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Possibility of Humid Municipal Wastes Hygienisation Using Gliding Arc Plasma Reactor

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Abstract: Sterilization of municipal waste for a raw material for the production of refuse-derived fuel and to protect surface and ground waters against biological contamination during transfer and storage creates a lot of problems. This paper evaluates the antimicrobial potential of non-equilibrium plasma in relation to the selected groups of microorganisms found in humid waste. The proposed research is to determine whether mixed municipal waste used for the production of alternative fuels can be sterilized effectively using low-temperature plasma generated in a gliding arc discharge reactor in order to prevent water contamination and health risk for working staff. This work assesses whether plasma treatment of raw materials in several process variants effectively eliminates or reduces the number of selected groups of microorganisms living in mixed municipal waste. The presence of vegetative bacteria and endospores, mold fungi, actinobacteria *Escherichia coli*, and facultative pathogens, i.e., *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *Enterococcus faecalis* and *Clostridium perfringens* in the tested material was microbiologically analyzed. It was found that the plasma treatment differently contributes to the elimination of various kinds of microorganisms in the analyzed raw materials. The effectiveness of sterilization depended mainly on the time of raw materials contact with low-temperature plasma. The results are very promising and require further research to optimize the proposed hygienization process.

Keywords: humid wastes; non-thermal plasma; RDF; water protection; hygienisation

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1. Introduction

The concept of plasma decontamination was proposed in the 1960s. Plasma methods are characterized by low toxicity for working staff. Despite the fact that the number of scientific papers and devices involved in plasma sterilization continues to grow, the development of industrial solutions based on non-thermal atmospheric pressure plasma is still a great challenge [1–4].

Among many solutions offered by modern technologies, plasma-based technologies are considered promising for removal of pollutants from gases and liquids [5–7]. Low-temperature plasma can be used to remove chemical and biological contamination in a variety of applications such as gas, water, soil treatment and decontamination of abiotic and biotic surfaces such as skin and seeds [8–21]. Non-equilibrium plasma was used to modify the surfaces of materials by changing the angle of surface tension, improving the properties of thin coatings, preserving and renovating archaeological features [22–30] and activation of liquids [6,31].

Cold plasma decontamination can be successfully utilized where high temperature is not recommended due to the thermolability of substrate materials. The antimicrobial properties of low-temperature plasma allow variety of applications in medicine and implantology (sterilization of surgical and dental instruments, disinfection and wound healing

support, stimulation of cells); in food industry (food sanitation and preservation, water treatment) and in situations where it is desirable to remove or eliminate microorganisms both in the vegetative and endospore form [1,11,18,32–34]. Reactive oxygen species play a crucial inactivation role when air or other oxygen-containing gases are used. With strong oxidative stress, cells can be damaged by lipid peroxidation, enzyme inactivation, and DNA cleavage [35].

The plasma sterilization properties result from the reaction between oxygen, nitrogen and water vapor, as a result of which reactive forms of oxygen and nitrogen (i.e., hydrogen peroxide, ozone, various radicals) with very strong antimicrobial activity are formed. An additional factor that kills microorganisms is the UV radiation emitted during plasma generation, which also contributes to their inactivation [1–3,11,18,33,34,36]. The mechanism of plasma action on microorganisms consists of several stages and involves mainly the permanent damage to the cell wall, cytoplasmic membrane and intracellular structures, genetic material and enzyme system [36].

There is a diverse range of microbiological challenges facing the food, healthcare and clinical sectors. The increasing and pervasive resistance to broad-spectrum antibiotics and health-related concerns with many biocidal agents drives research for novel and complementary antimicrobial approaches. The possibility of tailoring cold plasma to control specific microbiological challenges is apparent. Microbiological issues in relation to food and healthcare-associated human infections have been tested and described in [37].

Investigation focused on the effect of Modified Atmosphere Packaging (MAP) gas mixtures on reactive species generated, their efficacy and mechanism of inactivation against *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* were presented in [38]. Study [39] investigated the effects of dielectric barrier discharge atmospheric cold plasma treatment on the inactivation of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, and *Tulane virus* (TV) on Romaine lettuce, assessing the influences of moisture vaporization and post-treatment storage on the inactivation of these pathogens. Five types of microorganisms, which are commonly associated with nosocomial infections, including *Escherichia coli*, *Pseudomonas alcaligenes*, *Staphylococcus epidermidis*, *Micrococcus luteus* and *Serratia marcescens* were tested at four airstream velocities, ranging from 2 to 7 m/s, and two different relative humidity levels. The inactivation efficacies varied from 20% to 70%. It is interesting to note that the inactivation efficacy increased with gas velocity. No detectable inactivation effect was found for *M. luteus* and *S. marcescens*. Experiments showed that cold plasma technology has high potential to be used as an energy efficient method for disinfection. Limitations of application were also broadly discussed [40].

The contamination of nut products, like almonds, with human pathogens is a recurring concern in the food industry. In the study [41] the inactivation of *Salmonella enteritidis* PT 30 (ATCC BAA-1045) on the surface of unpeeled almonds by cold atmospheric pressure plasma was investigated. The maximum achieved inactivation depended on the process gas.

Cold plasma used in wound therapy inhibited microbes in chronic wound due to its antiseptic effects, while promoting healing by stimulation of cell proliferation and migration of skin cells [42]. In the study, two types of plasma systems were employed to generate cold plasma: a parallel plate dielectric barrier discharge and a capillary-guided corona discharge. Chronic wound that failed to heal were often infected by multidrug resistant organisms thus, more recalcitrant to healing. Effect of low temperature plasma treatment on several microorganisms: *Penicillium chrysogenum*, *Cladosporium cladosporioides*, *Aspergillus fumigatus*, *Fusarium culmorum*, *Escherichia coli* and *Staphylococcus aureus*, deposited on polylactide (PLA) surface, was investigated in [43]. After the PLA surface was modified, fungal spores were counted and the degree of bacterial reduction was determined. Images of specimens' surface, reflecting the effect of the low temperature plasma on particular microorganisms, were obtained by using a digital camera, polarizing microscope, epifluorescence microscope, and scanning electron microscope revealing that low temperature plasma caused cell membrane cracking.

These specific antimicrobial properties of plasma allow for new plasma applications. The use of plasma technologies for waste sterilization [44,45] and exhaust gas treatment [46] are considered promising. Therefore, attempts to apply high-temperature plasma in waste destruction have been made for several years [47]. There are already some waste treatment companies on the market which conduct studies on the implementation of plasma technologies [44], but such solutions are usually based on high-temperature plasma. On the other hand, the use of low-temperature plasma in municipal waste treatment plants is still a developing and new idea. What speaks in favor of the use of cold plasma in waste treatment, according to Winnicki and Tuźnik [48], is the fact that it is one of clean technologies in terms of emission and rational in terms of the economics. Non thermal plasma generated at the atmospheric pressure in gliding arc reactor is one of possible options [49]. Plasma based techniques also include low temperature plasma formed in corona and barrier discharge, which were widely investigated by various research groups: [50–54].

The lethal and sublethal effect of dielectric barrier discharge atmospheric cold plasma on *Staphylococcus aureus* were investigated in dependence on the treatment times, applied input powers and gap distances [55]. Another cold plasma devices based on DBD for food processing systems were used for reducing a microbiological contamination, denaturing food proteins [56,57]. Cold plasma was also used to reduce microbial load in tomato juice [58].

The real case municipal wastes treatment technologies are still quite limited. The most popular way of mixed municipal wastes handling is their placement in the landfills or incineration (with or without energy recovery). Recycling, composting or anaerobic fermentation are possible processes for the selected fractions of wastes (plastics, metals, biodegradable). Safety of staff and inhabitants around the places of waste transfer stations and waste storage and disposal places is crucial. In spite that hygienization is an additional step, which increases utilization cost, especially if it requires dry substrate; the modern society is willing to recognize surplus cost for the sake of epidemiological safety. There are several methods tested for hygienization of municipal wastes such as self-heating during storage in prisms, mixing with calcium oxide or ozonation. Each of them is affected in the reduction of microbial load, but this also has some drawbacks, such as the possibility of self-ignition; necessity of mixing and reorganizing prisms; addition of chemical compounds, which may possibly affect further technological processes; efficiency dependence on the moisture content [59–62].

Presented study is focused on the possibility of low-temperature plasma application for hygienization of humid, mixed municipal waste. Research was performed in the framework of project focused on management of raw materials used for the production of refuse-derived fuel (RDF). One of the project tasks was the enhancement of handling and storage safety of humid municipal wastes. It was necessary to investigate whether plasma treatment process would effectively reduce or eliminate microorganisms living in raw materials used for the production of RDF.

2. Material and Methods

2.1. Non-Equilibrium Plasma Treatment

The study was carried out with the use of a mini glide-arc plasma reactor (GAD) with the samples of real humid municipal waste used for the production of refuse-derived fuel (RDF), which were supplied by "EKO-BIOMASA" Sp. z o.o.

Mini Glide-arc plasma reactor was single phase, two electrodes plasma reactor with the gas supply system [31]. The scheme of the plasma reactor is shown in Figure 1.

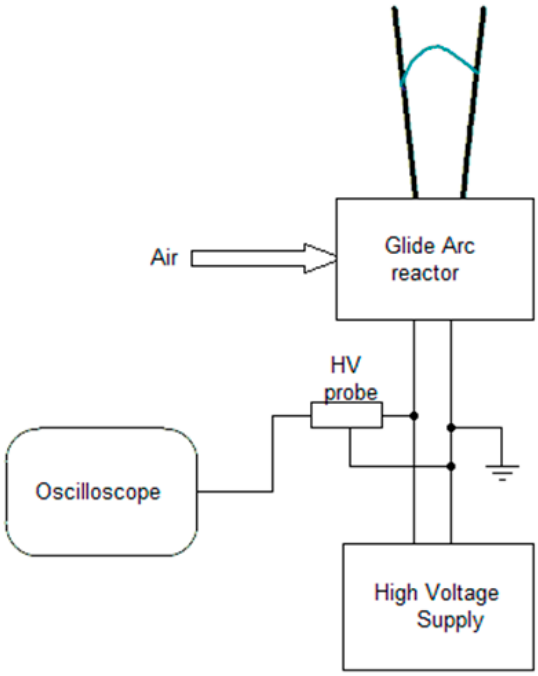

Figure 1. Scheme of the gliding arc plasma reactor.

Based on the previous experiments, the following parameters of reactor's geometry were proposed: copper electrodes 1.5 mm thick, 10.4 cm long and with 5 cm distance between the electrodes in the upper part and 3 mm in the lower part.

High voltage power supply system provided continuous functionality of discharge ignition and stable operation of the plasma reactor. The reactor cycle was periodic. The power supply parameters are listed in Table 1.

Table 1. Parameters of power supply system for plasma reactor.

Apparent power drawn from power grid	52 VA
RMS (Root-Mean-Squared) voltage (primary windings)	230 V
RMS (Root-Mean-Squared) voltage (secondary windings)	612 V
Max. voltage (secondary windings)	3760 V
Frequency (primary and secondary windings of the transformer)	50 Hz

2.2. Tests Involving the Use of Low-Temperature Plasma on Municipal Waste Samples

The waste sent by "EKO-BIOMASA" Sp. z o.o. in a collective packaging was divided into portions using laboratory scales. A batch sample of 15 g of mixed humid waste was placed in a disinfected glass chamber with the mini glide-arc reactor electrodes over the waste's surface. According to the previous findings and also from economy point of view, the process gas was air of 480 dm^3/h flow. Non-thermal plasma was used for waste treatment for 5, 15 and 45 min, respectively. Samples were numbered according to experimental parameters (Table 2). Three repetitions were performed for each case. The control samples were also prepared by exposing waste to air with a predetermined gas flow without plasma and separately, to the slow rise of temperature. Mechanical mixing was not implemented. A small movement of light fractions was the result of the gas flow. The location of the waste in the chamber and the location of the plasma reactor and the thermocouple were shown in Figure 2.

Table 2. Sample temperatures for the selected experiment condition and duration.

No.	Treatment	Time [min]	Sample (15 g)	Temperature [°C]
1.	Plasma + Air	5	I	40.5
2.	Plasma + Air	5	II	40.7
3.	Plasma + Air	5	III	42.0
4.	Plasma + Air	15	I	61.7
5.	Plasma + Air	15	II	55.0
6.	Plasma + Air	15	III	56.4
7.	Plasma + Air	45	I	64.3
8.	Plasma + Air	45	II	71.4
9.	Plasma + Air	45	III	69.1
10.	Air	5	Control I	22
11.	Air	5	Control I	21
12.	Air	5	Control I	21
13.	Ambient air	45	Control II	71.5
14.	Ambient air	45	Control II	71.5
15.	Ambient air	45	Control II	71.5

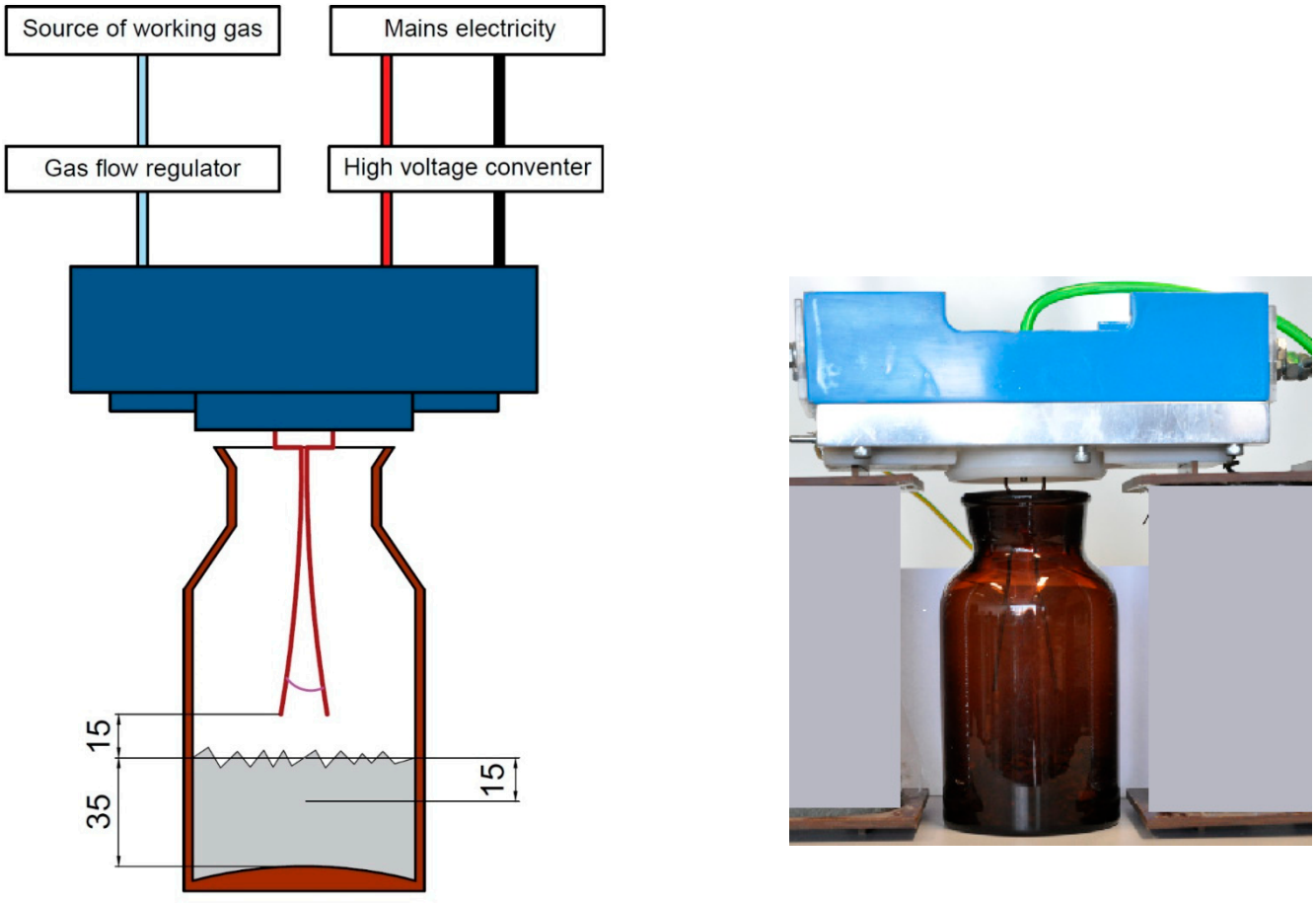

Figure 2. Experimental setup (units in mm).

The wastes were stored at 23 °C. The temperature was measured using a DT-847U temperature meter with Type K thermocouple (Yu Ching Technology Co., Ltd., Taipei, Taiwan). The thermocouple was placed inside the sample subject to plasma activity at a distance of about 1.5 cm from the waste surface. Tables 2 and 3 presents temperatures of the samples for the selected experiment duration. The temperature increased with the duration of the experiment but also depended on the composition of the waste. The gas flow impact on the temperature of control sample was minimal.

Table 3. Average sample temperatures for the selected experiment duration.

Time [min]	Av. Temperature [°C]
5	41.1
15	57.7
45	68.3

When plasma treatment was completed, samples were packed into sterile bags, assigned a description label (No. 1–9 test samples subject to plasma treatment; No. 10–12—control sample of 3 mixed batches and No. 13–15 temperature control samples) and immediately sent to a microbiological laboratory for further analysis.

In addition, carbon and nitrogen oxides and ozone concentration generated in plasma were measured using the MX6 iBrid (Industrial Scientific Corporation. Pittsburgh, PA USA) and Eco Sensors A-21ZX (Eco Sensors, Newark, CA, USA) measuring instruments, respectively. The reactor worked in the flow system, and the process gases, after passing through the reactor, were removed through the ventilation system. The measured gases concentrations are presented in Table 4. The results of the pH measurements of the samples are shown in Table 5.

Table 4. Measured gases concentrations.

Air Flow Rate [dm^3/h]	iBrid MX6 [ppm]			Eco Sensors A-21ZX [ppm]	Time [s]	Mode
	NO_2	NO	CO	O_3		
480	0	11 ± 2	10 ± 1	0.10 ± 0.06	10	With the sample
480	0.5 ± 0.1	28 ± 3	19 ± 2	0.16 ± 0.04	120	
480	0.5 ± 0.1	35 ± 5	19 ± 5	0.20 ± 0.06	300	
480	0	12 ± 2	0	0.16 ± 0.04	10	Without the sample
480	0.4 ± 0.1	31 ± 4	2 ± 1	0.24 ± 0.02	120	
480	0.6 ± 0.1	39 ± 6	5 ± 2	0.31 ± 0.04	300	

Table 5. Average number of microorganisms (10^6 CFU/g RDF) and average pH of tested samples of raw material subject to plasma process (CFU-Colony-Forming Unit, RDF-Refuse-Derived Fuel).

Sample	Plasma Treatment Time [min]	Vegetative Bacteria	Endospores	Mold Fungi	*Staphylococcus* spp.	*E. coli*	*Salmonella* spp.	*E. faecalis*	pH
1.	5	4.12 ± 0.01 ab	0.11 ± 0.01 ab	1.39 ± 0.04 ab	24.82 ± 0.06 ab	0.54 ± 0.01 ab	0.07 ± 0.01 ab	4.11 ± 0.01 ab	8.03
2.	5	11.19 ± 0.03 ab	0.19 ± 0.02 ab	2.84 ± 0.01 ab	27.92 ± 0.07 ab	0.49 ± 0.01 ab	0.02 ± 0.01 ab	11.18 ± 0.01 ab	7.71
3.	5	6.28 ± 0.01 ab	0.08 ± 0.01 a	0.95 ± 0.05 ab	24.38 ± 0.07 ab	0.41 ± 0.01 ab	0.01 ± 0.001 a	6.29 ± 0.02 ab	7.74
4.	15	2.21 ± 0.03 a	0.13 ± 0.03 a	0.41 ± 0.01 a	23.14 ± 0.05 a	0.04 ± 0.01 a	0.01 ± 0.001 a	2.22 ± 0.02 a	7.47
5.	15	0.93 ± 0.01 a	0.16 ± 0.03 a	0.11 ± 0.02 a	27.11 ± 0.04 ab	0.05 ± 0.01 a	0 ± 0 a	0.94 ± 0.01 a	7.41
6.	15	0.61 ± 0.01 a	0.07 ± 0.01 a	0.09 ± 0.02 a	24.87 ± 0.08 a	0.08 ± 0.01 a	0 ± 0 a	0.61 ± 0.01 a	7.29
7.	45	0.49 ± 0.01 ab	0.12 ± 0.01 ab	0 ± 0 a	0.09 ± 0.01 a	0.01 ± 0.01 a	0 ± 0 a	0.49 ± 0.01 ab	7.27
8.	45	0.14 ± 0.01 ab	0.03 ± 0.01 a	0 ± 0 a	0.12 ± 0.01 ab	0 ± 0 a	0 ± 0 a	0.14 ± 0.01 a	6.98
9.	45	0.22 ± 0.01 ab	0.02 ± 0.01 ab	0 ± 0 a	0.07 ± 0.01 a	0 ± 0 a	0 ± 0 a	0.22 ± 0.01 a	7.33
10.	Control I	4.04 ± 0.02 a	0.13 ± 0.02 a	0.38 ± 0.02 a	27.64 ± 0.09 ab	0.18 ± 0.01 a	0.03 ± 0.01 a	4.04 ± 0.01 a	7.31
11.	Control I	3.82 ± 0.02 a	0.15 ± 0.02 a	0.41 ± 0.02 a	25.53 ± 0.09 ab	0.23 ± 0.01 a	0.05 ± 0.01 a	3.24 ± 0.01 a	7.32
12.	Control I	4.24 ± 0.02 a	0.23 ± 0.02 a	0.38 ± 0.02 a	27.64 ± 0.09 ab	0.19 ± 0.01 a	0.07 ± 0.01 a	3.94 ± 0.01 a	7.34
13.	Control II	3.14 ± 0.02 a	0.13 ± 0.02 a	0.17 ± 0.02 a	18.84 ± 0.09 a	0.21 ± 0.01 a	0.06 ± 0.01 a	2.14 ± 0.01 a	7.84
14.	Control II	3.58 ± 0.02 a	0.18 ± 0.02 a	0.21 ± 0.02 a	15.23 ± 0.09 a	0.33 ± 0.01 a	0.07 ± 0.01 a	2.34 ± 0.01 a	7.54
15.	Control II	3.08 ± 0.02 a	0.15 ± 0.02 a	0.25 ± 0.02 a	16.48 ± 0.09 a	0.34 ± 0.01 a	0.04 ± 0.01 a	2.58 ± 0.01 a	7.61

Mean ± standard error of mean (n = 3). The different letters within a column indicate a significant difference at $p < 0.05$ according to Tukey's test.

3. Microbiological Analyzes

All samples exposed to low-temperature plasma (1–9) and the control samples (10–15) were subject to a microbiological analysis. 10 g of each sample was taken for isolation of microorganisms. Isolation was performed by serial tenfold dilution method according to Koch using microbiological growth media [63,64]. Temperatures of samples (inside the flask) and of water vapor over the samples were measured using DT-847U temperature meters with Type K thermocouples thermocouple (Yu Ching Technology Co., Ltd., Taipei, Taiwan). The following groups of microorganisms were identified: general vegetative bacteria and endospores (MPA agar, BTL, Lodz, Poland, cultured at 37 °C, 24 h), mold fungi (MEA maltose agar, BTL, Lodz, Poland, cultured at 28 °C, 5 days), actinobacteria (Pochon's agar, BTL, Lodz, Poland, cultured at 28 °C, 7 days). The presence of facultative pathogens was also studied: *Staphylococcus* spp. (Chapman's agar, BTL, Lodz, Poland, cultured at 37 °C, 24 h), *Escherichia coli* (TBX agar, BTL, Lodz, Poland, cultured at 44 °C, 24 h), *Salmonella* spp. and *Shigella* spp. (SS agar, BTL, cultured at 37 °C, 24 h) *Enterococcus faecalis* (Slanetz Bartley growth medium, BTL, Lodz, Poland, cultured at 37 °C, 48 h), *Clostridium perfringens* (agar with sulfate and cycloserine SC, BTL, Lodz, Poland, cultured at 37 °C, 24 h, Agar 3% was used on top of the culture to ensure anaerobic conditions). pH of wastes was measured with a CP-105 Elmetron pH meter (Elmetron, Zabrze, Poland), samples (5 g) were suspended in 25 mL of saline (Sigma-Aldrich Chemicals, Warsaw, Poland) and mixed. The number of vegetative bacteria and endospores shows the abundance of nutrients easily assimilated by microorganisms in the analyzed raw materials. A numerous occurrence of bacteria, mold fungi and actinobacteria also indicates favorable conditions (temperature, substrate reaction, humidity) for the growth and development of microorganisms. The presence of facultative pathogens (*Staphylococcus* spp., *E. coli*, *Salmonella* spp., *Shigella* spp., *E. faecalis*, *C. perfringens*), which may pose an epidemiological threat, is an important indicator of microbial contamination. The serial dilution analysis was carried out in three repetitions and the reaction of the samples was also measured. The number of colony-forming units (CFU) of microorganisms was determined with the use of the dilution culture method by calculating the test result per one gram of the test raw material. For the initial identification of microorganisms isolated from the specimens, Gram-stained

bacteriological preparations and mycological preparations in Lugol's iodine (Coel, Krakow, Poland) were prepared.

The statistical analysis of the obtained results was made with the use of the Statistica v.13.0 software (StatSoft Polska, Krakow, Poland). A two-factor analysis of variance was used to check the significance of the variation in the number of selected microbial groups in the samples, depending on using plasma and exposure time.

4. Results and Discussion

According to the literature, low-temperature plasma was successfully used to inactivate bacteria (*Bacillus subtilis*, *Escherichia coli*, *Geobacillus stearothermophilus*, *Staphylococcus cusus*, *Salmonella enteridis*) and fungi (*Aspergillus brasiliensis*, *Alternaria alternata*) as well as their toxic metabolites [65–69]. The analysis of the gas concentration during the plasma treatment indicated that O_3 and NO took an active part in the hygienisation process as their concentration decreased when the sample was present. On the other hand, CO could be one of the reaction products as concentration with the sample was much greater that without the sample. From the microbiological point of view, assessment of selected microbial groups' occurrence in the raw materials used for RDF production subjected to the plasma treatment is depicted in Table 5. The existence of high microbial biodiversity was ascertained. Such a phenomenon should not be surprising because, according to the studies by Fernández et al. [70] on biofilm deactivation, the elimination of mixed populations of microorganisms is extremely troublesome because the plasma limiting factor is a multi-layer microbial packing that makes plasma deactivated cells a specific barrier to microorganisms found in deeper layers within the sterilized material. Particular attention should be paid to the presence of facultative pathogens in the analyzed material (*Staphylococcus* spp., *E. coli*, *Salmonella* spp., *E. faecalis*), which may pose an epidemiological risk. At present, there is no agreement as to the scale of the effect of cell wall construction on the efficiency of low-temperature plasma. According to Ermolaeva and Gintsburg [71], the effectiveness of hot plasma depends on the species and source of isolation of the strain. The tested Gram-negative bacteria exhibited similar plasma sensitivity, unlike Gram-positive strains, among which, low-sensitivity isolates were identified. On the other hand, Daeschlein et al. [72] stated that Gram-positive bacteria are much more sensitive than Gram-negative. Based on the analysis of the data in Table 5, it was found that pH of the samples was in the range of 6.98–8.03 and oscillated within the range of neutral reaction, except for sample 1, which showed slightly alkaline pH. Therefore, it can be considered that the pH of the humid raw materials used for the production of alternative fuels had no effect on the change in the number of tested microorganisms as it remained almost stable. The results on the number of Shigella spp., actinobacteria and C. perfringens were not included in the table because these microorganisms were not detected in any of the analyzed samples.

The number of vegetative bacteria exceeded the number of endospores. While the number of vegetative bacteria (Table 5) in the analyzed samples indicated a strong decreasing trend (the longer the plasma worked, the less the number of microorganisms), the results were not clear for endospores (Table 5). The reason for this may be that bacteria in the form of endospore are capable of producing protective structures (sheaths, capsules) that allow them to survive under unfavorable conditions.

The number of mold fungi and *Salmonella* spp. increased compared to the control group after short plasma exposure (5 min), and then decreased (15 min) and reached zero (45 min). As a result of the mycological analysis, the following types of mold fungi were identified, including toxigenic fungi: *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhizopus* and *Alternaria*. They can pose a risk to the health of people who have contact with raw materials used for the production of alternative fuels because they produce mycotoxins and cause allergic reactions [73].

As far as staphylococci are concerned, the results present strong uniform trends, which show that the longest (45 min) contact of raw materials with plasma was the only variant

guarantying full elimination of these microorganisms. Shorter contact times did not affect the number of *Staphylococcus* spp.

The number of *E. coli* increased compared to the control group after a short plasma contact time, and then decreased (15 min) and dropped to almost zero (45 min), depending on the sample.

The interpretation of changes in the number of *E. faecalis* in the analyzed samples it is similar to the other microorganisms studied in this paper. Their concentration increases for short treatment time but then decreases for longer treatment.

It should be noted that waste is a very specific raw material from the microbiological point of view. Due to its composition, structure and nutrient content, waste is a very friendly environment for microorganisms. According to Fernández et al. [70] the performance of low-temperature plasma depends on the level of surface structure differentiation of the treated materials. It was found that it was more difficult to remove microorganisms with the use of plasma from fruit and vegetables with a rougher surface. This phenomenon was explained by the differentiation of surface construction: its unevenness and roughness became a place where bacterial cells might "hide", and therefore their exposure to active species was reduced. The limitation of hygienization with the use of plasma technologies is low efficiency in the decontamination of porous materials, which have a structure similar to that of mixed municipal waste [33,74]. Direct comparison with other hygienization methods such as temperature, ozone and CaO is difficult. In the most of cases mechanical mixing was required and many results concern only selected types of wastes, for instance, only dry, solid fractions [61,62,73]. The main drawback of plasma application without the mixing of wastes might be long contact time required. Conducted studies indicated that the number of all analyzed microorganisms decreased (in some cases to zero) after 45 min contact of the samples of raw materials with plasma. Particular attention should be paid to the fact that short (5 min) contact of raw materials with plasma led to intense growth of, for example, *E. coli* or mold fungi (Table 5). Although there is some mild thermal effect in microorganism inactivation, the results show that the effects at longer treatment time are due to the plasma treatment (a synergy between UV, IR and RONS (reactive oxygen and nitrogen species) and not only due to the temperature (Figure 3). Based on this, it can be stated that it is extremely important to correctly select the plasma treatment process parameters, which will be effective and will not have the opposite effect to the expected one.

5. Conclusions

Humid mixed wastes were treated with non-thermal plasma generated in the GAD reactor for hygienization purposes. For practical reasons, taking into account the operating costs of potential installation, the process gas was air.

Based on the obtained results, the best decontamination effects were observed when the longest (45 min) plasma contact time with raw materials used for RDF production was applied. Five-minute plasma exposure seemed to give an undesirable stimulating effect, whereas the results obtained during 15 min of the process were insufficient to effectively reduce the number of some microorganisms, for example, *Staphylococcus* bacteria and bacteria in the form of endospores. The activity of plasma was rather surface one and it is recommended to mechanically mix the waste in order to increase the efficiency of the process in the long run. High humidity mixed wastes contain populations of microorganisms that show high biodiversity, which in practice means that it is very difficult to deactivate them effectively. It is therefore reasonable to conduct further research to optimize the technological parameters of the plasma process, which may contribute to the implementation of low-temperature plasma technology in municipal waste treatment.

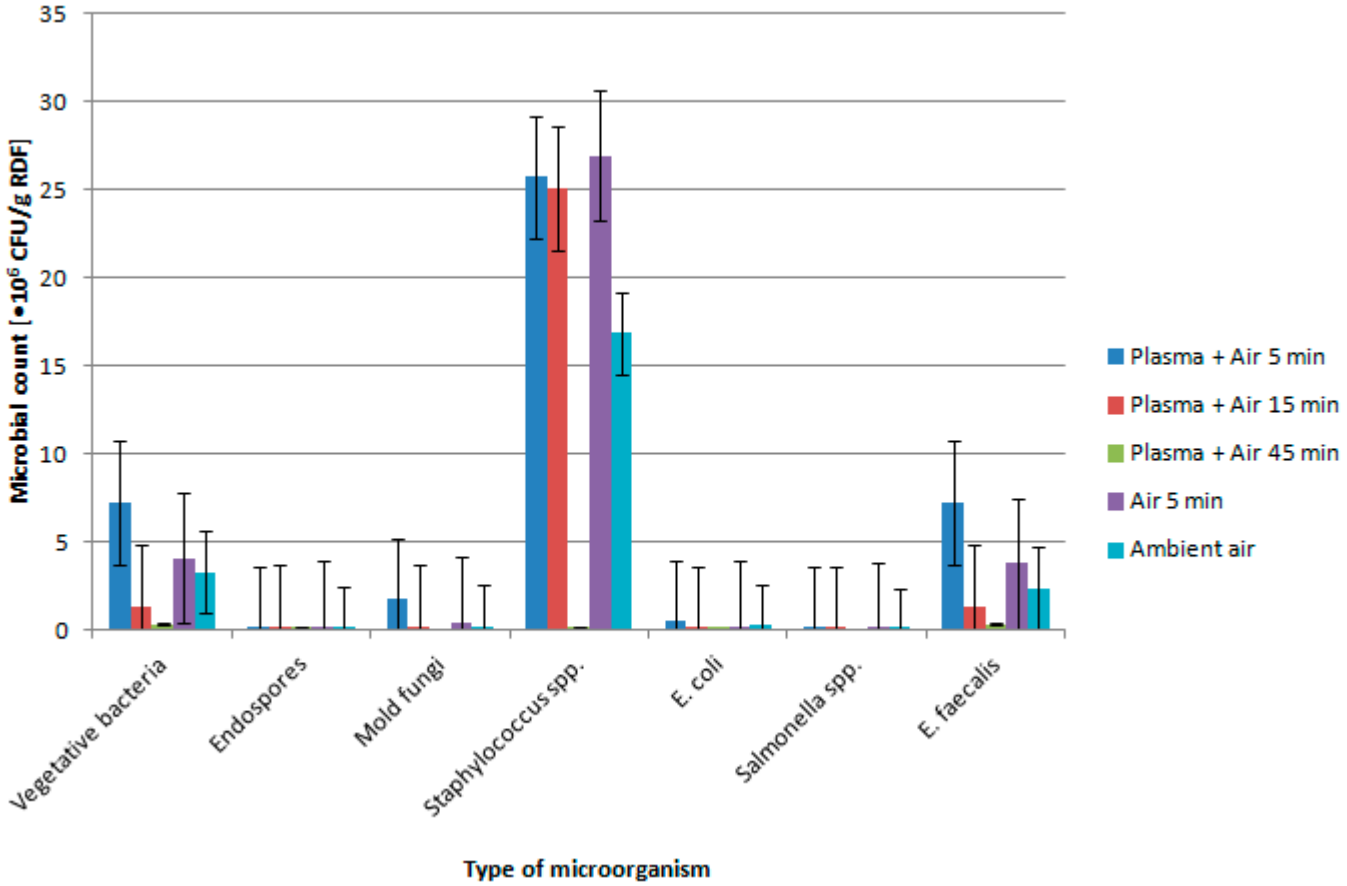

Figure 3. Averaged microbial count for batches 1–3, 4–6, 7–9 with control batches 10–12 and 13–15.

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