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Bioaerosol Emissions during Organic Waste Treatment for Biopolymer Production: A Case Study

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Abstract: Environmentally sustainable methods of waste disposal are a strategic priority. For organic waste management and innovative biological treatments present advantageous opportunities, although organic waste treatment also includes environmental drawbacks, such as bioaerosol production. This study aims to evaluate bioaerosol spread during an innovative experimental treatment. The process consists of two anaerobic steps: acidogenesis, which includes polyhydroxyalkanoate accumulation, followed by methanogenesis. Bioaerosol, PM10, and endotoxin concentrations were measured at three sampling points during different campaigns to evaluate: (1) the background levels, (2) the contamination produced in the pre-treatment stage, and (3) the residual contamination of the outgoing digested sludge. Environmental PM10 seemed to be generally quite contained, while the endotoxin determination was close to 90 EU/m^3. Significant microbial concentrations were detected during the loading of the organic fraction of municipal solid waste (fungi > 1300 CFU/m^3, Bacillus genus ($\approx$10^3 CFU/m^3), higher *Clostridium* spp. and opportunistic human pathogens such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), suggesting a significant contamination level. Such results are useful for hazard identification in the risk assessment of innovative processes, as they reveal contaminants potentially harmful to both workers' health and the environment.

Keywords: bioaerosol; anaerobic digestion; OFMSW; biological risk; endotoxins

1. Introduction

The amount of waste produced worldwide is rapidly rising due to population growth and urbanization. The global production of municipal solid waste (MSW) is likely to double in the next decade, more than 40% of which is expected to be organic matter, higher in developing countries than higher-income countries [1,2]. More attention needs to be paid to waste management to safeguard both the environment and human health. Therefore, it would be desirable to rely more on environmentally sustainable methods for waste disposal to prevent organic waste from entering landfills. To that aim, the European Union (EU) has proposed that member states reduce biodegradable waste entering landfills [3] to 10% by 2035 [4]. Another priority of the EU consists of a transition toward a circular economy, producing added-value commercial products and energy to minimize waste generation and thus the environmental impact [5].

An example of high-value production of biological origin is the extraction of polyhydroxyalkanoates (PHAs). PHAs are a type of polyester that are synthesized mainly by

Gram-positive, Gram-negative, and anaerobic photosynthetic bacteria through sugar and lipid fermentation [6]. The use of PHAs is a more profitable way to recover organic matter than traditional anaerobic digestion technology. The recovery process offers the opportunity to retrofit existing waste facilities or wastewater treatment plants [7]. Moreover, replacing synthetic plastics with biodegradable PHAs would be extremely advantageous for society and the environment. However, PHAs have a higher production cost than conventional plastics (about 15 times more expensive than polymers derived from petroleum, such as polypropylene). Therefore, commercializing and industrializing them on a large scale are still limited. More efforts are required to improve the PHA synthesis process and reduce the cost [7,8]. One option could be to include PHA production in the anaerobic digestion of organic waste.

Anaerobic digestion (AD) is an economically advantageous technology for the treatment of organic waste and its management. It is a biological process that involves catalysis by anaerobic and microaerophilic microorganisms (typically thriving in 2–10% O_2). It allows the degradation of organic biomass, leading to the production of biogas and digestate. The latter consists of the leftover indigestible matter and the microorganisms' mass. AD has several noteworthy advantages, such as low production of sludge, low energy requirement, low operating costs, and potential energy recovery [9,10]. Anaerobic digestion technologies can be classified into continuous or batch operation, wet (total solids (TS) content <20%) or dry (TS content >20%) moisture levels, mesophilic (20–45 °C temperature range) or thermophilic (50–55 °C temperature range) conditions, and single- or two-stage digestion [10]. For PHA production, separation of acidogenesis and subsequent methanogenesis is needed. PHAs are synthesized in bacteria by using intermediates of the β-oxidation of alkanoic acids and accumulated in intracellular vacuoles during acidogenesis, then the growth conditions for selected PHAs producing microorganisms can be optimized [7]. Generally, for the organic fraction of municipal solid waste (OFMSW), thermophilic treatment can be used to guarantee higher effectiveness [11] and adequate management to guarantee pathogen reduction [12].

Although organic waste treatment is a preferable process with respect to disposal, it is not risk-free and must be properly assessed and managed. One of the weaknesses of the organic waste treatment process is bioaerosol dispersion. Bioaerosol, also known as primary biological airborne particles (PBAPs), includes all airborne particles from a biological source ranging from 0.001 to 100 μm in size. Pathogenic or non-pathogenic and viable or non-viable microorganisms can exist in bioaerosol [3,13]. Bioaerosol components that are considered potentially dangerous are bacteria, archaea, fungi, fungal spores, viruses, endotoxins, microbial fragments, β (1,3) glucans, mycotoxins, pollen, and others [13,14]. Biological particles can remain suspended in the air for extended periods and potentially cover distances >250 m from their source due to their small size and light weight [15]. Bioaerosol can cause adverse health effects, including a wide range of acute and chronic illnesses, in occupational environments and even in nearby residential communities subjected to high exposure levels [3,16,17].

PBAPs are part of the airborne total suspended particles (TSP). Particulate matter is a key indicator of air pollution and is generated as a consequence of natural and human activities. The finest PM can remain suspended for a long time and travel long distances in the atmosphere, potentially causing a wide range of health problems, such as heart attacks, aggravated asthma, decreased lung function, and airway irritation [18].

The World Health Organization (WHO) defines particles suspended in the air according to their equivalent aerodynamic diameter. PM10 has been defined as particles among which 50% have an equivalent aerodynamic diameter smaller than 10 μm [19,20] and can be considered one of the reference parameters to evaluate air quality. European Directive 2008/50/EC set the environmental limit for PM10 emissions at 50 μg/m^3 daily, and this value should not be exceeded more than three times in a calendar year [21].

This work aims to provide, through microbial characterization, a method for monitoring environmental air microbiological during the emissions of an AD pilot plant producing

PHAs starting from OFMSW. The evaluation considers an experimental process in the absence of containment measures. The diffusion in the air of any pathogens is assessed to define measures to be taken to contain the risk.

2. Materials and Methods

2.1. Sampling Campaigns

Three sampling campaigns were conducted to characterize the composition of bioaerosol exposure in a pilot-scale facility for PHA and biogas production using urban organic biowaste. The pilot plant was located in a wastewater treatment plant (WWTP) in the northeast of Italy and was previously described [5,7]. The anaerobic treatment of OFMSW occurred in separate phases under thermophilic conditions (55 °C), with fermentation and methanogenesis conducted in 2 distinct reactors. The sampling campaigns were performed in March, May, and November of 2016. They were carried out at 3 points (P0, P1, and P2) in the pilot plant, as shown in Figure 1.

- P0 was located in the area where the process of shredding OFMSW is self-managed and the pre-treated OFMSW enters the plant. The pre-treatment process included shredding or squeezing and was conducted at the pilot plant itself (3 and 4 May) or by an external company.
- P1 was the intermediate phase where the liquid phase coming from acidogenesis was separated by centrifuge for subsequent enrichment of PHA producers (22 November).
- P2 was located where the methanogenesis of the resulting biosolids process took place.

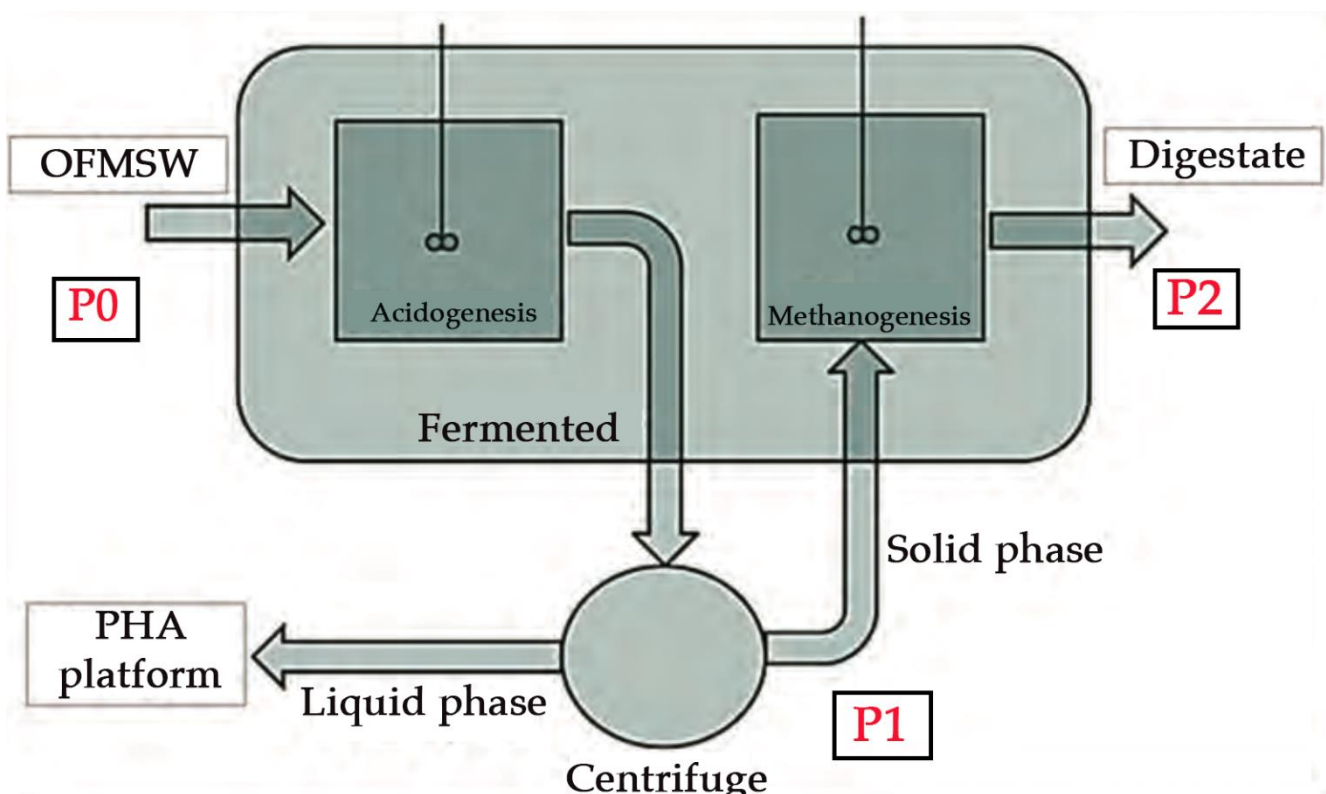The sampling points were very close to each other (just 10 m).

Figure 1. Three sampling locations (P0, P1, and P2) at the pilot plant. P0: Area dedicated to preparation of shredded organic fraction of municipal solid waste (OFMSW); P1: position next to entry point of OFMSW pre-treated by shredding or squeezing; P2: location of final methanogenesis.

The sampling campaigns were conducted as follows:

1. Background definition (10 and 11 March 2016): the first campaign evaluated the background contamination levels of the pilot plant, sampling all points (P0, P1, P2).
2. AD process including pre-treatment of the OFMSW (3 and 4 May 2016): the second campaign assessed the potential impact of the OFMSW shredding phase, which is self-managed and somewhat automated, analyzing points P0 and P1. During this phase, to limit worker exposure, no collective protective equipment was employed, only personal equipment.
3. AD process without pre-treatment of OFMSW (22 November 2016): the third campaign evaluated the residual contamination when AD was conducted using OFMSW liquid partly squeezed and homogenized by an outsourcing plant [7]. In this phase, P1 and P2 were sampled.

Both PM10 and bioaerosol sampling were performed in all 3 campaigns. Meteorological conditions, such as wind speed and temperature, were also collected.

2.2. PM10 Sampling and Analysis

An AirFlow PM10 high-volume sampler (HVS) (AMS Analitica, Pesaro, Italy) was employed for PM10 monitoring. It provides a suction flow of 1.27 m^3/min, which perfectly complies with the UNI EN 12341 requirements. Its operating principle relies on the entrance of dispersed airborne particles into a high-volume pre-selector cascade impact system. The PM10 samplings were conducted for at least 4 h, and the normalized volume of each sampling was annotated as the meteorological variables detected by the sampler sensors. Five glass microfiber filters (203 × 254 mm; Pall Corporation, Port Washington, NY, USA) were used. These were conditioned before and after the samplings by placing them in a dryer with silica gel for 48 h to reduce humidity. Gravimetric analysis of the filters was conducted using a precision weighing scale to determine the PM mass concentration [22].

2.3. Endotoxin Content Assessment

Endotoxins were extracted from 1/8 of the HVS filters. Each section of the filter was cut into smaller pieces and placed in pyrogen-free 50 mL centrifuge tubes containing 15 mL of RPMI-1640 medium (Sigma Aldrich, St. Louis, MI, USA) and 0.025% of Tween-20 (Sigma Aldrich, USA). The centrifuge tubes were then put into an ultrasonic bath for 10 min and vortexed for an additional 5 min 3 consecutive times. The samples were centrifuged at 5000 RPM for 10 min to remove the filter. The supernatant was collected and stored at −20 °C. The endotoxins were analyzed using the LAL (limulus amebocyte lysate) chromogenic endpoint assay (QLC-1000 n. 50-648U, Lonza) at 37 °C. Microplates were read at 405 nm with a Tecan Infinite 200 PRO spectrophotometer and i-control (version 1.10) software, following the manufacturer's instructions. *E. coli* 0111:B4 endotoxin was used as standard due to its ability to catalyze LAL proenzyme activation, thus allowing the release of p-nitroaniline. The latter produces a yellow color that can be quantitatively detected by a spectrophotometric read at 405 nm. *E. coli* is used as a standard because it is economical and an easily cultivable microorganism. The standard curve was obtained through 6 known endotoxin concentrations (starting at 50 EU/mL) suspended in RPMI-1640 medium (Sigma) and diluted 1:10 with autoclaved MilliQ water (pH ranging from 6.0 to 8.0). The regression coefficient of the curve should be at least 0.98. The limit of detection (LOD) is 0.01 EU/mL. Only the values quantified between the first and last curve points were considered valid [23].

2.4. Bioaerosol Sampling and Culture-Dependent Analysis

Bioaerosol sampling was performed with the SAS Super 180 dual surface air system (PBI International, Milan, Italy), a high-volume sampler that allows microbial air monitoring through air contact on Petri dishes (RODAC contact plates, VWR, Radnor, PA, USA) set up with specific media. Culture media were prepared in the laboratory following the manufacturer's protocol. The heads of the SAS sampler were sterilized in an autoclave at

121 °C for 20 min before sampling. Table 1 lists the investigated microbial parameters, the respective culture media, and the appropriate incubation temperature.

Table 1. Microorganisms investigated and technical information on culture media used.

Investigated Microorganisms	Culture Media	Incubation Temperature
Bacterial count at 22 °C	Plate Count Agar (Merck VM361263 202)	22 °C
Bacterial count at 37 °C	Plate Count Agar (Merck VM361263 202)	37 °C
Bacterial count at 55 °C	Plate Count Agar (Merck VM361263 202)	55 °C
Fungi/yeasts	Sabouraud Glucose Agar with 4% Chloramphenicol (Sigma 89579)	24 °C
Pseudomonadaceae	Cetrimide (Merck VM077084 930)	37 °C
Bacillus spp.	Hi-crome Bacillus Agar (Fluka 92325)	30 °C
Clostridium spp.	mCP (SIFIN TN1288)	44 °C
Gram-negative bacteria	MacConkey Agar (Biolife 4016702)	37 °C
Salmonella/Shigella	XLD-D (bioMérieux 51049)	37 °C
Actinomycetes	Starch Casein Agar (US Biological S7968-25)	55 °C

An antimycotic (cycloheximide 0.2 g/L) was added to all media except Sabouraud to avoid fungal contamination. Bioaerosol samples were repeated from 3 to 4 plates for each biological target for at least 3 different concentrations (air volume sampling). The count of colonies formed by the microorganisms (colony-forming unit (CFU)) allowed the microbial contamination of the sampled air (most probable number (MPN)/m^3) to be determined. Rapid biochemical tests (API, bioMérieux) were also performed for the isolation and identification of microorganisms.

The Global Index of Microbial Contamination (GIMC), proposed by Dacarro et al. [24], was calculated as the sum of total microbial counts determined for mesophilic bacteria, psychrophilic bacteria, and fungi in all sampled areas. The Index of Mesophilic Bacterial Contamination (IMC) can be obtained by calculating the ratio between the values measured for mesophilic and psychrophilic bacteria at the same sampling point.

The bioaerosol sampling points for each campaign were as follows:

- Background definition (10 and 11 March) at P0, P1, and P2.
- AD process, including pre-treatment of OFMSW (3 and 4 May) at P0 and P1.
- AD process without pre-treatment of OFMSW (22 November) at P1 and P2.

2.5. Data Elaboration and Statistics

Statistical analysis was conducted using SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA). The following statistical analyses were performed: (a) log transformation was applied to nonnormally distributed data; (b) Spearman's rank correlation coefficients were calculated to evaluate relationships between variables; (c) Student's t-test was employed to compare means; and (d) ANOVA was used for multivariate analysis assuming equal variance, followed by Tukey's post hoc test for multiple comparisons. The mean differences and correlations were considered statistically significant when the *p*-value was <0.05 and highly significant when the *p*-value was <0.01.

3. Results

The PM10 values were rather low and consistent with what was expected for a suburban area (about 7 km from the city center) located in a green area. Moreover, they were in line with PM10 environmental data validated by the local environmental protection agency (ARPAV), ranging from a mean of 8 to 28 µg/m^3 at the four local sampling stations. The highest level observed was 37.53 µg/m^3 (afternoon/evening of 10 March 2016), and the lowest level was 5.97 µg/m^3 (morning of 4 May 2016) under conditions of moderate wind (13.5 km/h). No statistically significant differences were observed among the three sampling campaigns or the sampling points.

Endotoxin values varied; the highest detected concentration was 85.62 endotoxin units (EU)/m^3 (11 March 2016), and the lowest was 24.11 EU/m^3 (10 March 2016). Endotoxin levels did not increase significantly following the use of OFMSW and were not affected by wind speed or temperature. The limited available data may have a negative influence on the significance of PM10 and endotoxin levels. Table 2 reports the PM10 and endotoxin concentrations.

Table 2. PM10 and endotoxin concentrations divided by campaign.

Campaign	Date	PM10 (μg/m^3)	Endotoxin (EU/m^3)	Wind Speed (Km/h)	Temperature (°C)
First	10 March	37.53	24.11	8	10
	11 March	9.38	85.62	7	10.5
Second	3 May	11.89	58.05	9	23
	4 May	5.97	37.19	13.5	17.5
Third	22 November	29.97	36.61	7.6	12.5

During the use of OFMSW, fungi concentrations were underestimated because the sampled plates showed confluent growth even with the lowest sampling volume (50 L). In the second sampling campaign, an accurate evaluation of the 55 °C count and Actinomycetes was not possible due to the spread of *Bacillus* spp., which showed crusty colonies that inhibited the growth of other microorganisms. Plates containing *Bacillus* spp.-selective media were confluent in the second sampling campaign (shredding of OFMSW). Table 3 shows the results of bioaerosol monitoring.

Table 3. Microbial concentrations measured in all three sampling campaigns. CFU, colony-forming unit.

Indicators CFU/m^3	First Campaign (P0, c1, P2)		Second Campaign (P0, P1)		Third Campaign (P1, P2)		Analysis of Variance
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	*p*-Value
Bacterial count at 22 °C	187.78	97.54	68.33	60.12	78.00	0	n.s.
Bacterial count at 37 °C	89.33	17.24	71.00	45.08	43.00	4.24	n.s.
Bacterial count at 55 °C	24.00	27.78	0	0	5.00	1.41	n.s.
Fungi/yeasts	207.78	73.96	1,307	0	1,307	0	<0.01
Pseudomonadaceae	0.33	0.58	13.00	24.09	1.50	2.12	n.s.
Bacillus spp.	4.33	1.53	369.75	729.01	73.33	33.00	<0.05
Clostridium spp.	0.33	0.58	7.25	5.03	3.00	2.83	<0.05
Gram-negative bacteria	0.33	0.58	12.75	12.06	9.50	12.02	n.s.
Salmonella/Shigella	0	0	30.25	29.02	3.50	3.54	n.s.
Actinomycetes	3.67	3.06	0.50	0.58	4.50	3.54	n.s

Mean differences for each parameter, considering the three sampling campaigns, showed statistically significant results for the following:

- Fungi (ANOVA *p* < 0.01): background concentration was lower than the other two sampling campaigns with OFMSW (207.8 CFU/m^3 vs. >1307 and >1307 CFU/m^3).
- *Bacillus* spp. (ANOVA *p* < 0.05): background concentration was lower than the campaign with manually shredded OFMSW (4.3 CFU/m^3 vs. 369.8 CFU/m^3).
- *Clostridium* spp. (ANOVA *p* < 0.05): background concentration was lower than the campaign with manually shredded OFMSW (<limit of quantification CFU/m^3 vs. 7.3 CFU/m^3).

The background level was low but not negligible for a nonconfined site, although protected by a canopy and with limited dispersion due to the surrounding buildings on two sides. GIMC registered 1455, 5785, and 2856 CFU/m^3 in the first, second, and third campaigns, respectively. The contamination level markedly increased during the OFMSW shredding phase, and in this phase, GIMC increased from 485 to more than 1400 CFU/m^3.

The IMC increased with manually shredded and squeezed OFMSW by 2.31 and 1.22 times, respectively, indicating worsening sanitary conditions. The *Bacillus* spp. background concentration was lower than 3 CFU/m^3 (first sampling campaign). The concentration reached 1307 CFU/m^3 during the OFMSW shredding phase (second campaign) and 50 CFU/m^3 with squeezed OFMSW (third campaign). The growth of Enterobacteria in selective media was also detected. Some pathogens and many opportunistic microorganisms, mainly transmissible by air, contact, and the oral–fecal route, were isolated and identified through rapid biochemical tests (API, bioMérieux). Table 4 lists the identified microorganisms; most of them were also previously detected at a similar plant [25]. Among them, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which are classified as class 2 pathogens, were observed. Regarding the indicators, only *Clostridium perfringens* was found during the third sampling campaign, while *Escherichia coli* was never detected.

4. Discussion

The registered PM10 and endotoxin concentrations were not conditioned by the OFMSW treatment, probably due to the outdoor location of the pilot plant and the small number of available measurements. PM10 samplings performed in the afternoon (10 March and 3 May) registered higher concentrations than those performed in the morning (11 March and 4th May). These levels were lower than the value established by the European Air Quality Regulation (Directive 2008/50/EC) as a daily mean of 50 μg/m^3 for PM10, which should not be exceeded more than three times in a calendar year. Observed PM10 levels were also significantly lower than threshold limits for respirable PNOC in occupational environments proposed by the American Conference of Governmental Industrial Hygienists (TLV/TWA 3 mg/m^3), even if such limit refers to personal sampling [26]. The data collected on March 10, which were closer to the environmental limit, do not refer to the pilot-scale impact but to the background contamination (campaign 1) due to the presence of constantly active wastewater treatment impacting the same area.

In general, the afternoon data were influenced by environmental accumulation due to anthropic activities and heating, while the morning data were influenced by the dispersion effect of the night. Endotoxin levels were consistent with the literature in similar environmental conditions [27–30]. Additionally, the endotoxin content was below the lowest proposed value in the workplace (OEL of 90 EU/m^3, suggested by the Dutch Expert Committee on Occupational Standards in 2010) [3,31]. However, the number of samples collected was low, and they were limited to pilot plant activities, which cannot be extrapolated to full-time activities. In fact, a higher value of 86 EU/m^3 was observed on March 11. This was probably due to contamination caused by the normal activity of the wastewater treatment plant hosting the pilot plant.

Bioaerosol data showed relatively low *Clostridia* concentrations and high *Bacillus* concentrations. These results are in line with what was expected and reported in the literature [13,33,34], except for airborne Salmonella (maximum 30 CFU/m^3), which is not commonly detected in bioaerosol monitoring [13]. This could be due to the limited containment measures taken at pilot-scale plants for bioaerosol generation and dispersion. The sampled bioaerosol represents the surrounding contamination of the pilot plant, so even if it was affected by the anaerobic contribution effluents at the pilot plant, the bacterial and fungal species sampled were both anaerobic and aerobic.

Table 4. Microorganisms isolated through rapid biochemical tests (API, bioMériux) in all three sampling campaigns. % indicates percentage identified.

Campaign	Isolated Microorganism	%	Relevance to Human Health [32]	Main Transmission Route/Vehicle	Environmental Dispersion	General Description
First	*Pseudomonas aeruginosa*	99.5	Opportunistic pathogen (class 2)	Aerosol	Ubiquitous	Gram-negative, *Pseudomonadaceae*
	Serratia rubidaea	81.2	Opportunistic pathogen	Parenteral	Water, soil	Gram-negative, *Enterobacteriaceae*
	Photobacterium damselae	95.0	Pathogen	Oral–fecal	Water, food	Gram-negative, *Vibrionaceae*
	Pseudomonas fluorescens	75.2	Opportunistic pathogen	Oral–fecal, parenteral	Soil, surface water	Gram-negative, *Pseudomonadaceae*
Second	*Hafnia alvei*	99	Opportunistic pathogen	Oral–fecal	Soil, food	Gram-negative, Enterobacteria
	Serratia marcescens	98	Opportunistic pathogen	Direct contact	Ubiquitous	Gram-negative, Enterobacteria
	Proteus vulgaris	99.9	Pathogen (class 2)	Contact	Soil, fertilizers, sewage	Gram-negative, Enterobacteria
	Serratia liquefaciens	90.3	Opportunistic pathogen	Direct contact, oral–fecal	Soil, waste, wastewater	Gram-negative, Enterobacteria
	Serratia odorifera	99.9	Opportunistic pathogen	Direct contact, oral–fecal	Ubiquitous	Gram-negative, Enterobacteria
	Pseudomonas luteola	82.6	Opportunistic pathogen	Aerosol	Ubiquitous	*Pseudomonadaceae*
	Klebsiella pneumoniae	98	Opportunistic pathogen (class 2)	Aerosol	Wet environment, water facilities	*Enterobacteriaceae*
	Klebsiella oxytoca	99.6	Opportunistic pathogen (class 2)	Direct contact	Ubiquitous	*Enterobacteriaceae*
	Bacillus cereus	99.9	Pathogen	Oral–fecal	Soil, dust, food	Gram-positive, *Bacillaceae*
	Pantoea spp.	99.8	Opportunistic pathogens	Parenteral	Water, soil	*Enterobacteriaceae*
	Bacillus licheniformis	99.9	Opportunistic pathogen	Aerosol	Soil	Gram-positive, *Bacillaceae*
	Citrobacter freundii	76	Opportunistic pathogen	Oral–fecal	Water, soil	*Enterobacteriaceae*
Third	*Pantoea* 1 spp.	98.8	Opportunistic pathogens	Parenteral	Water, soil	Gram-negative, *Enterobacteriaceae*
	Enterobacter cloacae (2)	99.0;98.7	Opportunistic pathogens	Contact/aerosol	Water, ubiquitous	
	Pseudomonas aeruginosa	99.0	Opportunistic pathogen (class 2)	Aerosol	Ubiquitous	*Pseudomonadaceae*
	Pseudomonas luteola (2)	98.6;89.0	Opportunistic pathogen	Aerosol	Ubiquitous	*Pseudomonadaceae*
	Pseudomonas putida (2)	98.8;91.0	Opportunistic pathogen	Aerosol	Nosocomial	*Pseudomonadaceae*
	Cintrobacter freundii	97.8	Opportunistic pathogen	Oral-fecal	Water, soil	*Enterobacteriaceae*
	Enterobacter aerogens	99.0	Opportunistic pathogen	Contact/aerosol	Water, ubiquitous	
	Klebsiella pneumoniae	97.9	Opportunistic pathogen (class 2)	Aerosol	Wet environments, water	*Enterobacteriaceae*
	Proteus vulgaris	99.0	Pathogen (class 2)	Direct/indirect contact	Soil, fertilizers, sewage	*Enterobacteriaceae*

In the first sampling, bioaerosol monitoring showed low concentrations overall, as measured by the GIMC value, indicating a limited bioaerosol presence and persistence in

the background area where the pilot plant was located. However, in the second sampling campaign, higher concentrations were detected for both fungi and *Bacillus* spp. Significant contamination levels were also supported by the GIMC values for the second and third sampling campaigns. Notably, the levels of microorganisms belonging to the Bacillus genus, which are important for their ability to produce spores [35] and withstand the chemical–physical conditions of anaerobic digestion, were especially high. The Bacillus genus could be representative of microbial contamination related to OFMSW and could be used as a potential indicator [36]. The Bacillus genus is also relevant because it encompasses pathogenic species such as *Bacillus cereus* and *Bacillus anthracis*. The former infects humans, causing food-borne illness, while the latter causes anthrax [35]. *Bacillus subtilis* and *Bacillus cereus* are also involved in biofilm production, allowing them to survive even the most aggressive environmental conditions [37,38].

Another finding was the detection of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* through culture-dependent methods. *Pseudomonas aeruginosa* is a Gram-negative bacterium, ubiquitous in the environment and commonly associated with soil and water. It is an opportunistic human pathogen that can cause a wide range of acute and chronic infections, especially in immunocompromised individuals. It is particularly hazardous because it is responsible for severe nosocomial infections, such as pneumonia. It is also able to adapt to, survive, and resist various classes of antibiotics, such as carbapenems. For all of these reasons, *P. aeruginosa* is considered a rising threat to public health and a cause of great concern in the scientific community [39–41]. *Klebsiella pneumoniae*, another opportunistic Gram-negative bacterium, was also identified in this study. Similar to *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* mainly affects individuals with compromised immune defense. It is hypervirulent and multi-drug resistant due to the diversity of its antimicrobial resistance (AMR) genes, its varied DNA composition, and its high plasmid burden. It is the causative agent of a variety of infections, including in the respiratory tract, urinary tract, and bloodstream [42,43]. It is one of the most frequent causes of nosocomial and community-acquired infections, representing a major hazard to human health [43]. Unlike endotoxins and PM10, significant differences were observed in bioaerosol dispersion due to both the presence of OFMSW and its treatment methods. The level of biological contamination can be considered intermediate, according to the National Institute for Insurance against Accidents at Work (INAIL) classification in 2010, since the GIMC was always higher than 1000 CFU/m^3 but always lower than 10,000 CFU/m^3.

As reported in the literature, the activity with the most impact is farming, in which animals and biomasses are present contemporaneously as bioaerosol sources. Generally, wastewater treatment plants present lower air contamination levels [44] with respect to farming [45] and composting plants [46]. In this study, the background bioaerosol of the wastewater treatment plant was increased by the impact of the organic waste treatment at the pilot plant. Such increase is not negligible, even if not so critical.

Finally, it should be noted that all analyses conducted in this study involved a pilot facility that employs techniques that cannot always be applied to an industrial-scale plant. Therefore, the results obtained cannot be directly extrapolated to an industrial-scale plant. However, this research gives some valuable insights into airborne microbial species released during organic waste treatment processes and provides a qualitative assessment of bioaerosol contamination levels. At the pilot plant, no collective protection was used, allowing the possibility to see what could be worker exposure risk in the case of a lack of protection. At an industrial-scale plant, quantitative evaluation of the actual bioaerosol levels emitted during organic waste disposal would be essential, considering that the volume of treated organic waste will increase and the technical solutions applied will differ. The occupational risk associated with bioaerosol exposure should be minimized by managing the work activity and using collective protective equipment (e.g., biofilters) and personal protective equipment (e.g., glasses, helmets, masks, gloves, safety shoes, protective suits), along with other protective devices (e.g., respiratory protection and efficient ventilation systems) [43,47,48].

5. Limits

The monitoring campaign was performed in a pilot plant employing techniques that cannot always be applied to industrial-scale plants. In this scenario, each step must be subjected to risk assessment and reorganized to improve efficiency and safeguard both workers and the environment.

Occupational risk assessments are usually performed with personal samplers, and in this study, high-volume environmental samplers were used, which can detect what was dispersed in a particular area but not exactly what and how much people would be exposed to. However, in this case, we considered an experimental plant, which differed from an industrial plant where there is a different duration of exposure, and individual and collective protective devices are routinely used.

The lack of correlation and significant differences among PM10, endotoxins, and bioaerosol data may be due to the outdoor location of the pilot plant and the small number of available measurements. There were few repetitions because the microbial characterization was not the main focus of the facility; on the other hand, the results obtained in this monitoring campaign show the effect of the self-managed pre-treatment process, which is the most contaminating stage.

The use of only culture-based methods underestimated the microbial contamination levels since the culturability of environmental bacteria is generally low [43]. Consequently, some microorganisms that are not cultivatable under certain specific growth conditions could have been undetected. Biomolecular techniques such as real-time polymerase chain reaction (PCR) and next-generation sequencing (NGS) are more sensitive, provide greater microbial resolution and diversity, and have much higher quantification limits [3]. Nevertheless, these methods cannot discern if the genetic material belongs to a viable or non-viable microorganism, potentially leading to overestimation [25]. Using a combination of both techniques could better represent bioaerosol contamination levels [13].

6. Conclusions

The results obtained in this study showed higher microbial contamination produced when the organic waste treatment in the plant was included, especially fungi, *Bacillus* spp., and opportunistic pathogens. Such air contamination is potentially harmful to both workers' health and the environment, in particular when pre-treatment of raw organic waste was involved. Consequently, regular monitoring of critical parameters can be auspicial. On the other hand, no particular impact can be attributed to the other pilot plant activities for the production of PHAs. However, for PM10 and endotoxins, the detected levels in such an outdoor environment can be considered acceptable. A complete risk assessment requires a specific quantitative evaluation of the mass flows emitted at each processing step and cannot disregard full-scale monitoring. Finally, an industrial-scale process, with both manually shredded and squeezed OFMSW, should consider the findings of this study to achieve appropriate risk containment.

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