

Emission of bacteria and fungi in the air from wastewater treatment plants – a review

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1. ABSTRACT

An increase in global population, coupled with intensive development of industry and agriculture, has resulted in the generation and accumulation of large amounts of waste around the world. The spread of pathogenic microorganisms, endotoxins, odours and dust particles in the air is an inevitable consequence of waste production and waste management. Thus, the risk of infections associated with wastewater treatment plants (WWTPs) has become of a particular importance in recent decades. Sewage and unstable sludge contain various pathogens such as viruses, bacteria, and human and animal parasites. These microorganisms can be transmitted to the ambient air in wastewater droplets, which are generated during aeration or mechanical moving of the sewage. Bioaerosols generated during wastewater treatment may therefore pose a potential health hazard to workers of these plants or to habitants of their surroundings. The degree of human exposure to airborne bacteria, fungi, endotoxin and other allergens may vary significantly depending upon the type and the capacity of a plant, kind of the facilities, performed activities and meteorological conditions.

2. INTRODUCTION

Recent awarenesses about the risks posed by airborne microorganisms are the reason for the enormous development of aeromicrobiology. However, it seems that there is no internationally accepted threshold limit value for biological contamination of air (1). This is a complex problem involving many interactions, among others, individual immunity system. Therefore, the ascertainment of microorganisms presence, especially as indicators in the air at a given site, is generally accepted as synonymous with the term “range emission” of the tested facility and an estimation of the potential risk zone (2). Atmospheric air, having limited self-purification ability, is an important component of the environment, so that there is a need for its maximum protection. It should consist primarily in reducing emission into the atmosphere, since air pollutants occurring there may be transferred by the wind over very large distances (3-11). According to Griffin *et al.* (5), bioaerosols can be transported within and between continents on upper air currents. Some culturable microorganisms have been detected as high in the earth’s atmosphere as 20,000 m. Papke *et al.* (12) showed that microbes were viable even after being transported several thousand kilometres and were capable of causing an infection (e.g. the epidemic of meningitis, which spread from the African belt to Scandinavian countries).

The microorganisms found in the air are usually accidental and commensal. They appear as the large number of sporulating forms, such as bacteria’ endospores and spores of fungi. Less numerous are the pathogenic microorganisms; however, they pose a direct threat to human and animal health. Contamination of the air by microorganisms, including pathogenic ones, generates from various sources, both natural, such as water, soil or rotting plants and animal remains, and anthropogenic, including municipal landfills and sewage treatment plants. Pathogens, mainly found in excreta (13,14), and secretions of patients are transferred in general by sewage and municipal waste from households and hospitals, creating unspecified health hazard in the surroundings of WWTPs. The generation, treatment, and disposal of the human and animal waste contribute to the increase in the production of bioaerosols containing a wide variety of microbial pathogens and related pollutants.

Bioaerosols might be a vehicle for the dissemination of human and animal pathogens from wastewater. Their presence in the air might pose a potential epidemiological threat. This review is intended to summarize the information on bioaerosols and highlight the significance of bioaerosols emitted during municipal waste treatment for public health and condition of the

environment. Comparing the degree of contamination with bioaerosols generated by WWTPs which use different types of sewage treatment systems, seems to be particularly important. The determination of the spreading range of bioaerosols allows defining the size of the potential health hazard zone to workers of WWTPs and inhabitants of the surrounding areas.

3. AIR POLLUTION

Air pollution is an inherent complex, containing particulate matter of varied sizes and composition, inorganic gases, and myriad volatile organic compounds intermingled with biological materials such as pollens, spore and fungi fragments, viruses, bacteria and others (15,16). They can act as cloud condensation and ice nuclei at relatively warm temperatures and influence the formation of precipitation, the hydrological cycle, and climate. Moreover, fungi might influence the chemical composition of cloud and rain water by metabolic transformation of organic trace substances (17,18). The contaminants of ambient air arise from a variety of natural and anthropogenic sources (19) and the latter are dominated by emission from the combustion of fossil fuels (20).

Aerial dispersal is a natural facet of the life-cycle of many microorganisms, required for reproduction and for the colonization of new sites. Especially the fungi have developed intricate mechanisms by which they actively eject their spores in great numbers into the air. Fungi, bacteria and algae which colonize soil, bodies of water, plant surfaces, rocks and buildings are readily released into the air by wind and splashing water. They can be aerosolized as individual spores or cells, rafted on dust and soil particles or associated with insects and protists. The potential roles of bioaerosols in sick building syndrome, in occupational illness in animal handling, and in solid/liquid waste management industries are a major concern. Therefore, the responsibility of air quality management has been designated to units, which have been authorized to identify goals for the protection of the environment and public health, to identify emission sources contributing to the air pollution, the criteria of pollutants and to establish a coordinated system of measures to attain acceptable air quality and identification of pollutants [e.g. U.S. Environmental Protection Agency (EPA) in the U.S.A.].

3.1. Bioaerosol

Bioaerosols is a term commonly used to describe viable and non-viable airborne biological particles, such as fungal spores, bacteria, pollen, and viruses and their fragments and by-products, like bacterial endotoxins, mycotoxins, peptidoglycans, (1-3)-beta-D glucans, which may affect living organisms infectiously, allergically, toxigenically or pharmacologically (21,22). Fungal spores, bacteria, and pollen are typically 1–30, 0.2.5–8 and 17–58 μm in diameter, respectively, while viruses generally have diameters $<0.3 \mu\text{m}$ (23). Their concentrations in the atmosphere are significant. Matthais-Maser *et al.* (24) suggested that up to 28% (by volume) of the particulate matter suspended over remote land surfaces comprises of biological particles. Womiloju *et al.* (25) and Jaenicke (18), concluded that fungal cells and pollen accounted for 4–11% of the total mass of airborne particulate matter $<2.5 \mu\text{m}$. Bioaerosols are typically associated with particulate matter or surrounded by a thin layer of water, having an aerodynamic diameter range of 0.5–100 μm (21). Although the atmospheric air is a natural environment where a variety of microorganisms might occur, the adverse physical and chemical characteristics and lack of nutrients cause the air to become a way for a transfer of microorganisms, rather than a habitat for their existence. The largest concentration of microbes in the air is stated directly above the soil surface, especially in populated areas, during dry summers and moderately strong wind. Precipitation removes microorganisms from the air only temporarily. They may become a component of the bioaerosol again after drying by micro-convection currents (26). Microorganisms can get released into air through micro-droplets ejected along with secretions of nasopharyngeal or oral origin, while talking, sneezing or coughing. For example, during a cough, millions of tiny droplets of water and mucus are thrown with great speed (about 100 m/s).

3.2. Meteorological factors and bioaerosol concentration

The survival of bioaerosols and the extent of bioaerosol dissemination are dictated by biotic factors, which control the viability of the aerosolized organisms, as well as the abiotic factors limiting release, transport, and dispersion of organisms. The size, density, and shape of the droplets or particles are the most important physical characteristics, while the magnitude of air currents, relative humidity, and temperature are the significant environmental parameters. The transport of bioaerosols can be defined in terms of distance and time. Submicroscale transport involves very short periods of time, under 10 min, as well as relatively short distances, under 100 m. This type of transport is common within indoor environments. Microscale transport ranges from 10 min to 1 h and from 100 m to 1 km and is the most frequent and significant type of bioaerosol transport from a human health standpoint (27).

The composition, size and concentration of the microbial populations comprising the bioaerosol vary with the source, dispersal mechanisms in the air, and more importantly with the environmental conditions prevailing at the particular site (28,29). Bioaerosols generated from water sources (such as during splashing and wave action) are different from those generated from soil or nonaqueous surfaces. They are usually formed with a thin layer of moisture surrounding the microorganisms and consist of aggregates of several microorganisms. Bioaerosol particles are subjected to Brownian motion, gravity, electrical forces, thermal gradients, electromagnetic radiation, turbulent diffusion, inertial forces, oxygen concentrations, and relative humidity (30). The extent to which bioaerosols respond to these forces varies depending on the physical properties of bioaerosols, such as size, shape and quantity (31). Additionally, there are biotic factors, such as the type of organism, viability status, growth phase, and inherent resistance to electromagnetic radiation, that ultimately determine the bioaerosol characteristics. Brownian motion of bioaerosols arises because of their constant bombardment by molecules of the surrounding medium. It increases with the rise of the

temperature and decreases with particle size. For bioaerosols bigger than 1 μm , gravitational settling is a much more influential factor than Brownian motion (21). The gravitational effect on a bioaerosol particle is countered by the drag or frictional force exerted on that particle. When the two forces are equal, the particle reaches its final or terminal velocity. Thus, during bioaerosol transport downwind, the concentration decreases with time, not only because of biological inactivation, but also because of gravitational settling. Diffusion of bioaerosols from regions of higher concentration to regions of lower concentration is a significant factor that operates in outdoor environments. Since bioaerosol particles have a net charge on them (depending on the source characteristics), electrical forces could have an effect on the deposition rates and, thereby, bioaerosol concentrations over time and space. Nicholson *et al.* (32) reported that endospores of *Bacillus subtilis* are extremely resistant to a variety of electromagnetic radiation. Bioaerosol particles generally move down thermal gradients from regions of warmer temperatures to cooler regions. In general, increasing temperature has a deleterious effect on aerosolized organisms.

Naturally occurring culturable bioaerosols have been shown to exhibit both diurnal and annual cyclic patterns in relation to the meteorological conditions of the examined area. Karra *et al.* (33) observed the maximum number of airborne bacteria at the WWTP in the late afternoon and after sunset in the summer season. On an annual basis, Gotkowska-Płachta *et al.* (34) and Korzeniewska *et al.* (35) found the largest number of airborne microorganisms at the WWTPs area during early spring. Grisoli *et al.* (36) and Fang *et al.* (37) reported that the fungal contamination of air at WWTP area was higher in summer than in winter. However, Korzeniewska *et al.* (38) examining the presence of *Enterobacteriaceae* family bacteria in the air samples, collected near aeration chambers, ascertained the greatest number of these bacteria in the winter season. This could be due to the high wastewater evaporation, resulting from the difference in sewage and ambient temperature.

In the environments of high humidity, fungal spores can be released into the air, which could cause infections or allergic reactions by humans (39). Peccia *et al.* (84) have shown, using microscopy and culture methods, that when aerosolized bacterial cells, such as *B. subtilis*, *Serratia marcescens* and *Mycobacterium* sp., are exposed to a relative humidity exceeding 50%, they tend to demonstrate increased water sorption, which protects the cells from UV-induced inactivation. The bacterial cells are found to absorb water from the atmosphere when the relative humidity ranges between 20 and 95%. Peccia *et al.* (40) have also shown that when aerosolized bacterial cells, such as *Mycobacterium parafortuitum*, are exposed to a relative humidity ranging between 4 and 95%, UV-induced photoreactivation protects the cells from UV-induced inactivation. Their results suggest that unlike the UV damage noticed in bacterial cells suspended in water, cyclobutane thymine dimers are not the most significant form of UV-induced DNA damage in aerosolized bacteria. Since the relative humidity affects the density of the bioaerosols, which in turn will dictate the settling velocities and ultimately the potential exposure, the issue of photoreactivation needs to be taken into consideration when evaluating risks associated with pathogenic bioaerosols. Tseng and Li (41) studied the effect of UV dose, type of virus nucleic acid, and RH (relative humidity) on the effectiveness of ultraviolet germicidal irradiation (UVGI) to deactivate airborne viruses. For airborne virus deactivation, the effectiveness of UVGI strongly depended on the type of virus nucleic acid. Viruses with dsRNA or dsDNA were significantly less susceptible to UV inactivation. For 90% airborne virus inactivation, the UVGI dose for dsRNA and dsDNA viruses was approximately 2 times higher than ssRNA and ssDNA viruses, respectively. The microorganism susceptibility factor was highest for the viruses, similar to that for fragile bacteria, but 13–20 times higher than that for endospore bacteria or fungal spores. The susceptibility factor for the viruses was higher at 55% RH than that at 85% RH, possibly because when RH is increased, water sorption on the virus surface might provide protection against UV-induced DNA or RNA damage. Short-wave ionizing radiation (X rays, gamma rays, and electron beams) can cause indirect damage to nucleic acids. Hughes (42), basing on studies at sewage treatment plants in Antarctica, reported that environmental stresses, such as desiccation and solar UV, can be detrimental to the viability of aerosolized organisms. His study has also shown that upon deposition the Antarctic terrestrial environment is inhospitable for airborne faecal coliform bacteria. Within one hour of initial deposition, faecal coliform viability declined up to 99.8% and 99.9.8% under desiccation and solar radiation stresses, respectively. When solar radiation and desiccation stresses are combined, faecal coliform survival in the air is likely to be reduced further. Evans *et al.* (43) analyzing heterotrophic bacteria against meteorological parameters, found an inverse relationship to average dry interval humidity and positive correlation with wind speed, especially during storm events. This emphasizes the possibility of microbiological contamination of the air, particularly risk of human pathogen infection.

3.3. Assessment methods for bioaerosol

Evaluation methods of air contamination might be divided into: culture-based, non-culture-based and other methods. Sampling of culturable bioaerosols is based on impactor (microorganism are collected directly on a culture medium), liquid impinger (microorganisms are collected in liquid collection fluid) or air filtration methods (microorganisms are collected on a filter). After samples collection, colonies of bacteria and fungi are incubated on culture media at a defined temperature over a 3–7 day period. Colonies are counted manually or with the aid of image analysis techniques. Counting of culturable microorganisms has some serious drawbacks including poor repeatability, selection for certain species due to chosen culture media, temperature etc. and the fact that dead microorganisms, cell debris and microbial components are not detected, while they too, may have toxic and/or allergenic properties. On the other hand, counting of culturable microorganisms might be a very sensitive technique and many different species can be identified (44).

Non-culture-based methods enumerate organisms without regard to viability. Sampling of non-culturable bioaerosols is generally based on air filtration or liquid impinger methods. Microorganisms can be stained with a fluorochrome, e.g. acridine orange, and counted with an epifluorescence microscope (45). Possibilities of classifying microorganisms taxonomically are

limited because too little structure can be observed. Bacteria collected with impingers or filters can be counted by flow cytometry after staining with 4',6-diamino-2-phenylindole (DAPI) or by applying fluorescent *in situ* hybridization (FISH). FISH involves the use of fluorochrome-labelled nucleic acid probes to target rRNA within morphologically intact cells. This method allows taxonomic determination from kingdom to species (46). The main advantage of microscopy or flow cytometry is that both dead and living microorganisms are quantified. Disadvantages include laborious and complicated procedures, high costs per sample and unknown validity.

Instead of counting culturable or non-culturable microbial cells, constituents or metabolites of microorganisms can be measured as an estimation of microbial exposure. Toxic (e.g. mycotoxins) or pro-inflammatory (e.g. endotoxin) components can be measured but also non-toxic molecules may serve as markers of either large groups of microorganisms or of specific microbial genera or species. The use of advanced methods, such as polymerase chain reaction (PCR)-based technologies and immunoassays, have opened new capabilities for detection and speciation regardless of whether the organisms are culturable or not. Some markers for the assessment of fungal biomass include ergosterol measured by gas chromatography–mass spectrometry (47) or fungal extracellular polysaccharides measured with specific enzyme immunoassays, allowing partial identification of the mould genera present (48). Other agents such as (1-3)-beta-D glucans (49) and bacterial endotoxin are being measured because of their toxic potency.

4. WASTEWATER TREATMENT PLANTS AS A SOURCE OF MICROORGANISMS

The production of urban wastewater and sludge is increasing on a global scale, because more cities are being connected to wastewater treatment plants. Domestic and industrial wastewaters are collected by an extensive network of sewer lines and treated at municipal plants. Commercial and industrial establishments have to pretreat their wastewater to varying degrees before they are released into the sewer lines. In order to eliminate the microorganisms present in the sewage (especially in the case of the effluent from hospitals with infectious diseases wards) disinfection processes are performed. They can be divided into physical methods (ultrasound, UV) and chemical (chlorine gas, sodium hypochlorite, chlorine dioxide, ozone). At the treatment plant, the wastewater undergoes: preliminary treatment - floatables, grit and grease removal; primary treatment - gravity sedimentation to remove suspended solids; secondary treatment - biological treatment to reduce biochemical and chemical oxygen demand (BOD and COD respectively) and remove suspended solids; and in many cases tertiary treatment - biological removal of nitrogen, mainly chemical or biological removal of phosphorus, disinfection. The solid components accumulated at each treatment stage are generally referred to as sludge or biosolids. The quantity and the characteristics of the sludge depends on the type and volume of wastewater and the treatment kind used (50). Sludge undergo treatment at the wastewater treatment plant before they are used or disposed of. Two common treatments are dewatering followed by stabilization. The dewatering procedures are air-drying, vacuum filters, centrifugation, and belt filter presses. Stabilization processes, such as lime stabilization, anaerobic and aerobic digestion, composting and or heat-drying, are used to reduce organic matter, pathogen levels and odours in sludge. Recycling the sludge as an organic fertilizer is environmentally friendly, but among the large diversity of microorganisms found in urban wastewater, some pathogens can be present (viruses, bacteria and parasites) (51) and such microorganisms are concentrated in sludge during the treatment of wastewater. Furthermore, some of these pathogens are known to survive for several months in the environment (52). Chun-Ming *et al.* (53) observed that some pathogenic bacteria such as *E. coli* O157:H7 cells survived in composting process even at 54 to 67°C. Therefore, monitoring of pathogens during wastewater and sludge treatment enables to evaluate the efficiency of the process in terms of sanitization (54).

4.1. Bacteria, moulds and yeasts in the sewage and sludge

The degradation of organic substances in WWTPs is mainly a result of the activities of aerobic and facultative anaerobic heterotrophic bacteria and heterotrophic fungi. Numerous saprophytic and opportunistic organisms, and sometimes pathogenic or potentially pathogenic microorganisms occur in the raw wastewater of all types of treatment plants, regardless of the origin of sewage (55,56,57). The microflora of wastewater is as varied as the composition of pollutants. The highest amounts and the most diverse of microorganisms are found in a domestic sewage along with human and animal excreta, which may include bacteria: *Aeromonas*, *Acinetobacter*, *Campylobacter*, *Clostridium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Mycobacterium*, *Pantoea*, *Pseudomonas*, *Serratia*, *Staphylococcus*, *Salmonella*, *Shigella* and *Vibrio* (34,35,38,54,57-61), as well as filamentous fungi from genus *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Trichoderma* and numerous yeasts and yeast-like fungi like *Candida*, *Cryptococcus*, *Geotrichum* and *Rhodotorula* (57,62-65). Elimination of microorganisms in the process of sewage treatment is the result of a combination of physical (sedimentation, filtration, adsorption), chemical (redox potential, toxicity, changes in the pH value) and biological factors (competition for nutrients, grazing by protozoa, lytic activity of bacteria and bacteriophages, the production of bacteriocins) (66,67).

As Korzeniewska *et al.* (57) reported the numbers (CFU – colony forming units – in 1 cm³) of heterotrophic mesophilic bacteria, *Enterobacteriaceae* bacteria, moulds and yeast/yeast-like fungi in untreated wastewater ranged from up to 1.9×10⁵–6.4×10⁷, 2×10⁵–4×10⁷, 1.0×10³–3.0×10³ and from 8.5×10³ to 5.0×10⁴, respectively. Therefore treatment of sewage could be not only a source of emission of chemical compounds, but also many bioaerosols which pollute atmospheric air and might become a threat to human health (58,62,68). The character and range of the environmental effects produced by a WWTP depend on the initial concentration of microorganisms in sewage as well as their growth phase, emission threshold level, sewage treatment technology, aeration techniques (69,70,71), meteorological and environmental conditions (35,57,61,72).

4.2. Pathogenic and potentially pathogenic microorganisms in the sewage

Pathogens that are present in raw wastewater are also present, in concentrated amounts, in unstable sludge. Concentrations and types of pathogens in treated sewage and biosolids depend significantly on the origin of waste and the type of their purification process (34,38,55,57,60). Although aerobic and anaerobic wastewater treatment processes reduce bacteria number in the sewage, some pathogens can remain in the sewage outflow and the final biosolid product. Studies have shown that aerobic and anaerobic treatment units remove faecal coliforms from sewage with efficiency up to 90–99.9% (55,73). Jones (74) has also reported a significant reduction of *Campylobacter* bacteria when the sewage undergoes activated sludge treatment. The reduction of pathogens during treatment processes can vary; depending on how precisely the process is controlled. Even with a 1–2 order of magnitude decrease in bacterial and viral numbers, the actual concentration of microorganisms in the treated wastewater and biosolids can still be significantly high. As Filipkowska (55), Espigares *et al.* (59) and Kay *et al.* (56) reported, although the sewage purification system was efficient and reduced the contamination load to the low level and removed a great percent of indicator bacteria (even above 99%), the purified sewage could be a source of many pathogenic bacteria in the inland waters. These bacteria are often characterized by multiple resistance, showing a cross-resistance to multiple antibiotics simultaneously (75,76). Examples include bacteria belonging to the family *Enterobacteriaceae* (77). Among them, bacteria from *Salmonella*, *Shigella*, *Escherichia*, *Klebsiella*, *Serratia*, *Enterobacter* or *Proteus* genera deserve a special attention. Since the 80s, an increase in the number of infections caused by these bacteria has been observed. They have been found to be one of the most important etiological agents of systemic infections (78–81). *E. coli* is a common cause of urinary tract infections, *Klebsiella* spp. and *Enterobacter* spp. cause pneumonia, while all *Enterobacteriaceae* are associated with blood infection (sepsis), peritonitis, and gastrointestinal infections. Bacteria of the genus *Salmonella*, which produce toxins are responsible for typhoid and paratyphoid fever. The natural habitat of these bacteria is the gastrointestinal tract of humans and animals and the entries of infections are mainly gastrointestinal, respiratory, urinary, biliary, wound and soft tissue. *E. coli*, which has the ability to encode genes of multiple resistance, is a physiological component of the microflora in the colon and naturally inhabits the gastrointestinal tract of humans and animals, both sick and healthy. Feuerpfeil and Stelzer (82) found that 80.5% of the faeces samples of healthy people contained coliform bacteria resistant to some antibiotics, the microorganisms were frequently resistant to several antibiotics simultaneously. Along with these excrements, microbes get into the domestic and municipal sewage. After having been collected in treatment plants and even in well-functioning biological plants, huge quantities of these bacteria get to the environment with treated sewage (83). Reinthaler *et al.* (84) ascertained as many as 10^2 CFU/ml resistant coliform bacteria in the effluent of a large treatment plant. About 17% of those bacteria had a six-fold resistance to antibiotics. Together with purified sewage, they can penetrate the soil, surface water, rural groundwater supplies, municipal drinking water and also accompanied by bioaerosols - the air. Their presence is an underlying cause of an increasing public health problem.

4.3. WWTPs as a source of bioaerosols

Raw wastewater is a potential carrier of pathogenic microorganisms (35,58,85) and may pose a health threat, especially, when those microorganisms become aerosolized during aeration. Microorganisms which are transferred from sewage to the air in the form of bioaerosol are subjected to certain conditions which can inhibit their development. Some die rapidly mainly from desiccation, exposure to excessively high or low temperatures or are annihilated by solar radiation (42). However, some microorganisms are equipped with specific mechanisms which enable them to combat the unfavourable environmental conditions that could inhibit their biological activity (86,87). Thus, number of microorganisms in the air seems to be one of the major indicator of atmospheric pollution from WWTPs.

Some wastewater treatment facilities, such as aeration chamber, biofilters and grit chambers (especially blown) can disperse wastewater droplets containing various microorganisms that are transmitted along with the wind, sometimes over long distances (12). Therefore, droplets produced might contain varying numbers of pathogenic microorganisms, some of them having the ability to infect a person through the respiratory system, contact or swallowing (88). The potential hazard posed by bioaerosols depends on the pathogenicity of a specific microorganism as well as other factors. The environmental conditions which determine the survival of the microorganisms in the air, the meteorological conditions (30,89) (especially wind speed and direction) which govern airborne dispersion from the emission sources the pathway to come into the body and also the immunological response of the potential receptor are considered the most important factors. The main pathways for the transmission of microorganism to humans are: by direct contact with contaminant source (through mucous membranes or skin), by ingestion (through hands or accidentally) and by inhalation. Most of the bacteria-carrying particles in the air of a WWTP have an aerodynamic diameter below 4.7. μm . Hung *et al.* (90) observed that most *E. coli* containing droplets generated by the bubbles were between 3.3. and 4.7. μm , with count median aerodynamic diameters of around 4.5. μm . The small size of these particles means that they can enter the lungs easily if inhaled, becoming a potential cause of infections in immunocompromised people and causing allergic responses in others. In addition, these small particles can be very easily carried by the wind to distances ranging from a few hundred metres to several kilometres, posing a potential biological hazard not only to site workers but also to local residents (91).

The transfer of the microorganisms from wastewater to the air occurs during the different phases of the process in wastewater treatment plants, particularly in those containing moving mechanisms (such as in the influent to the primary and final settling tanks, and in the grit tanks) and where forced aeration of wastewater is performed (34,60,69,70,89,92–94). This study also confirmed by Filipkowska *et al.* (68) and Sánchez-Monedero *et al.* (71), who reported that the pre-treatment, biological treatment

and sludge thickening were the processes which generated the highest amount of bioaerosols. Korzeniewska *et al.* (35,57,60) and Filipkowska *et al.* (69) observed the highest numbers of heterotrophic and *Enterobacteriaceae* bacteria (see Table 1), ranged up to 3.9×10^4 and 5.0×10^2 CFU/m³ near grit/grate chambers and 2.4×10^4 and 2.2×10^3 CFU/m³ near aeration chambers respectively. These facilities were monitored in WWTPs as a potential source of bioaerosols since mechanical agitation of treated wastewater caused a turbulence that may lead to the generation of airborne particles. These results are in agreement with the results of other authors working under similar operational conditions (58,95). Medema *et al.* (96) detected *Legionella* spp. and *L. pneumophila* in air samples at 3 out of the 5 sewage treatment plants tested. Samples of air above trickling filters, aeration tanks, the screen and the belt press were positive for *Legionella*. The concentration ranged from 0.5.6 - 56 per m³ of air (identification by PCR).

When bubbles of aerated sewage reach the surface they burst and little film drops are ejected up to 15 cm above the surface. Splashing and bubble bursting that occur as a result of forced aeration in activated sludge processes are very often responsible for producing large bioaerosols. Surface-active particles, such as bacteria, concentrate at surface microlayers and are dashed up by the bursting bubbles. As Blanchard and Syzdek (97) reported, the highest contribution to emission of aerosols can be attributed to a thin surface layer (a few millimetres in thickness) of sewage, in which inorganic and organic substances along with microorganisms are concentrated. They showed in their experiments that bacterial concentrations in the drops ejected from the bubbles were 10–1000 times higher than those of the wastewater source, depending on the drop size. The number of airborne microorganisms increases rapidly with bubble size (90,98) so that the type of the aeration system greatly influences the production of aerosols (58,89,95,98). Wastewater aeration by aerators, diffusers, sprinklers and dipper wheels might cause an increase in the probability of transport of microorganisms from wastewater to the air. Spreading of microorganisms caused by underwater aeration is more limited than in cases of surface aeration (Table 1). One of the most promising solution seems to be the fine -bubble diffused-air aeration system (26,30), inducing only minor turbulence in the tanks, and emitting aerosols to a much smaller degree than mechanical aerators with vertical or horizontal axis (38). The second one is covering grit tanks and aeration chambers (57,70,99,100).

Sánchez-Monedero *et al.* (71) studied three different aerations systems: air diffusion, horizontal rotors and surface turbines, used for the activated sludge biological treatment in six WWTPs in order to compare the level of bioaerosol emission. They found that aeration systems based on horizontal rotors produced the highest amount of airborne mesophilic bacteria, in the range from 3.3×10^3 to 4.5×10^3 CFU/m³, measured 3 m and 5 m downwind the rotors, respectively, while the lowest amount of mesophilic bacteria was generated by the fine bubble diffusers and ranged from 22 to 57 CFU/m³. Filipkowska *et al.* (69) affirmed that aeration systems based on horizontal rotors produced the highest amount of airborne haemolytic and mesophilic bacteria ranged from 3.6×10^4 to 3.9×10^4 CFU/m³ respectively. Filipkowska *et al.* (101) and Korzeniewska *et al.* (38) reported a remarkable decrease in the levels of airborne microorganisms when a plant was converted from a conventional activated sludge process using coarse bubble aeration into a biological nutrient removal system using fine bubble aeration. Similar results were obtained by Fernando and Fedorak (70). They affirmed that the bioaerosol levels recorded above the fine bubble aerated tank were very similar to those recorded at background locations (33). Brandi *et al.* (102) investigated airborne bacteria and fungi at a distance of 2 and 10 m downwind from the aeration tanks of two wastewater treatment plants with different aeration systems. They affirmed that fixed-film reactor generates less microbial emission than the activated sludge plant. Microbial concentrations were higher in aerosols generated by the mechanical aeration system (5.6×10^2 CFU/m³ bacteria and 1.1×10^3 CFU/m³ fungi) than in aerosols emitted by the fine bubble diffused air system (2.2×10^2 CFU/m³ bacteria and 1.9×10^2 CFU/m³ fungi).

According to particle size measurements, the microorganisms containing aerosol are in the size range of <2.0. µm, which enables them to reach the alveoli of the lung. Korzeniewska *et al.* (35,38,57,60) and Filipkowska *et al.* (63,64) identified a lot of pathogenic and potentially pathogenic bacteria, moulds and yeast in this inhalable range (Table 2). They affirmed that bacteria of genera: *Citobacter*, *Enterobacter*, *Klebsiella*, *Serratia*, *Pantoea* were predominant in the air samples collected near mechanical and biological treatment sites, while *Salmonella*, *Escherichia* or *Shigella* were isolated rarely. In the air samples collected in WWTPs' surroundings only, *Pantoea* and *Serratia* were identified. Among moulds *Absidia*, *Actinomucor*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Mucor* and *Penicillium* were predominant irrespective of site of air collection. Filamentous fungi are natural inhabitants of soil and water and probably these environments were the source of moulds in the air of WWTPs' surroundings. In the air near grit/grate chamber and aeration chamber, yeasts and yeast-like fungi from genera *Candida*, *Cryptococcus* and *Rhodotorula* were observed. Yeasts, occurring sporadically in air sampled in WWTPs' surroundings, should be regarded as typical microflora of sewage.

5. BIOAEROSOLS AND HUMAN HEALTH RISK

The atmospheric life expectancy of primary biological aerosol particles can range from a near indefinite time frame for some of the smallest virus particles (size from 10 nanometres) to a few hours for the larger pollen particles (to 100 micrometers) (21) Microorganisms do not typically colonize the air, although a wide variety of them can be found in the atmosphere. Pathogenic bacteria dispersed into the outdoor air from natural phenomena or human activities (e.g. wastewater treatment) are a vital factor affecting public health, agriculture, ecological conditions and international security. Inhalation of bioaerosols can cause a variety of inflammations, hypersensitivity, and allergic responses in lung (91), especially in sensitized individuals. Turner *et al.* (1) believe that all the microorganisms present in the atmosphere should be considered as potentially harmful. Bioaerosol

particles with a diameter of 1–5 μm caused the most serious concern since they are readily transported into the lungs, with the greatest retention of the 1–2 μm particles in the alveoli (103,104). The microbial component of respirable bioaerosols contributes significantly to the pulmonary diseases associated with inhalation of agricultural dusts (105). Airborne biological allergens, fungi, thermophilic actinomycetes, endotoxin and (1-3)-beta-D glucans are associated with non-infectious airway diseases such as allergies, asthma, and hypersensitivity pneumonitis (106). Many gram-negative bacteria produce lipopolysaccharides (LPS) as a part of the outer membrane of their cell wall. These potentially toxic LPS are also referred to as endotoxins and are released upon cell lysis. While LPS are comprised of three covalently linked subunits (i.e. lipid A, core polysaccharide, and O-antigen or -polysaccharide), it is the lipid A portion that is responsible for toxicity. Exposure to airborne endotoxins can cause chronic fatigue or/and acute fever and inflammatory reactions in the respiratory tract, accompanied by cough, chest tightness, shortness of breath and wheezing (107,108). Chronic exposure to endotoxins in organic dusts from occupational settings can lead to decreased lung function, chronic bronchitis and byssinosis (109,110). The exact thresholds for adverse health effects due to exposure to endotoxins, glucans, airborne bacteria and fungi are not known. In background ambient environments, inhalable, thoracic, and respirable endotoxin concentrations are generally <10 endotoxin units (EU/m^3) (111,112). However, exposure to relatively low ambient concentrations of 50–100 EU/m^3 has been found to cause respiratory effects (72,113).

Due to overwhelming urbanization trend in some crowded areas of the world, quite often WWTP and related sewage works, originally located away from urbanized areas, become surrounded by new residential and/or shopping districts. In such situation the question of hygienic sustainability of WWTPs site location arises not only in terms of frequent noxious odours, but also in terms of intermittent enteric illness and related syndrome of unknown origin among nearby residents (96,114,115). In many studies concerning sewage workers health, a particular type of disease is mentioned, probably of viral origin, which infects workers at WWTPs, and is referred to as “Sewage worker’s Syndrome”. Its symptoms are general discomfort, weakness, acute rhinitis and fever. Some studies show a significant connection between cases of respiratory and intestinal diseases of workers of WWTP and habitants of the nearby areas and viral species (characteristic to sewage) in bioaerosols (61,116). Airborne viruses may require a low infective dose, a single virus particle may be enough to infect a person, especially without immunity (115,117). Medema *et al.* (96) obtained an annual average probability of infection (characterized by general malaise, weakness, fever, occurrence of gastrointestinal symptoms and nausea striking WWTP workers) equal to 0.011 for Enterovirus, 0.18 for *Cryptosporidium* and 0.0032 for *Campylobacter*. Westrell *et al.* (115) found average probability of infection values of 1.0 for Rotavirus and 0.16 for *Cryptosporidium* (these assessments did not consider the presence of immunity in the exposed population). This means that workers at WWTPs would quite certainly become infected during one year, unless they were already immune or suitably protected. Bunger *et al.* (91) affirmed that the exposure to organic dust at workplaces of composting facilities is associated with adverse acute and chronic respiratory health effects, including mucosal membrane irritation (MMI), chronic bronchitis, and an accelerated decline of forced vital capacity (FVC%). The pattern of health effects differs from those at other workplaces with exposures to organic dust, possibly due to high concentrations of thermo-tolerant/thermophilic actinomycetes and filamentous fungi at composting plants (118,119). A significant risk is also posed by microbial allergens and endotoxin (106,107). Smit *et al.* (120) found a positive dose-dependent connection between endotoxin exposure and adverse respiratory effects by human, such as wheezing, shortness of breath and cough. A causal relationship between exposure to non-infectious airborne biohazards [i.e. endotoxins, (1-3)-beta-D glucans, allergens of bacteria and fungi] and the occurrence of gastrointestinal symptoms, fever, respiratory symptoms, skin disorders, eye irritation, headache, fatigue and nausea by the workers of sewage treatment plants has also been considered by many authors (44,91,121,122). A significant connection between exposure to rod shaped bacteria and the occurrence of fatigue and headache by sewage treatment workers has already been demonstrated (95). Douwes *et al.* (44), Buche (123) and Brooks *et al.* (124) also recorded that a wide variety of health problems, including infectious diseases, acute toxic effects, allergies, cancer, respiratory symptoms and lung function impairment of workers were related with exposition to bioaerosols in their occupational environment.

Considering regulations of safety in the workplaces, the assessment of the risk of infection associated with wastewater treatment plants (WWTPs) takes on a new significance (125). For most biological agents, safe exposure levels or threshold limit values - i.e. below which no negative health effect is observed - could not be established yet, making it impossible to set Occupational Exposure Limits (OELs). There is a surprising lack of information concerning the infectivity of aerosolized microbial pathogens, especially the enteric pathogens. Recent studies have shown that environmental stress conditions such as osmotic shock, heat, and low pH could stimulate the infectivity and virulence of enteric pathogens (126,127). Some authors recommend the measurement of annual and daily level of particulate matter (PM) as an indicator of the air pollution. PM consists of solid and liquid particles that vary in their physical and chemical properties and that are classified by particle diameter. When inhaled, PM_{10} particles (with a diameter of less than 10 μm) penetrate deep into the respiratory system. Then, finer particles (with a diameter of less than 2.5 μm) go on to penetrate the lungs and pass into the bloodstream and are carried into other body organs. Concerns about these particles and a wide range of associated health impacts, led WHO (World Health Organization) to develop guidelines addressing their risks. According to WHO, level of particulate matter $\text{PM}_{2.5}$ should not exceed in annual average and 24-hour (not to be exceeded >3 days/year) exposure 10 and 25 $\mu\text{g}/\text{m}^3$ respectively (104). Long-term average exposure to PM is associated with both the risks of chronic effects on human health, such as impaired development of lung function, and the frequency of acute effects, such as the aggravation of asthma or incidence of respiratory symptoms. The risk increases linearly with the concentration of pollution, and there is no evidence to suggest a threshold for PM below which no adverse health effects would occur (103,104). Polymenakou *et al.* (128) detected a large fraction of the clones at respiratory particle sizes (< 3.3 μm in size) which were phylogenetic neighbours of human pathogens. They have been linked to several

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diseases such as pneumonia, meningitis and bacteraemia or suspected to induce pathologic reactions such as endocarditis. Raisi *et al.* (129) observed, however, that concentrations of airborne bacteria and fungi outdoors were not correlated with the particle number or particle mass concentration.

Performing a risk assessment without OELs as reference point is possible e.g. by comparing the actual concentration level with the usual environmental level or with the concentrations in different workplace settings. Polish proposal for OELs for bioaerosols at industrial settings polluted with organic dust include mesophilic bacteria, gram-negative bacteria, thermophilic actinomycetes, fungi and bacterial endotoxin with threshold limit value 1.0×10^5 , 2.0×10^4 , 2.0×10^4 , 5.0×10^4 CFU/m³, and 2.0×10^3 EU/m³ respectively (130). In contrast to chemical hazards, biological agents are living organisms that are able to grow and to multiply in the workplace if the living conditions they need are prevailing. Investigations must include determination of the main sources of aerosols and a careful monitoring of their potential to spread diseases, both in quantitative and qualitative ways, depending on the pathogen isolated. Therefore precise quantitative exposure assessment methods seem to be very crucial.

6. SUMMARY

The transfer of the microorganisms from wastewater to the air occurs mainly during the mechanical (moving of raw sewage) and biological (aeration of wastewater in bioreactor) phases of sewage purification. Allergic rhinitis and asthma, chronic bronchitis, extrinsic allergic alveolitis, and organic dust toxic syndrome (ODTS) are major groups of respiratory diseases associated with exposure to bioaerosols from WWTPs (44). The exposure of sewage workers and habitants of WWTP surroundings to airborne bacteria, fungi and endotoxin may vary depending upon the type and capacity of the facility, performed activities and weather conditions. The flow rate and the composition of the sewage and air humidity play a predominant role in increasing the concentrations of the bioaerosols. According to many authors, the sites of pre-treatment and the primary clarifiers, as well as those sites containing moving mechanical equipments for water aeration, are the steps with the highest emission of bioaerosols. The aeration system used in the biological process greatly affects the amount of bioaerosols generated. Moreover, wind speed and its direction are important factors governing the bioaerosol dispersion once they are airborne. Consequently, workers of these sites may be exposed to harmful levels of bioaerosol. Therefore, in order to eliminate emission of bioaerosol and significant decrease of the number of airborne microorganisms, covering grit tanks, section of raw sewage's influent to the primary settling tanks and aeration chambers seems to be necessary (57,69,99,100).

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Abbreviations: WWTP: wastewater treatment plant; BOD: biochemical oxygen demand; COD: chemical oxygen demand; CFU: colony forming unit; LPS: lipopolysaccharides; EU: endotoxin units; ODTs: organic dust toxic syndrome; RH: relative humidity; UVGI: ultraviolet germicidal irradiation; dsRNA: double-stranded ribonucleic acid; dsDNA: double-stranded deoxyribonucleic acid; MMI: mucosal membrane irritation; FVC%: forced vital capacity; DAPI: 4',6-diamino-2-phenylindole; FISH: fluorescent *in situ* hybridization; OELs: Occupational Exposure Limits; PM: particulate matter; WHO: World Health Organization.

Key Words: Bioaerosol, Wastewater treatment plant, Pathogens, Human health risk, Review

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Table 1. Range of microbial concentrations (CFU/m³) in air samples (collected by impaction method using MAS-100 *Eco* Merck) at WWTPs and surrounding sites

Microorganisms	Plant	Control site	WWTPs area		Surroundings		References	
			Mechanical treatment ¹	Biological treatment ²	(100 m from the fence of WWTPs)	(200 m from the fence of WWTPs)		
Heterotrophic bacteria (HPC) (CFU/m ³)	A ³	2.0.×10 ² -7.0.×10 ²	1.8.×10 ² -3.9.×10 ⁴	7.5.×10 ³ -1.3.×10 ⁴	6×10 ²	-	(68)	
	B ⁴	0-1.0.×10 ³	1×10 ² -8.5.×10 ³	5.3.×10 ² -3.7.×10 ³	-	1.3.×10 ² -2.2.×10 ³	50-1.5.×10 ³	(57)
	C ⁵	1.0.×10 ² -8.6.×10 ⁴	34-5.7.×10 ³	58-4.6.×10 ³	33-3.4.×10 ³	29-1.6.×10 ⁴		(35)
	D ⁶	0-4.9.×10 ³	2.7.×10 ² -1.3.×10 ⁴	4.9.×10 ² -2.4.×10 ⁴	6.0.×10 ² -6.9.×10 ³	-		ud ⁷
<i>Enterobacteriaceae</i> bacteria (CFU/m ³)	A	0-9	0-8	1.4.×10 ² -2.2.×10 ³	26	-		(69)
	B	0-17	0-1.6.×10 ²	5-9.0.×10 ²	0-1.8.×10 ²	0-50		(57)
	C	0-17	0-5.0.×10 ²	0-79	0-17	0-1.1.×10 ²		(35)
	D	0-30	0-3.5.×10 ²	0-50	0-50	-		(60)
Moulds (CFU/m ³)	A	1.8.×10 ² -3.3.×10 ³	5.7.×10 ² -6.8.×10 ²	1.0.×10 ³ -1.1.×10 ³	2.4.×10 ³	-		(69)
	B	0-1.3.×10 ³	50-5.6.×10 ³	26-1.1.×10 ⁴	0-1.9.×10 ³	0-4.5.×10 ³		(57)
	C	0- 1.0.×10 ⁴	8-8.1.×10 ³	1.0.×10 ² -4.7.×10 ³	0-1.0.×10 ⁴	0-1.1.×10 ⁴		ud
	D	8- 1.1.×10 ⁴	0-7.1.×10 ³	0-1.3.×10 ⁴	0-1.2.×10 ⁴	-		(63)
Yeasts (CFU/m ³)	A	47-6.0.×10 ²	3.6.×10 ² -1.4.×10 ⁴	4.3.×10 ³ -4.7.×10 ³	1.3.×10 ²	-		(69)
	B	0-25	0-79	0-7×10 ²	0	0-2×10 ²		(57)
	C	0-2.0.×10 ²	29-2.5.×10 ²	0-2.9.×10 ²	0-2.4.×10 ²	0-9.4.×10 ²		ud
	D	0-2.5.×10 ²	0-3.5.×10 ²	0-1.5.×10 ²	0-2.4.×10 ²	-		(63)

¹Plant A,C,D - grit chamber and Plant B - grate chamber ² Plant A,C,D - aeration tank and Plant B - inside the bioreactor ³WWTP with aeration sewage by horizontal rotors ⁴WWTP with aeration sewage by membrane plate diffusers in the reactor (BIO-PAK closed system) ⁵WWTP with fine-bubble diffused-air aeration system ⁶WWTP with activated sludge tanks aerated by CELPOX devices ⁷unpublished data
Microorganisms isolated on: Bulion-agar medium at 26°C/72 h (HPC), Chromocult medium at 37°C/24 h (*Enterobacteriaceae*), RBC medium at 26°C/3 to 7 days (Moulds and Yeasts)

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Table 2. Most frequently identified microorganisms in air samples (collected by the impact methods using MAS-100 *Eco Merck*) of WWTPs area and its surroundings sites

Microorganisms	Control site	WWTPs area		Surroundings		References
		Mechanical treatment ¹	Biological treatment ²	(100 m from the fence of WWTPs)	(200 m from the fence of WWTPs)	
<i>Enterobacteriaceae</i> bacteria (CFU/m ³)	<i>Citrobacter freundii</i> , <i>Enterobacter amnigenus</i> , <i>Klebsiella pneumoniae ozaenae</i> , <i>K. ozaenae</i> , <i>Pantoea spp.</i>	<i>Enterobacter aerogenes</i> , <i>E. asburiae</i> , <i>E. cloacae</i> , <i>E. sakazakii</i> , <i>Citrobacter farmeri</i> , <i>C. freundii</i> , <i>Escherichia coli</i> , <i>E. coli</i> 1, <i>Klebsiella oxytoca</i> , <i>K. ornithinolytica</i> , <i>K. pneumoniae</i> , <i>K. terrigena</i> , <i>Pantoea sp. 2</i> , <i>Pantoea sp. 3</i> , <i>Serratia fonticola</i> , <i>S. liquefaciens</i> , <i>Stenotrophomonas</i> , <i>S. malthophila</i>	<i>Citrobacter braakii</i> , <i>C. freundii</i> , <i>C. youngae</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>K. terrigena</i> , <i>Kluvera sp.</i> , <i>Morganella morgani</i> , <i>Pantoea sp.</i> , <i>Pantoea sp. 3</i> , <i>Serratia ficaria</i> , <i>S. liquefaciens</i> , <i>S. rubidaea</i> , <i>Shigella sp.</i> , <i>Yersinia enterocolitica</i>	<i>Klebsiella pneumoniae ozaenae</i> , <i>Pantoea sp.</i> , <i>Pantoea sp.3</i> ,	<i>Pantoea sp.3</i> , <i>Providencia rettgeri</i> , <i>Serratia rubidaea</i> , <i>S. plymuthica</i>	(35,57,60)
Moulds (CFU/m ³)	<i>Absidia</i> , <i>Actinomucor</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Botrytis</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Fusarium</i> , <i>Geotrichum</i> , <i>Gliocadium</i> , <i>Mucor</i> , <i>Oidium</i> , <i>Penicillium</i> , <i>Phialophora</i> , <i>Phoma</i> , <i>Scopulariopsis</i> , <i>Trichoderma</i> , <i>Trichothecium</i>	<i>Absidia</i> , <i>Actinomucor</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Chaetomium</i> , <i>Cladosporium</i> , <i>Cunninghamella</i> , <i>Curvularia</i> , <i>Fusarium</i> , <i>Geomyces</i> , <i>Geotrichum</i> , <i>Mucor</i> , <i>Nigrospora</i> , <i>Oidium</i> , <i>Penicillium</i> , <i>Phoma</i> , <i>Rhizopus</i> , <i>Scopulariopsis</i> , <i>Trichoderma</i> , <i>Trichothecium</i> , <i>Verticillium</i>	<i>Absidia</i> , <i>Actinomucor</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Blastomyces</i> , <i>Chaetomium</i> , <i>Chrysosporium</i> , <i>Cladosporium</i> , <i>Diplosporium</i> , <i>Geomyces</i> , <i>Geotrichum</i> , <i>Mucor</i> , <i>Oidium</i> , <i>Paecilomyces</i> , <i>Penicillium</i> , <i>Scopulariopsis</i> , <i>Trichoderma</i> , <i>Zygorhynchus</i>	<i>Absidia</i> , <i>Actinomucor</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Botrytis</i> , <i>Chaetomium</i> , <i>Chrysosporium</i> , <i>Cladosporium</i> , <i>Cunninghamella</i> , <i>Diplosporium</i> , <i>Fusarium</i> , <i>Geotrichum</i> , <i>Mucor</i> , <i>Oidium</i> , <i>Penicillium</i> , <i>Pullularia</i> , <i>Scopulariopsis</i> , <i>Trichothecium</i> , <i>Ulocladium</i>	<i>Absidia</i> , <i>Actinomucor</i> , <i>Alternaria</i> , <i>Aspergillus</i> , <i>Botrytis</i> , <i>Chaetomium</i> , <i>Chrysosporium</i> , <i>Cladosporium</i> , <i>Cunninghamella</i> , <i>Diplosporium</i> , <i>Geotrichum</i> , <i>Mucor</i> , <i>Penicillium</i> , <i>Scopulariopsis</i> , <i>Trichoderma</i> , <i>Trichothecium</i>	(57,63,64)
Yeasts (CFU/m ³)	<i>Candida spp.</i> , <i>Cryptococcus laurentii</i> , <i>Pichia spp.</i>	<i>Candida sp.</i> , <i>C. calliculosa</i> , <i>Cryptococcus sp.</i> , <i>C. laurentii</i> , <i>Rhodotorula sp.</i> , <i>R. mucilaginoso</i>	<i>Candida sp.</i> , <i>C. quilliermondi</i> , <i>Cryptococcus laurentii</i> , <i>C. albidus</i> , <i>Rhodotorula sp.</i> , <i>R. glutinis</i>	<i>Candida sp.</i> , <i>Cryptococcus sp.</i> , <i>C. humicolus</i> , <i>Rhodotorula sp.</i> , <i>Saccharomyces sp.</i> , <i>S. cerevisiae</i> , <i>Sporobolomyces salmonicolor</i>	<i>Cryptococcus sp.</i> , <i>C. humicolus</i> , <i>Rhodotorula sp.</i>	(57,63,64)

¹grit chamber/grate chamber ²aeration tank/inside the bioreactor