al., 2004). Restaino also showed that ozone was capable of inactivating yeasts (*Candida albicans* and *Zygosaccharomyces bacilli*) as well as spores of *Aspergillus niger*.

The objective of this work was to assess the efficacy of different technologies, i.e., mechanical in tandem with chemical and ozonation, on the production of medicated bedding straw.

Materials and methods

The microflora levels of straw were assessed during two types of treatment. Firstly, the effect of an industrial process (including the mechanical and chemical treatments) and secondly the effect of a lab scale process of ozonation on the reduction of straw microflora were investigated.

Straw industrial processing line (including the mechanical and chemical treatments)

Following the harvest, the straw is collected and stored under cover until it is further processed (Raw material, R shown in Figure 1). The straw is then milled to increase absorbency. The milled straw is screened and aspirated (cleaning step) to remove most of the dust and microflora present while treatment with bactericides and mould inhibitors is then applied. Chemicals such as propionic acid and formaldehyde (bactericide and mould inhibitor) are used in order to decontaminate straw.

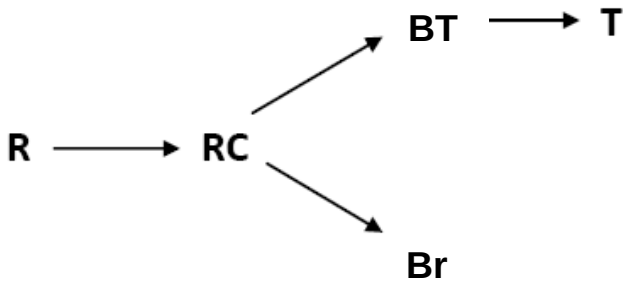

Figure 1. Straw industrial treatment process: R (Raw material), RC (Raw cut material), BT (Straw after cleaning but before treatment), Br (Dust + Briquette), T (Straw after treatment T).

Samples of straw from the various stages of the industrial operation (RC, R, Br, BT and T) were collected in order to quantify the levels of microflora at the different stages of the industrial process. Microbial analysis was performed as described in “Microbiological analysis”.

Ozone Treatments

A series of lab tests were performed in an ozone column (100 ml volume). The ozone had to be produced on site by an ozone generator (Ozone Services, Burton, B.C., Canada) operating with an electrical (corona) discharge method. The levels of the produced ozone were measured by an ozone gas analyzer (Ozone Services, Burton, B.C., Canada). In order to assess the disinfection efficacy of ozone various flow rates were tested. 5 g of straw sample was loaded into the 100 mL ozone (Figure 2). Three types of treatment were applied: (i) a treatment that focuses on the reduction of the microflora of the raw cut (RC) material (ii) treatment that assesses the effect of the residual ozone during the storage of the product (iii) treatment that assesses the effect of the ozone on the post-aerated (P-A) straw. The operation conditions of these treatments were as follows:

1. RC material was treated at a flow rate: 0.5 L/min, with an average ozone concentration: 36 µg/mL (Table 1). Treatment times were: 0, 15, 30 min, All tests were conducted in duplicate.
2. P-A straw samples were treated with ozone (Flow rate: 0.5 L/min. ozone concentration: 36 µg/mL.) (Table 1) for 30 min. then left in a sealed environment for a period (4 hrs or 18 hrs) before microbiological analysis to study the effect of residual ozone treatment.
3. P-A straw was subjected to a range of treatments at various flow rates (0.125, 0.031 L/min) and various ozone concentrations (36, 99, 150 µg/mL). (Table 1) Treatment time: 0, 5, 15, 30 mins.

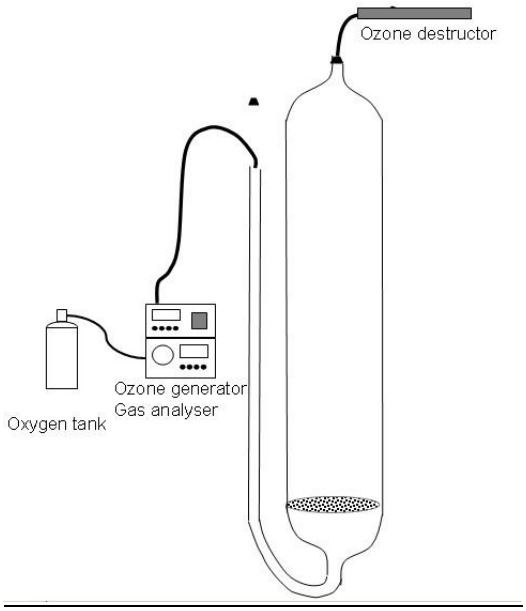

Figure 2. Ozone bubble column.

Microbiological analysis

Sample material was transferred to a sterile stomacher® bag and MRD (Maximum Recovery Diluent, Oxoid, CM0733, Basingstoke, Hampshire, England). The bag was then placed in a Stomacher® 400 Circulator, laboratory paddle blender (Seward, 4a Southdownview Way, Worthing, West Sussex, BN14 8NL, England) for a period of 3 minutes at 230 rpm. 1 ml was then extracted and placed in 9 ml of Ringer solution (Oxoid BR00526) (Oxoid, Basingstoke, Hampshire, England). Diluted samples were spread on different nutrient agar plates depending on the microflora under investigation.

Straw was screened based on three nutrient media:

- PCA (Plate Count Agar, Oxoid CM0325) (Oxoid, Basingstoke, Hampshire, England) non-selective medium. Incubation conditions were: 1-2 days at 30°C.
- VRBGA (Violet Red Bile Glucose Agar, Oxoid CM0485) (Oxoid, Basingstoke, Hampshire, England), selective medium which only enables *Enterobacteria* to grow. Incubation conditions were: 24 hours at 30°C.
- MEA (Malt Extract Agar, Oxoid CM0059) (Oxoid, Basingstoke, Hampshire, England) nutrients agar for moulds and yeasts. 4 ml of lactic acid was added to the agar before pouring the plates. Incubation conditions were: 7 days at 30°C.

Three replications were performed and data were expressed in CFU/g.

Results and Discussion

Critical Control Point (CCP) in straw processing

A Critical Control Point is a stage in a process in which specific controls can be applied in order to reduce a food safety hazard to acceptable levels. The first is at the initial acceptance of the raw material non cut CCP 1 and then when the straw is cut is CCP 2. The third (CCP 3), is at the stage the straw is mechanically processed. The fourth and final Critical Control Point, CCP 4 is at the exit of the final product (Figure 3).

- CCP 1: at the entry of the raw material (non cut).
- CCP 2: at the entry of the raw material (cut).
- CCP 3: at the stage of the mechanically processed straw.
- CCP 4: at the exit of the final product.

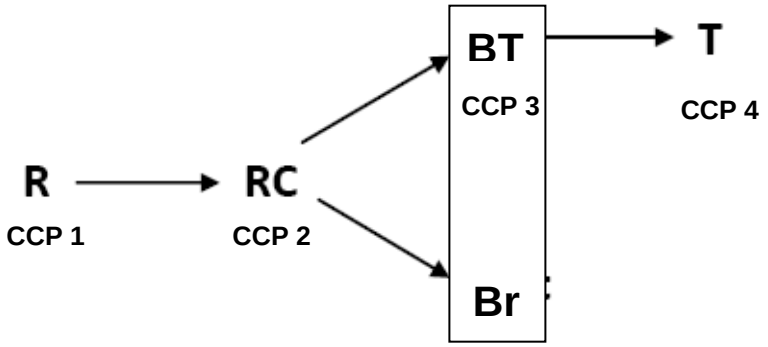

Figure 3. Critical control points (CCP) of the medicated bedding straw industrial processing line

Microbiological analysis

From the PCA media (Figure 4) it was observed that the use of propionic acid and formaldehyde chemicals on straw reduced the microbial level from the raw material to the final product by 1.143 logs. The total counts were lowered at each of the processing steps (cutting and chemical treatment), 0.773, and 0.327 logs, respectively. The BR value (6.574 logs) appears to be higher than the initial concentration recorded at R (5.87 logs). This is to be expected as the product at this stage consists of the concentrated dust of the raw material.

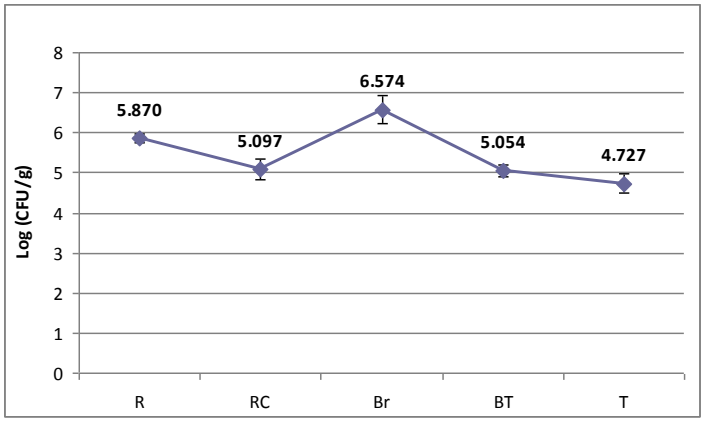

Figure 4. Quantification of straw microflora in PCA media at the different industrial processing steps

With respect to the *Enterobacteriaceae* substantial reductions were observed following the cleaning (BT) and chemical treatment (T) stages where the microbial levels were reduced 0.796 and 1.597 logs, respectively, when compared with the raw material (Figure 5).

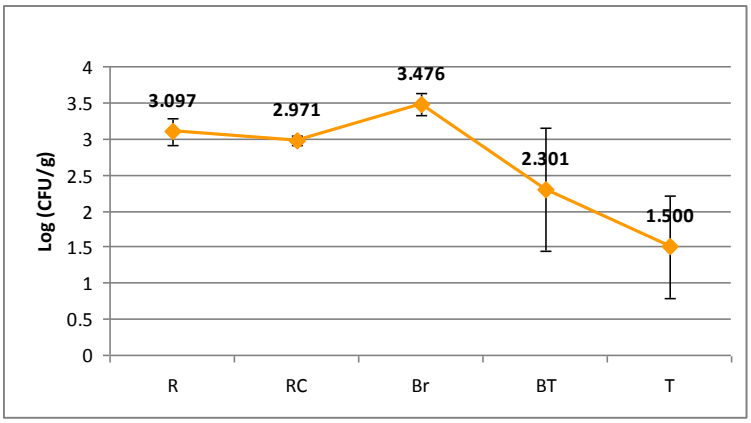

Figure 5. Quantification of *Enterobacteriaceae* in VRBGA media.

As a result of the cutting process (RC), the CFU was reduced by 0.69 logs. The greatest reduction however was observed following the treatment phase where the CFU was reduced by 1.251 logs. The 1.984 logs yeasts and moulds reduction from the initial raw material to the final product was due to the use of propionic acid and formaldehyde (Figure 6).

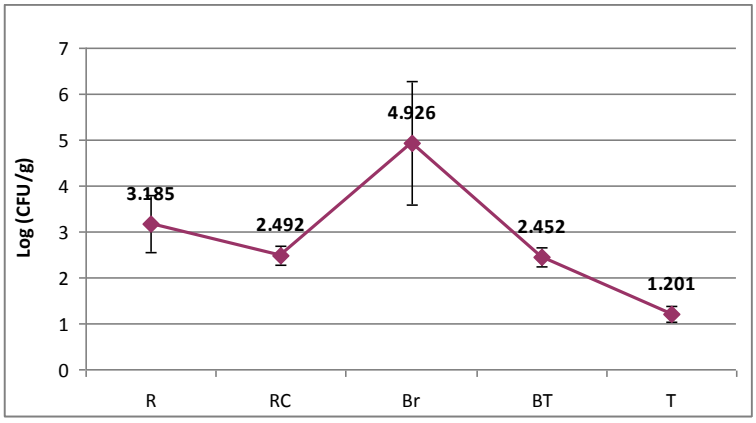

Figure 6. Quantification of yeasts and moulds in MEA media.

Ozone Treatments

Treatment of RC (raw cut) material

As mentioned in the “material and methods”, there were three treatment types studied. The first ozone treatment type focused on the reduction of the microflora of the RC (raw cut) material. This was achieved by using a flow rate of 0.5 L/min in turn generating an ozone concentration of 36 µg/mL. Results (Figure 7) show that the use of ozone (for treatments that lasted 15 and 30 minutes) on straw reduced the initial microbial level of the raw material by 1.082 and 1.359 logs, respectively. This indicates that the initial 15 minutes exposure was more effective than the subsequent 15 minutes.

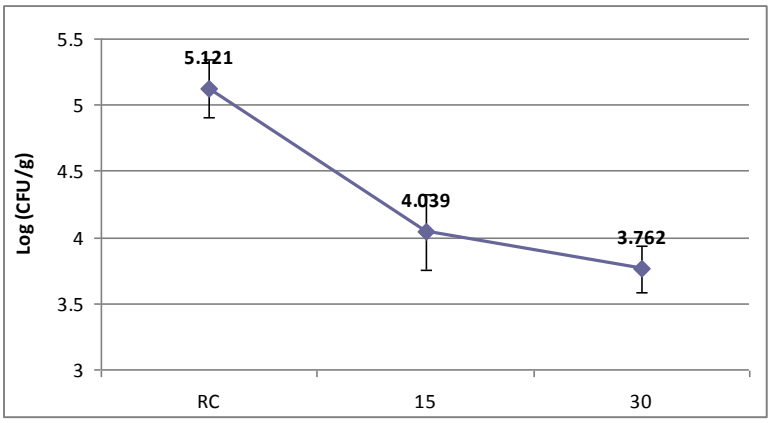

Figure 7. Quantification of straw microflora in PCA+ media as a result of ozone treatment of RC straw for either 15 or 30 minutes

The *Enterobacteriaceae* levels were reduced by 1.745 and 1.82 logs from the raw material following ozone treatment (Figure 8).

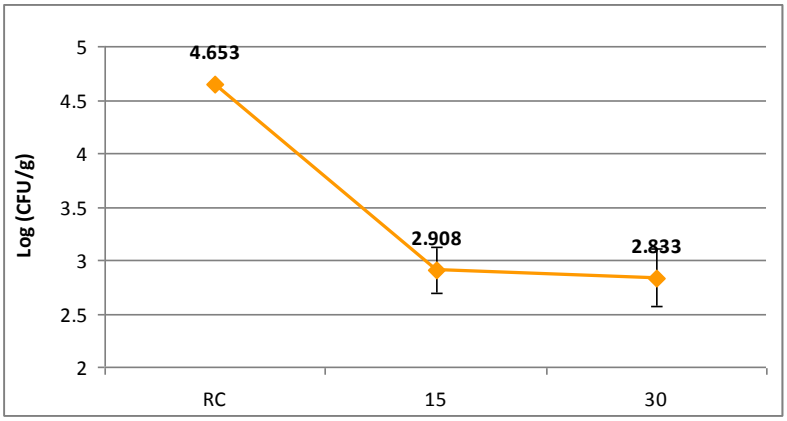

Figure 8. Quantification of *Enterobacteriaceae* in VRBGA media as a result of ozone treatment of RC straw for 15 or 30 minutes (Ozone conc: 36 µg/mL)

From the MEA media (Figure 9) it was observed that the use of ozone on straw reduced the mould levels from the raw material by 3.224 logs.

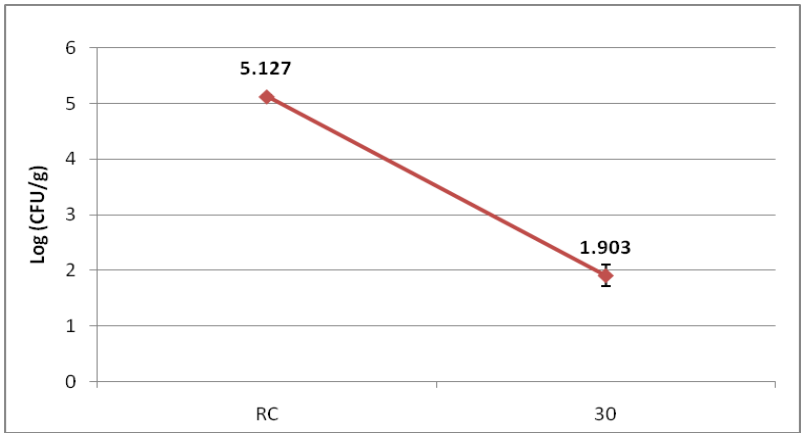

Figure 9. Quantification of yeasts and moulds in MEA media as a result of ozone treatment of RC straw for 30 minutes (Ozone conc: 36 µg/mL)

From all the obtained results it can be clearly seen that the use of chemicals on straw reduced the microbial levels from the raw material to the final product. The first ozone treatment was conducted on raw straw material after it had been chopped. The material at this stage has a greater surface area so would enable better interaction between the straw and the ozone treatment. The treatment method was tested over 3 different exposure times, 0, 15, 30 minutes. It is obvious from Figure 7 (PCA media), Figure 8 (VRBGA media) and Figure 9 (MEA) that there is a substantial reduction in the microflora levels as a result of the ozone exposure. The reduction is even more impressive when compared to the results from the initial straw microbial analysis from the various process steps (Figure 4, 5 & 6). PCA log reduction for ozone was 1.359 logs as opposed to 1.143 logs for all the process stages. However only 0.327 logs of that 1.143 log reduction can be attributed to the chemical treatment step. It is a similar situation for both the VRBGA and the MEA analysis.

Effect of residual ozone

The second ozone treatment type was designed to assess the effect of the residual ozone during the storage of the product. Again an ozone concentration: 36 µg/mL was used with a treatment time of 30 minutes. Following the initial treatment the sample was left in a sealed environment (for either 4 or 18 hrs) to study the residual ozone effect. From the PCA media (Figure 10) it was observed that the use of ozone treatments (4 and 18 hrs) on straw reduced the initial microbial level of the raw material by 1.214 and 1.124 logs.

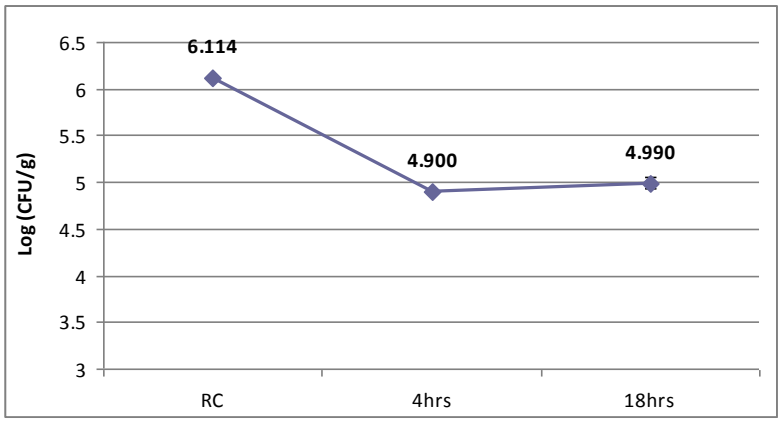

Figure 10. Quantification of straw microflora in PCA+ media as a result of ozone treatment of RC straw for 4 or 18 hours. (Ozone concentration: 36 µg/mL)

From the MEA media (Figure 11) it was observed that the use of ozone treatments on straw reduced the microbial levels from the raw material up to 1.033 logs.

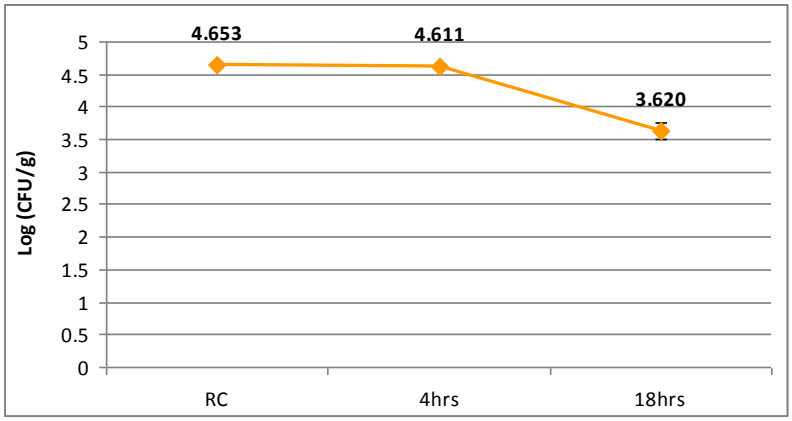

Figure 11. Quantification of *Enterobacteriaceae* in VRBGA media as a result of ozone treatment of RC straw for 4 or 18 hours. (Ozone concentration: 36 µg/mL)

Treatment of BT (straw after dust removal) material

The third ozone treatment type assessed the effect of the ozone on mechanically treated straw. Several flow rates (0.5, 0.125, 0.031 L/min) were used to generate different ozone concentrations (36, 99, 150 µg/mL). From the PCA media (Figure 12) it was observed that the use of ozone treatments at 0.5 L/min (15 and 30 minutes) on straw reduced the microbial level of the straw material (after dust removal) by 0.611and 0.905 logs.

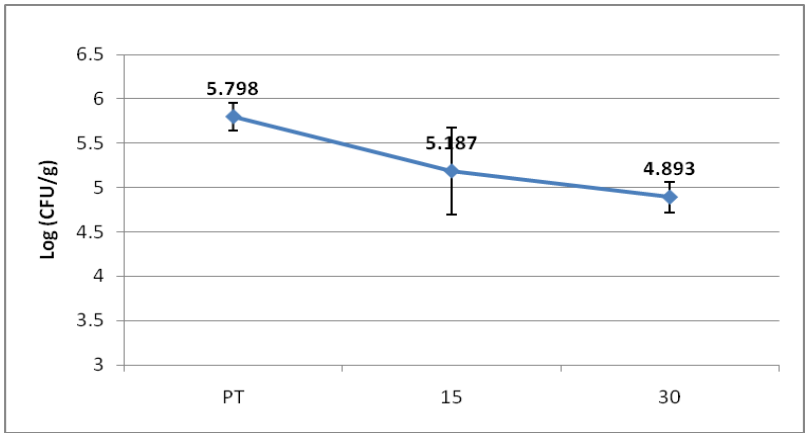

Figure 12. Quantification of straw microflora in PCA media as a result of ozone treatment of straw (after dust removal) for 15 or 30 minutes. (Ozone concentration: 36 µg/mL)

The *Enterobacteriaceae* levels on the VRBGA and MEA media from the P-A material following ozone treatment resulted in high microbial variation and results are not shown.

From the results of the PCA media (Figure 13) it was observed that the use of ozone treatment, ozone concentration: 99 µg/mL (5, 15 and 30 mins) reduced the microbial level of the straw material post aeration by 0.777, 1.654 and 2.393 logs. A linear relationship between exposure time and CFU reduction was observed for these type of data.

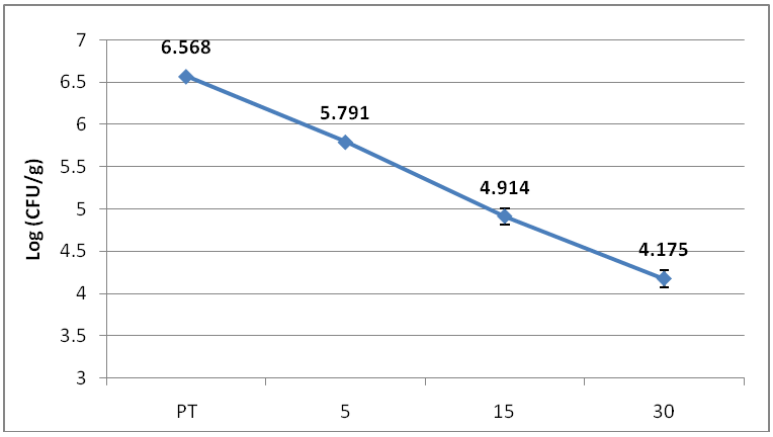

Figure 13. Quantification of straw microflora in PCA media as a result of ozone treatment of straw (after dust removal) for 5, 15 or 30 minutes. (Ozone concentration: 99 µg/mL)

The *Enterobacteriaceae* levels in VRBGA media. (ozone concentration: 99 µg/mL) were reduced by up to 0.247 logs from the raw material following ozone treatment. While results are promising, ongoing research is underway to examine ozone performance in more concentrations.

The PCA media results (Figure 14) indicate that the use of ozone treatment (ozone concentration: 150 µg/mL) on straw reduced the microbial level of the straw material with the greatest reduction observed with the initial 5 minutes of treatment.

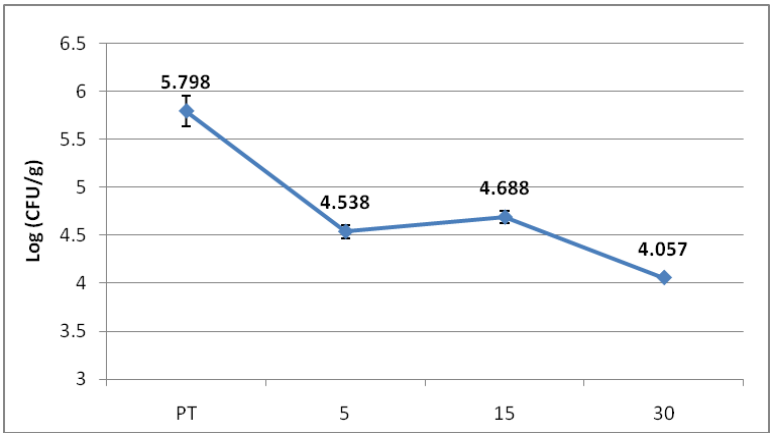

Figure 14. Quantification of straw microflora in PCA media as a result of ozone treatment of straw (after dust removal) for 5, 15 or 30 minutes. (Ozone concentration: 150 µg/mL)

The *Enterobacteriaceae* levels were reduced by 0.164 logs from the raw material following ozone treatments of 15 minutes (Figure 15). The results from the 5 minute exposure trial were excluded due to excessive variance.

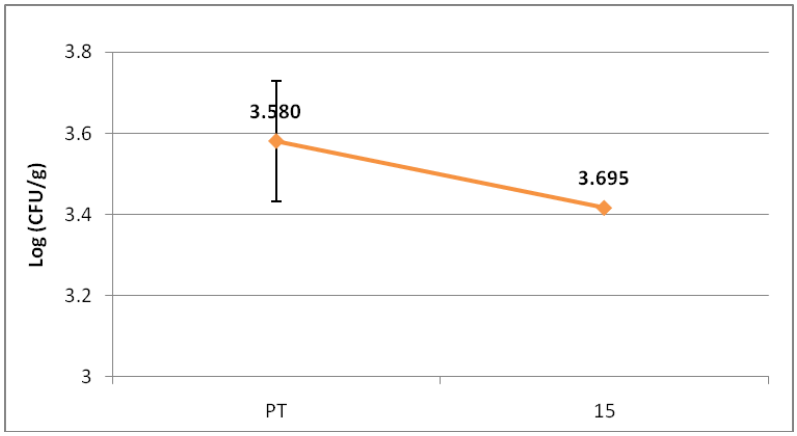

Figure 15. Quantification of *Enterobacteriaceae* in VRBGA media. media as a result of ozone treatment of straw (after dust removal) for 15 minutes. (Ozone conc: 150 µg/mL)

Conclusions

The aims of this study were to identify the most dominant microflora in straw used for animal bedding by macroscopic bacteria fungi analysis and to evaluate ozone treatments as a potential alternative to current chemical treatment methods. Four critical control points (CCPs) of a processing line for straw disinfection were identified. CCP 1 and 2 were identified at two

locations at the entry stage of the raw material (cut and non cut). The third (CCP 3) was deemed to be at the stage that the straw was mechanically processed with the final critical control point (CCP4) established to be at the exit of the final product. The use of antifungal chemicals (e.g. propionic acid and formaldehyde) appear to be more effective on fungi than on bacteria. Results from the ozone treatments trial indicate that this method is a very effective alternative to current chemical practices in achieving significant microbial reduction. Ozone was found to perform well in all the studies and exhibited a tendency to be equally effective over short exposure times as longer time frames.

Early indications would appear to support the theory that ozone is a suitable and appropriate alternative to traditional chemical controls and treatments. The tendency for O_3 to spontaneously reconstitute back to O_2 is a very useful trait which could have major positive benefits in terms of controlling safety aspects in industry. Further tests are required to clarify the extent to which ozone can perform in this capacity.

Acknowledgements

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