School Experiments on the Biotoxicity of Metal and Metal Oxide Nanoparticles

Isabel Böselt[1], Timm Wilke[2], Thomas Waitz[3]
Department of Chemistry Education, Georg-August-University (Germany)
[1]iboesel@gwdg.de, [2]twilke2@gwdg.de, [3]twaitz@gwdg.de

Abstract

The use of metal and metal oxide nanoparticles, such as silver and zinc oxide in everyday life objects, is increasing which is why it can be expected that the entry of nanoscale materials into the environment is steadily growing. Even though there are currently only a few studies of the effects of nanomaterials on humans and the environment, current research reveals that some of these materials present certain risks [1]. For example, current studies prove an increased biotoxicity of silver nanoparticles on microorganisms such as Escherichia coli [2].

Apart from the synthesis of silver and zinc oxide nanoparticles, we present an experiment inspired by Kurt Winkelmann [3] to demonstrate the possible effects of metal and metal oxide nanoparticles on microorganisms for use in both high school and university laboratory classes. The experiment will demonstrate the influence of the nanoparticles on the alcoholic fermentation of Saccharomyces cerevisiae, also known as baker's yeast. In conclusion, we will give some examples of how to implement these experiments in chemistry school classes.

1. Background

The possible effects of metal and metal oxide nanoparticles on human beings and the environment become more and more important to consider, due to the increasing usage of nanotechnology in our everyday life. In comparison to bulk materials, nanoparticles show distinct characteristics that result from their smaller size and bigger surface area [4]. The size enables them to enter and pass physiological barriers harming living organisms [4]. With the increasing use of nanoparticles in our society, one frequently comes in contact with them which reveals how important it is to integrate nanotechnology and its risks in chemistry education. Especially zinc oxide and silver nanoparticles, which are known for their antimicrobial effects, are worth investigating. It is proven, for example, that both have antibacterial effects on Escherichia coli probably by affecting factors such as the permeability (ZnO) and the stability (Ag) of the bacteria's membrane [4]. Furthermore, studies revealed that nanoparticles are able to cause oxidative stress generating reactive oxygen species (ROS) which harm cells by damaging their DNA, proteins or lipids [4]. Various semiconducting metal oxide nanoparticles like titanium dioxide or zinc oxide create ROS in photocatalytic reactions (see Fig. 1).

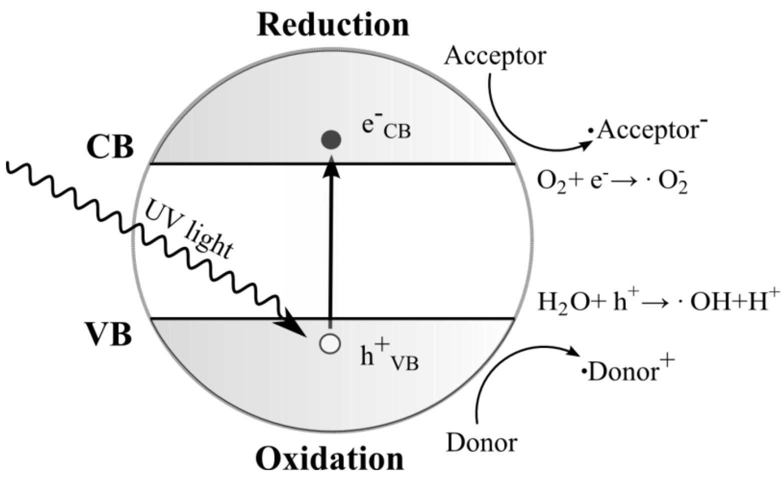

Fig. 1: *Model of photocatalytic reactions. An electron in the valence band is promoted into the conduction band by UV light. As a result, an electron-hole is formed in the valence band. Subsequently a redox reaction takes place leading to the formation of several radical species, e.g. ROS [6].*

Moreover, silver and zinc oxide nanoparticles find many applications. Silver nanoparticles are nowadays used in socks, shampoo, washing machines, sprays, toothpaste and cellular phones to name only a few. Zinc oxide nanoparticles are mainly used in sunscreen and in the cosmetic industry[4].

2. Didactic and Methodical Considerations

The many applications of silver and zinc oxide nanoparticles already described as well as the question of whether or not daily products like sunscreen, cosmetics, toothpaste or shampoo harm the user make the toxicity of nanoparticles relevant for students' lives.
For this, we present experiments that enable high school and university students to conduct an independent examination of the biotoxicity of diverse nanoparticle suspensions after having synthesized them. Silver and zinc oxide nanoparticles are comparatively simple to synthesize which allows for an easy application in school settings. For the experiments, we chose predominantly harmless chemicals which are readily available and normally found in school chemical collections. To demonstrate the effects of metal and metal oxide nanoparticles on living organisms or the environment, we use the unicellular microorganism Saccharomyces cerevisiae, also known as baker's yeast, as a model. Saccharomyces cerevisiae is classified as part of the fungi kingdom and is a eukaryotic microorganism, 5-6 µm in size [5]. Its use in chemistry education contains various advantages. First of all, yeast is easily accessed. In addition, it has a simple structure allowing for simplified examination of the effects of nanoparticles on it. Furthermore, yeast finds many applications in our everyday life, such as in the production of beer and wine [5] which is important for providing a daily relevance to students. Moreover, it is possible to create a connection between chemistry and biology classes, *e.g.* to explain and examine cell structures using conventional school microscopes. Finally, in comparison with more complex living organisms such as mice, the examination of yeast is ethically justifiable.
On the basis of the presented experiment, students can not only learn about the toxic effects of nanoparticles and the structure of eukaryotic cells, but also about the process of alcoholic fermentation and the functioning of enzymes. To sum up, the experiments were elaborated so that basic knowledge and experimentation skills can be developed while showing the risks metal and metal oxide nanoparticles can entail.

3. Experiments

3.1. Synthesis of Nanoparticles in School Chemistry Education

3.1.1. Synthesis of Zinc Oxide Nanoparticles in Ethanol

Zinc oxide nanoparticles are synthesized by precipitation of zinc acetate dihydrate with sodium hydroxide in ethanol (2) [6]. A 0.2 M solution of sodium hydroxide is prepared by dissolving 0.28 g in 35 ml ethanol and heating the solution to 40°C. At the same time, a solution of 2.2 g zinc actetate in 100 ml ethanol is prepared and heated to 90°C to achieve the complete dissolution of zinc acetate. Once the solution has cooled down to 60°C, the first solution is slowly added.

$$Zn(CH_3COO)_2 \cdot 2\,H_2O + 2\,NaOH \rightarrow ZnO + 2\,CH_3COO^- + 2\,Na^+ + 3\,H_2O \qquad (2)$$

After synthesis, the size of the nanoparticles (5.5 – 7 nm) is verified by showing their fluorescence by irradiating the suspension with UV light.
Since the background of the connection between the size of the zinc oxide nanoparticles and their fluorescence is quite complex and not yet completely verified, it seems adequate to restrict the theory of fluorescence for chemistry classes in general. In this context, the (simplified) energy-band model can be applied [6].

3.1.2. Synthesis of Silver Nanoparticles with Glucose

Silver nanoparticles are synthesized by a polyol process with PVP (polyvinyl pyrrolidone) as the stabilizing agent and glucose as the reducing agent (3) [7]. First, a solution of 0.5 g PVP and 1 g glucose in 40 ml demineralized water is prepared and heated to 80°C. Then, a 0.18 M solution of silver nitrate is prepared by dissolving 0.2446 g in 8 ml of demineralized water. Afterwards, it is added to the solution of glucose. This solution is stirred for one hour at 80°C and finally cooled down to interrupt the reaction [7].

$$2\,AgNO_3 + C_5H_{11}O_5COH + H_2O \rightarrow C_5H_{11}O_5COOH + 2\,NO_3^- + 2\,H^+ + 2\,Ag \qquad (3)$$

3.2. Experiment on the Biotoxicity of Nanoparticles

To examine the biotoxic effects of zinc oxide and silver nanoparticles on yeast, an experiment feasible for school settings was elaborated (see Fig. 2):

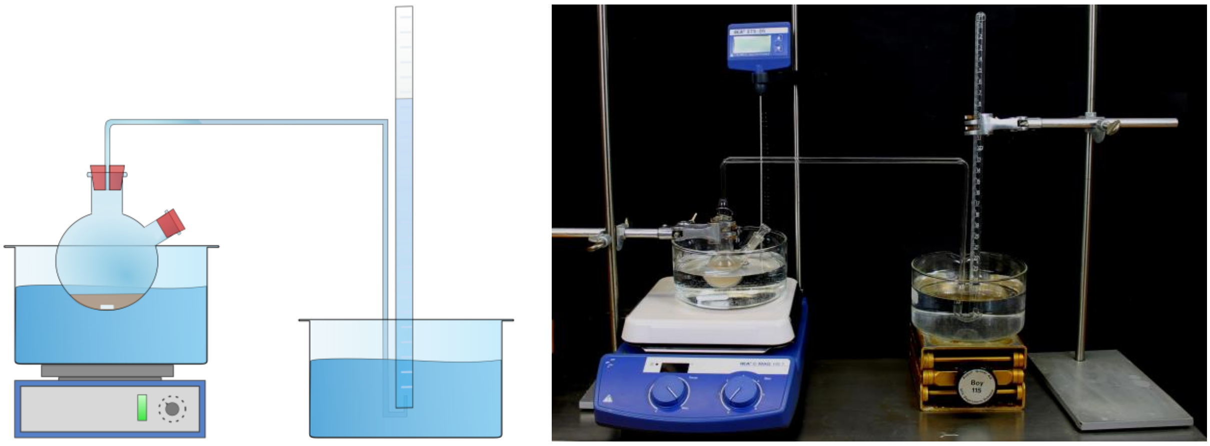

Fig. 2: *Experimental setup for the pneumatic collection of carbon dioxide. On the left of each illustration is the water bath (35°C) in which the two-necked, round-bottom flask with the reaction mixture is emerged. The flask is connected to the second water bath via a glass tube. At the opening of the glass tube the carbon dioxide gas is collected by a 25 ml burette.*

The volume of carbon dioxide produced by alcoholic fermentation is measured over time with and without the presence of nanoparticlces. For the measurement of the rate of alcohol fermentation without nanoparticles, 20 g of baker's yeast are dissolved in 100 ml demineralized water. Then, 1 g glucose is dissolved in 100 ml demineralized water to create a 1% solution of glucose. 5 ml of the solution of yeast and 10 ml of the solution of glucose are given into a two-necked, round-bottom flask in a water bath and heated to 35°C. The volume of carbon dioxide is measured every 5 min for 70 min.

3.2.1. Influence of Zink Oxide on the Rate of Alcohol Fermentation

The procedure is repeated four times with addition of 0.5 ml, 0.7 ml, 0.9 ml and 1.0 ml of zinc oxide nanoparticles. The results are presented below (see Fig. 3).

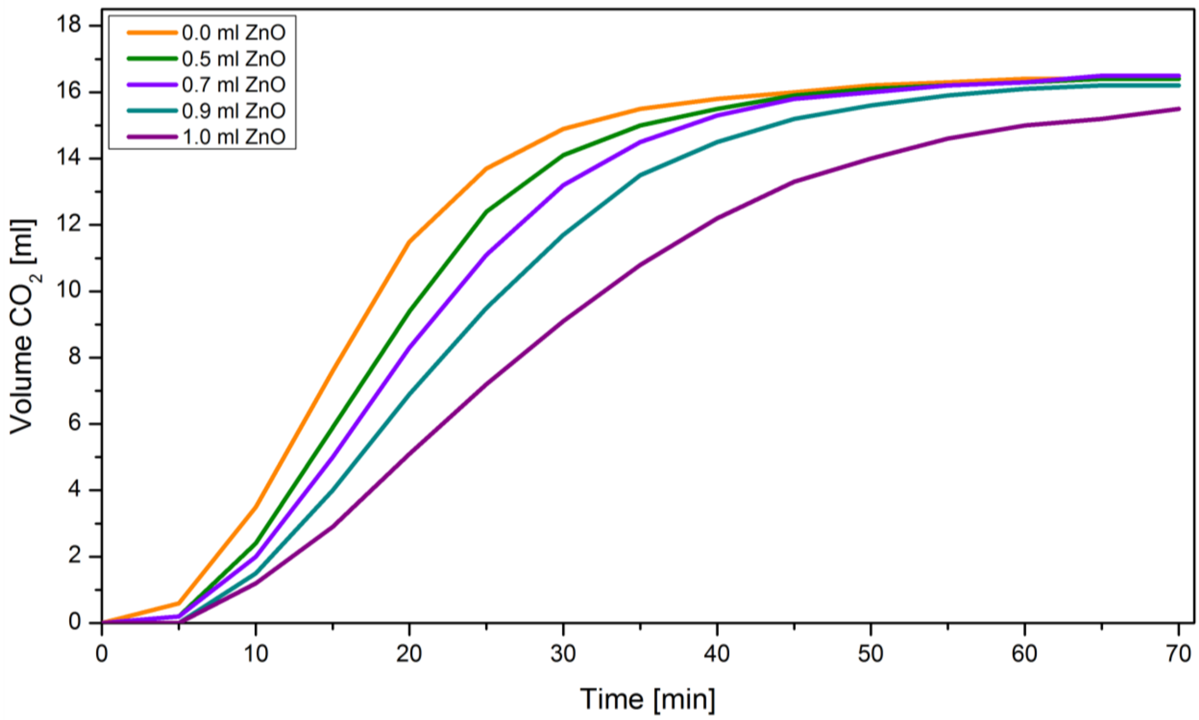

Fig. 3: *Rate of alcohol fermentation in dependence of zinc oxide nanoparticles*

The diagramm (see Fig. 3) shows that the carbon dioxide production per minutes is faster without nanoparticles than with the addition of a zinc oxide nanoparticles. The more suspension added, the lower the velocity (volume per minute). As illustrated in the diagram, after a certain time the velocity decreases before reaching the maximum production of carbon dioxide (about 16.5 ml). In a complete fermentation, about 28.07 ml carbon dioxide would be produced. Therefore, a carbon dioxide volume of 16.5 ml correspond to a glucose conversion of 58.8%.

3.2.2. Influence of Silver Nanoparticles on the Rate of Alcohol Fermentation

In another experiment, the fermentation was repeated with addition of 0.1 ml, 0.2 ml, 0.3 ml and 0.4 ml of silver nanoparticle suspension. The results are presented in Fig 4.

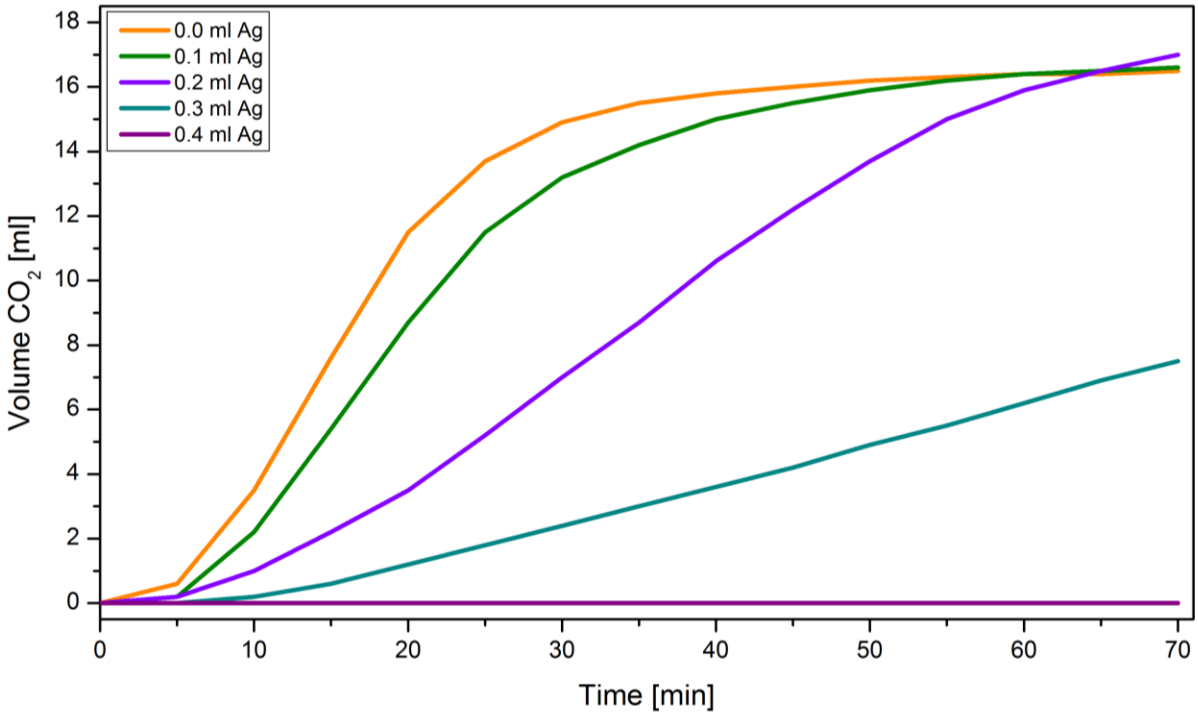

Fig. 4: *Rate of alcohol fermentation in dependence of silver nanoparticles*

The observed effect of the addition of nanoparticles is similar for zinc oxide and silver nanoparticles. The more nanoparticle suspension is added, the smaller the production of carbon dioxide per minute. As visible in Fig. 4, the maximum volume of both the orange and green line is about 16.5 ml.
Since the number of zinc oxide and silver nanoparticles in the suspension cannot be determined easily, the influence of both cannot be compared. What can be said though is that both have an effect on the velocity of alcohol fermentation.

3.2.3. Evaluation of the Results

The simplified alcoholic fermentation with yeast is as follows [8]:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \quad \Delta G_0 = -235\ kJ/mol$$

In this anaerobic process, sugar is metabolized by yeast to produce ethanol and carbon dioxide. Assuming carbon dioxide acts as an ideal gas, a calculation shows that from 1 g of glucose about 28.07 ml of carbon dioxide could be produced. Since a certain percentage of ethanol is toxic for cells, only 58.8% (16.5 ml CO_2) of glucose gets metabolized before the reaction is inhibited [9]. However, the fact that some gas was dissolved in water has to be considered. In school or university settings, this fact could be used to allow students to practice how to proceed in an error analysis.
Once nanoparticles are added to the reaction, the velocity decreases which indicates that a part of the yeast might be damaged or “inactivated”. Since with the presence of nanoparticles fewer yeast cells are intact, the fermentation needs more time to reach the maximum of the glucose metabolism. Because the production of carbon dioxide stops completely when 0.4 ml of the silver nanoparticle sus-

pension is added, it can be assumed that nanoparticles have a toxic effect on yeast. However, this experiment cannot resolve exact processes of how zinc oxide and silver nanoparticles affect yeast cells.

4. Outlook

A great variety of possible school experiments can be conducted to better understand the processes of the presented experiment and to examine the effects of different nanoparticles on, for instance, alcoholic fermentation and different microorganisms.
In addition to the measurement of the volume of carbon dioxide produced over time during alcohol fermentation, it would be possible to create a Michaelis-Menten diagram in order to analyze the kinetics of the reaction. Furthermore, in upper school grades, the growth of yeast according to the influence of nanoparticles could be examined photometrically. Here *e.g.*, it would also be possible to use other microorganisms and to vary the number of added nanoparticles as well as the nanoparticles themselves (*e.g.* copper).
To understand how nanoparticles can harm microorganisms, their diffusion through cell membranes or rather diaphragms can be investigated. An option could be to investigate diffusion through a film of n-octanol or to microscope cell membranes in nanoparticle surroundings. Another simple experiment which shows the biotoxic effects of metal and metal oxide nanoparticles on yeast is a viability assay with methylene blue. Dead cells would be colored by the dye but living cells wouldn't since cell membranes of living cells are able to reduce the dye to leucomethylene blue, which is colorless [10].

References

[1] H. Schmundt, M. Verbeet, *Spiegel*, *2008 (24),* **2008**.
[2] H. Schwegmann, *Wechselwirkungen zwischen anorganischen Nanopartikeln und Mikroorganismen,* Dissertation, Karlsruhe **2013**.
[3] Florida Institute of Technology, http://www.fit.edu/faculty/ profiles/profile.php?tracks=kwinkel [25.11.2014].
[4] C. Buzea, I. I. Pacheco Blandino, K. Robbie, *Nanomaterials and nanoparticles: Sources and toxicity,* in Biointerphases, vol. 2, issue 4 **2007.**
[5] P. Dersch, *Mikrobiologie: 43 Tabellen*, Taschenlehrbuch Biologie, Thieme, Stuttgart [u.a.] **2008**.
[6] T. Wilke, K. Wolf, A. Steinkuhle, T. Waitz, *Experimente mit fluoreszierenden Zinkoxid-Nanopartikeln*, **Submitted**.
[7] S. Kittler, *Synthese, Löslichkeit und biologische Aktivität von Silber-Nanopartikeln,* Dissertation, Essen **2009**.
[8] H. Cypionka, *Grundlagen der Mikrobiologie*, 4th ed., Springer-Lehrbuch, Springer, Berlin, Heidelberg **2010**.
[9] Massachusetts Institute of Technology, *Boosting biofuel: Yeast made to tolerate high levels of ethanol, making them more productive*, http://www.sciencedaily.com/releases/2014/10/141002141803.htm [02.01.2015] **2014**.
[10] K. Götz, C. Seliger, *Effektexperimente: Blue Bottle*, http://daten.didaktikchemie.uni-bayreuth.de/experimente/effekt/effekt_bluebottleh.htm [02.01.2015] **2010**.