Study of the effects of essential oils on microbes present in ventilation systems

M.-C. Pibiri[a,*], C. Seignez[b], C.-A. Roulet[a]

[a]*LESO-PB, Swiss Institute of Technology, EPFL, Lausanne, Switzerland;* [b]*LBE, Swiss Federal Institute of Technology EPFL, Lausanne, Switzerland*

ABSTRACT
Many studies have shown that ventilation systems often host bacteria and fungi that may be dangerous for the health of people exposed to them. This problem may be particularly acute in hospitals, where exposed people are more sensitive. The paper presents the methodology developed to study the effectiveness of some plant's extract, the essential oils to hinder microbial growth, in order to propose an indoor air purification method based on the germicidal and odorant properties of the essential oils. First results are encouraging.

INDEX TERMS
Indoor air; Ventilation systems; Airborne microorganisms; Essential oils

INTRODUCTION
Many studies (Burge, 1995; Flückiger, 1999; Bluyssen *et al.*, 2002) have shown that numerous germs, bacteria, fungi and moulds are hosted in air ducts and dispatch in the whole building envelope through air circulation. These airborne microorganisms can have negative effects on the health of exposed occupants. This problem may be particularly acute in hospitals, where exposed people are more sensitive to these forms of microbiological contamination (Bardana and Anthony, 1996).

Extracted from different parts of plants, essential oils have interesting characteristics: antiseptic, bactericidal, fungicidal and antiviral among others (Franchomme *et al.*, 1990). Several essential oils inhibit certain metabolic functions of microorganisms, such as growth and multiplication (Hermal, 1993; Dusart, 1998). They have been well studied for therapeutic uses and their tolerances are well described in the literature. Allergic reaction or toxic effects are mostly due to improper usage like too high doses in concentration or too long uses in time. These side effects happen mostly through skin contact or ingestion (Franchomme *et al.*, 1990). Essential oils have been used traditionally in religious and therapeutic ceremonies. They are still considered as rare and precious raw materials in the perfumery industry. They are mostly well accepted, since their 'nature-like' odours can be mixed to diffuse pleasant and discrete smells.

The final aim of the project is to propose an indoor air purification method based on the antimicrobial and odorant properties of the selected essential oils. This method should help buildings' occupants and should not harm the environment.

METHOD
The research on essential oils is very much centred on medical and pharmaceutical applications. Methods described to show their activity are mainly based on liquid phase contact. Air applications through the use of the vapour phase of essential oils have been

* Corresponding author.

commonly used, but scientific studies are seldom done. This issue has been already studied twice (Sarbach, 1962; de Billerbeck, 2000). In his thesis, Sarbach had tested 54 essential oils on six bacteria with three different methods. The number of experiments was numerous and gives an exhaustive classification of the essential oil's activity for each method. Unfortunately, results cannot be compared because of the diversity of the protocols. Two methods were based on direct contact in a liquid or agar medium. The other one ('méthode des atmosphères') corresponds partially to our application. It is based on the vapour effect through diffusion of the essential oil in the volume of a Petri dish. This method is very useful for a first screening to select active essential oils, and to have a first idea of the effect of an essential oil on a specific microorganism. It is not suitable for our application because the experimental conditions vary much from those found in ventilation systems: the volume of air is extremely low and the bacteria are lying on a nutrient medium.

de Billerbeck's (2000) work is based more on the effect of the volatile phases of the essentials oils. The microorganism observed is a fungus, *Aspergillus niger*, that is responsible for manuscript and paint degradation. The objective of her work was 'the conservation, seeing the disinfections of museums and library's archives'… Therefore, she tested the same three methods and then developed two bioreactors of 2 and 16 l. This work shows the effect of the essential oil she chose, C*ymbopogon nardus*, commonly called citronella or lemon balm. She observed by light microscopy, scanning electron microscopy and transmission electron microscopy morphological alterations of treated samples. Unfortunately, 'among all the tests with the vapour phase no correlation between the quantity of essential oil settled and the volume of the Petri dish or the bioreactors could be established'. She concludes that 'the higher activity of the volatile components of the essential oils tested in vapour phase rather than in liquid phase indicates that the diffusion of these components is better in vapour phase. This statement justifies the use of theses products for clean air processes'.

With these results in mind, we decided to find a methodology more adapted to our problem in the ventilation systems.

PRELIMINARY ATTEMPTS

Inspired by previous work, we still needed to make several choices:

1. to define representative microorganisms;
2. to develop an effective method to quantify the effect of the essential oils;
3. to select efficient essential oils.

First, we had to define which microorganisms we intend to observe: airborne microorganisms sampled in ventilation systems or standard strain collections of bacteria or fungus? Air samples were chosen for our experiments.

Among the several existing sampling methods (centrifugation, liquid impinge, electroprecipitation, air filtration), we have used an air impactor on Petri dishes, the MAS 100 Eco from MBV Switzerland (Figure 1), in several air-handling units.

We observed a wide and diversified variety of microorganisms, with the constraint to define them in order to count their colonies after incubation and contact with the essential oils ('méthode des athmosphères' described in Sarbach and de Billerbeck). Since collecting air samples inside ventilation systems is not very convenient and did not give satisfying results (too many different germs), we envisaged two settings shown in Figure 2, in order to better control the process and the strains.

Figure 1 The air impactor used.

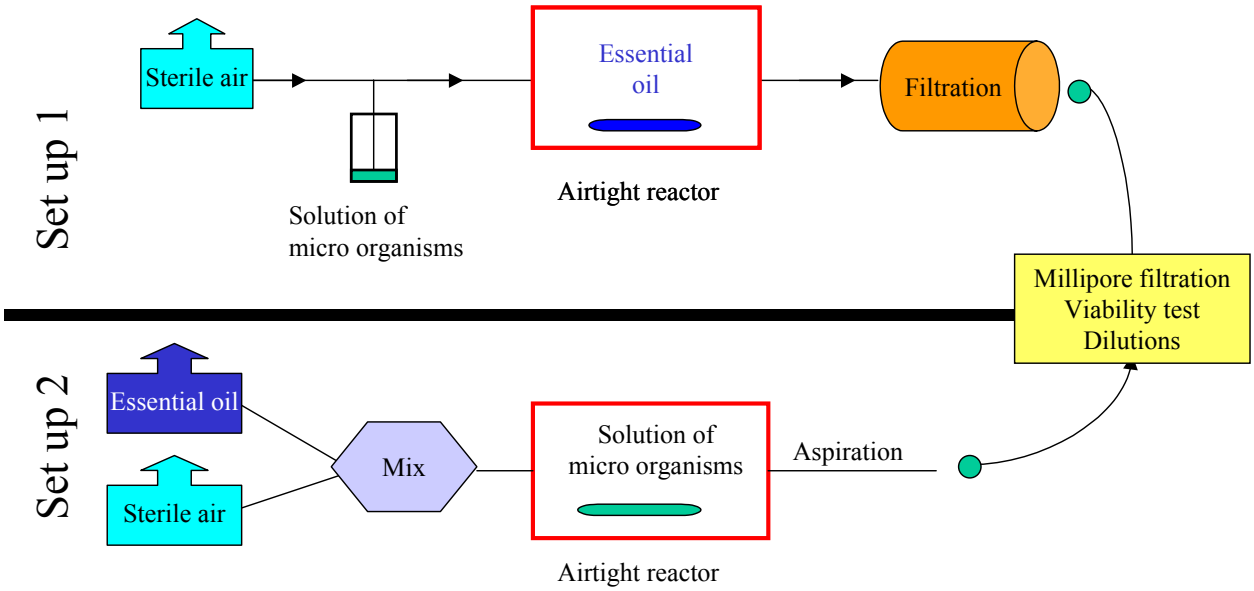

Figure 2 Experimental set-up.

In set-up 1, sterile air is charged by bubbling in a solution of the defined microorganisms before transfer in a watertight reactor containing vapours of the essential oil. Sampling is done by air filtration. In set-up 2, the air mixed with vapours of the essential oil, is ducted trough a reactor containing a solution of microorganisms. Sampling in done by aspiration. Three enumeration techniques are possible:

1. the number of colonies can be counted on a Millipore filter;
2. colonies can be determined according to the dilutions method;
3. microorganisms can also be directly determined by viability measurements technique.

We choose the third method for the precision of the results. In order to demonstrate the effect of the essential oils on microorganisms, we should be able to differentiate the active entities from the dead or inactivated ones; a total count is not informative enough. The Backlight (Molecular probe…) coloration technique gives us this information. The reactive method

colours differently bacteria with damaged membrane structure and the bacteria with undamaged structures. Count is performed using an epifluorescence microscope. We tested three different bacteria in broth solutions, before and after they have been in contact with a strongly antibacterial essential oil, *Cinnamonum zeylanicum*, or cinnamon (Sarbach, 1962; Billerbeck, 2000).

We observed a lower proportion of viable bacteria in the solution treated with the cinnamon. This method was nevertheless abandoned because of the difficulty to enumerate the bacteria and get reproducible results. Some bacteria like *Micrococcus pyrogenes* stand in aggregates making it difficult to count and others like *Pseudomonas putida* showed unstable coloration during the observation time.

RESULTS

Even if the different methodologies are insufficiently adapted to our application, we were able to see qualitatively the effect of essential oils. First, the vapour phase of essential oils has an inhibition effect on growth of colonies of microorganisms found in ventilation system, as shown in Figure 3.

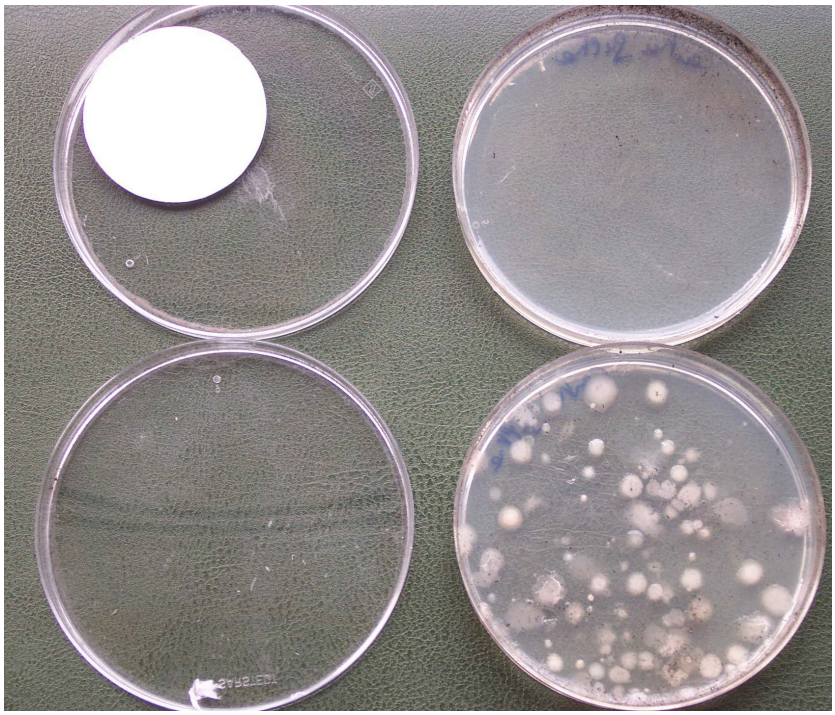

Figure 3 Qualitative effects of essential oils observed on microorganisms. 'Méthode des atmosphères': effect of *Aniba rosaedora*, or rose wood oil on microbes found in a ventilation system. Left: with, right: without the presence of impregnated filter placed on the lid of the Petri dish.

Second, we observed different activities of the selected essential oils, as shown in Figure 4. This contact method is called the Aromatogram technique (an essential oil version of the traditional antibiograms). Results of several tests are shown in Table 1. Savory, origano and thyme showed the highest activities and will be selected for further work.

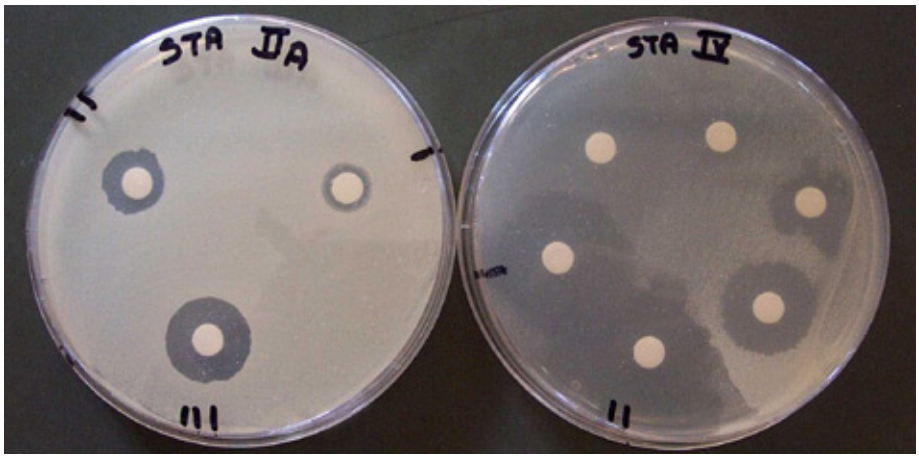

Figure 4 Qualitative effects of several essential oils observed on a strain of *Staphylococcus aureus* by diffusion of oil on agar medium in a Petri dish.

Table 1 Aromatograms: different inhibition diameters observed on a strain of *Staphylococcus aureus* in contact with 2 µl of several essential oils dropped on 6 mm diameter discs

Essential oil (Latin denomination)	Cinnamon (*Cinnamonum zeylanicanum*)	Clove (*Eugenia caryophyllata*)	Savory (*Satureja montana*)	Origano (*Origanum compactum*)	Thyme (*Thymus vulgaris*)
Inhibition diameter (cm)	2.4	2.1	7.3	7.5	7.6

FUTHER WORK

We decided to work with well-known strains from airborne microorganisms to have quantitative results also. We have chosen bacteria to start with. For this study we were inspired by the methods used to asses the efficiency of disinfectants in hospital areas. Bactericidal effect corresponds to the first level of disinfections according to the European standard EN 1040 (CEN, 1997). We selected two test strains recommended by this standard: *Staphylococcus aureus* (German and American collection numbers DMS 799 and ATCC 6538, respectively) and *Pseudomonas aeruginosa* (DMS 939 and ATCC 15442). However, in this standard procedure the tests are performed in the liquid phase. As we want to demonstrate the destructive effect in the gaseous phase, we will apply another French Standard NF T72-281 (AFNOR, 1986) adapted to gaseous disinfections processes.

The reactor for these experiments consists of an airtight and sterilized polycarbonate box filled with a controlled mix of air and essential oil vapour. Bacteria are immobilized on watch glasses and exposed during different times in the reactor. Temperature and relative humidity inside the reactor can be varied.

Once we have show the effect of the essential oil and oil mixes following the protocol of NF T72-281 (AFNOR, 1986), it is intended to expose the two microbial strains to various concentrations of several essential oil and oil mixes, using either the experimental set-up 1 or 2. This should allow us to select efficient oil mixes and determine their critical concentration.

In a second step, onsite experiments will be performed in several ventilation systems to validate the method.

CONCLUSIONS AND IMPLICATIONS

If the germicide effect of essential oils by direct contact in solid or liquid medium is rather well known, the effects of the vapour phase was determined only on a fungus, *Aspergillus niger*. We designed experimental set-ups and choose a procedure and standard strains to assess the effect of essential oil and oil mixes on Gram-positive and Gram-negative bacteria. First tests are encouraging.

ACKNOWLEDGEMENTS

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