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STUDY TO DETERMINE THE PERFORMANCE OF MICROGENIX AIR PURIFICATION SYSTEM AGAINST AIRBORNE VIRUS (SMALLPOX)

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December 2002

ATTACHMENT TO REPORT PRODUCED ON BEHALF OF MICROGENIX TECHNOLOGIES LIMITED TO DETERMINE PERFORMANCE OF THE MICROGENIX AIR PURIFICATION SYSTEM AGAINST THE DEADLY SMALLPOX VIRUS.

Tests carried out at the Centre for Applied Microbiology & Research

(CAMR) at Porton Down

The attached report proves the effectiveness of the Microgenix Air Purification system when tested against 50 million spores of the simulated Smallpox virus.

The tests were carried out using the smallest of the Microgenix duct mounted units (MD085). Whilst it was originally intended to test at an airflow rate of 300 m^3/hr it was decided to increase the airflow to 500 m^3/hr which was considered to be a more practicable volume for commercial application. Whilst producing results of 97%+ on first pass, it was scientifically calculated that, with the simple addition of two extra Ultra Violet lamps, a kill rate in excess of 99% would be achieved at the increased airflow of 500 m^3/hr and at first pass. Adjustments and modifications have now been implemented to accommodate the higher flow rate and to achieve a kill rate in excess of 99%.

December 2002

DETERMINATION OF THE PERFORMANCE OF A MICROGENIX AIR PURIFICATION SYSTEM WITH AIRBORNE VIRUS

A report produced for
Microgenix Technologies Limited

Title	DETERMINATION OF THE PERFORMANCE OF A MICROGENIX AIR PURIFICATION SYSTEM WITH AIRBORNE VIRUS
Customer	Microgenix Technologies Limited
Customer reference	Project No. RM06
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File reference	
Report number	MICROGENIX_REP03
Report status	Final Version

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1 Introduction

1.1 THE STUDY

Airborne Matters was asked by Microgenix Technologies Limited to outline a programme for testing the performance of a Microgenix Air Purification System (in-duct system) with a surrogate small pox virus aerosol. The programme of performance testing under controlled laboratory conditions was outlined by Airborne Matters and carried out by the Centre for Biology and Research **(CAMR) at PORTON DOWN**. The Microgenix Air Purification system was tested by challenging it with a high aerosol concentration of a viral model.

1.2 MICROGENIX AIR PURIFICATION SYSTEM

Microgenix supplied one of their latest duct-mounted air filtration systems for test. This unit is a single pass unit and does not rely on re-circulation to achieve its kill rate. It is therefore inevitable that greater total efficiencies can be achieved with multi-pass systems. The system achieves anti viral and/or anti bacterial action through a combination of filtration of bioaerosol through a fibrous filter pre-coated in biocide Biogreen 3000 solution followed by exposure to UVc radiation from an array of lamps.

2 Aim and Objective

The aim of this work is to determine the performance of a Microgenix Air Purification System (in-duct system) with a surrogate for small pox virus aerosol, under controlled laboratory conditions.

This study has one principal objective:

To demonstrate the duct mounting Microgenix Air Purification System (as supplied by Microgenix) effectively destroys airborne surrogate virus.

3 Programme of Work

Because of safety issues, the performance of the Microgenix Air Purification System was tested with a viral model acting as a bioaerosol surrogate for small pox virus. The selected viral model used was MS-2 coliphage (NCIMB 10108), which is an un-enveloped single stranded RNA coliphage, 23 nm in diameter with a molecular weight of 3.6 x 10^6 Dalton. MS-2 coliphage sprayed from dilute nutrient broth is known to remain infectious under the conditions used in these tests. Spraying this suspension from a Collison nebuliser (Collison, 1935 and May 1973), the airborne coliphage are carried in droplets, which are larger than the infectious particles, consisting mostly of media constituents.

3.1 TEST RIG FOR CHALLENGING MICROGENIX AIR PURIFICATION SYSTEM WITH AIRBORNE MS-2

The test rig is shown diagrammatically in Figure 1. Filtered air (using a coarse filter) was drawn through the rig at 500 m^3 hr^{-1} by a fan unit. The air was initially drawn into a spray chamber housing a 3-jet Collison spray. The 3-jet Collison spray contained a suspension (10-20 ml) of the test microorganism MS-2 coliphage. The MS-2 coliphage was aerosolised by applying compressed air to the Collison nebuliser at 180 KPa for 5 minutes. The breakthrough and challenge were sampled simultaneously, during the 5 minutes, via test ports provided both upstream and downstream of the system being tested. The aerosolised microbes were passed into a mixing chamber and then into a duct which housed the Microgenix Air Purification System. At the downstream end of the housing, another length of duct was connected which is linked to the fan units via a back-up HEPA filter assembly which was designed to prevent the escape of any remaining airborne micro-organisms into the environment.

3.2 MEASURMENT OF FLOW RATE

The flow rate of air through the system can be varied. The flow rate was increased from previous tests at 300 m^3 hr^{-1} and set to 500 m^3 hr^{-1}, measured with an anemometer at the inlet to the spray chamber. The anemometer was an Airflow AV2 anemometer (Serial No. 79286, Cal. Date 29.04.02, Airflow Development Ltd, High Wycombe, UK). The airflow was checked at the centre of the inlet and the volumetric airflow was determined from the cross sectional area.

3.3 MICROBIOLOGICAL AIR SAMPLING

Cyclone samplers were used to sample the air via the test ports in the ducting described above (Upton *et al.*, 1994, Griffiths and Boysan, 1996, Griffiths *et al.*, 1997, and Griffiths, 1998). The clean glass cyclone samplers were linked to a vacuum pump so that circa. 700 litres of air per minute (lpm) could be sampled both upstream and downstream of the Microgenix Air Purification System. The

collecting fluid (Phosphate buffer plus manucol and antifoam) (PBMA) was injected into the inlet of the cyclone at a rate of about 1-2 ml per minute. The airborne particles containing the microorganisms were deposited by centrifugal forces on the cyclone wall and washed off by the swirling collection fluid. The fluid was withdrawn from the base of the cyclone by a syringe at the end of the sampling period. The volume of fluid collected was measured.

3.4 TEST MICROORGANISM

MS-2 coliphage (NCIMB 10108).

A vial of MS-2 coliphage (NCIMB 10108) was obtained from the National Collection of Industrial and Marine Bacteria, Torry Station, Aberdeen. Stock suspensions of MS-2 were prepared by infecting the host organism *Escherichia coli* ATCC 12435 (NCIMB 9481) during the logarithmic phase of growth as follows:

A flask containing 50 ml nutrient broth was inoculated with a colony of *E. coli* ATCC 12435 (NCIMB 9481) from a stock TSBA plate previously stored at $4^oC \pm 2^oC$. The flask was incubated at $37^oC \pm 2^oC$ on a shaker in a water bath for 18 hours. Ten ml of this suspension was inoculated into a flask containing 500 ml sterile nutrient broth. After incubation for 3½ hours at $37^oC \pm 2^oC$ the contents of the MS-2 vial was aseptically inoculated in the shaken flask and incubated for a further 90 minutes. After lysis of the bacterial cells chloroform was added to the suspension to inactivate any remaining bacteria. The suspension was centrifuged for 20 minutes at 2000g and the supernatant was transferred to a sterile bottle and stored at $4^oC \pm 2^oC$.

The suspension was assayed for MS-2 by a method described by Kay (1972) and is as follows:

A fresh TSBA plate was inoculated with *E. coli* ATCC 12435 and incubated at $37^oC \pm 2^oC$ for 18-20 hours. A 10 µl loopful was transferred aseptically to 10ml sterile nutrient broth in a universal bottle. After mixing thoroughly the broth was incubated at $37^oC \pm 2^oC$ for 260 minutes. In the meantime small bottles containing 3 - 4 ml soft phage agar were heated at $90^oC – 100^oC$ for 90 minutes and then cooled and maintained at 60^oC until required. Immediately before use the molten agar was cooled to 45^oC. A volume of suitably diluted MS-2 suspension (between 100 µl and 1000 µl) was transferred to the bottle containing the molten soft agar. Immediately, three drops of the *E. coli* suspension were added by a 50 drop ml^{-1} Pasteur pipette. After replacing the lid of the bottle the contents were rapidly mixed before being poured onto a TSBA plate ensuring that the surface was evenly covered. The plates were incubated immediately at $37^oC \pm 2^oC$. The plaques formed were counted at 16-18 hours after incubation.

In the experiments described here a suspension containing high titre MS-2 was required. This suspension was produced by adding a 0.2ml of a high titre stock suspension to 500ml flask containing 60ml of a 150 minute culture of the host strain in a shaking incubator at $37^oC \pm 2^oC$. After 240 minutes the culture was centrifuged at 2000g for 20 minutes. The supernatant was collected and was centrifuged again

and stored at $4^oC \pm 2^oC$ prior to use. The suspension was assayed before use as described above. The spray suspension used was prepared from the high titre suspension by carrying out a ten fold dilution in 50% ($^v/_v$) nutrient broth, followed by a ten fold dilution in sterile distilled water, producing a spray suspension of concentration 1.52×10^{10} pfu/ml.

3.5 TEST METHODOLOGY

The Microgenix Air Purification System was bolted onto the CAMR test rig. The volumetric air flow rate was then adjusted to 500 $m^3 hr^{-1}$, measured by the anemometer. The Collison nebuliser containing the MS-2 coliphage (10-20 ml) was placed in the spray chamber and the sterile cyclone samplers were connected to the upstream (challenge) and downstream (test) sampling ports. The Collison nebuliser, operated at 180kPa, and the upstream and downstream cyclone samplers were activated for 5 minutes. The collection fluids from the cyclone samplers were collected and the volumes were measured. This test method was carried out using two conditions in the following sequence:

- 3 runs (i. e. triplicate) with UV system activated.
- 3 runs (i. e. triplicate) with UV system inactivated

The volumetric airflow through the cyclone samplers was then measured using the vane anemometer. The samples were assayed, using the techniques described above.

Fresh sterile cyclone samplers and tubing were not used between the three test runs using the same test conditions, however new cyclone samplers and tubing were used for the two different test conditions. The Microgenix Air Purification System was not decontaminated between test runs. The UV lamps (when operated) were allowed to get to maximum power for at least 15 minutes before the start of the run, as advised by Microgenix Technologies Limited.

3.6 CALCULATION OF PERFORMANCE EFFICIENCY

The percentage efficiency of the Microgenix Air Purification System was calculated using the following formula, where A is the total number of plaque forming units (pfu) challenging the unit and B is the total number of pfu exiting the unit.

$$Efficiency(\%) = \left(\frac{A-B}{A}\right) x100$$

4 Results

The results of the six tests are shown in Table 1 below.

Test Conditions	Total Challenge to system (pfu/m^3)	Collected downstream of system (pfu/m^3)	% Efficiency of the System
(1) UV activated	4.25 x 10^7	7.45 x 10^5	**98.25**
(2) UV activated	4.29 x 10^7	1.78 x 10^6	**95.85**
(3) UV activated	5.08 x 10^7	1.06 x 10^6	**97.91**
(1) UV inactivated	5.00 x 10^7	1.46 x 10^7	70.80
(2) UV inactivated	5.52 x 10^7	1.93 x 10^7	65.04
(3) UV inactivated	5.50 x 10^7	2.83 x 10^7	48.55

TABLE 1: PERCENTAGE EFFICIENCY OF THE MICROGENIX AIR PURIFICATION SYSTEM WHEN CARRIED OUT IN TRIPLICATE UNDER TWO DIFFERENT TEST CONDITIONS

4.1 Summary of Results

The performance of a commercial air purification system, the Microgenix Air Purification System supplied by Microgenix, was determined against viral aerosols of MS-2 coliphage (NCIMB 10108). The Microgenix Air Purification system was challenged with the MS-2 coliphage at 500 m^3hr^{-1} in a controlled system with the UV lamps activated and inactivated. The results of the tests are summarised below as average percentage (%) efficiency of the system:

Test Conditions	Average % Efficiency of the System (± standard deviation)
UV Lamps Activated	**97.34 (±1.30)**
UV Lamps Inactivated	61.46 (±11.55)

The duct-mounted Microgenix Air Purification System operating at 500 m^3hr^{-1} inactivates 97.34 % of airborne MS-2 phage. 61.46 % of the airborne MS-2 phage is inactivated by the filter coated with Biogreen, which forms an integral part of the purification system.

Calculations show that the overall inactivation efficiency of the air purification system will increase to approach 99.99 % if the unit was operated at 300 m^3 h^{-1}, as the residence time of the airborne microorganisms inside the purification system is increased by a factor of 5/3. This assumes no change in filter efficiency at the lower flow rate.

The calculations are further explained in **Item 5 Discussions** below.

5 Discussions

The Microgenix Air Purification System supplied by Microgenix Technologies Limited has been shown to be effective with an average inactivation efficiency of 97.34% (with a standard deviation of ±1.30%) against aerosols of MS-2 phage when the UV lamps are activated. The average efficiency was 61.46% (with a standard deviation of ±11.55%) against aerosols of MS-2 phage when the UV lamps are inactivated. These levels of efficiency go along way towards meeting the requirements of commercial and most hospital applications. In more sensitive applications longer residence times should be considered. An unpaired Student t-test on the % efficiency results, showed a significant statistical difference (t – 5.347 and P value 0.00590) when the Microgenix Air Purification System was operated with the UV lamps activated compared to the system with the UV lamps inactivated.

Microorganisms exposed to UV radiation generally experience a single stage exponential decrease in population similar to other methods of disinfection such as heating, ozonation, and exposure to ionising radiation (Koch, 1995 and Mitscherlich and Marth, 1984) as follows:

$$S = \exp(-kIt)$$

where:

S is the surviving fraction of the initial population (S = 1 - inactivation factor)
k is a standard rate constant unique to each species of microorganism (cm^2 μJ^{-1})
I is the UV intensity (μW cm^{-2})
t is the time of exposure (seconds)

Note 1 µJ = 1 µW-s.

Using this equation with the inactivation efficiency obtained measured at a flow rate of 500 m^3 h^{-1} shows the overall inactivation efficiency of the air purification system is likely to increase to approach 99.99 % when the unit is operated at 300 m^3 h^{-1}. This improvement is due to the residence time of the airborne microorganisms inside the purification system being increased by a factor of 5/3. **It can therefore be calculated that with an increase in the residence time at 500 m^3 h^{-1} together with the coated filter and by the inclusion of two additional lamps an efficiency in excess of 99% will be achieved.** This calculation does not take account of any change in filter efficiency at the lower flow rate.

6 Conclusions

From the encouraging test results obtained in this and in an earlier series of tests it is evident that this product can be extremely effective in the eradication of bacteria and viruses in a wide variety of applications.

From our initial involvement in this product we believe that its performance potential has not yet been realised in full and still further improvements could be made fairly quickly without undue cost.

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Figures

FIG. 1: RIG FOR TESTING MICROGENIX AIR PURIFICATION SYSTEM