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Bactericidal Technologies for Clean Rooms and Indoor Air Quality Applications

by

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Bio burden is *the* most important contamination control parameter for pharmaceutical and biotechnology clean rooms. Additionally, in the general indoor air environment many sick building syndromes are attributable to microbial contamination. This article reviews the important technologies related to control of bacteria in indoor air. The scope is restricted to anti bacterial technologies for the reduction of airborne bacteria and thus involves aerobiology and bio-aerosols.

Although there are specific FDA requirements and guidelines for bio burden in various classes of clean rooms and for various pharmaceutical operations and processes, one of the main obstacles in achieving the required bio burden levels, is that the measurement of bio burden is time consuming. Typically, bio burden measurement involves sampling, incubation and counting of colonies (colony forming units - CFUs). Recently, however, UV fluorescence (cf. Seaver and Eversole [1] Pinnick et al. [2]) technology has made it possible to achieve real time monitoring of particles of biological origin. Typically lasers with wave lengths of between 240 - 550 nm are used for this purpose. Pinnick et al. [2] used a pulsed laser system at 266 nm. This research work is based on the fact that several primary compounds in cells, such as amino acids tryptophan (350 nm) and tyrosine (300 nm), reduced nicotinamide adenine dinnucleotides (NADH - 450 nm) and flavins (540 nm) exhibit UV fluorescence. This makes it possible for instruments based on this technology to distinguish between non biological and biological particles and simultaneously to measure the particle size. More recent work shows that this technology has the potential to distinguish between at least some type of bacteria.

One commercial instrument (TSI's (St. Paul, MN) FLAP2 - 1997 R&D100 award winner) is already available. TSI claims that this instrument measures "the intrinsic fluorescence of particles containing living organisms", and therefore is able to distinguish between particles containing viable organisms and other non viable particles. While this technology may not directly measure the bio burden (in terms of colony forming units (CFUs) per unit volume of air), it will find increasing use in the real time monitoring of air in hospitals, clean rooms and military nuclear, biological and chemical (NBC) warfare protection systems - as a real time supplement to the standard methods of determining bio burden. *As this happens more attention will be focused on clean room contamination control systems - currently mainly mechanical filtration.*

Mechanical Filtration

This is *the* predominant technology currently in use in terms of controlling air borne particles - whether biological or not - in clean rooms and indoor air quality applications. Mechanical HEPA filters form the mainstay of clean room contamination control systems. Since bacteria are much larger than the sub micrometer size particles that are

well controlled by HEPA filters (these are at least 99.97% efficient at 0.3 Um), they are easily removed by the clean room HEPA filters. Virus particles on the other hand can be much smaller - as small as 2 nm. Although it is reasonable to expect that most viruses will be present as aggregates or will be attached to other particles, a small fraction may be expected to be in the fundamental state - as a single virus. Depending on the virus, this may be dangerous or detrimental to the product. Most viruses are not expected to survive for long in the absence of moisture. However, some studies show that the common cold virus (*rhinovirus)* may survive for as long as 2-4 hours in normally low humidity winter indoor environments. Dr. Elliot Dick's (cf. [3])work at the University of Wisconsin has shown that the primary transmission of the common cold (in adults) occurs through air - and not through direct contact - including kissing!

One problem with mechanical filters is that under certain conditions common bacteria caught on the filter can start growing on the filters, grow through the media and start shedding into the room. The well known case of the *Legionnaire's* outbreak at the veterans convention in Philadelphia has been attributed to this phenomena. In that case the filters were supposedly in a wet state. Generally, it is accepted that bacteria is difficult to grow on clean glass fiber filter media, used in HEPA filters, under normal humidity conditions. Many filtration engineers claim that bacteria growth is not a problem "unless bacteria bring with them their own food". However, since the function of these filters is to capture all particulate contamination, filters eventually get dirty. Additionally bacteria and other microbes are often attached to other dirt particles. This dirt becomes food for the bacteria. Consequently even in normal environments bacteria can survive or grow on the filters. As the trend towards using HEPA clean room filters for longer periods (based on pressure drop constraints) continues, the possibility of bacterial growth on the filter, and thus the rise in the air bio burden, also increases. The following experiments conducted by Jaisinghani et al. [4] show that very little contaminant is needed for growth of *Staphylococcus epidermidis* and *Escherichia coli* on HEPA glass filters.

Clean 6"x6" x2" deep glass mini pleat (with ribbon separators) filters were subjected to *E. coli* aerosol. Following this the air flow (without the aerosol) was continued for 4 hours. The air temperature was maintained around 70^0 +/- 5^0 F with a relative humidty (RH) at 50% +/- 5%.Then the filters were cut up, bacteria extracted, plated on growth medium and then incubated for 24 hours. Very little of the *E. coli* survived on the clean glass filter, keeping in mind that *E. coli* is not a hardy organism. Next about 1 gm of colloidal kaolin was added to the *E. coli* solution that was to be aerosolized. This time the recovery of *E. coli* was about 10^4 - 10^5 CFU/square inch of the filter media. Similar tests with *S. epidermidis* recovered more *S. epidermidis* than with *E. coli* even without the colloidal kaolin, due to the more hardy nature of *S. epidermidis.* With 1 gm of colloidal kaolin in the 25 ml *S. epidermidis* solution (in tryptic soy broth) the recovery of *S. epidermidis* was about 10^5 - 10^6 CFU/square inch of filter media. Tests with air flow continued for 7 hours did not result in any significant reduction in bacteria recovery. Keeping in mind that the dirt holding capacity of a filter of this size (to a point such that the filter pressure drop increases to double the initial value) is about 40 g, *clearly very little (1 g colloidal kaolin) of contaminant is necessary for sufficient growth or*

survivability of bacteria. This clearly illustrates that we should expect common bacteria to survive and grow on glass HEPA filters, even under normal temperature and RH conditions. Thus these filters, instead of being contamination control devices, can become the cause of the problem!

In order to alleviate this some HEPA filter manufacturers offer filter media treated with biocides. These chemicals can be effective in killing many common indoor microorganisms - provided they are in contact with the filter (Hoenig et al. [5] and Rhodes et al. [6]). Once a layer of contaminant builds up on the filter, the bactericides are usually ineffective (Tolliver [7] and Hoenig et al. [5]). Another concern regarding biocide impregnated filters is that many of the biocides (especially fungicides) are carcinogenic and thus filters need to be handled with care. Although most of the chemicals used have low vapor pressure, there may be some concern with some types of biocides stripping off into the air flow.

Ionizing Electrically Enhanced Filtration (EEF)

Jaisinghani [8,9,10] has played a significant role in the commercialization of EEF technology. The most recent version (see Figure 1) of this technology maintains the filter under an *ionizing* (as opposed to a simple electrostatic field) field. Another higher intensity ionizing field charges incoming particles, stabilizes the electrical fields and increases the safety and reliability by ensuring that no spark over can occur towards the filter. This method (1997 R&D 100 award winner) provides two fundamental benefits:

1. *Bacteria are killed as they pass through a first high intensity ionizing field and then killed as they are subjected to continuous ionizing radiation when they are trapped on the filter. This inhibits growth of bacteria on the filter.*
2. *The same ionizing fields enable the filtration performance(penetration reduction) to increase by about three orders of magnitude.*

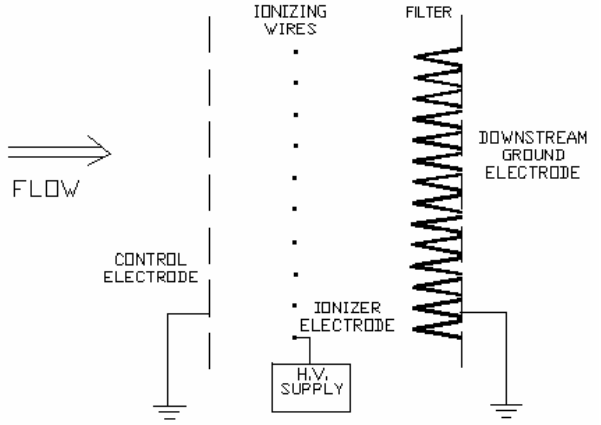
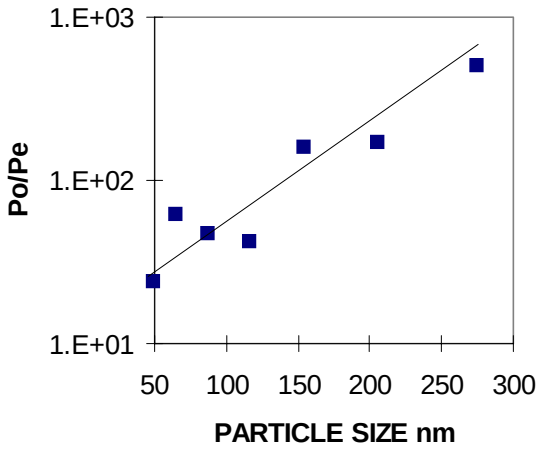


Figure 1 - Technovation's Ionizing EEF Technology

Since the cost of the additional electrical components is partially offset by the increase in filtration performance (either higher flow at the same pressure drop and filtration efficiency or lower pressure drop at the same flow and efficiency, as compared to mechanical filtration of the same size) this is a highly cost effective way to achieve a higher level of bio burden control. Figure 2 shows the ratio of the fractional filter penetration (for various particle sizes in nanometers i.e. a thousandth of a micrometer) without electrical enhancement, P_0, to the penetration of the same filter, with electrical enhancement, P_e. [Penetration is 1 - fractional efficiency, i.e. the fraction of particles not captured by the filter.] The higher the

penetration ratio, P_0/P_e, the higher is the effectiveness of the EEF technology. The ionizing EEF achieves about three orders of magnitude improvement, at 0.3 Um, in filtration performance at a very high flow velocity (about 600 fpm). A commercial air handler unit based on this ionizing EEF technology is currently being marketed by Technovation, Midlothian, VA.



The bactericidal properties of the Technovation's EEF were evaluated under laboratory and field conditions. Laboratory tests similar to the ones described above (Mechanical Filtration) were conducted with *S. epidermidis*. The results (Table I) show that the EEF killed almost 100% of the bacteria as compared to the control tests (without electrical enhancement). The viable *S. epidermidis* were reduced from about one million CFU/square inch of filter media, to almost zero! Similar results were obtained with *E. coli.* For more details refer to Jaisinghani [11].

Figure 3 - Filtration Improvement due to the EEF

Table I - EEF Bactericidal Test Summary using *S. epidermidis*

FILTER	INCUBATION TIME	EEF EXPOSURE TIME	EEF FIELD STRENGTH	AVERAGE COLONIES	COMMENT
control or EEF	hours	hours	(v/d1) kv/cm	#/sq inch	
control	24.00	0.00	0.00	1.00E+06	No Additional Growth
control	24.00	0.00	0.00	1.02E+05	After 24 Hours
EEF	24.00	4.00	4.64	0.00E+00	100% KILLED
EEF	24.00	4.00	3.99	3.44E+02	99.93% KILLED
EEF	24.00	4.00	4.24	0.00E+00	100% KILLED

A field study related to the efficacy of the EEF, with respect to conventional filtration was conducted by one of the users (Encelle) of Technovation's EEF system. A small laboratory, previously equipped with four conventional HEPA fan filter units, was equipped with one Technovation Model 1001 EEF HEPA, resulting in about the same total flow rate. The bio burden was reported as a complex weighted variable - the

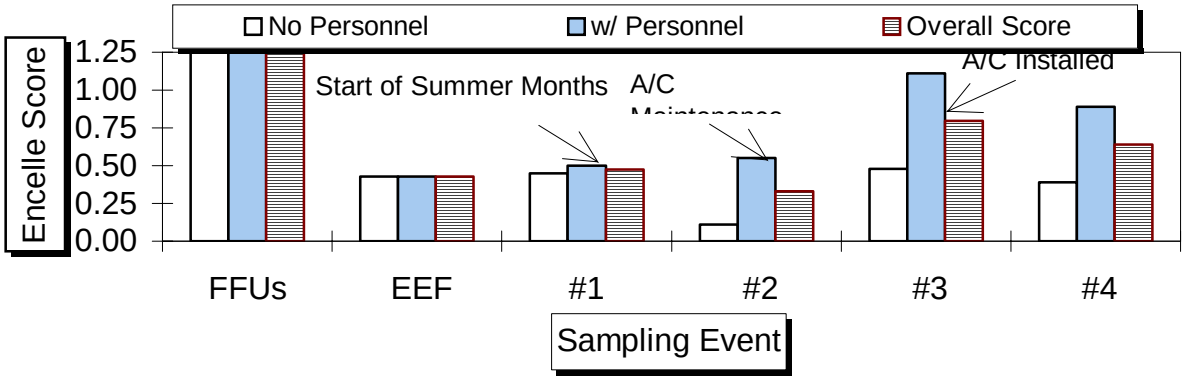


Figure 4 - Encelle Air Quality (Bio Burden) Results

Encelle score. For details refer to Jaisinghani [11]]. The results (Figure 4) show that, on an average, as a result of the EEF, the Encelle Score (bio burden) decreased by about 60%, as compared to conventional HEPA filters.

UV Radiation

UV radiation is commonly used for sterilization applications. Recently, this technology has been incorporated into fan powered systems for HVAC applications (UV Technologies Inc.,Greenwich, CT). In combination with filtration, Sarpino and Jensen [12] have shown that this technology can achieve inactivation/kill efficiencies in the high 90% range. The UV radiation requires a minimum residence time, and therefore the equipment is larger compared to the EEF equipment. Additionally, the cost of the UV lamps cannot be offset by other advantages, such as improved filtration performance as in the case of the EEF. Although, not as cost effective as the EEF, this technology should find application in critical medical and other specialized applications.

Another form of UV technology is the use of UV lamps in quarantine and other infectious disease control rooms. Miller-Leiden et al. [13] have studied the effectiveness of UV lamps in rooms. In order for the UV radiation to not be detrimental to occupants in the room, the UV lamps need to be ceiling or wall mounted with some sort of shielding such that the radiation at the personnel level is with safe limits. This limitation significantly lowers the potential of this technology. In time based tests Miller-Leiden et al.[13] achieved 30-100% reductions with *Micrococcus luteus, Bacillus subtilis* and *E. coli.*

In summary recent research has resulted in improved methods for detection and control of biological aerosols. This should in turn result in better specification, monitoring and control of bio burden in clean rooms, safer medical facilities and healthier indoor environments.

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