

Anti –bacterial and fungistat properties
of microfine zinc oxide

Literature data

1. Tests in Neurospora Crassa and Aspergillus Oryzea

The high surface area of nano zinc oxides maximizes its anti-bacterial and fungistat properties compared to regular zinc oxides and organic UV-absorbers.

The table below shows the growth inhibition results of microfine zinc oxide incorporated into nutrient containing agar. The test organisms are “Neurospora Crassa” (a red bread mould – a common food spoilage organism) and “Aspergillus Oryzae” (a soil organism).

Nano ZnO concentration	Colony diameter growth (mm) for Neurospora during 15 days	Colony diameter growth (mm) for Aspergillus during 15 days
Blank	60	60
0.25%	10	38
5 %	5	18

2. Anti-bacterial property test of microfine zinc oxides (halo test)

Test bacteria : Staphylococcus Aureus, Colibacilli, Salmonellae, Pneumobacillus and Pseudomonas aeruginosa

Culture medium : ordinary agar, ordinary broth

Test method : Each of the test bacteria was cultivated on an ordinary broth culture medium for 24 hours, and diluted to 100. 0.1 ml of the diluted bacteria liquid was planted on an ordinary agar culture medium. Each of the test materials was placed on the ordinary agar to which the bacteria were planted. After 48 hours of cultivation, the width of inhibitory halo observed around a test material was measured.

Ordinary agar :

Meat extract : 3 g
Peptone : 10 g
NaCl : 5 g
Agar : 15 gr

Ph = 7 1 litre

Ordinary broth

Meat extract : 3 g
Peptone : 10 g
NaCl : 5 g

pH = 7 1 litre

Table 2 : anti-bacterial property test with microfine zinc oxide added (unit : mm)

Bacteria type		Growth with microfine ZnO added
Gram positive bacteria	S. Aureus	0.9-1.4
	B. subtilis	6.7
Gram negative bacteria	E. Coli	1.6-2.0
	Salmonellae typh.	1.8-2.0
	K. pneumon.	1.6-2.4
	P. aerugi	*

* : although no inhibitory band was observed, bacteria did not grow on the contacting border

3. Minimum inhibitory concentration of microfine zinc oxide

	Staphylococcus aureus	Bacillus subtilis	Escheria coli	Salmonella tiphymurium	Klebsiella Pneumoniae	MRSA
Microfine zinc oxide	125	62	500	500	125	160*
Regular zinc oxide	1000	125	> 2000	>2000	500	Not measured

* shake flask method

Table 3 : minimum inhibitory concentration of microfine zinc oxides, compared to regular zinc oxide

4. Anti-bacterial property test of PET films applied with microfine zinc oxide

Test material

- film applied with microfine zinc oxide
- Base : PET film, application method : bar coater, thickness of application after drying : 4 microns

Application samples :

- Acrylic binder : (25% zinc oxide in solids ingredients)
- Acrylic binder : (75% zinc oxide in solids ingredients)
- PVC binder : (25% zinc oxide in solids ingredients)
- PVC binder : (50% zinc oxide in solids ingredients)

Test bacteria : Staphylococcus Aureus

Culture medium : ordinary agar, ordinary broth, and physiological buffer solution added with phosphoric acid

Test method : the bacteria solution was cultivated on an ordinary broth culture medium for 24 hours, and diluted with physiological buffer solution added with phosphoric acid to make test bacterial liquid. A strip of film applied with microfine zinc oxide (4 cm x 4 cm) was put in an Elken screw cup, to which 0.1 ml of the test bacterial liquid was added. Immediately after the addition, and after the 24 hours maintained at 37°C, it was rinsed with aseptic physiological saline and the number of live bacteria was measured.

Film	Number of live bacteria (cell/ml)	Rate of reduction (%)
Film without application of ZnO (0 hours)	1.55×10^6	
Film without application of ZnO (24 hours later)	1.96×10^6	
Acrylic 25% (24 hours later)	2.29×10^4	30.7%
Acrylic 75% (24 hours later)	1.02×10^2	68.1%
PVC 25% (24 hours later)	3.30×10^4	28.2%
PVC 75% (24 hours later)	5.10×10^2	57.0%

Rate of reduction (%) = (log number of live bacteria before comparison - log number of live bacteria in tested sample)/log number of live bacteria for comparison x 100
effective > 26%

6. Antifungal property test of microfine zinc oxide

Test bacteria : Asp. Nigger, Clad. Cladospo., Cheatomium sp., Trichoderma sp., Trichophyton ment., Candida albicans, Saccha. Serevisiae

Culture medium : potato dextrose agar + CM, Sabouraud's agar

Test method : the test bacteria was pre-cultivated on a PDA culture medium for 14 days, and spores were suspended in physiological saline added with phosphoric acid (0.02 % TWEEN 80) to make spore suspended liquid. The test material of a given concentration (1%, 2.5 %, 5% and 10%) was mixed to PDA (pr Sabouraud's agar) culture medium, and one loop of spore suspended liquid was inoculated to the culture medium. Candida albicans and Saccha. Serevisiae were observed 3 days later, Trichophyton ment. Was observed 7 days later, and Asp. Nigger, Clad. Cladospo., Cheatomium sp. And trichoderma Sp. were observed 14 days later.

Remarks :

- the figures indicate the results of the MIC (minimum inhibitory concentration) for reference (in ppm)
- Description of the codes : - : no growth of fungi observed, +/- : slight growth of fungi observed, + : growth of fungi observed

Results : antifungal property test of microfine zinc oxide (2.5% concentration)

- Asp. Nigger : +/- (> 2000)
- Clad. Cladospo : - (2000)
- Cheatomium sp : +/- (500)
- Trichoderma sp. : - (500)
- Trichophyton ment. : - (1000)
- Candida albicans : +/- (2000)
- Saccha. Serivisae : - (1000)

7. Deodorant property test of microfine zinc oxide

1. Deodorant property test with isovalerate

Test material : microfine zinc oxide, grains of activated carbon for comparison

Reagents and instruments : Odor bags, Isovalerate, Gas detecting tube

Test method : 1 gram of the test material was put in the odorbag (25 cm x 25 cm) and the bag was heat-sealed. Then, 1 liter of nitrogen based isovalerate (approx. 50 ppm) was inserted in the bag. The bag was left in the room temperature and the change of residual gas concentration within the bag with time was measured by the gas detecting tube. The test of a bag to which no test material was put was also conducted in the same procedures.

Table 6. Deodorant property test of microfine zinc oxide with isovalerate

Product/Time elapsed	5 minutes elapsed	30 minutes elapsed	60 minutes elapsed	180 minutes elapsed
Microfine zinc oxide	9 ppm	7 ppm	7 ppm	5 ppm
Bag with no test material	55 ppm	43 ppm	42 ppm	40 ppm
Grains of activated carbon	23 ppm	9 ppm	7 ppm	6 ppm

Residual gas concentration in ppm

2. Deodorant property test with ammonia

Test material : microfine zinc oxide, grains of activated carbon for comparison

Reagents and instruments : Odor bags, Isovalerate, Gas detecting tube

Test method : 1 gram of the test material was put in the odorbag (25 cm x 25 cm) and the bag was heat-sealed. Then, 1 liter of ammonia (approx. 50 ppm) was inserted in the bag. The bag was left in the room temperature and the change of residual gas concentration within the bag with time was measured by the gas detecting tube. The test of a bag to which no test material was put was also conducted in the same procedures.

Table 7. Deodorant property test of microfine zinc oxide with ammonia

Product/Time elapsed	1 hour elapsed	3 hour elapsed	6 hour elapsed	24 hour elapsed
Microfne zinc oxide	300 ppm	250 ppm	240 ppm	200 ppm
Bag with no test material	500 ppm	500 ppm	500 ppm	400 ppm

Residual gas concentration in ppm

8. Deodorant property test of films incorporated with microfine zinc oxide (elimination of the odor of isovalerate)

Test materials :

- film incorporated with microfine zinc oxide : LLD-PE (3% of microfine zinc oxide added)
- film not added with the test material : LLD-PE (without additive)

Reagents and instruments :

- odor bags
- isovalerate
- gas detecting tube

Test method :

Heat seal was applied to the test material to form it into a bag of 20 cm x 30 cm. Then 1 litre of nitrogen based isovalerate (approx. 50 ppm) was inserted into the bag. The bag was left in the room temperature and the change of the residual gas concentration within the bag with time was measured by the gas detecting tube. The test of an odor bag (20 cm x 30 cm) to which no test material was put was also conducted in the same procedures.

Results

	1 min	30 min	60 min	180 min	360 min
Bag with no test material	48 ppm	43 ppm	42 ppm	40 ppm	35 ppm
PE	23 ppm	17 ppm	10 ppm	5 ppm	2 ppm
PE + ZnO	12 ppm	9 ppm	5 ppm	3 ppm	2 ppm

Table 8. Residual gas concentration of isovalerate in time