Synthesis and Characterization of ZnO Film on Stainless Steel Wire and Bioaerosol Antimicrobial Activity Evaluation

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Abstract: ZnO thin films were prepared by electrophoretic deposition on stainless steel wire sieve, using zinc acetate as a precursor. The film was sintered and characterised by Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD) and chemisorption of oxygen. A culture bacterial consortium composed by gram negative rod-shaped microbes was prepared in a liquid agar in a flask. It was transported by air through a reactor equipped with a UV lamp with 3 and 5 sieves of a stainless steel wire coated with ZnO film. It was exposed in continuous in five experiments to photocatalytic advanced oxidation. The experiments showed a total efficiency for colony forming unit reduction of a maximum of 99.66% for a residence time of 20 seconds with 5 stainless steel wire where exposed in continuous to UV. Also they were evaluated at 7.5 seconds, observing that the contribution of residence time and amount of catalytic for the CFU reduction was quite similar. Variance analysis showed that the efficiency was significant with the no parametric Kruskal-Wallis test with $P < 0.05$. This technology could be used to clean indoor air of closed environments such as hospitals, crowded buildings or public transportation systems where airborne bacteria has been documented.

Key words: Photocatalytic oxidation, ZnO thin film, antibacterial activity, ZnO film on stainless steel wire.

1. Introduction

The study of ZnO has acquired great importance because of its antimicrobial and bactericidal properties and potential to eliminate microorganisms through photocatalytic oxidation when ZnO is irradiated with ultraviolet light in the presence of water and oxygen [1-3]. The ZnO has shown a multifunctional inorganic particle with antimicrobial properties [4].

ZnO has been recognized as a preferable material for a variety of environmental applications due to its high photosensitivity, non-toxic nature, low cost and

chemical stability [5]. ZnO is a wide band gap semiconductor, and is considered as suitable alternative to TiO_2 due to its non-toxicity and relatively lower preparation cost [3].

ZnO has a high UV absorptivity and can absorb more light in the UV spectrum than TiO_2 semiconductors [6], which has higher photocatalytic activity than titanium dioxide [7], and possesses superior durability, and heatresistance [8].

ZnO has been investigated for its qualities as an antibacterial agent in the absence of light since as early as the 1950s, and has since been shown to be effective for the inactivation of *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis* and

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Staphylococcus aureus among others [9].

Nanocomposites of ZnO/Fe₃O₄ were tested against *Staphylococcus aureus* and *Escherichia coli* and the antibacterial activity of these nanoparticles was found to be dependent on the weight ratio (Zn)/(Fe), the higher the ratio, the higher the antibacterial activity [10], while for nanocomposites of ZnO/ZnAl₂O tested against *Escherichia coli*, the inactivation rate increases with time [5].

ZnO with ultraviolet light irradiation, in the presence of water and oxygen, overcomes the band gap between the Valence Band (VB) and Conduction Band (CB). This generates the electron (e⁻) hole (h⁺) pairs which react with O₂ and H₂O to form superoxide anion radicals in the cathodic sites (O₂⁻), and radicals (OH) at the anodic sites. This leads to the production of other reactive species such as hydrogen peroxide. These oxidative species (h⁺, OH, O₂ and H₂O₂) are highly reactive, and considered to be the dominant oxidative species contributing to the mineralisation of microorganisms [9]. The oxidation is initiated by the attraction of oxidisable material to the semiconductor ZnO surface, where the charge of the pair-hole is transferred to the adsorbed species [11] and the OH is oxidised to CO₂ and H₂O [12]. The effect of the photocatalytic oxidation on the bacteria was evaluated by monitoring cell cultivability, cell wall integrity and nucleic acid stability [13].

There have been several bactericidal mechanisms proposed, one of which stated that the sorption of ions onto the negatively charged bacterial cell wall causes deactivation of cellular enzymes, and the disruption of membrane permeability, leading to eventual cell lysis and death. Also, the interaction of reactive species with enzymes in the respiratory chain reaction results in the uncoupling of respiration from the synthesis of ATP and oxidation of the intracellular Coenzyme A (CoA), which inhibits cell respiration and subsequently causes cell death [9, 13]. Peroxidation of the lipid membrane and the eventual oxidation on the membrane proteins will cause a rapid leakage of

potassium ions from the bacterial cell resulting in direct reduction of cell viability [14].

As a consequence of disorder in the cell wall permeability, drainage of the intracellular constituents and alterations of the protein structure occur [15], also, electrostatic interactions within the cytoplasmic membrane of the cell wall, breaks the lipid bilayer of bacterium and fungus, draining the cytoplasm content [13]. Most of the research indicates that the destruction of the cell membrane that protects the cytoplasm is an important process for inactivation [16].

Air polluted by microorganisms in closed areas such as buildings and hospitals can affect the health and welfare of exposed individuals [17, 18]. *Bacillus* is a typical bacteria that is viable in the air; this genus has been isolated from external air as the predominant microorganism in Oregon in rural and urban environment and Beijing [19], and as the predominant microorganism in indoor air in an Indian hospital, reported as Gram Negative *Bacillus* (GNB) [20]. Microorganisms carried in indoor atmospheres are a public health problem [21-23]. In the past, this problem has been treated using UV light, promoting the formation of dimers or double molecules of the nucleotides cytosine-cytosine, thymine-thymine, cytosine-thymine and uracil-uracil, thus, inhibiting the replication of DNA and RNA and limiting reproduction of the microorganism [24]. However, UV-induced processes suffer from a lack of residual effect as the chlorine [9].

Related to the support materials to photocatalytic reaction, AISI 304 steel and wire mesh-supported materials have recently been used as monolithic catalysts for different reactions such as the photo degradation of methylene blue solutions under ultraviolet irradiation [25, 26]. Also SS 304 steel sieves 100 meshes and 300 meshes coated with TiO₂ thin films deposited by sol-gel and dipping methods have been used to deactivate airborne microbial cells [27].

In order to design the experiment, it was considered

that the photocatalytic oxidation reaction performance is a function of the source light wavelength and intensity, the reaction surface or photocatalyst amount, the concentration of bacteria (Colony Forming Units), humidity, temperature, oxygen and residence time as the most important [28, 29].

The novelty of this work is the application of electrophoretic deposition of zinc oxide to achieve uniform films of ZnO on a Stainless Steel (SS) 304 US 100 wire sieve. This film is then irradiated with UV light to degrade airborne bacteria by continual photocatalytic oxidation in a one litre reactor. The degradation efficiency as a function of time and amount of photocatalyst, once deducted the degradation caused by the UV light alone was evaluated. The results hypothetically expected were a significant reduction in the Colony Forming Units (CFU).

This technology could be used to clean indoor air of closed environments such as hospitals, crowded buildings or public transportation systems [18], where the presence of airborne bacteria has been documented as a problem [30, 31] and has been considered a factor responsible for inducing sick building syndrome [13, 32].

2. Material and Methods

2.1 Preparation of Substrate

The SS 304 US 100 wire mesh sieve substrates were prepared by removing all of the impurities with an abrasive paper, cutting the wire sieve into circles of 7.5 cm of diameter with a hole of 2.5 cm in order that they could be inserted into a quartz tube of 2.5 cm of diameter.

The wire sieves were ultrasonically cleaned in acetone by two hours and rinsed with deionized water.

2.2 Preparation of Thin Film Coating

A stainless steel 304 wire sieve was exposed in an electrodeposition cell to a solution of 0.004 M of zinc acetate $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ with a purity of > 97% and a MW of 219.53 g/mol, the latter was dissolved in distilled water. The electrodeposition cell was

comprised of a Nalgene jar (Fig. 1), with a zinc acetate solution where the SS wire sieve was coated with $\text{Zn}(\text{OH})_2$ using a galvanic source MR PG-3VE with 20 Volts for 2 minutes. In the cell, the stainless steel wire sieve was the cathode and a stainless steel plate was the anode; a constant voltage was applied between the two electrodes (Fig. 2).

2.3 Thermal Treatment of $\text{Zn}(\text{OH})_2$

The ZnO films obtained were calcined from room temperature to 500 °C with a heating rate of 10 °C/min and maintained at this temperature for 2 hours in order to obtain the ZnO, before being cooled in an oven.

Fig. 1 Electrodeposition cell.

Fig. 2 Substrate coated with ZnO.

The agglomerates gels formed in the electrodeposition process and not attached to the cathode, remain suspended in the solution of zinc acetate in the electrodeposition cell. They were filtered and calcined at 500 °C, and then characterized by SEM and XRD to verify that it was obtained ZnO, and by chemisorption to measure active sites.

2.4 Chemisorption of Oxygen

The volumetric apparatus (Fig. 3) containing: a cell of known volume made of stainless steel, two digital sensors of low and high pressure and a high vacuum system and furnace for pretreatment of samples were used.

These samples were heated to 300 °C for 2 h under high vacuum (1×10^{-3} torr) to remove the adsorbed gases. The oxygen used in the chemisorption was of high purity 99.99% (Infra). Once the sample was at 25 °C, an injection of O₂ (589 torr) was made and allowed to stabilize for 4 h to occur the O₂-chemisorption-physisorption, then vacuum (1×10^{-3} torr) was applied for 10 min and again 589 torr of O₂ were injected to know the O₂ physisorption. The difference between these two adsorption isotherms allowed to determine the amount of O₂ chemisorbed.

2.5 Scanning Electron Microscopy

The coating morphology and composition of ten samples were analysed by Scanning Electron Microscopy (SEM) with a SEM SUPRA 55VP instrument (Zeiss). It was a high resolution SEM that used a Schottky-type field-emission electron source. A beam booster was used to optimise electron optical performance at all accelerating voltages. The optical column had no crossovers and utilised a magnetic/electrostatic objective lens to minimise beam imperfections at low voltage by reducing the chromatic aberration. The qualitative and quantitative chemical analyses were obtained by coupling the energy dispersive spectroscopy of X-ray (EDS) to microscope.

Fig. 3 Volumetric apparatus to measure the O₂ chemisorption.

2.6 X-Ray Diffraction

An X-ray D-500 Siemens diffractometer equipped with a copper anode tube source, which generates a Cu radiation with a $K\alpha$ ($\lambda = 1.54 \times 10^{-8}$ Å), comparable to the spacing among atoms. The beam is collimated (forced into parallel rays), shaped (so that it has a definite “shadow”) and directed onto the sample, which is in the form of a flat plane, taking advantage of Bragg-Brentano geometry: the entire sample diffracts the beam toward the same detector, enabling a larger sample and a larger beam size to be used as copper tube and a nickel filter. The diffractometer can identify crystalline compounds by matching diffraction patterns to a database.

2.7 Culture Preparation

A bacteria culture consortium was prepared, using microbial strains cultivated from viable native bacteria from air samples, collected from the atmosphere in a station of the atmospheric monitoring network from the Mexico State in the border of Mexico city. The bacteria were rod-shaped and showed a negative response to Gram staining. Nutrient broth was used to induce massive growth of these bacteria, which were incubated at room temperature for 48 hours and stored in a one litre vacuum flask.

2.8 Photoreactor

A cylindrical reactor with a one litre of capacity was adapted to carry out the photocatalytic reaction. It comprised a container with a germicide UV lamp light source, Light Source Inc. model GPH254T5L/4 MI-50-93, (254 nm wavelength), protected with a quartz tube that support inserted three or five stainless steel 304 wire sieves, covered with ZnO.

The reactor was equipped with two sampling ports to sampling before and after the photocatalytic reaction. It was integrated in a train consisting of the vacuum flask attached to the reactor joined to two flasks in series with acidulated water (0.1 M sulphuric acid). This destroys any bacteria that were not inactivated in the reactor before the final outlet, through the pump that extracted the microbes from the vacuum flask (Fig. 4). The sieves were inserted into the quartz tube to be irradiated directly on the reaction surface (Fig. 5) passing the airborne bacterial through them.

2.9 Bacteria Degradation

The photocatalytic oxidation was a continuous process, where the following parameters were maintained constant: humidity at 30%, UV wavelength of 254 nm and intensity of 2.9 mWs/cm² and temperature of 26 °C.

The variables studied were the surface reaction area and the residence time.

In the experiment, the bacterial consortium was carried out from the vacuum flask through the reactor with a vacuum pump (Emerson model S3MYHCB-1452), where samples of 100 cm³ of air were extracted with a syringe at the entrance and at the reactor outlet through the sampling ports. These samples were injected into a Petri dish containing a standard liquid culture medium, using an impactor equipped with a diffusion plate, where the samples were retained for 5 minutes. The Petri dishes containing the bacterial consortium were incubated for 48 h and the CFU in the samples were analysed.

Fig. 4 Reactor train arrangement.

Fig. 5 Reactor interior view.

The experiments were conducted five times with different residence time of 7.5 seconds and 20 seconds and with three and five coated films, having 120 cm² of surface reaction/1,000 cm³ of volume and 200 cm² surface reaction/1,000 cm³ of volume respectively, which inserted to observe the effect of the amount of photocatalytic material and residence times on the reduction of CFU (Table 2). In order to eliminate the effect of ultraviolet light on bacterial degradation, it was also evaluated this condition, taking five samples at the inlet and outlet of the reactor.

3. Results and Discussion

3.1 Scanning Electron Microscopy

The presence of the film on the network was observed by identifying the ZnO as well as its composition by EDS. Micrographs are presented at low magnification 50 X and at 40,000 X in Figs. 6 and 7. In these images, it is possible to observe the nanostructure of the ZnO film. The elemental composition of the film and the SS-wire sieve is shown in Fig. 8. An average composition of 2.52 wt% of Zn was obtained. This means that 3.13 wt% of ZnO was present. The presence of Fe (61.72 wt%), Cr (15.46 wt%), Oxygen (11.02 wt%), Ni (6.92 wt%), Mn (1.66 wt%) and C (0.66 wt%) were also observed.

The films obtained were homogeneous as others recently reported on stainless steel sieves with surface coated TiO₂ thin films to degrade bacteria with similar efficiencies [26].

The SS wire sieve from the second experiment M2, had the elemental composition shown in Fig. 8 in percent of weight obtained with the SEM SUPRA 55VP Bruker Nano GMBH instrument, whereas the other samples had similar compositions.

3.2 X-Ray Diffraction

The XRD confirmed the presence of crystal of ZnO on samples obtained from several SS-wire < sieves. Fig. 9 shows the X-ray analysis pattern of a sample of ZnO powder as zincite obtained from the agglomerate gel filtered and calcined at 500 °C and reported as the zincite phase, as previously described [33]. Fig. 9 also shows a sample representative of the X-ray pattern of the ZnO electrodeposited on the SS as a film, where ZnO was in the hexagonal zincite phase. The sample showed the Zn and Fe (austenite) together with Zn and ZnO reflexions.

3.3 Chemisorption of Oxygen

In average, the amount of ZnO deposited on the sieve surface was of 0.000135 g/cm² of sieve. The O₂

Fig. 6 SEM image of the SS wire sieve covered with ZnO 50 X magnification.

Fig. 7 SEM image of the SS wire sieve covered with ZnO 40,000 X magnification.

Fig. 8 Composition of the SS wire sieve by EDS.

Fig. 9 X-ray of ZnO powder as zincite and ZnO supported on SS wire sieve.

isotherm of the SS-wire sieve showed very low O_2 chemisorption (Fig. 10 and Table 1) while the ZnO powder showed a typical O_2 isotherm (Fig. 12). The sample M2 of ZnO on SS-wire sieve showed an O_2 chemisorption larger than the O_2 chemisorption of the ZnO powder. This result shows that the ZnO film supported on the wire sieve had more vacancies O_2 on its surface after H_2 reduction process than the ZnO powder.

3.4 Photocatalytic Activity

The photocatalytic activity was determined as a function of the bacterial CFU reduction. Table 2 shows the efficiency of destruction when the airborne bacterial were exposed to UV alone as well as the efficiency with UV and SS wire sieves coated with the ZnO film. Table 3 shows the efficiency increment

with residence time and amount of photocatalyst.

The data shows, that when the amount of photocatalytic material increment, the CFU reduction is slightly more than when the residence time increment, and that the CFU reduction tends to 100% when both parameters are incremented.

3.4 Variance Analysis

With these data, it was carried out a variance analysis to establish the significance of the experiment, with the hypothesis that the two experiments are different and that the differences are significant, as an alternative hypothesis, it was proposed that the experiments are not different.

Applying the Shapiro-Wilk test, it was confirmed that the data do not follow a normal distribution with a Probability value ≤ 0.001 (Table 4).

Fig. 10 O₂ isotherm of the SS-wire sieve at 25 °C, chemisorption + physisorption (F + Q).

Fig. 11 O₂ isotherm of the ZnO powder at 25 °C, chemisorption + physisorption (F + Q).

Fig. 12 O₂ isotherm of the ZnO supported on SS-wire sieve M4, at 25 °C, chemisorptions + physisorption (F + Q).

Table 1 O₂ chemisorption for the ZnO film supported on SS-wire sieves.

Sample	ZnO content (%)	O ₂ chemisorption (O/ZnO) (g atom of oxygen/mol ZnO)
SS-wire sieve	0	0
ZnO powder	100	0.0189
ZnO supported on SS-wire sieve	0.2542	0.7153

Table 2 Efficiency reduction of CFU Gram negative rod-shaped microbes with UV light and the ZnO film photocatalyst.

No.	Conditions	Residence time (sec)	SS wire sieve coated with ZnO film	Average UFC/100 mL before reactor	Average UFC/100 mL after reactor	CFU destruction efficiency by UV (%)	CFU destruction efficiency by UV plus SS sieves
1	Only irradiation with of ultraviolet light (blank)	7.5	0	59	2.15	96.36	---
2	Irradiation with ultraviolet light plus coated SS wire sieve with ZnO film	7.5	3 (120 cm ²)	61	0.67		98.89
3	Irradiation with ultraviolet light plus coated SS wire sieve with ZnO film	7.5	5 (200 cm ²)	57	0.58		98.98
4	Only irradiation with of ultraviolet light(blank)	20	0	58	1.87	96.77	----
5	Irradiation with ultraviolet light plus coated SS wire sieve with ZnO film	20	3 (120 cm ²)	60	0.63		98.93
6	Irradiation with ultraviolet light plus coated SS wire sieve with ZnO film	20	5 (200 cm ²)	63	0.21		99.66

Table 3 Photocatalytic efficiency of CFU reduction as function of residence time and number of sieves.

Disinfectant material	Residence time (sec)		Efficiency increment (%)
	7.5	20	
UV	93.6	96.72	
UV + 3 ZnO covered sieves	98.89	98.93	0.04
UV + 5 ZnO covered sieves	98.98	99.66	0.68
Efficiency increment (%)	0.09	0.73	

Table 4 Normality test (Shapiro-Wilk) equal variance test: failed (P < 0.050), normality test (Shapiro-Wilk) failed (P < 0.050).

Group name	N	Missing	Mean	Standard deviation	Standard error of the mean
Inlet (blank)	5	0	59.000	42.626	19.063
UV irradiation	5	0	2.200	1.304	0.583
UV + ZnO irradiation	5	0	0.600	0.548	0.245

Source of variation	DF	SS	MS	F	P
Between groups	3	12,565.600	4188.533	9.209	< 0.001
Residual	16	7,277.200	454.825		
Total	19	19,842.800			

Since the distribution was not normal, it was applied the Kruskal-Wallis test in a range that was applicable to non-parametric data (Table 5).

With the Kruskal-Wallis test, it was confirmed that

the differences are significant, since P = 0.004 is less than 0.050, and the differences in the median values among the treatment groups are greater than would be expected by chance.

Table 5 Kruskal-Wallis one way analysis of variance on ranks, normality test (Shapiro-Wilk) failed ($P < 0.050$).

Group	N	Missing	Median	25%	75%
Inlet (blank)	5	0	60.000	21.000	96.500
UV irradiation	5	0	2.000	1.000	3.500
UV + ZnO irradiation	5	0	1.000	0.000	1.000

* $H = 13.468$ with 2 degrees of freedom and ($P = 0.004$).

According to these results, the differences between the treatment with UV light and the treatment with ZnO are significant.

4. Conclusions

The following conclusions can be obtained from this study:

(1) With electrophoretic deposition of $\text{Zn}(\text{OH})_2$ and a subsequent calcination, the ZnO film obtained covered the entire sieve with a homogenous morphology, where zincite is present as the ceramic with photocatalytic activity;

(2) The electrophoretic deposition does not require controlled conditions of pressure and temperature, and occur at ambient conditions;

(3) The chemisorption of oxygen shows the presence of active sites where oxidation could occur and are potential sites for photocatalytic activity;

(4) The chemisorption of oxygen on the sieve covered with ZnO and the ZnO powder shows that the surface reaction area is higher in the sieve, such condition potentially increase the efficiency in destruction of the microorganism;

(5) ZnO ceramic possess significant antibacterial properties for this consortium;

(6) The experiment shows that the amount of photocatalytic material contributes more than the residence time to increase the destruction efficiency, and approaches to total destruction when the amount and time increases;

(7) The experiment shows that this technology could be extrapolated to control airborne bacteria in an indoor environment in a short time;

(8) The photocatalytic process is more cost-effective than other technologies actually used

with similar bacterial destruction efficiencies, since the former only needs energy to operate and the semiconductor catalyst is inexpensive and capable of mineralising airborne microbes.

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