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Testing, validation and dissemination of an innovative biotechnology for air and water treatment.

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Esame finale anno 2017

To my family, always with me, even when long gone, supporting and motivating me every step I take, giving me the real sense behind the words "Difficulties are just things to overcome after all." (Ernest Shackleton)

To my little Ohana: my big two, three and four-legged loves.

To my dear friends, the family we choose for ourselves.

To people I walk with, I laugh with, I drink with.

The Faith of Men: "Life is not always a matter of holding good cards, but sometimes, playing a poor hand well.

— Jack London —

"For scientific discovery, give me Scott; for speed and efficiency of travel, give me Amundsen; but when you are in a hopeless situation, when you are seeing no way out, get down on your knees and pray for Shackleton"

— Raymond Priestley —

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Abstract

The present study is aimed to follow the start-up in the Italian and European framework of an American biotechnology for environmental decontamination and it focused on field testing stage for air treatment application, in parallel with a bench/pilot scale application on industrial wastewater treatment.
The biotechnology applied is based on immobilized cell bioreactors, where air is ventilated and water is recirculated to provide the optimal conditions for the development of a mixed bacteria consortium, growing on contaminants captured from contaminated media (i.e. air or wastewater).
The technology proposed has been studied from different perspectives, i.e. emission risk, overall sustainability and remediation performance.
Several pilot installations have been accomplished for air treatment application, in different areas of interest. In particular, in the healthcare sector (hemodialysis unit, operatory room, intensive care unit and anatomo-pathological laboratory), where the protection against microbial and chemical agents is perceived as a necessity, both for operators and patients, the biotechnology displayed remarkable results, particularly on VOCs and bacterial count.
In order to try and address one of the most challenging issue for air treatment, i.e. odor containment, two major pilot applications have been performed, on waste and wastewater treatment plant, with promising, but still unsteady results.
A new opportunity for application was envisaged in radioactivity contaminated indoor environments and a preliminary impact assessment has been outlined, based on results obtained in different fields.
For wastewater treatment, a single pilot scale plant was implemented and silk manufacturing effluent was object of the experimental remediation attempted. In a cost-effective perspective, the implementation of this system appears to be suitable for several solutions, i.e. within the framework of a multi-stage treatment process or as independent and easily implementable wastewater technology for cottage-scale manufacturing or small communities.

PART I: Introduction

Chapter 1 – Introduction

1.1. Objective of the study

The present study is aimed to follow the start-up in the Italian and European framework of an American biotechnology for environmental decontamination.

The technology was developed by Sam Sofer, PhD, PE, based on extensive research performed at the New Jersey Institute of Technology. Idea stage and basic research were accomplished in the US, together bench scale pilot tests and promising results obtained lead U-Earth Biotechnologies s.r.l. to bring bioreactors on the market.

The Ph.D. research work, therefore, focused on field testing stage for air treatment application, in parallel with a bench/pilot scale application on industrial wastewater treatment. The main goal was to accomplish the validation of the performance, starting from data obtained with the case studies implemented and trying to envisage application in new areas, where available technologies could be supported and improved (e.g. healthcare and industrial facilities), contamination issues are not addressed for the lack of proper solutions (e.g. fugitive gases treatment) or more sustainable solution for local scale application would be required (e.g. wastewater treatment for small communities or specific environments). Information derived from available publication about the early stages of development and first pilot applications were used to set an appropriate procedure for monitoring and validation of the performance, based on international standards and guidelines.

The formulation of a model for sizing and optimization of the biotechnology with adjustment for the different areas of interest has been studied, based on benchmark technologies and pilot applications.

1.2. Structure of the study

The research work performed will be presented in Part I, starting from problem identification, i.e. a short summary on air and water quality issues, followed by possible solutions and benchmark technologies survey, focusing, in particular, on environmental biotechnologies.

The technology under study will, then, be presented in Part II, in terms of working principle and technical solution, following literature available and primary data collected. Monitoring methods and protocols applied for the performance assessment will be discussed in detail, considering both international standards and new solutions implemented. The risk assessment activities carried out in cooperation with Fresenius Medical Care (FMC) and Synlab will be described in terms of emissivity evaluation, in presence of contaminated air and water. A screening Life Cycle Assessment, complemented with CO_2 equivalent calculation, has been performed to verify the overall sustainability of the technology proposed.

Part III will report pilot installation accomplished for air treatment in different areas of interest, i.e. healthcare facilities, where several installations has been feasible thanks to cooperation with FMC and industrial facilities, i.e. waste treatment and wastewater treatment plants. A new opportunity for application was envisaged in radioactivity contaminated indoor environments and a preliminary impact assessment has been outlined, based on results obtained in different fields. For wastewater treatment, a single pilot scale plant was implemented and silk manufacturing effluent was object of the experimental remediation attempted.

Concluding remarks will be presented in Part IV, focusing, in particular, on sustainability in technology implementation and research issue, limitations and obstacles found in performance assessment and modeling, and overall considerations regarding the consistency and relevance of results obtained.

1.3. Problem identification

1.3.1. Air quality issue

In the contemporary world, we have witnessed a growing concern about health, especially in developed countries, which is strongly related to both the evolution of health systems and the increased awareness of citizens' rights (Campos et al. 2010, Datta et al. 2005, Le Cloirec et al. 2005).

The scientific community progressively awakened on the effect of air pollution on human health, making it a research topic since the industrial revolution (APA 2010). Despite the progress achieved in this area, with technological improvement and updated regulations, air quality remains a critical aspect, given the repercussions that pollutants may have, regardless of their nature (physical, chemical or microbiological), on human health and ecosystems (EEA 2013, WHO 2014, Héroux et al. 2015).

Air pollution is, in fact, still a major concern of modern society, due to its long-term effects and it is considered a major cause of mortality and morbidity in the world, related to respiratory, cardiovascular and cancerous diseases. Together with adverse effect on human health, air pollution is recognized as primary cause of several negative impacts both on ecosystems and climate (EEA 2015, WHO 2013).

Air is the most fundamental element for the survival of the human being. Every day, about 10,000 liters of air enters the lungs, so the quality of the air breathed is a determining factor for human health (Vijayan 2015). According to the World Health Organization (WHO), air pollution poses a threat to the well-being and health of the population. A high number of fatalities are recorded every year, due to the exposure to air pollution; 3.7 million from exposure to outdoor pollutants and 4.3 million as a result of exposure to indoor air pollutants (WHO 2015).

On the other hand, indoor and outdoor air pollution also lead to considerable global economic impacts, related to the occurrence of premature deaths, rising health care costs, and reduced productivity (EEA 2015). Due to the social changes that have taken place over the last few years, particularly in urban areas, about 80 to 90% of time is spent indoors (e.g. housing, workplaces, transportation, cultural and social activities), thus justifying the increasing relevance that Indoor Air Quality (IAQ) has acquired in contemporary societies (WHO 2010, Fekadu et al. 2015).

Data gathered within SEARCH project (School Environment and Respiratory Health of Children, 2007-2010), coordinated by REC- Hungary (Regional Environmental Center for Central and Eastern Europe), demonstrated how typical outdoor airborne pollutants are actually found in equal or higher concentrations in indoor environments, aggravated by contaminants directly related to the specific environment, i.e. formaldehyde in case of schools, offices and healthcare facilities, Volatile Organic Compounds and solvents for industrial facilities etc.

Volatile Organic Compounds (VOC) are defined as

- “compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates and ammonium carbonate, which participates in atmospheric photochemical reactions, except those designated by EPA as having negligible photochemical reactivity” or “organic chemical compounds whose composition makes it possible for them to evaporate under normal indoor atmospheric conditions of temperature and pressure” (Code of Federal Regulations, EPA);
- “any organic compound having at 293,15 K a vapour pressure of 0,01 kPa or more, or having a corresponding volatility under the particular conditions of use” (Dir 1999/13/EC).

In outdoor environment, VOCs are mostly related to photochemical smog and, consequently, tropospheric and ground-level ozone formation. While coarse particulate is generally formed due to combustion processes, fine particles derive from several coagulation and nucleation phenomena occurring in the atmosphere due to gases interaction. Typical particulate precursors are SO_x (sulfur oxygenated compounds) and NO_x (nitrogen oxygenated compounds), but VOCs are also included in the class.

In indoor environment, where VOC may be not only dispersed by specific activities (painting, dry cleaning, disinfections etc.), but also released by surfaces (i.e. furniture, coatings, textiles etc.), VOCs may react with the indoor ozone (coming from office equipment, like printers, from air purifier, such as ionizers, or from the outside) even in low concentrations (i.e. below public health standards). As stated by EPA, these chemical reactions give rise to sub-micron sized particles and by-products "that may be associated with adverse health effects in some sensitive populations". In addition to this, it should be considered that several VOCs are listed among carcinogens compounds (e.g. formaldehyde, methylene chloride, acetaldehyde, perchloroethylene etc.) and pose a consequent thread on human health on their own.
Typically occurring in indoor environment, fine particles may pose serious threat to human health, since they are able to linger in the air for long period of time (Utrup et al. 2003) and, therefore can travel long distances (Zarra et al. 2008, Schiffman et al 2000). Moreover, their small size allows them to enter the respiratory tract, down to pulmonary alveoli.
Commonly regarded as a simply disturbing occurrence, odors may actually be listed among typical airborne contamination. ISO 5492:2008, prepared by Technical Committee ISO/TC 34, Food products, Subcommittee SC 12, Sensory analysis defines odour as "sensation perceived by means of the olfactory organ in sniffing certain volatile substances". The definition implies directly

- a physiological process, activated by receptors electrically linked to specific parts of human brains, responsible for signal decoding and substances identification;
- a psychological component, activated by personal experience associated to the specific odor.

In presence of persistent disturbing odours, the quality of life may be affected in terms of psychological stress and insomnia (Wilson et al 1980, Brennan 1993, Zarra 2008), even when physiological impacts are not directly triggered. According to World Health Organization (WHO, http://www.who.int/about/mission/en/) definition of "health", it is a "complete, physical, mental and social well-being and not merely the absence of disease or infirmity": in this sense, odours may pose a serious threat to community's health.
As reported by Zarra et al. (2008), different mechanisms are involved into odour impact, related to: high odorant concentrations, with consequent irritation of respiratory tract; synergic effect of different VOCs, which, combined, may exceed the protection value; odorant effect enhanced by airborne particulate, carrying odour on its surface.
The general air quality of an environment, especially when indoor, should, then, be carefully considered and assessed to protect human health, applying monitoring and, where necessary, sustainable treatment solutions.

1.3.2. Wastewater issue

Earth surface is covered by water for about 70%. This relative abundance of water resource is, nevertheless, counterbalanced by the scarcity of freshwater available for drinking purpose, due its chemical quality. In fact, about 97.5% of global water is saline (96.5% stored in oceans and about 1% between groundwater reservoir and lakes), while only 2.5% is freshwater. Among freshwater reservoirs, the most abundant is polar ice, counting for about 2/3 of the total, while groundwater store 30% and surface water bodies store about 1% of total freshwater available (Gleick 1996). Considering the distribution presented, combined with irregular allocation in the different geographical regions, protection of freshwater reservoirs, in terms of quantity and quality, should be a priority of our age.
Several indicators have been developed to assess threads posed to water resources, such as Water Footprint, Virtual Water Balance, Water Stress, mostly within the framework of international projects, such as Aqueduct, promoted by World Research Institute (WRI). In the following figure (Fig.1.1), current water stress ranking is proposed, in terms of ratio between water availability and usage. Projection have been developed

for the next decades on the same indicator, in order to support stakeholders and public authorities in adopting proper measure to fight or prevent water scarcity.

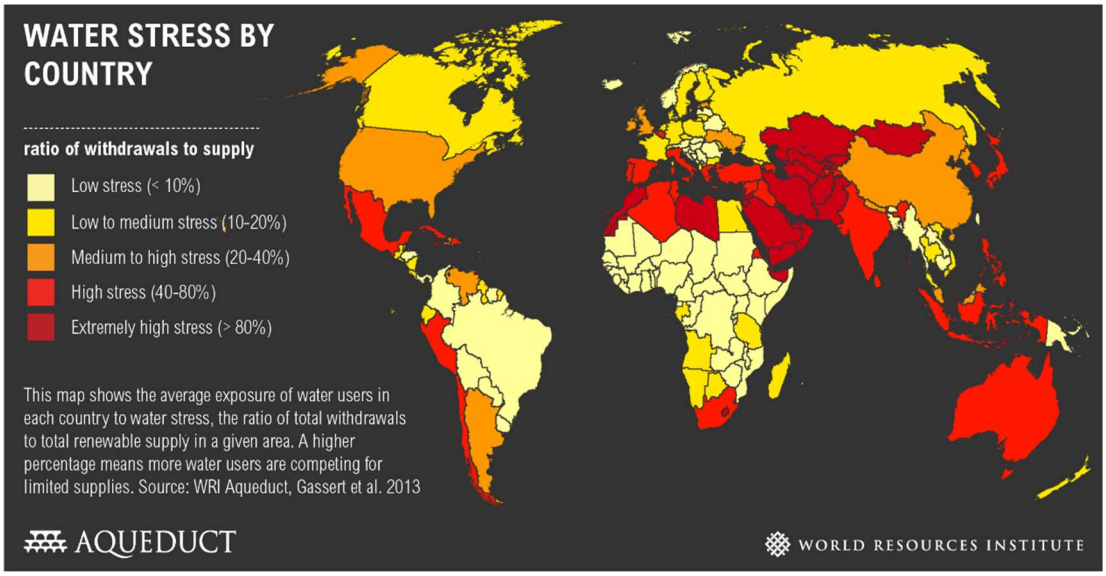


Fig. 1.1.: Water stress by country (Aqueduct, World Resource Institute).

For these reason, researches and technologies have been recently developed to promote efficient use of water, water saving and recovery.

Due to high availability of salt or brakish water, desalination solutions have been extensively studied and they are currently applied to obtain freshwater in arid and semi-arid regions (e.g. United Arab Emirates or California, US). About 40% of desalination products worldwide, i.e. distilled water, derives from thermal treatments, requiring either high energy content processes or large space availability (in case of solar pond production).

Urban green technologies offer a range of integrated solutions to fight water scarcity in metropolitan and residential areas, such as rainwater and stormwater harvesting for direct internal building application (e.g. sanitary water), which are widely regarded as promising techniques. Nevertheless, chemical and biological quality of water supplied ought to be monitored and adjusted accordingly to the intended use, e.g. to prevent microbial contamination or integrate possible missing minerals (Vasundevan et al. 2000, Vasquez et al. 2003, Kahinda et al. 2007, Sazakli et al. 2007).

For industrial areas, three most promising options are typically investigated to be ideally implemented:

1. Multiple use of water: in an industrial symbiosis perspective, a whole industrial area is involved in creating an Industrial Ecosystem, with symbiotic relationship between several activities, where the same water is fed to different production phases of different industries, based on the specific requirements (e.g. spring water could be used for washing vegetables in food industry, then fed to a manufacturing company for cleaning purpose and, finally, as thermal buffer for condenser or heating system). An actual example of this best practice sis currently implemented in Kalundborg (Denmark).
2. Water reuse: water is treated within the facility and re-fed to the same production cycle, to create an almost closed loop. This technique is currently applied, for example, in several quarrying sites in Emilia Romagna (Italy).
3. Water recycling: after the primary water use is accomplished and an effluent produced, wastewater treatment is performed to allow further utilization of water for a different activity (e.g. landscape watering, Tchobanoglous 2002).

Domestic wastewater contains a high load of organic matter which, untreated, while decomposing, may lead to malodorous gases and, therefore, nuisance to the surroundings. Moreover, pathogenic microorganisms, nutrients and toxic, mutagenic and carcinogenic compounds may be included or develop within wastewaters. Therefore, appropriate removal and conveying into sewage system is necessary, followed by properly designed treatment plants (Tchobanoglous 2002).
Industrial wastewater treatment represents a major environmental issue, both for human health and ecosystem protection, as testified by international regulations, setting restrictive limitations for wastewater discharge. Since industrial wastewaters are rarely segregated on the basis of their composition or contamination level, wastewater treatment plants typically face not only heavily, but even variously contaminated media (Zahn 1993). While domestic wastewater typically displays a quite homogeneous and constant composition, even if characterized by diurnal and seasonal variation patterns, industrial wastewater composition reflects process peculiarity both from the chemical and physical point of view (Droste 1997, Tchobanoglous 2002). Common contaminants generally found in industrial wastewater may include immiscible floating materials (e.g. oils), suspended solids, soluble hazardous and non-hazardous organic materials, soluble inorganic materials (e.g. ammonia, nitrates, etc.), volatile materials.
Unstable composition, frequently characterized by high organic and toxic load or extreme conditions (e.g. pH, temperature, salinity etc.), causes increased fragility of treatment performance and working conditions, followed, consequently, by rising management cost. In this sense, the implementation of wastewater treatment solutions within the same production site would be crucial to optimize both treatment and recovery opportunities.

1.4. Environmental biotechnologies

Biotechnologies are defined by UN Convention on Biological Diversity (UN 1992) as “technological application that use biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.”.
Environmental biotechnology, in particular, in accordance with International Society for Environmental Biotechnology, "the development, use and regulation of biological systems for remediation of contaminated environments (land, air, water), and for environment-friendly processes (green manufacturing technologies and sustainable development)" (Zylstraa et al. 2005).
Therefore, their applications range from remediation of contaminated land and groundwater, air and wastewater treatment, agriculture etc., supported by a sustainable approach, considering both environmental and economic aspects of the process.
For the aim of the present study, a focus on applications for air and wastewater treatment is presented.

1.4.1. Air treatment applications

As stated by several authors, biotechnologies currently represent a sustainable alternative to conventional air pollution control techniques, both for their environmental impact and remarkable cost-effectiveness (Mudliar et al. 2010, Shareefdeen et al. 2005). Different technological solutions have been designed and implemented during the last decades, based on different characteristic of pollutants to be treated, in terms of chemical nature, load of contamination and quality requirements to be met by the final effluent gas, i.e. different removal efficiency necessary.
The ability of microorganisms to degrade chemical compounds depends on their metabolic activity and the possibility to find into chemical pollutants either Carbon or energy source. The composition and survival of the microbial community depends on nutrients available, as well as process contour conditions (Singh et al. 2005), such as humidity, pH, oxygen and temperature (Fig. 1.2.).

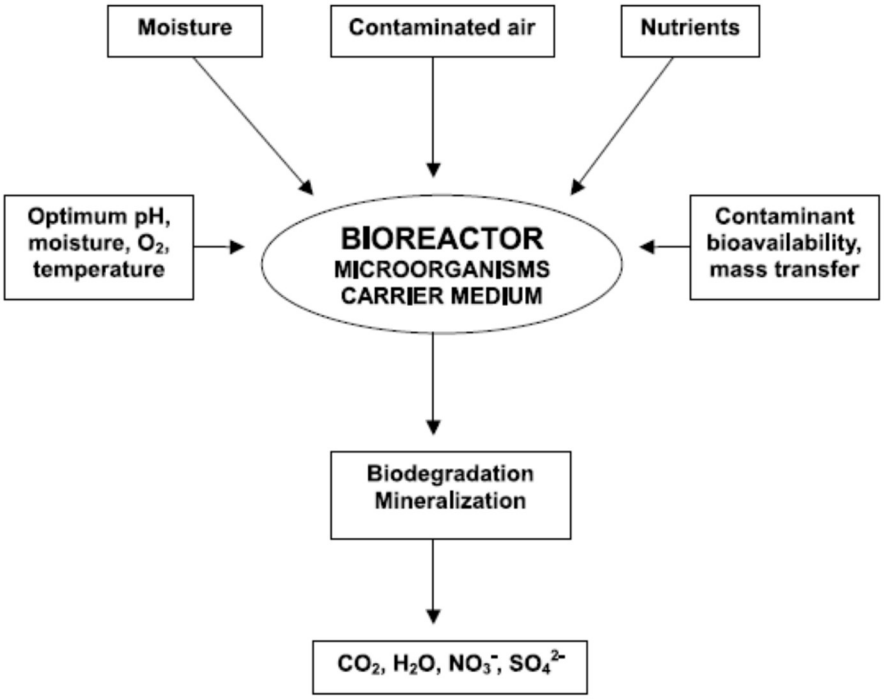


Fig. 1.2.: Biodegradation process in waste gas treatment reactors (Sing et al. 2005)

Mixed cultures of bacteria, actinomycetes, fungi, algae and protozoa are typically regarded as able to address waste gas treatment issues. The degrading activity is constituted by the breakdown of chemical bonds within the compounds, with bio-transformation into less complex metabolites, until complete mineralization into inorganic compounds (e.g. H_sO, CO_2, CH_4 etc.) (Singh et al. 2005).

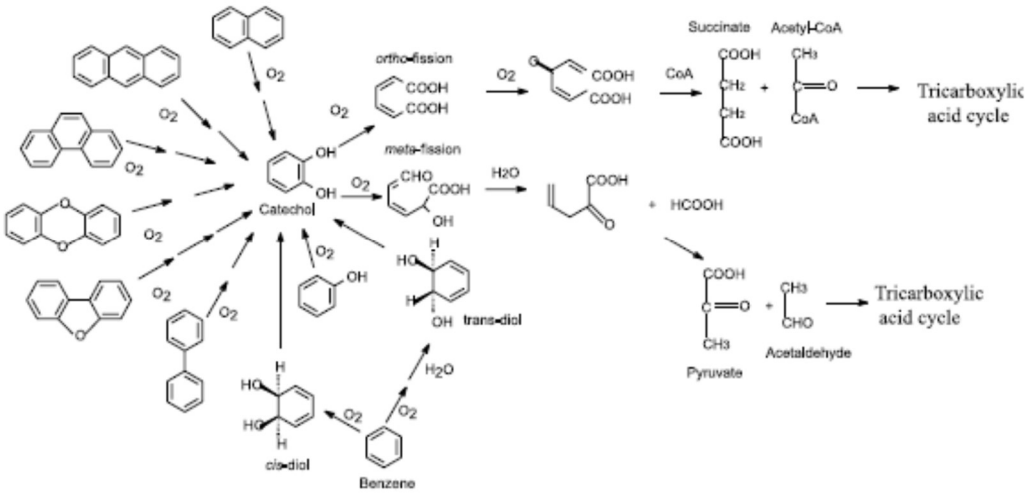


Fig. 1.3.: Incorporation of oxygen into the aromatic ring by the dioxygenase enzyme, followed by meta or ortho cleavage (Le Cloirec et al., 2005).

A variety of different chain-reactions are required to achieve the degradation of pollutants, most of the time represented by quite complex compounds, as shown in the example in Figure 1.3.

Standard air phase biological reactors include biofilters, biotrickling filters and bioscrubbers, albeit recently developed bioreactors involve membrane technology (Shareefdeen et al. 2005, Kumar et al. 2008a, 2008b, 2009, Mudliar et al. 2010).

In biofilters, waste gas flows through a bed of porous medium where a microbial population grows on contaminants adsorbed on the medium surface. This configuration has been used since late '50s (Pomeroy 1957) to treat gaseous effluents and it is characterized by the presence of mixed microbial consortia, typically organized in biofilm configuration, which leads microbial cells to be exposed to different environmental conditions (i.e. temperature, pH, oxygen, carbon dioxide, air contaminants, and metabolites from other organisms as well as cell components and endotoxins from dying and lysing organisms), related to different positions in the depth of the biofilm (Singh et al. 2005). Biofilters may be either open system (Fig. 1.4.) or closed systems (Fig. 1.5.).

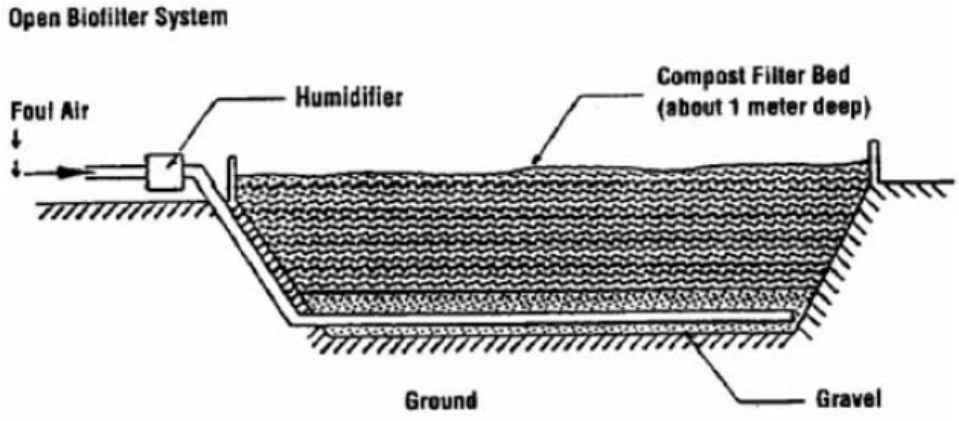


Fig. 1.4.: Open biofilter system (Williams et al. 1992).

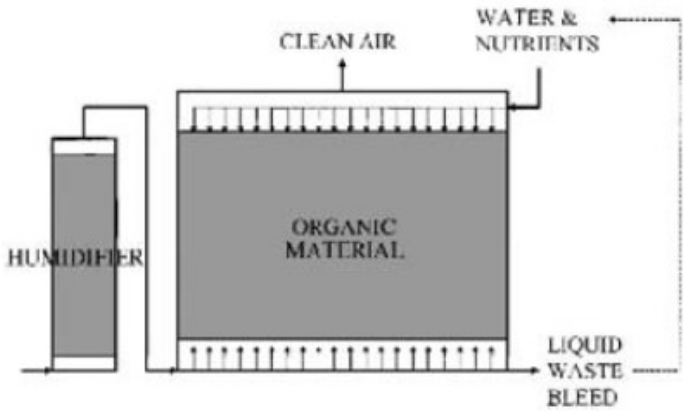


Fig. 1.5.: Closed biofilter system (McNevin et al. 2000).

Characteristic of the packing media, pressure drop within it and medium depth, moisture content, temperature, pH, oxygen and nutrients availability are among the most relevant parameters affecting biofilters efficiency.

Where biofilters are typically packed with organic materials, biotrickling filters are almost exclusively packed with specifically manufactured media, of inorganic nature, and a liquid phase is allowed to trickle down the bioreactor (Fig.1.6.). The trickling liquid act as a buffer to control several reaction parameters, such as pH, salt, metabolite concentration, and to add nutrients to the microbial population performing the degrading activity over the waste gas (Deshusses et al. 2005, Oh et al. 1997, Mpanias et al. 1998; Cox et al. 2000; Gabriel et al. 2003).

Accurate maintenance of packing material, as well as careful trickling liquid phase control are key factor for the effectiveness of the technology.

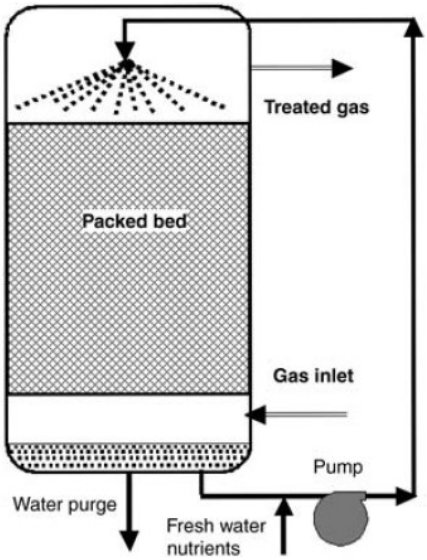


Fig. 1.6.: Biotrickling filter (Revah et al. 2005)

Bioscrubbers, or "suspended-growth bioscrubber" (Ockeloen et al. 1996, Koe and Yang 2000, Singh et al. 2005), are three-phase, fluidized-bed bioreactor systems, where the pollutant compounds (in particular VOCs) to be treated are physically separated/absorbed in liquid phase and, then, undergo a second stage of biological treatment in a liquid-phase bioreactor (Fig. 1.7.). The first stage (gas/liquid contact) is normally performed through a packed-bed column, working in counter-current mode, while the liquid-phase bioreactor is a simple aerated tank.

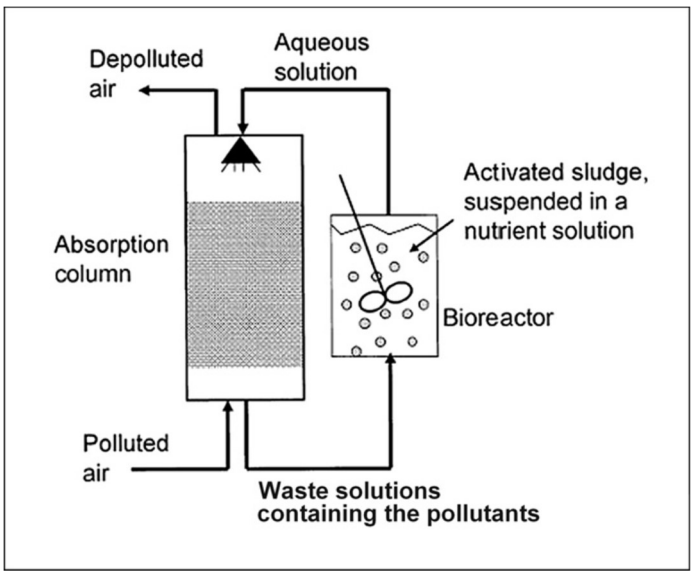


Fig. 1.7.: Bioscrubber (Mudliar et al. 2010).

Key parameters for process optimization are oxygen concentration, nutrients, temperature and pH (Singh et al. 2005). Typical bioscrubbers implement activated sludge, derived from wastewater treatment plants, as starting inoculum (Ottengraf, 1987). Where necessary, i.e. in presence of specific contaminants and

controlled gas flow conditions, the bioreactor may be inoculated directly with dedicated microbial strains, with a known metabolic activity and, therefore, more predictable performance.

1.4.2. Wastewater treatment applications

Biotechnological solutions for wastewater treatment are generally widely applied, more than physical, chemical or mixed techniques. They, in fact, take advantages of naturally occurring biocatalytic activity and are able work in ambient temperature and pressure (sensibly lowering the treatment costs) over even large volumes of effluents. Domestic wastewater is efficiently treated, as well as several industrial wastewaters, when bio-available contaminants are present (e.g. phenol, mono- and di-chlorophenols, benzene, nitro-benzene, toluene, ethylbenzene, naphthalene).

Typical wastewater treatment plants are designed on a three stage-process (Fig. 1.8.), with:

1. Pre-treatment for removal of suspended and floating materials (sedimentation, oil separation and floatation) and pH adjustment (equalisation and neutralisation) as primary treatment;
2. Secondary treatment, commonly performed in activated-sludge bioreactors, where microorganism able to grow on organic compounds (i.e. using them as Carbon source, electrons acceptors or energy source) degrade chemicals;
3. Final removal of residual contaminants (chemical or biological) by sand filtration, reverse osmosis, adsorption, phytoremediation, electrodialysis and/or disinfection. This tertiary treatment phase is applied only where specific effluent quality is required (e.g. for water reuse or discharge in sensible water bodies).
4. A complementary treatment for solid residual, i.e. settled material, dried sludge etc., is necessary before the final disposal in landfill (e.g. incineration or anaerobic digestion).

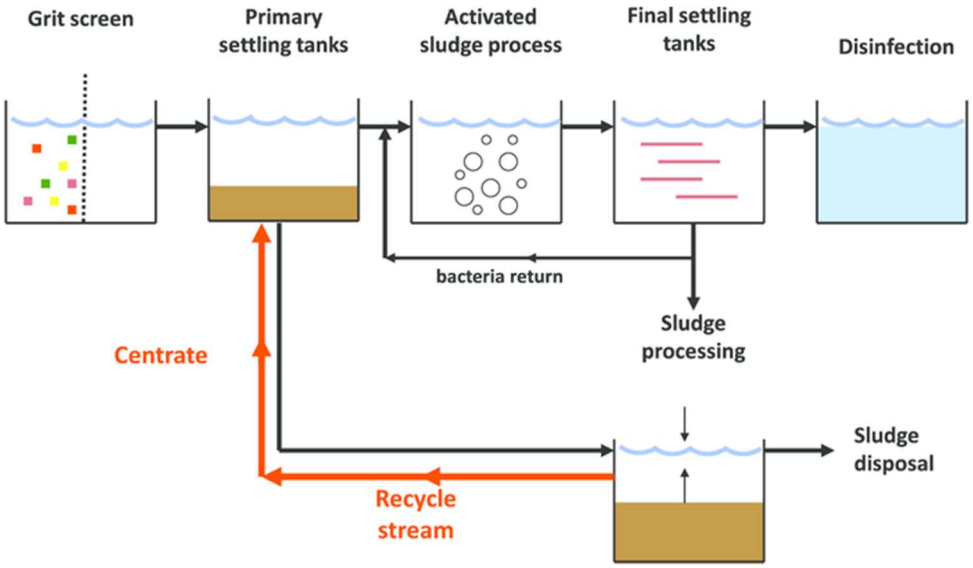


Fig. 1.8.: Typical wastewater treatment schematic in municipal wastewater treatment plant (Zhou et al. 2011).

Several bacterial population are generally involved in secondary treatment stage, together with protozoa in aerobic reactors, forming aggregates of flocs, tending to sediment at the bottom of the tank.

Most of the available biological treatment are aerobic, due to the large bacterial consortia involved, operating in parallel on broader range of contaminants (such as organic compounds, nitrogen and phosphorous), offering, therefore, a highly versatile treatment. In addition to this, aerobic bacteria display a very esoergonic metabolism, with high growth rate, resulting in a stable process, shorter residence time

required and faster treatment (or smaller reactors needed). Typical drawbacks of aerobic treatment are: energy requirement to ensure proper aeration, consequent stripping of volatile, possibly hazardous, compounds, heavy sludge production, impossibility to degrade some contaminants (e.g. nitrate and sulfate, non-ionic surfactants, high molecular weight hydrocarbons and polyhalogenated chemicals).

Anaerobic treatments are typically applied to degrade or bio-transform compounds not managed by aerobic ones and to overcome issues presented. Nevertheless, in a complementary perspective, anaerobic processes display slower and lower degradation ability over soluble organic compounds due to low metabolism and growth (this implies, evidently, longer residence times and lower stability of the process) and high sensitivity to environmental conditions and toxic compounds. Moreover, anaerobic bacteria consortia work in series, with a limited number of compounds entering the population's metabolism, depending on bacteria responsible of the first step of biotransformation.

In order to address complex wastewater compositions, several composite configurations are currently implemented at industrial scale, such as: aerobic, continuous, well mixed dispersed biomass; aerobic, continuous, plug flow + immobilized biomass; anaerobic, continuous, dispersed biomass; anaerobic, continuous, partially dispersed biomass; anaerobic, continuous, plug flow + immobilized biomass; integrated anaerobic-aerobic , continuous, dispersed biomass; integrated anaerobic-aerobic , continuous, immobilized and dispersed biomass.

Standard wastewater treatment systems are, anyway, generally not able to perform a quick nor complete biodegradation of several xenobiotic compounds, defined as "recalcitrant", which tend to leave the plant either with final effluent (e.g. dichlomethane, dichloroethane, trichlorophenols) or through volatilization (e.g. chlorobenzene, chloroform, tetrachloroethane, trichloromethylene, vinylchloride) or sorption to solids, like particles or sludge (e.g. hydrophobic compounds, such as anthracene, fluorene, pyrene, chlorobenzene, hexachlorobenzene, Chlordane and Endrin). Specific solutions have been developed to address such recalcitrant contaminants, such as Granular Activated Carbon (GAC), Powered Activated-Carbon Treatment process (PACT process), expanded bed granular activated-carbon (GAC) anaerobic reactor, Membrane Extractive Reactors, photodegradation with TiO2 and UV. (Rittman et al. 2001).

PART II: The technology

Chapter 2 – Presentation of the technology

2.1. Introduction

The technology applied for the present study has been developed following two decades of researches by Sam Sofer, PhD, PE in the industrial biotechnology field and it is based on immobilized cell bioreactors.
Immobilized activated sludge was originally evaluated by New Jersey Institute of Technology (Rus, 1992) as a potential biosorbent for the removal of heavy metals from liquid waste streams. The immobilization method consists of microorganism entrapment in calcium alginate beads. A bench-scale system was developed and tested to optimize the biosorption process, gaining promising results for heavy metals, such as lead and chromium. The very same bacteria consortium, mixed with selected, but non-engineered strains, fungi and enzymes, has been included into a biomass preparation already on the market (U-ox®), which is expected to act as immobilizing and treating agent for airborne contaminants.
Since early '80s, (Shamat and Meier 1980, Tabak et al. 1981) activated sludge have been applied for biodegradation of organic compounds and, therefore, as biomass source for wastewater treatment.
Traditional activated sludge treatment stage normally includes free microorganisms in an aeration tank which promotes conversion of organic load in wastewater into biomass and carbon dioxide (Bonoli 2014a, 2014b). But where, typically, free cell microorganisms are used in the activated sludge process, immobilized microorganisms may instead be used, as suggested by several studies (Woods 1995, Lakhwala 1988, Lakhwala 1992), enhancing wastewater treatment potentiality. Immobilized biomass presents some remarkable advantage, compared with the free cell, such as (Mattiasson 1983, Westmeier and Rehms 1985 and 1987, Dwyer et al 1986, Lewandowski 1988, Chien and Sofer 1985, Sofer et al. 1990):

1. washout phenomena are avoided in continuous flow configuration,
2. the biomass could be recovered and reused if necessary,
3. the bioreactor presents great operational flexibility,
4. the cell configuration improves mass transfer and, therefore, microbial activity (i.e. pollutants removal through metabolic ways) with a self-regulated microbial growth (Lakhwala 1988, Lakhwala 1992),
5. cell density is higher, providing a higher biodegradation per unit volume of the reactor,
6. immobilized cell microorganism display higher resistance to high concentration of toxic compound (Sofer et al. 1990, in fact, confirmed what reported by Rehm, Crawford et al., i.e. that, when immobilized configuration is provided, microbial activity is not inhibited even at concentrations of chemicals typically fatal for the same population)

As proved by many authors, in fact, biofilms support a large biomass and, therefore, high microbial activities, but some degrading processes, such as mineralization, are promoted by high localized-solute concentrations, pH conditions and redox potential related to limited mixing in the vicinity of the bacterial cell (Lawrence 1998).
Immobilized cell on inert support involves active and passive attachment mechanism (Lakhwala 1988, Lawrence 1998, Loosdrecht 1987). As a first step, the passive attachment forms a mono-layer of bacterial film, thanks to weak Van der Waals forces between the bioreactor's surface and the bacteria. On the initial layer, the active attachment performed through exo-polysaccharides secreted by the same bacteria contributes to multi-layer biomass development (Wollersheim 1989).

2.2. The bioreactor

The bioreactors tested and currently applied on air treatment (patented and commercially known as AIRcel) are classified as "Immobilized cell bioreactors" and work with a combination of convection and biological digestion of materials captured, as leading mechanism.

The bioreactors, in analogy to bioscrubber technology, both fixed-film and suspended-growth (Singh et al. 2005, McNevin 2000), consist of three phases in close contact (Fig. 2.1.):

1. a solid phase, which is the bioreactor itself,
2. a liquid phase, i.e. water,
3. a gas phase, that is air (in case of air treatment application, it corresponds with the polluted medium to be treated).

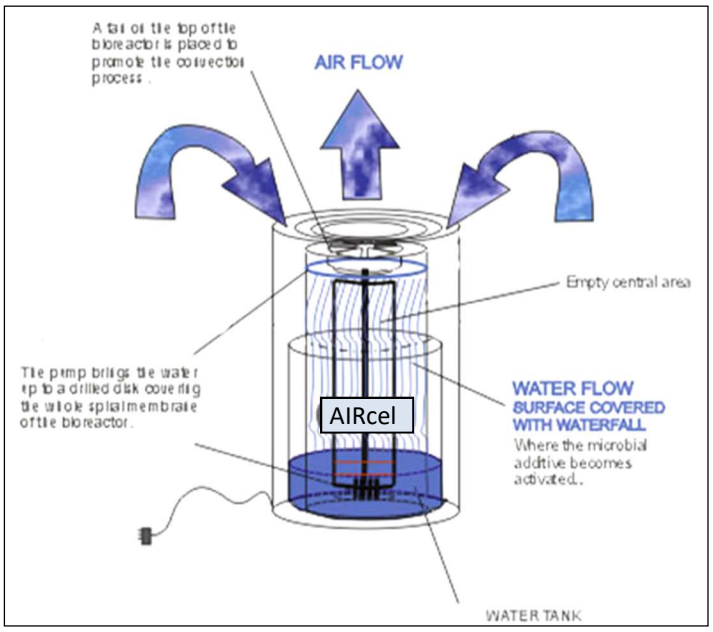


Fig. 2.1.: Simplified outline of an AIRcel bioreactor (U-Earth Biotechnologies s.r.l.).

As in common biofilters, the physical support for biomass growing is offered by a solid medium, but, in this case, a plastic patented bioreactor is provided with optimized configuration (Bonoli 2014a, 2014b). Biological oxidation is, in fact, enhanced by the design of the internal concentric vertical cylinders in plastic sheets, providing a lower pressure drop and resulting in less channelling than packed-bed bioreactors.

A water reservoir is maintained inside the bioreactor and refilled on a regular basis. The water is continuously pumped from the bottom of the bioreactor to the top plate, where a number of holes allow it to trickle down along the reaction surface, i.e. the cylindrical plastic sheets which are immersed in the water tank only for a half (Fig. 2.1. and 2.2.). This is analogy with biotrickling filter technology presented in Chapter 1.

Fig. 2.2.: Exploded graphic of an AIRcel bioreactor (U-Earth Biotechnologies s.r.l.).

This peculiar configuration, with large empty space inside the bioreactor, opposite to the classical support material filling, allows to overcome some typical drawbacks of the immobilized cell bioreactors, i.e. physical breakage of beads, channeling etc. In the first stages of development, Sofer et al. (1990) developed a recirculation bioreactor (Fig. 2.3), with longer bead-life and duration run due to aeration performed into the reservoir, instead of into the reactor itself.
In this case, aeration is provided by the fan at the top of the bioreactor in combination with water trickling along the cylindrical surfaces, forming a thin film that support its oxygenation.

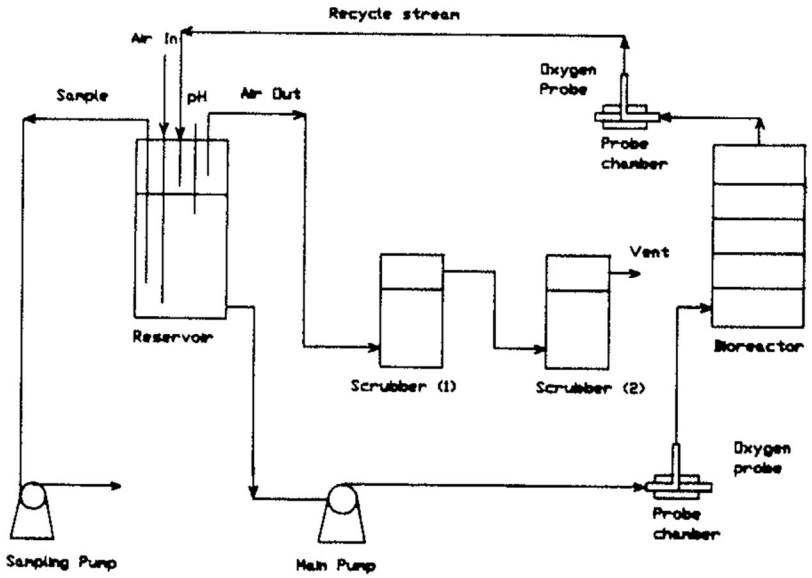


Fig. 2.3.: Recirculation reactor (Sofer et al. 1990).

Apparently small reaction surface and relatively high empty volume inside the bioreactor answer to a design principle based on potential energy provided by, on one hand, dissolved oxygen and contaminants gradient and, on the other hand, by bacterial activity in biofilm configuration.
The leading mechanism of the bioreactor, i.e. the driving force of microbial growth is the biological digestion of the pollutant materials attracted, which represents the only substrate provided.
While initially applied to water effluents treatment (Rus 1992, Borkowsky 1995), the main application indagated with the present study was on air treatment.
When applied to air contamination treatment, in fact, these miniaturized plants proved to be able to capture and degrade various pollutants, such as gases, volatile organic compounds (VOC) and odors by bio-oxidation, and remove particulates and heavy metals.
Air pollutants are first captured by the bioreactor and subsequently bio-digested, applying a principle defined by Sofer (2006) as "bio-hygienics".
As reported in Table 2.1, the airflow supplied by the fan is quite limited, but the number of total volume exchange within the bioreactor is relatively high.

	BIOREACTOR (AIRCEL85)
REACTION SURFACE (M^2)	0.55
AIR FLOW (M^3/H)	37
AVERAGE AIR VOLUME (L)	37
N. OF INTERNAL AIR VOLUME EXCHANGE/H	1000
WATER FLOW (M^3/H)	0.66
AVERAGE WATER VOLUME (L)	32
N. OF TOTAL WATER VOLUME RECIRCULATION /H	18

Tab. 2.1.: Bioreactor's operational specs

Sofer (2006) suggests that electrical field and gradient generated by microbial metabolic activity may trigger and enhance a diffusion effect, attracting contamination to the bioreactor (Fig. 2.4), this without additional ionization, nor electrostatic charging of particles, but through the exploitation of their natural surface charge. The grounding effect provided by the continuous water flow support the attraction effect on charged particles (Lakhwala 1991, Shim 1995, Sofer 2006).

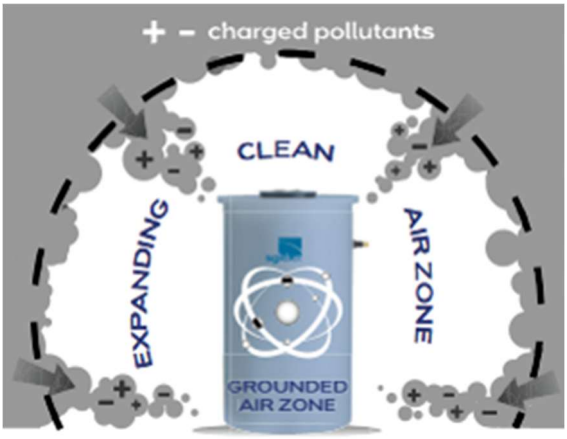


Fig. 2.4.: Clean air zone generated by bioreactor's activity (source U-Earth Biotechnologies s.r.l.).

Considering the scrubbing effect provided on airflow by water film trickling down the cylindrical surfaces, it may be assumed that a local contaminants concentration gradient is created at the interface between air and water flow. This promoting molecular diffusion among the different media involved, i.e. from air to water and from water to biofilm. In particular, since air is generally contaminated by relatively low concentrations of chemicals and particles (e.g. part per million, ppm, to part per billion, ppb), the system operated with dilute solution, allowing to hypothesize a diffusion chain, i.e. gas-liquid interface diffusion (air-water) followed by a thin film diffusion (air-water-biofilm), under Fick's law conditions (dilute solution, one-dimensional predominant diffusion and negligible convection in the same direction).
As in common diffusive media, the contaminants in the gas phase diffuse toward the support for immobilized active microbial culture (U-ox). Being the medium poorly porous and covered with a thin film of water, no air flow through it is allowed. The diffusive flux of the contaminant is, therefore, perpendicular to the direction of gas flow, and, as stated by Govind et al. (2005), its value depends on the gradient of concentration between the bulk and the interface (in this case, the two interfaces). Typically, the transport rate depends on air flow velocity, but here a more complex system is built up on air and water flow.
Diffusion coefficients of gases in water are typically in the range of 10^{-5} cm^2/s at 25°C (Cussler 1997), therefore long contact times are required to promote the diffusion to the biofilm. For this reason, a high number of air volume exchanges inside the bioreactor, together with water recirculation are required.
Shim's work (1995), focusing on a spirally wound polymeric membrane immobilized bioreactor to degrade a model VOC (ethanol) in air with a mixed bacterial culture, determined a direct relationship between air flow and reaction rate, increasing in parallel thanks to enhanced mass transfer, until the sloughing of the biomass from the polymeric support, or as a result of substrate inhibition (Borkowski 1995). Currently applied bioreactors air flow is tuned accordingly.

2.3. The biomass

Many authors stated the effectiveness of specific bacteria strains in degrading different contaminants (McNevin 2000), such as Chemoheterotrophic bacteria to promote Organic Carbon oxidation (from VOC to CO2 and H2O), Nitrifying bacteria for nitrification (from NH4+ to nitrite and nitrate), Sulfur oxidizing bacteria to achieve Sulfide oxidation (from H2S to S0 and sulfate) (all in aerobic environment) and Denitrifying bacteria, to promote Denitrification (from nitrate to gaseous nitrogen) in anaerobic conditions.
Since the biomass representing the core of the AIRcel technology is a proprietary formulation (u-ox), in which the claim is that no genetically manipulated microorganism, no specific information is available for the public, but, based on the evidences gathered, it appears to be a quite composite bacteria and enzymes consortium. The biomass proposed is, in fact, able to attack and digest compounds different in nature, degradation process, contour conditions requirements and inhibitors, final products and reaction by-products.
The biomass is provided in water suspension and the immobilization on the inner reaction surface is achieved progressively by water recirculation.
Thanks to cooperation with Fresenius Medical Care and Synlab, biomass concentration in AIRcel process water has been tested at different timing for a qualitative assessment of adhesion attitude during the first days after the setting-up. A similar experiment, with bioreactor applied to wastewater treatment has been performed and is presented into Chapter 7 of the present document.
The AIRcel bioreactor was set-up with a single dose of U-ox (i.e. 100 ml), dispersed into 40 litres of tap water. In addition to this, an opportunistic human pathogen, i.e. Pseudomonas aeruginosa, typically found in hospital water pipelines, was spiked in quite high concentration (2000 CFU/100 ml), during the setting-up and verified by cetrimide-agar plate count, in order to verify relative abundance of the two population and to assess ecological dynamics within the bioreactor. The test was conducted at standard room temperature of 20°C. A general bacterial count was performed on CASO-agar plate, which was defined as representative

of U-ox bacteria consortium. As prescribed by manufacturer, a second U-ox mono-dose was supplied to the system after about 15 days from the start-up.
AIRcel was, then, switched on and allowed to work into a test chamber with filtered inlet air (see Chapter 4). As inferable from results presented into Table 2.2., the biomass replicates inside the process water starting from the very first hours of application. It is presumable that, meanwhile, biofilm is forming along the vertical surfaces of the bioreactor.

AIRCEL WATER SAMPLING (H)	CONCENTRATION OF BACTERIA ON CASO-AGAR [CFU/ML]	CONCENTRATION OF PSEUDOMONAS AERUGINOSA ON CETRIMID-AGAR [CFU/100ML]	RATIO: OVERALL BACTERIA / PSEUDOMONAS AERUGINOSA
0	1.850	1.730	107
2	2.400	1.700	141
4	2.250	1.220	184
6	2.650	1.270	209
8	3.000	1.240	242
10	1.400	1.420	99
12	2.300	1.590	145
24	14.100	2.640	534
26	22.500	2.400	938
28	39.500	2.800	1411
30	51.600	6.640	777
32	63.150	7.920	797
34	96.800	11.040	877
36	90.000	18.560	485
51,5	172.000	76.600	225
57	370.000	> 100.000	< 370
75,5	340.000	> 100.000	< 340
81	5.170.000	440.000	1175
224	3.500.000	375.000	933
248	950.000	45.000	2111
316	415.000	34.000	1220
340	445.000	17.500	2542
364	605.000	6.500	9308
504	70.000	70	100.000
528	320.000	>10	> 3.200.000

Tab. 2.2.: Concentration of bacteria in suspension into AIRcel process water.

The concentration peak detected on the third day from the start-up was detected both for the general bacterial (Fig. 2.5), regarded as representative of U-ox biomass, and Pseudomonas aeruginosa (Fig. 2.6), i.e. the biological contaminant added to the solution. Considering the direct sampling performed, i.e. without homogenization of the water medium to avoid system's perturbation, this may lead to conclude that a detachment of biofilm occurs periodically, raising the concentration of dispersed biomass. Provided, then, that bacterial concentration detected during the following days showed a significant decrease, it is assumable that a dynamic equilibrium is established inside the bioreactor, with constant detachment of old biofilm and new biofilm formation ensured by continuous water flowing along the vertical surfaces (Fig. 2.7). Moreover, as reported by Borkowski (1995), biomass possibly stripped from the reactor support surface may enter the

process water (i.e. the reaction solution), continuing the contaminants degradation (i.e. substrate) as in free cell bioreactor.

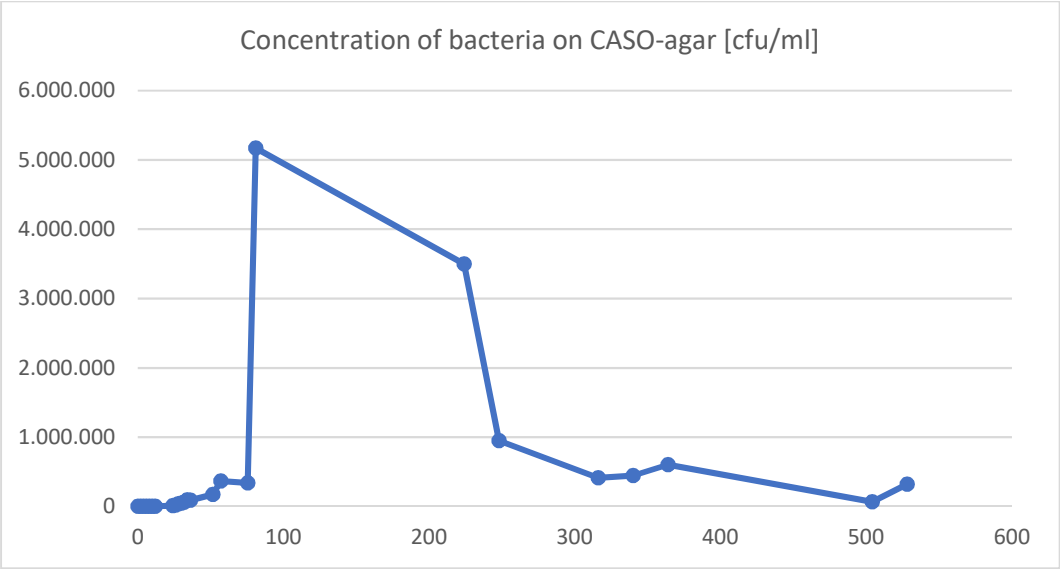


Fig. 2.5.: Concentration of bacteria developing on CASO-agar, i.e. general bacteria (representative of U-ox).

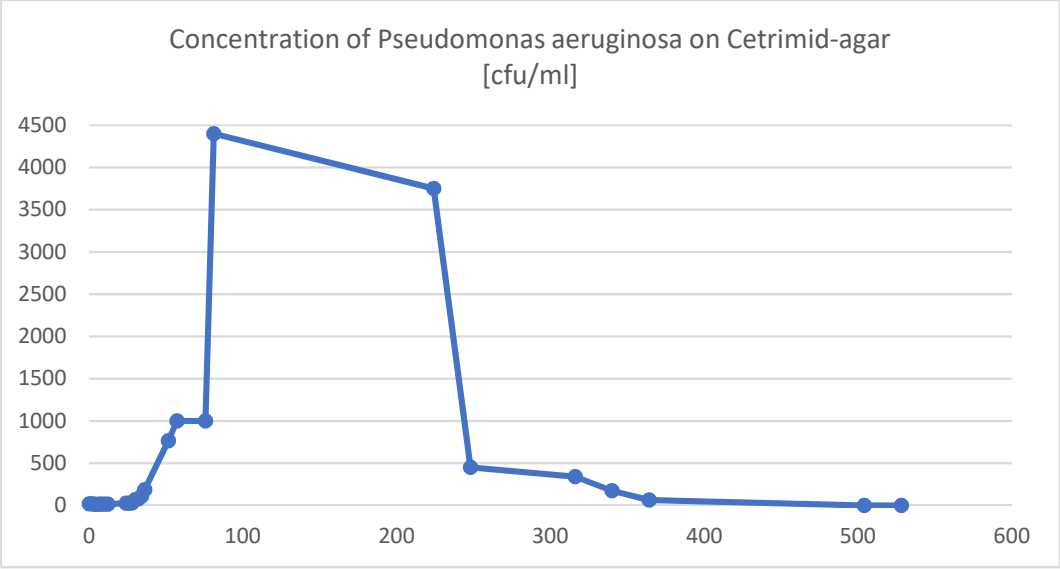


Fig. 2.6.: Concentration of bacteria developing on Cetrimid-agar, i.e. Pseudomonas aeruginosa.

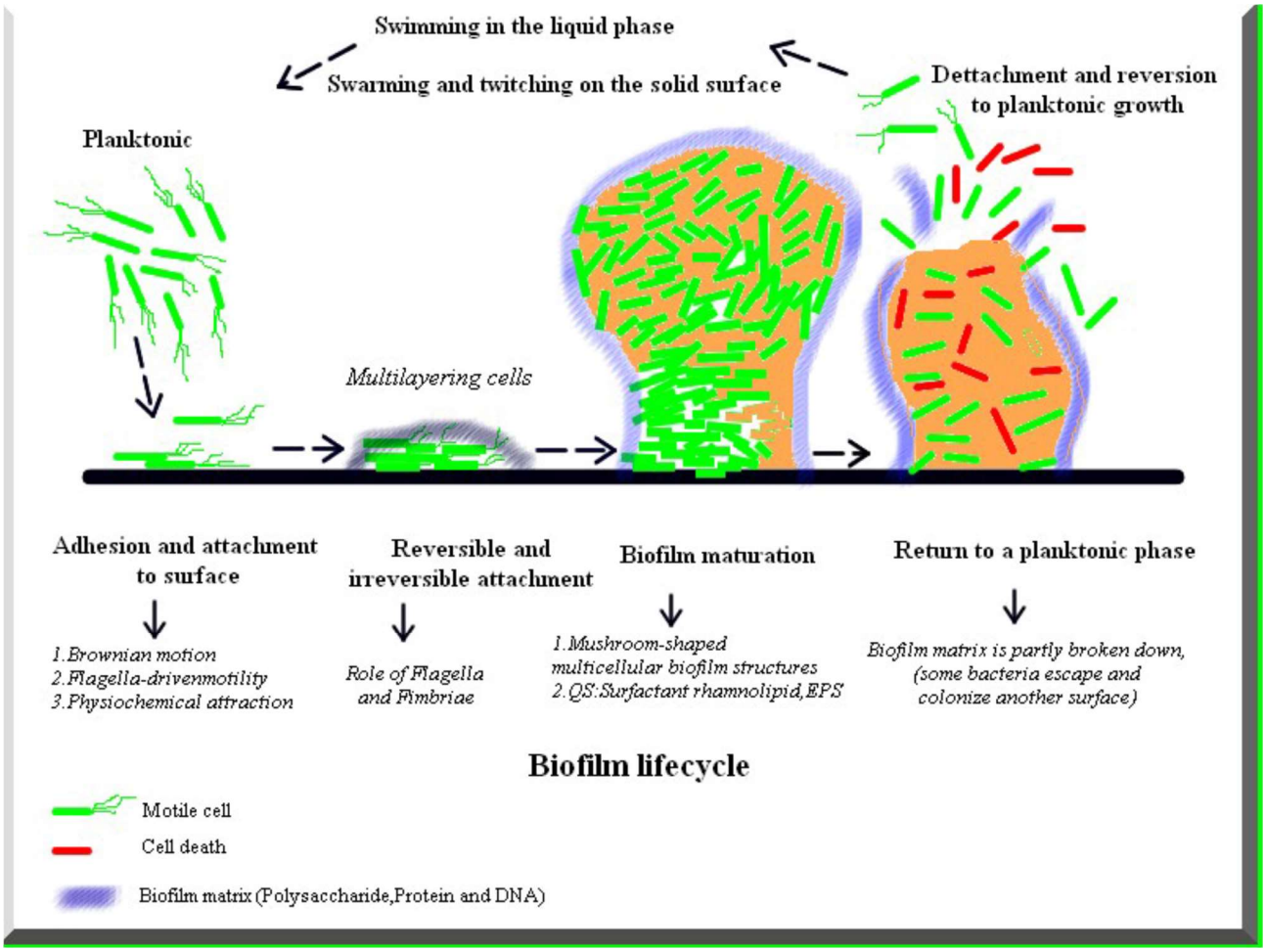


Fig. 2.7.: Biofilm life cycle, with initial attachment, where bacteria adhere via Brownian motion, flagella-driven motility and physiochemical attraction (van der Waals interactions), reversible and irreversible attachment, where Flagella and Fimbriae permanently anchor the bacteria to the surface, biofilm maturation through cell division and an extracellular matrix, and, finally, dispersal stage, where the biofilm matrix is partly broken down and returns to planktonic phase (Meliani et al. 2015).

Considering the ratio between the general bacteria count and biological contaminant, it can be observed that the general bacterial population, regarded as representative of the U-ox biomass supplied to the system and, therefore, autochthonous for the system, developed in a ratio ranging from 10^2 to 10^3, compared with the contaminant population until day 15. With the second U-ox mono-dose addition, the ratio between the two populations raised rapidly to values ranging from 10^5 to 10^6 (Fig. 2.8). This allows to conclude that possible opportunistic bacteria contaminations into the bioreactor and biofilm can actually be controlled by the periodical biomass addition and consequent augmentation of the degrading strains.

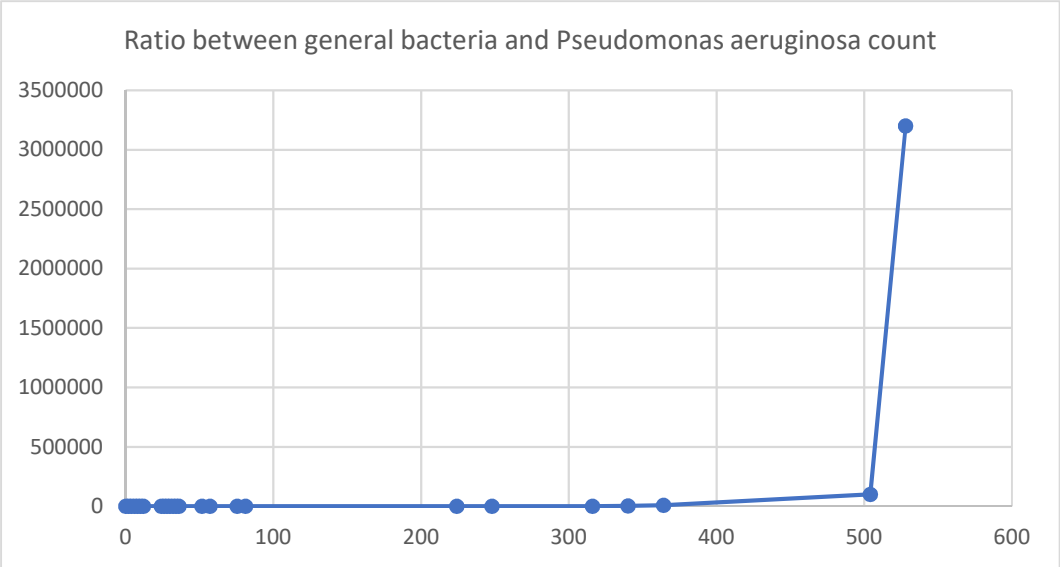


Fig. 2.8.: Ratio between general bacteria count and Pseudomonas aeruginosa.

In several applications, it has been observed that process water typically tends to take the colour of possible airborne contaminants (e.g. purple shade in presence of purple ink for pathological analysis), but it has been reported by Borkowski (1995) that yellowish shade in process water, in absence of specific contaminants (e.g. limonene, i.e. cyclic terpene), may indicates the release of extracellular enzymes carried out by bacteria during the degradation process.

2.3. The system

Based on evidence gathered during the experimental phase performed, a single unit is generally able to deal with low level of contaminations, while the combination of a number of bioreactors, actually build up a real air treatment system.

In real scale application, the system is based on stand-alone bio-oxidizers that provide internal air-mixing within the facility and capture particulates and gases by attracting them to an air zone with low concentration of contaminants generated by its action. In presence of high load of contamination attracted to a single bioreactor, then, the degrading capacity displayed by the biomass may decrease due to substrate inhibition. Since the system is modular, the correct sizing and positioning of bioreactors may, therefore, increase the global effectiveness the air treatment, by overlapping the individual unit's effect and offering larger reaction surfaces and biofilm activity. This gives space to anticipate a positive outcome in application for capturing and containing airborne contamination in indoor environment, even in presence of low ventilation.

Three sizes of bioreactors are currently manufactured by U-Earth Biotechnology and their technical specs are reported into Table 2.3.

As already mentioned, for most applications reported in the present document, AIRcel85 (i.e. the smaller size bioreactor) was used. Technical drawings provided by the manufacturing company are presented in Fig. 2.8 and 2.9.

	AIRCEL85	**AIRCEL600**	**AIRCEL5000**
COVERED AREA (M^2)	15÷150	50÷500	100÷1.500
CONTAMINANTS UPTAKE (KG/DAY)	3,5	11,5	76
MONTHLY U-OX REFILL (ML)	100 (200 as start-up dose)	500 (1.000 as start-up dose)	1.000 (2.000 as start-up dose)

	AIRCEL85	AIRCEL600	AIRCEL5000
AIR FLOW (M^3/H)	37	580	1030
VOLTAGE (V)	12	230	230
FREQUENCY (HZ)	50	50	50
POWER MAX (W)	45	310	840
ENERGY CONSUMPTION (KWH/D)	1,08	7,44	20,16
SOUND PRESSURE (DB)	31	36	38
WATER RESERVOIR MAX. (LT.)	31	170	600
SIZE (MM)	355x390x800	1200x800x1260	1200x1000x2110
EMPTY WEIGHT (KG)	20	95	165
FULL WEIGHT (KG)	51	265	765
ENERGY SUPPLY CABLE	2 mt. Cable with Schuko plug	2 mt. Cable with Schuko plug	2 mt. Cable with Schuko plug
WATER FEED (MM)	Polyuretane pipe Øe6xØi4	Polyuretane pipe Øe12xØi8	Polyuretane pipe Øe12xØi8
MAX WATER PRESSURE (BAR)	6	10	10

Tab. 2.3.: Technical specs of the three bioreactors size currently manufactured.

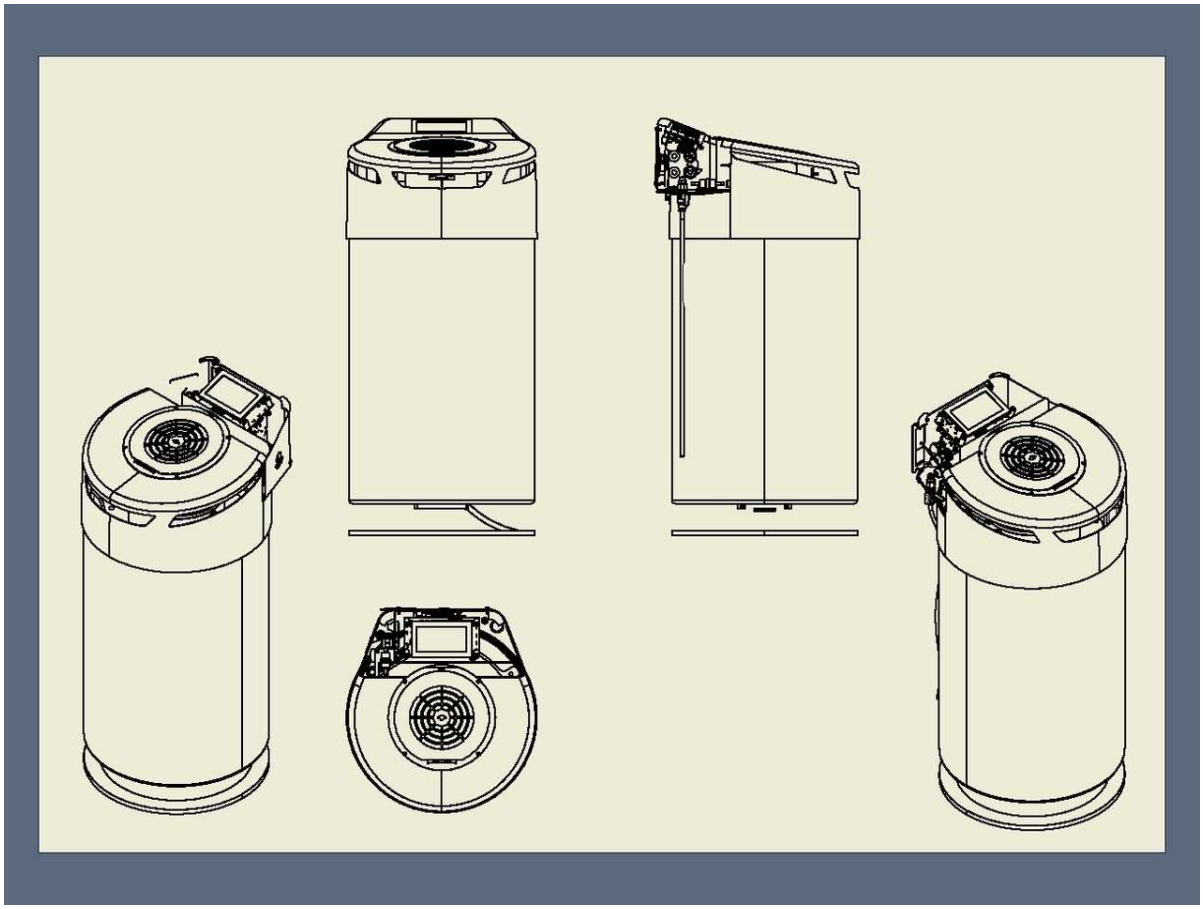


Fig. 2.8.: AIRcel 85 technical drawings, outside (U-Earth Biotechnologies s.r.l.).

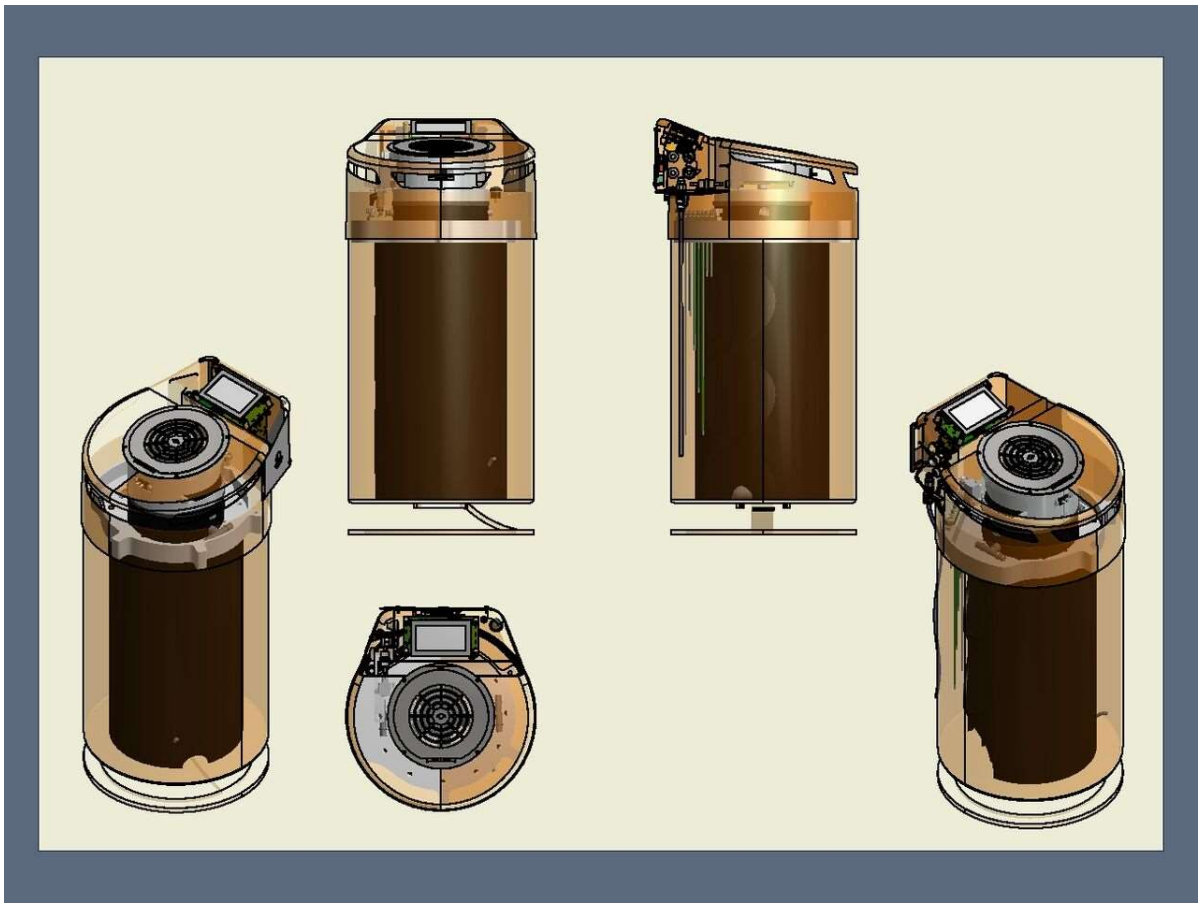


Fig. 2.9.: AIRcel 85 technical drawings, inside (U-Earth Biotechnologies s.r.l.).

The system can be accounted as a sustainable technology, particularly when compared to standard air treatment systems, since it does not require elevated temperatures (as post-burners) or pressures (as membrane filters) or excess energy (as any ventilation system) to operate. In Chapter 5 of the present document a more detailed analysis of technology sustainability would be provided.

Chapter 3 - Performance evaluation methods

A crucial aspect in the process of validation of an environmental biotechnology is the performance evaluation and assessment.
Provided the peculiar working principle of the technology, specific tools have been applied to assess the effectiveness of the different applications and gather empirical data for modeling.
In particular, since recirculation of air and water is the leading force of the remediation, standard monitoring methods have been adapted to meet the specific issue. For air treatment, a specific monitoring protocol has been developed and a wireless technology extensively applied, together with process water test; for wastewater treatment, in the other hand, key parameters have been tested on the basis of a defined schedule, with fast and easily manageable laboratory procedures.

3.1. Air treatment application

3.1.1. Air monitoring

Since the AIRcel technology operates in open air, it breaks with the typical outline of traditional air treatment technologies, i.e. ducting the exhaust air, filtering and emission. This undermines the standard efficiency assessment, based on the difference in concentration of the specific contaminant between outlet and inlet. For this reason, different methodologies have been applied to evaluate the effectiveness of the AIRcel technology, based on difference in contaminants' concentration before and after system's installation, to verify its impact on the specific environment.
Where possible, international standards have been applied (e.g. UNI EN ISO 7726 e UNI EN ISO 7730) and, in particular, sampling methodologies developed for Clean Rooms and controlled environments (UNI EN ISO 14644:2015; UNI EN ISO 14698-1:2004), hospital environments (National Health Service, ISPESL)) and Working place exposure evaluation in general (UNI EN 13205 – 1:2014 - Workplace exposure. Assessment of sampler performance for measurement of airborne particle concentrations. General requirements; UNI EN 13098:2002).

3.1.1.1. Monitoring protocol

A specific document has been developed, particularly devoted to as a result of Technical Standards, Guidelines and Regulations, together with previous testing experience, coordinated by Prof. Alfonso Andretta, Environmental Engineer from University of Modena and Reggio Emilia, Italy, in 2011 into Saronno Hospital, and Sam Sofer, PE PhD, professor and researcher for several years in New Jersey Institute of Technology, USA.
General rules have been set:

1. Baseline testing should be performed BEFORE installing the units (if same day of installation before putting water and biomass in the units). Three rounds of baseline would be preferable in order to obtain a database which could be statistically processed.
2. Similar contour conditions should be pursued for the different testing time, either always at rest, or always during the same shift, and similar contamination production conditions, or number of people occupying the room.
3. The best time for testing is a few days after biomass addition. Biomass mono-dose should be added on day 1 (installation), on day 15, on day 30 and every 30 days thereafter.

4. Note specific unusual conditions which occurred around the testing time, like room clean-up (detergents emissions), open windows (dust or outdoor contamination), lack of oxygen in the room due to lock up of rooms for long time (the system needs oxygen to work efficiently).
5. The monitoring performance is evaluated on the abatement of contaminants in the room in respect to the baseline data, and not by the actual air inlet VS air outlet of the units.
6. Preliminary activities comprise:
   6.1. Acquisition, when possible, of the Risk Assessment Document, the floor plan layout of the facility including HVAC ducting layout, and accident registry.
   6.2. Preliminary assessment of environmental contamination conditions of the area to be tested, examining historical data, where available.
   6.3. Site inspection and detailed analysis of the production cycle, of the areas in which specific activities are carried out, number of people usually in the area and possible sources, characteristics and mode of transmission of risk agents.
   6.4. Choice of the sampling points in different locations around the room and sampling spots must be kept the same for every test run. However, the sampling point should never be close to the AIRcel device. A minimum distance of 1,5 m must be kept from the AIRcel. The minimum number of sampling points must be defined as follows:
   • Area ≤ 30 m^2: n ° 1 sampling point in the middle of the area, 1.5 m above the ground;
   • Area > 30 m^2: n ° 2 sampling points along the diagonal equidistant between each other, 1.5 m above the ground;

Four classes of contaminants are involved in air monitoring:

1. **Microbial count**: Italian Guidelines "Microbiological monitoring on workplaces - Sampling and Analysis" (INAIL 2010) are followed, compatible with European Community Standards (such as UNI EN ISO 14698-2:2004 - Cleanrooms and Associated Controlled Environments - Biocontamination Control). The monitoring of the bio-aerosol is performed using an impact sampler and performing a triple sampling, for each point identified above, at a standard volume air sampling value of 1000 litres. A sampler compliant with national and international standards for environmental air monitoring, including those set by ISO 14698 (Cleanrooms and associated controlled environments), is to be used in order to sample a known amount of air, conveying it with proper flux, onto the Petri plates. The head of the instrument should be sterilized or disinfected after each single sample and subjected to autoclaving after each sampling cycle. A single contact plate will be used for each sampling, which will be stored in a refrigerator at standard temperature of +4 ° C in an inverted position and then transported as soon as possible, max within 24 hours, at a laboratory that will provide for subsequent incubation and analysis of the bacterial cultures. In addition to standard Total Viable Count analysis (counting colonies growing on Tryptic Soy Agar after 48h incubation at ± 1°C), selective analysis (both quantitative and qualitative, for the isolation of specific strain of interest) on colony grown after specific period of time in mesophilic conditions can be performed in order to identify pathogenic bacteria, relevant on nosocomial infections or post-surgical infections, such as:
   1.1. Gram + bacteria
   1.2. Gram – bacteria
   1.3. Staphylococcus aureus (with active sampling on specific culture medium, such as Baird Parker plates with 1% Tellurium and egg yolks solution)
   1.4. Pseudomonas aeruginosa
   1.5. Legionella: unfortunately, no standard procedure is commonly implemented for detection of such pathogen in air. Accordingly to CDC laboratory practice manual (National Center for Infectious

Diseases, 2005), impingement in liquid using an all-glass impinger or impaction on solid media using an Anderson sampler are suggested methods in order to obtain the most representative samples in this particular issue. No application has been carried out.

1.6. Viruses: specific methodologies to be defined for different strains of interest (filter/real-time qPCR, for example). No application has been carried out.

2. **Airborne particulates**: UNI EN ISO 146444:2015 and UNI EN 13205-1:2014 represent the standard for such monitoring, noting for each size class from 0.3 to 5 microns, the number of particles per unit volume.
3. **Volatile Organic Compounds** (VOC): VOC screening can be performed using a PID, like the Tiger VOC detector, a hand-held instrument for rapid, accurate detection of volatile organic compounds. Its photoionisation detection (PID) capabilities utilize a patented Fence Electrode technology, a 3-electrode format, resistant to humidity and contamination. The detection range from 1 parts per billion (ppb) to 20,000 parts per million (ppm). As an alternative, Flame Ionisation Detectors (FIDs) may be used or, following UNI EN 1076:2010, pumped samplers and analytical methods.
4. **Specific gaseous contaminants** of interest (e.g. Formaldehyde), where required. In particular, passive detection devices are used (e.g. Radiello). Passive or diffusive sampling relies on the unassisted molecular diffusion of gaseous agents (analytes) through a diffusive surface onto an adsorbent cartridge. Unlike active sampling, passive samplers require no electricity and they are simple to use. Passive sampling is quiet, non-flammable and does not represent an explosion hazard. It can be performed by anyone, anywhere and at a very low cost. Moreover, it is not susceptible to sample breakthrough. After sampling, the analytes are chemically desorbed by solvent extraction or thermally desorbed and analyzed. The passive samplers can be placed in the room, on a steady surface and away from the position planned AIRcel units, or on operator. Following the general rules previously stated, at least one sample should be taken before AIRcel installation, in order to detect a baseline either in general operating condition or during a specific moment of contamination exposure (eg. Anethstetic gas use during surgery), and 30-60-90 days after installation, to monitor exposition to a specific contaminant and possible effect provided by the AIRcel system.

For the completeness of scientific data and better interpret results, microclimatic conditions (i.e. temperature and relative humidity) must be tested with thermos-hygrometers, placed on the very same sampling points.

3.1.1.2. U-Monitor

With the aim of gather air quality data as representative as possible of the real environmental condition, a faster and easier monitoring method has been implemented.

A monitoring platform (U-Monitor) has been developed for U-Earth Biotechnologies by Gabriella Motta, PhD, and applied in several case studies. The electronic platform offers four slots for contaminants concentration sensors, together with two microclimatic sensors, for temperature and humidity.

In particular, sensors currently mounted on U-Monitor are:

- *Environmental parameters*:
  - Temperature [°C]: [-40°C to 125°C], ±2°C worse accuracy;
  - Humidity [%RH]: [0-100% RH], ±4%RH accuracy;
- *Air contaminants/Odorous gases* (Figaro TGS2602) [ppm]: [1 to 30 ppm], $C_6H_5CH_3$ (Toluene), H_2S (Hydrogen Sulphide), CH_3CH_2OH (Ethanol), NH_3 (Ammonia), H_2 (Hydrogen);
- *Solvent gases/VOC* (Figaro TGS2620) [ppm]: [50 to 5000 ppm], CH_3CH_2OH (Ethanol), H_2 (Hydrogen), C_4H_{10} (Isobutene), CO (Carbon monoxide), CH_4 (Methane);

. *Particulate matter* (Shinyei PPD42NS) [pcs/l]*:*
  + PM 1: [0 to 28000 pcs/l], detectable particle size ≥ 1 µm;
  + PM 2.5 [0 to 280000 pcs/l], detectable particle size ≥ 2.5 µm.

Each sensor collects data every 5 minutes, but it can be set to detect concentration and environmental data on 5 minutes to 1 hour basis. Thanks to a Wireless Sensor Network (WSN) technology, it able to communicate data to a server via Wifi, GSM network or Ethernet, every determined interval, making them available almost in real time for displaying (Fig. 3.1.).

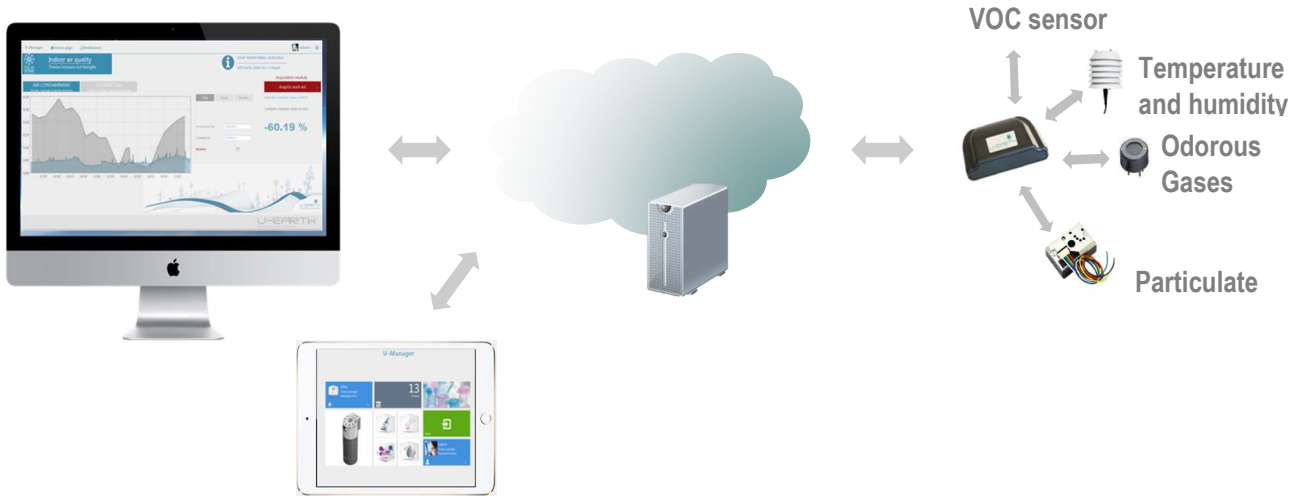


Fig. 3.1.: U-Monitor system (data collection, cloud record and on-line displaying) (U-Earth Biotechnologies s.r.l.)

Semiconductor sensors, as Figaro TGS2602 and Figaro TGS2620, consist of two elements: a first one, made of tin dioxide (SnO_2) with crystalline structure, and a second one acting as heater. The first one presents and excess of electrons, due to the charging effect triggered by the second unit, making it sensitive to different gases concentrations. Once electrically supplied, resistance detected at the sensor (precision of ±1% in the whole range) is proportional to gases concentration. Through software conversion, gas concentration is displayed as part per million (ppm) on the web platform. Since sensors are non-selective, i.e. they are sensible to groups of gases, not to single ones, they are calibrated both with single and mixture of gases (ethanol, hydrogen, control mixture UNI EN 12619:2002, i.e. methane 2,0 mg/m^3, ethane 1,5 mg/m^3, toluene mg/m^3, benzene 0,5 mg/m^3, methylene chloride 05 mg/m^3, with oxygen 11%, carbon dioxide 10%, carbon monoxide 50 mg/m^3 and nitrogen as complementary). The calibration performed and cross-referenced against a single device calibrated through standard method returns a final accuracy of ±15%, including resistance detection and calibration errors.

The laser scatter sensor Shinyei PPD42NS (Shinyei Technology), a low-cost and off-the-shelf product, detects particulate matter (PM), classifying them by size ranges, thanks to light beam alteration. A 100 Ω resistors provides a thermal plume, allowing particles inside the detection chamber, without ventilation. Through the projection of an infrared light beam, the sensor indirectly estimates the number of PM suspended in air by the scattering of the beam itself hitting particles traveling across the chamber. Photons are scattered with an approximate 45° angles, focused by a lens into a specific region where, finally, they are detected by a photodiode, translating light into pulse signal (Fig. 3.2.), which is proportional to particles concentration (Allen, Shinyei Technology). In this way, the measured parameter is the air opacity (*opacity percentage or Low Pulse Occupancy*), defined as the percentage of time (relative to a predefined time interval, e.g. 1 second) in which particles are detected by the photo diode sensor. The particle detection threshold of the sensor is determined by a *pass-band* filter, removing part of the background noise and identifying the range

of particles counted (Compagnoni 2016). In particular, the two channels allow two size ranges count (i.e. 1-10 microns and 2.5-10 microns) with accuracy related to PM concentration (±1.5% for concentrations below 5%; ±2.5% for concentrations above 15%, i.e. sensor's saturation limit), with no indication whether could be related to difference in the total mass, size distribution, or optical properties of the lens, or a combination of these phenomena (Gaia).

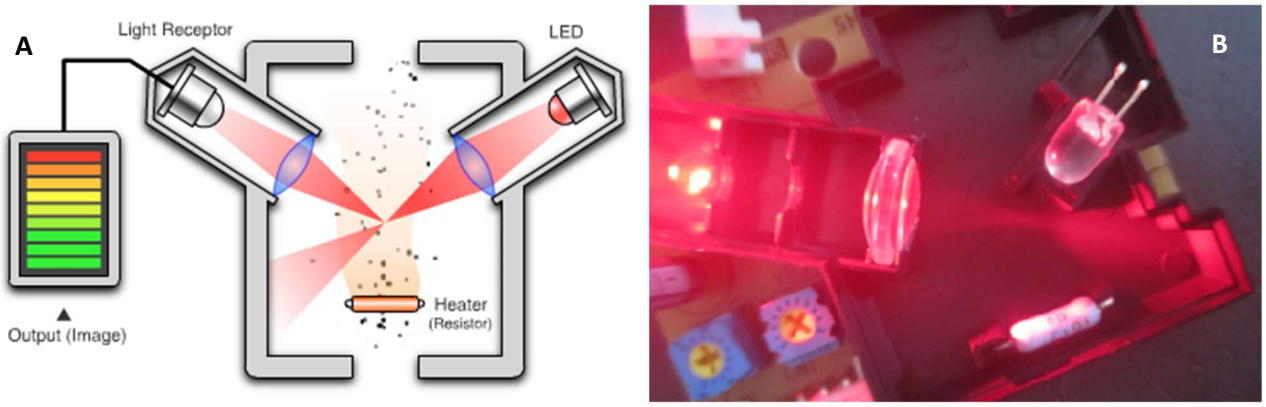


Fig. 3.2.: Particle sensor Shinyei PPD42NS – working principle (A); plan view (B) (Compagnoni 2016).

All sensors present a remarkable drawback (Holstius et al. 2014), but it can be overcome in two ways:

1. statistical analysis of the broad population of data collected (1 sample every 5 minutes per sensor, makes 288 concentration data per each sensor per day);
2. evaluation of trends and possible repetitive patterns in contamination: rather than treating data as absolute values, they are elaborated in comparison to a baseline.

The output interface displays, almost in real time (Fig. 3.3.), the measured data as:

- *Temperature* [°C];
- *Humidity* [%RH];
- *CO_2 equivalent* [ppm];
- *PM 1* [pcs/l];
- *PM 2.5* [pcs/l];
- *Odorous Gases* [ppm];
- *Volatile Organic Compounds (VOC)* [ppm].

Raw data can be extracted by a web platform as Excel files and they are statistically elaborated to evaluate:

- average values on a hour, day, week basis
- contamination conditions before and after AIRcel installation
- recovery time of the system after contamination events.

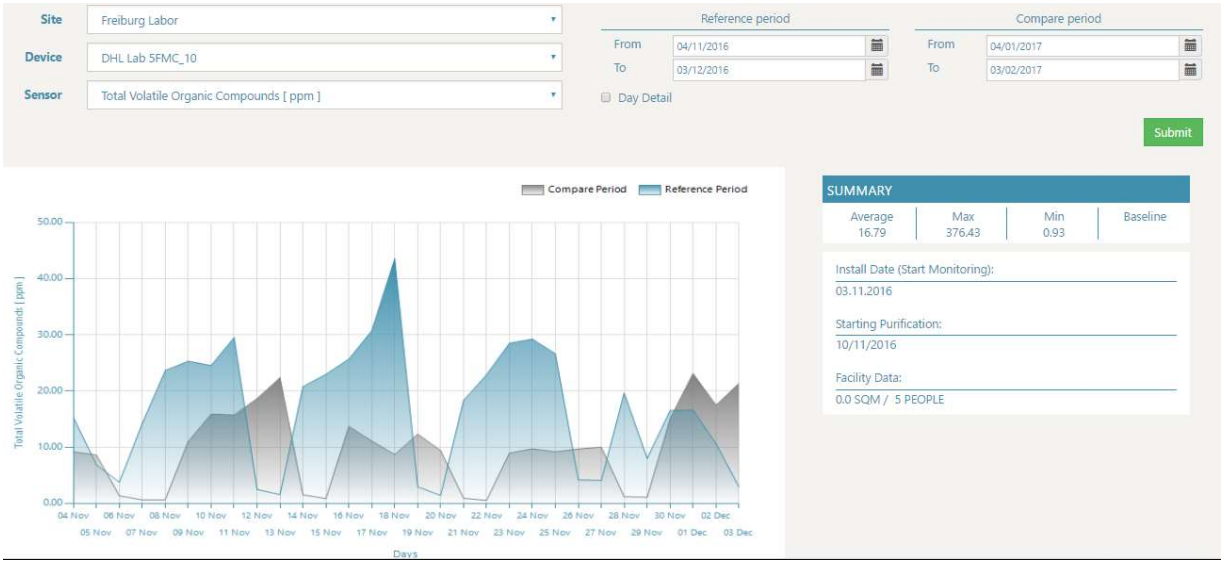


Fig. 3.3.: U-Monitor on-line display (screenshot from http://zzmsysuearthsql.cloudapp.net/DWH_Portal/).

3.1.2. Water monitoring

As support or countercheck for air monitoring, in the perspective of performance evaluation, process water inside the AIRcel can be tested.

The water is expected to result clean or sparsely populated with captured pathogenic bacteria/viruses. If any pathogens or undigested compounds are detectable, they are supposed to be captured and under treatment, i.e. in recirculation inside the bioreactor. In this case proof of concept could obtained by waiting for a proper interval (e.g. a week) without changing the water and then repeating the same test.

Each elemental base/metal etc. of the captured compounds would be found in the tank as sediment. The bottom sludge would be ultimately formed by product of bio-oxidation: e.g. H_2S gas would be digested in $H_2O + CO_2 + S$ and traces of sulfur element, while benzene C_6H_6, instead, should be completely digested. Therefore, an analysis of dry solids and composition of bottom sludge from the AIRcel water, would provide information about compounds captured from the air, such as H_2S, particulate matter, radioactivity, heavy metals, and other non-degradable matters like glass, silver, arsenicum ect.

In order to provide reliable samples on process water quality and, therefore, reliable data, a correct sampling is crucial and it must be performed

1. into a currently running Unit;
2. sampling water recirculating into the external tank, since that is regarded as the last treatment stage, while water into the core cylinder is still under process;
3. sampling as far as possible from the Units surfaces (both side and bottom surfaces), in order to avoid biomass perturbation and residual material;
4. always agreeing in advance with analysis lab the right container to be used;
5. keeping it clean and sealed before and after the sampling, away from direct light and sudden temperature leaps, possibly into thermostatic container at 4°C;
6. delivering the samples as soon as possible (ideally within 24 hours) to the laboratory to be analysed

This procedure would provide data to be compared to feed water quality (i.e. tap water, most of the time) in order to understand the treatment performance and can be related to air monitoring results to trace down the fate of major contaminants and microbiological agents, which should be absent into process water.

Samples of the bottom sludge, on the other hand, must be taken following a different procedure:

1. avoiding water refill for at least 4-5 days before sampling, in order to decrease the water level without compromising the unit's functionality;
2. switching off the unit;
3. waiting for suspended materials to sediment at the bottom of the tank;
4. emptying the AIRcel unit into a clean tank, where the sediments could be mixed and homogenised;
5. taking a sample into a clean and sealable container to be delivered to the laboratory at the earliest (ideally within 24 hours), keeping it clean and sealed before and after the sampling, away from direct light and sudden temperature leaps, possibly into thermostatic container at 4°C.

3.2. Wastewater treatment application

For application on wastewater treatment, a specific set-up of the AIRcel unit has been designed, accordingly to Sam Sofer indications and experience, working in batch with multiple recirculation over time (see Chapter 7).

Monitoring steps for the key performance parameters have been set to different time interval from 0 to 72 hours after wastewater addition.

Temperature and pH have been tested during the whole trial period, as well as target contaminants. They have been identified in Anionic Surfactants, Chemical Oxygen Demand, Chlorides, Free Chlorine and Phosphorous based first on the wastewater characteristic and remediation target and monitoring and budget efficiency reasons as well.

Environmental conditions have been tested with Temperature/Humidity probe, together with water temperature into air treatment unit, wastewater treatment unit and tank.

For target contaminants evaluation, different devices have been used:

1. HI 98172 Portable pH/ORP/ISE Meter with specific Chlorides sensor
2. Hach-Lange spectrophotometer DR 2800 and specific cuvette tests for different contaminants (Anionic Surfactants, Chemical Oxygen Demand, Free Chlorine and Phosphorous). In particular:
   2.1. COD has been selected as representative of the oxidizing capability and biodegradability of organic pollutants in wastewater. According to ISO 15705, COD is the volume of oxygen equivalent to the mass of potassium dichromate that reacts with the oxidable substances in water under the working conditions of the method (reaction time is 2 hours at 148 °C). Mercury sulfate, silver sulfate and sulfuric acid are accepted auxiliary reagents. The LANGE cuvette tests (LCK 1014 Hach cuvettes) work on the standard reaction principle, but using 90% less reagents (silver sulfate as catalyst and mercury sulfate to mask chloride) and applying a photometric evaluation method (the green coloration of Cr3+ is evaluated) instead of a titrimetric one.
   2.2. Anionic surfactants (LCK 332 Hach cuvettes) react with methylene blue to form complexes, which are extracted in chloroform and evaluated photometrically.
   2.3. Phosphate ions (LCK 350 Hach cuvettes) react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex, which is reduced by ascorbic acid to phosphomolybdenum blue.

Every measure has been taken in threefold repetition, in order to work on consistent data.

Water level has been checked and registered, in order to evaluate water consumption by evaporation.

Chapter 4 - Technology Risk Assessment

For a technology, which is candidate to be applied in particularly delicate environments, such as immunocompromised patients' wards in healthcare facilities, possible risk evaluation and assessment is a key element for validation. On these premises, the **AIRcel** has been evaluated as a market product and as a operating device, for its possible side effect, i.e. possible emission of pollutants or by-products.
As devices manufactured in Italy and commercialized in the European Economic Area (EEA), AIRCel are marked as "CE", therefore meeting all the legal requirements related to safety, health, and environmental protection.
AIRcel is also compliant with Restriction of Hazardous Substances Directive 2002/95/EC, (RoHS 1), short for Directive on the restriction of the use of certain hazardous substances in electrical and electronic equipment, as all its components are.
The **biomass (U-ox)** is produced in the US and it is imported with a CAS number correspondent to water, since no chemicals are added to the natural solution of water and bacteria. In this sense, a registration to REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals, Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006, appears not reasonable. REACH addresses the production and use of chemical substances, to evaluate their potential impacts on human health and environment safety.
It has been, nevertheless, checked at a preliminary stage of emissivity test for possible microbial threads and no critical growth of micro-organisms resulted detectable. The biomass, currently under proprietary recipe, consists of common environmental micro-organisms, non-human-pathogenetic and without remarkable population of molds inside.
Emissivity test in full operational mode and during starting-up hours have been performed with the cooperation of different partners:

- Fresenius Medical Care (FMC), Technical Product Management Hygiene/Water Quality Service
- Else-Kroener-Strasse 1, 61352 Bad Homburg – Germany;
- GmbH Deutsches Beratungszentrum für Hygiene (BZH), Schnewlinstraße 10, 79098 Freiburg im Breisgau – Germany;
- Synlab Umweltinstitut GmbH Niederlassung Leipzig/Markkleeberg Hauptstr. 105, 04416 Markkleeberg – Germany; Laboratoriumsmedizin MVZ Leverkusen Medizinisches Versorgungszentrum Paracelsusstraße 13 Leverkusen – Germany;
- Labor Dr. Rabe HygieneConsult, Frillendorfer Str. 154, 45139 Essen – Germany.

4.1. Emissivity test in full operational mode

The aim of the emissivity test was to identify possible threads coming from AIRcel working in full operational mode, in terms of possible increase in particles, bacteria and mould concentration into the treated environment.
The test activities are presented in Table 4.1.

Phase	*Goal*	*Scope*	*Method*
1	Evaluate possible emission from the AIRcel in regular working conditions	n.1 AIRcel unit working into a dedicated aerosol chamber	Addition of specific contaminants into air inlet and air monitoring on defined intervals

Phase	*Goal*	*Scope*	*Method*
2	Evaluate possible emission from AIRcel after 90 days, i.e. maximum interval between general cleanings of the system	n.2 AIRcel units, running for 90 days in different environments, i.e. aerosol test chamber and hospital laundry and consequent implementation into aerosol test chamber	Implementation of each AIRcel unit into aerosol chamber with constant HEPA filtered airflow and air monitoring on defined intervals

Table 4.1. Summary of emissivity test in full operational mode activities

4.1.1. The Test Chamber

4.1.1.1. Phase 1

For emissivity test to be performed on the AIRcel, a test chamber typically used for device validation.
The aerosol test chamber with internal dimensions L 1040 x W 960 x H 960 mm (0,958 m^3) has a double-walled stainless steel housing with smooth inner walls.
On the front, a large door with a viewing window is installed. In the middle of opposing walls air inlet and outlet pipes are placed. Through the inlet, air is forced into the chamber and it may be extracted through the air outlet by a fan. An HEPA filter can be installed at the air inlet, to ensure a good quality air coming into the chamber. A cross flow fan and air baffles in the outer edges ensure a proper air mixing within the chamber.
The U-Earth Aircel unit has been placed within the chamber.
The AIRcel 85 air outlet was initially covered using plastic and aluminum tubing then directly connected to the air outlet of the chamber (Fig. 4.1.). The air was drawn from the air inlet into the chamber, passed through the device and blow out through the AIRcel outlet, through the duct into the exhaust pipe (Fig. 4.2.).
This set up was aimed to try and detach air inlet and outlet of the AIRcel, in order to monitor different air flux, accordingly with the procedure typically applied to standard filtering devices.
Following the operational instructions, the unit has been checked daily and biweekly filled with tap water up to the optimum level.

Figure 4.1 AIRcel into the aerosol test chamber, first test run.

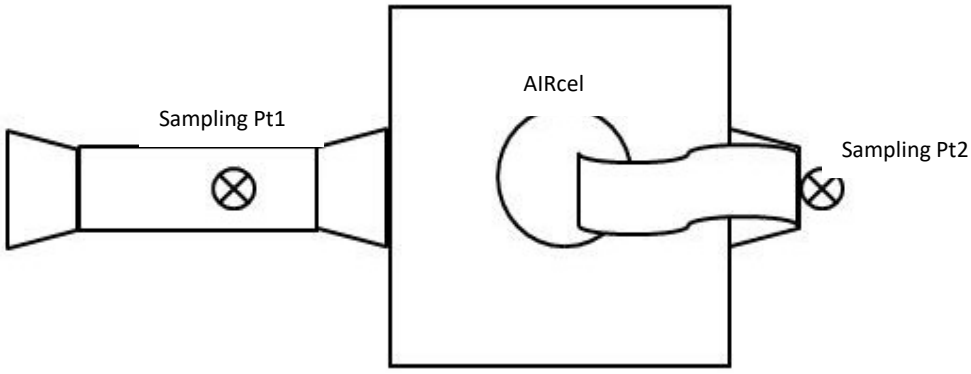


Figure 04.2 Test chamber outline, plan view

As anticipated, the set up proved to be inefficient and detrimental for the equipment: without proper air recirculation, in fact, the fan failed soon, due to excessive pressure disequilibrium and consequent stress.

In the second experiment run the AIRcel ran without any attachments. To ensure an even flow of air through the chamber, the outlet of the chamber has been equipped with a 3m long pipe and an extraction fan at the end. The fan speed was controlled in order to approximately match the inlet/outlet airflow with the AIRcel fan capacity. A plastic plate placed 10 cm from the outlet creates a complex canalization to prevent the air being drawn directly through the chamber into the outlet pipeline.
In this modified set-up, however, no clear separation of the inflow air and the air recycled through the AIRcel, was possible. Therefore, three different sampling points (marked with crossed circles in the following Fig. 4.3) proved to be necessary for the air monitoring:

1. Inlet: placed in the intake duct, about 30 cm out of the chamber,
2. Chamber: arranged within the chamber, near to the AIRcel (Fig. 4.4.),
3. Outlet: placed in the outlet pipe, approximately 2 m out of the chamber.

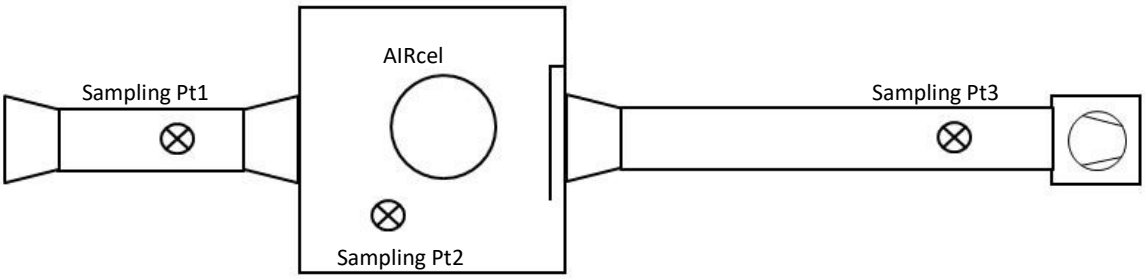


Figure 4.3. Test chamber outline, plan view, phase 1, revised

Figure 4.4. AIRcel in the aerosol test chamber, with sampler on the right, second test run

5.1.1.2. Phase 2

The second phase of the test has been dedicated to verifying possible emission from the AIRcel of airborne contaminants previously captured. In order to accomplish to the task, two different AIRcel units have been tested:

1. the unit previously tested into the aerosol test chamber for 90 days during phase 1 experiments,
2. an AIRcel unit installed for 90 days into a hospital laundry, running as single unit (i.e. undersized, in term of air treatment system) to be heavily loaded with contamination.

After 90 days, each unit has been installed into the aerosol chamber and tested.

At the beginning of each device test, the chamber has been cleaned and disinfected with a solution of 70% ethanol. The cleaning agent has been sprayed on all surfaces of the chamber and it was wiped off after a few minutes. The pipes have been dismantled and treated in the same way. For this purpose, a rod was used with cleaning cloths at one end.

A H13 Filter was mounted upstream of the inlet pipe of the test chamber (Fig. 4.5.). The filter has a theoretical efficiency above 99,95% on particles with a size of 0.3 microns or above, accordingly to UNI EN 1822. Therefore, in principle, all detected particles and microorganisms should originate from the device or from leakage of the chamber and the pipes. The leakage was regarded as low, but, nevertheless, not negligible and necessarily taken into account.

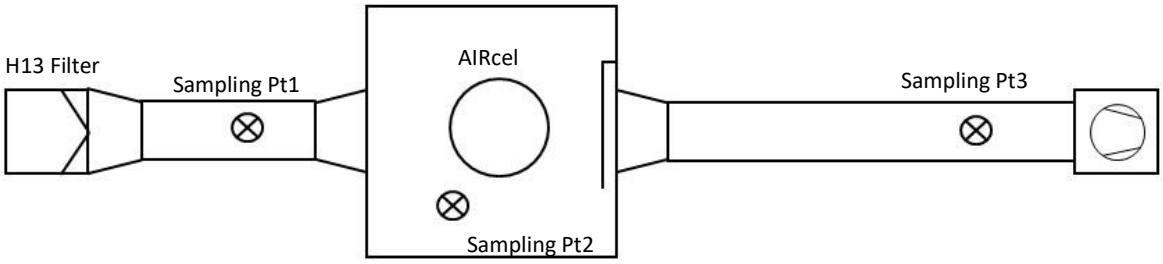


Figure 4.5. Test chamber set up for phase 2

4.1.2. Testing procedure

4.1.2.1. Phase 1

The AIRcel unit has been placed inside the chamber and switched on. From Week 2, a sample of house dust, with known composition and defined mould content was dispersed daily into the chamber. In order to increase the bacterial content of the air, a bacterial suspension was nebulized into the intake pipe.
The tests were carried out after a minimum of 3 hours after the control dust sample had been nebulized: in this way, adequate recirculation time was ensured.

4.1.2.2. Phase 2

Both devices loaded with contamination, i.e. the AIRcel unit tested into the aerosol chamber and the AIRcel unit installed into hospital laundry, were tested after 90 days of working in the controlled conditions in Essen for the final test. The time span corresponds to maximum interval between two general cleanings of the device, in accordance to manufacturer's indication on the user's manual.
Process water has been tested as wastewater at the end of the test phase.
All tests (except endotoxin and inner surfaces) were performed in threefold repetition.

4.1.3. Analysis methods

The analysis methods are based on national and international regulations and are listed in detail below.

4.1.3.1. Air samples quantitative analysis on bacteria

According to DIN EN ISO 16000-17, -18 and VDI 4300 Bl. 10, as reported by Synlab:

- Sampling the air with an impaction air sampler (Klotz Fh5/6) on special agar plates
- Air samples for bacteria and mould spores were collected by impaction with the air sampler Klotz Fh5 or Fh6.
- Incubation of the plates for 48 hours at 30 °C.
- Repeated counting the colony forming units during the incubation period.
- MALDI TOF. Some studies its reliability in specific bacterial strains identification compared with the traditional one. Although, in fact, the overall reliability is very high, controversial data and quite uncertain results have been found on particular species (for example, Bizzini et al. 2010, stated a 58% of reliability on Klebsiella identification) and only extraction procedures could improve results on controversial identification.

4.1.3.2. Air samples analysis on mould

According to DIN EN ISO 16000-17, -18 and VDI 4300 Bl. 10, as reported by Synlab:

- Sampling the air with an impaction air sampler (Klotz Fh5/6) on special agar plates
- Incubation of the plates for 7 days at 25 °C.

- Repeated counting the colony forming units during the incubation period.
- Determination of individual mould species (genera) based on the macroscopic characteristics of the mould colonies: size, shape, colour, special characteristics, as well as by production of cut and teased preparations by the microscopic characteristics of spores, sporophores and mycelium.
- Contact samples for bacteria and mould and adhesive film samples for mould were taken on the inner surfaces of the Aircel units.

4.1.3.3. Particle distribution

- According to Synlab, no evaluation criteria/limit or guideline values are available,
- A clean room condition is recommended,
- The particle size distribution in the air was measured by the particle counter Klotz Abakus mobil air.

4.1.3.4. VOC-measurements

According to DIN ISO 16000-5 and -6, as reported by Synlab, Volatile Organic Carbons (VOCs) and aldehydes were sampled using Gilian pumps onto activated carbon tubes.

4.1.3.5. Endotoxin measurements

According to BIA 9450, as reported by Synlab, air samples were collected on fiberglass filters by the Sartorius MD8.

4.1.3.6. Adhesive film samples

According to VDI 4300 Bl. 10, as reported by Synlab, samples were taken:

- Pressing a special adhesive film on the surface to be examined.
- Peeling of the adhesive film and transferring to a glass slide.
- Staining of the adhesive film preparation with Lactophenol blue solution.
- Light microscopic analysis of spores after spore types and additional mould units and mineral and organic particles were performed, together with distinction between spore deposition and fungal growth.

4.1.3.7. Contact samples

According to Synlab's report, several operations were performed:

- Sampling of surfaces with contact slides DG18 for moulds / yeasts and CASO for bacteria.
- Incubation of culture media over 6-7 days (moulds / yeasts) at 25 ° C and 44 ± 4 hours (bacteria) at 30 ° C.
- Repeated counting the colony forming units during the incubation period.

4.1.3.8. Wastewater samples

The wastewater was mixed before samples were taken, due to quick separation of suspended solids out of wastewater, as reported by Synlab.

Step-by-step procedure for the microbiological analysis of the biomass and the wastewater is reported in Fig.4.6:

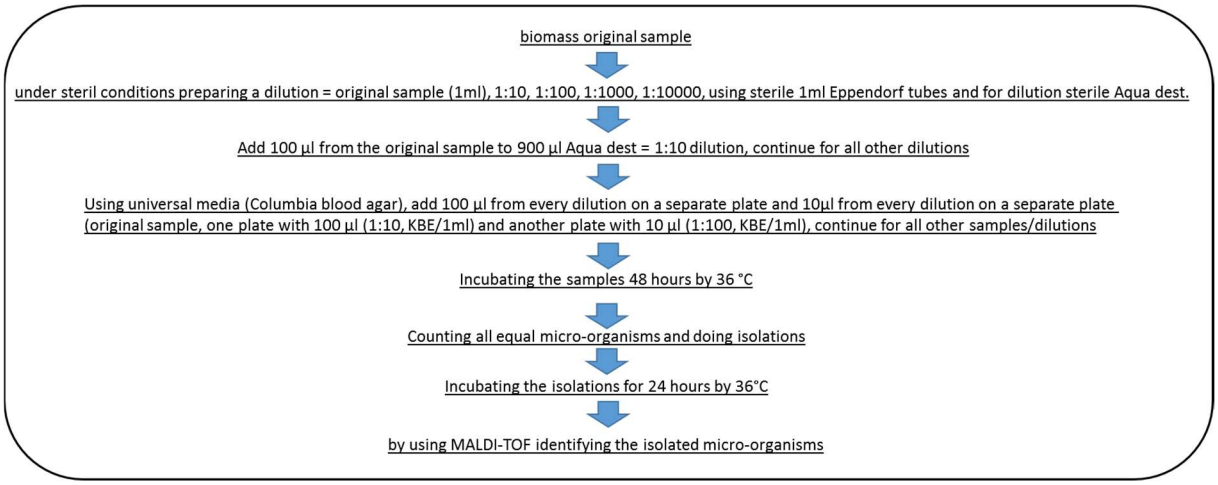


Figure 4.6. Step-by-step procedure for microbiological analysis of biomass and wastewater (Synlab).

4.1.4. Evaluation Criteria:

Results obtained have been evaluated on the basis of standards and guidelines commonly applied. Synlab's report has been summarized in the following paragraphs.

4.1.4.1. Volatile Organic Compounds (VOC/TVOC)

The Indoor Air Hygiene Commission of the Federal Environment Agency toxicological reference values for a number of airborne pollutants (IRK 2012, Breuer et al. 2016). Indoor air contains several organic compounds, deriving from cleanser, furniture and building materials emissions, but, unfortunately, available benchmarks are focused on relatively few single-contamination disposals. Indoor Air Hygiene Commission, with the aim of filling the gap, has developed standards for indoor air quality assessment based on the sum of volatile organic compounds (Total Volatile Organic Compounds, TVOC). In particular, for long-stay rooms:
- upper level of TVOC which should not be exceeded is identified between 1,000 and 3,000 µg/m³
- optimum range of TVOC to be reached is from 200 to 300 µg/m³ or below, if possible.

4.1.4.2. Endotoxin

No internationally accepted standard is available for endotoxin evaluation, since different monitoring procedures applied give rise to non-comparable results. For this reason, within the framework of the present work, literature recommendations have been assumed as quality level. Experimental and epidemiological studies have shown dose-response relations based on acute and chronic pulmonary effects with effect thresholds (Non-observed-effect-level NOEL) of 90-1800 EU/m³. In particular, Rylander assumes that toxic pneumonitis may arise with airborne endotoxin concentration of 2,000 EU/m³, while inflammation of the respiratory tract is possible starting from endotoxin levels of 100 EU/m ³.

4.1.4.3. Adhesive film samples

Evaluation criteria have been set from the laboratory as follows (Table 4.2.), based on what can be observed on the adhesive film.

OBSERVATION OF THE ADHESIVE FILM	ASSUMED CONDITION AT THE SAMPLING POINT	NOTES
ONLY ISOLATED FUNGAL SPORES AND NO PARTS OF MYCELIUM OR SPOROPHORES	no fungal growth	normal spore deposition

MORE FUNGAL SPORES BUT NO PARTS OF MYCELIUM OR SPOROPHORES	no fungal growth	if a mould source exists in the vicinity, it could lead to a secondary contamination at the sampled point
BOTH FUNGAL SPORES AND MYCELIUM AND / OR SPOROPHORES	fungal growth	
A LOT OF FUNGAL SPORES, TOGETHER WITH MYCELIUM AND SPOROPHORES	strong fungal growth	
EXTREMELY LARGE NUMBER OF FUNGAL SPORES (WITHOUT FURTHER FUNGAL INGREDIENTS)	strong fungal growth	

Table 4.2. Evaluation criteria for adhesive film samples.

4.1.4.4. Contact samples

The germ density on surfaces may not be assessed immediately in terms of hygiene. Nevertheless, densities remarkably higher than normal, most likely indicate a contamination of the sampled area. The assumed normal value for germ density, derived by several years of experience in the field of measurement and consulting activities is set on 25 cfu /25 cm^2.

4.1.5. Results

4.1.5.1. Phase 1

Results obtained during emissivity test in full operational mode, as reported by Synlab, are presented in different sections, as for different contaminants tested, and discussed in the following.

4.1.5.1.1. Bacteria and mould

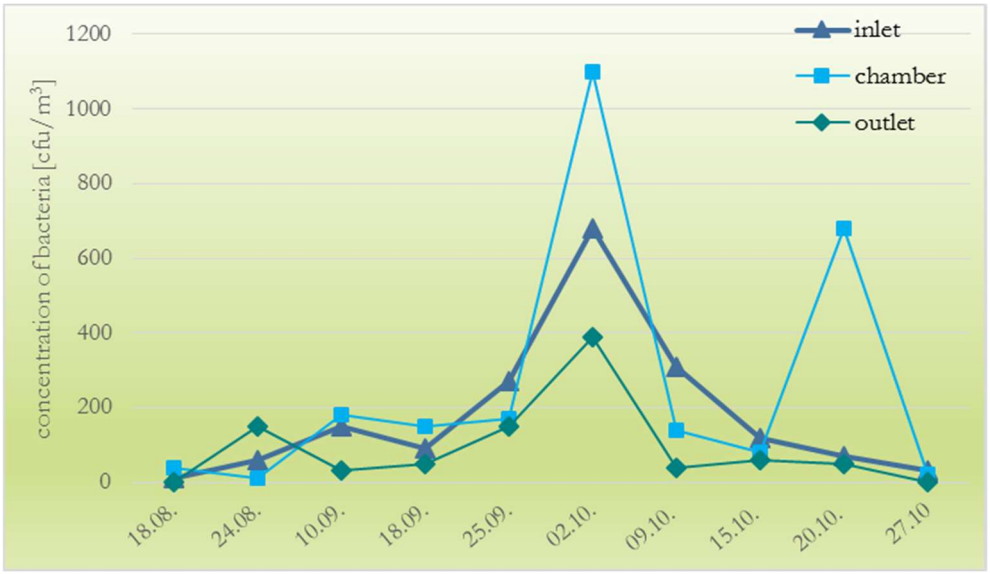


Figure 4.7. Concentration of bacteria in inlet, chamber and outlet samples, Phase 1 (Synlab).

As shown in Fig. 4.7., there is no statistically significant difference between the points of measurement. Two concentration peaks were detected (October, 2nd and 20th), but their cause couldn't be clearly determined. The difference in concentration could be attributed to a transitory failure in the device as well as an elevated fluctuation in the environmental conditions.

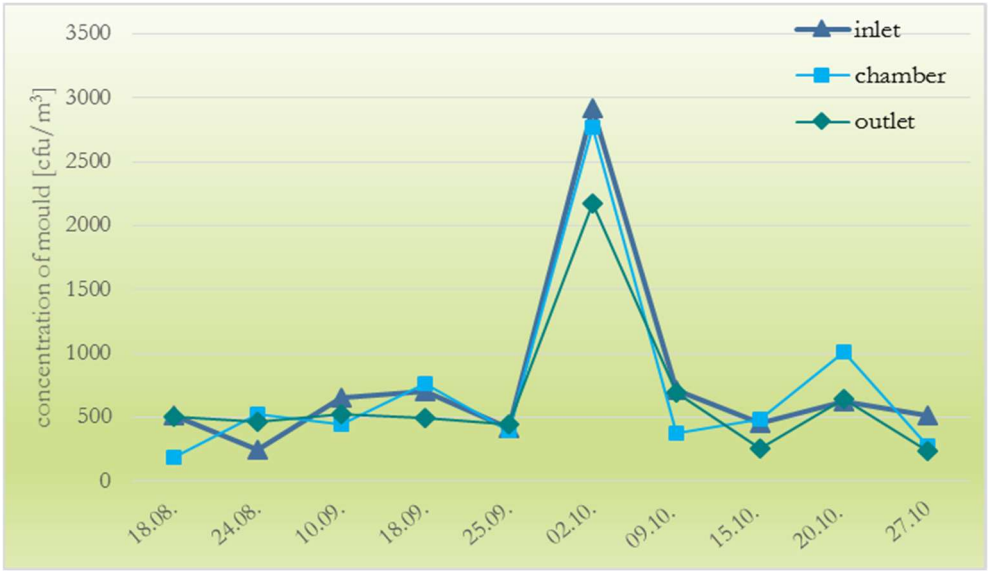


Figure 4.8. Concentration of mould in inlet, chamber and outlet samples, Phase 1 (Synlab).

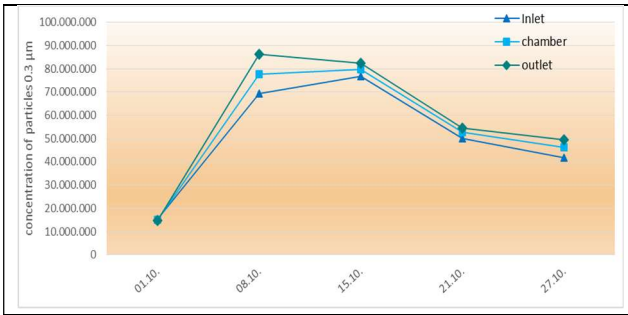
Approximately the same anomaly (October, 2nd and 20th) were detected on mould concentrations (Fig. 4.8), but here concentration within the chamber simply replicate concentration at the inlet. This seems to support the hypothesis of environmental-related event. Except for the two anomalies, data shows no statistically relevant difference in concentration.

For both contaminants classes, no significant increase in concentration appears to be related to the bioreactor's activity and, in addition to this, in most cases, concentrations detected into outlet air resulted lower than in the inlet.

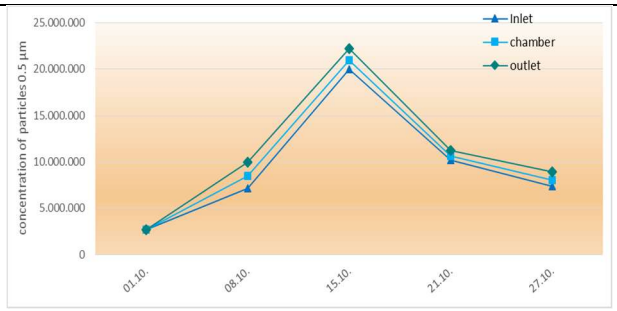
4.1.5.1.2. Particles

Six different size classes of particulate (0.3 μm, 0.5 μm, 1 μm, 2.5 μm, 5 μm and 10 μm) have been tested and results are reported into following Table 5.3.

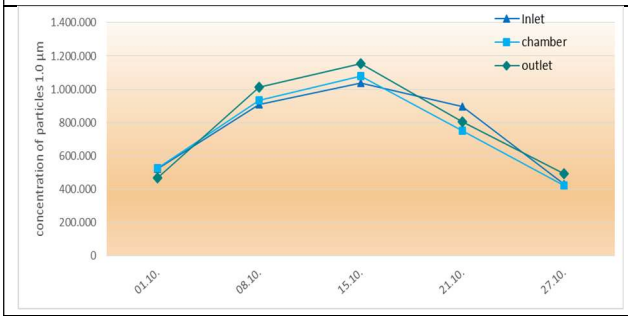
The three bigger size classes are typically regarded as representative of bacterial and mycological contamination in air.



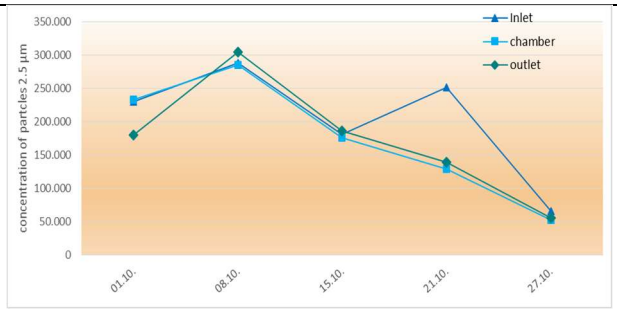
0.3 µm class



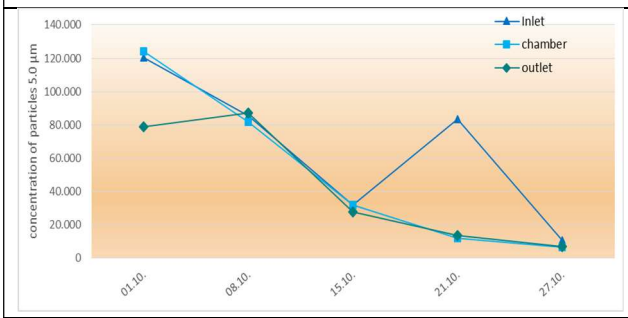
0.5 µm class



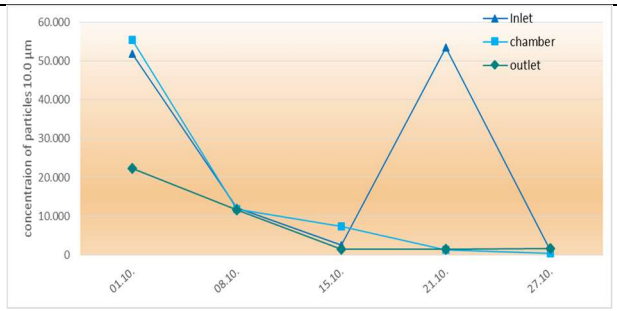
1.0 µm class



2.5 µm class



5.0 µm class



10.0 µm class

Table 4.3. Particle counts for different size ranges at the inlet, chamber and outlet samples Phase 1 (Synlab).

No significant increase in particle count has been detected within the chamber, nor in the outlet air, even in presence of higher particles concentration measured at the inlet. The same can be assessed for particles distribution.

4.1.5.2. Phase 2

During the second phase of the emissivity test, two AIRcel units run separately into the aerosol test chamber with filtered air inlet, after 90 days of working. One unit had been working into the same test chamber to perform phase 1 of the test, indicated as “Essen” in the following figures, the other into a hospital laundry, indicated as “Leverkusen”.

4.1.5.2.1. Bacteria and mould

Data presented in the following Figure 4.9 and 4.10 resulted from threefold repetitions of air samples, performed in the same conditions for both devices.

AIRcel85 “Leverkusen” had been exposed to a strong bacterial flora into the hospital laundry, with high bacterial pollution and, potentially, typical human pathogens. Nevertheless, and similarly to “Essen” device, no statistically significant increase of bacteria concentration at the outlet was detected (data showed relatively relevant standard deviation as well).

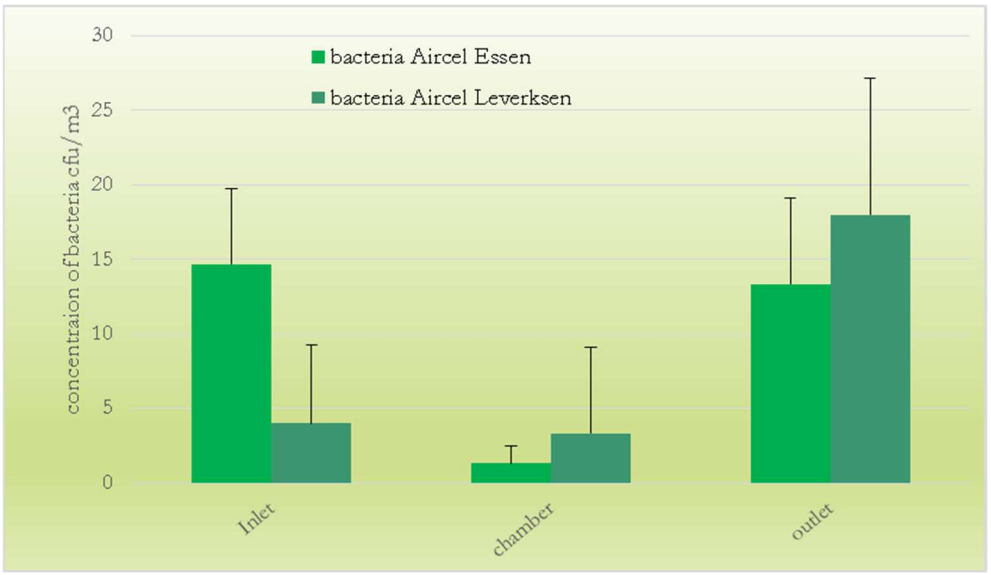


Figure 4.9. Concentration of bacteria in inlet, chamber and outlet air samples, Phase 2, average of a threefold repetition with standard deviation (Synlab).

Mould concentrations showed a trend quite different form bacterial ones, with a slightly significant increase detected at the outlet for AIRcel85 "Essen". Since this device had been tested for 90 days into the same test chamber with opposite results (Fig. 4.8), the relevance of this data has been evaluated within the framework of statistical fluctuations.

Figure 4.10 Concentration of mould in inlet, chamber and outlet air samples, Phase 2, average of a threefold repetition with standard deviation (Synlab).

4.1.5.2.2. Total Volatile Organic Compounds

Total Volatile Organic Compounds (TVOC) have been tested for both devices in inlet, chamber and outlet air and results are presented in the following figure.

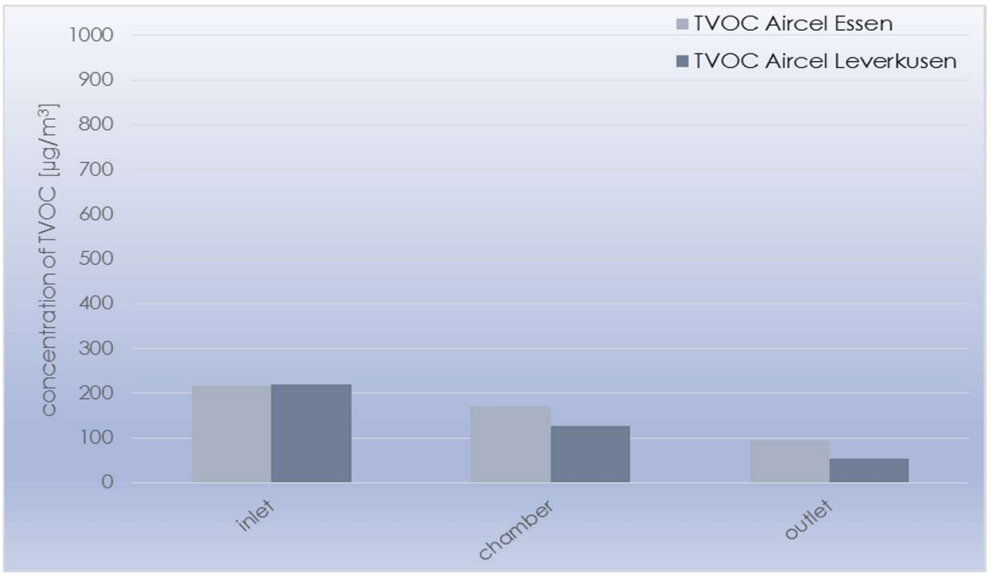


Figure 4.11. Concentration of TVOC for the two AIRcel units, Phase 2.

As displayed in Figure 4.11, no increase in TVOC was registered.

4.1.5.2.3. Endotoxin

Endotoxin have been tested for both devices in inlet, chamber and outlet air, as typical by-product of microbial contaminants degradation and results are presented in the following Figure 4.12. Results have been evaluated as non-statistically relevant.

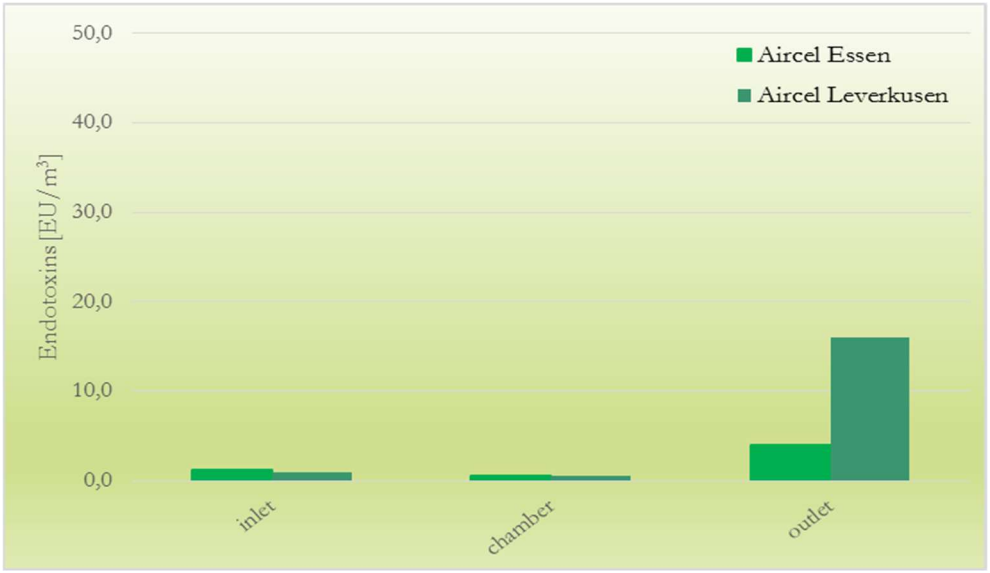
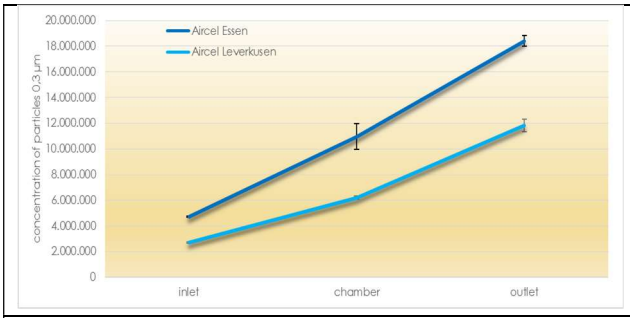
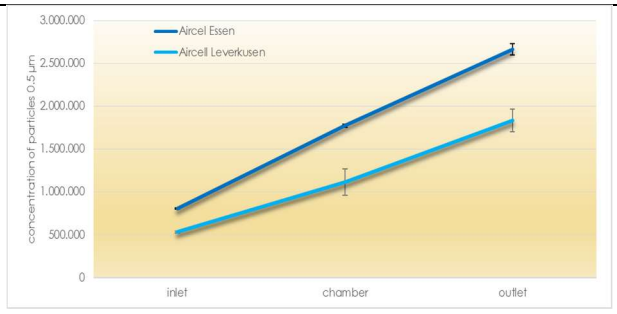


Figure 4.12 Concentration of Endotoxin for the two AIRcel units, Phase 2 (Synlab).

4.1.5.2.4. Particles

In the following Table 4.4, particles count for different sizes are presented. In this case, a tendency towards a higher number of particles is discernible at the outlet, but it has been evaluated as not significant by the laboratory and possibly related to turbulence at the sampling point.

<table>
<tr><td> 
</td><td> 
</td></tr>
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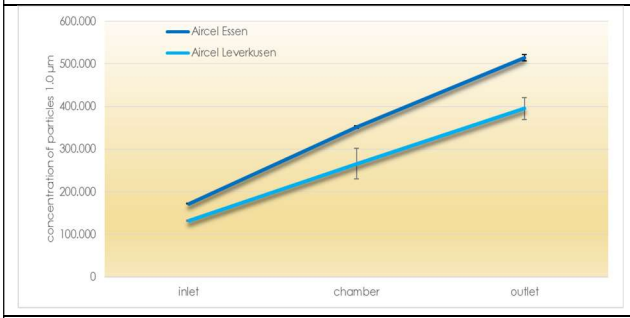
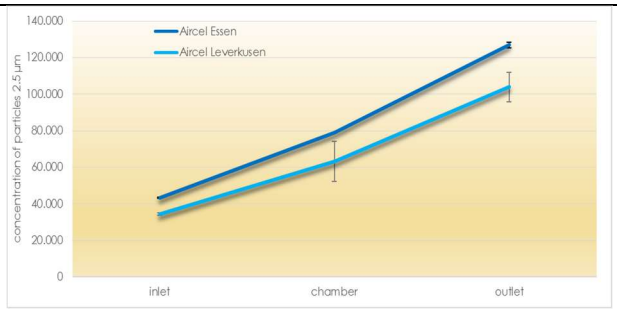
<tr><td> 
</td><td> 
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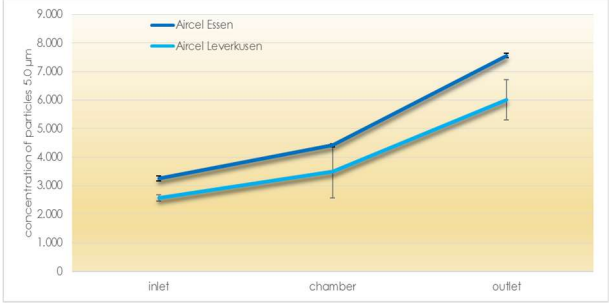
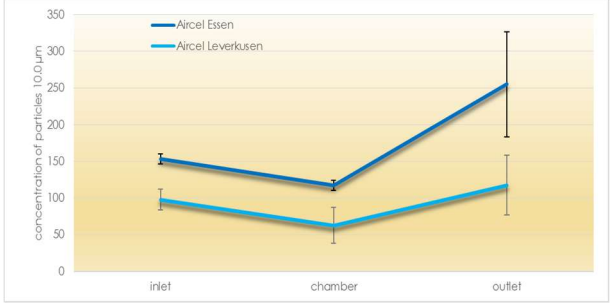
<tr><td>1.0 µm class</td><td>2.5 µm class</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>5.0 µm class</td><td>10.0 µm class</td></tr>
</table>

Table 4.4. Particle counts for different size ranges in inlet, chamber and outlet samples Phase 2 (Synlab).

4.1.5.2.5. Process water

Process water has been sampled at the end of Phase 2 into both AIRcel devices.

In the following Table 4.5., results of toxicity evaluation on dilution tests of the two samples are reported, proving the general safety of wastewater from the ecological point of view. In fact, the first dilution procedure was sufficient to eliminate toxicity for ecological population, except for luminous bacteria in AIRcel "Essen", for which a second dilution was necessary. The wastewater from both samples resulted, therefore, perfectly compliant with discharge regulations (Directive 2000/60/EC).

PARAMETER	UNIT	AIRCEL85 ESSEN	AIRCEL85 LEVERKUSEN	METHODOLOGY
TOXICITY FOR LUMINOUS BACTERIA	GL-value	2	1	DIN EN ISO 11348-2 (L 52)
TOXICITY FOR FISH EMBRYO	GE-value	1	1	DIN EN ISO 15088 (T6)

TOXICITY FOR DAPHNIA	GD-value	1	1	DIN 38 412-L 30
TOXICITY FOR ALGA	GA-value	1	1	DIN 38412 L33

Table 4.5. Toxicity values for process water of the two AIRcel devices.

Additional tests were performed to identify bacteria possibly collected and survived from installation into hospital laundry. As reported in the following Figure 4.13, micro-organism-spectrum resulted in normal environmental bacteria, non-human-pathogenic (Bacillus cereus, for example, can be commonly found in soil and food), even though possible endotoxin-releasing germs. Results obtained on endotoxins release are, therefore, most important in confirming the specific non-harmfulness of the technology.

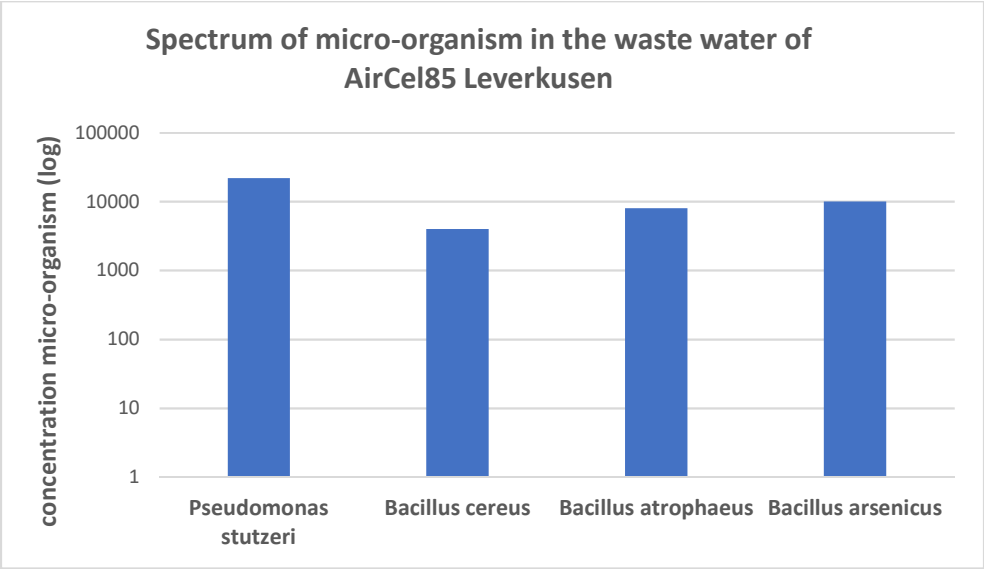


Figure 4.13. Micro-organism-spectrum from wastewater or AIRcel85 "Leverkusen" (Synlab).

4.2. Emissivity test during first 24 hours

On the basis of results obtained from the previous runs of risk assessment tests performed by Synlab in Essen laboratory and certified by BZH and issue remaining open from tests results and possible applications, further runs for technology test have been planned and recently performed.

Since the first trial period has been dedicated to verifying the possible emission of airborne contamination generated o re-emitted by the AIRcel, the prosecution of test activities has been devoted to identity and, possibly, quantify the risk of volatilization of microbial contaminants coming from supply water recirculating into the AIRcel. The aim of the test phase is to detect possible release of specific pathogens, i.e. infectious agents of high interest in hospital environments for immunocompromised patients, during the early hours of AIRcel application, in presence of feed water contaminated with different bacteria concentrations (drinkable or not-drinkable water). Therefore, the main focus of the test is on air monitoring, with the very same methodology applied during the previous tests.

Figure 4.14. Set-up of the test chamber.

Figure 4.15. AIRcel into the test chamber.

This has been accomplished by dosing contamination into process water, and sampling the water itself in different timing. In particular:

- different sampling time were required to describe the evolution of U-ox activity and contamination into process water,
- running the test was regarded as an opportunity to understand the role of the bacterial consortium in water contamination management,
- two different microbial contaminants representing a typical thread for hospital environment were initially considered, i.e. Pseudomonas aeruginosa and Legionella pneumoniae, which are infectious agents of high interest in hospital environments for immunocompromised patients.
- different microbial concentrations were proposed to be administrated to the device, in order to allow the identification of possible threshold limit value for technology management of contaminated water.

Concentration of bacteria spiked into AIRcel process water was defined to correspond to typical contamination conditions detected into tap water system. As reported by Mena and Gerba (2009) concentrations of Pseudomonas aeruginosa up to 2,300 mL (-1) (Allen and Geldreich 1975) or, more often, at 3-4 CFU/mL can be regarded as typical and, therefore, has been initially proposed for the test. In particular, 4 CFU/mL and 8 CFU/mL could be considered as representative concentration of bacteria for average tap water. On the basis of WHO report on Legionella, a threshold value of 1000 CFU/L is applied for Germany, therefore the test has focused on this order of magnitude of concentration, to be confirmed by a preliminary laboratory phase.

This preliminary activity was oriented to develop a method to univocally identify Pseudomonas aeruginosa and Legionella pneumoniae in a suspension of water and U-ox, in the same concentration as into the AIRcel. This allowed following AIRcel process water test in different times after the bacteria addition, which would be useful to understand behavior of the microbial population in time. Since the same growing medium is to be applied both for water suspension and air samples, results of the pre-test are crucial for the test-chamber phase too.
The first run of pre-test returned unclear results: in fact, applying the standard method for Pseudomonas aeruginosa detection, with Cetrimid as growing medium, as prescribed by DIN EN 16266 Water quality - Detection and enumeration of Pseudomonas aeruginosa -, bacteria grew poorly (when diluted in water) or by no means (when mixed with U-ox and water). Test has been repeated with different strains, returning reasonable results, both in water and in U-ox and water suspension. This proved that previous test was reasonably affected by the weakness of the strain used.
Pseudomonas test concentration is set to 2000 CFU/100ml, as proposed by Mr. Oldenburg (Synlab), based on his experience of high concentrations reasonably occurring in drinking water pipelines.

A preliminary test proposal was defined (Table 4.6) and modified in accordance with the research team. In particular:
- samples were taken every 2 hours during the first 2 days (7 am-7 pm);
- in the following days, just 2 samples were taken to define a trend in contamination, if present.
The same test chamber set up for the first test phase has been used.
Air monitoring focused on Pseudomonas aeruginosa only, due to lack of standard method for Legionella detection in air.
Pseudomonas test in the air must be performed on large volumes, to ensure more reliable results. For this reason, each sample was taken over 500 L of air, with threefold repetition. The following picture (Fig. 4.15) shows the sampling device implemented into the outlet pipe of the test chamber.
Each air sampling required 5 minutes. At the end of each monitoring series, the AIRcel was taken out of the test chamber and process water was sampled as well.

Figure 4.15. Air sampler mounted on the outlet pipe of the test chamber.

In order to simulate real environmental conditions, temperature of air inflow has been tuned with the implementation of a small heating system at the inlet, as shown in Fig. 4.16.

Figure 4.16. Heating system installed at the air inlet.

Final results were not available at the time of release of the present document, therefore, they will be object of further elaborations.

Table 4.6. Summary of test proposal for Emissivity test over the first 24 hours.

SUMMARY OF TEST PROPOSAL							
AIM					**VERIFY THE POSSIBILITY OF VOLATILIZATION FROM THE AIRCEL WITH THE PRESENCE OF U-OX, IN ORDER TO SIMULATE TYPICAL STARTING CONDITIONS OF THE TECHNOLOGY ADAPTATION OF PATHOGENS POSSIBLY COMING IN FROM SUPPLY WATER INTO AIRCEL AT NORMAL CONDITIONS**		
PHASE	**AIRCEL**	**U-OX**	**WATER**	**CONTAMINANT**	**TEST**	**TEST TIMING**	**N. OF SAMPLES PER EACH SAMPLING LOCATION**
1	Unit 1	Supplied at the beginning of each test	Tap water, supplied at the beginning of each test and spiked with	Pseudomonas aeruginosa, 4 CFU/mL	Pseudomonas concentration in AIRcel water tested:	every 3 hours during the first day (from t0 to t24)	9 (=27 with threefold repetition)
						every 6 hours during the following 24 hours (from t24 to t48)	4 (=12 with threefold repetition)
				Pseudomonas aeruginosa, 8 CFU/mL	Pseudomonas concentration in AIRcel water tested:	every 3 hours during the first day (from t0 to t24)	9 (=27 with threefold repetition)
						every 6 hours during the following 24 hours (from t24 to t48)	4 (=12 with threefold repetition)
				Total		2 days	36 (=108 with threefold repetition)
2	Unit 2	Supplied at the beginning of each test	Tap water, supplied at the beginning of each test and spiked with	Pseudomonas aeruginosa, 4 CFU/mL	Pseudomonas concentration in AIRcel water tested:	every 2 hours during the first day (from t0 to t24)	13 (=39 with threefold repetition)
						every 6 hours during the following 24 hours (from t24 to t48)	4 (=12 with threefold repetition)
				Pseudomonas aeruginosa, 8 CFU/mL	Pseudomonas concentration in AIRcel water tested:	every 3 hours during the first day (from t0 to t24)	9 (=27 with threefold repetition)
						every 6 hours during the following 24 hours (from t24 to t48)	4 (=12 with threefold repetition)
				Total		2 days	36 (=108 with threefold repetition)
3	Unit 1	Supplied at the beginning	Tap water, supplied at the beginning	Legionella pneumophila, 10^3 CFU/mL	Legionella concentration in AIRcel water tested:	every 3 hours during the first day (from t0 to t24)	9 (=27 with threefold repetition)
						every 6 hours during the following 24 hours (from t24 to t48)	4 (=12 with threefold repetition)

SUMMARY OF TEST PROPOSAL

AIM **VERIFY THE POSSIBILITY OF VOLATILIZATION FROM THE AIRCEL WITH THE PRESENCE OF U-OX, IN ORDER TO SIMULATE TYPICAL STARTING CONDITIONS OF THE TECHNOLOGY ADAPTATION OF PATHOGENS POSSIBLY COMING IN FROM SUPPLY WATER INTO AIRCEL AT NORMAL CONDITIONS**

PHASE	AIRCEL	U-OX	WATER	CONTAMINANT	TEST	TEST TIMING	N. OF SAMPLES PER EACH SAMPLING LOCATION
		of each test	of each test and spiked with	Total		2 days	13 (=39 with threefold repetition)
4	Unit 2	Supplied at the beginning of each test	Tap water, supplied at the beginning of each test and spiked with	Legionella pneumophila, 10^3 CFU/mL	Legionella concentration in AIRcel water tested:	every 3 hours during the first day (from t0 to t24)	9 (=27 with threefold repetition)
						every 6 hours during the following 24 hours (from t24 to t48)	4 (=12 with threefold repetition)
				Total		2 days	13 (=39 with threefold repetition)
						TOTAL NUMBER OF SAMPLES	98 (=294 with threefold repetition)
						TOTAL TIME	8 days (=24 with three repetition per each test)

Chapter 5 – Sustainability assessment

5.1. Introduction

The main function of the technology under study is, as presented in Chapter 2, air treatment. In addition to this, a new opportunity, albeit with minor development and documented applications, is represented by wastewater treatment. In both cases, decontamination may be regarded as an environmental benefit, focusing on the local scale. Nevertheless, in a global perspective, the overall eco-efficiency of the system must be evaluated.

The eco-efficiency concept was defined by World Business Council for Development (WBCSD, 2000) and it "is reached by the delivery of competitively priced goods and services that satisfy human needs and bring quality of life, while progressively reducing ecological impact and resource intensity throughout the life cycle, to a level at least in line with the earth's estimated carrying capacity".

In this sense, environmental impact indicators, such as Carbon Footprint (CF) and Life Cycle Assessment (LCA) represent powerful decision supporting tools.

LCA, in particular, results a comprehensive assessment of environmental performances. Society of Environmental Toxicology and Chemistry provided with a definition of LCA in 1993: "Life Cycle Assessment is a process to evaluate the environmental burdens associated with a product or process by identifying and quantifying energy and materials used and wastes released to the environment". Developed in compliance with UNI EN ISO 14040:2006 and UNI EN ISO 14040:2006, it is generally applied for the assessment of eco-efficiency and environmental impact of product and processes, allowing the quantification of environmental impacts generated throughout the whole life cycle, already during the design phase.

In the eco-design perspective, the implementation of LCA tool enables to tackle the 80% of the overall environmental impact of a product/process. LCA has been only recently used to evaluate innovative remediation solutions for contaminated groundwater and contaminated sites (Bonoli et al. 2013, Antonozzi 2014), due to difficulties, on one hand, in adaptation of software tools developed to evaluate product, rather than process (such as SimaPro and Gabi), and, on the other hand, in quantification of environmental benefit granted by the clean-up intervention. Timing to meet the remediation goals and lifespan of the technique applied are also critical elements, strongly affecting the tuning of the model.

Within this framework, a screening LCA has been developed to evaluate the biotechnology under study. SimaPro software, version 7.3.3., by Pré Sustainability (2006), has been applied, as compliant with ISO 14040-14044 standard and assessment procedure proposed by JRC (2007) in International Reference Life Cycle Data System (ILCD) Handbook e General Guide for Life Cycle Assessment and Detailed Guidance.

The goal of the present study is to outline an assessment of environmental performance of the biotechnology proposed and, in particular, of the device applied in the major pilot application that will be presented in the following chapters, i.e. AIRcel85. For the scope of the study, a single AIRcel85 unit was defined as functional unit, over supposed 5 years of activity. Due to the lack of specific information and pilot stage of applications followed, end-of-life phase was not taken into account and it will be subject of further iterations and refinements of the assessment. Considering the longer expected life-span of the technology, the choice of recyclable materials from the supply chain (plastic and metals) and the modular design, this assumption appeared reasonable, at a screening phase. Moreover, no benefit, i.e. positive impact, has been accounted for environmental remediation.

Where no primary data were available from the manufacturer, literature data has been implemented. In order to improve consistency of data, only one database has been used, among different provided by SimaPro software, i.e. Ecoinvent.

Two different calculation methods have been applied, in order to obtain, on one hand, an overall impact assessment, considering different impact categories and, therefore, effects on the environment, provided by IMPACT 2002+, and on the other hand, a single-issue evaluation, focusing on carbon dioxide, both directly and indirectly, and global warming, with Green House Gases Protocol method.
As screening LCA, significant issues have been identified, in terms of impact categories affected by AIRcel technology production and use and key processes, triggering the most relevant contributions to environmental impact. In the eco-design perspective, results have been shared with the manufacturing company, in order to implement conclusions from the present study into production activity.

5.2. Inventory

Compiling Life Cycle Inventory (LCI) represents a major effort, in terms of data collection, processes evaluation, material and energy flows identification and quantification, details definition and simplification decision. Inventory compilation and analysis, in fact, require an extensive data collection and accurate calculations to quantify relevant inputs and outputs of a product system, related to specific functional unit and system boundaries.
During this study, both primary data (i.e. collected in the production site or provided by the manufacturing company) and secondary data (i.e. collected from manuals, databases and technical specs of similar product currently on the market) have been used.
In order to work on data from literature as coherent and significant as possible, Ecoinvent has been used as only source of database information. As declared by Swiss Centre for Life Cycle Inventory (Frischknecht et al 2007), Ecoinvent is aimed to provide a set of generic and unified data, all relevant, reliable and transparent. This to allow easier Life Cycle Assessment, providing public with credible and widely acceptable results.
Life Cycle Inventory was compiled, for the present study and within the framework of a screening LCA, as reported in the following. Several assumption and cut-off decision were necessary in this phase and they will be subject to revision in future iterations of the analysis.
In particular, the process representing the functional unit, i.e. one AIRcel 85 (Fig. 5.1.), has been built up on 10 sub-processes:

1. AIRcel85_tank
2. AIRcel 85_head
3. AIRcel85_biostack
4. AIRcel85_fan
5. AIRcel85_pump
6. AIRcel85_electronics
7. AIRcel85_packaging
8. AIRcel85_transport
9. AIRcel85_energy consumption
10. U-ox monodose

Fig. 5.1.: AIRcel 85 exploded graphics (U-Earth Biotechnologies s.r.l.).

As a matter of fact, it proved to be impossible to model all the components with Ecoinvent processes directly. Therefore, Tables 5.1 to 5.10 report assumption and modeling choices made during the simulation, for each sub-process. Further details on the processes selected are reported into Annex I.

SUB-PROCESS 1: AIRCEL85_TANK	**RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS**	
MATERIALS	HDPE – structural	PVC - coating
MODEL EQUIVALENT	polyethylene, HDPE, granulate	polyvinylchloride
QUANTITY	Surface area= 1.0675 m^2 Thickness= 0.5 cm Volume= 5.337 dm^3 Weight (based on average HDPE density of 0.93 kg/dm^3) = 4.964 kg	50 g - estimation
PROCESSING	Thermoforming, with calendaring. Specific efficiency= 0.977 Consequently, the amount of HDPE required for the process is 5.081 kg.	Cut-off
ELABORATION	5.081 kg of HDPE, subject to thermoforming	0.05 kg of PVC

Tab. 5.1.: Sub-process 1, relevant data, calculations and assumptions

The AIRcel tank is made of granular HDPE, thermoformed in a cylindrical shape, open at the top. The tank is, then, varnished with a PVC layer as decalcomania. Since no specific primary data was available, a rough estimation was performed (Tab. 5.1.), to be elaborated during further iteration of LCA procedure.

SUB-PROCESS 2: AIRCEL85_HEAD	**RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS**
MATERIALS	Steel
MODEL EQUIVALENT	Steel, low-alloyed
QUANTITY	Primary data from manufacturer Weight= 4.5 kg
PROCESSING	Deep-drawing Weight= 4.5 kg Powder coating Surface area= 0.48 m^2
ELABORATION	4.5 kg of Steel, subject to deep-drawing and powder coating over 0.48 m^2

Tab. 5.2.: Sub-process 2, relevant data, calculations and assumptions

The "head" part of the AIRcel has been modeled as a top cover, of cylindrical shape, of steel, manufactured by deep-drawing and finished with powder coating (Table 5.2). Materials and processes are taken from Ecoinvent database and data are calculated on the basis of manufacturer primary data.

SUB-PROCESS 3: AIRCEL85_BIOSTACK	**RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS**	
MATERIALS	HDPE – structural	polyurethane
MODEL EQUIVALENT	polyethylene, HDPE, granulate	Polyurethane, rigid foam
QUANTITY	Primary data from manufacturer Weight= 2 kg	Primary data from manufacturer Weight= 0.25 kg
PROCESSING	Extrusion Specific efficiency= 0.996 Consequently, the amount of HDPE required for the process is 2.008 kg	Injection moulding Specificic efficiency= 0.994

		Consequently, the amount of PUR required for the process is 0.2515 kg
ELABORATION	2.008 kg of HDPE, subject to thermoforming	0.2515 kg of PUR subject to injection moulding

Tab. 5.3.: Sub-process 3, relevant data, calculations and assumptions

The inner structure, called "biostack", has two primary functions, with different parts and materials involved (Table 5.3.):

1. support for biomass growth, by two cylinders of HDPE with different diameter and holes,
2. water circulation, by top plate of polyurethane, with different holes to allow water trickling down on vertical surfaces, and pump outlet.

The mechanical parts of AIRcel devices are fan (Table 5.4.) and pump (Table 5.5.), providing air and water circulation, respectively. They have been modeled by assimilation with processes already implemented into Ecoinvent, referring to average products available on the market.
Focusing on the time-span of the study, i.e.5 years, maintenance occurrences are expectable and, therefore, n.2 fans and n.5 pumps have been included into the inventory.

SUB-PROCESS 4: AIRCEL85_FAN	**RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS**
MATERIALS	mixed
MODEL EQUIVALENT	fan, used in a power supply unit of a Desktop PC
QUANTITY	Primary data from manufacturer Weight= 0.218 kg
PROCESSING	-
ELABORATION	Fan of 0.218 kg

Tab. 5.4.: Sub-process 4, relevant data, calculations and assumptions

SUB-PROCESS 5: AIRCEL85_PUMP	**RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS**	
MATERIALS	mixed	Polyvinylidenchloride - pipe
MODEL EQUIVALENT	pump 40W	Polyvinylchloride, granulate
QUANTITY	1 pump	Primary data from manufacturer Weight= 0.07 kg
PROCESSING		extrusion, plastic pipes, with a specific process efficiency of 0.996
ELABORATION	1 pump, 40 W	0.0703 kg of PVC

Tab. 5.5.: Sub-process 5, relevant data, calculations and assumptions

For AIRcel electronics simulation, several assumptions were necessary:

1. Touchscreen was simulated as a LCD screen of equivalent size, since no specific process is present on Ecoinvent version 2.2 (Table 5.6A);
2. Wiring board was modeled on the basis of size and average weight data (Table 5.6A);
3. The power adapter implemented is a laptop power adapter, since it is a multifunctional device (Table 5.6B);
4. Supplementary electronics are simulated as electric cable and clamp connector (Tab. 5.6B);
5. The stick water level sensor has no direct equivalent into Ecoinvent database, therefore a model was built up over a potentiometer and electric steel sticks (Tab. 5.6C).

SUB-PROCESS 6.A: AIRCEL85_ELECTRONICS	RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS	
	Touchscreen	Wiring board
MATERIALS	mixed	mixed
MODEL EQUIVALENT	LCD module	Printed wiring board
QUANTITY	Primary data from manufacturer Weight= 0.065 kg	Primary data from manufacturer Size= 13.5 cm X 7 cm Assumption: Printed circuit standard board, multilayer and Aluminium PCBS Weight= 0.037 kg
PROCESSING		-
ELABORATION	0.065 kg of LCD screen	0.037 kg of printed wiring board

Tab. 5.6A.: Sub-process 6, relevant data, calculations and assumptions

SUB-PROCESS 6.B: AIRCEL85_ELECTRONICS	RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS		
	Power adapter and plug	Connectors for cables	Cable connection among components
MATERIALS	mixed	mixed	mixed
MODEL EQUIVALENT	power adapter, for laptop	connector, clamp connection	cable, connector for computer, without plug (synonym: Electric cable)
QUANTITY	1 adapter	Primary data from manufacturer n. of connectors= 16 Assumption: similar shape and size The dataset is based on an average weight of 9 gram/unit Weight= 0.144 kg	Assumption Length= 1 m The dataset is based on an average weight of 0.065 kg/m
PROCESSING	-	-	-
ELABORATION	1 power adapter	0.144 kg of connectors, clamp connection	0.065 kg of electric cable

Tab. 5.6B.: Sub-process 6, relevant data, calculations and assumptions

SUB-PROCESS 6.C: AIRCEL85_ELECTRONICS	RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS	
	Water level sensor – core	Water level sensor, sticks
MATERIALS	mixed	steel
MODEL EQUIVALENT	Potentiometer (assumption, since no specific process is present)	Steel, electric, chromium steel (assumption, since no specific process is present)
QUANTITY	n.3 potentiometers are included The dataset is based on an average weight 6.1 gram/unit. Weight= 0.183 kg	Estimated volume= 6.359 cm^3 Average steel density= 7.85 kg/dm^3 Weight= 0.050 kg
PROCESSING	-	Cut off
ELABORATION	Potentiometer, 0.061 kg	0.050 kg of electric chromium steel

Tab. 5.6C.: Sub-process 6, relevant data, calculations and assumptions

The AIRcel packaging has been modeled on corrugated cardboard box, shockproof material and plastic film. Due to lack of primary data, several estimations were necessary; moreover, since quantities derived were negligible and it is currently a hand-made operation, a cut-off was applied on packaging process.

SUB-PROCESS 7: AIRCEL85_PACKAGING	RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS		
	Box	Shockproof packaging	Packaging film
MATERIALS	corrugated board	polyurethane	LDPE
MODEL EQUIVALENT	packaging, corrugated board, mixed fibre, single wal	polyurethane, flexible foam	packaging film, LDPE
QUANTITY	Primary data Box total surface area= 2.205 m^2 Corrugated board average density= 0.5 kg/m^2 Weight= 1.103 kg	Estimated weight= 0.1 kg	Estimated= 0.05 kg
PROCESSING	-	Cut off: forming	Cut off: wrapping (hand-made)
ELABORATION	1.103 kg of corrugated board	0.1 kg of polyurethane flexible foam	0.05 kg of plastic film

Tab. 5.7.: Sub-process 7, relevant data, calculations and assumptions

Since no specific data on each single part supply chain was available, and being the manufacturing company still in a start-up phase, with consequent work-in-progress approach to production, a generic transport process has been outlined, considering the overall weight of AIRcel (Tab. 5.8), as declared on shipping documents, as transported for average distance normally considered for European Union (i.e. 200 km).

SUB-PROCESS 8: AIRCEL85_TRANSPORT	RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS
MATERIALS	mixed
MODEL EQUIVALENT	transport, lorry >28t, fleet average
QUANTITY	Assumption 10 kg of materials transported for 200 km (average EU)= 2 tkm
PROCESSING	-
ELABORATION	2 tkm

Tab. 5.8.: Sub-process 8, relevant data, calculations and assumptions

The energy consumption of the AIRcel has been taken into account for the entire use phase expected, i.e. 5 years (as defined, conservatively, as time-span of the project). The energy production mix implemented is considered as Italian (since first application of the technology were mainly set in Italy) (Tab. 5.9). This proved to be a conservative assumption, compared with the average European mix. Further iteration could focus to specific application and geographical framework.

SUB-PROCESS 9: AIRCEL85_ENERGY CONSUMPTION	RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS
MATERIALS	mixed
MODEL EQUIVALENT	Electricity, low voltage, at grid
QUANTITY	Primary data from manufacturer Energy consumption= 0.71 kWh/day Energy consumption (time span of the technology: 5 years) = 1295.75 kWh
PROCESSING	-
ELABORATION	1295.75 kWh of Electricity, low voltage

Tab. 5.9.: Sub-process 9, relevant data, calculations and assumptions

The biomass fed to the AIRcel system (i.e. U-ox) is a proprietary recipe, therefore only partial information was available to be included into the present study (Tab. 5.10):

1. the biomass is strictly of natural origins;
2. no GMO (i.e. Genetically-Modified Organism) is included within the bacterial consortium;
3. no chemical is added;
4. water is the main constituent of the suspension;
5. production site is located in New Mexico, US. This allowing to estimate air transport in about 9000 km.

In addition to this, it must be considered that the amount of U-ox added to the system is typically 1.2 l per year, except for the first year, when two additional mono-dose are necessary for the start-up. Considering the time-span proposed for the study, it corresponds to 6.2 l, i.e. In this perspective, and, in comparison with air transport, a cut-off of the elaboration process is acceptable.

SUB-PROCESS 10: U-OX_MONODOSE	**RELEVANT DATA, CALCULATIONS AND ASSUMPTIONS**		
	Road transport from manufacturer to airport and from airport to headquarter	Packaging (bottle)	Air cargo (US- EU)
MATERIALS	mixed	PET	mixed
MODEL EQUIVALENT	Transport, lorry >28t, fleet average/CH U	Polyethylene terephthalate, granulate, bottle grade, at plant/RER U	Transport, aircraft, freight, Europe/RER U
QUANTITY	n. of U-Ox monodose necessary over the life-span of the technology (5 years) = 62. Assumption: Weight (U-ox monodose) = 7.5 kg 7.5 kg transported over 200 km (average) = 1.5 tkm	Estimated weight= 0.02 kg per each bottle n.62 monodose for the time-span=	n. of U-Ox monodose necessary over the life-span of the technology (5 years) = 62. Assumption: Weight (U-ox monodose*5 years) = 7.5 7.5 kg transported over 9000 km (average) = 67.5 tkm
PROCESSING	-	Cut off	
ELABORATION	Road transport, 1.5 tkm	1.24 kg of PET bottle	Air transport, 67.5 tkm

Tab. 5.10.: Sub-process 10, relevant data, calculations and assumptions

5.3. Impact assessment

As required by ISO 14040 and ISO 14044, comprehensive Life Cycle Impact Assessment shall include several mandatory elements, such as the selection of impact categories and characterization models, the assignment of results obtained with LCI to the specific impact categories (i.e. Classification phase) and consequent quantification of category indicators (i.e. Characterization). For the present study, additional elaborations have been performed, by the application of Normalization and Weighting, in order to obtain results easier to communicate and share with manufacturer, to promote design effort to improve environmental efficiency of the AIRcel system.

Two calculation methods have been used: a single-issue calculation method (Greenhous Gases Protocol v.1.01) and a typical impact assessment method (IMPACT 2002+).

5.3.1. Greenhouse Gas Protocol

The Greenhouse Gas Protocol (GHG Protocol) method was developed by the World Resources Institute (WRI) in cooperation with the World Business Council for Sustainable Development (WBCSD). This method is based on the draft report on Product Life Cycle Accounting and Reporting Standard (WRI 2009, Frischknecht et al. 2007). It may be assimilated to the Carbon Footprint, defined as the sum of GHG emissions and removals, expressed as net impact on global warming in terms of CO_2 equivalent (JRC 2007, Proietti et al. 2016).

The time horizon of the calculation method is 100 years. Global Warming Potential (GWP) is, therefore, related to 100-year IPCC and it takes into account time-integrated radiative characteristics of well mixed greenhouse gases over the specific period. Carbon dioxide equivalents (CO2eq) of non-CO2 gases (CH_4, N_2O, SF_6, HFCs, CFCs) are calculated and attributed to four impact categories:

1. GHG emissions from fossil sources
2. Biogenic carbon emissions
3. Emissions from land transformation (optional)
4. Carbon uptake (optional).

Results of the impact assessment are reported in the following Figures 5.2 and 5.3 for Characterization and Weighting, respectively. It is immediately evident how energy consumption in the use phase is responsible for the major impact. In fact, about 82% of the total kg CO_2 equivalent are attributable to energy consumption, affecting in particular the emission from fossil fuel.

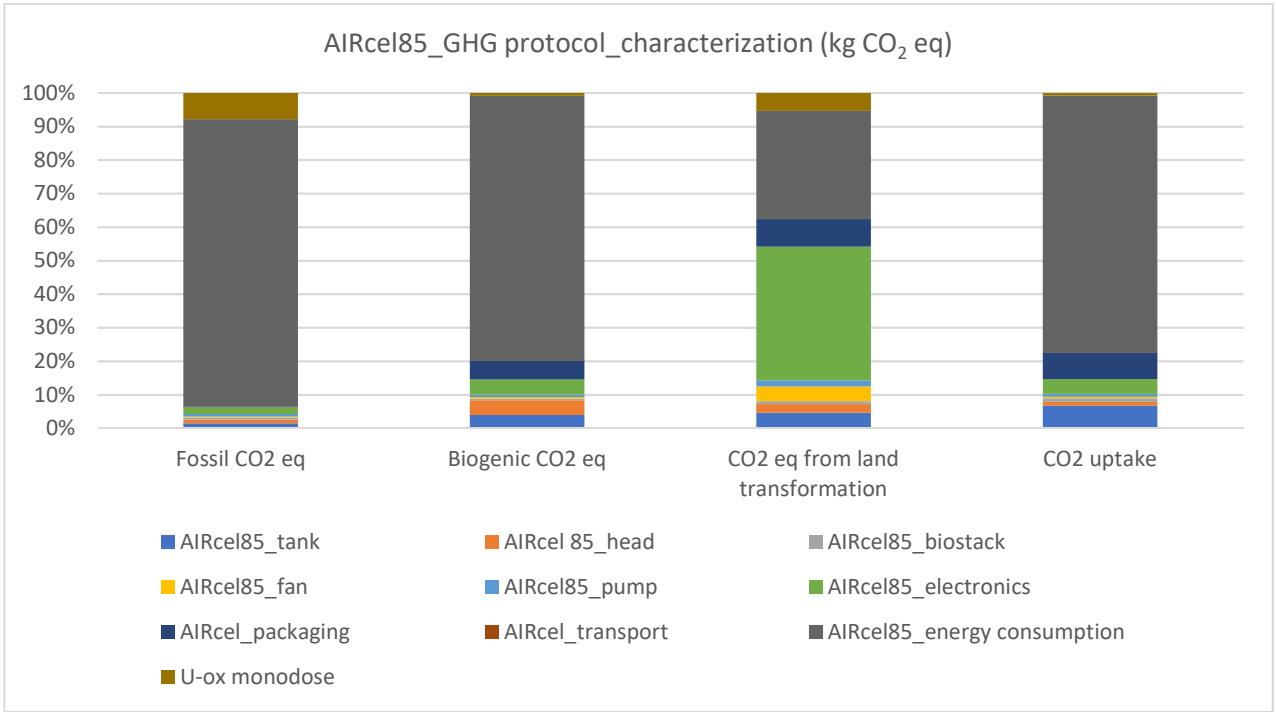


Fig. 5.2.: Impact assessment results, GHG Protocol, Characterization.

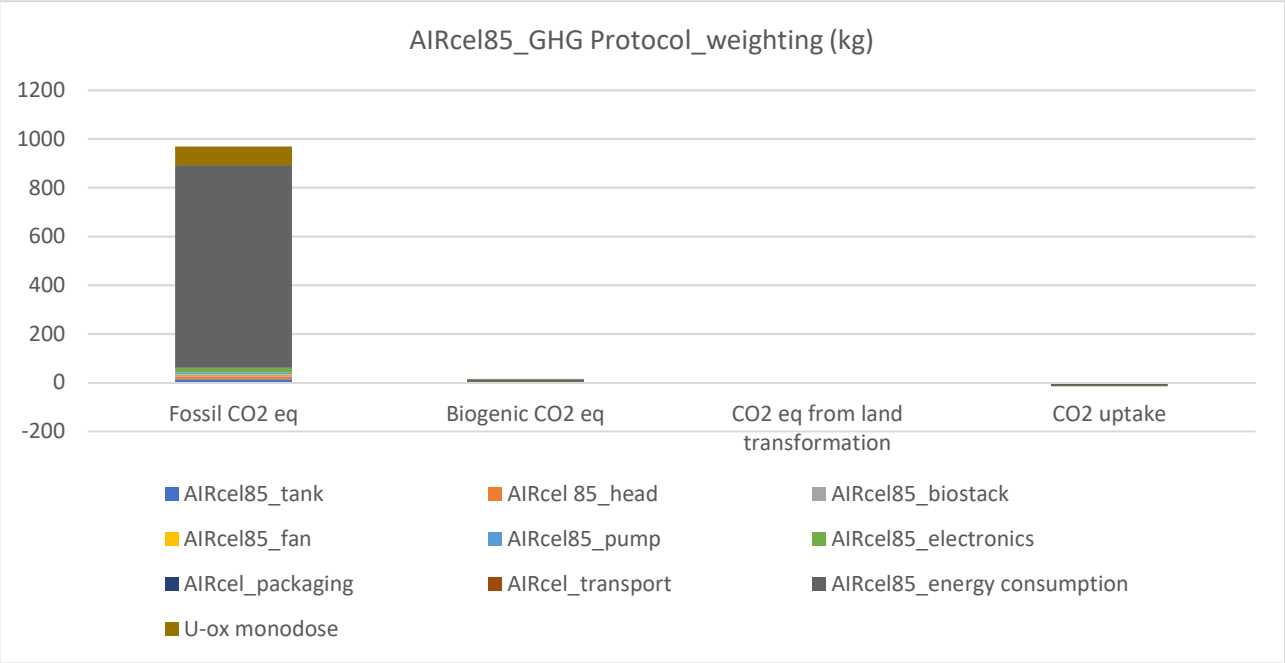


Fig. 5.3.: Impact assessment results, GHG Protocol, Weighting.

	CHARACTERIZATION				WEIGHTING				
IMPACT CATEGORY	Fossil CO2 eq	Biogenic CO2 eq	CO2 eq from land transformation	CO2 uptake	Total	Fossil CO2 eq	Biogenic CO2 eq	CO2 eq from land transformation	CO2 uptake
UNIT	kg CO2 eq	kg CO2 eq	kg CO2 eq	kg CO2 eq	kg	kg	kg	kg	kg
TOTAL	968,3169	14,14716	0,007549	14,36436	968,1073	968,3169	14,14716	0,007549	-14,3644
AIRCEL85_TANK	13,99533	0,55506	0,000348	0,972098	13,57864	13,99533	0,55506	0,000348	-0,9721
AIRCEL 85_HEAD	11,41595	0,621346	0,000198	0,163009	11,87449	11,41595	0,621346	0,000198	-0,16301
AIRCEL85_BIOSTACK	6,015079	0,075552	6,04E-05	0,141388	5,949303	6,015079	0,075552	6,04E-05	-0,14139
AIRCEL85_FAN	2,537156	0,06331	0,000333	0,069171	2,531627	2,537156	0,06331	0,000333	-0,06917
AIRCEL85_PUMP	7,324993	0,109177	0,000139	0,12431	7,31	7,324993	0,109177	0,000139	-0,12431
AIRCEL85_ELECTRONICS	19,08679	0,630135	0,003013	0,637639	19,0823	19,08679	0,630135	0,003013	-0,63764
AIRCEL_PACKAGING	1,866576	0,783271	0,000616	1,145126	1,505336	1,866576	0,783271	0,000616	-1,14513
AIRCEL_TRANSPORT	0,273272	0,000767	2,87E-06	0,000862	0,273179	0,273272	0,000767	2,87E-06	-0,00086
AIRCEL85_ENERGY CONSUMPTION	830,0772	11,18372	0,002435	10,99074	830,2726	830,0772	11,18372	0,002435	-10,9907
U-OX MONODOSE	75,72464	0,124826	0,000404	0,120016	75,72986	75,72464	0,124826	0,000404	-0,12002

Tab. 5.11.: Impact assessment results, GHG Protocol, Characterization and Weighting.

5.3.2. IMPACT 2002+

The IMPACT 2002+ (IMPact Assessment of Chemical Toxics) calculation method has been developed by Swiss Federal Institute of Technology - Lausanne (EPFL, now Ecointesys-life cycle systems). Its most distinctive feature is the implementation of a combined midpoint/damage approach, relating LCI results (i.e. elementary flows) to 4 main damage categories through 14 midpoints indicators (Fig. 5.4).

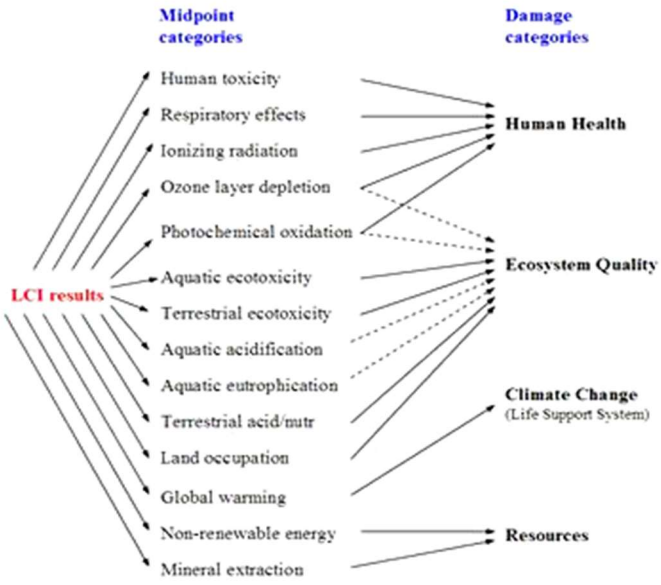


Fig. 5.4.: Impact categories (Jolliet et al. 2003)

Typically, during Characterization phase, LCI results are converted, by the application of specific factors, to a common unit and aggregated for impact categories.

Applying SimaPro software, a modified methodology is used: characterization factors for human toxicity and aquatic and terrestrial ecotoxicity, in fact, are taken from directly IMPACT 2002+, while factors for other categories are adapted from other methods (i.e. Eco-indicator 99, CML 2001, IPCC and Cumulative Energy Demand) and human toxicity is split up in 'Carcinogens' and 'Non-carcinogens'.

Normalization phase is focused on defining the relative magnitude for each indicator result of the product system: each categories' results is, therefore, referred to a reference information, making them dimensionless and, consequently, allowing comparisons.

The Weighting phase involves numerical factors to be attributed to each category on the basis of choice-value.

The following Figures 5.5 and 5.6 report impact assessment results for Characterization and Normalization, listed in Table 5.12.

As evidently displayed by graphics, Global Warming and Non-renewable energy utilization, together with respiratory inorganics category account for more than 90% of the overall impact and the process describing the energy consumption during the use phase is directly responsible for the most part of them (about 80%). These results are confirmed by Weighting phase and consequent attribution to each single process (i.e. Single Score, Fig. 5.7).

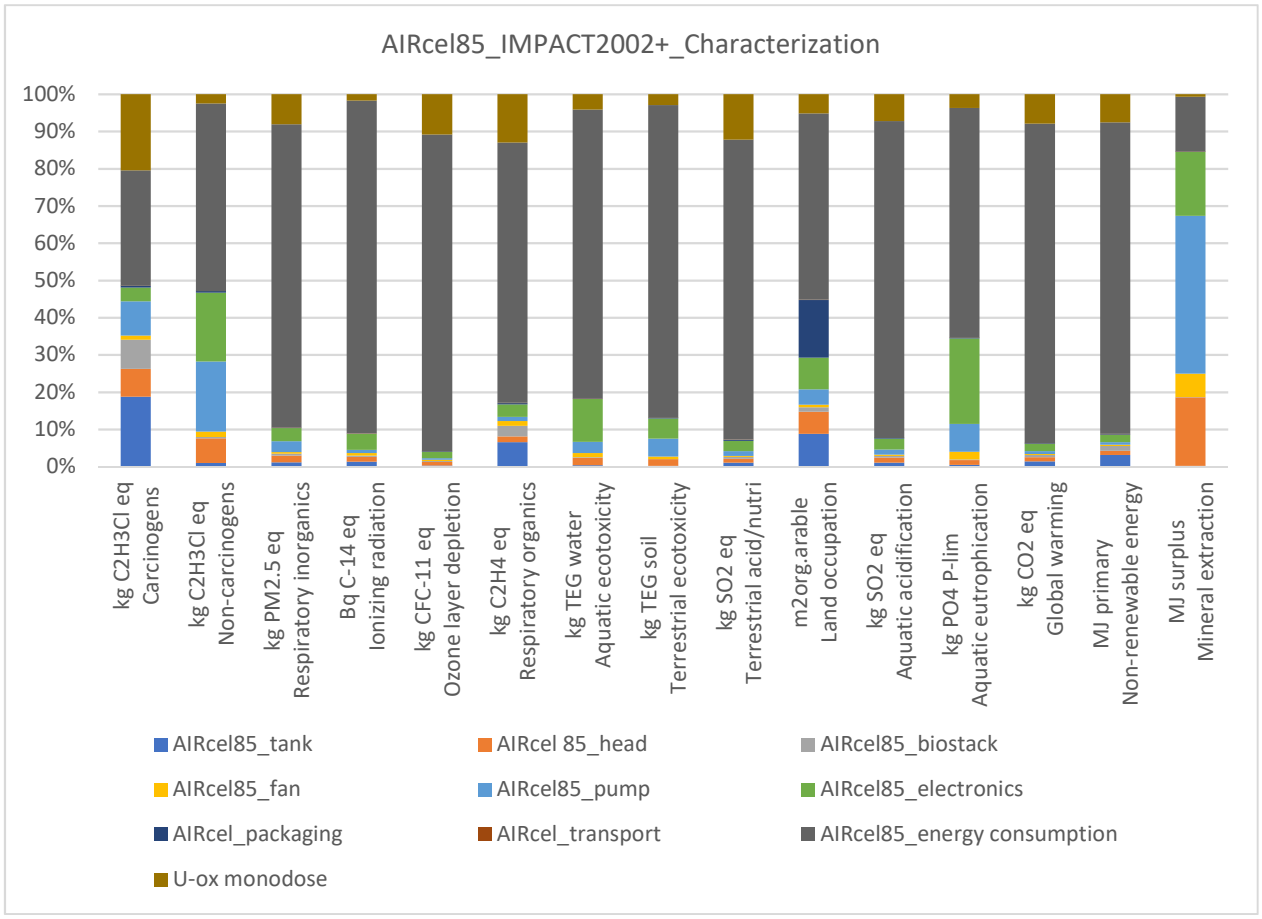


Fig. 5.5.: Impact assessment results, IMPACT2002+, Characterization.

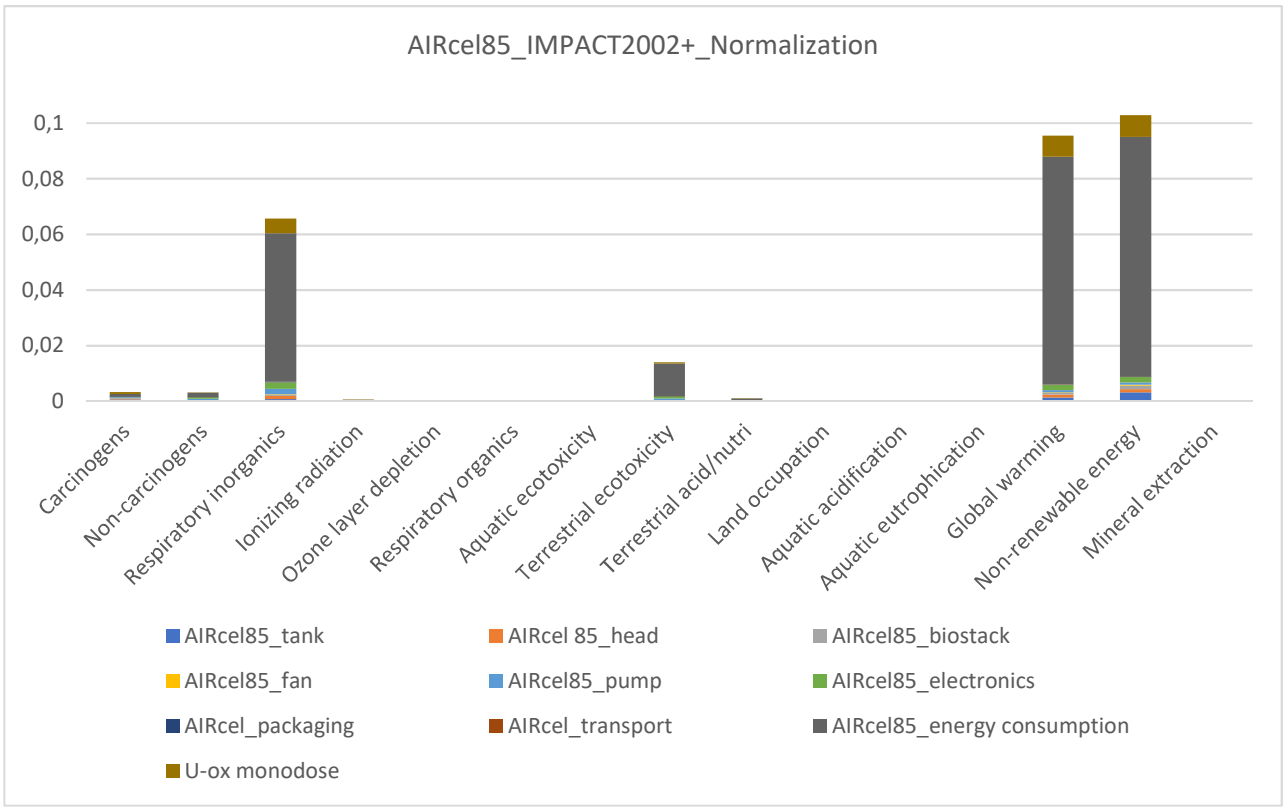


Fig. 5.6.: Impact assessment results, IMPACT2002+, Normalization.

IMPACT CATEGORY	UNIT	TOTAL	AIRCEL85_ TANK	AIRCEL 85_ HEAD	AIRCEL85_ BIOSTACK	AIRCEL85_ FAN	AIRCEL85_ PUMP	AIRCEL85_ ELECTRONICS	AIRCEL_ PACKAGING	AIRCEL_ TRANSPORT	AIRCEL85_ ENERGY CONSUMPTION	U-OX MONODOSE
CARCINOGENS	kg C2H3Cl eq	8,40352	1,578479052	0,628703496	0,659342424	0,088317007	0,776425936	0,310004406	0,043336292	0,002177669	2,595523552	1,72121
NON-CARCINOGENS	kg C2H3Cl eq	7,932215	0,075465286	0,527113608	0,026685013	0,115334118	1,495464906	1,465516471	0,030249105	0,002713648	3,998834156	0,194839
RESPIRATORY INORGANICS	kg PM2.5 eq	0,665263	0,007398909	0,012437738	0,003340695	0,002476864	0,019454756	0,023627291	0,001409419	0,000384916	0,540905714	0,053827
IONIZING RADIATION	Bq C-14 eq	17667,69	236,2781509	217,5896803	68,59827899	113,2552927	152,6006483	759,1363296	28,17202724	2,483545967	15791,41122	298,166
OZONE LAYER DEPLETION	kg CFC-11 eq	8,57E-05	2,17137E-07	8,28285E-07	2,42429E-07	2,80876E-07	3,5688E-07	1,48549E-06	1,32354E-07	4,53217E-08	7,28792E-05	9,25E-06
RESPIRATORY ORGANICS	kg C2H4 eq	0,229418	0,014998327	0,003622933	0,006421764	0,00305428	0,002630842	0,007503598	0,000814059	0,000249427	0,160425996	0,029697
AQUATIC ECOTOXICITY	kg TEG water	73330,14	285,7447726	1453,523953	128,6394211	770,1402963	2254,464156	8460,015074	120,7630075	15,65639073	56807,41767	3033,775
TERRESTRIAL ECOTOXICITY	kg TEG soil	24275,24	43,88948394	436,7127809	14,77578044	151,2688936	1178,078311	1303,190846	49,99122257	9,280165964	20377,95183	710,0983
TERRESTRIAL ACID/NUTRI	kg SO2 eq	15,14972	0,166144146	0,154362868	0,072559434	0,046598422	0,183669022	0,426306394	0,031396888	0,012230581	12,21090701	1,845544
LAND OCCUPATION	m2org. arable	2,502185	0,220620328	0,148722198	0,028493651	0,017052667	0,104329243	0,212659053	0,386615584	0,001971156	1,252287485	0,129434
AQUATIC ACIDIFICATION	kg SO2 eq	4,658435	0,04990753	0,062180145	0,02196889	0,014786493	0,064630969	0,136101792	0,007107798	0,001819266	3,965532999	0,334399
AQUATIC EUTROPHICATION	kg PO4 P-lim	0,226344	0,001136341	0,002729028	0,000493315	0,004663876	0,016836905	0,05195599	0,000493597	2,89498E-05	0,139723638	0,008283
GLOBAL WARMING	kg CO2 eq	944,9109	12,91382604	11,13121953	5,496611893	2,441763483	7,121278934	18,55177765	1,769434188	0,268059464	810,2545517	74,96234
NON-RENEWABLE ENERGY	MJ primary	15620,14	481,2920433	178,5520388	202,4229592	51,73238041	112,122061	302,571818	34,97602902	4,684432202	13074,35448	1177,429
MINERAL EXTRACTION	MJ surplus	21,91092	0,044293218	4,023491833	0,024551563	1,368737312	9,293971526	3,75961634	0,028812213	0,002782528	3,211558949	0,153103

Table 5.12.: Impact assessment results, IMPACT 2002+, Characterization.

IMPACT CATEGORY	TOTAL	AIRCEL85_TANK	AIRCEL 85_HEAD	AIRCEL85_BIOSTACK	AIRCEL85_FAN	AIRCEL85_PUMP	AIRCEL85_ELECTRONICS	AIRCEL_PACKAGING	AIRCEL_TRANSPORT	AIRCEL85_ENERGY CONSUMPTION	U-OX MONODOSE
CARCINOGENS	0,003318	0,000623184	0,000248212	0,000260308	3,48676E-05	0,000306533	0,00012239	1,71092E-05	8,59744E-07	0,001024713	0,00068
NON-CARCINOGENS	0,003132	2,97937E-05	0,000208104	1,05352E-05	4,55339E-05	0,00059041	0,000578586	1,19423E-05	1,07135E-06	0,00157874	7,69E-05
RESPIRATORY INORGANICS	0,065661	0,000730272	0,001227605	0,000329727	0,000244466	0,001920184	0,002332014	0,00013911	3,79912E-05	0,053387394	0,005313
IONIZING RADIATION	0,000523	6,9962E-06	6,44283E-06	2,0312E-06	3,35349E-06	4,51851E-06	2,2478E-05	8,34174E-07	7,35378E-08	0,000467584	8,83E-06
OZONE LAYER DEPLETION	1,27E-05	3,21472E-08	1,22628E-07	3,58917E-08	4,15837E-08	5,28361E-08	2,19927E-07	1,9595E-08	6,70988E-09	1,07898E-05	1,37E-06
RESPIRATORY ORGANICS	6,89E-05	4,50445E-06	1,08808E-06	1,92865E-06	9,17292E-07	7,90121E-07	2,25356E-06	2,44486E-07	7,49104E-08	4,81807E-05	8,92E-06
AQUATIC ECOTOXICITY	0,000269	1,04714E-06	5,32658E-06	4,71412E-07	2,82226E-06	8,26171E-06	3,10026E-05	4,42548E-07	5,73744E-08	0,000208176	1,11E-05
TERRESTRIAL ECOTOXICITY	0,014017	2,53431E-05	0,000252171	8,53198E-06	8,73472E-05	0,000680258	0,000752501	2,88664E-05	5,35865E-06	0,011766841	0,00041
TERRESTRIAL ACID/NUTRI	0,00115	1,26137E-05	1,17192E-05	5,50871E-06	3,53775E-06	1,39442E-05	3,23652E-05	2,38365E-06	9,28546E-07	0,000927052	0,00014
LAND OCCUPATION	0,000199	1,75548E-05	1,18338E-05	2,26724E-06	1,35688E-06	8,30148E-06	1,69213E-05	3,0763E-05	1,56845E-07	9,96445E-05	1,03E-05
AQUATIC ACIDIFICATION	-	-	-	-	-	-	-	-	-	-	-
AQUATIC EUTROPHICATION	-	-	-	-	-	-	-	-	-	-	-
GLOBAL WARMING	0,095436	0,001304296	0,001124253	0,000555158	0,000246618	0,000719249	0,00187373	0,000178713	2,7074E-05	0,08183571	0,007571
NON-RENEWABLE ENERGY	0,102781	0,003166902	0,001174872	0,001331943	0,000340399	0,000737763	0,001990923	0,000230142	3,08236E-05	0,086029252	0,007747
MINERAL EXTRACTION	0,000144	2,91449E-07	2,64746E-05	1,61549E-07	9,00629E-06	6,11543E-05	2,47383E-05	1,89584E-07	1,8309E-08	2,11321E-05	1,01E-06

Table 5.13.: Impact assessment results, IMPACT 2002+, Normalization.

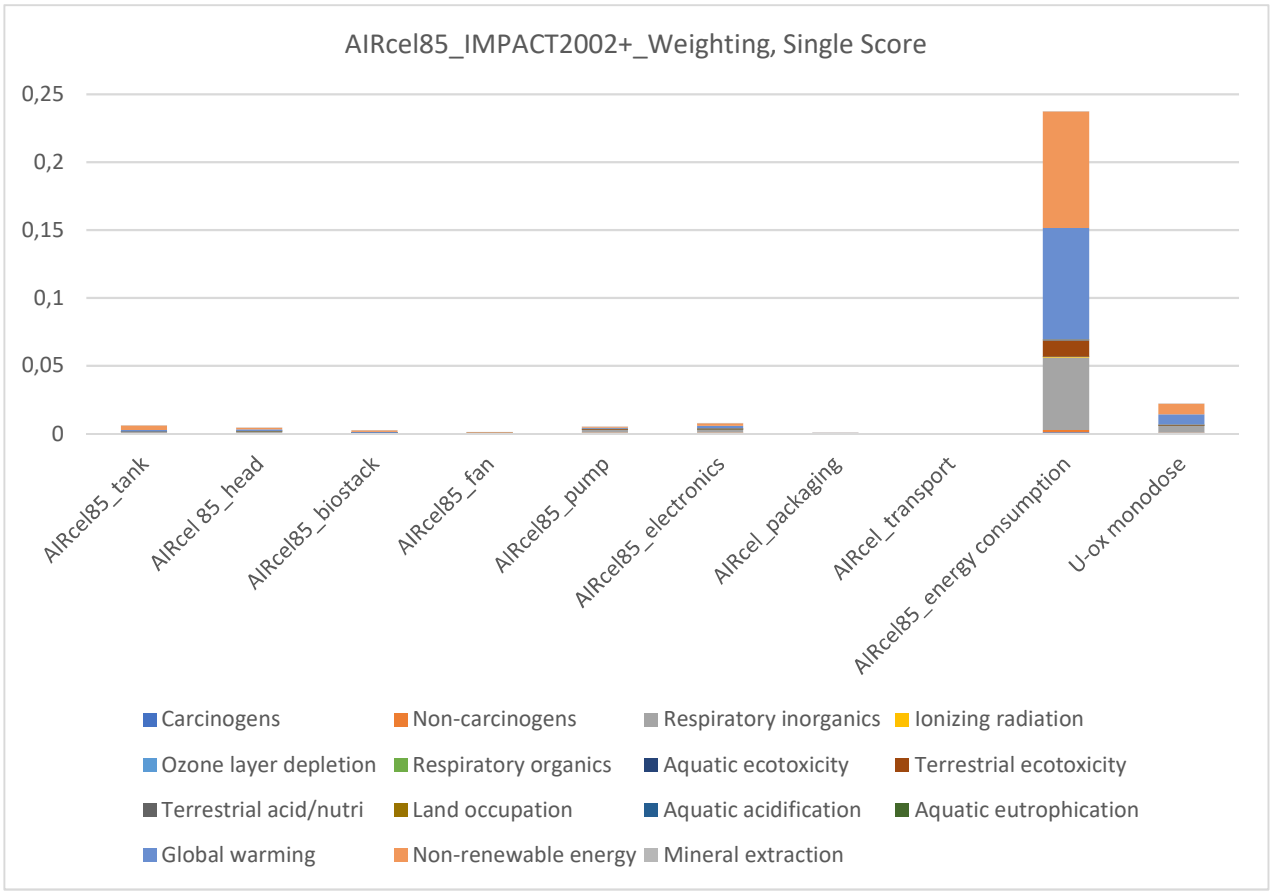


Fig. 5.7.: Impact assessment results, IMPACT2002+, Weighting, Single Score.

5.4. Interpretation of results

The present study focused on production and use phase of the AIRcel85, constituting only the first screening LCA on the specific technology, without taking into account end of life of components and materials. This aspect would be subject to further elaboration, but, at present, it is considered as counterbalanced by not considering the environmental benefit derived by the air treatment performed by the system.

Results obtained into the Impact Assessment phase showed a significant contribution of energy consumption process to the overall impact, in general, and on Global Warming Potential, emission from fossil fuels and respiratory contaminants, in particular.

This is evidently related to the specific energy production mix and could change with different county of application of the technology. Based on evidences provided by model's results, about 80% of the environmental impact generated by AIRcel85 could be tackled and reduced by coupling the air treatment system with renewable energy supply system, already in place or to be implemented. Thus, promoting an improvement of the overall sustainability of the company/facility installing the AIRcel.

The second process promoting the environmental impact of the technology resulted the biomass, i.e. U-ox, which is produced in the US and imported in Italy by the manufacturing company. As expected, intercontinental transport via aircraft impacts remarkably on the overall environmental performance, as shown in Fig. 5.8. As already stated, information on biomass production could not be included in the study, since proprietary recipe covers the item, therefore a cut-off was necessary on the issue.

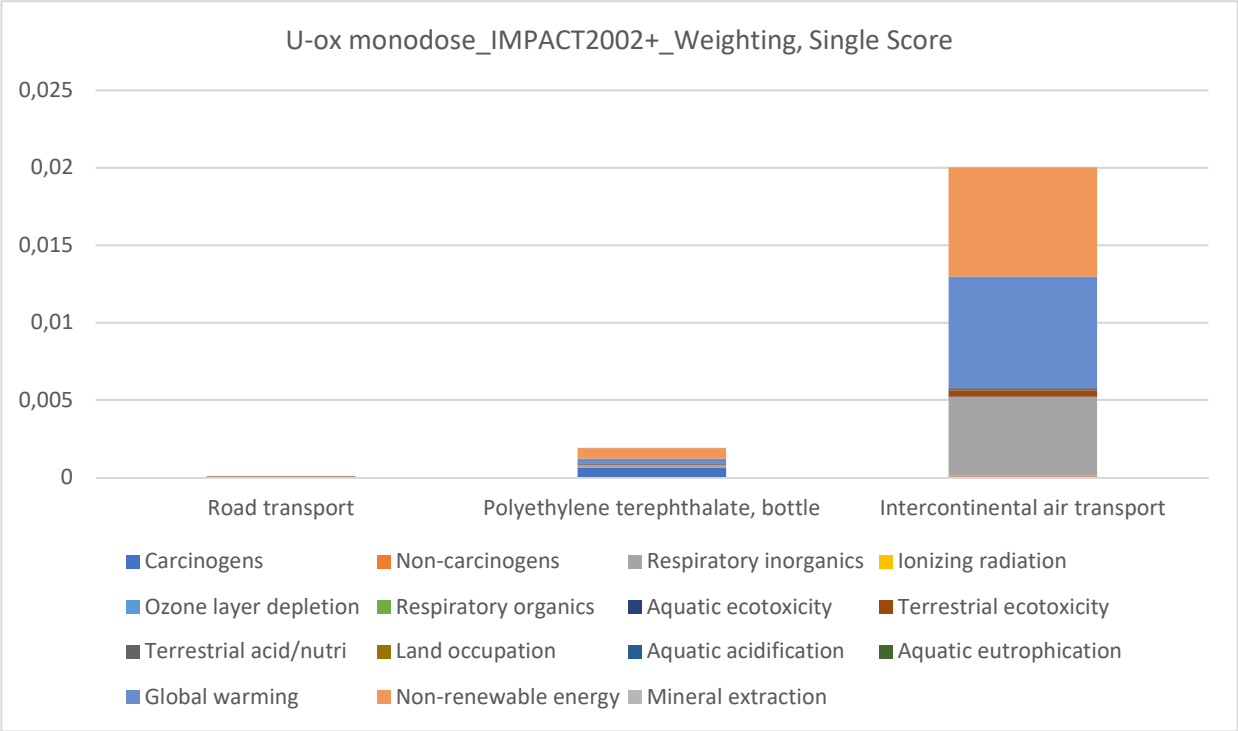


Fig. 5.8.: Impact assessment results for U-ox monodose process, IMPACT2002+, Weighting, Single Score.

Focusing on the CO_2 equivalent impact, results of the two methodologies applied identified AIRcel contribution in about 1 ton of CO_2 equivalent over the 5-year time-span accounted to the technology.
In order to try and assess the environmental burden actually posed by the AIRcel implementation, at present (i.e. with energy consumption affecting the overall performance by 80%), the benefit deriving from air treatment performed should be considered.
Based on information provided by the manufacturer, each AIRcel85 can capture up to 3.5 kg of airborne contaminants per day. Considering a theoretical scenario, with air pollution represented only by VOC, it could be assessed that a single AIRcel unit should be able to treat up to 3.5 kg of VOCs per day. With a rough estimation, i.e. attributing to the VOCs an average Global Warming Potential related to CO_2 equal to 1 (which is a rather conservative assumption, as reported by IPCC 2007), the AIRcel should be able to sequestrate up to 1277.5 kg CO_2 equivalent per year.
This result, compared with the impact calculated, i.e. 193.6 kg CO_2 equivalent per year, returns a net CO_2 equivalent uptake of about 1083.9 kg per year, which can be regarded as quite a remarkable result, even considering limitations of the present study.
An additional comparative evaluation may support the definition of the overall environmental performance, i.e. comparison of the estimated CO_2 equivalent uptake of AIRcel with trees, as typical carbon sinks. In fact, as clearly defined by several studies, they are able to absorb carbon dioxide from the atmosphere, releasing it only partially through night respiration and storing the rest in various organic compounds. Based on most recent results presented by Proietti et al. (2016), trees may store from 4.048 (e.g. oak) to 25.391 (e.g. walnut, poplar) kg CO_2 per year per plant, over the a 14-year time-span, considering both standing and accumulated biomass.
These data, compared with results obtained for the AIRcel allow to compare the biotechnological system performance with a number of trees ranging from 43 (42.68), in case of high growth rate species (e.g. walnut, poplar), to 268 (267.76) in case of low growth rate trees (e.g. oaks).

Considering an average tree density spanning from 230 to 455, as proposed by Khan and Chaudhry (2007) (Tab. 5.14), it could be assessed that the CO_2 net uptake of 1 hectar of properly spaced (i.e. about 300 trees/ha) trees is equivalent to:

- n. 1 AIRcel 85, in case of low growth rate species,
- n. 7 AIRcel 85, if high growth rate species are involved.

TREES DENSITY/HA	HIGH GROWTH RATE SPECIES (WALNUT, POPLAR)	LOW GROWTH RATE SPECIES (OAK)
230 (3.7X12.2 M)	5.4 AIRcel 70	0.9 AIRcel 70
455 (3.7X6.1 M)	10.7 AIRcel 70	1.7 AIRcel 70

Tab. 5.14.: AIRcel70/trees equivalence.

5.5. Conclusion

A screening LCA was accomplished considering production and use phase of a single AIRcel 85 unit. The assessment, even with several assumptions, simplifications and cut-off, returned interesting results, both in terms of impact categories affected and processes triggering the major environmental impacts.

In particular, energy consumption of the device during the use phase proved to develop the major environmental burden (around 80% of the total), confirmed by GHG Protocol and IMPACT 2002+ method. Therefore, the energy production mix used in the specific context of application, is crucial to define the overall impact and measures could be taken accordingly, as to obtain a remarkable reduction.

At the same time, biomass transport from the production site, located in New Mexico, US, heavily affects the global warming effect, as well as respiratory contaminants release, suggesting that a modifyied logistic would ensure a better environmental performance at the production stage.

In an attempt to properly interpret results obtained in the perspective of a remediation technology, i.e. considering the environmental benefit provided by its application, a rough comparison has been performed between net impact, i.e. combining negative and positive impacts, in terms of CO_2 equivalent, provided by AIRcel unit and typical carbon sinks, i.e. trees with different growth rate. Results obtained suggest that a single AIRcel unit could act as carbon sink equivalent to a number of trees ranging from 43, in case of high growth rate species (e.g. walnut, poplar), to 268, in case of low growth rate trees (e.g. oaks).

5.6. Annex I – LCI: Ecoinvent processes documentation

AIRCEL 85_TANK

PROCESS NAME	**polyethylene, HDPE, granulate, at plant/kg/RER**	**thermoforming, with calendering/kg/RER**	**polyvinylchloride, emulsion polymerised, at plant/kg/RER**
TRANSLATED NAME	Polyethylen-Granulat, HDPE, ab Werk	Tiefziehen, mit Kalandrieren	Polyvinylchlorid, Emulsions-Polyvinylchlorid, ab Werk
INCLUDED PROCESSES	Aggregated data for all processes from raw material extraction until delivery at plant. CAS number 009002-88-4. Synonims HDPE, PE, HD-PE.	This process contains the auxillaries and energy demand for the mentioned convertion process of plastics. The converted amount of plastics is NOT included into the dataset.	Aggregated data for all processes from raw material extraction until delivery at plant. CAS number 009002-86-2. Synonyms: PVC
REMARKS	Data are from the Eco-profiles of the European plastics industry (PlasticsEurope).	1 kg of this process equals 0.977 kg of thermoformed, calendered plastic sheets.	Data are from the Eco-profiles of the European plastics industry (PlasticsEurope). Not included are data for recyclable wastes, amount of air / N2 / O2 consumed, unspecified metal emission to air and to water, mercaptan emission to air, unspecified CFC/HCFC emission to air, dioxin to water. The amount of "sulphur (bonded)" is assumed to be included into the amount of raw oil. Waste data are taken from the respective report from 1998 - due to a lack of a similar table in the 2006 report of PlasticEurope.
GEOGRAPHY	24 European production sites	information from different European and Swiss converting companies	10 European plants
TECHNOLOGY	polymerization out of ethylene under normal pressure and temperature	present technologies	production by emulsion polymerization of vinylchloride
TIME PERIOD	time to which data refer	time to which data refer	time to which data refer
VERSION	2.2	2.2	2.2
ENERGY VALUE	Undefined	Undefined	Undefined
PRODUCTION VOLUME	4.31 Mt (1999)	unknown	530 kt (1998)
LOCAL CATEGORY	Kunststoffe	Kunststoffe	Kunststoffe
LOCAL SUBCATEGORY	Polymere (Granulate)	Verarbeitung	Polymere (Granulate)
SOURCE FILE	01829.XML	01856.XML	01842.XML

AIRCEL 85_HEAD

PROCESS NAME	**steel, low-alloyed, at plant/kg/RER**	**deep drawing, steel, 650 kN press, single stroke operation/kg/RER**	powder coating, steel/m2/RER
TRANSLATED NAME	Stahl, niedriglegiert, ab Werk	Tiefziehen, Stahl, 650 kN Presse, Einzelhub-Betrieb	Pulverbeschichten, Stahl
INCLUDED PROCESSES	Mix of differently produced steels and hot rolling	This dataset encompasses the process of deep drawing a part	Phosphating of the steel sheet, powder coating and heat curing. No transports of the steel sheet to the coating plant or back are inventoried.
REMARKS	represents Average of World and European production mix. This is assumed to correspond to the consumption mix in Europe	The reference for deep drawing is 1 kg of metal formed by deep drawing. As there is a large variation from factory to factory with regard to the LCI, it is advised that in case this dataset becomes important in the results, it has to be investigated further if the rough estimations made are applicable or not.	The powder inventoried is mainly used in exterior architectural applications. The coating thickness is 80 µm. The heat consumption is calculated for a sheet of 2 mm thickness.
GEOGRAPHY	Data relate to plants in the EU	Geographical coverage encompasses the industrialised countries.	Swiss waste treatment assumed
TECHNOLOGY	technology mix	Average technology	Phosphating, coating and heat curing. Treatment of the waste water is assumed.
TIME PERIOD	unknown	unknown	unknown
VERSION	2.2	2.2	2.2
ENERGY VALUE	Undefined	Undefined	Undefined
PRODUCTION VOLUME	unknown	0.0	Unknown
LOCAL CATEGORY	Metalle	unknown	Metalle
LOCAL SUBCATEGORY	Gewinnung	Metalle	Verarbeitung
SOURCE FILE	01154.XML	Spanlose Bearbeitung	01167.XML

AIRCEL
85_BIOSTACK

PROCESS NAME	**polyethylene, HDPE, granulate, at plant/kg/RER**	**thermoforming, with calendering/kg/RER**	**polyurethane, rigid foam, at plant/kg/RER**	**injection moulding**
TRANSLATED NAME	Polyethylen-Granulat, HDPE, ab Werk	Tiefziehen, mit Kalandrieren	Polyurethan, Schaum fest, ab Werk	Spritzgiessen
INCLUDED PROCESSES	Aggregated data for all processes from raw material extraction until delivery at plant. CAS number 009002-88-4. Synonims HDPE, PE, HD-PE.	This process contains the auxillaries and energy demand for the mentioned convertion process of plastics. The converted amount of plastics is NOT included into the dataset.	This dataset contains the transports of the monomers as well as the production (energy, air emissions) of the PUR foam. CAS number 009009-54-5	This process contains the auxillaries and energy demand for the mentioned convertion process of plastics. The converted amount of plastics is NOT included into the dataset.
REMARKS	Data are from the Eco-profiles of the European plastics industry (PlasticsEurope).	1 kg of this process equals 0.977 kg of thermoformed, calendered plastic sheets.	Dataset represents just one possible composition for a rigid PUR foam.	1 kg of this process equals 0.994 kg of injection moulded plastics.
GEOGRAPHY	24 European production sites	information from different European and Swiss converting companies	typical composition for European conditions	information from different European and Swiss converting companies
TECHNOLOGY	polymerization out of ethylene under normal pressure and temperature	present technologies	Present technology used in Europe. Transport and infrastructure - average values added.	present technologies
TIME PERIOD	time to which data refer	time to which data refer	date of publication	time to which data refer
VERSION	2.2	2.2	2.2	2.2
ENERGY VALUE	Undefined	Undefined	Undefined	Undefined
PRODUCTION VOLUME	4.31 Mt (1999)	unknown	5 Mt (Total PUR consumption 1990)	unknown
LOCAL CATEGORY	Kunststoffe	Kunststoffe	Kunststoffe	Kunststoffe
LOCAL SUBCATEGORY	Polymere (Granulate)	Verarbeitung	Polymere (Granulate)	Verarbeitung
SOURCE FILE	01829.XML	01856.XML	01839.XML	01853.XML

AIRCEL 85_FAN	
PROCESS NAME	**fan, at plant/kg/GLO**
TRANSLATED NAME	Ventilator, ab Werk
INCLUDED PROCESSES	This dataset contains the material inputs that build up all together a fan, used in a power supply unit of a Desktop PC. Requirements and emissions of the assembly process (energy, emissions, waste) are not included.
REMARKS	This dataset is a rough estimation of the material composition of a fan used in a PSU that can be found in a desktop PC.
GEOGRAPHY	Data used has no specific geographical origin (see Sampling Procedure).
TECHNOLOGY	The dataset represents the material composition of a fan, as used in PSU.
TIME PERIOD	unknown
VERSION	2.2
ENERGY VALUE	Undefined
PRODUCTION VOLUME	unknown
LOCAL CATEGORY	Elektronik
LOCAL SUBCATEGORY	Modul
SOURCE FILE	10806.XML

AIRCEL 85_PUMP PROCESS NAME	**pump 40W, at plant/p/CH/I**	**polyvinylidenchloride, granulate, at plant/kg/RER**	**extrusion, plastic pipes/kg/RER**
TRANSLATED NAME	Umwälzpumpe 40W, ab Werk	Polyvinylidenchlorid-Granulat, ab Werk	Extrudieren, Kunststoffrohre
INCLUDED PROCESSES	Production and disposal of a water pump. Including materials. Not including energy use of production and infrastructure for factory.	Aggregated data for all processes from raw material extraction until delivery at plant. CAS number 009002-85-1.	This process contains the auxillaries and energy demand for the mentioned convertion process of plastics. The converted amount of plastics is NOT included into the dataset.
REMARKS	Water pump Grundfos UP 15-35x20 with a capacity of 40W for use in solar collector systems.	Data are from the Eco-profiles of the European plastics industry (PlasticsEurope). Not included are the values reported for recyclable wastes, amount of air / N2 / O2 consumed, unspecified metal emission to air and to water, mercaptan emission to air, unspecified CFC/HCFC emission to air, dioxin to water. The amount of "sulphur (bonded)" is assumed to be included into the amount of raw oil.	1 kg of this process equals 0.996 kg of extruded plastic pipes.
GEOGRAPHY	Pump produced in CH.	4 European producers	information from different European and Swiss converting companies
TECHNOLOGY	Circulating pump for heat exchange fluid in solar collector systems for a one family dwelling.	production out of vinylidenchloride	present technologies
TIME PERIOD	Time of weighting the different parts of the pump.	time to which data refer	time to which data refer
VERSION	2.2	2.2	2.2
ENERGY VALUE	Undefined	Undefined	Undefined
PRODUCTION VOLUME	Not known	unknown	unknown
LOCAL CATEGORY	Sonnenkollektoranlagen	Kunststoffe	Kunststoffe
LOCAL SUBCATEGORY	Herstellung Komponenten	Polymere (Granulate)	Verarbeitung
SOURCE FILE	01865.XML	01844.XML	01851.XML

AIRCEL 85_ELECTRONICS PROCESS NAME	**potentiometer, unspecified, at plant/kg/GLO**	**steel, electric, chromium steel 18/8, at plant/kg/RER**	**connector, clamp connection, at plant/kg/GLO**	**power adapter, for laptop, at plant/p/GLO**	**LCD module, at plant/kg/GLO**	**Printed wiring board, mounted, LAPTOP PC mainboard, at plant/GLO**	**cable, connector for computer, without plug**
TRANSLATED NAME	Potentiometer, unspezifisch, ab Werk	Elektrostahl, Chromstahl 18/8, ab Werk	Anschluss, Klemmverbindung, ab Werk	Netzteil, für Laptop, ab Werk	LCD Modul, ab Werk	Leiterplatte, bestückt, Laptop PC Mainboard, ab Werk	Kabel, Stromkabel für Computer, ohne Stecker
INCLUDED PROCESSES	This dataset covers raw material input and production efforts for the production of potentiometers (the variable resistor type).	Transports of scrap metal and other input materials to electric arc furnace, steel making process and casting.	This dataset covers raw material input, energy consumption, infrastructure and transport efforts for the production of clamp type connectors.	Describes the production of a typical power adapter for a laptop computer. Calculated per 1 unit of power adapter, as most of them are equally long and of similar shape. Included are the materials steel, copper, HIPS and PVC with their respective manufacturing processes. The infrastructure is calculated via the proxy "electronic component	This module includes the various parts of the complete LCD module. All further efforts (auxiliaries, energy, emissions, waste, …) are included in a separate module – that is linked to this module here.	This dataset represents a mix of Pb-containing and Pb-free soldered laptop PC mainboards. It includes processes of components mounting using lead and lead free solder technology.	Describes the production of a typical electric cable (connector) for a desktop computer. Calculated per 1 m of connector. Included are the materials copper, TPE (elastomere) and PVC with their respective manufacturing processes. The infrastructure is calculated via the proxy "electronic component production plant". Further inventoried are

AIRCEL 85_ELECTRONICS PROCESS NAME	potentiometer, unspecified, at plant/kg/GLO	steel, electric, chromium steel 18/8, at plant/kg/RER	connector, clamp connection, at plant/kg/GLO	power adapter, for laptop, at plant/p/GLO	LCD module, at plant/kg/GLO	Printed wiring board, mounted, LAPTOP PC mainboard, at plant/GLO	cable, connector for computer, without plug
				factory". The electricity for the assembly of the power adaptor is considered to be 25% of the one for the computer mouse. The road and rail transportation for input materials from the regional storage to the production site plus the disposal of the power adapter are part of the dataset.			the electricity for the assembly of the connector, the fuel oil, propane and water input, the disposal of plastic and rubber parts, the VOC and methanol emissions created during the processing, plus the road and rail transportation for input materials from the regional storage to the production site. The accumulated hazardous waste is also reported.
REMARKS	The data represent a typical potentiometer, used in the information and	This process produces secondary steel. Only scrap is used as iron bearing input.	The data represent a current clamp type connector for three wires used in the	This dataset can be applied to describe the production of a typical power adapter for	Data are based on information in a US-EPA study about computer screens. The data represent a	Data are based on own assumption - assuming that in 2005 70% of all laptop PC mainboards are	This dataset can be applied to describe the production of a typical power cord/connector

AIRCEL 85_ELECTRONICS PROCESS NAME	**potentiometer, unspecified, at plant/kg/GLO**	**steel, electric, chromium steel 18/8, at plant/kg/RER**	**connector, clamp connection, at plant/kg/GLO**	**power adapter, for laptop, at plant/p/GLO**	**LCD module, at plant/kg/GLO**	**Printed wiring board, mounted, LAPTOP PC mainboard, at plant/GLO**	**cable, connector for computer, without plug**
	communication technology. Material data are taken from the literature. Infrastructure and production efforts are based on own assumptions. The dataset represents 1 kg of potentiometers with a weight of 6.1 gram/unit.		electronics industry in general. Material data are taken from product sheets of a producer. The energy demand for the production is from literature. Infrastructure and transport efforts rough estimations. The dataset represents 1 kg of clamp type connectors with a weight of 9 gram/unit	laptops. The total weight of the power adapter is 0.357 kg (without the belonging cables, which weigh an additional 0.174 kg). The information for this dataset is based on a study carried out with a leading laptop producer.	current 15-inch LCD computer screen at the end of the 90s.	produced, using Pb-free solder materials.	for computers. The total weight of 1 m of connector is 0.065 kg (without the belonging plugs). The information for this dataset is based on weighing, measurements and analysis at EMPA Laboratories.
GEOGRAPHY	Data represent a global valuable composition of potentiometers.	Data relate to plants in the EU	Data represent a global valuable composition of 3-wired clamp type connectors.	The data describes a typical power adapter for a laptop in 2003. The kind of laptop power adapter is produced by a leading	Data from literature – used as data for the global composition average	Own estimation - used for the global average	The data describes a typical computer cable in 2006. The kind of cable is produced by international manufacturers and available all

AIRCEL 85_ELECTRONICS PROCESS NAME	**potentiometer, unspecified, at plant/kg/GLO**	**steel, electric, chromium steel 18/8, at plant/kg/RER**	**connector, clamp connection, at plant/kg/GLO**	**power adapter, for laptop, at plant/p/GLO**	**LCD module, at plant/kg/GLO**	**Printed wiring board, mounted, LAPTOP PC mainboard, at plant/GLO**	**cable, connector for computer, without plug**
				international computer manufacturer and available all over the world. Therefore a global dataset is justifiable.			over the world. Therefore a global dataset is justifiable.
TECHNOLOGY	Average technology for the production of resistors and potentiometers - comprising termination printing, resistive body printing, glass coat printing, laser trimming, substrate stripping (SMT) resp. ceramic rods, deposition processes, ageing, capping (THT) resp. printing,	EU technology mix (mainly furnace with 4th hole, partly with additional evacuation of building atmosphere). An average for different alloys is represented.	average connector production technology, based on literature and assumptions	The manufacturing process is inventoried through plastic extrusion (for HIPS and PVC), rolling of steel into plates, and wire drawing for copper.	a-Si TFT LCD module	Dataset represents the mix of lead and lead-free mounting of motherboards.	The manufacturing process is inventoried through wire drawing and extrusion of insulation and jacket (TPE, PVC) around wire. Synonims: Power cord, Mains cable, Power cable, Mains lead, Flex, Electric cable, Line, String, Wire, Strand

AIRCEL 85_ELECTRONICS PROCESS NAME	**potentiometer, unspecified, at plant/kg/GLO**	**steel, electric, chromium steel 18/8, at plant/kg/RER**	**connector, clamp connection, at plant/kg/GLO**	**power adapter, for laptop, at plant/p/GLO**	**LCD module, at plant/kg/GLO**	**Printed wiring board, mounted, LAPTOP PC mainboard, at plant/GLO**	**cable, connector for computer, without plug**
	curing, punching, revetting, molding (potentiom.)						
TIME PERIOD	unknown	unknown	unknown	unknown	unknown	unknown	unknown
VERSION	2.2	2.2	2.2	2.2	2.2	2.2	2.2
ENERGY VALUE	Undefined	Undefined	Undefined	Undefined	Undefined	Undefined	Undefined
PRODUCTION VOLUME	unknown	unknown	unknown	unknown	unknown	unknown	unknown
LOCAL CATEGORY	Elektronik	Metalle	Elektronik	Elektronik	Elektronik	Elektronik	Elektronik
LOCAL SUBCATEGORY	Bauteile	Gewinnung	Bauteile	Bauteile	Modul	Modul	Bauteile
SOURCE FILE	07071.XML	01152.XML	07082.XML	07117.XML	07000.XML	07103.XML	07017.XML

AIRCEL 85_PACKAGING

PROCESS NAME	**packaging, corrugated board, mixed fibre, single wall, at plant/kg/RER**	polyurethane, flexible foam, at plant/kg/RER	packaging film, LDPE, at plant/kg/RER
TRANSLATED NAME	Wellkartonverpackung, gemischte Fasern, einwellig, ab Werk	Polyurethan, Schaum flexibel, ab Werk	Verpackungsfolie, LDPE, ab Werk
INCLUDED PROCESSES	This module includes the production of boxes out of corrugated board. It contains the steps of cutting, folding and printing. Besides the input of corrugated board, inks and glues are considered as well as the electricity consumption.	This dataset contains the transports of the monomers as well as the production (energy, air emissions) of the PUR foam (CAS number 009009-54-5)	This process contains the plastic amount and the transport of the plastic from the production site to the converting site as well as the dataset "extrusion, plastic film"
REMARKS	This is an example for the production of boxes out of corrugated board. The user can establish himself further boxes based on the information in this step.	Dataset represents just one possible composition for a flexible PUR foam.	Example process for the utilization of the different converting modules in the database.
GEOGRAPHY	Estimation based on average data from European producers, collected from FEFCO.	Typical composition for European conditions	
TECHNOLOGY	Average of present used technology	Present technology used in Europe. Transport and infrastructure - average values added.	Average of present used technology
TIME PERIOD	unknown	date of publication	time to which data refer
VERSION	2.2	2.2	2.2
ENERGY VALUE	Undefined	Undefined	Undefined
PRODUCTION VOLUME	unknown	5 Mt (Total PUR consumption 1990)	unknown
LOCAL CATEGORY	Papiere & Karton	Kunststoffe	Kunststoffe
LOCAL SUBCATEGORY	Karton und Wellkarton	Polymere (Granulate)	Verarbeitung
SOURCE FILE	01698.XML	01838.XML	01854.XML

AIRCEL 85_TRANSPORT	
PROCESS NAME	**transport, lorry >28t, fleet average/tkm/CH**
TRANSLATED NAME	Transport, Lkw >28t, Flottendurchschnitt
INCLUDED PROCESSES	operation of vehicle
REMARKS	Inventory refers to the entire transport life cycle. For road infrastructure, expenditures and environmental interventions due to construction, renewal and disposal of roads have been allocated based on the Gross tonne kilometre performance. Expenditures due to operation of the road infrastructure, as well as land use have been allocated based on the yearly vehicle kilometre performance. For the attribution of vehicle share to the transport performance a vehicle life time performance of 2.39E05 pkm/vehicle has been assumed.
GEOGRAPHY	The data for vehicle operation and road infrastructure reflect Swiss conditions. Data for vehicle manufacturing and maintenance represents generic European data. Data for the vehicle disposal reflect the Swiss situation.
TECHNOLOGY	Petrol, various emission treatment standards
TIME PERIOD	unknown
VERSION	2.2
ENERGY VALUE	Undefined
PERCENTAGE REPRESENTATIVENESS	100.0
PRODUCTION VOLUME	not known
LOCAL CATEGORY	Transportsysteme
LOCAL SUBCATEGORY	Strasse
SOURCE FILE	01944.XML

AIRCEL 85_ENERGY CONSUMPTION	
PROCESS NAME	**Electricity, low voltage, at grid/IT U**
TRANSLATED NAME	STROM, NIEDERSPANNUNG, AB NETZ
INCLUDED PROCESSES	Included are the electricity production in Italy and from imports, the transmission network as well as direct SF6-emissions to air. Electricity losses during low-voltage transmission and transformation from medium-voltage are accounted for.
REMARKS	This dataset describes the transformation from medium to low voltage as well as the distribution of electricity at low voltage.
GEOGRAPHY	Data apply to public and self-producers. Geographical classification according to IEA. Assumptions for transmission network, losses and emissions are based on Swiss data.
TECHNOLOGY	Average technology used to distribute electricity. Includes underground and overhead lines, as well as air- and SF6-insulated medium-to-low voltage switching stations. Electricity production according to related datasets
TIME PERIOD	Time of publications.
VERSION	2.2
ENERGY VALUE	Undefined
PERCENTAGE REPRESENTATIVENESS	100.0
PRODUCTION VOLUME	183 TWh
LOCAL CATEGORY	Elektrizität
LOCAL SUBCATEGORY	Versorgungsmix
SOURCE FILE	00757.XML

U-OX_MONODOSE PROCESS NAME	**transport, aircraft, fright, europe/rer**	**transport, lorry >28t, fleet average/tkm/CH**	**polyethylene terephthalate, granulate, bottle grade, at plant/RER U**
TRANSLATED NAME	Transport, Luftfracht, Intercontinental	Transport, Lkw >28t, Flottendurchschnitt	Polyethylenterephthalat-Granulat, Flaschengrad, ab Werk
INCLUDED PROCESSES	The module calls the modules addressing: operation of aircraft; production of aircraft; construction and land use of airport; operation, maintenance and disposal of airport.	operation of vehicle	Average data for the production of bottle grade PET out of ethylene glycol, PTA and amorphous PET. The data include material and energy input, waste as well as air and water emissions. Missing sum parameters to water (DOC, TOC, COD), transport and infrastructure are estimated. CAS number 025038-59-9
REMARKS	Inventory refers to the entire transport life cycle. Airport infrastructure expenditures and environmental interventions are accounted for using the yearly transport performance at uniqueairport in Zurich (2'020'000'000 tkm/a). Aircraft manufacturing is allocated based on the total life span of an aircraft (5.59E+10) and its transport performance (25t/unit).	Inventory refers to the entire transport life cycle. For road infrastructure, expenditures and environmental interventions due to construction, renewal and disposal of roads have been allocated based on the Gross tonne kilometre performance. Expenditures due to operation of the road infrastructure, as well as land use have been allocated based on the yearly vehicle kilometre performance. For the attribution of vehicle share to the transport performance a vehicle life time performance of 2.39E05 pkm/vehicle has been assumed.	Data are based on the average unit process from the Eco-profiles of the European plastics industry
GEOGRAPHY	Data from Switzerland is employed as a first estimate for Europe.	The data for vehicle operation and road infrastructure reflect Swiss conditions. Data for vehicle manufacturing and maintenance represents generic European data. Data for the vehicle disposal reflect the Swiss situation.	Data from several European production sites
TECHNOLOGY	For aircraft operation merley passenger jets are included in the average data. For the manufacturing of aircrafts	The data for vehicle operation and road infrastructure reflect Swiss conditions. Data for vehicle manufacturing and maintenance represents	PET production out of PTA and ethylene gylcol

U-OX_MONODOSE PROCESS NAME	**transport, aircraft, fright, europe/rer**	**transport, lorry >28t, fleet average/tkm/CH**	**polyethylene terephthalate, granulate, bottle grade, at plant/RER U**
	modern production technologies are taken into account.	generic European data. Data for the vehicle disposal reflect the Swiss situation.	
TIME PERIOD	unknown	Petrol, various emission treatment standards	Date of publication
VERSION	2.2	unknown	2.2
ENERGY VALUE	Undefined	2.2	Undefined
PRODUCTION VOLUME	-	Undefined	933 kt (2000)
LOCAL CATEGORY	Transportsysteme	100.0	Kunststoffe
LOCAL SUBCATEGORY	Luft	not known	Polymere (Granulate)
SOURCE FILE	01894.XML	Transportsysteme	01828.XML

PART III: Applications

Chapter 6 – Air Treatment Applications

6.1. Healthcare

Indoor Air Quality (IAQ) may be regarded as a critical factor, in particular, in healthcare facilities, due to the multiplicity of areas, services and activities involved, as well as the high number and diversity of occupants, on which the presence of various pollutants may cause adverse effects (Fekadu et al. 2015, Leung et al. 2006). In order to optimize the IAQ management in healthcare facilities, a multidisciplinary approach (from the scientific, technical and political point of view) would be necessary for the identification, prevention and correction of possible issues. This approach is critical to ensure an IAQ that minimizes potential adverse effects on occupant health, provides comfort, well-being, favorable conditions both for patient recovery and health professionals' productivity, together with cost optimization (Piteira 2007, Berton et al. 2012, Helmis et al. 2008, Salonen et al.2013).

The market currently offers a set of technological solutions focused on reducing indoor air pollution in healthcare facilities. These technologies have proven to be effective at the experimental level, with positive results in real scale application, both in improving IAQ and in protecting occupants (Leung et al. 2006, Otter et al. 2011, Wan et al. 2011).

Healthcare units' environment, as mentioned above, present several factors influencing IAQ. Given the high daily number of occupants in each dialysis unit, its structural characteristics, the type of activities performed (e.g. chemicals used in the disinfection of hemodialysis machines and anesthetic gases) and technological solutions for indoor air purification currently available in the market, the need to evaluate and characterize the IAQ and to test the evolution of the same after the installation of a technological system for indoor air purification was identified. For this reason, different potentially critical environments were selected for the implementation of the air treatment bioreactors' system, in cooperation with Fresenius Medical Care.

Fresenius Medical Care is the world's leading provider of products and services for people with chronic kidney failure. FMC cares for nearly 300,000 patients in a global network of more than 3,400 dialysis clinics. At the same time, FMC operates 40 production sites on all continents, to provide dialysis products such as dialysis machines, dialyzers and related disposables. The Company strategy is geared toward sustainable growth, aimed to continuously improve the quality of life of patients with kidney disease by offering innovative products and treatment concepts of the highest quality.

The applicative research effort focused, in particular, on:

1. Hemodialysis Unit
2. Operating Theatre
3. Intensive Care Unit
4. Pathology Laboratory.

6.1.1. Case studies

6.1.1.1. Hemodialysis unit

The present study has been elaborated together with Ana Cristina Duarte de Resende, Rui Lucena (Fresenius Medical Care) and Professor Doutor António de Sousa Uva (Universidade Nova de Lisboa, Escola Nacional de Saúde Pública). It has been submitted for publication.

Abstract

Several technical solutions, supported by scientific research work, have been proposed for Indoor Air Quality (IAQ) management in healthcare facilities, aimed to minimize potential health risk carried by different pollutants, chemical and microbiological, both for patients and staff. The IAQ has a significant impact on the welfare, health, behavior and productivity of occupants.

The objective of present study was the evaluation of an IAQ management system consisting in a biotechnology (bioreactors) for indoor air treatment. The study was conducted in a dialysis unit located in Lisbon, and, in particular, on four dialysis rooms.

The research methodology applied involved IAQ characterization through the monitoring of several physical, chemical and microbiological parameters before and after installation of the bioreactors, as to assess their possible impact on IAQ.

The initial characterization showed that IAQ of the dialysis unit was affected by the presence of certain chemical pollutants, such as formaldehyde (CH_2O) and total volatile organic compounds (total VOCs), typically associated to the frequent use of disinfectants related to the specific medical practice. As major outcome of the installation of the bioreactors, a decrease in the average concentration of about 90% for CH2O, 80% for bacteria and 70% for filamentous fungi and yeasts was detected. At the same time an increase of relative humidity (RH) and suspended particles concentration (PM_{10}) was registered.

Given the results of this study, the biotechnological system may be considered as a potential solution, complementing HVAC systems, to improve IAQ in the dialysis unit.

Keywords: Air pollutants; Indoor air quality; Healthcare; Air purification

Introduction

In this context, a case study was developed in a dialysis unit in Lisbon, with the application of a biotechnological system (bioreactors) for air treatment.

The system installed is modular and based on stand-alone bio-oxidizers, providing internal air-mixing within the hospital and capturing airborne pollutants thanks to convection and diffusion (Bonoli et al. 2014a, 2014b).

The objective of the present study is to assess the impact of the biotechnological system implemented in the dialysis unit, through the characterization of indoor air, before and after its installation.

For this study, the most relevant environmental parameters for IAQ have been identified in:

physical, such as temperature (T) and relative humidity (RH);

chemical, such as formaldehyde (CH_2O), total volatile organic compounds (total VOCs), carbon monoxide (CO), carbon dioxide (CO_2), ozone (O_3), suspended particles – 10 microns (PM10);

microbiological, such as total bacteria at 36 °C, filamentous fungi and yeasts.

In particular, T and RH play an important role both in the thermal comfort of the occupants and on the growth of microorganisms, as well as on the durability of the building and its materials and the energy consumption (APA 2010). In indoor environments, building and decoration materials, together with the use of disinfectants, are regarded as the main sources of CH2O emission (Martinez et al 2006, Dales et al. 2008, APA 2015). The concentration of total VOCs in indoor spaces is associated with the use of detergents and disinfectants, anesthetic gases, computers, building materials and decoration, among others (APA 2010, Martinez et al. 2006). According to Martínez and Callejo (Martinez et al. 2006), the concentration of VOCs in indoor air can be up to ten times higher than in outdoor air. In order to deal with this situation, Proença and Cano (Proença et al. 2010) propose two strategies: to promote the ventilation of spaces and to control emission sources. The presence of CO is typically related to tobacco smoke, burning of fossil fuels (e.g. oil,

coal, wood, gas) and emissions from vehicles (e.g. garages, or outdoor air) (WHO 2010, APA 2010), while CO_2 in indoor environments is mainly due to human presence (respiration) and the burning of fossil fuels (APA 2010). The standard indoor sources of O_3 are, among others: electric motors, electrostatic air purifiers, exterior air (Martinez et al. 2006). PM may infiltrate indoor through HVAC systems or through natural ventilation. Their presence in these spaces is also due to specific emission sources, such as building and decoration materials (e.g. furniture, carpeting, coating and insulation materials), tobacco smoke and occupants' activities (APA 2010, Martinez et al. 2006). In indoor environments, the development of biological agents is attributed to poor ventilation and excess humidity. These microorganisms, such as bacteria and fungi, are responsible for the emission of spores, cells, fragments and VOCs (APA 2010).
Due to the application of this biotechnological system, a decrease in concentration of indoor air pollution was expected into the dialysis unit selected for the study and it has been achieved for several key parameters.

Materials and methods

Case study

The experiment has been carried out into a running dialysis unit in Lisbon. A dialysis unit is a healthcare facility, primarily dedicated to the outpatient treatment for chronic renal failure (Miguel et al. 2012).
Patients on chronic dialysis usually have three dialysis sessions per week, with each treatment lasting four to five hours (Miguel et al. 2012, Grassmann et al. 2000). The average dialysis unit performs two shifts a day and it operates six days a week, as each patient is treated three times a week (Monday-Wednesday-Friday or Tuesday-Thursday-Saturday).
The pilot application and indoor air quality monitoring were carried out over 10 months.
In order to meet to the goal of the study, i.e. impact assessment of an air treatment biotechnology into a dialysis unit, it has been organized in two distinct phases:
pre-installation of the bioreactors, for the characterization of the initial conditions;
post-installation of the bioreactors, to evaluate the impact of the technology on IAQ.
The pilot areas are located at ground floor and they are constituted of four dialysis rooms, where renal replacement therapy is performed. They have been selected because they present a higher occupancy rate compared to the other spaces of the dialysis unit and they are regarded as critical areas for infection control.

- The dialysis room A (DRA) has an area of approximately 211 m^2, with 25 dialysis stations.
- The dialysis room B (DRB) has an area of approximately 222 m^2, with 29 dialysis stations.
- The dialysis room C (DRC) consists of 7 dialysis stations and an area of approximately 60 m^2.
- The HBV dialysis room (DRHBV) has the capacity to treat 7 patients simultaneously and occupies an area of 56 m^2.

The biotechnological system for indoor air purification under study takes advantage of a natural mechanism to treat the air, i.e. biological oxidation or biodegradation. In general terms, this trigger the digestion of airborne pollutants, performed by microorganisms and enzymes (Bonoli et al. 2014a, 2014b).
This technology is constituted of two main components:
Bioreactors: portable equipment, whose operation consists of three phases in close contact: solid phase (the bioreactor itself), liquid phase (water) and gas phase (air to be treated);
Biomass: an additive that is introduced into bioreactors, composed of selected, non-pathogenic, non-genetically modified microorganisms and enzymes (Bonoli et al. 2014a, 2014b).
The bioreactors system set up has been defined accordingly to manufacturer's indications and experience on the number and size of units and their positioning (Figure 1). Architecture of the dialysis unit, dimensions of the rooms, location of Heating, Ventilation and Air Conditioning (HVAC) system, doors and windows and location of supply of water and electricity have been taken into consideration.

In DRA, as well as in DRB, two mid-size bioreactors have been placed, while in DRC and DRHBV two small-size bioreactors have been installed per each.
The biotechnological system was installed four days after initial characterization of IAQ and it operated in continuous mode. During the testing period, it run 24 hours a day, except for occasional malfunctions and bioreactors complete cleaning, carried out after four months from the installation, in order to ensure the proper functioning of the system.
The replacement of the biomass, responsible for the biological oxidation of indoor air pollutants, was carried out following a planned schedule:

- 1st refill: at the start-up of the bioreactors;
- 2nd refill: 15 days after the 1st refill;
- 3rd refill: 15 days after 2nd refill;
- Following refill: 30 days after the previous one.

A daily check of the water level inside the bioreactors proved to be necessary and occasional replenishment has been performed.

Sampling and analytical methodology

At the methodological and operational level, measurements and samplings of indoor air were carried out, considering several environmental parameters.
Both the sampling process and the laboratory analysis has been performed by an independent laboratory, i.e. Laboratory of Analyzes of the Instituto Superior Técnico (LAIST).
The sampling points have been selected according to the APA technical guide (APA 2010) considering the following aspects:

- architecture of the dialysis unit;
- 1 to 2 m distance from corners, windows, HVAC system, partitions and other vertical surfaces (e.g. cabinets);
- 1.5 ± 0.5 m above ground level (APA 2010).

The minimum number of sampling points in each pilot area was defined according to the APA technical guide (APA 2010), based on the following expression:
$Ni = 0{,}15 \times \sqrt{Ai}$
Ni: number of sampling points in zone i
Ai: area of zone i in m^2
As reported in Figure 6.1, two sampling points per each dialysis room have been identified.

Figure 6.1: Bioreactors and sampling point positioning.

Air sampling were performed at different times:

- t_0: before the installation of the bioreactors (for the characterization of the initial conditions);
- t_1: 15 days after t_0;
- t_2: 30 days after t_1;
- t_3: 90 days after t_2;
- t_4: 90 days after t_3;
- t_5: 75 days after t_4.

The various samplings were carried out on the same day of the week, i.e. on Sunday, due to the dialysis unit working schedule, which is organized over 6 days a week, excluding Sunday. In this way, indoor air sampling was not directly subject to interference and fluctuations of external factors related to dialysis unit activities (e.g. use of chemicals for hemodialysis machines disinfection or anesthetics, opening/closing of doors), which could add confusing variables to the analysis.

During sampling, the HVAC system was working at same conditions as in operating time of the dialysis unit and the occupation of the rooms was of three people.
For air monitoring, standards methods have been applied and the following sampling instruments have been used:

- For T, RH, CO_2 and CO: probe, testo 435 (Testo AG);
- For CH_2O: electrochemical, Formaldemeter™ htV (PPM Technology);
- For VOCs: photoionizer, PHocHeck +® (Ion Science);
- For O_3: electrochemical, Series 200 (Aeroqual);
- For PM_{10}: optical dispersion, DustTrak™ 8520 (TSI Inc.);
- For bacteria, filamentous fungi and yeasts: M.M. 9.6 (2011-05-30; LAIST standard), MAS-100® (Merck Millipore).

The physical and chemicals parameters were determined locally, during the sampling, with no need for further elaboration. Short-term measurements of approximately 15 minutes were performed using direct reading equipment.
For the determination of the microbiological parameters, samples of 0.5 m^3 of air were collected on a Petri dish with the appropriate semi-solid culture medium (i.e. *Tryptic Soy Agar* for bacteria; *Malt Extract Agar* for filamentous fungi and yeasts). After the sampling, the Petri dishes have been incubated at appropriate temperature (i.e. 36°C for bacteria and 27°C for filamentous fungi and yeasts) for different periods (i.e. 2 days for bacteria and 7 days for filamentous fungi and yeasts) and colonies have been counted for analysis.

Statistical analysis

After completing the sampling process and validating the results, a statistical analysis has been performed in order to assess the effect of the biotechnology on the IAQ. In this way, t_0 values have been compared to t_5 ones, using the t-Student parametric test for paired samples. Statistical tests were performed with SPSS Statistics software (v.22, IBM SPSS, Chicago, IL).
To optimize the analysis of results, the arithmetic mean of the values obtained at the two sampling points in each dialysis room has been calculated and used for the assessment.

Results

The different parameters tested (physical, chemical and microbiological) are presented as evolution of the mean values obtained in each dialysis room (DRA, DRB, DRC and DRHBV) over the different sampling times (t_0, t_1, t_2, t_3, t_4 and t_5).
Figure 6.2 shows the mean temperature (in ºC) and relative humidity (in %) values recorded in the pilot areas.

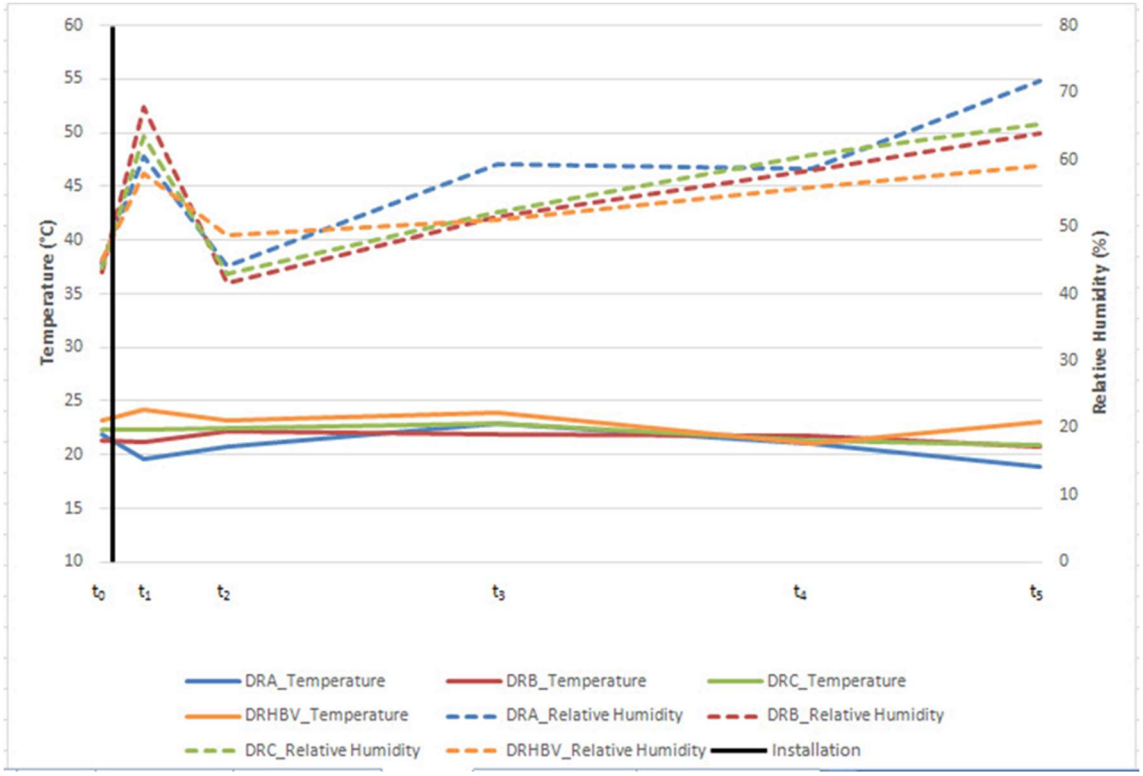


Figure 6.2: Average T and RH values for the pilot areas at the different sampling times.

At t_0 (pre-installation of the bioreactors), the average temperature value was 22.23ºC and at time t_5 (post-installation of the bioreactors), the average temperature value was 20,91ºC (difference of 1,313ºC between t_0 and t_5). The observed difference between the mean values of temperature (ºC) in the two sampling times is not statistically significant (p = 0.053).

In the dialysis rooms, at t_0, the mean value of HR was 44.18% and at time t_5 it was 65.08%, with an increase of 20.90% between t_0 and t_5. The difference observed between the mean HR values at the two sampling times is statistically significant (p <0.05).

Figure 6.3 shows the mean concentration of total CH_2O and total VOCs (both in $\mu g/m^3$) registered in the pilot areas.

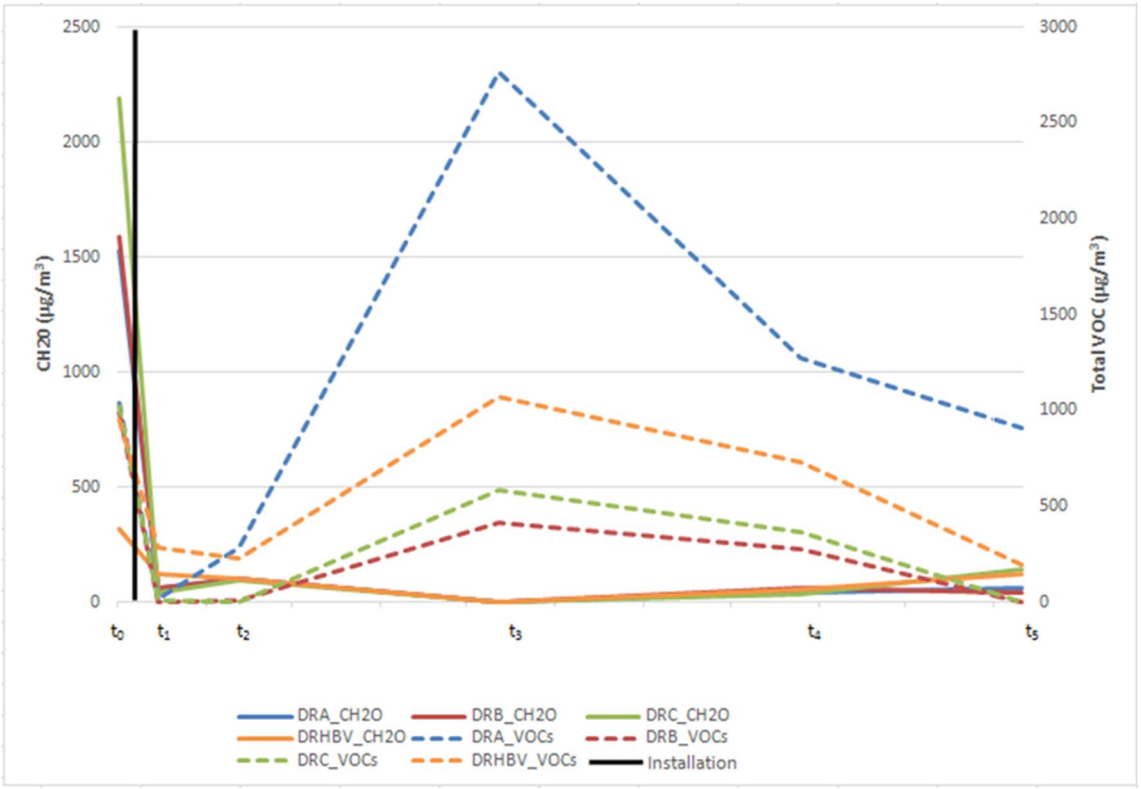


Figure 6.3: Average CH_2O and total VOCs concentrations for the pilot areas at the different sampling times.

At t_0, the mean value of CH_2O was 1406.25 µg/m³ and at time t_5 it was 90.00 µg/m³ (difference of 1316.25 µg/m³ between t_0 and t_5). The difference between the average concentrations CH_2O of the two groups (t_0 and t_5) is statistically significant (p = 0.004).

At t_0, the mean value of total VOCs was 996.75 µg/m³ and at time t_5 the mean value was 556.00 µg/m³ (difference of 440.75 µg/m³ between t_0 and t_5). The differences observed between the mean values of total VOCs of the two groups (t_0 and t_5) are not statistically significant (p = 0.326).

A peak in total VOCs on t_3 was observed.

During this investigation, only the determination of particulate class PM_{10} was performed, whose average concentration trend (in µg/m³) is represented in Figure 6.4.

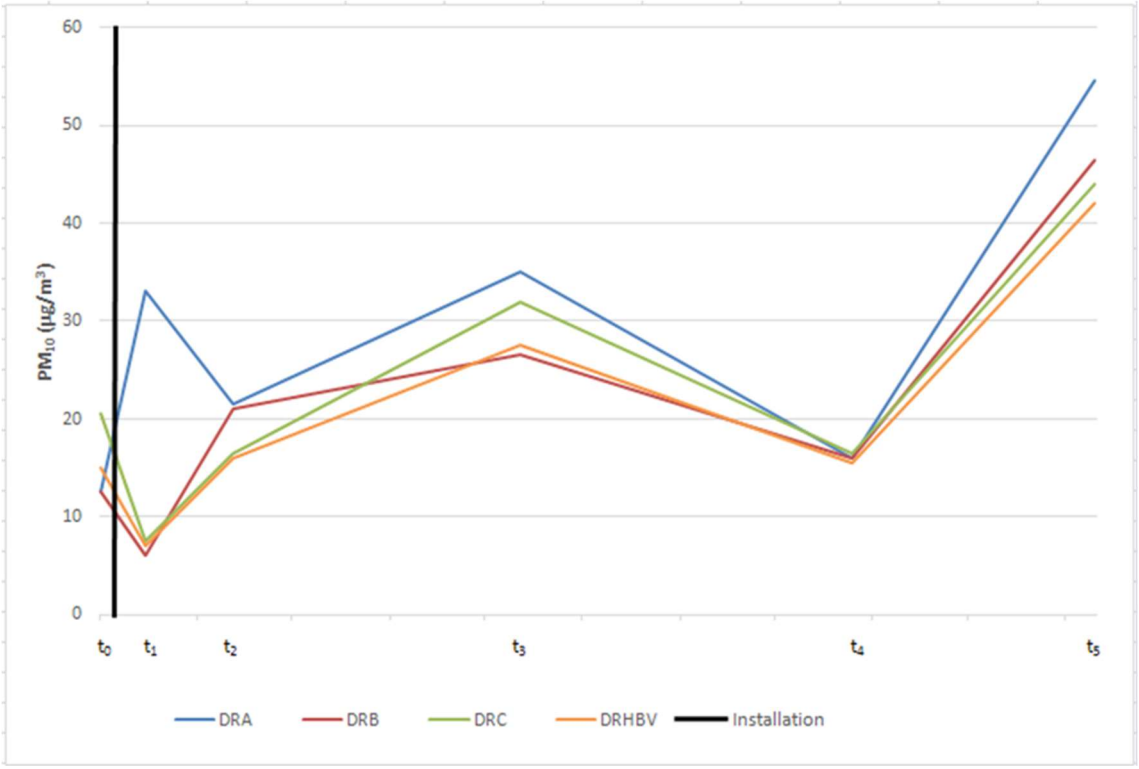


Figure 6.4: Average PM10 concentrations for the pilot areas at the different sampling times.

At t_0, the mean value of PM_{10} was 15.13 µg/m^3 and at t_5 it was 46.75 µg/m^3 (difference of 31.625 µg/m^3 between t_0 and t_5). The observed differences between the mean values of PM_{10} of the two groups (t_0 and t_5) are statistically significant (p <0.05).

The evolution, over the testing period, of the mean concentration of bacteria and filamentous fungi and yeasts (in CFU/m^3) is shown in Figure 6.5.

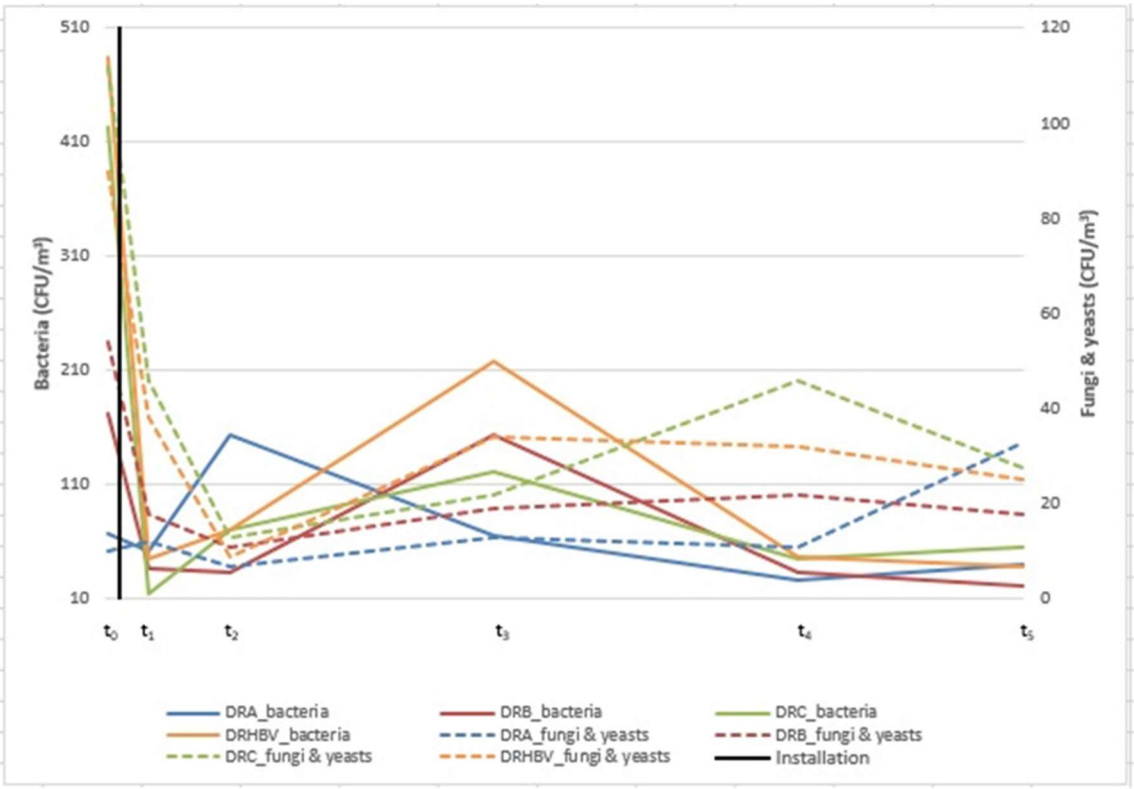


Figure 6.5: Average bacteria, filamentous fungi and yeasts concentrations for the pilot areas at the different sampling times.

From the analysis of Figure 6.5, it is verified that the highest average concentrations of the microbiological pollutants occur in the pre-installation period (t_0) in all the dialysis rooms, with the exception of filamentous fungi and yeasts detected into the DRA room (10 CFU/m^3 on t_0 and 33 CFU/m^3 on t_5).
On t_0, the average concentration of bacteria was 287 CFU/m^3 and on t_5 it was 39.12 CFU/m^3 (difference of 247.875 CFU/m^3 between t_0 and t_5). The difference between the mean number of bacteria of the two groups (t_0 and t_5) is statistically significant (p = 0.023).
At t_0, the mean concentration of fungi was 66.50 CFU/m^3 and on t_5 it was 25.88 CFU/m^3 (difference of 40.625 CFU/m^3 between t_0 and t_5). The difference observed between the average concentration of fungi in the two groups (t_0 and t_5) is not statistically significant (p = 0.061). Since the p-value was very close to 0.05, a unilateral test was performed and the result (p = 0.0305) proved that the average number of fungi at t_0 is higher than at t_5.
CO concentrations were determined only at sampling times t_0, t_1 and t_2, since all values proved to be lower than the minimum detection limit of the equipment (<1.0 mg/m^3), with no oscillations in the dialysis rooms before (t_0) and after (t_1 and t_2) installation of the bioreactors.
The differences observed on CO_2 concentrations before and after the installation of the bioreactors are very low and not statistically significant (p = 0.660).
O_3 concentrations, in parallel with CO, were performed only on t_0 (pre-installation of the bioreactors), t_1 and t_2 (post-installation of the bioreactors), presenting values below the detection limit of the equipment (<0.001 mg/m^3).

Discussion

The main objective of this study was to evaluate the impact of the IAQ management system implemented in a dialysis unit, through the characterization of the indoor air before and after the installation of the bioreactors. The results suggest that:

- The bioreactors appear to have no influence on the indoor temperature of the dialysis rooms (p = 0.053). In general, comparing the mean values of indoor temperature in t_0 and t_5 and considering that no external temperature readings were performed, it was not possible to associate the temperature variations recorded inside the dialysis rooms neither with the seasonal variations, nor with the bioreactors implementation.
- It was verified that the bioreactors could lead to an increase in the HR values (p <0.05), in the dialysis rooms, with a relative percentage increase of HR of 61.1% in the DRA room, 48.1% in the DRB room, 49.2% in the DRC room and 31.0% in the DRHBV room. It should be noted that this increase in RH was not associated to the results obtained on the microbiological parameters.
- A general decrease in the concentration of CH_2O in the dialysis rooms was observed during the testing period. The bioreactors may have contributed to this decrease in the mean concentrations (p = 0.004), with a relative percentage reduction of 96.1% in the DRA room, 97.5% in the DRB room, 93.6 % in the DRC room and 62.5% in the DRHBV room. Similar to the following total VOCs, and since CH_2O is actually a VOC, with the same emission sources, high average concentrations may result from the frequent use of disinfectants due to the medical practice in the dialysis rooms.
- Although the differences found were not statistically significant (p = 0.326), there was a reduction in the mean concentration of total VOCs (relative percentage reduction of 12.3% in the DRA room and 79.1% in the DRHBV room). Whereas bioreactors may lead to a reduction in the concentration of this pollutant. The observed peak in total VOC concentration on t_3 may come from the complete

cleaning of the bioreactors performed about a week before (between t_2 and t_3), which required to stop the system. A work done by Bonoli and Zanni (Bonoli et al. 2014a, 2014b), in an industrial context, applying the same air purification technology (bioreactors) obtained the same reading peak of total VOCs after cleaning the bioreactors.

- From the results obtained in t_0 and t_5 (pre and post-installation of the bioreactors) a generalized increase of the average concentration of PM_{10} in the dialysis rooms was observed, which may be related to bioreactors (p <0.05), with a relative percentage increase of 336.0% in the DRA room, 272.0% in the DRB room, 114.6% in the DRC room And of 180.0% in the DRHBV room. This evidence may be a consequence of the operation of the bioreactor fan, which could lead to increased indoor air circulation and, consequently, to the suspension of material deposited on the various surfaces (e.g., furniture, floor) of the dialysis rooms. The work of Bonoli and Zanni (Bonoli et al. 2014a, 2014b)[19], realized in an industrial context with the application of the same technology, also showed a great oscillation in PM concentration, which may be related to the specific activities. As observed for total VOCs and in line with the same study by Bonoli and Zanni (Bonoli et al. 2014a, 2014b), in fact, a peak in PM_{10} concentration was observed at t_3, after the complete cleaning of the bioreactors.
- Considering the results obtained on t_0 and t_5 (pre and post-installation of the bioreactors), a general decrease of the average concentration of bacteria in the dialysis rooms was observed. At bacteriological level, then, the bioreactors may have contributed to the reduction of the mean number of bacteria (p = 0.023), showing a relative percentage reduction of 41.2% in the DRA room, 87.2% in the DRB room, 86.9% in the DRC room and 91.9% in the DRHBV room;
- After installation of the bioreactors, there was a general decrease in the average concentration of filamentous fungi and yeasts in the dialysis rooms, except for the DRA room. Although the statistical difference was not significant (p = 0.061), the relative percentage reduction was 66.7% in the DRB room, 75.4% in the DRC room and 72.2% in the DRHBV room.
- Based on CO concentrations detected during t_0, t_1 and t_2, the absence of CO-emitting sources in the dialysis rooms was verified, as well as the indirect proof that the bioreactor *per se* appears not to be a source of emission. Thus, it was decided to suspend measurements of this pollutant in the dialysis rooms, after sampling time t_2.
- Since no significant changes in CO_2 concentrations were observed over the sampling period, it is possible to assess that, on one hand, the microbial activity inside the bioreactors did not contribute to the concentration of CO_2 and, on the other hand, the operation of the bioreactors appears to have no influence on the CO_2 concentration (p = 0.660). Thus, in line with a study by Ginja et al. (Ginja et al. 2012), the use of the CO_2 concentration as the baseline parameter for the assessment of the IAQ may not reflect the existence of sources of contamination responsible for high concentrations of other pollutants in indoor air.
- According to the results obtained on O_3 concentration, which were below the minimum detection limit (<0.001 mg/m^3), the absence of O_3-emitting sources in the dialysis rooms was proved and, indirectly, it may be assessed that the bioreactors did not constitute a source of emission of this pollutant *per se*. Thus, it was decided to suspend measurements of this pollutant in the dialysis rooms, after sampling time t_2.

In this research study the following limitations were identified:

- only four dialysis rooms in the same healthcare facility were analyzed, thus allowing nor extrapolation nor generalization of results obtained;
- sampling of the outdoor air was not performed, therefore no comparison of the IAQ data with external environmental conditions was accomplished;

- instant measurements were taken using direct-reading devices and not continuous measurements;
- no similar study was found in the specific scope of the present one, therefore making it difficult to validate the results obtained by comparison with other healthcare applications.

Conclusions

The IAQ management in healthcare facilities is complex and constitutes a critical part of environmental management protocols since the presence of specific pollutants in indoor air poses potential health risks to occupants.

The technical and scientific advances in this area are reflected in the offer of technological solutions for prevention and control of indoor air pollutants. Along with knowledge evolution in the fields, there is a need to evaluate and assess whether the adoption of these technologies is the solution that best fits the reality of healthcare sector.

On these premises, this study is aimed to contribute to the increase of the technical-scientific knowledge in this scope, namely, the contribution that a technological system for indoor air purification may present on IAQ.

The data compiled in the present research work focuses on IAQ of a dialysis unit, through the application of a biotechnological air treatment system, composed by bioreactors. The assessment has been performed by comparing results obtained with the characterization of the indoor air before (t_0) and after (t_5) the installation of the bioreactors. This allowed to draw several conclusions.

Before the installation of the bioreactors (t_0), remarkable concentrations of CH_2O and total VOCs were found. These chemical pollutants are typically associated with both the frequent use of disinfectants, due to the high medical practice, and the possible issues on air renewal through ventilation in the dialysis rooms.

After the installation of bioreactors (t_5) there was a decrease in the average concentration of CH_2O, total VOCs, bacteria, filamentous fungi and yeasts, but, on the other hand, an increase in RH and in the average concentration of PM_{10} in the indoor air. The functioning of the bioreactors appears to have no influence on the temperature and the average concentration of CO_2 in the dialysis rooms. The CO_2 concentration should be used with caution as IAQ indicator as it may not reflect the presence of sources of contamination responsible for even high concentrations of other pollutants in indoor air.

The remaining pollutants evaluated, i.e. CO and O_3, presented values lower than the minimum detection limit of the equipment from the first samplings, confirming the absence of specific emitting sources in the dialysis rooms and suggesting that the bioreactors do not represent a source *per se*.

At a percentage reduction rate of about 90% for CH_2O, 80% for bacteria and 70% for filamentous fungi and yeasts, the biotechnological system implemented may be considered as a potential solution for improving the IAQ, complementing the existing HVAC systems, in the dialysis unit.

6.1.1.2. Operatory theatre

The case study presented here in the following has been developed within the framework of the Climate Kic program “Pioneers into Practice”, it has been submitted for publication and it is currently under revision.

Abstract

The study has been developed within the framework of Climate Kic program, Pioneers into Practice, with the aim of setting a new standard for air treatment in Operating Theatres. The experimental application of a biotechnology working in environment air as support for traditional air venting and filtering system has been performed in three phases. The First and second phase have been focused on assessing the impact of the specific biotechnology in already treated environment with as key element the influencing of indoor air quality into Operating Theatres. The final experimental phase tested the opportunity, for the biotechnology proposed, to substitute traditional HVAC (Heating Venting and Air Conditioning) during non-operative time, whenever air volume exchange is not mandatory.

The use of AIRcel system (a low impact and very low energy demanding biotechnology for air purification) used during operational time is able to dynamically recover contamination spikes generated by perturbing events thus granting less contamination exposition during activity, and maintaining such recovery during HVAC shutdowns.

Allowing the HVAC system in the Operating Theatre to work only on operative time, and relying on AIRcel to treat the air also during shut down, the system generates better overall coverage meanwhile granting sensible energy saving, remaining in compliance with international standards of indoor air quality in hospital environment.

The adjustable shutdown schedule can span from 35% to 64% of the week, with correspondent energy savings.

Introduction

In the perspective of promoting sustainability in business, with potential direct effect on collective behavior, a project has been proposed for the application of the biotechnology under study in Operating Rooms. This to tackle one the most relevant energy consuming environment into the healthcare sector, due to ventilation and air treatment systems.

Since climate change is a global issue, a radical shift in production and consumption modalities is required (Markard, Raven and Truffer, 2012; Cappellaro and Bonoli 2014, Cappellaro 2015). In this sense, Sustainability Transitions (ST) offers both a theoretical framework (Technological Innovation Systems, TIS, MultiLevel Perspective, MLP, and Strategic Niche Management, SNM) and case history of good practices’ application. Transdisciplinary work and system approaches are key elements for Sustainability Transition, together with actors, networks and institutions involved into innovation (Cappellaro 2015; Geels, 2004; Weber, 2003;). The Climate Kic project, promoted by the EIT (European Institute of Innovation and Technology), provides the right habitat and program to support the process. As Pioneer, a 4-week placement has been undertaken. This period was designed to provide Pioneers with the opportunity to experience leading low carbon innovation initiatives in the Hessen Region (Germany) and develop generic capabilities as well as specific know-how through participation to a low carbon project. This in order to understand key systemic challenges in the transition to a low carbon economy, by exposing the Pioneer to an interdisciplinary experience and involve different socio-technical perspective and taking sustainability research into companies.

Fresenius Medical Care was the host company involved into the project and its corporate headquarters are in Bad Homburg v.d.H., Germany.

Provided the climate change effects are on a global scale, local actions may represent the real trigger to make transition happen. For this reason, niches, i.e. small-scale protected space for transition experiments

(Cappellaro 2015, Cappellaro and Bonoli 2014), are developed into incubator programs like Pioneers into Practice (PiP). The PiP international placement program, consists in a period of cooperation between a professional subject (a researcher, in the present case) and a company or institution to build up a transition experiment. In this case PiP offered the matching opportunity for real case application and validation of a biotechnology already on the market, tested into a very specific and energy demanding context, such as healthcare sector and Operating Rooms in particular.

The present study focused on the possibility to set a new standard for venting systems in hospital environment, including Operatory Rooms (OR).

International guidelines provide Indor Air Quality standards, quite different for different countries, as underlined by Melhado et al. (2006). In particular, specific range of comfort and safety are identified for temperature, humidity, airborne particles and gases by various institutions (CDC and HICPAC, DHHS and NIOSH, DGKH, VDI, ISPESL). The guidelines focus on ventilation system specifications (in terms of number of volume exchange per hour and outdoor air intake), efficiency requirements and air quality standards related to different surgery classes, similarly to cleanroom standards (ISO 14644) and referred to operative time.

The actual hospital practice is, nevertheless, to keep the venting and air treatment systems working on a 24/7 schedule, with possible lowering of the devices when the operating theatre is not in use (this practice is called 'setback' and it is applicable only in facilities with adjustable air flow systems). This widely accepted practice, while energy consuming, is adopted to counteract the possible contamination concerning Operating Rooms from the surrounding "grey" areas (such as pre and post-op rooms, corridors etc.) and from the air venting system itself, especially when the system is switched on again, generating a possible contamination peak (Traversari et al. 2016). Applied research efforts have been undertaken to propose the implementation of the innovative biotechnology under study for indoor air treatment (AIRcel) into the hospital environment, working as a substitute of traditional Heating Venting and Air Conditioning (HVAC) systems during non-operative time in OR and as support for standard venting systems during operating time. Several studies provided background references on the opportunity to work on new operating schedule for HVAC in operating theatres (Traversari et al. 2016, Babb 1995, Robert Koch Inst. 2000, Dettenkofer 2003). Waiting 15 minutes' after switching on ventilation before starting activity is considered sufficient as to ensure microbial safety of the Operating Theatre and prevent operatory infections (Hoffman et al. 2002), while 25 minutes have been evaluated by Traversari et al. (2016) as necessary to also restore standard air quality and climatic conditions to optimal. In order to take the researches results into real practice, making sustainable transition happen, long term monitoring has been performed.

Since HVAC energy consumption represents a major issue in the overall energy consumption budget of a hospital, demonstrating the possibility to lower or, better, switch off completely the HVAC system during non-operative time would be a strategic improvement in energy and costs management, and, at the same time, a step forward toward sustainability of healthcare facilities, thus tackling the climate change issue.

Materials and methods

The transition experiment consisted in placing a biotechnology for air treatment as support for HVAC standard system into an Operatory Room and Preparation Room, starting by beginning of the Summer until late Autumn 2015. The biotechnology implemented has been already applied in different sectors (Sofer 2006, Bonoli et al. 2014, Zanni et al. 2015) as an indoor air treatment system and it proved to be effective against airborne contaminants, both particles and gases.

An extensive collection and consequent analysis of primary data of indoor air quality in the Operatory Room have been performed in standard operating conditions, before and after the set-up of the proposed biotechnology in order to assess its performance into the specific environment. A low impact, low cost, wireless technology for indoor air monitoring has been applied, working in semi-continuous mode to collect

air quality data on particles count (2.5 to 10 microns and 1 to 10 microns by size) and gases (Volatile Organic Compounds - VOC and odorous gases) (U-Monitor, see Chapter 3).
The Collected data have been analyzed in order to identify the impact of the new technology implementation on indoor air quality in standard conditions, i.e. with HVAC system working on a 24/7 schedule. In particular, and in order to properly compare the performance matching with OR activity, a typical working and non-working day have been simulated, using average concentration data for the contaminants of concern, and calculated on an hourly basis. This approach allowed a useful comparison between the baseline period, with only traditional venting system working 24/7, and the following one, with HVAC and HEPA filters system supported by AIRcel bioreactors.
In a second phase of the experiment, a comparison between indoor air quality data and operatory activity of the room during a standard operation month has been carried out, in order to identify possible correlations between specific procedures and air quality data, therefore, validating the monitoring technology in the specific application thus allowing a backward analysis and correct interpretation of the data collected during the baseline, phase1 period and the monthly average contamination data. A statistical analysis of peaks in concentration of gases and particulate matter related to more invasive surgical procedure carried out during the study period has been performed. In particular, average concentration data have been calculated on an hourly basis in correspondence of endo prosthetic surgery for the entire duration of the procedure. Results have been, therefore, compared with the average calculated on hourly basis of the correspondent hour of the typical day.
Once defined the key factors causing direct effects on Operating Room air quality, a proposal for an innovative HVAC working schedule, with switching off period during non-operational time of the OR, has been outlined and applied during Autumn 2015 (phase 3). Indoor air data have been evaluated and their consistency verified against the baseline at the compliance point, identified in the opening time of the OR, thus ensuring proper quality for operation. Experiments during switch off time have been carried out during four different weekends and contaminants concentration data have been collected and elaborated in order to obtain, once again, an average trend, based on hourly average data, to be compared to weekends of regular ventilation schedule, taking into consideration both phase 1 and phase 2 of the study period.
Energy consumption related to HVAC and air filtering system has been evaluated on the basis of the energy bills and the technical data provided by the healthcare facility. This in order to quantify savings deriving from the shutting down schedule proposed and assess the feasibility of the supplementary installation of the biotechnology for air treatment, in a self-supporting perspective. Savings were calculated considering the installation of n.2 AIRcel bioreactors, one U-Monitor and a dedicated Wifi router and on the basis of electrical consumption cost supposed to be equivalent to 0,195 €/kWh, a yearly working schedule of 46 weeks (considering holidays and maintenance operations) and the longer shutting down performed scenario (including weekends and nighttime).

Results and discussion

Phase 1

This first phase consisted of the new technology implementation and of its impact evaluation on the indoor air quality in standard condition, i.e. with HVAC system working on a 24/7 schedule.
With the implementation of AIRcel biotechnology, a remarkable reduction of average values of gaseous contaminants was obtained within the first 3 months taking the VOC absolute values close to the lower detection limit of the sensor (Fig. 6.6).

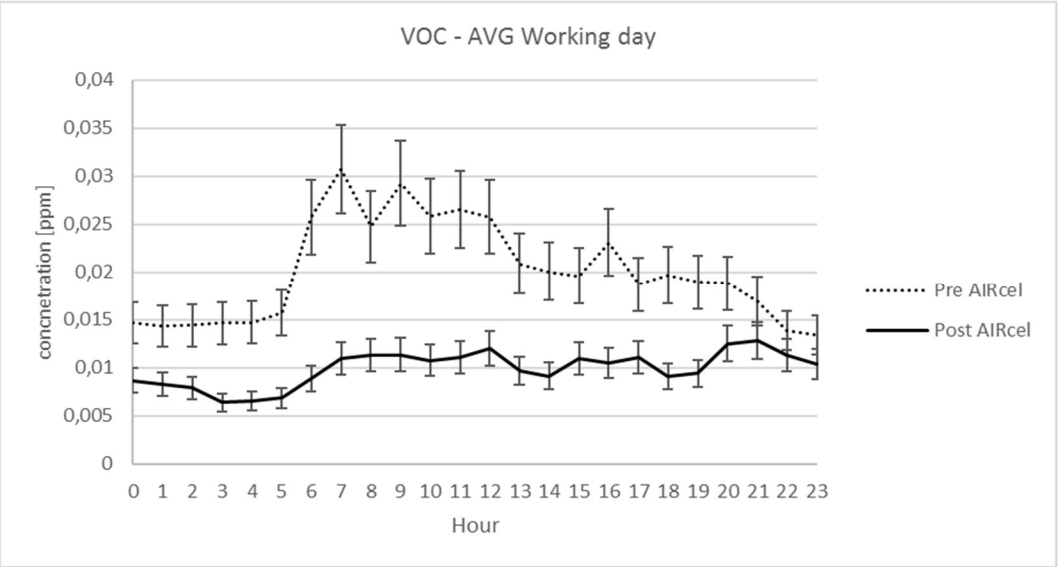


Fig. 6.6.: VOC concentration during average working day before and after AIRcel application

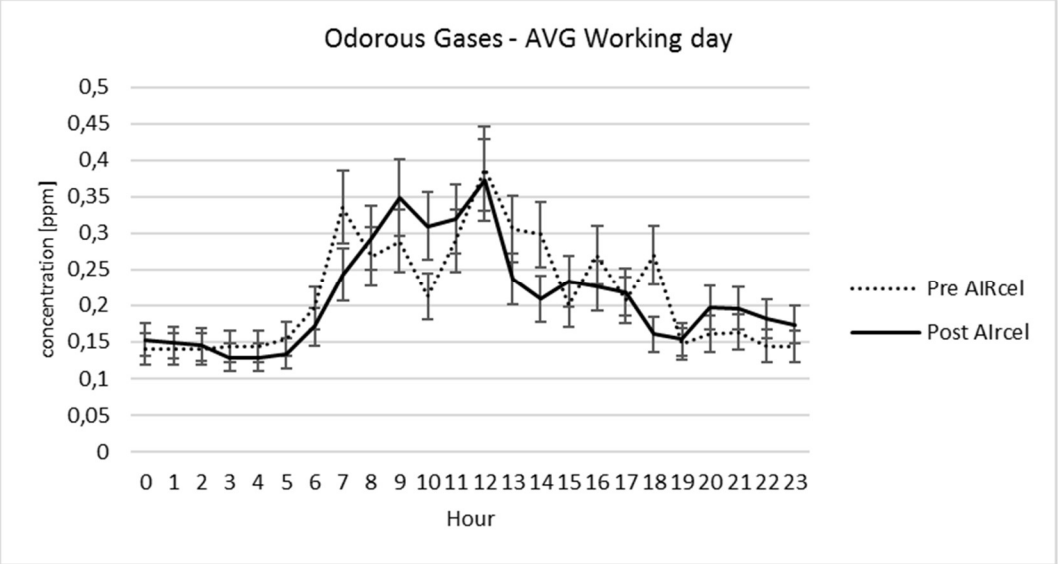


Fig. 6.7.: Odorous Gases concentration during average working day before and after AIRcel application

Due to different results obtained by the different gas sensors, with a general decrease in concentration detected by VOC sensor and a more time-dependent behavior in the Odorous Gases one, the activity log Operatory Room under study has been examined. In particular, the number of surgery performed at different hour of the day (percentage related to the total number of interventions of the month) have been matched with peaks in concentration of Odorous Gases detected, showing a direct correlation between the two aspects (Tab. 6.1.). For example, during September, considering a "Post AIRcel" period: the 63% of interventions starts from 8 to 11 am, when much of Odorous Gases peaks are detected, while the average trend is generally described by what already presented in Fig. 6.7.

	JUNE (BASELINE PERIOD)			**SEPTEMBER (PHASE 1)**		
START TIME	n. of interventions	Monthly share	Cumulative share	n. of interventions	Monthly share	Cumulative share

8	9	17,31%	17,31%	20	28,99%	28,99%
9	5	9,62%	26,92%	5	7,25%	36,23%
10	6	11,54%	38,46%	5	7,25%	43,48%
11	6	11,54%	50,00%	14	20,29%	63,77%
12	5	9,62%	59,62%	5	7,25%	71,01%
13	6	11,54%	71,15%	6	8,70%	79,71%
14	3	5,77%	76,92%	5	7,25%	86,96%
15	3	5,77%	82,69%	6	8,70%	95,65%
16	3	5,77%	88,46%	2	2,90%	98,55%
17	5	9,62%	98,08%	1	1,45%	100,00%
18	0	0,00%	98,08%	0	0,00%	100,00%
19	1	1,92%	100,00%	0	0,00%	100,00%

Tab. 6.1.: Number and percentage of interventions related to their starting time

Since smoke generated by laser or electro-surgery unit has been characterized by several studies, proving that the plume can contain gases and vapors such as benzene, hydrogen cyanide, and Formaldehyde (now recognized as carcinogenic type A), together with bioaerosols, dead and live cellular material, blood fragments, and viruses possible carried by the patient, any abatement in concentration must be regarded as a decreased risk for patient himself and surgical staff (Melhado et al. 2006, NIOSH, 1998;).
The dust in the operating theatre proved to be very thin, as the major fraction of the particles have size in the range between 2.5 and 1 micron (calculated as difference between detection of 10 to 2.5 and 10 to 1 microns sensors). Particle Matter trend was not clearly identifiable during the first phase of the study, while in the following months an abatement has been detected, accordingly to previous studies performed on the technology (Bonoli et al 2014a, 2014b).

Phase 2

The second phase of the experiment consisted of a comparison of indoor air quality data with operatory log of the room during a standard operation month, validating the monitoring technology in the specific application (Tab. 6.2.).
The airborne contamination in the operating room, in presence of different working programs, resulted to be greatly affected by the operations carried out during working days, with significant increases of gaseous contaminants (confirmed by both VOC and Odorous Gases sensors). In particular, invasive intervention, such as internal orthopedic prosthesis, proved to be highly impactful on air quality.
Remarkable peaks in concentration of VOC and Odorous Gases are easily traceable and remarkable in absolute value.
The contemporary increase in PM concentration was limited in absolute values and solved by the effect of the cleaning operation at the end of the working day, but resulted nonetheless significant.

	COMPARED TO AVG BASELINE VALUES				**COMPARED TO AVG SEPTEMBER VALUES**				**COMPARED TO AVG AFTER INSTALLATION (PHASE 1)**			
	VOC	OG	PM1	PM2.5	VOC	OG	PM1	PM2.5	VOC	OG	PM1	PM2.5
CONCENTRATION ABOVE CORRESPONDENT HOUR AVG	0%	22%	100%	100%	70%	55%	24%	7%	14%	12%	87%	4%
CONCENTRATION BETWEEN -10% AND	2%	5%	0%	0%	12%	14%	37%	20%	15%	14%	90%	19%

+10% OF THE CORRESPONDENT HOUR AVG												
CONCENTRATION ABOVE 150% OF CORRESPONDENT HOUR AVG	0%	14%	100%	94%	62%	31%	0%	2%	2%	8%	0%	2%
CONCENTRATION ABOVE 200% OF CORRESPONDENT HOUR AVG	0%	5%	18%	24%	55%	16%	0%	1%	1%	5%	0%	1%

Tab. 6.2.: AVG contaminants concentrations on hourly basis during endoprothesic surgeries compared to correspondent hour AVG referred to baseline period (i.e. before AIRcel installation), to the same month and to the whole test period with application AIRcel technology.

Phase 3

A series of experimental switching off of the HVAC system has been performed during non-operative time of the Operatory Room. In particular, in order to observe contaminants trend and recovery capacity of biotechnological system implemented in absence of forced air circulation, the switching off experiments were conducted during weekends. HVAC system has been shut down at Friday, 9 pm and switched on again on Monday, early morning, with different start-up times from 7 to 3 hours before the opening time of the operating theatre. Thus to ensure the establishment of standard indoor air quality for activity resume and patients' safety.

Gaseous contaminants, detected both by VOC (Fig. 6.8.) and Odorous Gases (Fig. 6.9.) sensors, show a peak at shutdown time. This condition is, however, managed and improved by AIRcel to values compatible with the resumption of operating activities, with little to no dependence on the restart time of the HVAC.

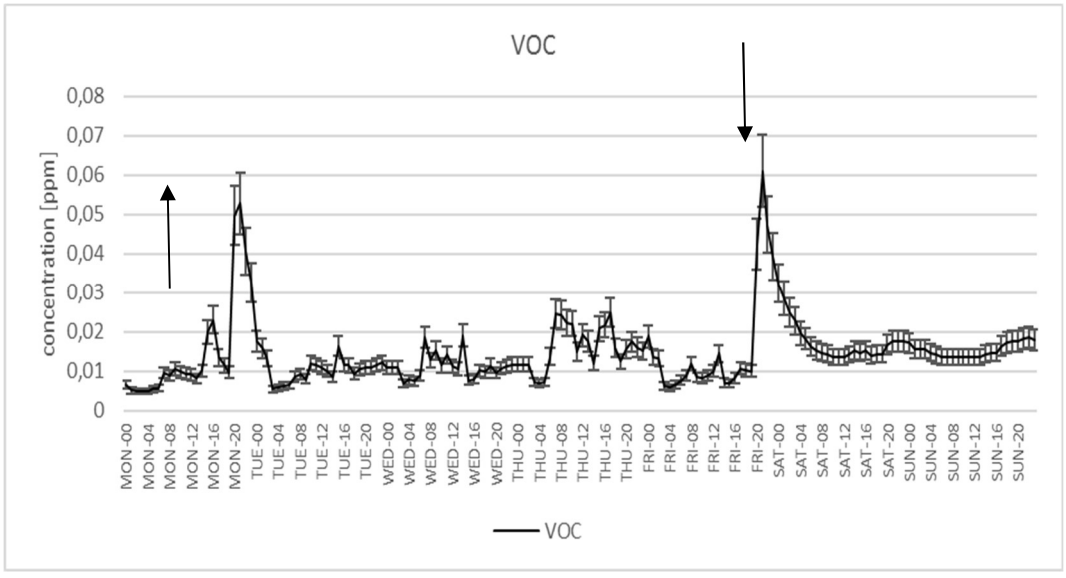


Fig. 6.8.: VOC concentration, AVG week during phase 2. The arrow up marks the switching on of HVAC and HEPA filtering system (Monday morning), while the arrow down marks its shutting down (Friday evening).

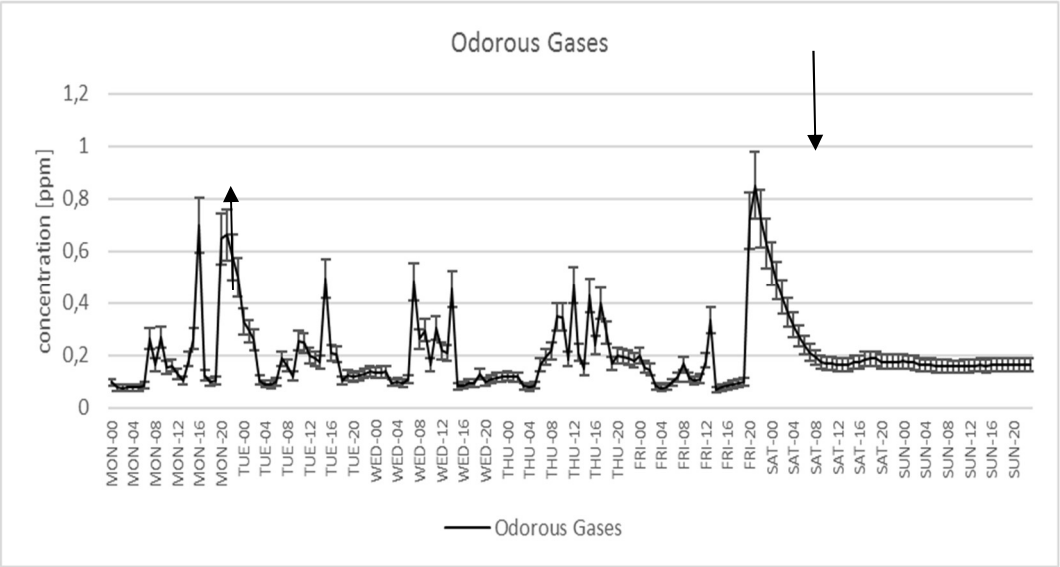


Fig. 6.9.: Odorous Gases concentration, AVG week during phase 2. The arrow up marks the switching on of HVAC and HEPA filtering system (Monday morning), while the arrow down marks its shutting down (Friday evening).

The finer particulate matter, i.e. 1-10 microns class (Fig. 6.10.), shows peaks of minor significance, while PM 2.5 trend appears affected by almost constant fluctuation (Fig. 6.11.). Relative unsteadiness of laser scatter sensor applied is to be regarded, in this case of smaller statistical database, as major responsible for difficult identification of trends.

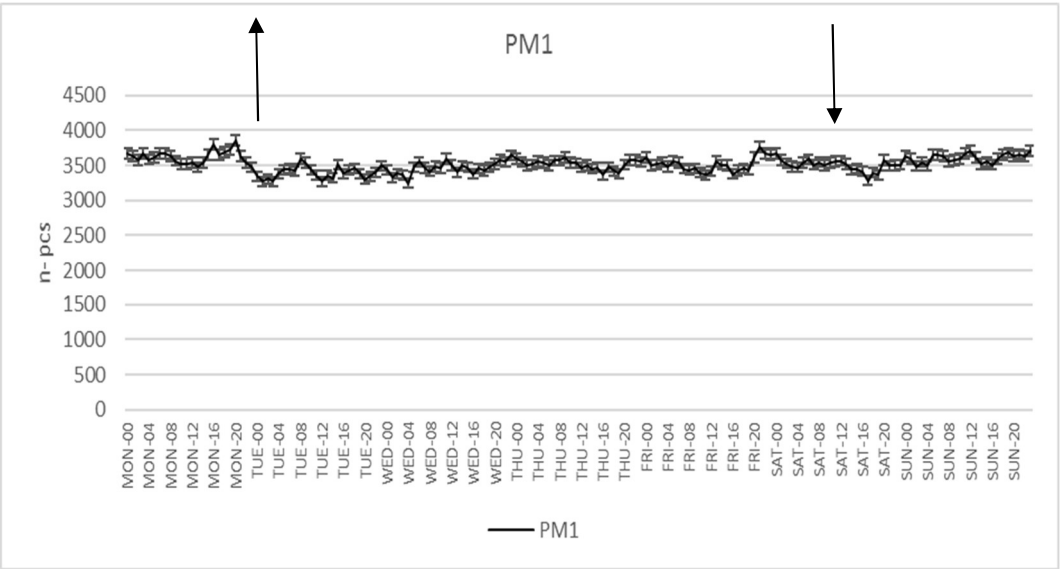


Fig. 6.10.: PM1 numbero of particles AVG week during phase 2. The arrow up marks the switching on of HVAC and HEPA filtering system (Monday morning), while the arrow down marks its shutting down (Friday evening).

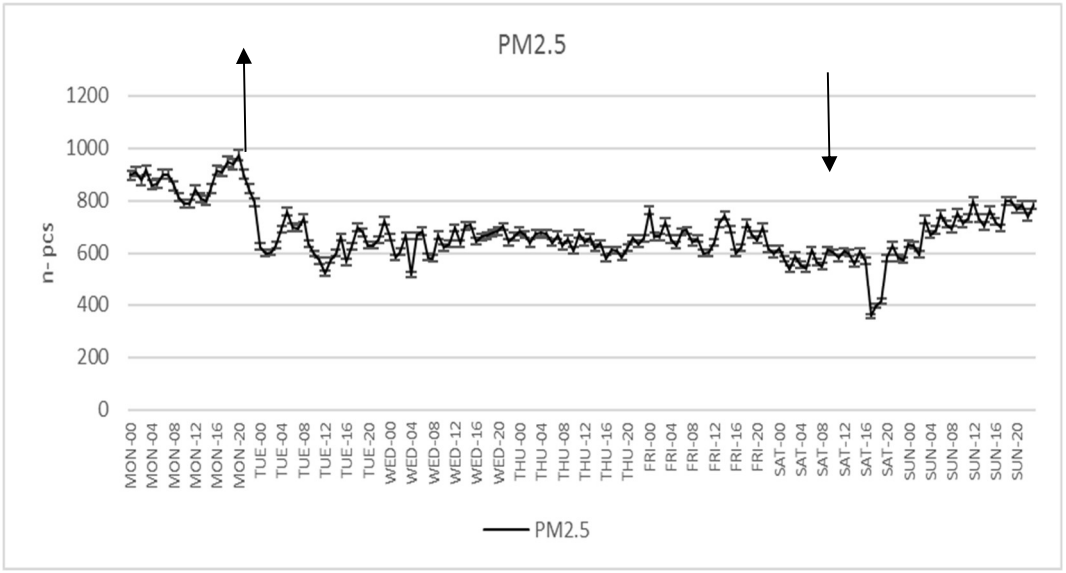


Fig. 6.11.: PM2.5, numbero of particles AVG week during phase 2. The arrow up marks the switching on of HVAC and HEPA filtering system (Monday morning), while the arrow down marks its shutting down (Friday evening).

Focusing on switching on time (Fig. 6.12. and Fig. 6.13.), the peak in concentration detected during the phase 2 test period is, anyway, contained below the average calculated for the whole test period. Compared with typical Monday morning trends, gas concentration is contained below the level registered without the shutting down.

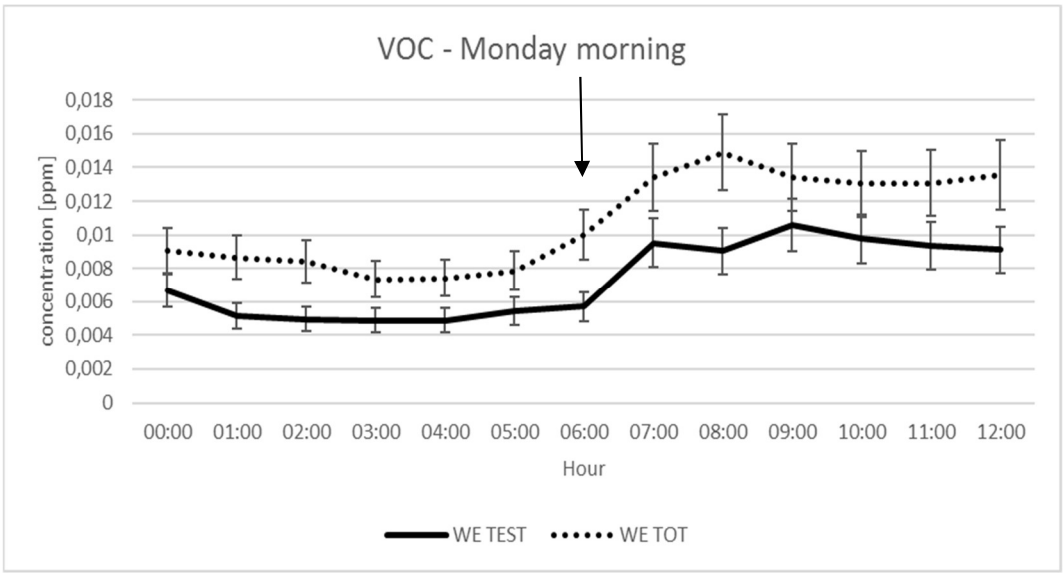


Fig. 6.12.: VOC concentration, Monday morning.

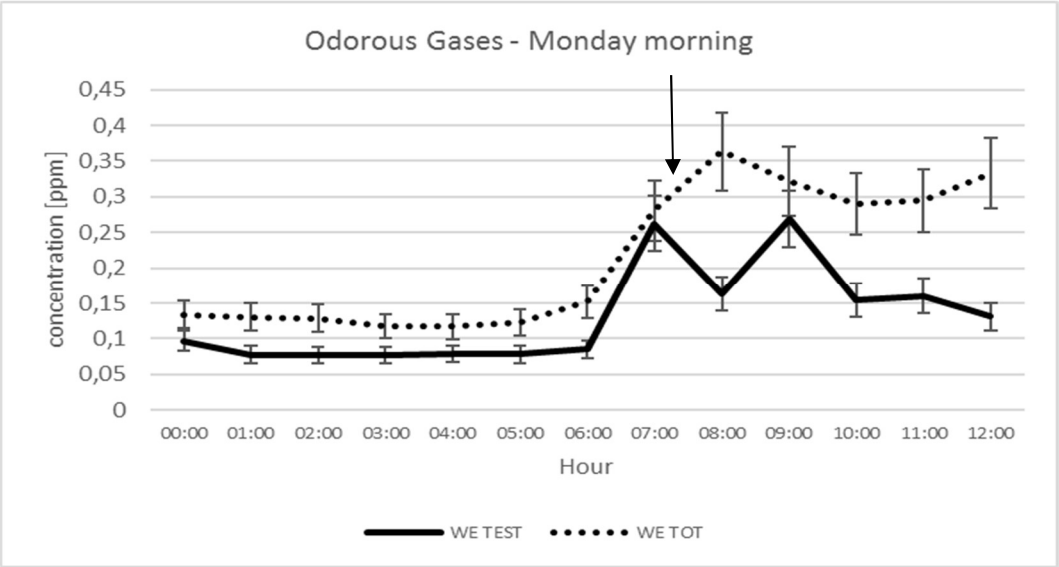


Fig. 6.13.: Odorous Gases concentration, Monday morning.

In order to verify the feasibility of the biotechnology installation in a self-supporting perspective, savings deriving from the shutting down schedule proposed (i.e. weekends and nighttime, for 64% of the week) have been calculated on a yearly basis and results (Tab. 6.3.) confirmed a positive outcome of the experiment. In particular, the investment, both for installation and maintenance, for biotechnology proposed appeared to be fully covered by savings obtained, within the first two years of application.

OPERATORY ROOM	UNIT	YEAR
AIRCEL ENERGY CONSUMPTION	kWh	259,15
U-MONITOR ENERGY CONSUMPTION	kWh	26,28
WIFI ROUTER ENERGY CONSUMPTION	kWh	105,12
HVAC ENERGY CONSUMPTION	kWh	35857,92
VARIABLE PART OF THE HVAC ENERGY BILL (EXCLUDING MAINTENANCE COSTS)	€	6.992,29
SAVINGS OBTAINED WITH THE FINAL SET UP (64% SWITCHING OFF)	€	4413,76

Tab. 6.3.: energy and cost savings calculation on the basis of the 64% shutting down proposed for the air venting and filtering system in the OR.

Conclusions

Indoor Air Quality (IAQ) in healthcare facilities is regarded as one of the major upcoming issue, due to high impact, both social and economic, of nosocomial infection, even in contexts fully compliant with hygiene standards.

The present study focused on the application of an air treatment biotechnology in Operatory Rooms, as support for standard HVAC and HEPA filtering systems. A semi-continuous monitoring of IAQ has been performed in order to assess the performance of the proposed biotechnology (AIRcel) in the specific application, comparing data collected before and after AIRcel installation. Remarkable results were obtained:

within the first 3 months, in fact, VOC concentration decreased to values close to the lower detection limit of the sensor.

Once defined a correlation between gaseous contaminants and operatory activities within the OR, internal orthopedic prosthesis has been identified as the most impacting operation, as foreseen by operators themselves. Possible contaminants accumulation during the day should be considered when compiling the surgery list to limit patients and staff exposition.

The behaviour of the contamination related to shutdowns of HVAC revealed that, with the implementation of AIRcel system (a low impact and very low energy demanding biotechnology), indoor air quality in operatory room is maintained and dynamically recovered after possible peaks related to switching off and on the HVAC. Thus, allowing to draw a shutdown schedule for the HVAC system during non-operative time in the OT, in compliance with international standards on indoor air quality in hospital environment and with sensible energy savings.

The adjustable weekly shutdown schedule can span from 35% of switching off time in the most conservative scenario (involving only 58 hours in the weekend) to 64% (with 60 hours in the weekend and 12 hours at nightime), confirming results obtained by previous researches (Babb et al 1995, Dettenkofer et al. 2003, Traversari et al. 2016). This improving the overall environmental performance of the health care facility, being the OR HVAC system one of the major energy demanding sector and, therefore, item in the energy bill. The air treatment biotechnology applied ensured a general containment of airborne contamination, with remarkable and immediate results on gaseous contaminants, while particulate matter decreased in longer times.

Accordingly to the applicative and business oriented research promoted by Climate Kic actions, the most challenging scenario of shutting down schedule has been evaluated in terms of cost saving and payback time for the initial investment. On the basis of the current energy costs in Italy, return time for the investment has been set into two years, considered acceptable and attractive from the market perspective.

6.1.1.3. Intensive Care Unit

As reported by Fresenius, patients admitted to the Intensive Care Units (ICUs) account for about 5-19% of total hospitalized patients. Based on data from Agenzia Sanitaria e Sociale (2008), patients into ICU develop approximately 25% of all hospital infections, presenting a risk of associated infections, 5 to 10 times higher than average hospital areas. The risk is typically related to intrinsic (e.g. immunosuppression, comorbidities and advanced age) and extrinsic (e.g. mechanical ventilation) factors. The mortality rate is tragically high, due to the frequent occurrence of resistant strains of bacteria and microorganisms causing this kind of infections. Several pathogens related to hospital infections may be airborne (Aspergillus fungi, Clostridium Difficile, Respiratory Syncytial Virus, Mycobacterium tuberculosis) and, therefore, travel from ICU to other hospital departments and patients.

The object of the study was to evaluate IAQ into an Intensive Care Unit (ICU), already implementing the best available hygienic procedure as a leading facility in the sector.

The bioreactors system has been installed after a period of baseline recording and the impact on several IAQ indicators has been assessed by comparing the baseline with the following three months of application:

- BEFORE installation period: September, 11th 2015 – October, 13th 2015
- AFTER installation period: October, 14th 2015 – January, 19th 2016

The pilot areas have been identified were:

1. Cardiological Intensive Care Units (UTIC), with up to 10 resident patients
2. Electro-physiology, with discontinuous occupancy rate, related to interventions' schedule

An air ventilation and treatment system was currently running into both pilot areas, with a declared air exchange rate of 16 and 10 total volume exchange per hour, respectively.

The main characteristics of the pilot areas are summarized into Tab 6.4.

PILOT AREA	**ICU**	**ELECTRO-PHYSIOLOGY**
ACTIVITY	Patients recovery after surgical procedures	Laparoscopic procedure performed on an irregular schedule
N. OF BEDS	10	1
N. OF AIRCEL UNITS INSTALLED	2	1
AIR VENTILATION SYSTEM	yes	yes

Tab. 7.4.: Pilot areas description

The IAQ monitoring has been performed through:

- application of U-Monitor platform
- microbial sampling and colonies counts for microorganisms developing at human body temperature (36°C). From September, 30th 2015 to January, 19th 2016, four sampling have been performed by a professional lab, in both areas, over 5 different points, located at 1.5 m from the bioreactors positions. 1,000 liters of air have been sampled each time, following the standard monitoring methodology, with a Surface Air System (SAS) and Petri dishes, with Tryptic Soy Agar (TSA), have been incubated at 36°C for 48 hours.

The overall contaminants' trend is presented in the following figure, recorded through several methods during the whole testing period (Fig. 6.14.). While in ICU the three main ICU parameters monitored (i.e. VOC, PM1 and microbial count) followed a similar trend, with decreasing concentrations displayed, in Electro-physiology performances appear reduced and microbial data resulted quite scattered.

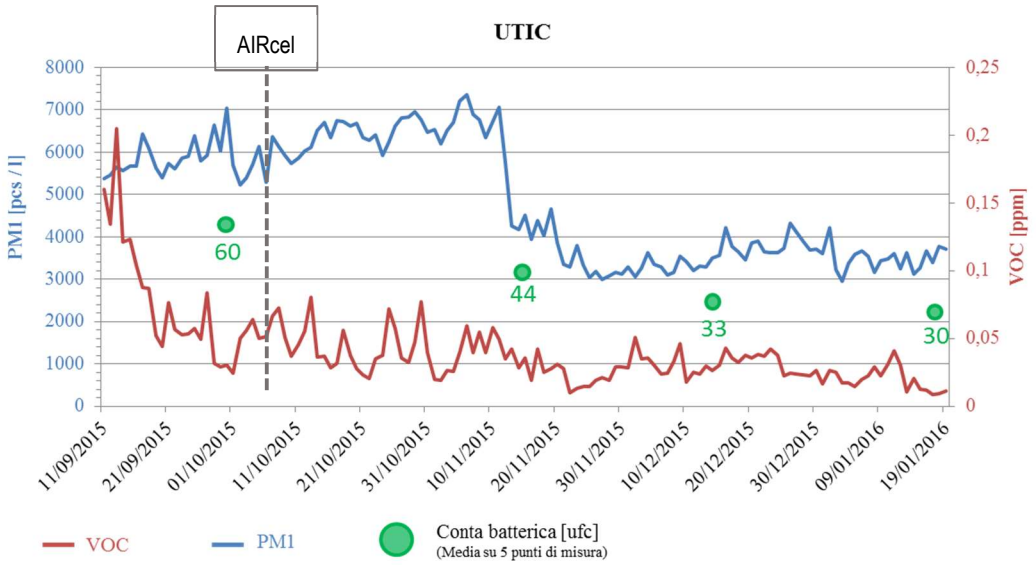


Fig. 6.14: Combination of the three main IAQ parameters tested (VOC, PM1 and microbial count) – Intensive Care Unit (ICU)

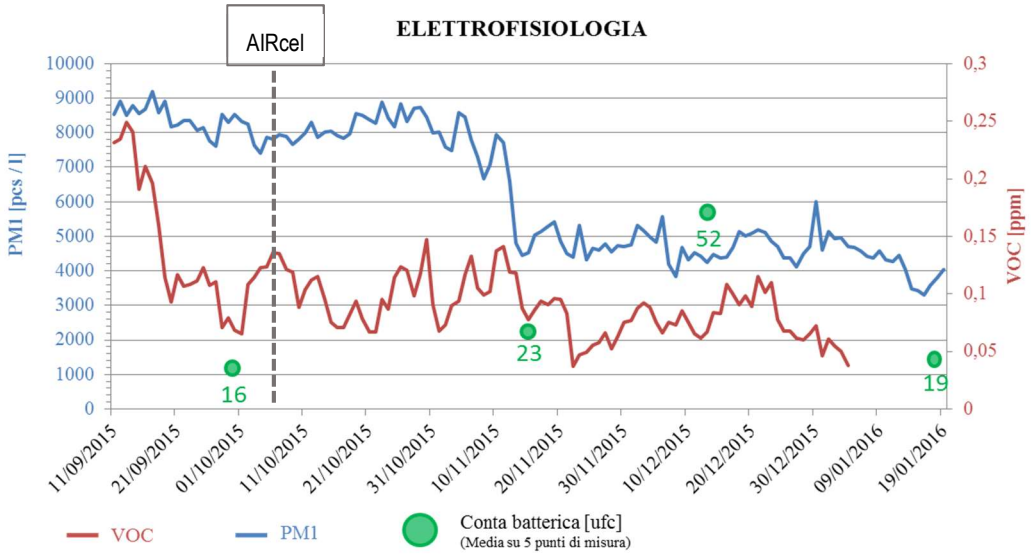


Fig. 6.15.: Combination of the three main IAQ parameters tested (VOC, PM1 and microbial count) – Electro-physiology

Hourly average values calculated are presented for total VOCs and PM1, building the "typical day" contaminants' trend and assessing the impact of AIRcel biotechnology implementation by comparing "before" and "after" conditions. The high frequency of sampling (i.e. every 5 minutes), in fact, returns concentration records over time scarcely intelligible and strongly affected by singular concentration peaks (Figg. 6.16, 6.17, 6.28, 6.29). Results obtained on IAQ parameters are presented into Table 6.5.

Daily average values compose the "typical week" contaminants trends, presented for the two environments and the two classes of pollutants (Figg. 6.18 and 6.19).

Concentration peaks have been evaluated, comparing the conditions BEFORE and AFTER bioreactors' installation, for both installation areas (Figg. 6.20 and 6.21).

As evident from Table 7.5. summary of results, abatement performances appear specular considering the two pilot areas, as well as the two main contaminants detected with the U-Monitor. In fact, in ICU, VOC concentration decreased for more than 50% (with 15% range between maximum and minimum), while PM1

decreased around 50% only in minimum values and maximum registered only minor abatements (-11%). For Electro-physiology, on the contrary, VOC concentrations decreased by 64% in minimum values, while average and maximum by 36%; PM1, on the contrary, registered a remarkable containment of the peaks (-57%) and a minor effect on average and minimum values (-31% and -23%).
From the microbiological point of view, average data into ICU decreased progressively to a 50% of the initial measurements, while in Electro-physiology concentrations increased during the first two monitoring time with AIRcel into the room and returned similar with the initial one. It must be noted that, except on the mid-December samples, all data from Electro-physiology remained within the limit proposed by Italian Health Authority (i.e. Istituto Superiore di Sanità) for operatory theatre in "at rest" conditions, i.e. 35 CFU/m^3, while in ICU, which is a residential ward, microbial count has been progressively taken to values typical of operatory rooms.

	RESULTS ON IAQ	**ICU**	**ELECTRO-PHYSIOLOGY**
VOC	Minimum values	-50%	-64%
	Maximum values	-65%	-36%
	Average values	-56%	-36%
	Notes	Faster recover from accumulation peaks	Faster recover from accumulation peaks
PM1	Minimum values	-49%	-23%
	Maximum values	-11%	-57%
	Average values	-23%	-31%
	Notes	Faster recover from accumulation peaks	Attenuation of the concentration peaks
MICROBIAL COUNT (36 °C)	average over n.5 samples	-41% -50% (final)	+95% +19% (final)

Tab. 7.5.: Results on Indoor Air Quality parameters (VOC and PM1).

For the completeness of the IAQ assessment, Odorous Gases sensors results have been evaluated over time (Fig. 6.28 and 6.29), trying to compare condition before and after AIRcel implementation. Unfortunately, data showed quite different trends, compared to VOC: after the first 40 days of application, in fact, in ICU maximum values tend to increase on an almost constant rate; in Electro-physiology, on the other hand, minimum values showed a slight increase and only a major sequence of higher peaks was registered.

Based on considerations exposed, a longer monitoring period would have been useful to better recognize consistent concentration trends for the IAQ parameters monitored into the two pilot areas and try to identify possible direct correlations with specific activities (i.e. interventions schedule, major cleanings etc.).

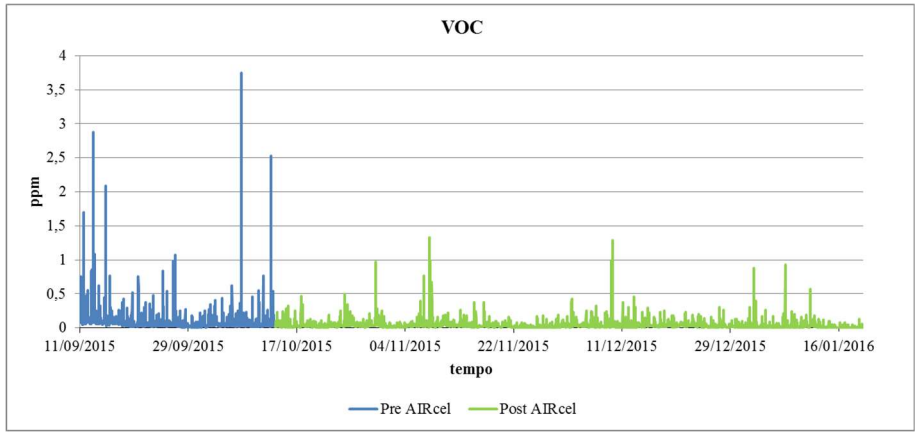


Fig. 6.16.: VOC concentrations record over time, ICU

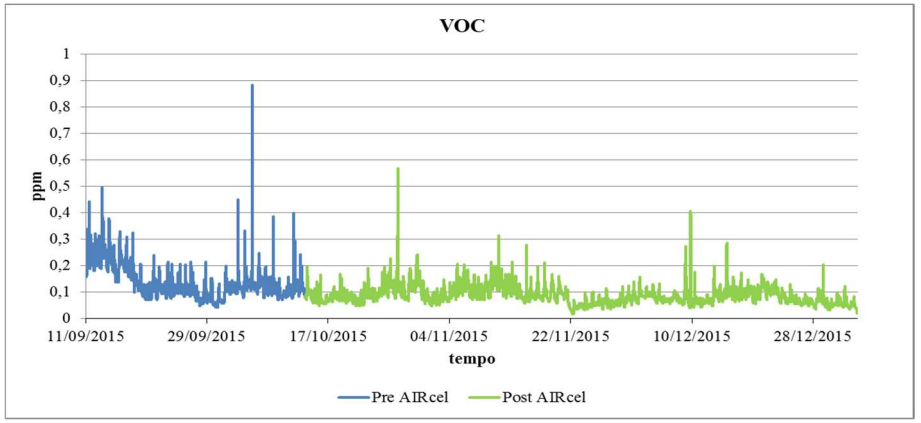


Fig. 6.17.: VOC concentrations record over time, Electro-physiology

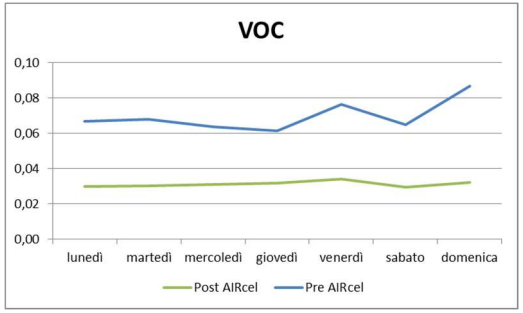


Fig. 6.18.: VOC concentrations in the "Typical week", ICU

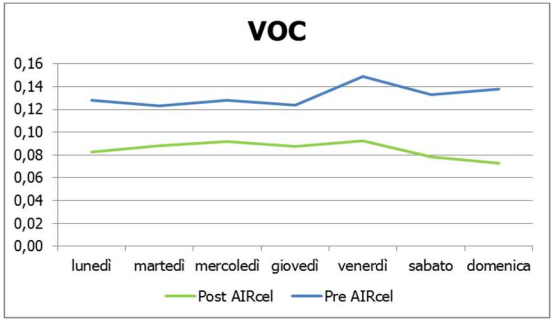


Fig.6.19.: VOC concentrations in the "Typical week", Electro-physiology

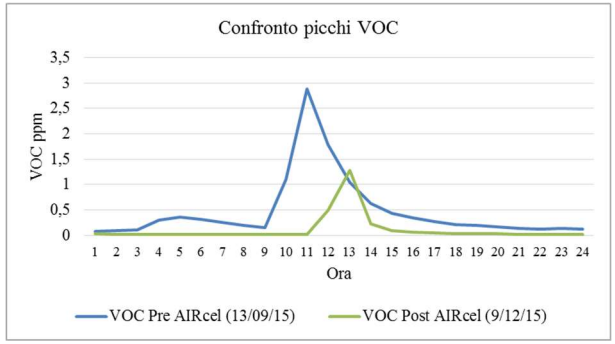


Fig. 6.20: VOC concentration peaks during the "Typical Day", ICU

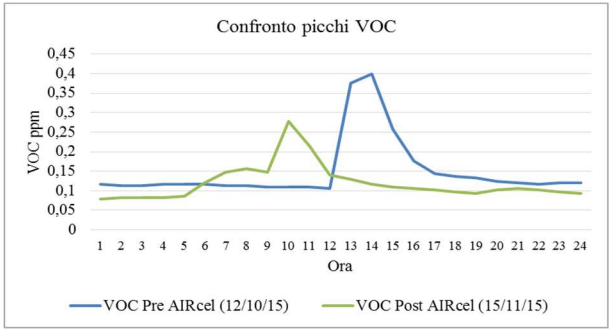


Fig. 6.21: VOC concentration peaks during the "Typical Day", Electro-physiology

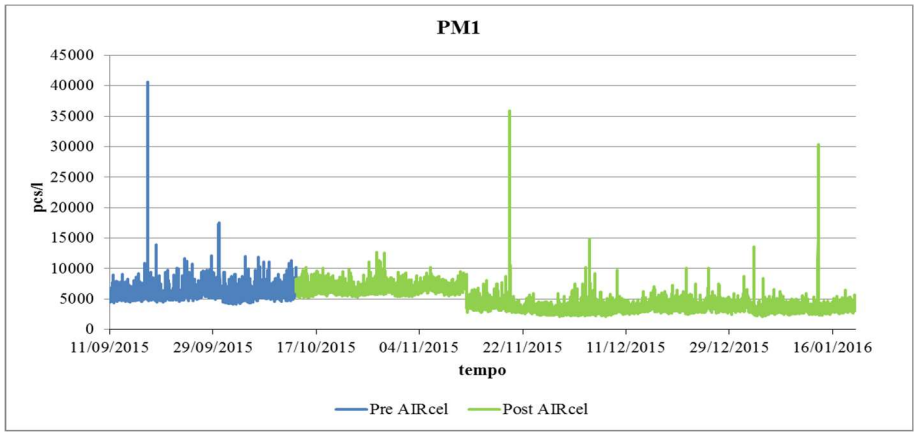


Fig. 6.22: PM1 concentration records over time, ICU

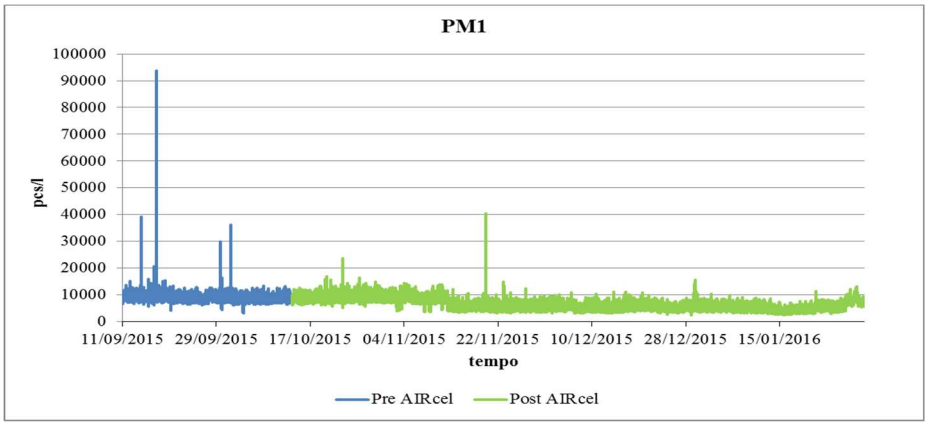


Fig. 6.23: PM1 concentration records over time, Electro-physiology

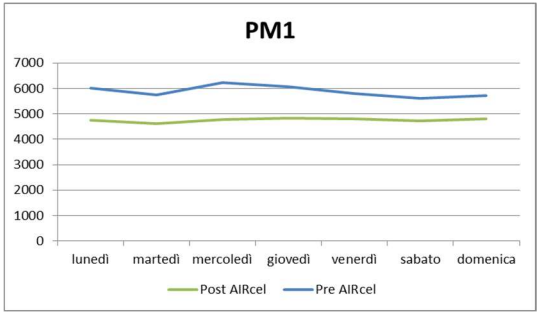


Fig. 6.24: PM1 concentrations in the "Typical week", ICU

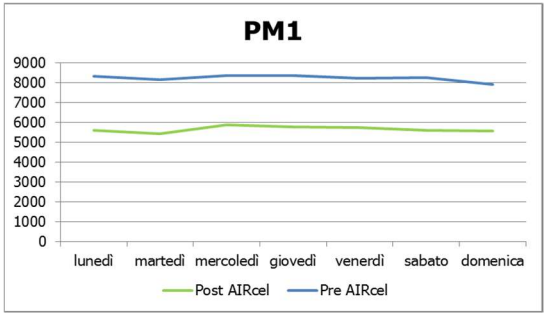


Fig. 6.25: PM1 concentrations in the "Typical week", Electro-physiology

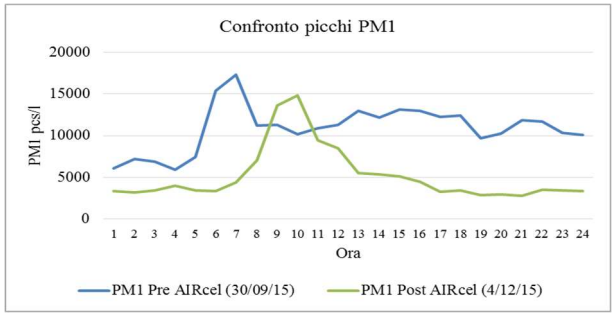


Fig. 6.26: PM1 concentration peaks during the "Typical Day", ICU

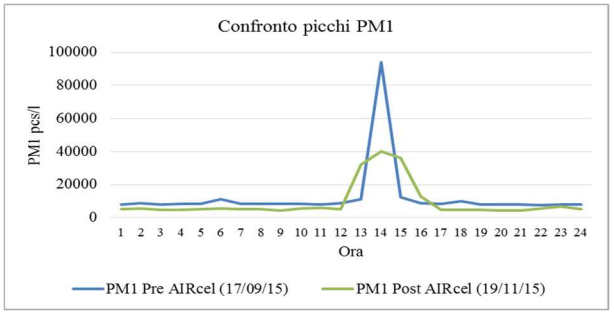


Fig. 6.27: PM1 concentration peaks during the "Typical Day", Electro-physiology

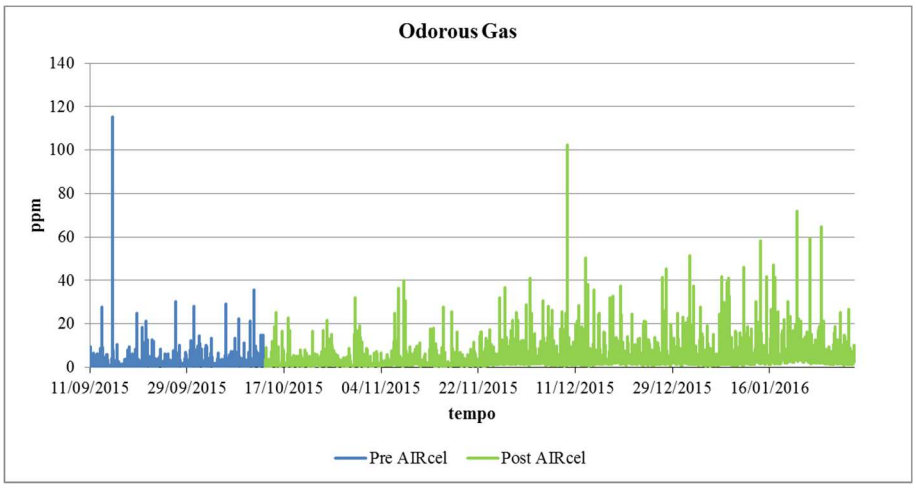


Fig. 6.28: Odorous Gases concentrations record over time, ICU

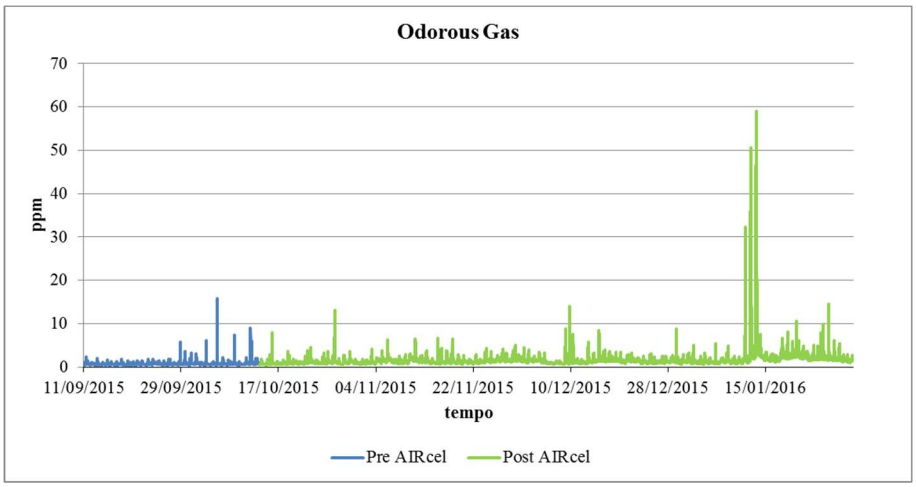


Fig. 6.29: Odorous Gases concentrations record over time, Electro-physiology

6.1.1.4. Anatomopathological laboratories

The objective of the study was to trace airborne contamination's patterns and assessing impact of AIRcel technology implementation into anatomopathological labs. Two different pilot installations have been performed, with different contour conditions (rooms' size, ventilation, working schedule etc.) and over different periods.

The airborne contaminant of major interest for IAQ in the specific environment was identified in Formaldehyde (CH_2O, CAS number 50-00-0), belonging to the VOC family and recently identified as carcinogenic 1/B (Regulation (EC) No 1272/2008; Commission Regulation (EU) 2015/491). This posing a protection issue toward workers exposed and necessity to implement technological solutions to improve IAQ in general and to decrease Formaldehyde concentration in air.

Based on previous experience in hospital application (i.e. paragraph 6.1.1.1. Hemodialysis unit), Formaldehyde results to be quite easily and steadily managed with AIRcel bioreactors. Therefore, the implementation as support for currently running air extraction and treatment system, or as complement where no direct prescription is present, appeared a reasonable and pro-active solution. For this reason, two pathological labs were selected and involved into experimental activities. The specific features of each lab are listed in the following table (Tab. 6.6).

PILOT AREA	**1**	**2**
ACTIVITY	Private lab, specialized in veterinary anatomopathological investigations and human dermatologic histology	Hospital lab, specialized in histological analysis of tissues
N. OF ROOMS	2 (labelled as Lab 2 and Lab 5)	2 (labelled as Samples preparation and Cytology)
N. OF U-MONITORS	2	2
APPROXIMATE SIZE	100 m^2	30 m^2
N. OF AIRCEL UNITS INSTALLED	7	3
AIR VENTILATION SYSTEM	no	yes

Tab. 6.6.: Pilot areas for pthological labs experimental application

Both pilot installations soon presented an unexpected maintenance issue: bioreactors, in fact, tend to clog with residual sludge with a far higher rate than normally experienced and, therefore to stop remediation activity. As shown in the following figures, taken 7 (Fig. 6.30) to 15 (Fig. 6.31) days after former cleaning and maintenance (i.e. four times to double the average cleaning rate expected during the first 3 months of activity), the amount of sludge was remarkable. In addition to this, it displayed a peculiar feature, i.e. a gel formation around tubes, trickling holes and in the peripherical region of the biofilm installed on the vertical surfaces of the inner cylinders.

Fig. 6.30.: Pilot area 1, sludge residual after 7 days.

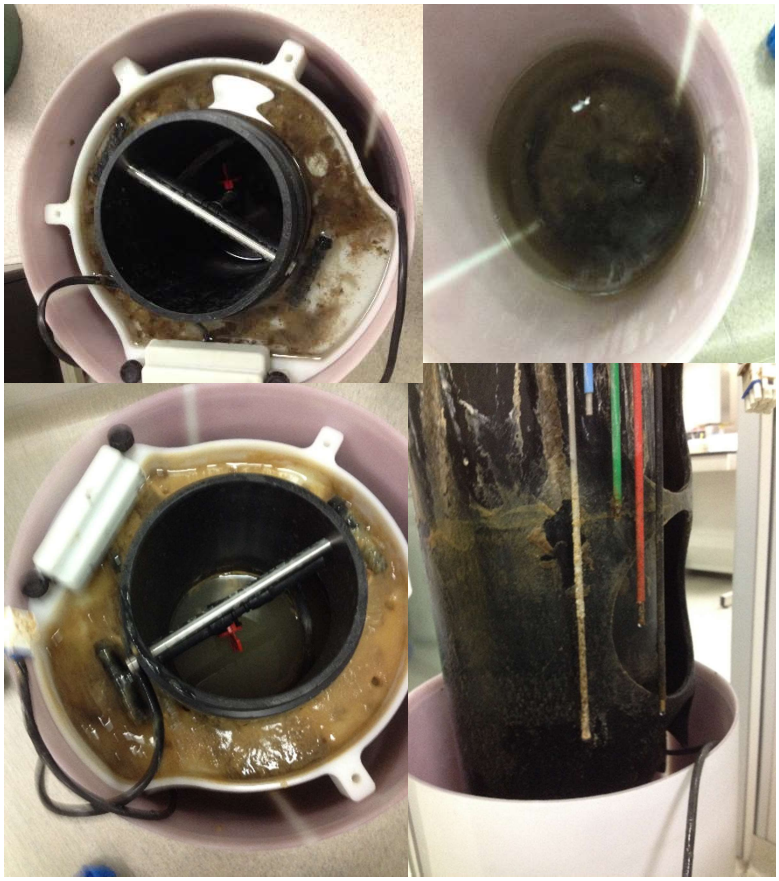


Fig. 6.31.: Pilot area 2, residual sludge after 15 days

Evidences gathered during the maintenance activities suggested that an additional remarkable issue was present and underestimated during the pilot design phase. Investigating the specific laboratory practice performed in the areas, a hydrocarbon-based compound candidates to clog bioreactors: paraffin wax.
Paraffin wax (CAS Number: 8002-74-2) typically used as support for anatomopathological samples is a complex hydrocarbon, consists of a mixture of hydrocarbon molecules, generally composed of alkanes, with chains built up on 16 (hexadecane), 18 (octadecane) and above (up to 36) carbon atoms, with the general formula C_nH_{2n+2}. (Addison, 1984; Kotlar et al., 2007).
In a pathology laboratory, paraffin wax is used to impregnate tissue prior to sectioning thin samples of tissue. This due to the unreactive nature of the paraffin, providing insulation and physical support to samples. The tissue to be analyzed is placed in paraffin wax for several hours and then set in a mold with wax to cool and solidify; sections are, then, cut on a microtome.
This operation could, therefore, represent a primary source of emission for both particulate matter and gases (Volatile Organic Compounds – VOCs) in the specific environment.
As a matter of fact, while VOCs are generally degraded within the AIRcel with low residuals and good abatement results, paraffin and wax particles proved to be easily captured, but hardly managed, in terms of digestion, by the technology. Their degradation by microbial populations present into U-ox, in fact, should be accomplished through specific functional aspects, i.e. direct oxidation for carbon and energy to CO_2, as it happens for other contaminants.
As reported by several authors, *n*-Alkanes can be effectively used a C-source by aerobic bacteria, both in form of paraffin wax (van Eyk et al., 1968; van Beilen et al. 2001; van Beilen et al. 2005; Hasanuzzaman et al. 2007; Sood et al. 2008, Sakthipriya et al. 2016, Tab.6.7.) and paraffin hydrocarbons in crude oil (Miget et al. 1969), even at standard environmental temperature.

Organism	Hydrocarbon	Incubation days	Temp °C	Degradation%
Acinetobacter bouvetii	C_{16}	15	30	72
Bacillus thermoleovorans B23	C_{13}–C_{23}	12	70	60
Bacillus	C_{16}	10	65	56
Consortium	C_{12}–C_{34}	14	28	78
Consortium	C_{12}–C_{21}	84	30	83–98
Geopbacillus kaustophilus	C_{20}	97	55	97
Pseudomonas aeruginosa WatG	C_{36}	14	30	25
Pseudomonas aeruginosa	C_{16}	15	30	75
Pseudomonas aeruginosa	C_{20}	12	28	70
Pseudomonas aeruginosa	C_{12}–C_{30}	7	30	>90
Pimelobacter simplex	C_{16}, C_{20}	60, 18	24	15
Rhodococcus cercidiphyllus	C_{16}, C_{20}	75, 18	24	15
Rhodococcus erythropolis	C_{16}, C_{20}	55, 10	24	15
Rhodococcus	C_{10}–C_{30}	7	30	37

Tab. 6.7.: Various microorganism and consortia for hydrocarbons degradation, with temperature conditions and results (Sakthipriya et al. 2016)

The study proposed by Zhang et al. (2015) demonstrated the ability of extracellular enzymes produced by six Aspergillus strains to degrade paraffin wax. In particular, the shape of wax crystals changed after degradation, displaying rough surface and a loose structure and gases (Co_2 and H_2) were released, together with organic acids (oxalate and propionate).
The peripheral metabolic pathway through which the Paraffin wax is typically degrade by microorganism, as reported by Sakthipriya et al. (2016), is presented in Fig.7.32.

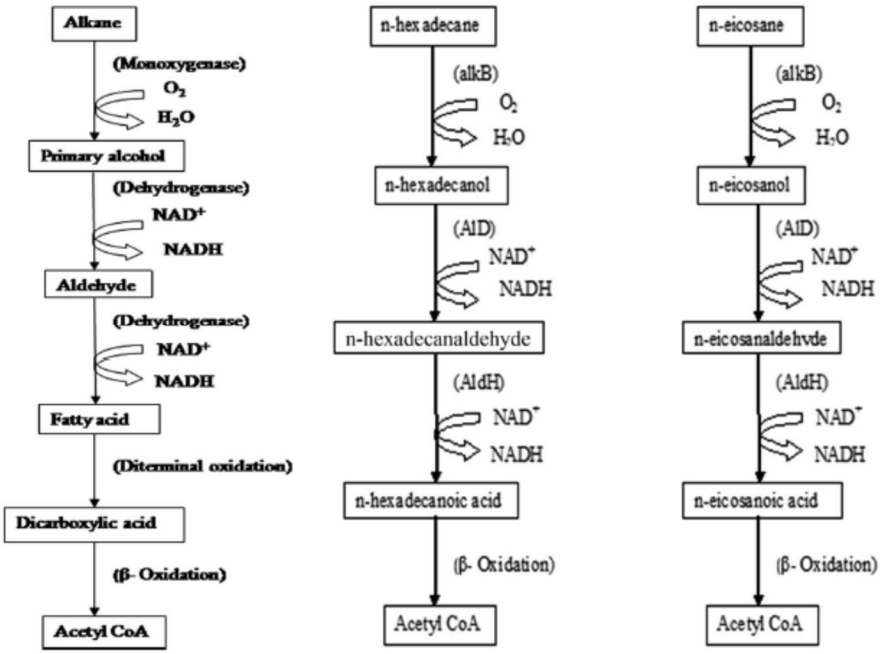


Fig. 6.32.: Peripheral pathway of Paraffin degradation (Sakthipriya et al. 2016).

Actually, residual material, presumably attributable to intermediate products from paraffin and wax degradation, tends to accumulate into AIRcel bioreactors, as the capture rate is evidently higher than the digestion capacity. This causing the clogging of water-plate holes and pump discharge tube, following the covering of the reacting surfaces, i.e. the cylindrical core and the inner tank surface, with a gel, most likely formed by intermediate metabolic products. For this reason, maintenance rate required resulted higher than expected from previous experience and practice generally ascribed to extraordinary maintenance operations (i.e. every 30-90 days) were necessarily performed weekly from the second month on. Only after the implementation of this management practice, the pilot could be considered as effective, as demonstrated by the following graphics (Fig. 7.33 and 7.34), related to VOC concentrations in Pilot area 1.
Periods presented in the following Fig. 7.33 are:

- November, 11st – 17th 2016: first week after installation. Maintenance was performed on the 17th.
- December, 9th – 15th 2016. Maintenance was performed on the 15th.
- February, 10th – 16th 2017. Maintenance was performed on the 15th.

As clearly visible, VOCs contaminants increase on a regular basis until the maintenance takes place. On the last maintenance, though, contamination decreases on the same day, showing that the system was, at the end of the third month of application, able to recover immediately after the cleaning and to work on the "instant" pollution (i.e. produced from everyday activities). This was not possible when the system was overwhelmed by the residual material trapped in and the initial saturation condition was to be broken, as during the first month of application.

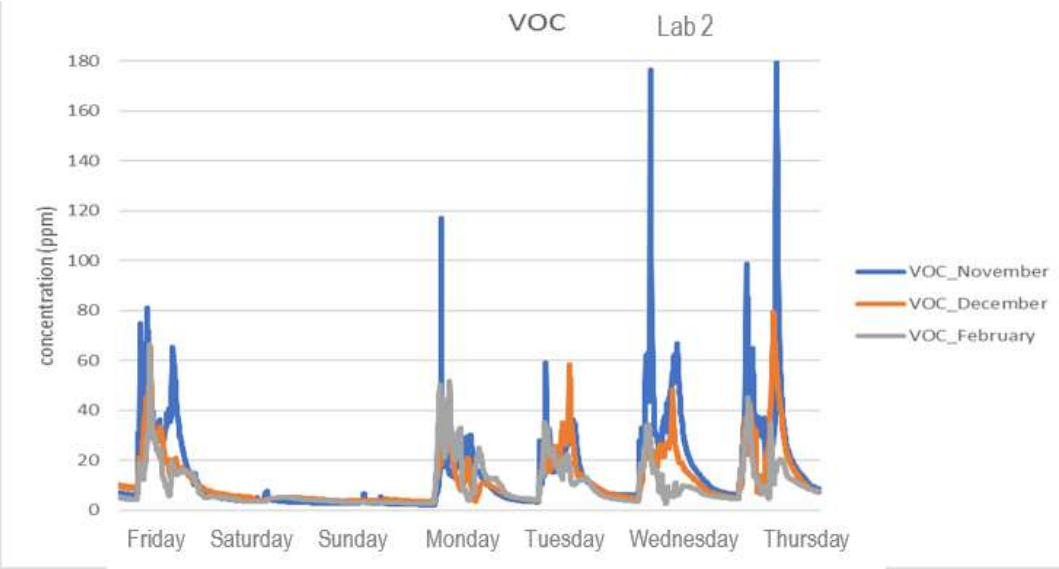


Fig. 6.33.: Pilot area 1, VOC concentrations in different weeks

The system performance increased during the last month, as shown in the following graphic (Fig. 7.34), comparing the first week of application in Pilot area 1, Lab 2, (11st – 17th 2016) with last week (February, 10th – 16th 2017), in terms of hourly average VOCs concentration (with an average decrease of 27%).

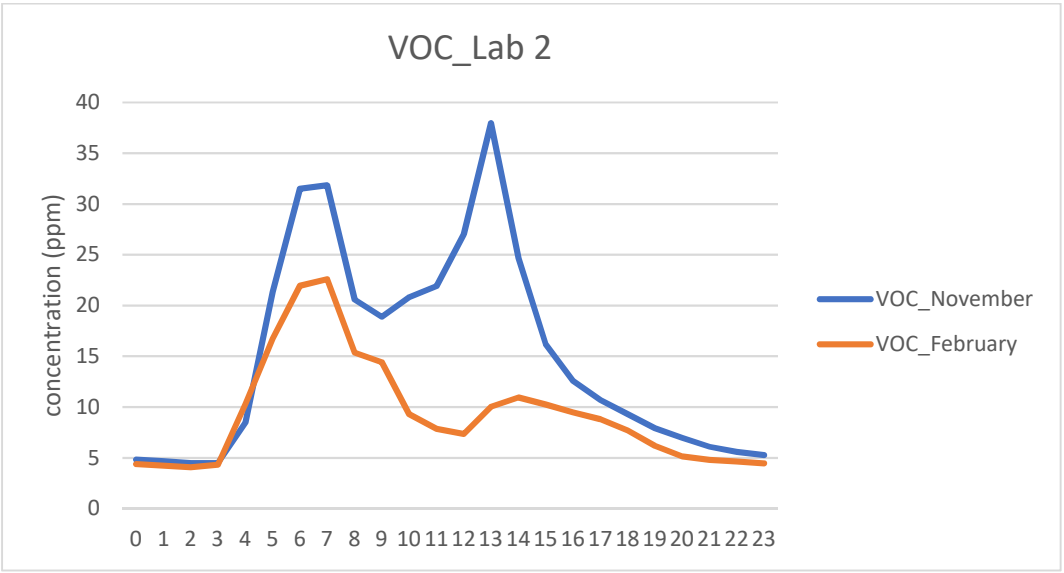


Fig. 6.34.: Pilot area 1, VOC concentrations in different weeks (November – beginning of the pilot test; February – end of the pilot test)

The pilot installations were organized as follows:

1. U-Monitors' installation, to register the average airborne contamination conditions in the areas, identify possible repetitive patterns and correlations between IAQ and specific activities. For Pilot area 1, unfortunately, only 1 week of data collection ("baseline") was possible, while on Pilot area 2, 6 weeks of data were collected (this to overcome the typical low activity rate registered in August, when the pilot started).

2. AIRcel installation, with U-Monitors still recording IAQ data, for 14 weeks in Pilot area 1 and 25 weeks in Pilot area 2.

As formerly mentioned, the high frequency of sampling performed by U-Monitors (i.e. every 5 minutes) returns concentration records over time scarcely intelligible and strongly affected by singular concentration peaks (Figg. 6.35, 6.36, 6.37, 6.38). Therefore, in order to assess the impact on IAQ parameters of bioreactors system under study, average values are calculated to compare "before" and "after" conditions. Hourly average values calculated are presented for total VOCs, which represented the objective of the biotechnology implementation, building the "typical day" contaminants' trend over working (Fig. 6.39, 6.40, 6.41, 6.42) and non-working days (Figg. 6.43, 6.44, 6.45, 6.46). Daily average values compose the "typical week" contaminants trends and they are presented in Figg. 6.47, 6.48, 6.49, 6.50.
Results obtained on IAQ parameters are presented into Table 6.8.

IAQ	PILOT AREA 1 – LAB 2	PILOT AREA 1 – LAB 5	PILOT AREA 2 - CYTOLOGY	PILOT AREA 2 – SAMPLES PREPARATION
VOC, CONCENTRATION RANGE (PPM)	0-4200	0-380	0-5	0-40
VOC, TYPICAL WORKING DAY	-31%	-21%	-29%	-44%
VOC, TYPICAL NON-WORKING DAY	-47%	-65%	-53%	-61%
VOC, TYPICAL WEEK	-34%	-25%	-35%	-48%
ODOROUS GASES, CONCENTRATION RANGE (PPM)	8,40-1040	10-630	0-320	0-540
ODOROUS GASES, TYPICAL WORKING DAY	-30%	-4%	+203%	+322%
ODOROUS GASES, TYPICAL NON-WORKING DAY	-36%	-23%	+105%	+181%
ODOROUS GASES, TYPICAL WEEK	-32%	-6%	+199%	+253%
PM1, RANGE (PCS)	3200-894725	2880-19675	2428-99205	4740-131670
PM1, TYPICAL WORKING DAY	+8%	+12%	+9%	-3%
PM1, TYPICAL NON-WORKING DAY	-7%	+11%	+8%	-2%
PM1, TYPICAL WEEK	+5%	+12%	+8%	-3%
PM2.5 RANGE (PCS)	205-7905	2-3170	124-4560	1650-14870
PM2.5, TYPICAL WORKING DAY	+9%	-6%	-8%	-8%
PM2.5, TYPICAL NON-WORKING DAY	+19%	+1%	-8%	-8%
PM2.5, TYPICAL WEEK	+12%	-4%	-8%	-8%

Tab. 6.8.: Results on Indoor Air Quality parameters, in the two Pilot areas

VOC time records analysis resulted difficult for both Pilot areas, due to the strong dependence of contamination from laboratory's activity, as demonstrated by the daily peaks displayed (Figg. 6.35, 6.36, 6.37, 6.38). The most evident results were registered for Pilot area 1, and into Lab 2 in particular, where, after the new maintenance schedule implementation (i.e. every week from the second month on), VOC trends were significantly lower, with three exceptions on 2-3-6 of February. In Pilot area 2, about 6 weeks after bioreactors installation, VOC decreased evidently, but, during the last 4 weeks of the pilot, new concentration peaks showed up, lowering the average performance recorded.
VOC concentrations range proved to be remarkably higher into Pilot area 1, due both to significantly higher use of solvents (e.g. Xylol) and Formaldehyde and absence of air extraction and ventilation system during the pilot test. Considering the Typical Working Day (Figg. 6.39, 6.40, 6.41, 6.42), average VOC abatement ranges from 21 to 44%, while, for the Typical Non-Working Day (Figg. 6.43, 6.44, 6.45, 6.46), results ranged from 47 to 65%, showing a better performance during recovery time for the system, i.e. when the area is at rest and air pollution to be managed is only residual and not renovated by ongoing activity. As evident from the graphics, during Working Day VOCs tend to accumulate during the central hours of the day, when the lab activity is hectic and, therefore, more solvents are released into the environment. Considering the Typical Week concentrations (Figg. 6.47, 6.48, 6.49, 6.50), in Pilot area 2 results registered during the biotechnology installation period are stably lower than during the baseline, while for Pilot area 1 a slight accumulation is identifiable on Friday.
Odorous gases displayed a peculiar behavior during the test period in Pilot area 2. In fact, concentration range appears to be two to three times higher into Pilot area 1 compared to Pilot area 2, but in the former, sensors registered 6% (Lab 5) to 32% (Lab2) abatement, while, in the latter, average concentrations raised from two to three times of the baseline values. In parallel, no specific change in odors perception has been pointed out: unfortunately, the non-selective nature of the gas sensors implemented makes it impossible to properly interpret the phenomenon. Apparently, some gases detected by Odorous Gases sensor and not by VOC sensor, has increased in concentration, therefore, a deeper investigation of laboratory practice would be recommended.
In Pilot area 1, where no air ventilation and treatment system was implemented, Particulate Matter of the two classes resulted higher than in Pilot area 2, but in the same order of magnitude. Considering instrument sensitivity and relative error, both differences between the two environments and before/after AIRcel installation appear scarcely significant.

In order to address the maintenance issue and better investigate the degradation dynamics within the bioreactors, samples of process water and residual sludge were taken at the end of test period in Pilot area 1 and in a different lab, as countercheck, and submitted to chromatographical analysis (Gas Chromatography – Mass Spectrometry, GC-MS) with the cooperation of a specialized lab. In particular, n.3 different samples were submitted to chromatography:

- Sample 1: it was taken from a bioreactor installed into an area were procedures do not involve the use of Paraffin wax (countercheck);
- Sample 2: it was taken from a bioreactor installed into the area were Paraffin wax is used for pathological analysis
- Sample 3: it was taken from a bioreactor previously installed into the area were Paraffin wax is used for pathological analysis, but moved to a balcony 48 hours before the sampling. This relocation was aimed to verify possible benefit from decreased exposition to airborne contamination, i.e. bioreactor's system ability to recover in outdoor conditions (with more oxygen available and lower contaminants concentrations).

Target contaminants to be identified with the analysis are paraffin wax and degradation metabolites, such as low volatiles hydrocarbons, alcohols and aldehydes, by the application of method proposed by Marino (1998).

Under the applied test conditions, different results were obtained for the three samples:

1. No evidences of hydrocarbons related to Paraffin wax;
2. Several Paraffine hydrocarbons clearly identified, such as:
   - Eicosane $C_{20}H_{42}$ (i.e. solid Paraffin),
   - Tetracosane$C_{24}H_{48}$,
   - Pentacosane $C_{25}H_{50}$,
   - Hexacosane $C_{26}H_{54}$
   - Octacosane $C_{28}H_{58}$

   In addition to this, the chromatograhical spectra displayed signals of fatty acids, such as as Myristic acid (C14), Palmitic Acid (C16) and Stearic acid (C18), and unsaturated acids (Oleic acid).

   The main peak eluted at a Retention Time of 7,85 min (correspondent to Hexadecanoic acid) and a significant peak was also detected at RT = 7,01 min (Myristic acid).
3. GC- MS spectra did not indicate any presence of Paraffine hydrocarbons, but fatty acids were detected, i.e. Myristic acid (C14), Palmitic Acid (C16), Stearic acid (C18), Oleic acid and Linoleic acid. The main signals elute at RT = 8,58 min (Oleic acid) and RT = 7.85 min (Palmitic acid)

As reported by Sakthipriya et al. (2016), the solubility of long chain hydrocarbons decreases with an increase in carbon number and a reduction in the temperature, due to effect on both viscosity and bioavailability of the medium. In order to obtain a remarkable degradation of Paraffin (77-93% in 24 hours), Sakthipriya's group applied a temperature of 35°C and two mesophilic strains of selected bacteria (Pseudomonas aeruginosa, a human pathogen, and Pseudomonas fluorescens). Therefore, provided the lower temperature of the medium and mixed consortium of bacteria forming the biofilm, together with real scale scenario (i.e. mixed airborne contamination to be treated), it is assumable that the bioreactors' system installed

- captured a remarkable amount of Paraffin particles and organic material from the air,
- accumulated intermediate products from paraffin wax degradation, as demonstrated by the presence of different hydrocarbons and acids, typically listed among degradation products of eicosane (Sakthipriya et al 2016),
- would have required longer recovery period (or lower exposition to the present load of contamination) to carry on the major or complete degradation of Paraffin, as demonstrated by the presence of fatty acids alone in Sample n.3, without residual long-chain hydrocarbons.

For these reasons, it is possible to conclude that the system was undersized for the specific pilot area, but, with the implementation of a higher number of bioreactors, possibly upgraded to reduce the clogging effects, it may represent an interesting solution for pathological laboratories.

Albeit paraffin wax is not reported as carrying inhalation risk for itself, except for a general "low hazard" indication related to wax fumes (MSDS, Edwards et al. 1984), particles dispersed in indoor air, especially when small and regardless of their chemical nature, may pose a health risk over chronic exposure. In addition to this, it should be considered potential health risk given by paraffin wax particles possibly reacting with solvents typically applied in pathological practice (e.g. Xylol): a higher solubility could, in fact, make the paraffin particles able to interact with biological tissues and, therefore, hazardous when inhaled.

Specific toxicity studies would be necessary to properly assess potential risk in pathological laboratories, but, based on results obtained and evidence gathered during the present study, the precaution principle should lead to take additional safety measures against paraffin wax issue.

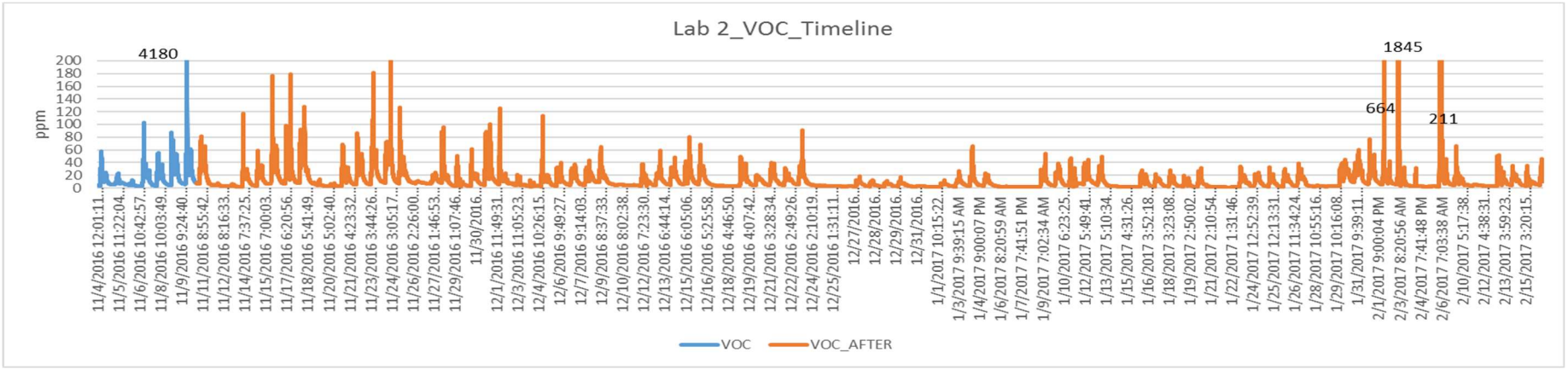


Fig. 6.35.: Pilot area 1, Lab 2 – VOC time record

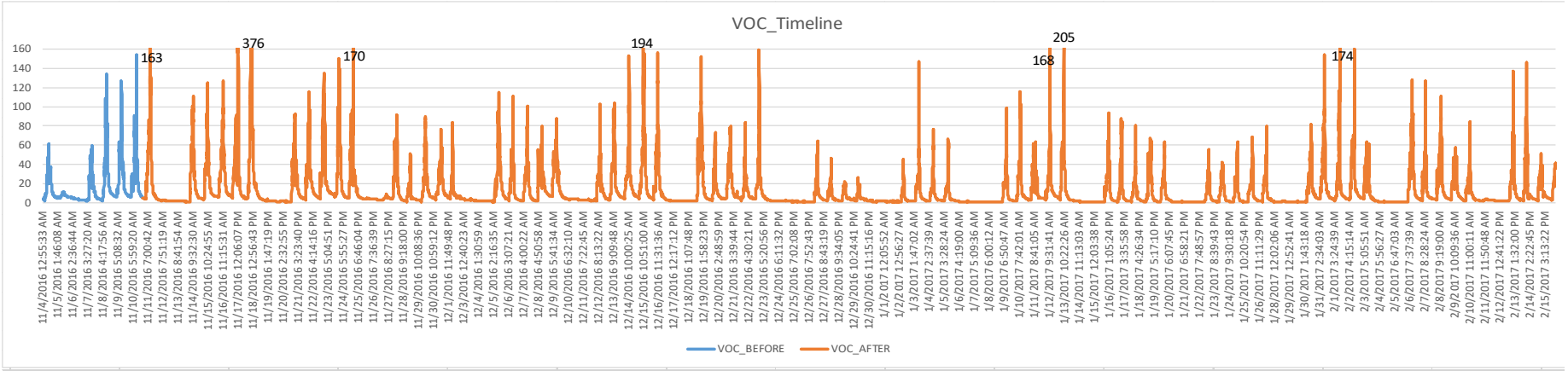


Fig. 6.36.: Pilot area 1, Lab 5 – VOC time record

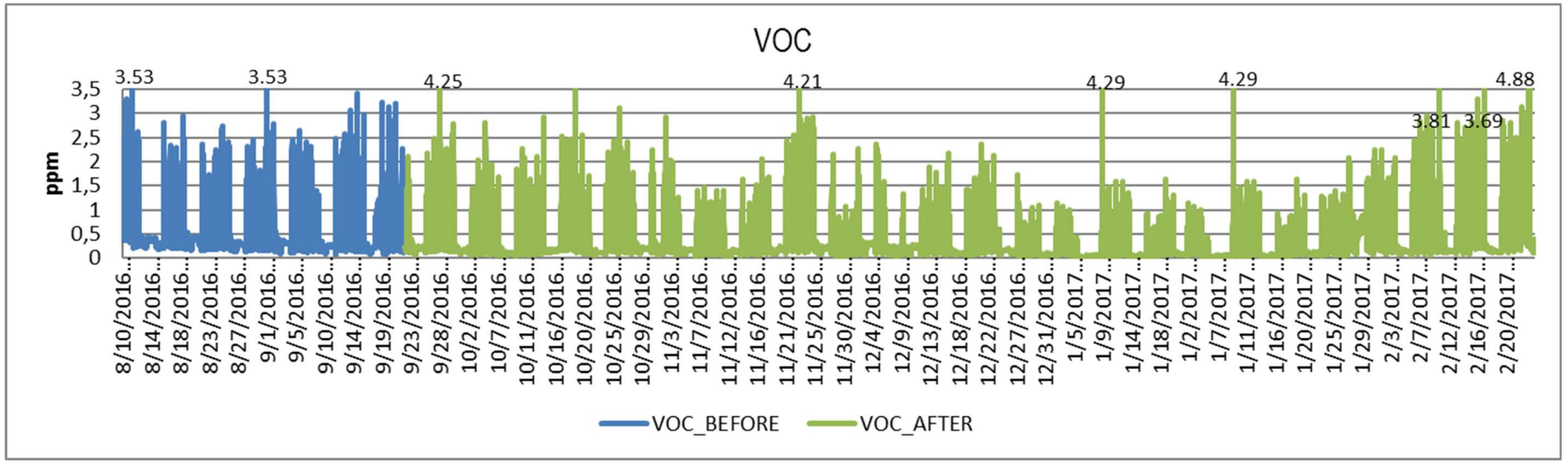


Fig. 6.37.: Pilot area 2, Cytology – VOC time record

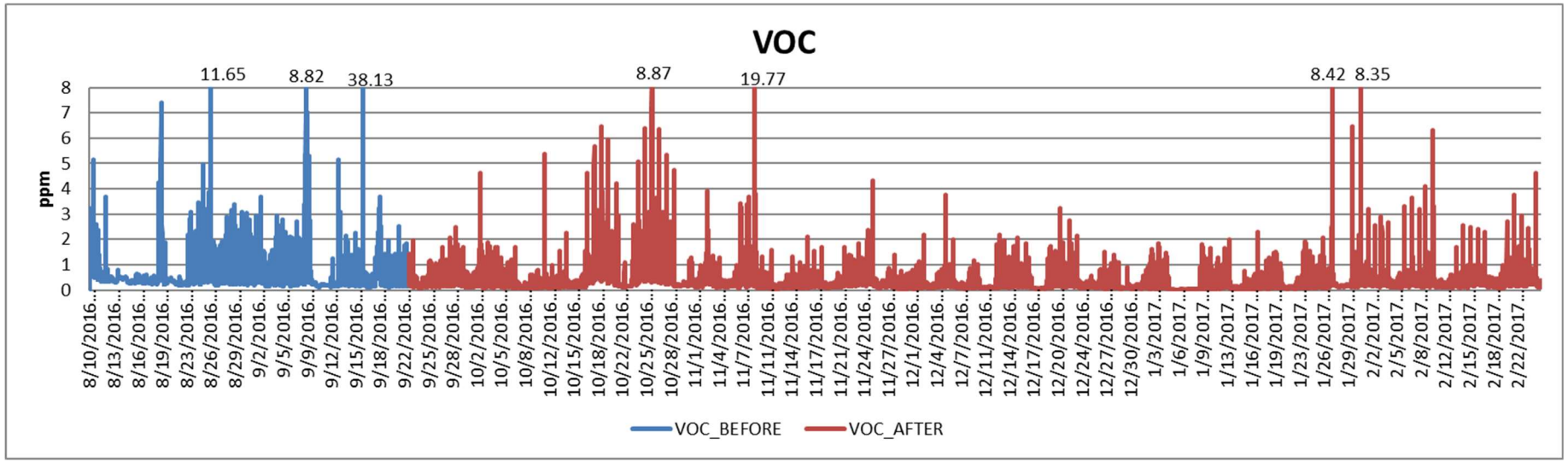


Fig. 6.38.: Pilot area 2, Samples preparation – VOC time record

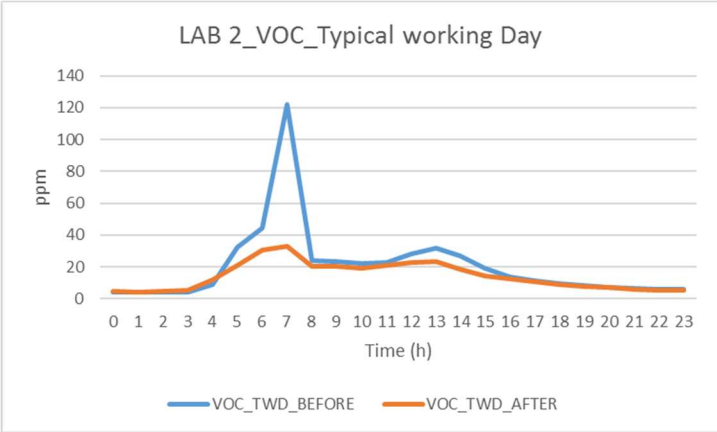


Fig. 6.39.: Pilot area 1, Lab 2 – VOC concentration on the Typical Working Day

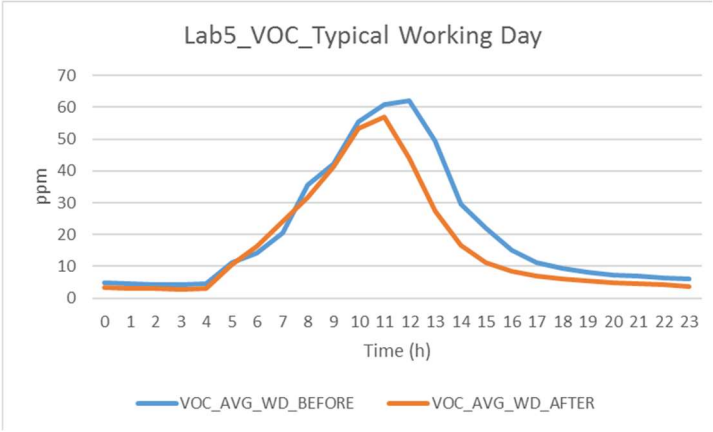


Fig. 6.40.: Pilot area 1, Lab 5 – VOC concentration on the Typical Working Day

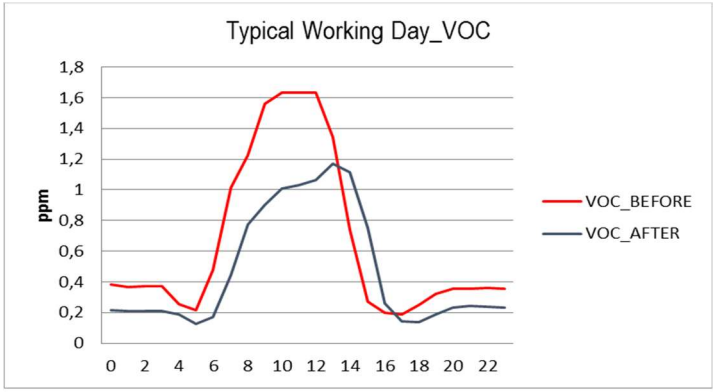


Fig. 6.41.: Pilot area 2, Cytology – VOC concentration on the Typical Working Day

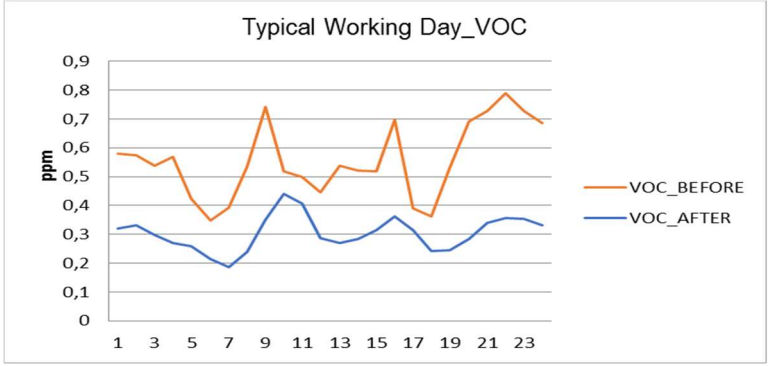


Fig. 6.42.: Pilot area 2, Samples preparation – VOC concentration on the Typical Working Day

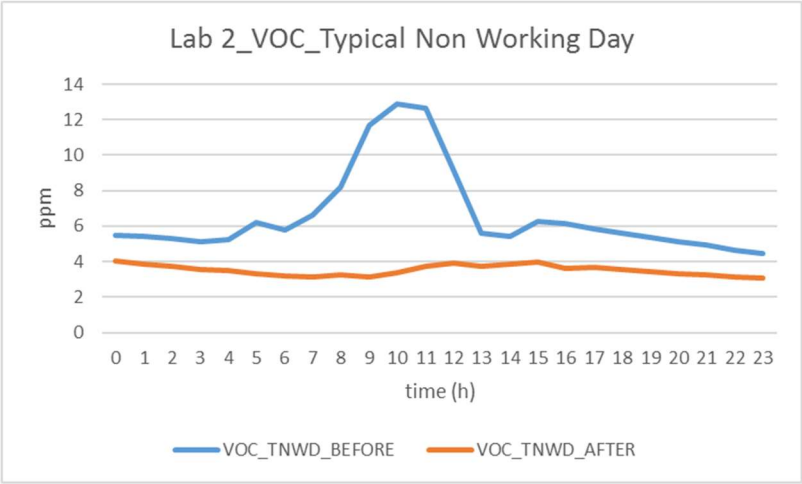


Fig. 6.43.: Pilot area 1, Lab 2 – VOC concentration on the Typical Non Working Day

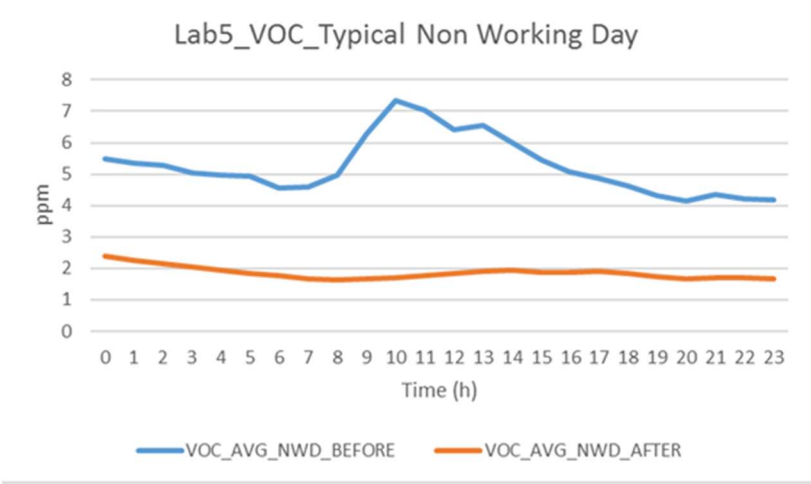


Fig. 6.44.: Pilot area 1, Lab 5 – VOC concentration on the Typical Non Working Day

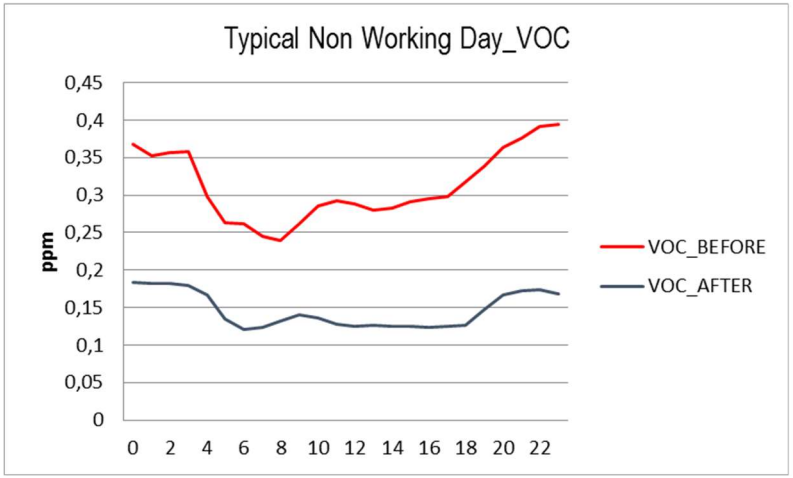


Fig. 6.45.: Pilot area 2, Cytology – VOC concentration on the Typical Non Working Day

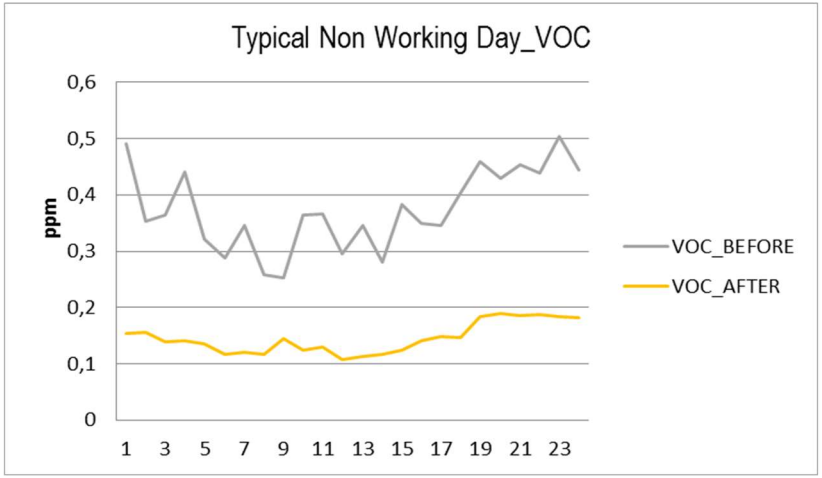


Fig. 6.46.: Pilot area 2, Samples preparation – VOC concentration on the Typical Non Working Day

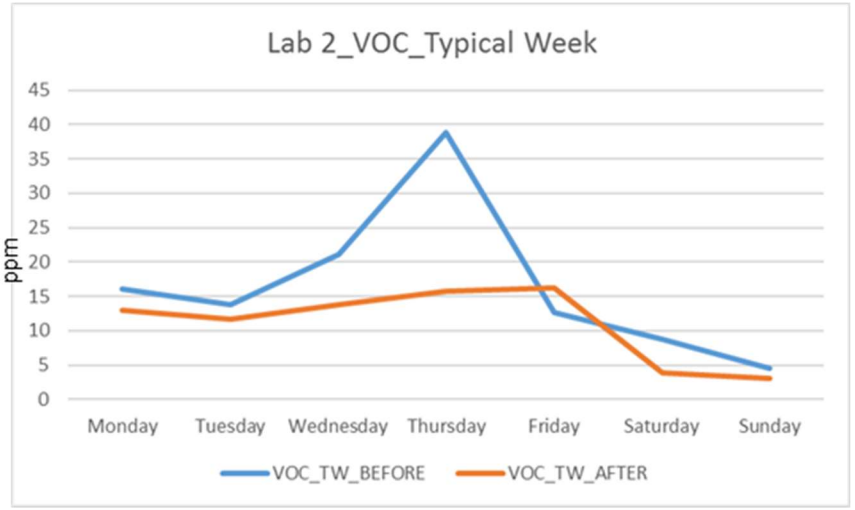


Fig. 6.47.: Pilot area 1, Lab 2 – VOC concentration on the Typical Week

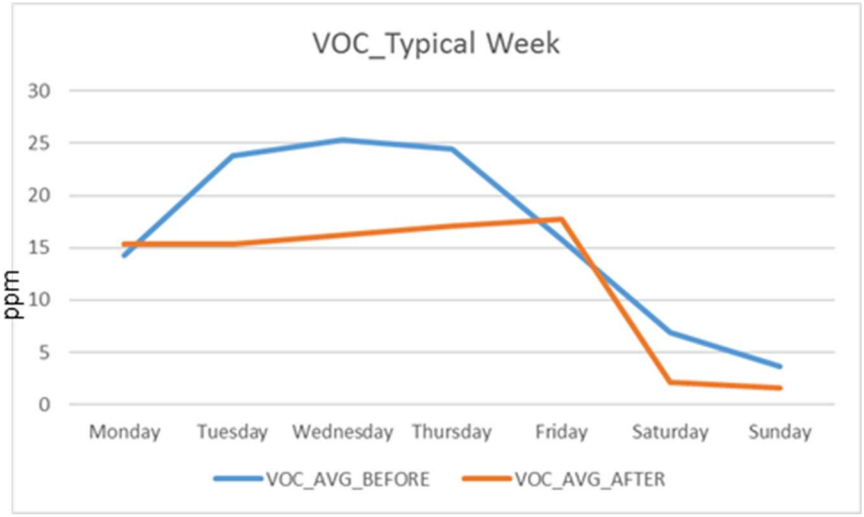


Fig. 6.48.: Pilot area 1, Lab 5 – VOC concentration on the Typical Week

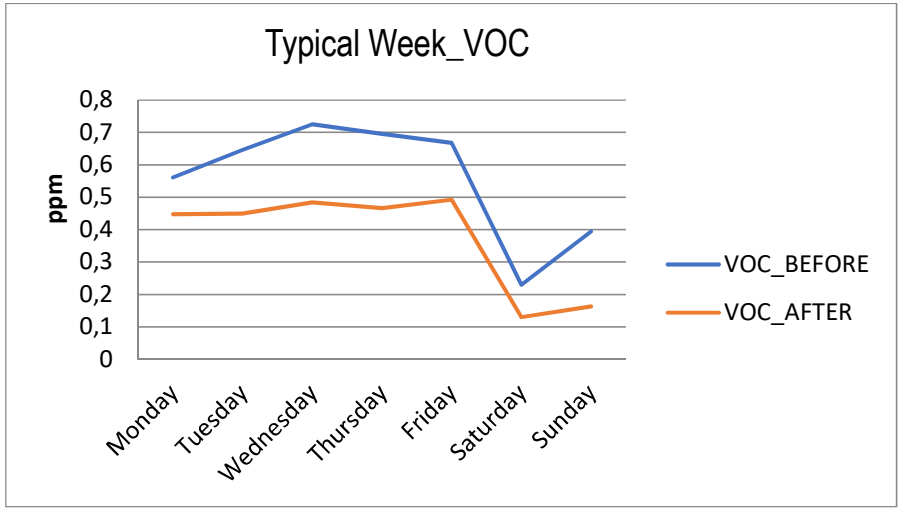


Fig. 6.49.: Pilot area 2, Cytology – VOC concentration on the Typical Week

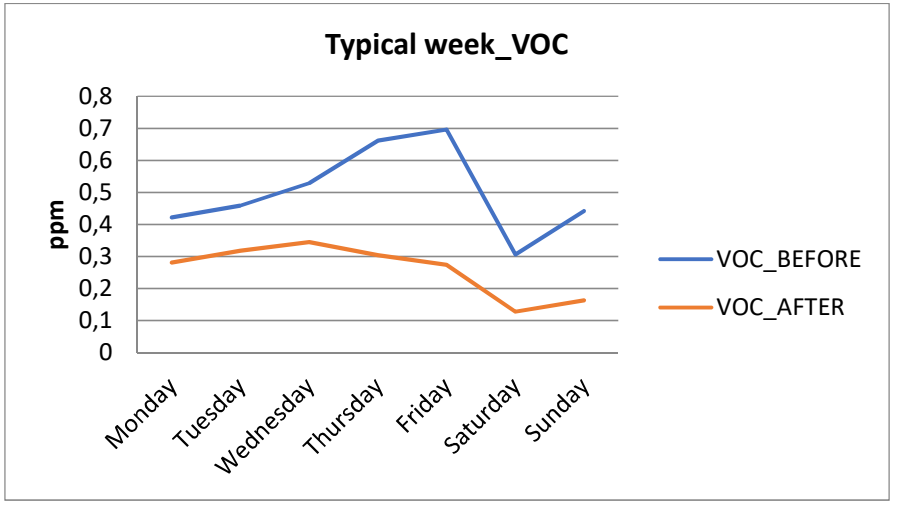


Fig. 6.50.: Pilot area 2, Samples preparation – VOC concentration on the Typical Week

6.2. Odor containment

In order to try and address one of the most challenging issue for air treatment, i.e. odor containment, two major pilot applications have been performed, on waste and wastewater treatment plant.
Of course, determining the effectiveness of a technology in this field is particularly tricky, due to the complex nature of phenomena related to odor spreading and perception.
In fact, as stated by many authors, the detection of relations between olfactory odors units and concentration of gaseous indicators in the air presents several criticalities, such due to the high number of odor producer compounds as to different olfactory effects generated in presence of antagonist or synergic elements.
The general approach to odor detection is commonly related to: a) concentration compared to odor detection threshold; b) intensity; c) physical-chemical characteristics d) hedonistic tone; e) quality.
Two main families of detection methods are normally applied to odor study: sensorial, i.e. based on human component (dynamic olfactometry and questionnaires), and analytical (Gas Chromatography-Mass Spectrometry or colorimetric methods), i.e. involving instrumental apparatus and chemical analysis. Both methods present, nevertheless, limits and uncertainties (Zarra et al.2008, Zarra et al. 2014):

- sensorial techniques, even when performed accordingly to EN 13725:2003, depend on sensibility of subjects included in the panel of evaluation and, being a group of trained testers, it is not necessarily representative of the population exposed to the specific odor;
- analytical methods rely on an initial screening of airborne contaminants, aimed to identify specific odor tracers and, therefore, may not consider synergic effects or adequately represent annoyance produced, while it can easily be affected by bias (meteo-climatic conditions, concurring contaminations etc.).

The procedure for characterization of odours and related impact assessment is not specifically regulated (Naddeo et al. 2012): therefore, several protocols have been proposed and applied. Trying to determine a direct correlation between odorimetric units and gaseous concentration of compounds identified as odor indicators could be both complicated and misleading, due to the number of odor producer compounds as well as to different olfactory effects generated in presence of antagonist or synergic elements (Mancini et al. 2010, Bonoli et al. 2014a, 2014b). As experienced during the two pilot test applications presented in the following, the real scale scenario may be too complex to be modeled and univocally interpreted.

6.2.1. Waste treatment

The present chapter has been published as a research paper on “WSEAS Transaction on Environment and Development” as “Indoor air quality in waste treatment: environmental issue and biotechnology application for air pollution containment, a case study”.

Abstract

A proper integrated management of municipal waste analyzes the entire life cycle of waste, from cradle to grave, i.e. the final stage of disposal or recycling, through which waste come back as a resource, as required by Waste European Directive. In this perspective, every possible impact factor should be taken into account and, therefore, air quality and odor control have to be addressed as crucial elements for sustainable waste management, as directly affecting quality of life of both workers and people living in the surroundings of waste treatment facilities. While the issue is generally regarded as a major concern in presence of incinerators (for air pollution control) and landfill (for odor, mainly), it is usually neglected when segregated dry waste treatment is involved, but it remains an element of concern for population and, therefore, public stakeholders. A modern segregated waste treatment plant, already compliant with regulations requirements regarding indoor air quality and human health, was taken as a case study to prove the effectiveness of a biotechnological treatment for air pollution and odor control. The system applied is based on stand-alone bio-oxidizers that provide internal air-mixing within the facility and capture particulates and gases by attracting them to a clean air zone generated by its action. In this paper, only the preliminary phase of application for the system is presented. It was preceded by a completion of analysis of air quality baseline, collected by a Wireless Sensor Network, which have been compared to the following five months of system activity, showing a consistent effectiveness in air pollutant containment and abatement. These results found confirmation in parallel independent laboratory analysis which showed comparable abatement trends. A comparison with a traditional biofiltration case study marked the great opportunity offered by the bioreactors' system implemented in an overall indoor air quality perspective.

1 Introduction

The approach of the European Community with regard to waste management is based on the following principles:

- Prevention;
- Recycling and reuse;
- Final disposal and related monitoring.

Technologies related to disposal phase, as well as recycling, are to be understood in the wide framework of an integrated approach to all stages of waste management in order to ensure adequate protection of health and environment (Bonoli et al. 2007).

The integrated waste management systems are designed to organize waste streams, methods of collection, treatment and disposal, with the goal of achieving environmental benefits, economic optimization and social acceptability.

A proper integrated management of municipal waste analyzes the entire life cycle of waste, from cradle, corresponding to the time when a product becomes a waste, to the grave, i.e. the final stage of disposal or recycling, through which waste ceases to be such and come back as a resource. It is, therefore, clear, on the basis of the Waste European Directive that recovery technologies must be encouraged, in particular by encouraging selective collection of municipal wastes. In fact, the collection phase plays a major role in the integrated waste management system, as it allows to promote recycling operation necessary in order to substantially reduce pollution, energy and raw materials consumption, together with waste production in a cost-effectiveness, efficiency and environmental protection perspective (Bonoli et al. 2007). In order to

match European requirement, then, an integrated approach to environmental impact related to waste management must be implemented, taking into account every possible impact factor during the entire life cycle. As suggested by many authors, waste treatment facilities, from landfill to incineration, may be associated with emissions of air pollutant, negatively affecting air quality in the surrounding areas (Hamoda 2006).

Even if municipal waste sorting and crushing plants carry a minor to negligible risk in terms of threat to public health, generating an number of contaminants (both gases and particles) lower by orders of magnitude when compared to, for example, incinerators, they nevertheless may represent a source of disturbing odor and air quality-related operational risk for employees, as air contaminants can be a major source of respiratory diseases.

Traditional air pollution control and prevention technologies include physico-chemical methods such as adsorption on activated carbon, thermal as well as catalytic oxidation. The effectiveness of these technologies is strongly related to the ability to provide the right working conditions (e.g. high oxidation temperatures or controlled air flux rates and large reaction surfaces for adsorbent bed), and, therefore, operational costs tend to grow together with required performances. Furthermore, these technologies may lead to several by-products which, being pollutants, appears as concerning as the ones removed (e.g. exhausted adsorbent bed and incineration ashes, both heavy and fly ashes, which have to be disposed as dangerous wastes in EU Countries) (Devinny et al. 1999) and present few to none application opportunity on diffusive sources of airborne pollutants (e.g. landfills or Biological Mechanical Treatment plants). Therefore, there is a need for alternative technologies for air pollution control that have the potential of replacing physico-chemical treatment technologies, stimulating the development of several solutions.

The removal of odors from air into wastewater and waste treatment plants is often effected through biological means in unit operations like biofilters, biotrickling filters and bioscrubbers, which can be generically referred as organic perfusion column (McNevin et al. 2000).

In Europe, together with chemical deodorization, biofiltration is by now a well-known and widely used technology for control of odors, air pollutants and volatile organic compounds (VOC - often related to disturbing odor issues) from different sources in industrial and public service sectors (Leson et al. 1991), but it remains difficult to apply, especially in urban area, because of the wide surfaces required and possible lowering in performance due to climate condition. Into a biofilter, in fact, a contaminated air flux is ducted to pass through a biologically enriched layer of a filter material (i.e. soil, wood chips, compost or mixed materials) where the pollutants are absorbed/adsorbed and biodegraded by the microbial population. Byproducts of microbial oxidation are primarily water, carbon dioxide, mineral salts, some volatile organic compounds and microbial biomass (Nicolai et al. 2001).

Microbial activity is regarded to be affected by moisture content, pH, nutrient limitation, temperature and microbiology of the biofilter medium (la Pagans et al. 2005) strongly relating the system performance to contour and working conditions.

2 Problem Formulation

It is well known that odor problems related to waste management system may originate from airborne or surface contaminants (i.e. bacteria and fungus growth, spores, chemical fumes or digestion vapor), so that a genuine health concern accompanies odors, even when intermittent or deriving from segregated waste treatment facilities, where organic fraction should be absent. Therefore, odor control remains one of the most significant challenges for waste treatment facilities today, even if materials come from segregated collection.

Since disturbing odor are usually caused by compounds with low odor thresholds, off-gas concentrations will often be in the low ppmv range (Leson et al. 1991), making their abatement rather difficult or very expensive, both under an economical and environmental perspective. Air treatment, in fact, requires a great amount of energy, especially when dealing with piped air. In addition to this, air extraction and ducting (similar to Pump-and-Treat system used in soil and groundwater remediation field), which is the most common technique applied for air treatment, do not guarantee problem solution, since odor are often carried by fine and ultrafine particulate, as well as by gases.
As the efficiency of odor and air pollutants treatment system in waste treatment facilities is widely regarded as unlikely to be sufficient for some volatile organics, more reliable tools are needed, in addition to commonly used technologies or in their replacement, in order to reduce such recalcitrant contaminants (Kim et al. 2013).
A modern segregated waste treatment plant was taken as a case study to prove the effectiveness of a biotechnological treatment working on motion of contaminant for concentration gradient and not through ventilation, providing a sustainable alternative to traditional air treatment techniques.
The waste treatment plant selected for the trial will be treated for 15 months with Immobilized cell Bioreactors, commercially known as AIRcel system, for the containment of odor problems alleged by the neighborhood and microbiological hazards possibly carried on wastes. Both issues are closely linked to the type of work performed within the facility, even though former air quality checks have shown that the plant is compliant to regulations for healthcare in workplaces. The company involved into the test has shown, however, interested in establishing a new standard of environmental quality within their facilities in order to prevent health and environmental risks both for workers and population living in nearby areas.
The effectiveness of the experimental application in terms of reduction of airborne contamination (gaseous, odorous and microbiological) shall be evaluated by monitoring performed by an accredited third party laboratory, a continuous wireless monitoring station and evaluation of the attitudes of people about the trend of odor emissions from the plant.
The preliminary phase of application for the system, presented into this paper, was preceded by a completion of analysis of air quality baseline: the control units (commercially known as U-Monitor) have been, in fact, installed the previous week the bioreactors system was set. The definition of the baseline was carried out during one week (July 14 to 20), excluding the first 3 days of installation (11-13 July), which showed high concentration values so abnormal compared to following days, and considered, conservatively, not significant for the construction of a term of comparison. The baseline is, therefore, been detected in a period of decreasing activity, up to the stop, of the plant for summer break.

2.1 Performance test

A precisely scheduled monitoring plan has been developed, as to cover the whole experimental period, i.e. 15 months after its inception, during which a continuous monitoring system will be kept operational and lab analysis, such as column air test for gases and odor and Petri plates count through Surface Air System sampling, will be repeated on a seasonal basis as a complement and countercheck. In order to reproduce as faithfully as possible the boundary conditions, the production cycle will be reconstitute, from time to time, very similar to the baseline.
During the technical inspections, spot measurements were made of Volatile Organic Compounds by PID (handheld photoionizer) which, although not bearing an absolute probative value, had completed the perceptual impressions collected. This kind of portable device, in fact, is a broad band detector, calibrated on using isobutylene, and other compounds may produce a response depending on concentration. Being not selective (it may virtually ionize every compound with an ionization energy less than or equal to the lamp

output) and sampling on an instant basis, this monitoring method has not been taken into account as a reliable performance test for the system.
In order to have continuous feedback on the effectiveness of the bioreactor system installed, two monitoring stations have been places in different location of the treatment facility (supply station and secondary shredder), equipped with a set of sensors that can detect a variety of contaminants, as well as explained in the next section.

2.1.1 Monitoring System

The monitoring system consists of a Wireless Sensor Network (WSN), designed to collect air quality data in the environments where the bioreactors are installed, and a software platform that is the control center, processing and visualization of the data collected.
The objective of the monitoring devices is to detect the presence of harmful gases and fine dust into the environment and, optionally, some environmental parameters, such as temperature and humidity.
The monitoring devices, physically realizing the WSN, are characterized by:

- sensors for the detection of
  - temperature
  - humidity
  - environmental contaminants (mainly toluene ($C_6H_5CH_3$), hydrogen sulfide (H_2S), ethanol (CH_3CH_2OH), ammonia (NH_3)
  - solvent gases (mainly alcohol, solvents, hydrocarbons, VOC)
- particle counters PM1 and PM2.5
- built-in WiFi module for wireless and real time communication of data
- time of collection of environmental data set to 15 minutes.

2.1.2 Baseline definition

The definition of the baseline of comparison is critical for evaluating the performance of the system. This baseline has, in fact, to include a sufficient number of days to constitute a proper statistical basis for the calculation of an average that can be representative of the period and the activity of the plant. The combination of the trial with the decrease in physiological activity of the plant for the summer necessitated a proper assessment of this aspect, but it was regarded, nevertheless, as a great opportunity to relate operational phases of the facility to air pollutant concentration, during this preliminary study.

Since the very first days of application of the monitoring system showed a significant gap in high concentration of all sensors, they have been discarded and the more representative trend displayed in the following week was assumed as baseline value (Table 6.8).

AVG VALUES	AIR CONTAMINANT	SOLVENT GAS	DUST (1-2,5 MICRON)
FEEDER	84 ppm	65 ppm	2341 part/dm3
SECONDARY SHREDDER	52 ppm	63 ppm	1820 part/dm3
WHOLE FACILITY	68 ppm	64 ppm	2080 part/dm3

Table 6.8. Baseline values

Evaluation of the data collected so far by the U-Monitor can not ignore the contextual consideration of production trends, as the baseline definition period and the installation of the bioreactors system took place during summer, when waste treatment proceeds in a cycle far different from the standard. At the same time, summer months, in the previous year, proved to be a critical period for disturbing odor emission, probably related to longer rest of residual material into storage tanks and anaerobic conditions establishing into them, giving space to sulfur compounds to develop and spread out.

3 Problem Solution

The air treatment system proposed to try to improve air quality standards into the waste treatment facility is constituted by stand-alone Immobilized cell Bioreactor, carefully sized and placed in order to empower the system effect and overlap influence area of the single units. No exhaust air pipeline has been installed, since the AIRcel system works on indoor containment of contaminants, preventing issue typically related with air ducting, such as high energy consumption for ventilation and air conditioning and difficulties in capturing pollutant which may be more affected from electrical surface field rather than air motion, because so fine that specific surface is overwhelming compared to mass and volume.

3.1 Technology applied

The system is based on stand-alone bio-oxidizers that provide internal air-mixing within the facility and capture particulates and gases by attracting them to a clean air zone generated by its action.

The bioreactors, in analogy to biofilters technology (McNevin et al. 2000), consist of three phases in close contact: a solid phase, which is the bioreactor itself, a liquid phase, i.e. water, and a gas phase, that is air to be treated. As in common biofilters, a physical support for biomass growing is offered by a solid medium, but, in this case, a plastic patented bioreactor is provided with optimized configuration in order not only to become growing support for biomass, but even to enhance its degrading activity.

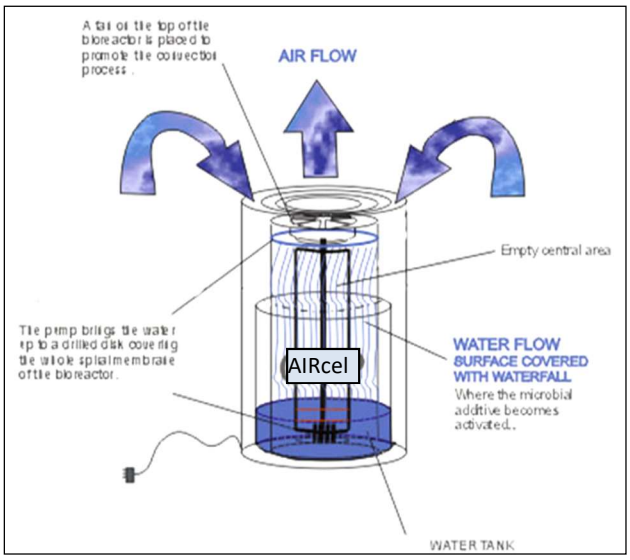


Fig. 6.51 - Simplified outline of an AIRcel bioreactor (U-Earth Biotechnologies s.r.l.)

The leading mechanism is the biological digestion of the hazardous materials attracted. These miniaturized treatment plants, in fact, utilize bio-oxidation to destroy gases, volatile organic compounds (VOCs), odors,

and remove particulates though bio-hygienics principles, i.e. the natural phenomena used to control IAQ, electrical as well as biological (Sofer 2006), thus airborne contaminants are first captured and subsequently digested biologically.

Many authors stated the effectiveness of specific bacteria strains in degrading different contaminants (McNevin et al. 2000) such as Chemoheterotrophic bacteria to promote Organic Carbon oxydation (from VOC to CO_2 and H_2O), Nitrifying bacteria for nitrification (from NH_4^+ to nitrite and nitrate), Sulfur oxydising bacteria to achieve Sulfide oxydation (from H_2S to S^0 and sulfate) (all in aerobic environment) and Denitrifying bacteria, to promote Denitrification (from nitrate to gaseous nitrogen) in anaerobic conditions. Nevertheless, since the biomass representing the core of the AIRcel technology is a proprietary formulation, in which the claim is that no genetically manipulated microorganism, it appears to be a quite composite bacteria and enzymes consortium The biomass proposed is, infact, able to attack and digest compounds different in nature, degradation process, contour conditions requirements and inhibitors, final products and reaction by-products.

Intimate gas-liquid mixing with electrically grounded water from the reservoir tank additionally grounds the clean air zone, attracting and capturing pollutants. Contaminants, along with the odors that they generate, are attracted to this clean air zone by concentration gradients (pollution moves from high to low concentration, both with mass and electrical charge), where the charged particles are removed by electrical grounding and the organic compounds are oxidized (Borkowski 1995, Lakhwala et al. 1991, Shim et al. 1995). This can be accounted as a sustainable technology, particularly when compared to standard air treatment systems, since it does not require elevated temperatures (as post-burners) or pressures (as membrane filters) or excess energy (as any ventilation system) to operate.

In the waste treatment facility offered as case study, n.8 AIRcel of the bigger size have been placed: n.6 inside the building and around the potential source of contamination (input waste material storage, treatment line and final product storage tanks); n.2 just outside, in order to cover the main exit of final product and guard the external border of the two storage tanks, accounted to act as major source of odor contamination. A simplified sketch of the treatment plant and system implemented is provided below, with the aim of showing the expected area of influence of the eight bioreactors.

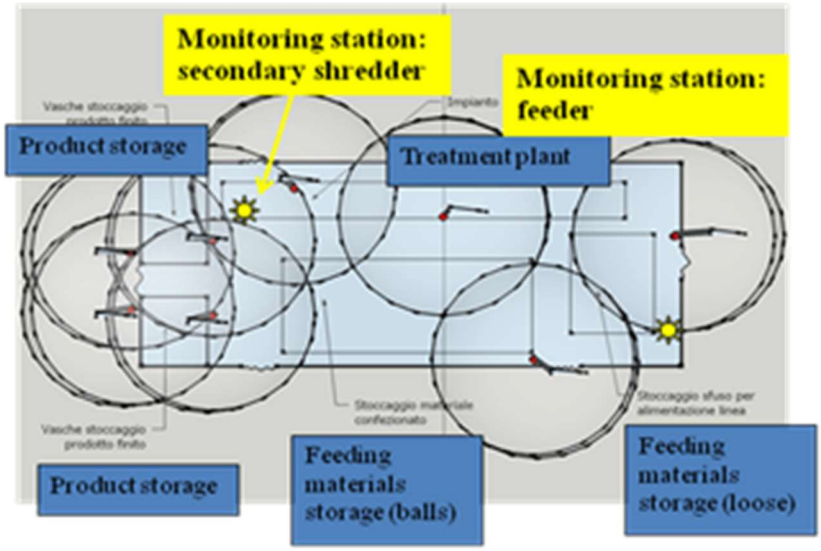


Fig. 6.52 - Outline of the system into the treatment facility

3.2 Results and discussion

The evaluation of the data collected by monitoring system has been divided between gaseous contaminants and particulate matter, which present different behaviors both in chemical and physical terms.
In order to provide an effective comparison between the contaminant concentration found during the first five months of the trial and what obtained as a baseline, some graphs are displayed in the following pages. In particular, results returned by the sensors "Air Contaminant" and "Solvent Gas" are reported first, as more closely related to the odor quality of the environment.

3.2.1 Gaseous contaminants

It is immediately evident how the system has responded to the initial saturation condition with expected developments of airborne pollutant concentration, the interpretation of which cannot, however, be abstracted from the evaluation of operational condition of the waste treatment plant:

- initial increase in the concentration of the contaminants monitored, although the peak contained 80% of the baseline value (Fig. 6.53), corresponded with the delivery of particularly smelly waste material. The highlighted peak is evident for both contaminant clusters detected. This event has come to engage on the phase of desaturation of the system that could not be still able to immediately treat the emergency;
- subsequent decrease of the concentrations of airborne contaminants, with a similar trend found by two different sensors and in the two positions of detection;
- secondary peak concentration (highlighted in violet in Fig. 6.53) detected during the second week of operation, appears as correspondent to the working phase of the system on the contamination immobilized on surfaces. This event, which tends to momentarily increase the concentration of pollutants in the indoor environment, has occurred concurrently with a maintenance issues on the machines;
- concentrations after the peaks are subsequently dropped, despite the recovery in the plant full capacity at the beginning of September: during the first 120 days of operation of the plant at full capacity, the airborne contamination continued its downward trend now that the system reached a state of equilibrium that allows the containment of pollutant events within a very short time;
- the next four peaks (highlighted in Fig. 6.53 and 6.54 in red) detected by the sensor "Air Contaminant" at the Feeder and have been related to maintenance work on the bioreactors, which required the stop of the air treatment system;
- in Fig. 6.54 and 6.55, a black arrow indicates a secondary peak in concentration detected by the Solvent Gas sensor, presumably due to the delivery of particularly smelly material;
- the concentrations found in the last weeks of full operational performance of the bioreactors' system, were maintained within 15% of baseline values, with a consequent reduction greater than 85% detected by both sensors, but Week 46 and 50-51 experienced major failure of the technology and, consequently, higher peak of contaminants concentration have been detected. In Par. 3.4 an attempted correlation between system's failures and secondary concentration's peaks was made, with the aim to proceed in the next months of the trial period with a deeper analysis of the phenomena.

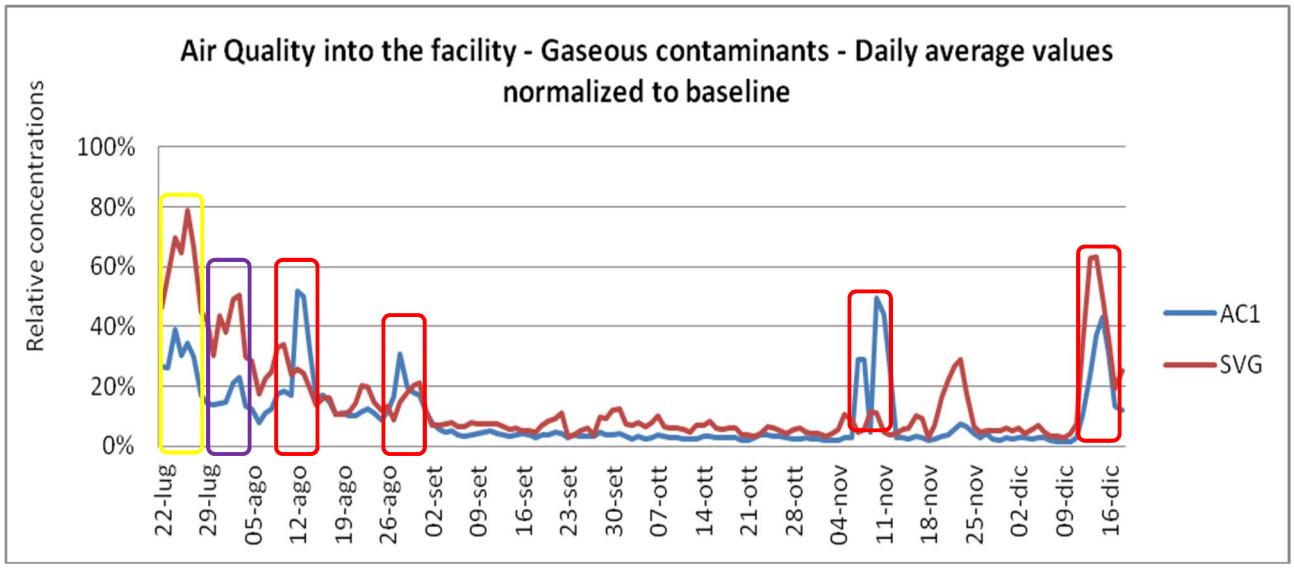


Fig. 3 - Daily avg values referred to baseline value, gaseous contaminants

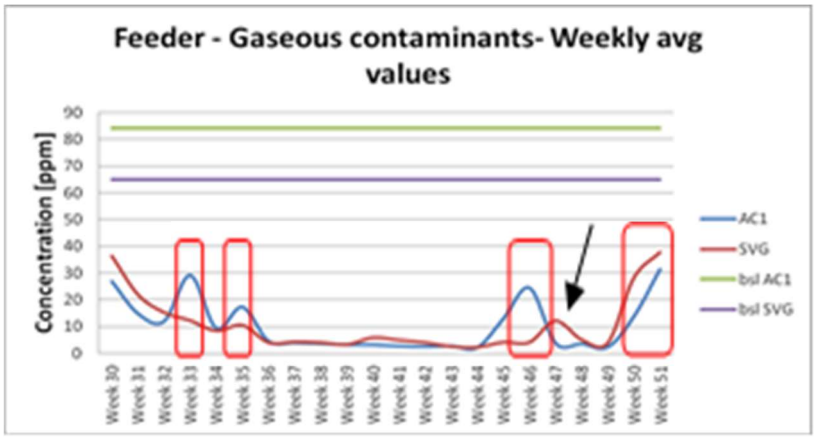


Fig. 6.54 - Weekly avg values, gaseous contaminant at the feeder section

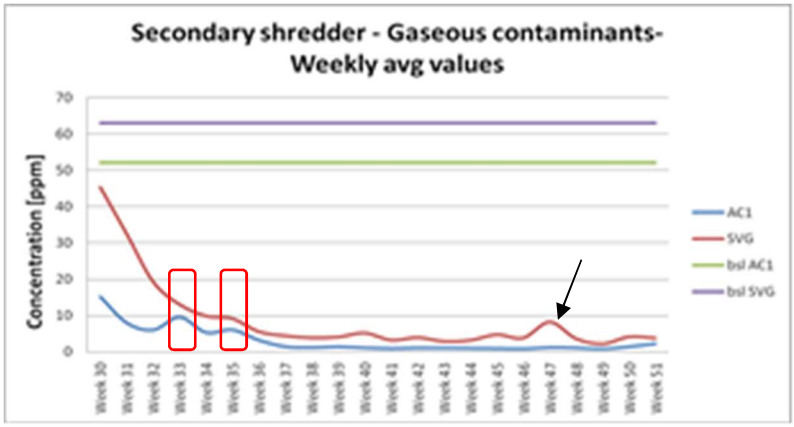


Fig. 6.55 - Weekly avg values, gaseous contaminant at the secondary shredder section

Relating concentration peaks detected by sensors with indication of intense or disturbing odor event recorded by workers (note that no odor events has been reported by people living in the surrounding area),

it can be considered that they proved to be related to gaseous concentration peaks detected at the feeder section, while no correspondence were detected with secondary shredder activity.
The peaks of gaseous contamination appear to be wider (so, longer lasting) and with a higher absolute value during the frst weeks of Bioreactors system application (July and August). During September, on the contrary, the peaks of contaminants are tighter (i.e. "shorter" in time) and reach an absolute value greatly reduced (6-8 times) than in previous months.
This trend shows that, following a first period of de-saturation (July and August) in which a large amount of contaminants (high peak values) travels slowly to the AIRcell system (broad peaks), after nearly two months of operation (from September on to December), contaminants move in small clouds (peak values slightly higher) that are attracted quickly toward AIRcell (narrow peaks) and do not spread into the surrounding environment.
The absence of abnormal measurements on device Secondary Shredder attest that the AIRcell can capture and remove contaminants that generate odors, preventing its spread to areas far from the source.

3.2.2 Particulate matter

The contamination related to particulate matter, perceived as "fine dust" characterizing the indoor air, has been detected in parallel with the gaseous contaminants already presented. In order to give a consistent interpretation of the results a few considerations are needed:
- The particulate contamination is necessarily influenced by the activity of the plant, since it is generated by the operations of opening the waste balls and consequent shredding of the waste for final sorting, alternated to moment spent cleaning of the conveyor belts, which, therefore, must be emptied and production line stopped. For this reason, the evaluation of the performance should be carried out in parallel with production notes provided by the Company and, in particular, it is necessary to divide the consideration of two different periods:
1. summer, characterized by partial and intermittent activity of the plant, with delivery of materials, waste treatment and maintenance works when needed (July, 22nd-August, 30th), as demonstrated by the different baseline values encountered (higher for feeder section, rather than secondary shredder area);
2. autumn, with recovery of full time production of the plant (September, 1st - December, 18th);
- particulates tend to move in the air in eddies and clouds with a different degree of concentration, rather than distribute evenly in the environment;
- the two areas where monitoring stations have been installed are characterized by very different work load of suspended particulate matter: while the burden on the feeder section is related to input of the vehicles and the opening of the "bales" of waste delivered, the secondary shredder undergoes waves of contamination from two possible sources:
1. the proper shredding activity
2. the cleaning of the conveyor belts by means of compressed air, carried out at time intervals dictated by the conditions of the same belts.
These issues explain a trend of concentrations quite different from what revealed on gaseous contaminants and it is crucial to consider separately the two monitoring stations.

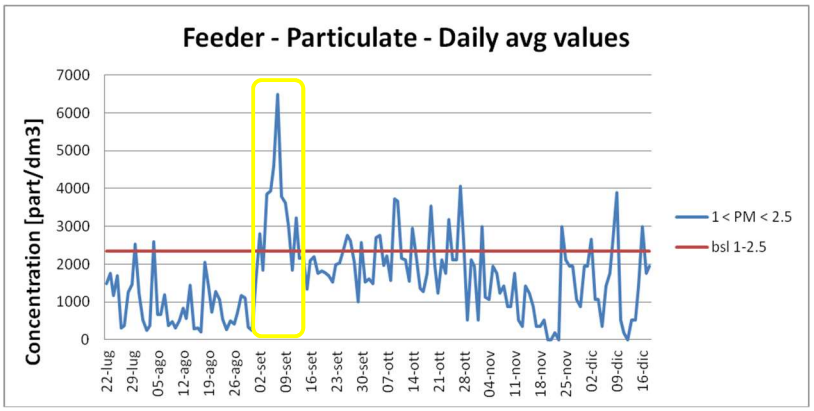


Fig. 6.56 - Daily avg values, particulate matter at the feeder section

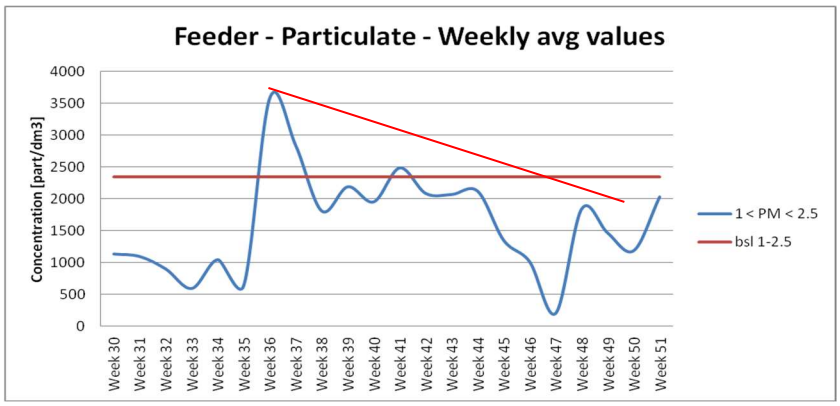


Fig. 6.57 - Weekly avg values, particulate matter at the feeder section

The first five weeks of AIRcel system activity are characterized by a reduction of particulate contamination in correspondence to the feeder section of the plant, as shown by weekly average values, which are below 40% of the baseline. Since the levels of operation of the plant have been kept low and close to the total rest in the period of the definition of the baseline and in the subsequent weeks, it can be stated that the system contained the contamination present in the plant during those weeks.

The only peaks that exceed the baseline value are so explainable:

- 07/29/2014 peak: maintenance of AIRcel units after two days of alarm due to a high load of dust which covered the air outlet, evidenced by the remarks quoted in the production notes. At the same time, the plant has been running for two shifts on July, 24th and 25th and one again on 28th;
- 08/05/2014 peak: maintenance of AIRcel units in the days immediately preceding it.

In correspondence of waste treatment plant coming back fully operational on September 1st, a peak concentration of particulate matter has been detected (marked in yellow in Fig. 6.56-6.58-6.60), due to the re-suspension of material trapped in machinery remained steady for weeks and the increase in the pollution load carried by the shredding of waste. During the following weeks, the peaks tend to decrease, returning to fluctuate around the baseline values at the feeder section. This corresponds to a satisfactory result in containment of dust contamination, since particulate concentrations are back to a diminishing trend, albeit in full operation of the plant (which implies trucks coming in and out of the building to discharge waste materials and bulldozer moving it from storage to feeder section), towards values that characterized a period of progressive switching off of the same and the peaks are progressively decreasing (red line in Fig. 6.57).

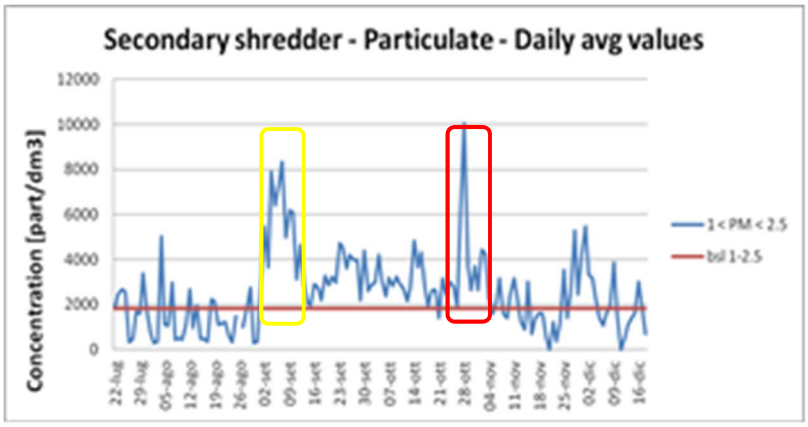


Fig. 6.58 - Daily avg values, particulate matter at the secondary shredder section

The area of the secondary shredder is evidently of more complex management (note that the facility is an open space, with no sects dividing operating sectors), due to the very nature of the processing, which tends to re-suspend periodically dust and particulate matter (even those deposited on the surfaces by gravity). Since the baseline has been defined in a period of partial processing inside the plant, values lower than the ones detected at the feeder section were provided for the same period; in contrast, the resumption of activities has meant that the peak concentrations are higher in this area, although chronologically corresponding to those already tested at the feeder (even during the shutdown, i.e. July 29th and August 5th).

The direct dependence from the operating schedule, or cleaning activities, is reflected in the performance of the most jagged peaks of concentration in daily average concentration (Fig.8). An evident peak was detected during and immediately after the maintenance work performed in late October (in red in Fig. 8 and 9). Over the forthcoming months, it will be determined the degree of correlation between these peaks and the cleaning of conveyor belts, in collaboration with the company, which is required to keep track of cleaning activities, as has been done for the production.

The line drawn in green color in Fig.6.59 shows how the concentration peaks are progressively decreasing, to demonstrate containment performed by the system even in conditions of full operation of the plant.

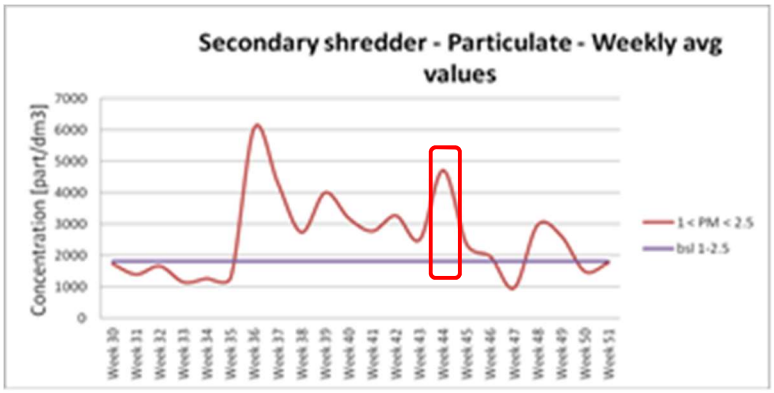


Fig. 6.59 - Weekly avg values, particulate matter at the secondary shredder section

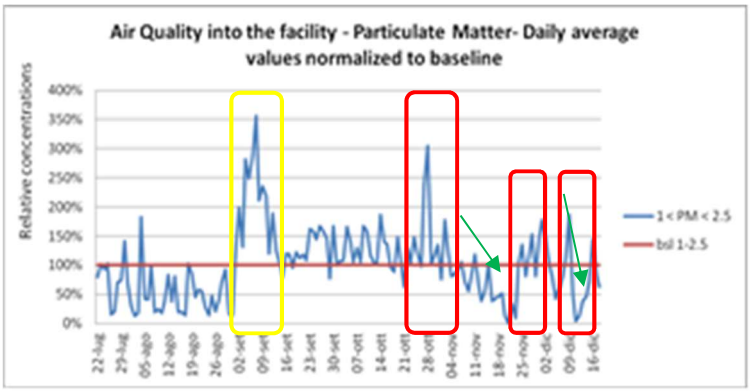


Fig. 6.60 - Daily avg values referred to baseline value, particulate matter

The concentration's trend at the two sections are, obviously, reflected by the overall air quality inside the facility (Fig. 6.60, with the re-starting of plant activity marked in yellow and major maintenance works in red), but, thanks to this elaboration, it becomes more evident how the maintenance and cleaning operation on the bioreactors affected positively the system's performance, providing a sensible decrease in particulate concentration (highlighted with green arrows following the steep of the decreasing trend).

3.3 Comparison with lab analytical data

As stated by many authors, the detection of relations between olfactory odors units and concentration of gaseous indicators in the air presents several criticalities, such due to the high number of odor producer compounds as to different olfactory effects generated in presence of antagonist or synergic elements (Mancini et al. 2010).

In this case, a combined use of olfactory methods and traditional chemical analyses has been applied to indicator compounds, procedure generally regarded as a useful mean of evaluation of odor impact on territory in the proximity of solid waste treatment or storage plants. The evaluation of odor effect with physical-chemical analysis, which appears as the most strict approach when compared to olfactometry, presents, nevertheless, several elements of concern in the monitoring planning phase, since even a single source of emissions could easily be carrier of multiple odor-promoter compounds. This concern is, obviously, enhanced when the possible source of air contamination is as heterogeneous as municipal solid wastes (even if derived by segregated collection, as material object of the present study).

The general approach to odor detection is commonly related to: a) concentration compared to odor detection threshold; b) intensity; c) physical-chemical characteristics d) hedonistic tone; e) quality. Trying to determine a direct correlation between odorimetric units and gaseous concentration of compounds identified as odor indicators could be both complicated and misleading, due to the number of odor producer compounds as well as to different olfactory effects generated in presence of antagonist or synergic elements (Mancini et al. 2010).

The odorimetric unit (1 O.U./m^3) is defined accordingly to the standard CEN TC 264, as "The amount of odorant that dispersed in 1 cubic meter of neutral air causes a sensation odor" and is considered as a measure of the concentration of odor (APAT 2013).

On the basis of Liu et al., odors from biostabilization processing of municipal solid waste after Biological Mechanical Treatment (BMT) have been analyzed by Gas chromatography–mass spectrometry (GC–MS) analysis and results showed that, among the total volatile organic compounds (VOCs), the main components of the produced gas were benzene, toluene, ethylbenzene and xylene (BTEX) along with other alkanes, alkenes, terpenes, and sulphur compounds (Liu et al. 2009). Methyl-mercaptan and dimethyl-sulphide are

also often regarded as characterizing parameter of odour emissions by guideline for solid waste treatment and storage plants (Lovely, 2006).
In the present study, a static sampling method has been applied, in order to obtain a more accurate outcome: the sample is, in fact, collected in a bag and analyzed within 30 hours; this sampling is used to odoriferous sources with concentrations varying in time due to wide reaction surfaces involved, as lagoons, tanks and landfill (APAT 2013). The instrumentation used for the sampling consists of a sample probe, distribution tube, a particulate filter upstream to the detecting system, a hood designed to provide turbulent airflow. The sample is collected only after the passage of a volume equal to 3 times shell volume into Nalophan™ bags with PTFE pipes, following the UNI EN 13725:2004 standard procedure.
Accordingly to international standards, for wide emitting surfaces or indoor environments, more than one sampling point are to be selected: for the present study, seven collection points have been identified (n.1 inside the waste treatment facility, n.4 outside the passage doors and n.2 on the roof of the building, one at the chimney of the extraction duct and one after the traditional air treatment system)
As stated by many authors and, in particular, on the basis of conclusions of Shaharuddin (Shaharuddin et al. 2009), meteorological parameters present great influence on airborne particulate behavior. Thus, meteorological data have been recorded during sampling collection, in order to correctly evaluate the results: this allowed a detailed analysis of the two baseline campaigns which led to the conclusion that only one presented the necessary weather, pressure, temperature and wind conditions to be accounted as representative of the average local conditions during summertime.
Different analytical methods have been applied, accordingly to international standards, for different contaminants to be found into the air column:

- Hydrogen sulphide - EPA method 15
- Aliphatic amines - Nalophan bag+CG-MS
- Mercaptans - Nalophan bag +CG-FPD
- Hydrocarbons and aldeydes - EPA TO 15 1999 mod. (Nalophan)

Among compounds tested, aliphatic amines and mercaptans remained below the detection limit value in all the sampling campaign, while a significant abatement has been recorded for total hydrocarbons and odor, as showed in Table 6.9.

SAMPLING	ODORS [O.U.]	TOTAL HYDROCARBONS [PPM]
07/03/2014	918	2036
10/30/2014	412	685
ABATEMENT PERFORMANCE	55%	66%

Table 6.9. Gaseous contaminants and odor abatement performance

Due to the peculiar working principle of the system proposed, a direct comparison with removal efficiency provided by benchmark technologies could be either difficult or misleading. This system, as discussed before, works on indoor air, treating it inside the facility, while all other systems applied on odor and emission from MSW treatment plant operate on extracted and ducted air. Nevertheless, a comparison with results provided by Liu (Liu et al. 2009) is presented in Table 6.10. In Liu's experiment, a compost biofilter was established in Shangai's BMT pretreatment and composting processes used for MSW disposal and its performance checked. The biofilter showed higher removal efficiency for alkanes with smaller molecular weights, compared to the higher molecular weight ones. As marked in the following table, performance obtained is comparable, but, considering that biofilters treat extracted air, a correction factor related to extraction fan and ducting

efficiency should be applied in order to evaluate the overall performance of the system in direct comparison with AIRcel's technology.

COMPOUND	AIRCEL TECHNOLOGY REMOVAL EFFICIENCY	LIU ET AL. REMOVAL EFFICIENCY
PENTANE	81%	87,4%
HEXANE	96%	85,8%
OCTANE	36%	91%
DECANE	75%	10%
AVG	72%	69%

Table 6.10. Alkanes abatement performance in comparison with results provided by Liu (Liu et al. 2009).

In parallel with gaseous contaminant detection, microbial contaminant counts have been performed during the same monitoring campaigns.
A STRAINBUSTER 60 sampler has been used in association with Petri plates. Samples have been taken at human height (1,5 m from the ground), as requested by international standards, in three different monitoring points inside the waste treatment facility, since the outdoor environment is not expected to be affected by microbial contaminant concentration inside, unlike what happens for gaseous and odor producer compounds.
Several microbial strains have been monitored, some of which resulted undetectable even in the baseline definition phase (i.e. *Fecal Streptococcus,Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella spp)*, but total count per temperature interval and some strains experienced sensible variations during the testing period, as reported in Table 6.11.

MICROBIAL INDICATOR	VARIATION FROM BASELINE
TOTAL BACTERIA COUNT AT 22°C	9% (-6%)
TOTAL BACTERIA COUNT AT 36°C	-38%
TOTAL COLIFORM BACTERIA	-98%
TOTAL MYCETIC COUNT	60% (-43%)
FECAL COLIFORM BACTERIA	-98%
ENTEROBACTERIA	-99%
OVERALL ABATEMENT PERFORMANCE	-48%

Table 6.11. Microbial contaminants abatement performance compared to baseline

Target potentially harmful bacteria (colifrms, fecal coliforms and enterobacteria) showed a decreasing in number of colonies above 98%, while the total count at 36°C (a mixed indicator, not specifically identifying harmful bacteria) diminished of about 38%. The total bacteria count at 22°C appears not sensibly affected by the system application, but is less significant in human health protection perspective than the 36°C count. The countertrend data seems to be the Fungi count, since it shows an increasing of about 60%, compared to

baseline. Nevertheless, this data can be explained by comparing it to the first baseline attempt monitoring, discarded for meteorological reason: climate conditions (i.e. low pressure, rainy day), in fact, where probably more similar from the first attempt baseline to the final monitoring time (late October) and this appears to affect the development of Fungal colonies overwhelming the system ability to treat them.

3.4 Overall considerations

To complete the evaluation of the data collected, perceptual aspects has been taken into consideration, being directly related to the conditions of odorous contamination and, in addition to correlations made in the previous paragraph, impressions of the facility's staff and complaints from the local residents have been recorded and it is inferred what follows:

1. since the summer, reported as critical for odor conditions for the surroundings in previous years, no alert for malodorous emissions has been reported. It can be considered as a preliminary, but crucial achievement of containment of airborne contamination. The summer period, in fact, is a critical time for the odor emissions on the one hand for weather and climate reasons (high temperature, in fact, promotes anaerobic digestion of residual waste), and secondly, due to the slowing down, until the total stop, of the plant activity. This operating mode favors the establishment of anaerobic conditions within the storage tanks of the residual material and, consequently, the development of anoxic sulfur compounds (eg hydrogen sulphide) with low odor threshold and, therefore, potentially disturbing in smell;

2. the staff notes that the smell is still present within the facility, related to the input of particularly odorous material and its processing, but at the same time, it tends to not propagate outside. This configuration corresponds to the action of the containment principle established by AIRcel system around the source of contamination and it is reflected in measurements provided by portable photoionization detector (PID), which detected

2.1 fluctuating values of volatile organic compounds within the plant, related to the nature of snapshot surveys the device performs, which appear to be less effective in open environment;

2.2 a decrease of the same values at the chimney, where the flow of air conveyed by local extractor makes the measurements more constant in time and, then, reliable, as far as possible: this data confirms the impressions of the staff and corresponds to the expected behavior of the contaminants, which are attracted by AIRcel more effectively than traditional aspiration.

An overall evaluation of the system performance cannot ignore the influence of units' malfunctions and difficulties in adapting such system to the peculiar environment of a segregated waste treatment plant. As noticed into results presentation section, a sensible influence on system's performance is exerted by maintenance conditions of the bioreactors. In occurrence of major failure of the machines, in fact, a decreased performance is offered by the whole system, proving its efficiency in contaminants capturing and removal while in full functionality. In Table 6.12, malfunctions of bioreactors units are reported, together with an estimated system's functionality left, in order to try and find a correlation between these occurrences and secondary concentration peaks in sensors' readings. The residual functionality is just an estimation, since no remote control of the bioreactors is provided so far and it is consequently impossible to guess how long the malfunction is lasted. This is particularly evident when considering the 13% of system functionality found during the early October maintenance work, which is, in fact, coupled with just a slight increase in contaminants concentrations, probably due to the short malfunction time. On the other hand, a 50% of residual functionality in earl December has led to a strong increase in gaseous contaminants (from a value lower than 10% of the baseline to about 50%). At the same time the more recent maintenance works are evidently related to dramatic abatement compared both to former contamination condition and baseline

values: this suggests an optimal cleaning schedule for the bioreactors of about 4-6 weeks, when applied in waste treatment facilities.

MAINTENANCE DATE	UNITS UNDER MALFUNCTION ALERT	SYSTEM FUNCTIONALITY LEFT
07/29-31/2014	n.5	38%
08/4-6/2014	n.3	63%
08/27-28/2014	n.2	75%
10/03/2014	n.7	13%
10/22/2014	n.1	88%
12/09/2014	n.4	50%

Table 6.12. Malfunctions log and residual functionality of the system

3.5 Outlook of the work

By the end of the testing period, several outcomes are expected:

1. Recognizing correlation between lab test and wireless monitoring system results, in particular aimed to find a possible correlation between particulate sensor readings and microbial count performed through impact sampler.
2. Identifying a link between particulate matter peaks at the secondary shredder section and conveyor's belt cleaning operations, in order to make them predictable.
3. Establishing an equilibrium state for the bioreactors system, with a stable containment of air pollution and predictable performance.
4. Defining a new state-of-the-art both for the AIRcel system, with a predictable sizing-related performance for this industrial sector, and for indoor air quality standards into modern segregated waste treatment plant.

4 Conclusion

The effectiveness of a biotechnological air treatment system (AIRcel) on improving air quality inside a modern and regulation compliant waste treatment facility is under investigation by the means of a fifteen months test period, during which different monitoring methods are applied in order to delineate the more comprehensive performance trial possible. The Immobilized Cell Bioreactor's system's working principle relies on motion of contaminant for concentration gradient and not through ventilation, providing a sustainable alternative to traditional air treatment techniques, both for the reduced energy consumption and lower potentially disturbing emissions. This would be a sensible improvement towards environmental, economic and social sustainability, since waste treatment facilities are widely associated with emissions of air pollutant, negatively affecting air quality in the surrounding areas

The baseline has been detected in a period of decreasing activity, up to the total stop of the plant for summer break, element which influenced application results in two ways:

1. providing a rest period for the crushing, and therefore, withdrawal of waste material, which tended to increase gaseous contaminants values due to anaerobic conditions established into the storage tanks;
2. showing a lower concentration of particulate matter due to shredder's stop, destined to be overwhelmed by working conditions, which, actually returned concentration peaks.

The gaseous contamination has been effectively treated with an immediate response to the intensification of work and events maintenance on the system, reaching a higher value decreased by 85% compared to baseline during the last weeks of full functionality of the system.
The particulate contamination is clearly influenced by the processing conditions of the waste treatment system, both from a chronological point of view: it is clearly recognizable, in fact, the time of resumption of full operational schedule, whose concentration peaks are gradually decreasing, and topographical, since the data at the secondary shredder are higher than the one detected at the feeder section, while during the definition of the baseline (when processing of waste material has almost stopped), an opposite behavior was found. The concentrations trend is, nevertheless, decreasing and fluctuating below the baseline values, showing a strong dependence on the machines' cleaning and maintenance conditions.
Both gaseous contaminants and particulate matter appear to be effectively captured and treated by the system, as demonstrated by stress test conditions provided by several units' malfunctions occurred during the trial period, during which the detected concentrations experienced peaks strictly related to system's failure and decreasing trends consequent to the maintenance works. The optimal cleaning schedule for the system is identified in 4-6 weeks, confirming the strong dependence of the performance from the recovery ability of the water system (in hospital environment application, in fact, the experienced optimal cleaning schedule was 10-12 weeks) related to the load of contaminants to be treated.
The peaks of smell recorded inside the facility are reflected in the surveys carried out.
The smell impressions gathered by the staff and the absence of complaints from the residents around the plant confirmed a reduction of odor emissions at their source, limiting the fugitive contaminants, despite the working conditions of the venting inside the facility have not changed.

6.2.2. Wastewater treatment

6.2.2.1. Introduction

Emissions typically associated to wastewater treatment plants derive from organic volatile compounds released from wastewater itself or sludge produced by the treatment processes. This is one of the major side effects of liquid effluents remediation process, which could impact local communities not only from the aesthetic point of view, but may lead to regulatory agencies involvement (Muga et al. 2008). Even though low concentrations and typical non-dangerous nature of the compounds emitted from wastewater treatment plant hardly lead to toxicological-sanitary risk (Zarra 2008), they are widely regarded as a major source of disturbance on surrounding population (Zarra 2008, Frechen 1988, Bidlingmaier 1997, Stuetz and Frechen 2001).

The object of the application was containment of unpleasant odors spreading into Como city from the municipal wastewater treatment plant. Due to poor urban planning, affected evidently by peculiar geography, with narrow valleys degrading to the Lake of Como and little space for proper urban development, the wastewater treatment plant is currently located into a mixed area, with shopping centers, residential blocks and small factories on the surrounding hill.

The issue was particularly disturbing for citizens and a committee had been organized, in order to debate it with the municipal company running the plant. As suggested by Tchobanoglous et al. (2003), an open dialogue with the surrounding community is crucial to properly address both health and environmental issues.

The wastewater treatment process performed into the plant is quite typical, with primary grinding and settling, secondary treatment with clarification, sludge separation, grinding, centrifugation, drying, storage and transportation to final disposal. Since, on one hand, several structural works were planned to cover the open settling tanks and, on the other hand, the most disturbing odor was reported as coming from the sludge treatment facility, this building was selected for pilot installation. An air treatment plant was already present into the building, aspirating air from centrifuges and sludge storage tanks and treating it by wet scrubbing with Ozone as oxidant, in order to contain both odor and microbial content. As widely reported (Muga et al. 2008; Fukuyama 2004), sludge processing facilities (i.e. storage, thickening, stabilization and dewatering) are key locations for odor control into a wastewater treatment plant, both for material elaborated and specific process applied (i.e. heating, drying and possible fermentation occurring). Airborne contaminants typically associated with wastewater treatment are ammonia (NH_3) and hydrogen sulfide (H_2S), produced by anaerobic bacteria growing on organic sulfur and dissolved into wastewater until turbulence occurs in the flow.

6.2.2.2. Pilot test performed

During the pilot test, n.6 AIRcel®5000 units have been installed into:

1. centrifuge room (n.2 bioreactors),
2. sludge storage room (n.3 bioreactors),
3. trucks entrance area (n.1 bioreactor).

Continuous monitoring of airborne contaminants was provided with n.3 U-Monitors, one per each of the areas.

Fig. 6.61. Centrifuges room

Fig. 6.62 Sludge storage room

Key Performance Indicators identified to evaluate the success of the project were threefold:

1. reduction of airborne contamination within the treated areas, measured by the U-Monitor stations. As reported by Zarra (Zarra 2014), continuous monitoring of airborne contaminants is necessary to evaluate odor emissions from wastewater treatment plants as wee to identify strategies to control their impact on local communities;
2. decrease of the bacterial population in air, measured by an external laboratory, periodically checking microbial airborne pollutant in the plant. For the assessment of microbial air quality and focusing, in particular, on potential pathogenic bacteria, the bacterial count at 36 ° C, with an incubation time of 24-48 hours, was defined as performance indicator. The agar plate count (Oxoid) was carried out on samples taken through a single stage impact with constant orthogonal flow, Surface Air System SAS 100 (PBI), with a suction flow of 100L/min on two consecutive samples of 330 and 600 l of air in clean weather conditions and the final accepted value is the average of the two;
3. odor perception, based on citizenship feedback.

In addition to the three KPI presented, the analysis of process water of different bioreactors was scheduled, with the aim of re-trace contaminants typically related to disturbing odor from wastewater treatment plant, i.e. Sulfur compounds (Hydrogen Sulfide or DiMethyl Sulfide - Zarra 2008).

The pilot test was planned on three different phases, with and intermediate step between phase 2 and 3, as reported in Figure 6.63.

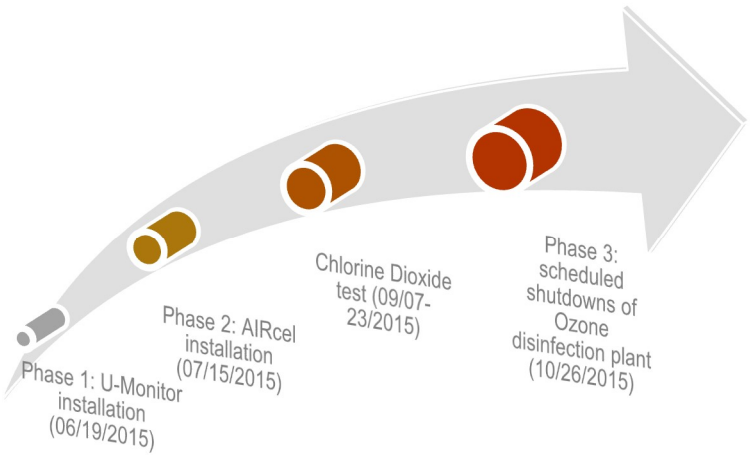


Fig. 6.63: Pilot plan

In particular:

1. Phase 1: installation of U-Monitors for "baseline" definition (June, 19th 2015) in the three different areas. Data collected during the first four weeks by the two gas sensors (VOC and Odorous Gases) has been used as reference to evaluate AIRcel system abatement performance. The truck entrance was regarded as the most representative of the odor spreading toward the city, since it was located at the interface between the sludge treatment facility and the courtyard area. On the contrary, U-Monitor positioned into the sludge storage room proved to be strongly affected both by sludge loading/downloading operations and data resulted illegible.
2. Phase 2: bioreactors installation (July, 14th, 2015). AIRcel bioreactor system run for about 17 weeks, during which several tests have been performed.
3. Chlorine Dioxide test: during phase 2, as blind test, the Municipal Company tested a chemical additive (Chlorine Dioxide) for sludge sterilization.
4. Phase 3: scheduled shutdowns of Ozone disinfection plant (10:00 am to 1:00 pm for five days, from October 26th to 30th; 10:00 am to 5:00 pm for five days the following week, from November 2nd to 6th).

6.2.2.3. U-monitor's results

Following figures present results obtained on Volatile Organic Compounds, as detected by U-Monitors in truck entrance (Fig. 6.64) and centrifuges room (Fig. 6.65). Bioreactors installation (Phase 2) and Chlorine Dioxide test have been marked, as both events showed effects on contaminants concentration registered.

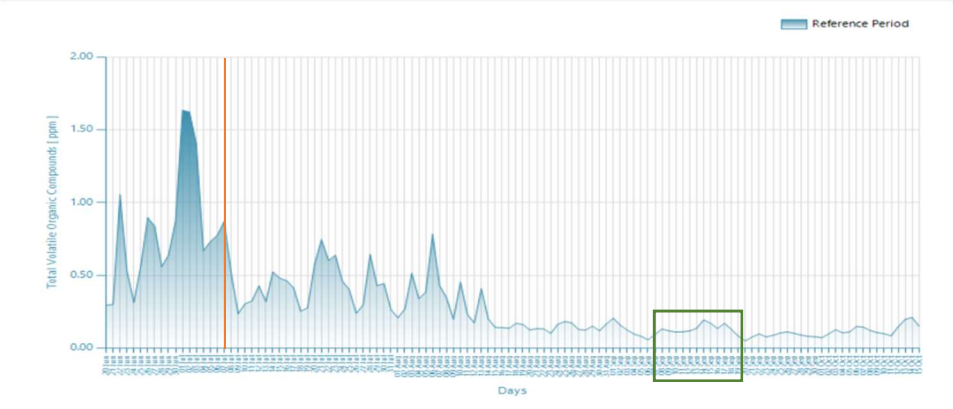


Fig. 6.64: VOC instant concentration, Truck Entrance

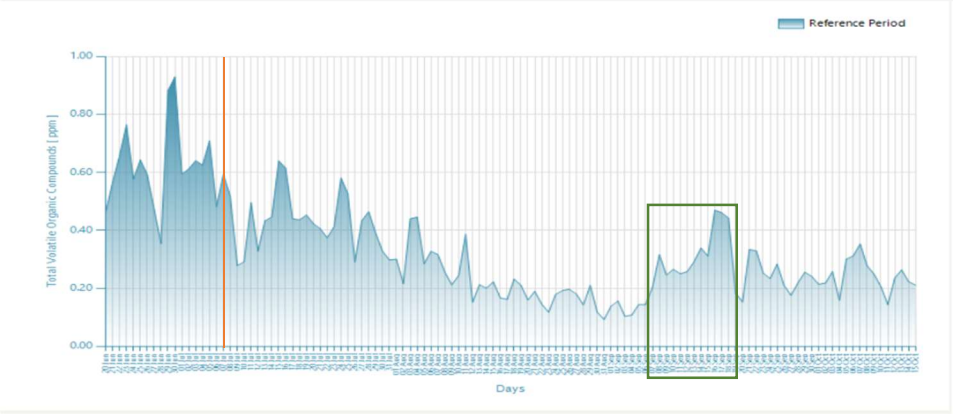


Fig.6.65: VOC instant concentration, Centrifuges Room

The following figures are built on comparison between two different periods' results, before (June, 20th to July, 13th) and after (September, 19th to October, 12th) bioreactors installation. The two period were defined as to be equal in time, but, of course, environmental conditions were naturally different (e.g. Daily Temperature differed for more than 10°C).

Concentration peaks affecting VOC concentrations at truck entrance during the baseline period (Fig. 6.66) have been reduced to values comparable to original background concentrations. A remarkable reduction of peaks of VOC contamination was obtained even in centrifuges room (Fig. 6.67).

VOC daily average concentrations were reduced by 85,5% (Fig. 6.68) in truck entrance and 56.5% in centrifuges room (Fig. 6.69).

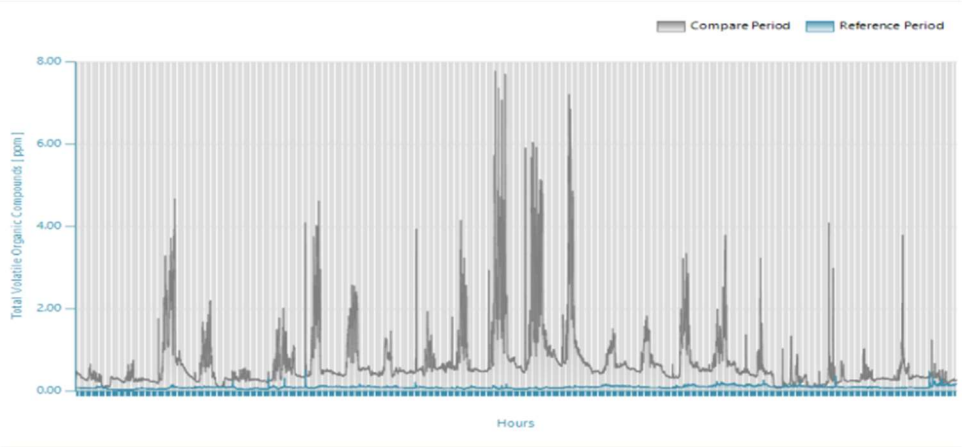


Fig.6.66: VOC instant concentrations in two different periods, Truck Entrance:

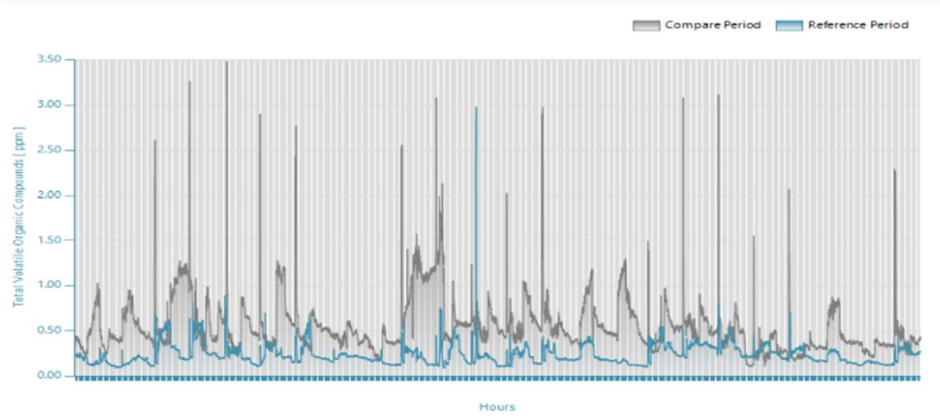


Fig. 6.67: VOC instant concentrations in two different periods, Centrifuges Room:

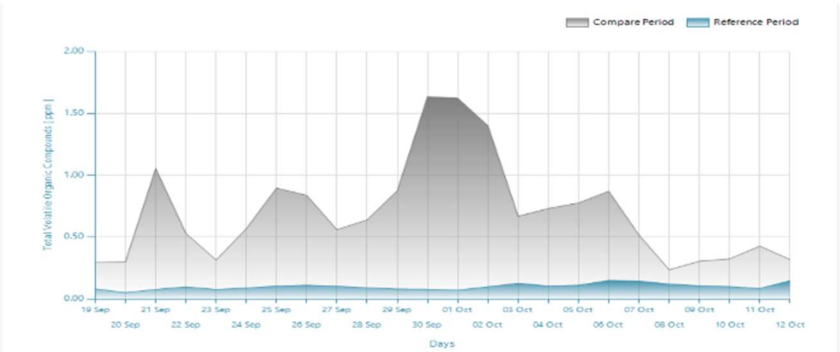


Fig. 6.68: VOC daily average concentrations in two different periods, Truck Entrance:

Fig.6.69: VOC daily average concentrations in two different periods, Centrifuges Room:

The odorous gases sensor, more sensible to Sulfur compounds, and, therefore, regarded as more representative of disturbing odor spreading from the wastewater treatment plant, returned results reported in the following figures. As reported for VOC, results are presented for truck entrance (Fig. 6.70-6.72) and centrifuges room (Fig. 6.71-6.73) for two different periods, before (June, 20th to July, 13th) and after (September, 19th to October, 12th) bioreactors installation. For both pilot areas, background values have been reduced (83% for truck entrance, Fig. 12, and 45.6% for centrifuges room, Fig. 6.73), as well as concentration peaks.

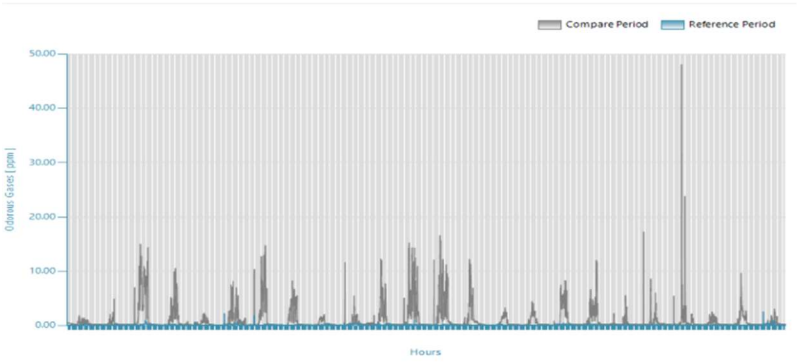


Fig. 6.70: Odorous Gases instant concentrations in different periods, Truck Entrance:

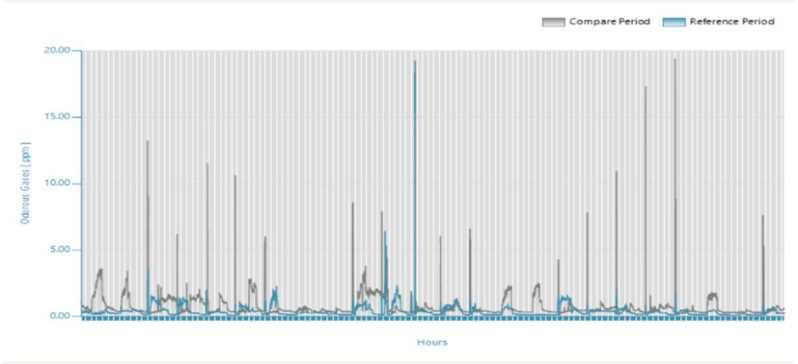


Fig. 6.71: Odorous Gases instant concentrations in different periods, Centrifuges Room:

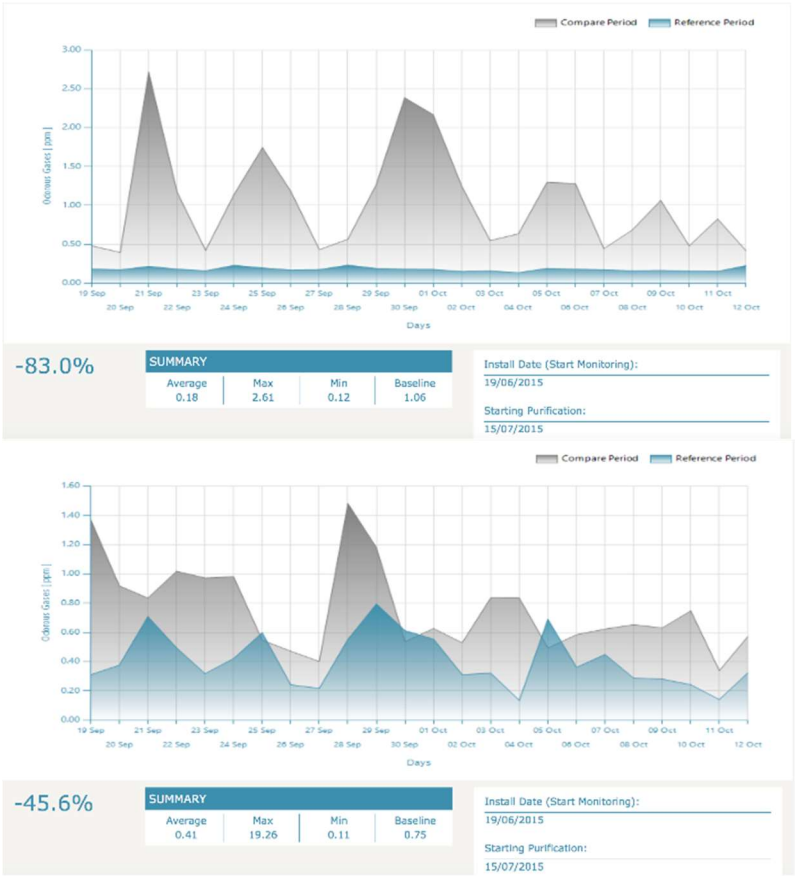


Fig. 6.72: Odorous Gases concentrations in different periods, daily average, Truck Entrance:

Fig. 6.73: Odorous Gases concentrations in different periods, daily average, Centrifuges Room:

Phase 3-first week (October, 26th to 30th) results are reported in the following, for VOC (Fig. 6.74 and 6.75) and Odorous Gases (Fig. 6.76 and 6.77), compared to a baseline week (June, 29th to July, 3rd).
Daily contamination patterns are clearly visible, as well as lower concentration registered during Phase 3-first week (i.e. with AIRcel bioreactors working full time and Ozone system switched off from 10:00 am to

1:00 pm), even if concentrations remained lower than during the baseline period (i.e. without AIRcel and with Ozone system working full time). In the centrifuge room, based on Wednesday results, doors have been opened on the sludge storage room, providing better oxygenation and, therefore, results on the remains of the week.

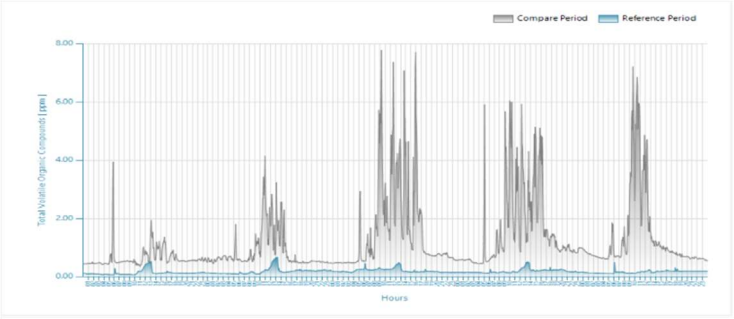


Fig. 6.74: VOC instant concentration, Phase 3-first week, Truck Entrance

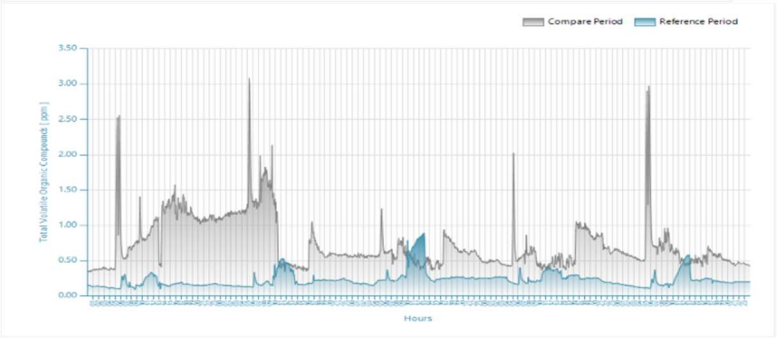


Fig. 6.75: VOC instant concentration, Phase 3-first week, Centrifuges Room

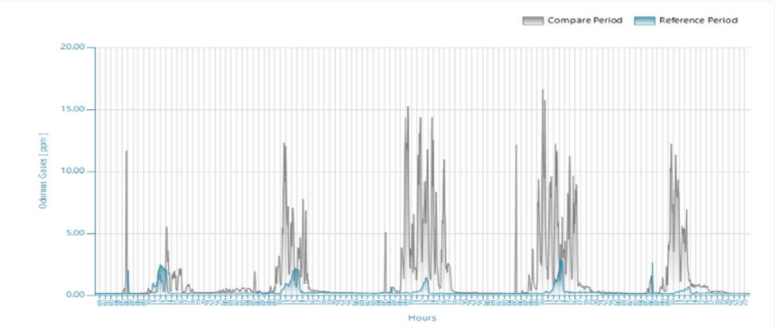


Fig. 6.76: Odorous Gases instant concentration, Phase 3-first week, Truck Entrance

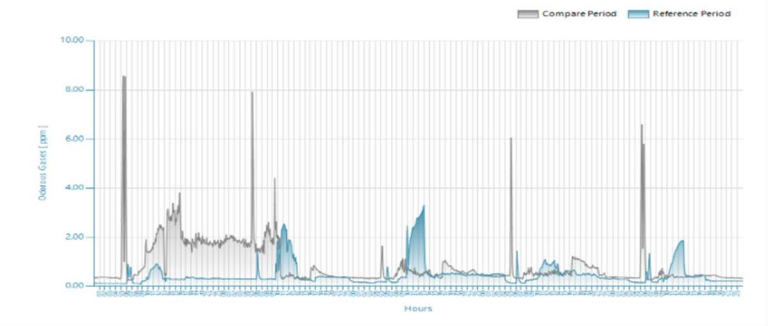


Fig. 6.77: Odorous Gases instant concentration, Phase 3-first week, Centrifuges Room

The pilot test has been interrupted at the beginning of Phase 3, second week, due to disturbing odor reported in the courtyards surrounding the plant and confirmed by U-Monitor's results. Unfortunately, it was not possible to verify the origin of the emission, most likely related to the abrupt decrease in night's temperature registered during the weekend, which could have affected both

1. bioreactors' performance

2. biological treatment basins, located outdoor, with a consequent sudden change in sludge characteristics and, therefore, odor.

The extremely delicate urban framework of the pilot test forced to an anticipated closing, giving no space for further tuning of the experiment.

6.2.2.4. Microbial test results

Microbial counts at 36°C have been performed in two different period, i.e. on AIRcel system installation (July, 14th 2015) and after 100 days (October, 23rd 2015).

Even though meteorological conditions were comparable, during the two sampling days, environmental temperature naturally differed, due to the changing season, for almost 20 °C, thus influencing the results obtained.

Table 6.13: Microbial count at 36°C

CFU/m3	*July, 14th 2015*	*October, 23rd 2015*
Centrifuges room	379	228
Sludge storage room	262	51
Truck loading	35	130

6.2.2.5. Process water results

As for similar application, the main purpose of water testing was to identify and quantify elements and compounds not degraded (or under degradation process, i.e. intermediate metabolites) by the biomass, especially metals. Since bioreactos are supplied with tap water, contaminants possibly found into process water are reasonably deriving from attraction of airborne pollutants and consequent elaboration provided by the biomass, as previously discussed. Water samples were collected 93 days (about 12 weeks) after AIRcel maintenance, during which only water and biomass refill had been accomplished.

Due to adjustable ventilation system present in the building, but no specific data available, only a limited backward assessment of contaminants captured was achievable (Zanni et al. 2015), therefore the main tangible outcome is a qualitative evaluation of chemicals found into water, in comparison with typical tap water values.

Table 6.14: Process water results for three bioreactors (B6, B3, B1)

		B6	*B3*	*B1*	*West Como typical value**	*Concentration limit in drinking water***
Cr	mg/l	<0.01	<0.01	<0.01		0.050
Cu	mg/l	<0.01	0.01	0.01		1.0
Fe	mg/l	0.02	0.02	0.03	0.0128	0.02
Ni	mg/l	0.01	0.01	0.01	≤0.01	0.01
Se	mg/l	<0.01	<0.01	<0.01		0.01
Zn	mg/l	0.15	0.1	0.11		0.12
Hg	mg/l	<0.002	<0.002	<0.002		0.001
As	mg/l	0.01	0.01	0.01	0.0047	0.01

		B6	*B3*	*B1*	*West Como typical value**	*Concentration limit in drinking water***
B	mg/l	0.51	0.48	0.47		1.0
Cd	mg/l	<0.01	<0.01	<0.01		0.005
Pb	mg/l	<0.01	<0.01	<0.01		0.01
Pb	mg/l	0.3	0.28	0.34		0.31
Al	mg/l	0.13	0.11	0.11		
Mn	mg/l	<0.01	<0.01	<0.01		
S	mg/l	175	155	144		

*https://reti.acsm-agam.it/acqua-como-zona2

** D. Lgs. 31/2001

For Sulfur, in particular, a dedicated analysis was performed on AIRcel input water and a value of 21 mg/L was detected, i.e. about 36% of the average concentration found into the bioreactors.
Considering:

- average water content into each bioreactor: 600 L
- average water consumption per week, per each bioreactor: 50 L

it is possible to estimate the amount of Sulfur deriving from water refill as:

600(L) * 21(mg/L) + 50(L/week) * 12(weeks) * 21(mg/L) = 25.2 g

This can be compared to the overall amount of Sulfur present into the three bioreactors tested (as average):

600(L) * 158 (mg/L) = 94.8 g

Results show that the amount of Sulfur actually found into bioreactors is about 3.76 times what expectable by concentration of water content. Therefore, it is reasonable to assess that each bioreactor captured about 0.733 g of Sulfur per day, corresponding to 0.021058 mol/day of Sulfur, or, if referred to a typical sulfur compound responsible for bad odors, i.e. Hydrogen Sulfide (H_2S), 0.022906 mol/day.

6.2.2.6. Conclusion

As mentioned before, the pilot test has been interrupted at the beginning of second week of Phase 3, due to disturbing odor reported in the courtyards surrounding the plant. Unfortunately, it was not possible to verify the origin of the emission, most likely related to the abrupt decrease in night's temperature registered during the weekend. This could have affected both bioreactors' and biological wastewater treatment basins performance, with consequent change in sludge characteristics and, therefore, odor, giving rise to disturbing emissions, even though, as reported by several studies (Muga et al. 2008) summer is normally regarded as the most difficult season. The extremely delicate urban framework of the pilot test forced to an anticipated closing, leaving no space for further tuning of the experiment and required repletion of the different monitoring campaign in parallel with changing seasons.

6.3. New opportunity for application – Radioactivity contaminated environments

Based on decontamination performance demonstrated by several applications of the biotechnology under study, new opportunities may be foreseen. Among different possible issue to be tackled (VOC fugitive emission in industrial facilities, toner powder and related explosion risk in recycling plants, Compagnoni 2016), radioactive particles containment could be regarded as one the most interesting for its potential impact on human health.

The present study has been preliminarily presented to International 8th International Conference on Environmental Engineering and Management – ICEEM08 (Iasi, 9th-12th September 2015). The following report has been submitted for publication and it is currently under evaluation.

6.3.1. Introduction

Radioactivity represents one of the major threats posed by anthropic activity, both related to warfare (nuclear weapon testing has been recently resumed, breaking the NPT - Non Proliferation Treaty), and accidents occurred in energy production. Post-Cold War generations have been marked by concerns related to accidental events in nuclear power plants with consequences on global scale. Names of nuclear power plants involved in catastrophic accidents are now worldwide known and they evoke ghosts of human errors, bad or brave choices, inadequacy of safety measures, silence about expected effects on ecosystems and human health, in different time and space scales. When natural disaster of gigantic proportions, as the earthquake that hit Japan on March 11st, 2011, involves a nuclear power plant, disastrous consequences are hardly containable. In the perspective of a global climate change and consequent increase in major environmental events occurrence, the possibility of a natural disaster should be taken into account in the assessment of related risks activities for nuclear power plants as well as nuclear spent fuel disposal sites and military repositories.

Risk on environment and human health, but also environmental, economic and social sustainability should become elements of concern when dealing with radioactivity (Rehdanz et al. 2015, Fortuna et al. 2011). Since the concept of sustainability is not usually assessed through a unique mathematical method, but rather evaluated by the means of different indicators (Bardos et al. 2009, Oughton et al. 2004) a special attention for this aspect should be considered while planning a remediation, also if applied to radioactivity contaminated media, in which social and ethical aspects must be included.

As suggested by NABIR Program report on Bioremediation of metals and radionuclides (Faison et al. 2003), microorganisms can interact with metals and radionuclides on the basis of the same principles, since the two classes of pollutants, often found together in contaminated environments, are not affected by biodegradation, but they can be, nevertheless, affected by biologically-mediated transformation. Microbial activity may, in fact, interact with the local environment, changing the contour condition and promoting a shift in the oxidation state, decreasing the solubility of the contaminants of concern, thus limiting their mobility among environmental matrices, or increasing the solubility, where an active remediation technology, like pump and treat system, is applied.

Bio-remediation tools are widely regarded as a new hope for complex remediation scenarios and recalcitrant contaminants. Demonstrations of the removal and immobilization potentiality of inorganic contaminants by microbial transformations, sorption and mineralization have been gathered show the potential of both natural and engineered microbes as bio remedial tools (Faison et al. 2003, Barkay ad Schaefer 2001,).

Different studies in laboratories and in natural environments have shown a high affinity of microbial biomass for heavy metals, as well as actinide elements and radionuclides. Published results (Sarró et al. 2005) suggest that biofilm communities in spent nuclear fuel pools are directly involved in the accumulation of

radionuclides from water. The two main mechanisms involved in microorganisms-radionuclides interaction are biosorption and bioaccumulation. The former is a metabolically independent physical process at the cell surface, which implies passive sequestration of metals and radionuclides, while the latter is an energy-dependent process involving intracellular accumulation. Biosorption is, at present, the most practical and widely used approach for the bioremediation of heavy metals and radionuclides (Barkay et al. 2001).
Sarró's results (Sarró et al. 2005) suggest that biofilms are able to proliferate on metallic solid support in water, in spite of the radioactive and oligotrophic conditions of the water, and they are able to retain radionuclides, which accumulate on the surface of metallic support sampled.
The radionuclides and toxic metals released into solution are immobilized by enzymatic reductive precipitation, biosorption and redistribution with stable mineral phases in the waste, making remediation goals achievable by first precipitation (Citrobacter sp., for instance, has been proved to successfully precipitate uranyl phosphate) and then immobilization of inorganic contaminants; second, concentration and thus reduction in volume of contaminated matrices; and third, compartmentalization of heavy metals or radionuclides to an environment matrix in which their harm is reduced (Barkay et al. 2001).
Microbial precipitation of metals and radionuclides as minerals of sulfide, hydroxide, phosphate and carbonate have potential and documented applications in bioremediation, while biosorption is regarded as an effective treatment for wastewater. Soil bacteria (such as Ralstonia and Burkholderia) have been classified by the Microbial Genomics Research, DOE (Department of Energy, USA) as microorganisms potentially useful for bioremediation of several compounds and elements, even radioactive ones.
The application of such solution is addressed to collect airborne radioactive particles into a concentrated medium, such as water. The liquid form media would become easier to be managed and, therefore, less harmful to environment and human health, by achieving a compartmentalization of radioactive contaminated media (Barkay et al. 2001). Due to the safety issue related to radiations, pilot-scale application involved heavy metals which behavior could be assimilated to radionuclides and give important information about the up-scaling of the system in real case scenarios.
In order to assess the applicability and possible effectiveness of the application of the air treatment biotechnology on airborne radioactive particles, a first step application on iron dust has been performed. In particular the study focused on airborne metal particles produced in precision mechanics industry, working on final reaming of Chromium-Molybdenum Steel barrels (Zanni et al. 2015).

6.3.2. Materials and methods

The present study has been carried out on two main pathways: on one side, on the basis of similarities in behavior between radionuclides and heavy metals, examining the opportunity represented by a biotechnology for air treatment, in order to capture and, therefore, contain radioactive particles, in a compartmentalized media; on the other side, by an evaluation of the risk reduction of possible diminished concentration of radionuclides in air.
Considering that radionuclides are physically unstable elements, decaying with the emission of energy and particles, their behavior is clearly not entirely replicable with a surrogate element. However, heavy metals result, nevertheless, the best option for research, at this stage, due to the similarities between the two contaminants families. In fact, both elements are sensible to chemical-physical transformation, both direct (changes in valence state, methylation, complexation with organic agents) and indirect (sorption/desorption from sediments). Moreover, they are reported to be not biodegradable (Faison et al. 2003), so they are typically accumulated into the environment and, consequently, human body, through ingestion, but, most of all, inhalation. In indoor environment they can both accumulate, and metal dust, in particular could pose health risk for workers or, in extreme conditions, lead to explosion risk (Khambekar 2013). This can occur

when powdered metals (e.g. iron, aluminum, and titanium) enter the scenario in concentrations exceeding European regulations limits - even occasionally - in presence of ignition, given specific conditions of oxygen, humidity and temperature.

Considering adverse health effects carried by airborne radionuclides and heavy metals, the main similarities are related to their ability to enter the body through ingestion and inhalation and their inclination to accumulate into tissue. Focusing, in particular, on inhalation process, particles' size results as a key element for the risk they carry (Zanni et al. 2015, Seaton et al. 1995, Gbadebo and Bankole 2007, Popoola et al 2012), since smaller particles may reach deep respiratory tract and, possibly, travel to different tissue coming into contact with blood circulation within the pulmonary alveoli. Particle size which range between 0.1 and 1 μm are typically deposited in the lungs, triggering inflammation and sensitization process [13, 14].

Airborne metal particles are involved in adverse health effects typically caused by particles depending on their size, even though direct impact of metal dust on human health is not easily assessable, due to multiple factors involved in long-term effects (as cancer development) (Gbadebo and Bankole 2007, Popoola et al. 2012).

Nevertheless, heavy metals are generally accumulated in fatty tissue, with potential effects on nervous or circulatory system and consequences on internal organs functions (Zanni et al. 2015, Hassan 2012, Waisberg et al 2003, Bocca et al. 2004).

Radioactive particles, on the other hand, once entered the body through breathing thus deposited in deep respiratory tract, may continue to emit energy in the form of rays, causing damage to living tissue. Several studies assess the existing link between the exposition dose and frequency of cancer events (Morawska 2003, Hahn et al. 1999, Raabe 2010). The risk carried by airborne particles, as damage magnitude multiplied by frequency of the exposure, is clearly enhanced by the tendency to linger in indoor air for different periods of time, longer as the size becomes smaller (Raabe 2015), regardless of the chemical nature of dust involved, thus suggesting that safety measures aimed to reduce the concentration of airborne radionuclides particle in air would consequently decrease the carcinogenic risk.

This assumption, in addition to similarities in behavior between heavy metals and radionuclides, suggests that an indirect assessment of airborne radioactivity containment and compartmentation opportunity is effectively possible.

In the perspective of a sustainable research activity and as a first step towards a comprehensive assessment of the applicability of the technology proposed on radioactive contaminated environment, an experimental application on airborne iron dust have been carried out in real scale.

Promising results have been obtained, regardless of the initial indoor air quality, which proved to be consistently compliant with the regulatory limits for safety of the working sites (Bonoli et al. 2014a and 2014b, Sofer 2006). Airborne iron dust has been captured by free standing immobilized cell bioreactors, installed in an open space sector of a larger building, where several mechanic processing were carried out.

The focus was set on the final and precision reaming of Chromium-Molybdenum Steel barrels (Zanni et al. 2015).

Metal dust is produced in mechanical precision industry by specific processing, generally involving mechanical friction and, therefore, volatilization of particles. In the case study, fine mist was generated by mechanical friction of the reaming machinery on the rough-cut barrel and consequent heat dissipated. Two main families of contaminants were generated in such operations: fine and ultrafine metal dust, from mechanical friction of the gun barrel and the reamer, and oil mist, arising from the cooling and lubricating fluids used in the metalworking process.

The facility where the case study has been conducted is sized about 14.100 m^3, while the overall bioreactors' system venting capacity was 6000 m^3/h. Therefore 2 hours and 20 minutes were necessary to have a total

recirculation of indoor air inside the system, thus only supporting the natural ventilation of the environment since no other forced ventilation system was present.
Non degradable pollutants can be found within the residual material at the bottom of the units, where spent biomass is stored as well. For this reason, process water analysis is a key element to define the technology's functionality for the specific application: in the case study, in fact, metals have been traced in water, thus proving the ability of the system to compartmentalize possible airborne radioactive particles.
Quality tests on process water have been performed during field scale application in order to assess residual material and outline strategies for its handling in the perspective of application on radioactive contaminated environment. Since metal concentration both in drinking water and in U-ox® additive is negligible, metal concentration detected into the process water and residual material at the bottom of the AIRcels® at the end of the testing period should be attributed only to air treatment. Concentration data have been transformed into total mass of airborne metal dust captured by assuming an average water content into the system equal to 40% of the total capacity, below which the biodegrading activity is compromised due to the low recovery capacity of the ecosystem inside the bioreactor.
In a full scale application in radioactive contaminated environment, air quality could be tested not only for radioactivity parameters, but even particles matter concentration, in order to assess the technology effectiveness. In addition to this, frequent tests on process water and precipitate should be scheduled to set a threshold radioactivity value to conclude the batch phase for each unit, discharge water and residual material and properly dispose them. In particular, precipitation of radioactive particles into the residual material is expected, as verified for metal dust, thus enhancing a further compartmentalization of radioactivity. Since the functionality of the system resulted mostly related to contaminants' concentration gradient, rather than conventional convection, the technology could be applied in real scale scenario with two possible approaches:
1) as containment of the primary source of contamination or
2) as protection of a sensible target.
In both cases, desired result can be acquired by placing AIRcel TM units at proper distance and with a belt-distribution around either the source of contamination or the target to protect. In the case study on airborne iron dust treatment, source containment was selected as the most suitable approach, but, for field application on radioactive particles, a reverse application would be preferable.
Outlining a possible application of the biotechnology proposed in real scale scenario, research work has focused on the most recent nuclear accident event related to a major environmental disaster (i.e. Dai-chi Fukushima Nuclear Power Plant), in order to understand possible dynamics (Fernandez-Moguel and Birchley 2015, Sevón 2015) and contaminants involved (Matsumoto et al. 2015, Arakawa et al. 2015). References examined has driven to exclude possible application of the bioreactors as air treatment for source containment during accident event, due to contour conditions and working principle of the technology itself. On the other hand, an application for sensible target protection has been evaluated, on the basis of information gathered about radionuclides contamination.
In particular, Cesium (as CsOH and CsI) and noble gases constituted the major fraction of emissions from the FNPP. Therefore, Cesium has been selected as main target contaminant for calculation of the technology's possible effect.
^{137}Cs presents a considerable environmental threat, related to several factors, such as high relative mobility in the soil–plant system, long-term bioavailability, high radiotoxicity and relatively long half-life (30.17 years), compared to human life (Aung et al. 2015, Rahman and Voitgt 2004, Konoplev et al. 2016). It equally poses concern for human health, since it tends to distribute in the whole body into soft tissue, through ingestion or inhalation, increasing cancer risk due to beta particles it emits, particularly toxic to bone marrow (Faison et al. 2003).

Features of each specific environment of release may influence radiation distribution and evolution with time. In Fukushima prefecture, for example, the prevalence of forested land with higher bioturbation (i.e. retention of radionuclides by organisms, fungi and bacteria in agricultural and forest soil ecosystems), on grasslands and prevalence of clay minerals has led to a high dispersion. Radiocaesium, in fact, tends to bound, in time, to clay minerals (Konoplev et al. 2016) and it can be transported with them in case of wind erosion of soil or resuspension of deposited materials. In addition to this, ^{137}Cs has been proved able to attach to environment aerosols, mainly inorganic one like sulfate aerosol (Kaneyasu et al 2012), ranging generally from 0.1 to 1 μm (Hatano et al. 1998, Evangeliou et al. 2013, Gonze et al. 2015) (2 μm in FNPP accident (Kaneyasu et al 2012)), thus providing an exposure pathway for human target, with soil and urban surface acting as secondary source of contamination, even in long term (Chumak 2013). Even though some studies, based on numerical modeling, considers as negligible the inhalation dose after the first months from a nuclear accident, evidences have been raised that the lack of application of decontamination countermeasures in forested areas may provide a pool of contamination to be released in time, thus causing an increase in air dose (Fesenko et al. 2001) even in the long-term perspective.

As stated by previous studies, an actual retrospective determination of exposure dose for population would require site-specific data, related to a number of factors, such as the radionuclides release conditions, meteorological data, characteristic of the built environment, and epidemiological approach (WHO 2013, Hooper 2011), but scientific-based considerations can be made on the basis of information available.

In the present simplified calculation, only the inhalation exposure pathway is considered, being the one mostly affected by possible application of the biotechnology tested (AIRcel TM air purifier). In order to outline possible benefits deriving from application on indoor environment decontamination from radionuclide particles, a backward assessment has been performed on the basis of the dose value indicated by Yasutaka (Yasutaka et al. 2013). Therefore, an average radioactivity value has been assumed equal to the threshold limit value for total decontamination measure application in ICA (Intensive Contamination Survey Areas) of Fukushima Prefecture, i.e. 5mSv. From that value, combined with literature data of inhalation dose coefficient, exposure time (Yasutaka et al. 2013) and breathing rate (Mori et al 2015), a calculation of the airborne concentration of ^{137}Cs in indoor environment has been made. Assuming, then, an abatement rate equal to the one reached by the system on the real scale application on metal dust, a final concentration of the radionuclide in indoor air has been calculated and the total inhalation dose has been modified accordingly. Several assumptions have been taken in account, such as same system sizing and performance as the field test, a single exposure pathway, a single radionuclide, and no daughter radionuclides nor decay products equilibrium has been taken into account, A simplified evaluation of the decreased risk factor would be quite complex, due to multiple elements contributing the assessment (Puncher 2014). First of all, the different approach in entry dose data, which are related to mass concentration in case of standard carcinogenic compound, while monitored and loaded as activity for radionuclides, implies a non-linear scalability from results obtained in proposed field experiment and possible application on decontamination in radioactive contaminated scenarios. Nevertheless, further research actions and model application could be implemented on the basis of these first, rather promising results. In particular, an evaluation has been attempted for influence of application of the technology for air treatment in radioactive contaminated areas, by applying a simplified calculation proposed by Hooper for inhalation intake determination of radionuclides (Puncher 2012). The inhalation intake of radionuclides (^{137}Cs, in the present study) is computed as

$$\text{Intake [g]} = \text{Airborne Radionuclide Concentration [g/m}^3\text{]} \times \text{Breathing Rate [m}^3\text{/h]} \times \text{Exposure Time [h]} \quad \text{(Eq.1)}$$

The mass intake can be converted into activity intake by multiplying the value obtained by the specific activity of the radionuclide (3. 215 TBq per gram, for Cs-137).
The Inhalation Dose can be calculated as follows:

$$\text{Inhalation Dose} = \sum i \text{ Intake } i \times D\ i \qquad \text{(Eq.2)}$$

where Intake_i is the Inhalation intake of radionuclide i [Bq]; D_i is the Inhalation dose coefficient for radionuclide i [Sv Bq^{-1}] .
Inhalation dose coefficients have been tabulated by U.S. Environmental Protection Agency (1988) and ICRP (2001), corresponding to specific dosimetric models. Such models have been recently questioned as carrying an uncertainty degree which could lead to improper estimations (Puncher et al. 2012, Puncher et al. 2014, Yasutaka 2015, UNSCEAR 2000), but they remain standard reference for dose calculations. For the present study, dose factor for male adult exposed to Cs-137 has been assumed as $4.80 \cdot 10^{-9}$ Sv Bq^{-1}, as proposed by European Nuclear Society and published in the Federal Gazette No. 160, 28th August 2001, referred to the dose in 24 organs or tissues and the effective dose for inhaled activity of 1 Bq and median particles aerodynamic diameters of 1 microns.

6.3.3. Results and discussion

In the following Table 6.15, analytical results of oil aerosols concentration obtained during the test period application are presented.

	BASELINE VALUE (T0)	END OF TEST PERIOD (T1)	AVG ABATEMENT
SP1	0,17	0,05	71%
SP2	0,21	0,15	29%
SP3	0,13	0,1	23%
SP4	0,23	0,05	78%
AVG	0,185	0,0875	53%

Tab. 6.15 – Aerosol abatement rate during preliminary field test. SP1, SP2, SP3 and SP4 are Sampling Points for air quality monitoring within the facility.

The indication of the values below the detection limit (0.1 mg/l of air) was reported and considered for the assessment as a concentration conservatively corresponding to half of this limit (i.e. 0.05 mg/l). The four sampling points are marked with a number code (SP, i.e. Sampling Point, 1-2-3-4) and related value reported in Tab.6.17 are the average results of multiple samplings.
The average abatement rate for aerosol shown by the system during the field test, i.e. 50-53%, has been assumed as typical for the applied sizing, conservatively applying a 5% error on the lower performance value.

On this premise a backward assessment of its ability to decrease the inhalation rate of radionuclides in post-accident scenario has been performed.
An initial concentration of Cs-137 has been considered as correspondent to an inhalation dose of 5 mSv. Considering an average of 16 hours spent in indoor environment and an Inhalation Dose Coefficient of $4.8 \cdot 10^{-9}$ Sv/Bq, as proposed by EURATOM, the Intake can be calculated. Therefore, through Breathing Rate and Exposure Time, airborne concentration of Cesium can be estimated. Once applied the biotechnology in indoor environment, provided that sizing and positioning of the modules would ensure the same efficiency, airborne Cesium concentration would decrease to a 55% of the initial concentration calculated. Through a backward assessment, the final Inhalation Dose has been estimated would results decreased by a 13%.

PARAMETER	UNIT	OUTDOOR	INDOOR	INDOOR(1)	TOTAL	TOTAL (1)
BREATHING RATE	m3/h			0.78		
CS-137 ACTIVITY	Bq/g			3,215E+12		
INHALATION DOSE COEFFICIENT	Sv/Bq			4,8E-09		
EXPOSURE TIME	h	8	16	16	24	24
INHALATION DOSE	Sv	3,57E-03	1,43E-03	7,86E-04	5,00E-03	4,37E-03
INTAKE	Bq	2,24E+03	2,98E+05	1,64E+05	1,04E+06	9,11E+05
INTAKE	g	2,31E-07	9,26E-08	5,09E-08	3,24E-07	2,82E-07
AIRBORNE RADIONUCLIDE CONCENTRATION	mg/m3	3,71E-08	7,42E-09	4,08E-09		

Tab. 6.16 – Backward assessment on airborne Cs-137 concentration, assuming inhalation as only exposure pathway, with an indoor air dose rate that is 40% of the outdoor air dose rate, on the basis of threshold limit value of 5 mSv, above which total decontamination has been performed in ICA (Intensive Contamination Survey Areas) of Fukushima Prefecture (Yasutaka 2015). Average Breathing rate is proposed by EPA (2011). Scenario (1) has been designed on the assumption of 45% efficiency in airborne Cs-137 abatement in indoor air performed by the biotechnology proposed, thus resulting in a decrease in total Inhalation Dose.

Considering the actual feasibility of biotechnology application in real scale on radioactive contaminated environment, the issue regarding the residual material must be addressed thoughtfully, since once the compartmentalization into the bioreactors is accomplished and the airborne radionuclides concentration is decreased, the radioactive burden is moved to the centrate inside the system.
During the field test on airborne metal dust contamination, process water testing has been performed on day 120 after installation, with the aim to identify and quantify elements and compounds not degraded by the system. Results showed a remarkable presence of metal dust (mainly iron), suspended solids (TSS), together with an increased COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand), clearly related to the biological activity.

CONTAMINANT	CHROMIUM (TOT)	NICKEL	IRON	TSS
CONTENT INTO THE WHOLE SYSTEM (N.7 AIRCEL UNITS)	2.7234 g	1.6295 g	884.4420 g	215699.4 g
AVG. DAILY ABATEMENT (120 DAYS EXPERIMENT)	0.0227 g	0.0136 g	7.3704 g	1797.495 g

Tab.6.17 - Total quantity of main contaminants found in the residual process water at the end of the test period (assuming an average water content of the system, corresponding to 40% of the maximum content).

Suspended solids concentration is related both to the airborne particulate matter captured and spent biomass.
Lead, copper and cadmium, were detected in traces (in concentrations around the detection limit values), while iron was found in remarkable quantities (Tab. 6.19). Metals detected are most likely deriving from airborne particles captured by the system, since tap water supply is certified metal-free. The mass of iron dust detected in the process water is equal to 0.4% of the TSS. A limited amount of Cs-137 is expected to be found dissolved in the aqueous phase, since the radionuclides presents a high adsorption capacity (Han et al. 2015, Shena et al. 2009), thus helping the further compartmentalization into residual solid material at the bottom of the tanks.

6.3.4. Conclusions

The present study has been carried out on two main pathways:
on the basis of similarities in behavior between radionuclides and heavy metals, examining the opportunity represented by a biotechnology for air treatment, in order to capture and, therefore, contain radioactive particles in a compartmentalized media. For this purpose, an immobilized cell bioreactors system has been applied in a manufacturing plant for containment of the airborne contamination related to the specific processing involved. The application offered promising results, both in abatement of aerosols and metal dust capturing ability.
performing an evaluation of the risk reduction due to diminished concentration of radionuclides in air possibly obtained. Several assumptions were necessary, such as a final abatement rate equal to the one verified on field test (condition that could be anyway reached by proper sizing of the system). Results are encouraging and they propose a new perspective for air treatment in radioactive contaminated media.

Removal of radionuclides from atmosphere have been identified as a major target for action in the after-accident scenario and countermeasures for preventing possible resuspension and continuous emission from surfaces have been applied (Yasutaka 2013). Taking as reference Fukushima's Daichi NPP accident, Cesium-137 has been taken into consideration as decontamination target. In this perspective, a preliminary assessment of the opportunity represented by the biotechnology under validation, can be regarded as first step toward real scale application and primary data collection on the specific contaminant.
As support for decontamination operations, air treatment technology should be applied in order to protect emergency workers and healthcare staff taking care of radiation-diseased patients (Han et al. 2015) during crisis period or to accelerate people's return in decontaminated indoor environments (homes, working places, schools).
Since the air dose rate, together with the cumulative dose, is one of the main factor to be considered in regard to the post-decontamination return time for civil population, the application of air treatment technology devoted to decrease this parameter could sensibly contribute to improve social sustainability of a cleanup intervention.
In real scale application perspective, a different sizing of the system, with a higher number of bioreactors per unit surface, for example, could help in reaching a customizable performance to the specific application and target. Several other factors may influence the effective exposure to radionuclides (building typology (Yasutaka 2013), anthropic activity, use of lands (Yasutaka 2013, Owen et al 1996) etc.), but low impact, low

energy demanding and flexible technologies for decontamination should be regarded as an actual and sustainable opportunity to operate in post-accident scenarios.

After the biodegradation process, the residual material (Rus and Sofer 1995), may be separated from clear water by sedimentation. In the preliminary application in metal dust polluted environment, metal recovery can represent an actual opportunity for secondary raw material collection by different means (Jha et al. 2004, Jha et al. 2008, Kentish 2001, Icopini and Long 2002, Waynert et al 2003, Nishijima 2000). While ion exchange reverse osmosis, solvent extraction, evaporation and precipitation appear more suitable to metal dust recovery, membrane separation has been accounted both for metals and radionuclides (Rana et al. 2013, Zakrzewska-Trznadel 2013).

As demonstrated by Horikoshi (Horikoshi et al. 1981), many microorganisms, such as microalgae, fungi and actinomycetes are capable of binding uranium and, when only physico-chemical adsorption at the cell surface is involved, release it under pressure of more suitable chelating agent (e.g. Ethylenediaminetetraacetic acid, EDTA). This opportunity should be taken into consideration and tested in the following step of application.

The bio-remediation of contaminated environmental media is to be acquired in compliance with local regulations and safety measures. It must be considered that compartmentalization, i.e. concentrating the radioactive polluted medium, could lead to higher emissions, if not properly managed, or redistribution of the radioactive burden, but it would nevertheless grant restoration of an environmental condition compatible with the ecosystem and life.

Possible recovery of radionuclides would represent the keystone for overall sustainability of the process. Compartmentalization and reduction in activity of the radionuclides are clearly remediation priorities, but solutions for centrate handling ought to be pursued in a circular economy perspective and in a global scenario of radioactive elements scarcity and poorly sustainable extraction.

Chapter 7 - Wastewater treatment

7.1. Case study

The experimental activity on wastewater treatment has been organized in two phases:

1. Phase 1: pilot plant set-up and first application. Preliminary results deriving from this phase have been presented to 6th European Bioremediation Conference in Chania, Crete, 28th June-3rd July 2015.
2. Phase 2**:** further experiments have been performed, with different treatment times and main target contaminants.

General conclusions have been drawn, considering both experiences.

7.1.1. Phase 1: first pilot application

"Bio-degradation of silk industry wastewater: a case study"

7.1.1.1. Abstract

BACKGROUND

Industrial wastewater treatment represents a major environmental issue, both for human health and ecosystem. The present study focused on an immobilized cell bioreactors system to be applied on silk manufacturing effluent, in order to accomplish an initial treatment phase, pursuing possible reuse within the same facility.

RESULTS

After 24 hours, the system processed 55% COD (final 72%). 25% of anionic surfactants have been abated despite typical inhibition effect they carry. Free Chlorine displayed a dynamic equilibrium with Chlorides, with a final loss to be attributed to volatilization. The removal efficiency on (ortho)phosphates is 92%, with apparent partial conversion to phosphorus, which had a final removal efficiency around 45%.

Water consumption (0,49-0,58 L/h to 0,11-0,17 L/h) is mainly related to evaporation.

CONCLUSION:

The system under study proved to be an effective solution for silk industry wastewater treatment to be implemented into a multi-stage process, displaying a high adaptability of the proprietary biomass and scalability of the system itself.

As easily and locally applicable wastewater treatment technology, with the opportunity to recover clean water from evaporation, the system could contribute to closed loop use of water, within the same facility or in an integrated district perspective of industrial symbiosis.

Keywords:

Industrial Wastewater, Silk, Textile, Bioremediation, Wastewater Treatment, Industrial Symbiosis

7.1.1.2. Introduction

Industrial wastewater treatment represents a major environmental issue to protect water bodies quality, both for human health and ecosystem, as testified by international regulations, placing restrictive limitations for wastewater discharge. Since industrial wastewaters are rarely segregated on the basis of their composition or contamination level, wastewater treatment plants are facing not only heavily, but even variously contaminated media (Zahn 1993). This element causes increased fragility of treatment performance and working conditions, followed, consequently, by rising management cost to be incurred by customers.

The present study has been performed with the aim of modifying the wastewater treatment methodology applied on an Italian silk manufacturing, in order to answer an existing need in the textile industry, where high costs and poor environmental performance put at risk, in the European economic framework, their very survival.

Silk is one of the oldest textile fibre known: first evidences of silk fibre production have been dated around 3500 BC in China, which is still, at present times, the first producer worldwide with 126 MT (metric ton) over 153 MT total (Basu 2015, International Sericulture Commission 2013).

Italy is the second exporter of weaved silk in the world after China. Export represents ¾ of the total sales volume, which was still growing in 2014 by a 1,8% on the previous year (Cerved Group data, Unicredit). Production of the silk fibre is usually performed in outsourcing in China, where labor and environmental regulation and, therefore, production costs are lower. In Italy, the weaving process is carried out by an heterogeneous complex of operators: from small specialized companies and contractors to vertically integrated and diversified companies. In particular, the province of Como, with its ancient origins, remains the most active business district of the sector (the same Bologna has been a leading district for silk production since the XIV century) (www.setarium.it). Within this framework, a silk manufacturing company historically settled in Como has been involved in the present study.

Silk presents mechanical characteristics that artificial fabrics rarely match, due to its peculiar chemical structure. Raw silk is composed of two major proteins, different in structure and, therefore, function. Fibroin, or the silk filament, where the amino acids (mainly glycine and alanine) are ordered to form a pattern of crystalline regions, with bundles of microfibrils, building a protein with quaternary structure. Sericin, or the gum, in which the amino acids are randomly arranged in amorphous regions (Basu 2015).

As natural polymer composed by several different amino acids, silk presents tails of amino and carboxylic groups (general formula $NH_2CHRCOOH$). Thus, the molecule is able to build salt bonds with anionic and cationic dyes, making the silk able to be dyed by acid and cationic chemicals, as well as through direct process, metal complex, acid mordant or reactive dyes (Basu 2015).

Most of the silk currently on the market comes from *Bombyx mori* (commonly known as mulberry silkworm). The silk processing from this silkworm includes several steps (Zahn 1993): reeling, weaving, degumming, dyeing or printing, and finishing. During wet reeling process, cocoons are cooked and poured into a reeling bath (Basu 2015), producing wastewater and a mass of filaments to be converted into silk yarn by spinning. Soap, soda, sulphuric acid, acetic acid are commonly used as reactant in silk production. The gum coating requires a quite demanding process to be removed. The degumming process is necessary to separate the two major proteins composing raw silk: fibroin and sericin, which is mainly discharged into wastewater, but could become a valuable by-product, if properly separated. A soap solution, hot bath and cold rinsing is the basic procedure, but, in most cases, various chemicals are added as bleaching solution (hydrogen peroxide, sodium silicate, hydrosulphite and diluted sulphuric acid or, alternatively, hydrogen peroxide, sodium pyrophosphate and EDTA), at specific condition of temperature and pH. Sericin, together with Sulfur compounds, Nitrogen, Chloride and surfactants deriving from silk treatment steps, represent major concerns for wastewater management.

The effluent, in fact, tends to decrease oxygen concentration in water in presence of hydrosulphides and it represents a thread for human health since dyes generally contains organically bound chlorine, which is typically carcinogenic. Heavy metals are typical textile wastewater pollutants as well (Malik et al. 2014).

7.1.1.3. Materials and Methods

The system applied is based on stand-alone bio-oxidizers, working in batch on wastewater to be treated, coupled with a free surface tank to provide additional oxygenation to the system and with a twin bio-oxidizer treating air and possible stripped contaminants.

When wastewater is treated with immobilized-cell spiral bio-reactions, clean air adds oxygen to the centrate containing bacteria, and enzymes. As the wastewater and biomass mix re-circulate via an internal pump through the bioreactor, the biomass, a consortium of bacteria and oxidative enzymes not genetically modified, but, still, under proprietary recipe, attacks the contaminants, degrading them to their components. With time, biomass is immobilized onto the internal surfaces of the bioreactor, enhancing the degrading ability of the system.
Since providing oxygen is crucial for immobilized cell technology, an additional external free surface tank has been implemented, as suggested by Professor Sofer, to recirculate wastewater through a 0,20 m cascade and obtain extra oxygen from turbulence generated. An air treatment device, equivalent with the bioreactor used for wastewater treatment, has been included into the system, in order to prevent contaminants to travel into air after vaporisation, related to turbulence in the external tank (Fig. 7.1).

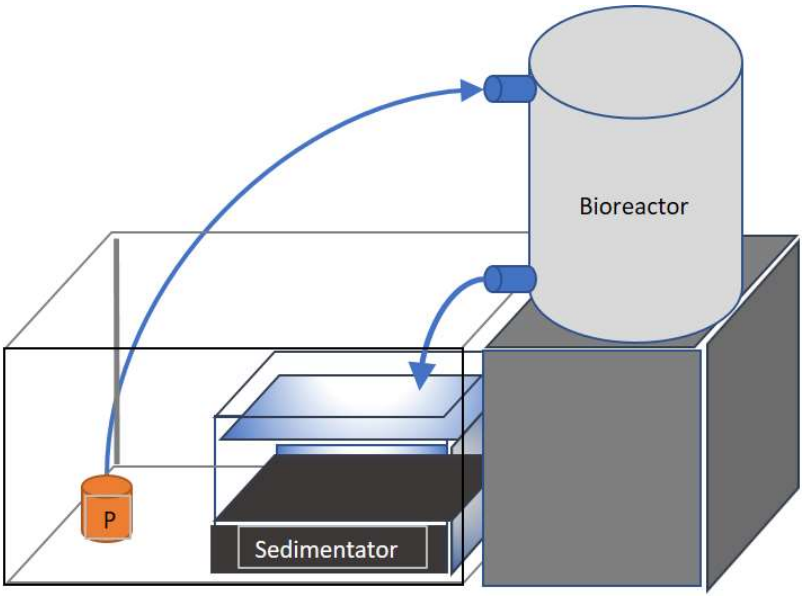


Fig. 7.1. Wastewater treatment unit, pilot scale, design.

The wastewater treatment plant has been built up in small pilot scale, using patented bioreactors for air treatment AIRcel 85, supplied by U-Earth Biotechnologies, and a 60 litres plastic tank with open surface (Fig. 7.2).
All materials used for the experiment are re-used and re-usable, in order to improve the overall sustainability of the research and contain costs. Only monitoring consumables have been disposed, while all other materials are ready for re-use.
Wastewater samples coming from silk production and dyeing carried out by an Italian company have been supplied to the system. Different effluents, extracted by different production stages have been treated in batch mode, in 24, 48 and 72 hours' tests.
After a starting run with clean water and a biomass dose, aimed to obtain an initial immobilization of bacteria on the reaction surface, highly diluted wastewaters (0,5% and 10%) from the degumming bath were added, in order to make the biomass develop on chemicals available. Then, a single dose (100 ml) of biomass was added in time 0 and 35 L of wastewater to final discharge supplied to the system.

Monitoring steps for the key performance parameters have been set in different timing, from 0 to 72 hours. Temperature and pH of the wastewater have been tested during the whole trial period, as well as target contaminants, as presented in Chapter 4. They have been identified in Anionic Surfactants, Chemical Oxygen Demand, Chlorides, Free Chlorine and Phosphorous based first on the case wastewater characteristic and remediation target and monitoring and budget efficiency reasons as well. Environmental conditions have been tested with Temperature/Humidity probe, together with water temperature into air treatment unit, wastewater treatment unit and tank.
Water level has been checked and registered, in order to evaluate consumption through evaporation.

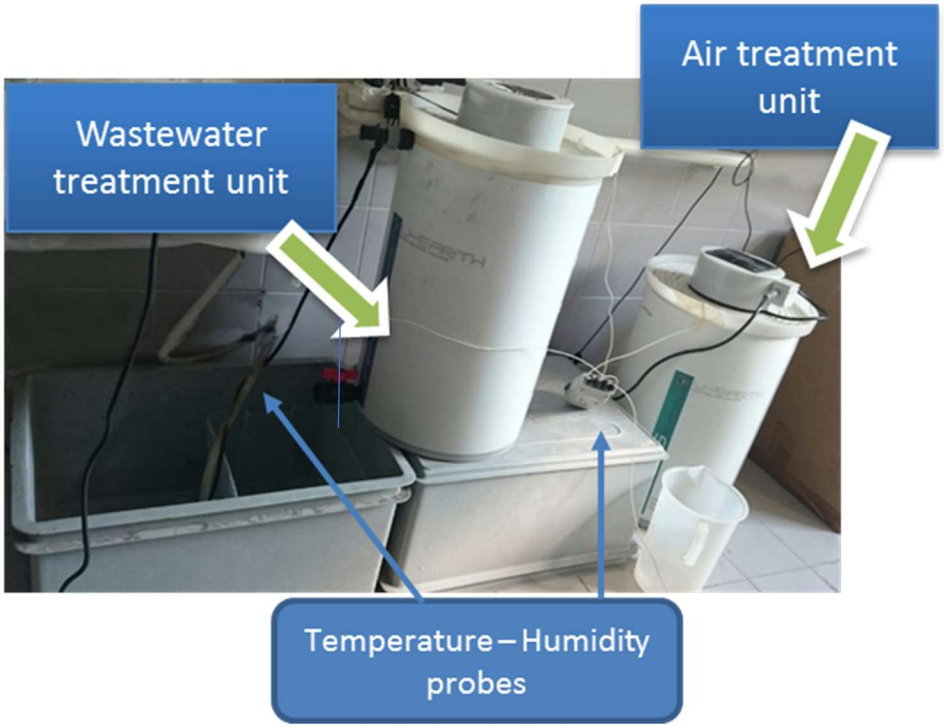


Fig. 7.2. Wastewater treatment unit, pilot scale, installed.

7.1.1.4. Results and discussion

Initial run with diluted wastewater from the degumming bath, with a high content in sericin, anionic surfactants, softeners and chelating agents, led to biomass immobilization on the inner surfaces of the bioreactor. A strong decontamination of the first dilution proposed (over 90% abatement of free Chlorine, over 88% of COD, 55% of Phosphorus) has been accomplished and less efficient treatment of the second (10% dilution), where the complex pollution led to less robust results. Over a 72-hour run, in fact, a 64% removal of free Chlorine was obtained, together with 39,64 % decreased COD, 29,7% abatement over Anionic Surfactants, but with an increase of 12,48% of Chlorides and 28,99% Phosphorus, to be presumably attributed to concentration over a decreased wastewater volume.
The wastewater to final discharge, which the present study was mainly focused on, is constituted by a mix of the degumming bath and following treatment, adding less polluted water, soap and trypolyphosphate to the solution. This leading to a different effluent mix and, therefore, different reaction and performance offered by the system.
The overall Chemical Oxygen Demand has been tested as the main target of this preliminary phase of proposed application on silk industry, since COD concentration represents not only a major environmental issue, but even an economic one for industry, being a key element for discharge fee composition. After 24

hours, the system had processed more than 55% of initial COD, mainly due to bacterial activity and system's enhanced oxygenation and the 72 hours run proved to be sufficient to decrease the concentration by 72% (Fig. 7.3). Thus leading the effluent within the limit value defined by Italian regulation (i.e. 500 mg/L, D.Lgs. 152/06).

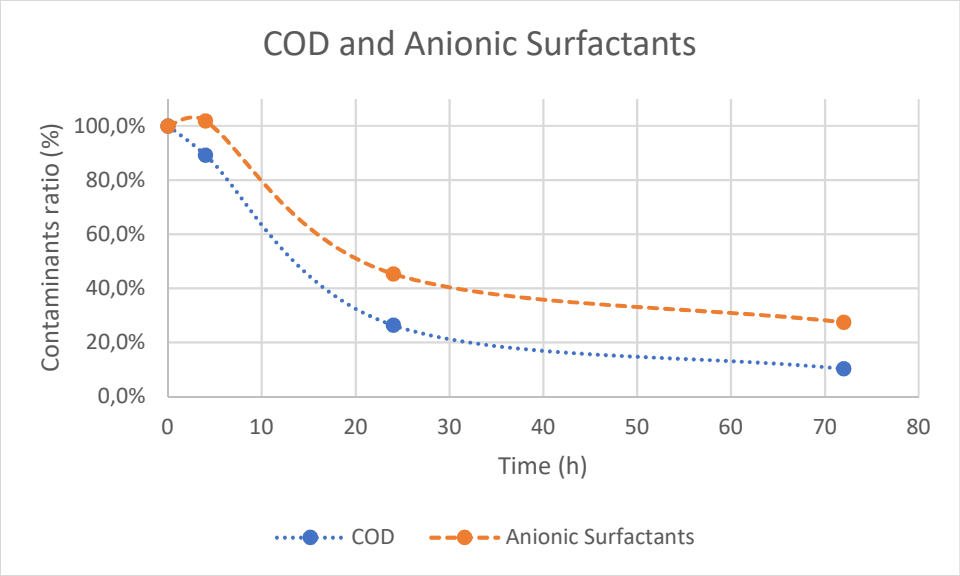


Fig. 7.3: COD and Anionic Surfactants content in the system, compared to the initial batch feed.

Anionic surfactants are typical pollutants from textile industry, extensively used due to their chemical properties, related to their configuration. They, in fact, presents a polar head, which is well solvated in water, and a nonpolar hydrocarbon tail, thus combining hydrophobic and hydrophilic characteristics (Hallman et al.). Surfactants degradation is commonly regarded as a twostep process: a primary degradation, corresponding to the loss of surfactant properties by the molecule, and an ultimate degradation step, leaving only final products on the ground (CO_2, CH_4, H_2O, minerals and new biomass). Linear alkylbenzene sulphonates (LAS), in particular, are considered largely biodegradable, as demonstrated by wastewater treatment plant data presented by several authors, under aerobic conditions. They can also precipitate in the presence of polyvalent metal ions (e.g. Ca^{2+}) (Hallman et al., Mancini 2012, Borghi et al. 2011).
Since anionic surfactants are commonly regarded as carrying inhibition effect on activated sludge (Brunner et al 1988), which appears to be related to the proprietary biomass used, the overall performance, with a 72,5% abatement of concentration in the treated wastewater, the bacteria consortium appears to be quite adaptive and the system efficient in removing even these recalcitrant contaminants.
Free Chlorine in the system, while initially decreased, tended then to raise again, in dynamic equilibrium with Chlorides (as shown in Fig. 7.4), with a final removal of 58,8% and part of the loss possibly related to volatilization. Air treatment unit coupled to the wastewater treatment is, in this sense, strategic in order to avoid environmental dumping from water to air matrix. Chlorides removal efficiency is achieved during the first 24 hours and it corresponds to the 48,5%.

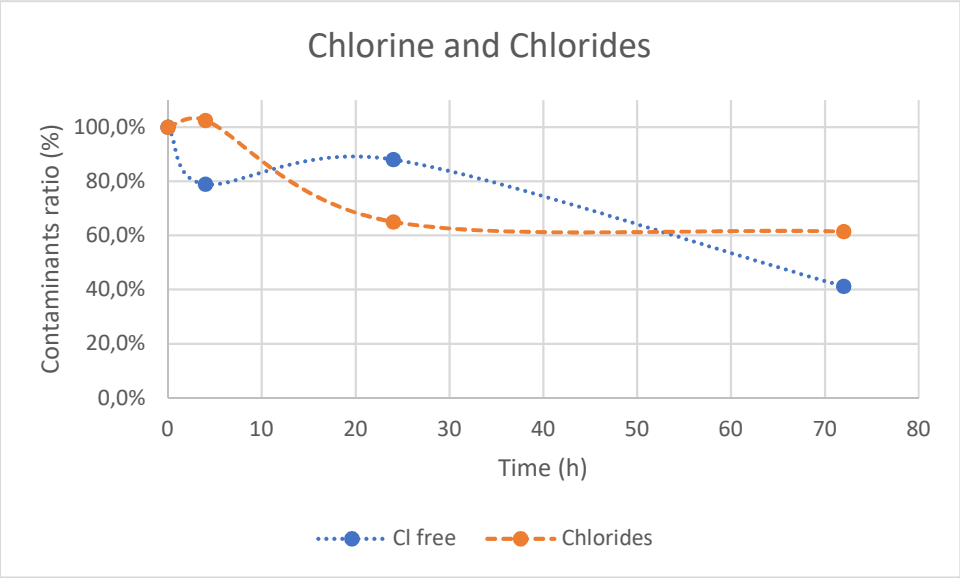


Fig. 7.4.: Chlorine and Chlorides content in the system, compared to the initial batch feed.

The system displayed a removal efficiency on phosphorus of about 75,9% during the first 24 hours and abatement of 97,1% of (orto)phosphates during the complete 72 hours run (Fig. 7.5), with apparent partial conversion to phosphorus. Since the initial concentration was no more than 40% of the discharge limit imposed by Italian regulation (D.Lgs. 152/06) in term of concentration, the abatement obtained is considered satisfying.

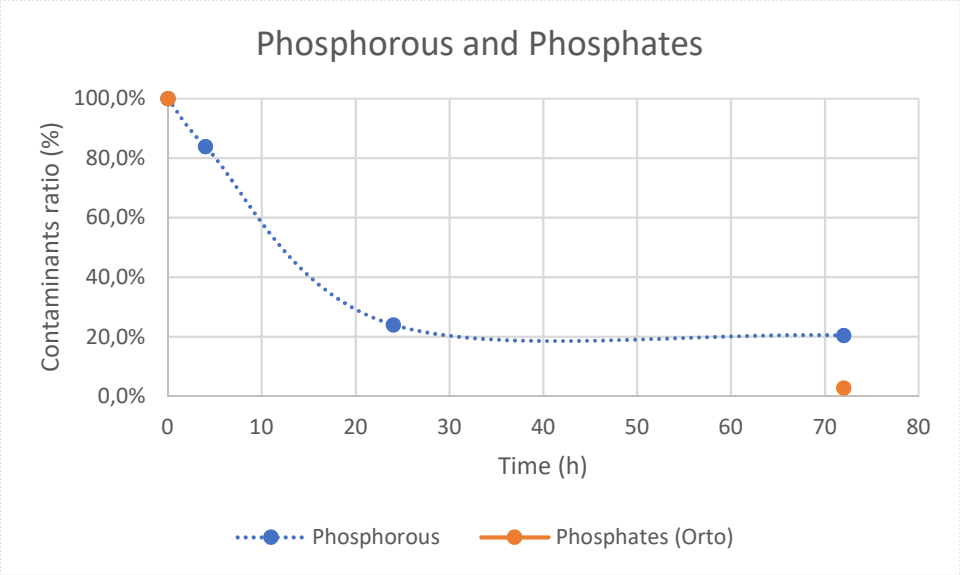


Fig. 7.5.: Phosphorous and Phosphates content in the system, compared to the initial batch feed.

Water consumption displayed by the system is mainly related to evaporation and, therefore, partially to room temperature. The hourly consumption ranged from 0,49-0,58 L/h for the first 24 hours, to 0,11-0,17 L/h for the following 48 hours (Fig. 7.6).

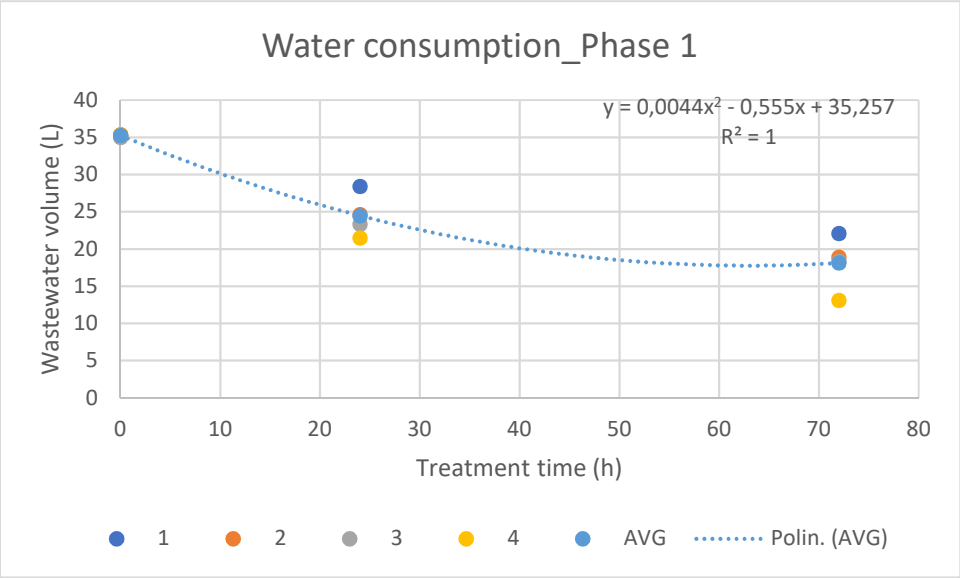


Fig. 7.6: Wastewater volume in the system

In a short-term perspective, results obtained, in terms of pollutants concentration coupled with wastewater volume decrease, could answer to a budget need for reduced discharge fees, where applied, and a reduced amount of contaminants into final effluent.

In a sustainable scale-up perspective, on the other hand, water consumption becomes a major issue and must be addressed accordingly, in order to promote water reuse and enhance the closed loop use of water in the industrial sector. Where, in fact, wastewater coming from treatment plant could present residual pollution level incompatible with highly specialized water demand (as in silk industry), evaporated water from the wastewater treatment system proposed could match the requirements. With the implementation of a condenser unit on the top of the system, the issue could find a suitable answer and it will be integrated into the scale-up design.

The following step of the research will focus on scale-up issue and overcoming of batch mode limitation in volume and overall flow. In order to match the treatment volume and residence time required by real scale industrial requirements, a sequenced multi-batch system could be the key for the scale-up design, while a modification of the inner design of the bioreactors will be avoided.

7.1.2. Phase 2: further experiments and elaborations.

Pilot scale plant set-up defined for Phase 1 application has been replicated for Phase 2.

7.1.2.1. Biomass attachment test

With the aim of defining typical attachment time for the biomass supplied to the system and, therefore, set the optimal timing for wastewater addition to the system, a series of attachment tests have been performed. A single dose of dispersed biomass (0,1 L) has been added to 35,5 L of clean tap water (i.e. average initial wastewater volume used for remediation experiments) and turbidity tests has been taken in different timing, in order to verify the settlement degree of the biomass.

Results obtained showed that 16 to 21 hours are necessary to allow total attachment of the biomass to the inner surfaces of the bioreactor and sedimentation of residual at the bottom of the aeration tank.

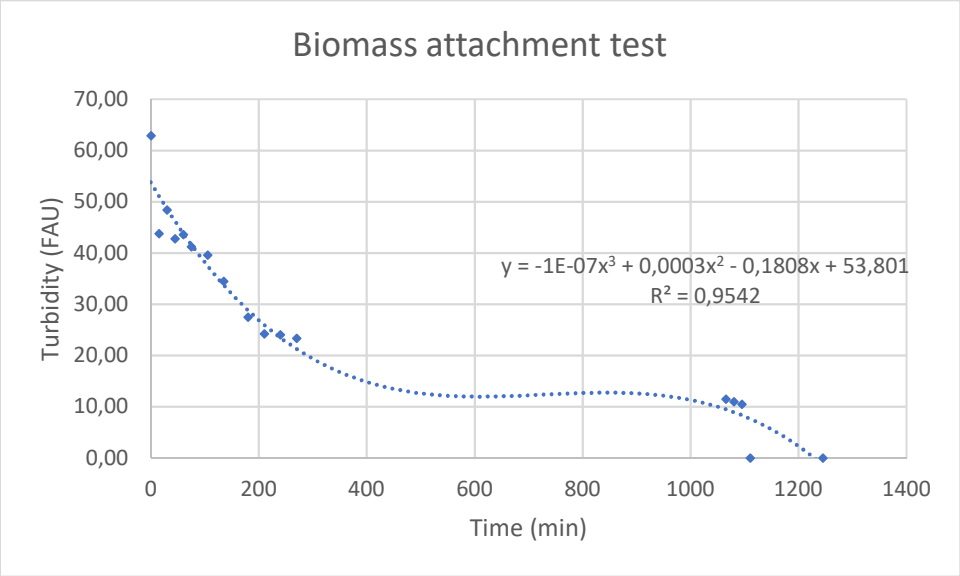


Fig. 7.7: Biomass attachment test

7.1.2.2. Remediation tests

The pilot plant has been installed into real environmental condition, in a semiconfined space outdoor. Different remediation experiments were performed on effluents from silk production industry. The three batches treatment experiment were prepared as reported in Table 8.1.

	BATCH 1	**BATCH 2**	**BATCH 3**
EFFLUENT	Untreated wastewater	Untreated wastewater	Effluent treated during Batch 2 experiment (75%) + untreated wastewater (25%)
EFFLUENT VOLUME (L)	30,18	31,01	28,1
RESIDENTIAL TIME (H)	48	48	48

Table 7.1: Treatment experiments, batches preparation.

The wastewater added to the system displayed an unstable and changeable composition, presumably related to the ongoing biological processes involving the high protein content (sericin). Several samples were taken at the beginning of each remediation experiment and analysed for ammonium, anionic surfactants and Chemical Oxygen Demand (Table 7.2).

	$N\text{-}NH_4^+$	ANIONIC SURFACTANTS	COD
B1_WWS_1	79,6	17,9	1358
B1_WWS_2	79,9	23,7	1357
B1_WWS_3	79,3	25,5	1357
B1_WWS_4	75,1	32,4	1357
B2_WWS_1	75,2	30,4	185,2
B2_WWS_2	75,3	28,8	197,8
B2_WWS_3	75,7	12,2	191,5
B3_WWS_1	76,0	12,4	295,6
B3_WWS_2	76,0	12,4	350,7
B3_WWS_3	76,0	12,9	323,2

Table 7.2: WasteWater Samples (WWS) composition (mg/L) for ammonium, anionic surfactants and COD in the three different batches (B1, B2, B3).

Based on Phase 1 experience, wastewater volume has been recorded at each monitoring step. Results showed a remarkable difference in wastewater evaporation between the two experimental phases, reasonably attributable to the different season and, therefore, environmental and water temperature. In fact, while during Phase 1 water temperature ranged from 18.4 °C to 22 °C, during Phase 2 it varied from 10.4 °C to 12.2°C. As presented in Table 8.3, wastewater consumption passed from 48.6% of Phase 1 to 9.4% of Phase 2. Previous applications performed by Sam Sofer with a different bioreactor's configuration showed evaporation volumes, after 24 hours processing, of about 70%, against 31% (Phase 1) and 3% (Phase 2) obtained here.

Due to sensible decrease in effluent volume, concentration results have been related to the initial batch volume. This allowed a comparison between the total estimated amount of contamination present into the batch at the beginning of the treatment and the result, considering the evaporated water as an opportunity for recovery, rather than simply a loss to the system. For this reason, results are presented in terms of percentage of contaminants remaining into the system at the different sampling times.

A limiting factor for water evaporation may be identified in the presence of surfactants. In the specific effluent treated into the pilot application, anionic surfactants were one of the key parameters to be investigated, since they are strictly related to the specific industrial process which produced the wastewater (i.e. silk manufacturing).

	0	24	48
P1_B1	100%	80,4%	62,5%
P1_B2	100%	69,7%	53,5%
P1_B3	100%	66,7%	52,5%
P1_B4	100%	60,8%	37,1%
P1_AVG	100%	69,4%	51,4%
P2_B1	100%	95,3%	90,7%
P2_B2	100%	95,3%	90,6%
P2_B3	100%	99,8%	90,4%
P2_AVG	100%	96,7%	90,6%

Table 7.3: Residual wastewater into the system, measured after 24 and 48 hours, during Phase 1 (P1, Batches 1, 2, 3, 4) and Phase 2 (Batches 1, 2, 3).

As presented into following graphics (Fig. 7.8, Fig. 7.9 and Fig. 7.10), the three batches experiments returned quite different results, with a decrease in remediation performance of about 40% from Batch 1 to Batch 2. Provided the similar average concentration for wastewater feed in batches 1 and 2, which should have led to similar outcomes, the main difference among the three batches is represented by environmental and, consequently, water temperature. Between Batch 1 and Batch 2, in fact, night temperature turned to near-zero values, while day temperature decreased enough to have water temperature around 10°C (about 2 °C lower than during Batch 1 test).

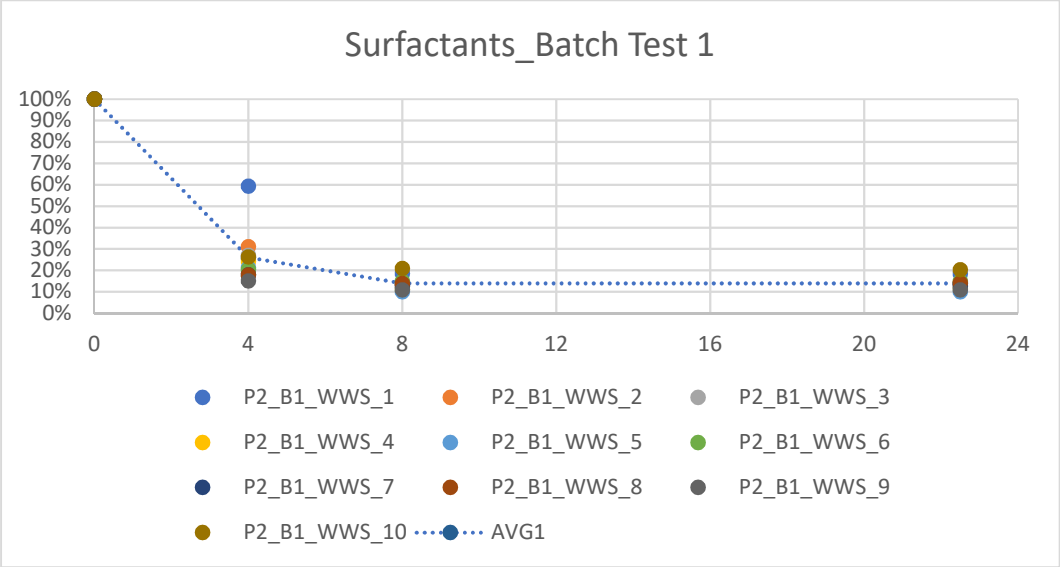


Fig. 7.8: Surfactants, first test run (Batch 1)

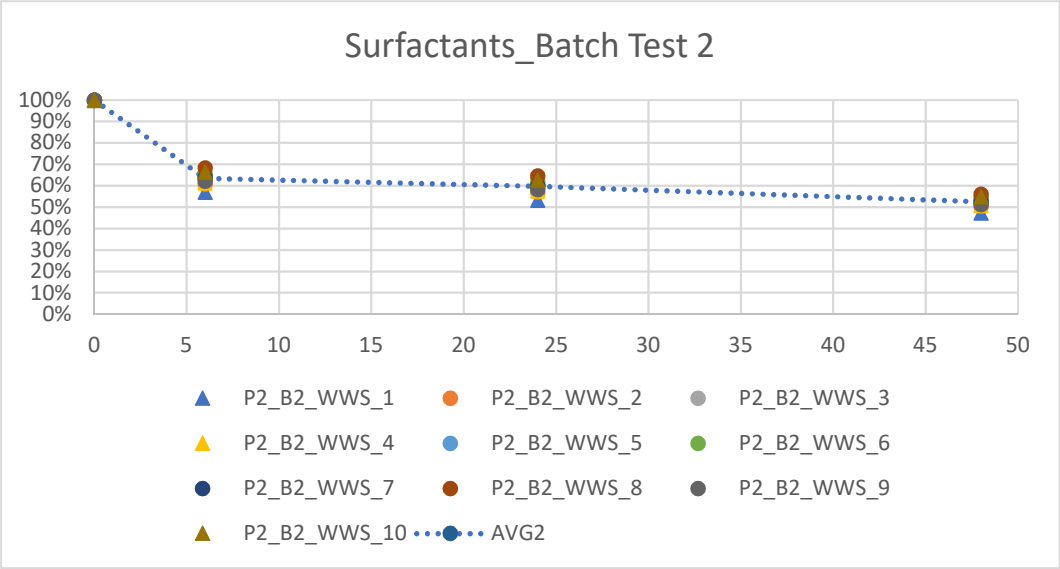


Fig. 7.9: Surfactants, second test run (Batch 2)

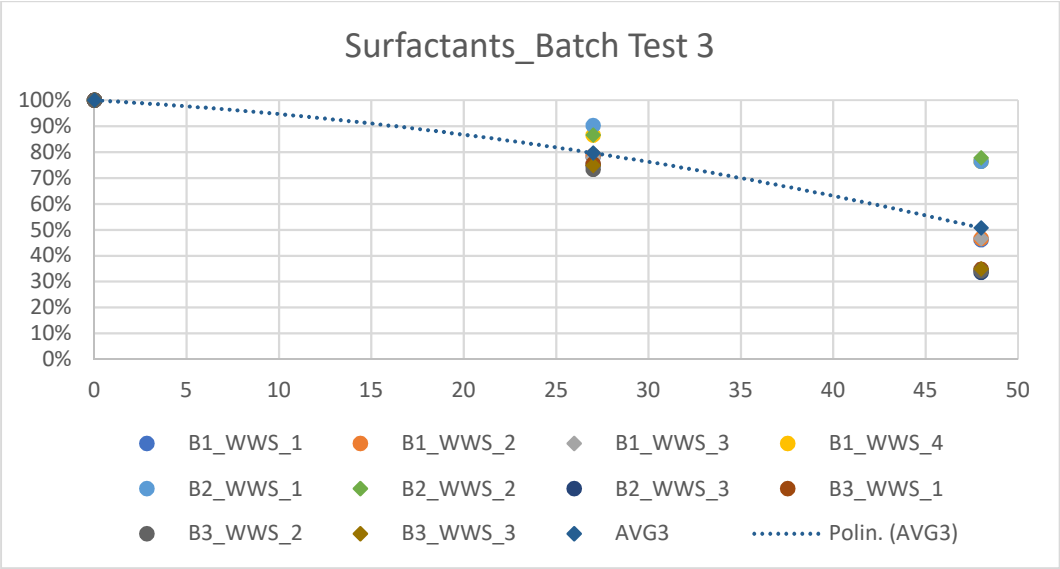


Fig. 7.10: Surfactants, third test run (Batch 3)

Together with anionic surfactants, a contaminant of interest for the pilot test has been identified in ammonium cation.

The three batch experiments returned quite results, in particular from Batch 1 to Batch 2 and 3, as highlighted regarding anionic surfactants. In fact, after the first 24 hours of treatment, a reduction of about 40% of the system content of ammonium was achieved during Batch 1 experiment, while less than 10% and 20% reduction was obtained with Batch 2 and Batch 3, respectively.

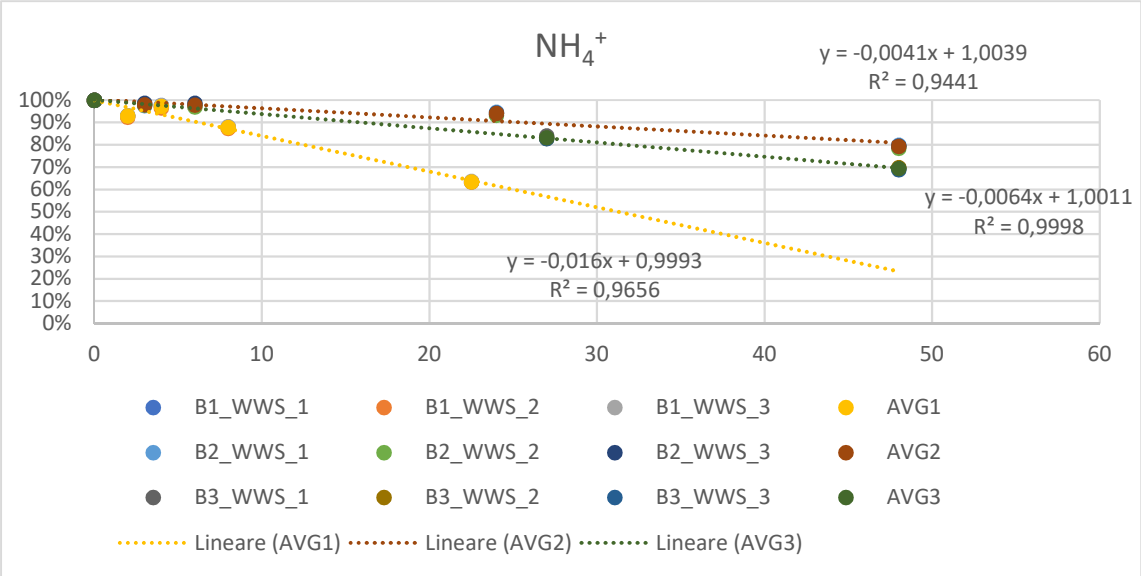


Fig. 7.11: Ammonium, Phase 2

As into Phase 1, Chemical Oxygen Demand (COD) has been effectively treated by the pilot system. Results obtained during the three batch experiments are reported in Figure 7.12, together with results from Phase 1. A remarkable similarity between Phase 1 and Batch 1 results is detectable, while Batch 2 and Batch 3 COD concentrations showed no significant decrease. This represented a minor issue, since COD values of the last two experiment were lower than discharge limit (D.Lgs 152/06) from the start, as demonstrated during characterization of the effluent (Table 7.2).

In accordance to trends displayed for other contaminants, different environmental conditions seemed to play a significant role on the treatment performance. In fact, concentrations trend for Phase 1 and Batch 1 were similar, but with different remediation performances. In terms of concentrations, remediation performances dropped from 57% to 22% after 24-hours treatment, while, relating the concentration to the overall amount of effluent, it is possible to verify that in Phase 1 a reduction of 73.6% was achieved, considering evaporation as water recovery opportunity, and during Batch 1, COD decrease accounted for only 25%. This data is, anyway, coherent with application reported by Sam Sofer.

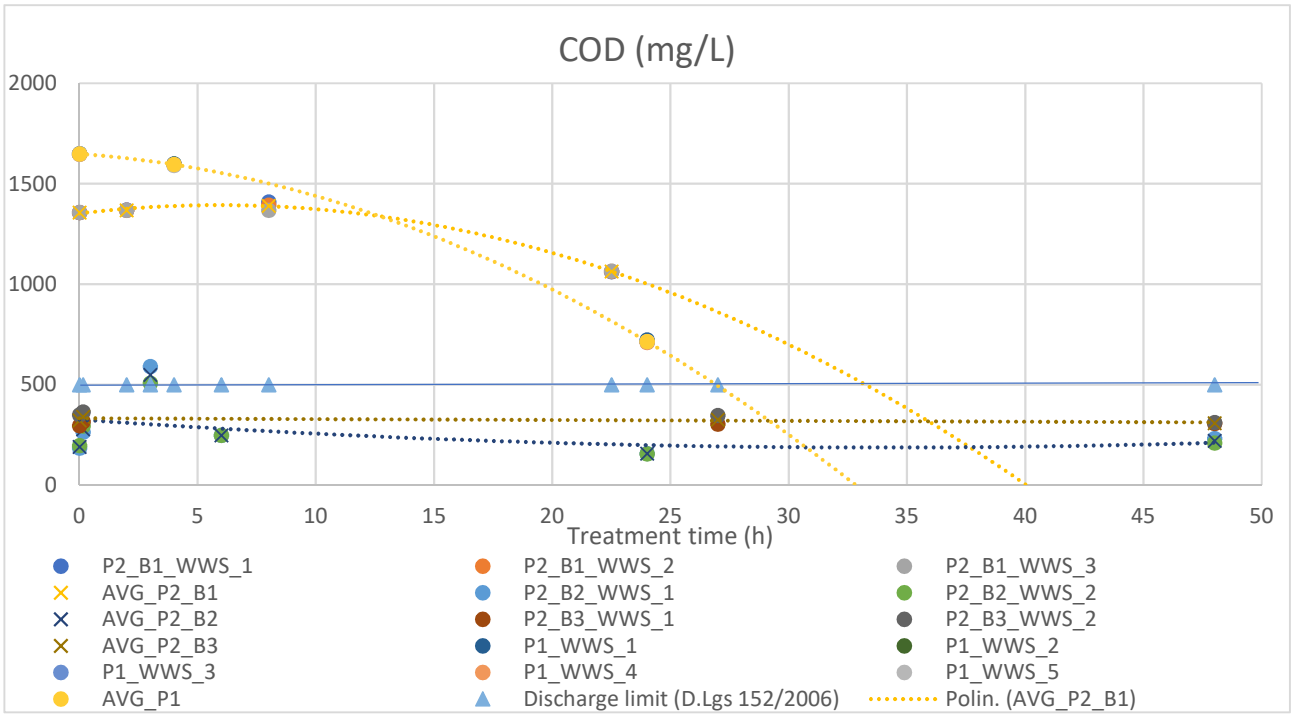


Fig. 7.12: COD concentration, Phase 2 (B1, B2, B3) and Phase 1 experiment (P1)

Treatment performances proved to be quite unstable and strongly dependent to environmental conditions. Longer residential time could be necessary in order to obtain satisfying results for lower water and environmental temperature. During the three batch experiments, water reached 12-10°C only during the day, therefore, even if in a semiconfined environment, it is likely expectable that, at nighttime, it has decreased by 2-3 °C at least. This suggesting that the optimal water average temperature range for biomass digestion performance could be set above 10 °C. Considering oxygen dissolved in water, which is clearly a limiting factor for biodegradation of contaminants, and typical temperature for mesophilic bacteria activity (Sipma et al. 2010, Morgan-Sagastume et al. 2003), an upper temperature value could be set on 30-35 °C.

7.1.3. Conclusions

The system proposed proved to be an effective solution for silk industry wastewater treatment, displaying a high adaptability of the proprietary biomass and scalability of the system itself (due to the modular design), even though strongly dependent on environmental conditions and, most of all, water temperature. This should be considered in a possible full-scale implementation, in order to prevent winter-time failures of the wastewater treatment, which represent a typical drawback of biological treatment system. As reported by Sipma (Sipma et al. 20109, in fact, temperature fluctuation and, in particular, winter temperature may lead to reduced microbial activity and, therefore, decreased treatment performances (Sipma et al. 2010, Morgan-Sagastume et al. 2003).

In a cost-effective perspective, the implementation of this system appears to be suitable within a multi-stage treatment process. As marked by many authors, for example, the sericin recovery should be pursued and ultrafiltration has been identified as a feasible technology (Fabiani et al. 1996). Over a worldwide production of silk worm cocoons of about 1 million tons (Aramwit et al. 2012), in fact, about 50.000 tons of sericin could be effectively recovered (Zhang 2002) and applied both in cosmetic and pharmaceutical industry (Aramwit et al. 2012, Zhang 2002, Padamwar et al. 2004), consequently decreasing the organic load on silk production wastewaters and, therefore, environmental impact. Where advanced recovery system would leave a high

COD in the final effluent (Fabiani et al. 1996, Turbiani et al. 2011), the present system could be integrated as low energy demanding and offering an opportunity in clean water recovery through evaporation.
An easily and locally implementable wastewater treatment technology, as the one under study, is the key to work on closed loop use of water, in the very same facility or in an integrated district perspective of industrial symbiosis (as proposed by Kalundborg Symbiosis experience, Chertow 2000). Where treatment performance would not ensure a water quality sufficient for reuse into the same cycle, in fact, less noble or, on the contrary, highly specialized (Vaithanomsat 2008) uses could be identified and promoted, into an integrated perspective. On the other hand, simple system modifications could lead to remarkable improvement in final effluent quality, turning an issue into a resource. The evaporated water recovery is, in this sense, one of the main design element for the scale-up. Thus, improving the overall environmental performance of silk production, which is largely spread in developing countries both in industrial and rural context ("cottage" production), where sustainable water management is strategic to ensure access to clean water resource Soulivanh 2007). In rural area, in particular, a sustainable wastewater treatment and possible cascade water use cycle should focus and take advantage on phosphorus recovery, addressing it as a resource for the local economy and global sustainability.

PART IV: Concluding remarks

Chapter 8 – Sustainability

8.1. Sustainability of the research work

Sustainable development, as defined by Brundtland Commission, i.e. "development that meets the needs of the present without compromising the ability of future generations to meet their own needs", should be the core focus of Environmental Engineering research. As stated by American Society of Environmental Engineering, this branch of Engineering works on protection both of environment and human population from adverse effect they have one on the other. Where, in fact, anthropic activities trigger impacts on the environment, their consequences on local and global scale may affect not only environmental quality, but even human safety, e.g. extreme natural events occurring as results of climate change.

For this reason, environmental researchers should pursue sustainable solution for engineering issues, not only by promoting the real transition to a sustainable society, but even raising awareness of political, economic and social stakeholders and applying sustainability principles to their research work.

Based on these principles, the present study has been developed as the second stage of research, i.e. field scale implementation, on a biotechnology for air and wastewater remediation. Except for the risk assessment (Chapter 5), where laboratories activities have been carried out with the support of media contaminated on purpose (both air and water), all other case studies and data presented resulted from genuine problem-solving applications, i.e. in presence of actual contamination conditions in real environments. In addition to this, monitoring activities were performed, when possible, through electronic sensors and probes and only where necessary, with the application of analysis involving consumables and analytes and, anyway, promoting the implementation of laboratory re-use practice.

8.2. Sustainability of the biotechnology proposed

In addition to this, an impact assessment tool, i.e. Life Cycle Assessment, has been applied to perform a screening of the environmental impact generated by the technology implemented and, therefore, assess possible weak points which could be object of improvement, in a further eco-design phase. This aspect has been analyzed following an approach already proposed within the framework of Minotaurus Project (Microorganism and enzyme Immobilization: NOvel Techniques and Approaches for Upgraded Remediation of Underground-, wastewater and Soil), coordinated by Fachhochschule Nordwestschweiz (Switzerland) (Hochstrat et al. 2015). The project, in fact, was developed with the aim of delivering innovative bio-processes, based on immobilized biocatalysts, for water and site remediation, together with support for evidence-based decision on their implementation, including Risk Assessment and Life Cycle Assessment, to evaluate the actual environmental benefit of solutions proposed.

Evaluating the present biotechnology, in particular, energy consumption proved to be the most critical factor affecting the environmental performance of the bioreactors and a focus on potential energy supply based on renewable resources has been suggested. Nevertheless, results obtained on air treatment should be considered from two different perspectives:

1. as already stated in Chapter 6, implementing into the assessment the environmental benefit provided by technology's application, i.e. the amount of pollutants removed from indoor air (based on manufacturer's data), a rough comparison has been performed between net impact, in terms of CO_2 equivalent, provided by AIRcel unit and typical carbon sinks, i.e. trees with different growth rate.

Results obtained suggest that a single AIRcel unit could act as carbon sink equivalent to a number of trees ranging from 43, in case of high growth rate species (e.g. walnut, poplar), to 268, in case of low growth rate trees (e.g. oaks);

2. when the system is implemented as complement for existing air treatment system, as in the case study accomplished within Climate Kic program "Pioneers into Practice", in cooperation with Fresenius Medical Care, and remarkable energy savings are acquired, this positive impact should be taken into account. A simulation has been performed, with the application of SimaPro software (vers. 7.3.3.) and results obtained in terms of kg of CO_2 equivalent/year are reported in the following Table 8.1, Fig. 8.1. and Fig. 8.2. In this case, the installation of n.2 AIRcel 85 units, together with a monitoring device with dedicated wifi hotspot for data transmission, allowed to draw a plan for traditional air ventilation and filtering system for about 64% of the year (considering non-operational time, i.e. nights and weekends, in compliance with international guidelines). Therefore, it has been possible to calculate the expected energy saving and, consequently, the positive impact generated, corresponding to about 14 tons of CO_2 equivalent per year for a single Operatory Room (OR) in a private clinic.

IMPACT CATEGORY	UNIT	TOTAL	AIRCEL SYSTEM	AIR TREATMENT SYSTEM_ENERGY SAVING
TOTAL	Kg	-14152,6	416,3056864	-14568,9
FOSSIL CO2 EQ	Kg	-14148,6	416,2077003	-14564,8
BIOGENIC CO2 EQ	Kg	-179,902	5,607612699	-185,509
CO2 EQ FROM LAND TRANSFORMATION	Kg	-0,04043	0,001220916	-0,04165
CO2 UPTAKE	Kg	175,9306	-5,510847529	181,4414

Table 8.1: Environmental impact (kg CO_2 equivalent) of proposed air treatment system configuration, focus on energy consumption, GHG Protocol.

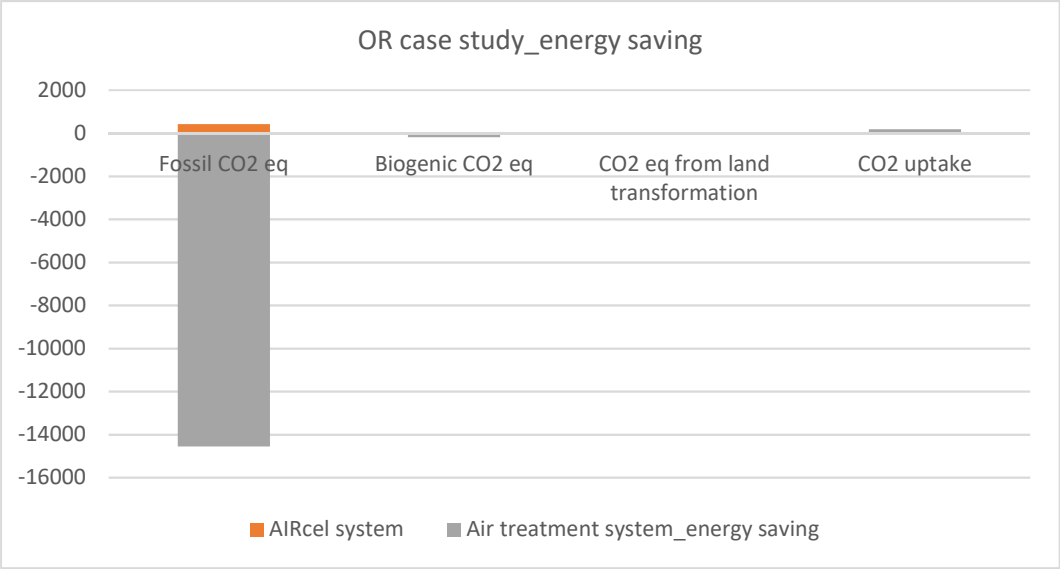


Figure 8.1: Environmental impact (kg CO_2 equivalent) of proposed air treatment system configuration, focus on energy consumption, GHG Protocol, Characterization.

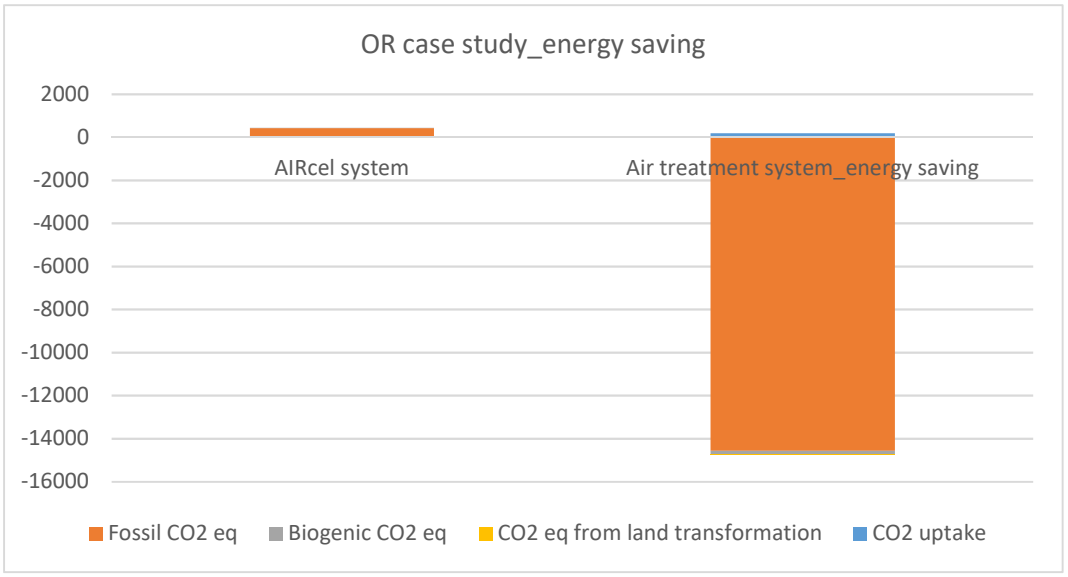


Figure 8.2: Environmental impact (kg CO_2 equivalent) of proposed air treatment system configuration, focus on energy consumption, GHG Protocol, Single issue.

Chapter 9 - Monitoring and performance assessment

9.1. Monitoring issue

A crucial aspect in the process of validation of an environmental biotechnology, which was one of the main goal of the present study, is the performance evaluation and assessment.
Provided the peculiar working principle of the biotechnology, specific tools have been applied to assess the effectiveness of the different applications and, in particular, standard monitoring methods have been adapted to meet the specific issue.
For air treatment, a specific monitoring protocol has been developed for healthcare facilities and process water test have been performed. In addition to these, a wireless technology was extensively applied, meeting the need for a low-cost, real-time and multi-parameter monitoring solution. Statistical consistency of data collected was considered as supplying for relative accuracy of the sensors.
For wastewater treatment, in the other hand, key parameters have been tested on the basis of a defined schedule, with fast and easily manageable laboratory procedures.
Field scale applications presented, nevertheless, several monitoring issues related to the number of uncontrolled variables and disturbing events possibly occurring during the test period, affecting the data set with bias difficult to eliminate.

9.2. Performance evaluation

9.2.1. Air treatment application

The research activity focused, in particular, on the healthcare sector, where the manufacturing company (i.e. U-Earth Biotechnologies) was supported by Fresenius Medical Care.
The main pilot test proposed returned promising results, reported in Table 9.1.
As evident from data presented, results on Particulate Matter are fluctuating and not significant, since, in most cases and regardless of increase or decrease of number of particles detected, they remain in the range of possible counting errors of the specific technology (U-Monitor).
More consistent results have been obtained on bacterial count (except in one case, in presence of a single AIRcel unit), Formaldehyde and Volatile Organic Compounds (VOC).

Table 9.1 - Legenda

	Contaminant's concentration increase >200%
	Contaminant's concentration increase >100%
	Contaminant's concentration increase 20-100%
	Contaminant's concentration fluctuating ±20%
	Contaminant's concentration decrease 20-49%
	Contaminant's concentration decrease 50-79%
	Contaminant's concentration decrease 80-100%

	DIALYSIS ROOM A	DIALYSIS ROOM B	DIALYSIS ROOM C	DIALYSIS ROOM HBV	OR	ICU	ELECTRO-PHYSIOLOGY	LAB 2 - PILOT 1	LAB 5 - PILOT 1	CYTOLOGY - PILOT 2	SAMPLES PREPARATION - PILOT 2
N. BIOREACTORS	6(eq)	6(eq)	2	2	2	2	1	2	2	2	1
SIZE OF THE PILOT AREA (M^2)	211	221	60	56	50	80	35	20	30	35	15
N. OF PEOPLE/BEDS	25	29	7	7	4	10	2	3	3	3	1
ACTIVITY SCHEDULE	24h/6d + 8h/1d	24h/6d	24h/6d	24h/6d	12h/5d	24h/7d	8h/5d	8h/5d	8h/5d	8h/5d	8h/5d
VENTILATION (VOL/HOUR)	6	6	6	6	16	10	16	no	no	6	6
AIR FLOW - VENTILATION SYSTEM (M^3/H)	3798	3978	1080	1008	2400	2400	1680			630	270
AIR FLOW - AIRCEL SYSTEM (M3/H)	222	222	74	74	74	74	37	74	74	74	37
AIR FLOW RATIO	17,11	17,92	14,59	13,62	32,43	32,43	45,41			8,51	7,30
PILOT TIME (MONTHS)	10	10	10	10	3	3	3	3	3	6	6
BACTERIAL COUNT	41	87	87	92		50	(+)19				
FILAMENTOUS FUNGI & YEASTS	(+)230	67	75	72							
VOC	12	100*	100*	79	61	56	36	34	25	31	49
FORMALDEHYDE	96	97	94	63							
ODOROUS GASES					13	(+)98	(+)104	32	6	(+)247	(+)268
PM1					(+)30	23	31	(+)3	(+)12	(+)8	3
PM2.5					(+)30	51	56	(+)12	4	8	8
PM10	(+)336	(+)272	(+)115	(+)180							

Table 9.1: Summary of installation data and results obtained in the healthcare sector.

* final concentrations were below the detection limit of the apparatus, i.e. <0.001 mg/m^3

In anatomopathological laboratories, where two distinct pilot were installed and both Formaldehyde and VOC appeared as the contaminants of major concern, evidences gathered during the maintenance activities suggested that an additional remarkable issue was present and underestimated during the pilot design phase. Investigating the specific laboratory practice performed in the areas, a hydrocarbon-based compound was identified into the bioreactors: paraffin wax. Residual material, in fact, attributed to intermediate products from paraffin and wax degradation, accumulated into AIRcel bioreactors, as the capture rate resulted higher than the digestion capacity. This causing the clogging of water-plate holes and pump discharge tube, following the covering of the reacting surfaces, i.e. the cylindrical core and the inner tank surface, with a gel, most likely formed by intermediate metabolic products. In order to address the maintenance issue and better investigate the degradation dynamics within the bioreactors, samples of process water and residual sludge were analyzed through Gas Chromatography – Mass Spectrometry, GC-MS. Several Paraffin hydrocarbons were identified (i.e. Eicosane, Tetracosane, Pentacosane, Hexacosane and Octacosane), as well as fatty acids (i.e. Myristic acid, Palmitic Acid and Stearic acid) and unsaturated acids (Oleic acid). It is, then, possible to assess that AIRcel system captured a remarkable amount of Paraffin particles and organic material from the air and accumulated intermediate products from paraffin wax degradation, as demonstrated by the presence of compounds typically listed among degradation products of eicosane (Sakthipriya et al 2016). Nevertheless, it would have probably required longer recovery time and lower exposition for complete degradation of Paraffin.
For these reasons, it is possible to conclude that the system was likely undersized for the specific pilot area, but, with the implementation of a higher number of bioreactors, possibly upgraded to reduce the clogging effects, it may represent an interesting solution for pathological laboratories.

In order to try and answer to one of the most challenging issue for air treatment, i.e. odor containment, two major pilot applications have been performed, on waste and wastewater treatment plant.
Determining the effectiveness of a technology in this field proved to be particularly tricky, due to the complex nature of phenomena related to odor spreading and perception.
Emissions typically associated to wastewater treatment plants derive from organic volatile compounds released from wastewater itself or sludge produced by the treatment processes. The extremely delicate urban framework of the pilot test performed on this issue forced to an anticipated closing, leaving no space for further tuning of the experiment and required repletion of the different monitoring campaign in parallel with changing seasons.
The fifteen-months application in a waste treatment facility, on the other hand, returned quite promising results. In fact, both gaseous contaminants and particulate matter appear to be effectively captured and treated by the system, as demonstrated by stress test conditions provided by several units' malfunctions occurred during the trial period, during which the detected concentrations experienced peaks strictly related to system's failure and decreasing trends consequent to the maintenance works. The optimal cleaning schedule for the system is identified in 4-6 weeks, confirming the strong dependence of the performance from the recovery ability of the water system (in hospital environment application, in fact, the experienced optimal cleaning schedule was 10-12 weeks) related to the load of contaminants to be treated.
The smell impressions gathered by the staff and the absence of complaints from the residents around the plant confirmed a reduction of odor emissions at their source, limiting the fugitive contaminants, despite the working conditions of the venting inside the facility have not changed.
Comparing the overall results obtained with evaluation proposed by Mudliar et al. (2010) (Table 9.2), and considering different test conditions, i.e. in-line operation of the benchmark technologies against the open-air configuration of the technology proposed, it is possible to assess a similarity in behavior with similar technologies (i.e. biofilters, biotrickling filters and bioscrubbers) for low concentrations of VOCs and highly

soluble compounds (e.g. Formaldehyde). No specific data are available on slightly soluble VOCs, while medium abatement performances have been reported on high concentrations of VOCs (high in case of Formaldehyde, but unstable in case of composite contamination conditions).
Capital and operational costs are estimated as quite low, compared with benchmark technologies.
The bioprocess control could be ranked as more similar to bioscrubbers than other benchmark technologies, thanks to the presence of water as buffer and the periodical augmentation of the biomass.

Bioreactor Type	Target VOCs and Odours conc. g/m³	Treatment efficiency for					Pressure drop	Capital cost	Operational cost	Bioprocess control[a]
		Low conc. of VOCs /Odours	High conc. of VOCs /Odours	High water soluble VOCs	Low water insoluble VOCs	Fluctuating feed conditions				
Biofilter	<1	High	Low	High	Low	Low	Low	Low	Low	Low
Biotrickling filter	<0.5	High	Low	High	Low	Low	Low	Low	Low	Low
Bioscrubber	<5	High	High	High	Low	High	Very low	Medium	Medium	High
Membrane reactor	High	High	High	High	High	Need long-term evaluation	Need long-term evaluation	High	High	Need long-term evaluation

Table 9.2: Comparative performance evaluation of bioreactors for VOC and odor control (Mudliar et al. 2010).

9.2.2. Wastewater treatment application

Provided the limited experimental experience, in comparison with air treatment application, the system proposed proved to be an effective solution for silk manufacturing wastewater treatment, displaying a high adaptability of the proprietary biomass and scalability of the system itself (due to the modular design), even though the performance was strongly affected by environmental conditions and, most of all, water temperature. This should be considered in a possible full-scale implementation, in order to prevent winter-time failures of the wastewater treatment, which represent a typical drawback of biological treatment system.
In a cost-effective perspective, the implementation of this system appears to be suitable for several solutions to be tested in further development of the present study, such as:

- as a phase within the framework of a multi-stage treatment process, possibly after a pre-treatment phase of the industrial effluents, thus improving the biomass degrading performance;
- as independent and easily implementable wastewater technology for cottage-scale manufacturing or small communities.

As easily and locally applicable wastewater treatment technology, with the opportunity to recover clean water from evaporation, the system could contribute to closed loop use of water, within the same facility or in an integrated district perspective of industrial symbiosis.

10. Conclusions and outlook of the work

As proposed by Chalmers University of Technology, the core results of sustainable research (https://www.chalmers.se/en/areas-of-advance/production/research/sustainable-production/Pages/Our-recipe-for-sustainable-research.aspx) should deal with the novelty of an idea or technological resource, its measured impact, in terms of burdens and benefits for environment and humans, and the increased awareness achieved (Fig. 10.1).

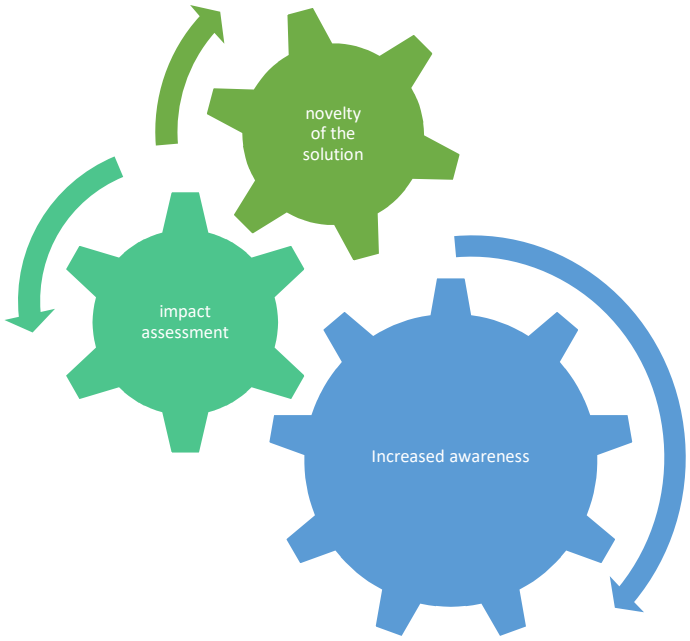


Fig. 10.1: Sustainable research (adapted from Chalmers University of Technology)

With the present study, an innovative biotechnology, based on immobilized-cell bioreactors, has been evaluated and tested in small field scale applications on air treatment in different environment, while a small-scale pilot plant has been implemented for wastewater treatment application. Air and water quality data have been collected to validate the remediation performance obtained with the application of the technology under study and, therefore, assess its positive environmental impact.

In parallel, a screening impact assessment has been accomplished, to identify major negative impact related to manufacturing and use phase of the bioreactors. The balance between environmental burdens and benefit, together with the many issue faced in setting up pilots and monitoring results, helped raising the awareness toward upcoming environmental concerns (e.g. Indoor Air Quality, wastewater management in small scale plants, remediation waste disposal etc.) and technological and cultural limitations to be overcome.

For air treatment application, for example, the innovative open-air configuration of the bioreactors, i.e. without standard air ducting and in-line filtration, offered ground to deep debates with air treatment professionals, proving that a cultural gap exists and ought to be bridged by experience and documented successful installations.

The opportunity offered for recovery of evaporated water is, on the other hand, one of the main design element to be promoted for the scale-up of wastewater treatment application. Thus, improving the overall environmental performance of the process and silk manufacturing in particular, which is largely spread in developing countries both in industrial and rural context (“cottage” production), where sustainable water

management is strategic to ensure access to clean water resource (Soulivanh 2007). In rural area, in particular, a sustainable wastewater treatment and possible cascade water use cycle should focus and take advantage on nutrients recovery (e.g. Phosphorous).

Together with an improved energy efficiency and supply, e.g. with solar generation, and larger access to the biomass to be inoculated (i.e. with the implementation of different and easily accessible bacteria consortia, as activated sludge), water recovery may candidate the biotechnology object of the present study as an appropriated technology for wastewater treatment. By the application of selected eco-designed tools, in fact, the bioreactor would represent a “decentralized, labor-intensive, energy-efficient, environmentally sound, and locally autonomous” technological solution, thus defining it as an appropriate technology (Hazeltine et al. 1999).

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