Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară
2021, **22** (1), pp. 037 – 046

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry
ISSN 1582-540X

ORIGINAL RESEARCH PAPER

PRELIMINARY INVESTIGATION OF BAKER'S YEAST FOR THE BIOSYNTHESIS OF METAL OXIDE NANOPARTICLES AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

Diana Rakhmawaty Eddy, Iman Rahayu, Retna Putri Fauzia, Safri Ishmayana*

Universitas Padjadjaran, Faculty of Mathematics and Natural Sciences, Department of Chemistry, Jln. Raya Bandung-Sumedang km. 21 Jatinangor, Sumedang, West Java 45363 Indonesia

*Corresponding author: ishmayana@unpad.ac.id

Received: November, 03, 2019
Accepted: March, 12, 2021

Abstract: The present study determined if metal ions (zinc sulphate, silver nitrate, copper sulphate, manganese sulphate and ferric chloride) can be used as precursors to prepare nanoparticles using a baker's yeast culture medium. The particles were characterized using Fourier transform infrared (FTIR), scanning electron microscope–energy-dispersive X-ray (SEM-EDX), transmission electron microscopy (TEM), and X-ray powder diffraction (XRD). The antibacterial activity against *Escherichia coli* and *Streptococcus aureus* was also evaluated. *Saccharomyces cerevisiae* strain A12 culture only synthesized nanoparticles using a silver nitrate solution. FTIR indicated that the protein might play a role in capping the nanoparticles, which formed large aggregates and contained silver, oxygen and a small amount of phosphorus. The nanoparticles were spherical, ranging in size from 9 to 85 nm, crystalline and characterized as Ag_2O, however, they demonstrated weak antibacterial activity.

Keywords: *antibacterial activity, biosynthesis, nanoparticles, Saccharomyces cerevisiae, silver*

INTRODUCTION

Nanoparticles are widely used in various applications including semiconductors [1, 2], photocatalysis [3-5], and antibacterial agents [6, 7]. They can be synthesized by various physical [8, 9], chemical [5] or biological methods [6, 10]. However, as most physical and chemical methods use toxic materials or extreme conditions, the development of biological methods is currently being explored [11-13]. Biological methods use milder conditions and non-toxic solvents, therefore are more environmentally friendly, fulfilling the green chemistry principles requirement.

Potential biological agents for the biosynthesis of nanoparticles include plants extract [6, 14] and microorganisms, such as bacteria [15], yeast [16] and algae [10]. A yeast that has been reported to have the ability to synthesize nanoparticles is *Saccharomyces cerevisiae* [17, 18], which has been used in the preparation of gold, silver [19] and manganese [20] nanoparticles. Other microorganisms, namely *Pichia fermentans* [16], *Aeromonas hydrophila* [21], and *Lactobacillus plantarum* [22] have been used in the preparation of zinc oxide nanoparticles. Since the ability to form nanoparticles may differ between yeast species or strain depending on both internal (i.e. genetic factor) and external (i.e. environmental) factors [12], there might be differences in the ability of a yeast strain to synthesize nanoparticles from different metal ions precursors [12, 23]. Therefore, the present study investigated the ability of a baker's yeast strain to synthesize nanoparticles using five salts as the source of different metal ions.

MATERIALS AND METHODS

Materials

All chemicals used including analytical grade zinc sulphate, silver nitrate, copper sulphate, manganese sulphate, ferric chloride, ammonium sulphate, potassium dihydrogen phosphate, and glucose were obtained from Sigma Aldrich (Singapore). Yeast extract, bacteriological peptone and bacto agar for growth media preparation were purchased from Difco Laboratories (Madison, USA).

Microorganism

The microorganism used was *Saccharomyces cerevisiae* strain A12, a baker's yeast strain [24] and a gift from A/Prof. Robert Learmonth, University of Southern Queensland. The yeast cells were maintained on slopes of a complete medium, yeast extract peptone (YEP), containing (w/v) 0.5 % yeast extract, 0.5 % bacteriological peptone, 0.3 % $(NH_4)_2SO_4$, 0.3 % KH_2PO_4, 1 % glucose and 1.5 % agar. Slopes were stored at 4 °C and sub-cultured every 6 months.

Preparation of media for nanoparticle biosynthesis

Cells were grown in YEP media containing (w/v) 0.5 % yeast extract, 0.5 % bacteriological peptone, 0.3 % $(NH_4)_2SO_4$, 0.3 % KH_2PO_4, and 1 % glucose. Starter cultures were inoculated from slopes and grown overnight (~16 h) at room temperature

and 180 rpm in an orbital shaker (DLab SK-O330-Pro), then used to inoculate 50 mL of growth media to give a final optical density of ~0.1 at 600 nm ($OD_{600\ nm}$). The cells were grown in a 250 mL Erlenmeyer flask sealed with an oxygen-permeable cotton wool bung. After 24 hours, the cells were separated from the growth media by centrifugation (Beckman TJ-6 centrifuge) at 8000 *g* and the supernatant was used for the biosynthesis of nanoparticles.

Biosynthesis of nanoparticles

Five salts were tested, namely zinc sulphate, silver nitrate, copper sulphate, manganese sulphate, and ferric chloride. The salt solutions were prepared to give a final concentration of 0.1 M and 25 mL was mixed with 10 mL of media from the previous step and 15 mL distilled water in a 250 mL Erlenmeyer flask The solution was then shaken at 180 rpm for 24 hours and the formation of nanoparticles was indicated by the presence of precipitate in the solution. The solution was centrifuged at 8000 g for 10 minutes and the precipitate was dried in a 60 °C oven for 24 h for further analysis.

FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to investigate the presence of chemical groups. Briefly, 0.5 mg of sample was ground with 50 mg of dried KBr, then pressed to form a pellet which was analyzed using an FTIR instrument (Perkin Elmer Spectrum-100, USA)

SEM-EDX Analysis

SEM-EDX analysis was performed to investigate the morphology and elemental composition of the nanoparticles. Around 0.5 g sample was attached to a tape and placed in the sample holder before scanning with an SEM-EDX instrument (Hitachi EDAX Team, Japan).

TEM Analysis

The morphology and size of the nanoparticles were analyzed by TEM (JEOL JEM-1400plus, USA) using a TEM Grid Cu 400 mesh.

XRD Analysis

XRD characterization was performed using Cu Kα ray radiation with a 2θ angle from 20 to 80°. The X-ray diffractogram was refined using Highscoreplus software to check the sample structure phase and crystallinity. Around 500 mg sample was spread evenly on the sample holder and analyzed with an XRD instrument (Bruker D8 Advance, Germany).

Antibacterial activity assay

The antibacterial activity of the nanoparticles was evaluated by the Kirby–Bauer test using paper discs (Ø = 6 mm) on a yeast extract-peptone-dextrose (YPD) agar plate. The concentration of particles tested was 100, 500 and 1000 ppm, with 100 ppm amoxicillin (PT. Kimia Farma, Indonesia) used as a positive control, while water was used as a negative control.

RESULTS AND DISCUSSION

Biosynthesis of nanoparticles

Five salt solutions were used as the source of metal ions for the biosynthesis of metal oxide nanoparticles and the results are shown in Table 1. Out of the five salts tested, the yeast strain used was only capable of forming nanoparticles with silver nitrate ($AgNO_3$). This result indicates that the yeast strain used in the present study has specificity for the particle biosynthesis. The present finding suggests that not all metal ion present in the media can be turned into metal oxide by the yeast strain used in this study.

Table 1. *Formation of particles from various salts using S. cerevisiae strain A12 growth medium*

Salt	Precipitate formed
$ZnSO_4$	-
$AgNO_3$	+
$CuSO_4$	-
$MnSO_4$	-
$FeCl_3$	-

Characterization of nanoparticles

The FTIR analysis of the nanoparticles is presented in Figure 1. The peak at 3239 cm^{-1} corresponds to the –OH stretching vibration and the peak at 1652 cm^{-1} corresponds to the –NH stretching vibration of the amide group, while the peaks at 1384 cm^{-1} and 1114 cm^{-1} correspond to the aromatic and aliphatic amines of C–N stretching vibration of protein [24]. This result indicates the presence of protein which might be responsible for the formation of the particle and agrees with previously published results which suggest that protein molecules have a role in the formation of nanoparticles as capping agents [25-27].

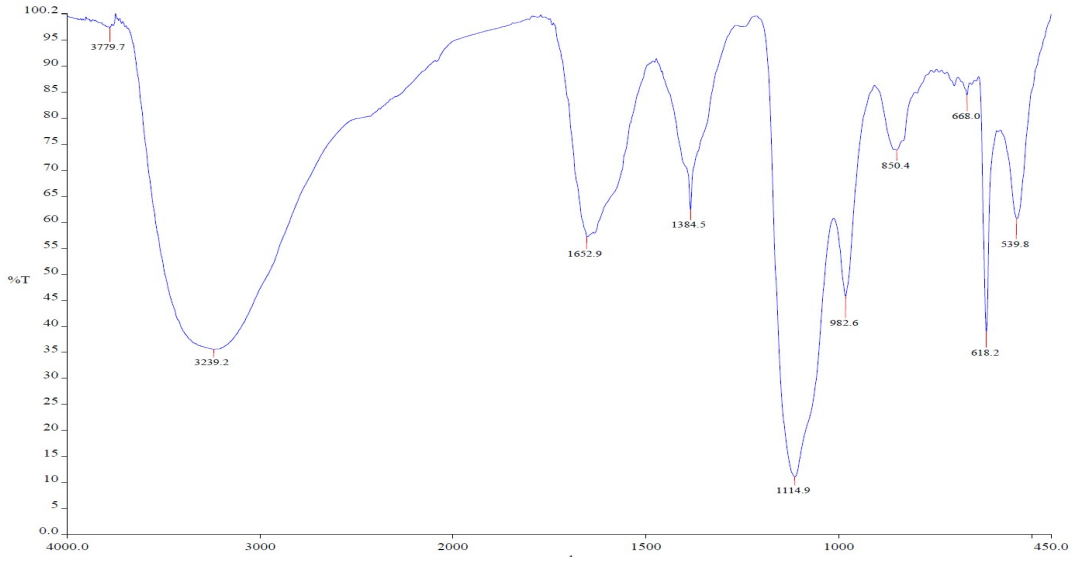
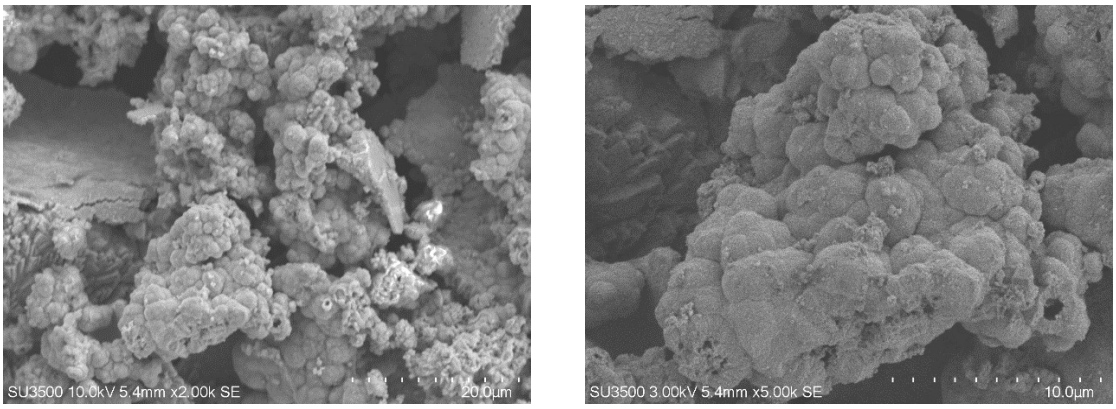


Figure 1. *FTIR spectrum of Ag_2O particles formed by culture medium of S. cerevisiae A12 using silver nitrate as a precursor*

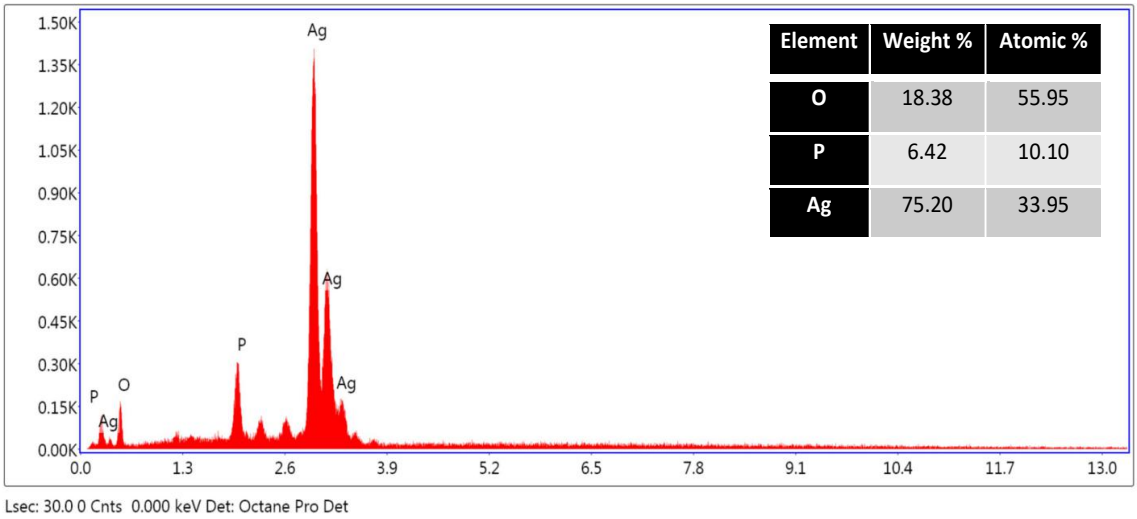
The morphology of the particles was examined by SEM-EDX, which can also determine the elemental composition of a particle. The SEM results are presented in Figure 2, while the EDX analysis is shown in Figure 3.



(**a**) (**b**)

Figure 2. *SEM micrograph of Ag_2O particles formed by S. cerevisiae A12 culture medium using silver nitrate as a precursor. (a) 2000 and (b) 5000 × magnification*

The SEM analysis indicates that the particles form aggregates, hence, have a larger particle size typical of nanoparticles synthesized by microorganisms [15, 28] and plant extracts [14] and are composed of silver (Ag), oxygen (O) and phosphorus (P), with Ag being the most dominant component. This indicates that the particle formed is silver with some impurities and the presence of phosphorus (P) is most likely because it is present in the yeast growth culture media and co-precipitates with the particle. TEM analysis was conducted to further investigate the aggregation phenomenon and the particle morphology and size (Figure 4).



Element	Weight %	Atomic %
O	18.38	55.95
P	6.42	10.10
Ag	75.20	33.95

Figure 3. *EDX spectrum of particles formed S. cerevisiae A12 culture medium using silver nitrate as a precursor. The inserted table contains the mass and atomic composition of the particles*

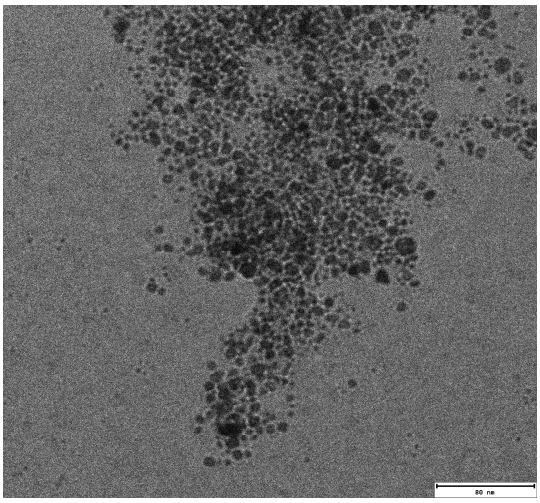


Figure 4. *TEM micrograph of Ag_2O particles formed by S. cerevisiae A12 culture medium using silver nitrate as a precursor*

The morphology of the particles is mostly spherical, with particles clumping together to form larger aggregates with protein most likely become a capping agent between the nanoparticles. This finding confirms the SEM results which showed aggregates of particles composed of smaller particles. This aggregation phenomenon was also reported in a previous study [14, 18, 28]. TEM also can be used to determine the size of the particle, revealing that the particle diameter ranged from 9 to 85 nm, with a mean value of 27 nm, hence can be categorized as a nanoparticle. The crystallinity was determined by XRD as shown in Figure 5, the sharp peaks indicating that the particles were in a crystalline state and characteristic of Ag_2O.

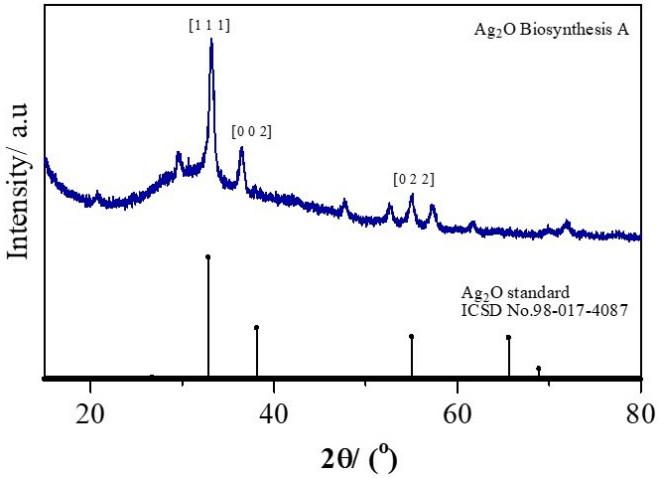
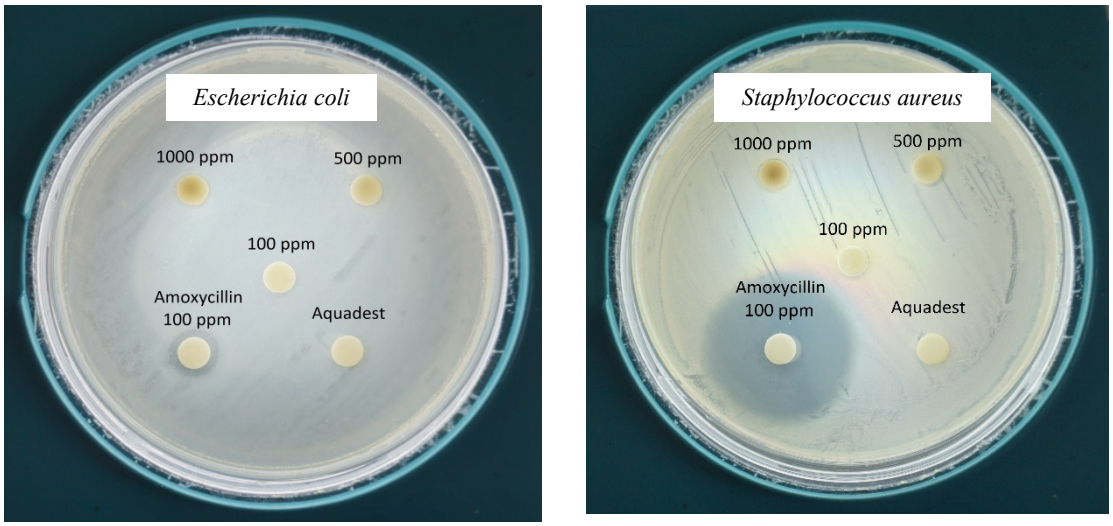


Figure 5. *Diffractogram of the Ag_2O particle prepared by S. cerevisiae A12 culture medium using silver nitrate as a precursor*

Evaluation of antibacterial activity

Silver nanoparticles are widely known for their antibacterial activity, so the particles synthesized in the present study were also evaluated for their antibacterial activity. The assay was conducted by the paper disc method using *Escherichia coli* ATCC 11229 (representing gram-negative bacteria) and *Staphylococcus aureus* ATCC 6538 (representing gram-positive bacteria) as presented in Figure 6 and Table 2.



(a) **(b)**

Figure 6. *Antibacterial activity of Ag_2O particles prepared by S. cerevisiae culture medium against E. coli ATCC 11229 (a) and S. aureus ATCC 6538 (b)*

Table 2. *Clear zone value obtained from the Kirby–Bauer test*

Sample	Clear zone (mm)	
	***E. coli* ATCC 11229**	***S. aureus* ATCC 6538**
Control (+)	10.33 ± 0.39	28.50 ± 0.00
Control (-)	0.00 ± 0.00	0.00 ± 0.00
Samples		
1000 ppm	6.53 ± 0.32	6.85 ± 0.28
500 ppm	6.18 ± 0.04	0.00 ± 0.00
100 ppm	0.00 ± 0.00	0.00 ± 0.00

The data is presented as a mean and standard deviation of three experiments

These results indicate that the synthesized particles had relatively weak antibacterial activity compared to the positive control (100 ppm amoxicillin), possibly due to the formation of large aggregates [29, 30]. As previous studies indicated the high antibacterial activity of silver nanoparticles [6, 25, 31], further studies could be conducted to reduce the formation of large aggregates and improve the antibacterial activity of the particles.

CONCLUSIONS

The baker's yeast strain used in this study, *S. cerevisiae A12,* can synthesize silver nanoparticles from a solution of silver nitrate. However, the nanoparticles formed large aggregates and had relatively weak antibacterial activity so further studies are required to optimize the synthesis conditions to reduce the formation of aggregates and improve the antibacterial activity.

ACKNOWLEDGEMENT

The present study was funded by the Directorate of Research and Community Services, Directorate General of Research and Development, Ministry of Research Technology and Higher Education, Republic of Indonesia through PDUPT research scheme, with contract number 2794/UN6.D/LT/2019. We also would like to acknowledge Dr Kristina Djanashvili from Department of Biotechnology, Delft University of Technology, The Netherlands for granting access to TEM instrument used in the present study.

REFERENCES

1. Guo, Z., Zhu, S., Yong, Y., Zhang, X., Dong, X., Du, J., Xie, J., Wang, Q., Gu, Z., Zhao, Y.: Synthesis of BSA - coated BiOI@ Bi_2S_3 semiconductor heterojunction nanoparticles and their applications for radio/photodynamic/photothermal synergistic therapy of tumor, *Advanced Materials*, **2017**, **29** (44), 1704136;
2. Gurinovich, L., Trotsiuk, L., Kulakovich, O., Sushko, N., Demir, H., Gaponenko, S.: Polarization properties of photoluminescence of anisotropic polymer films containing aligned Au nanorods and semiconductor nanoparticles of various shape, *Semiconductors*, **2018**, **52** (16), 2054-2056;

3. Lamba, R., Umar, A., Mehta, S., Kansal, S.K.: ZnO doped SnO_2 nanoparticles heterojunction photo-catalyst for environmental remediation, *Journal of Alloys and Compounds*, **2015**, **653**, 327-333;
4. Mansouri, M., Tanzifi, M., Lotfi, H., Nademi, M.: Investigation of UV/TiO_2-ZnO-Co photocatalitic degradation of azo dye (Reactive Red 120) by response surface methodology, *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2017**, **18** (2), 153-165;
5. Usman, M.R., Noviyanti, A.R., Eddy, D.R.: Photocatalytic degradation of diazinon using titanium oxide synthesized by alkaline solvent, *Indonesian Journal of Chemistry*, **2017**, **17** (1), 22-29;
6. Jayaprakash, N., Vijaya, J.J., Kaviyarasu, K., Kombaiah, K., Kennedy, L.J., Ramalingam, R.J., Munusamy, M.A., Al-Lohedan, H.A.: Green synthesis of Ag nanoparticles using Tamarind fruit extract for the antibacterial studies, *Journal of Photochemistry and Photobiology B: Biology*, **2017**, **169**, 178-185;
7. Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N.H.M., Ann, L.C., Bakhori, S.K.M., Hasan, H., Mohamad, D.: Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism, *Nano-Micro Letters*, **2015**, **7** (3), 219-242;
8. Low, Z.H., Chen, S.K., Ismail, I., Tan, K.S., Liew, J.: Structural transformations of mechanically induced top-down approach $BaFe_{12}O_{19}$ nanoparticles synthesized from high crystallinity bulk materials, *Journal of Magnetism and Magnetic Materials*, **2017**, **429**, 192-202;
9. Zhang, W., Li, J., Zhang, J., Sheng, J., He, T., Tian, M., Zhao, Y., Xie, C., Mai, L., Mu, S.: Top-down strategy to synthesize mesoporous dual carbon armored MnO nanoparticles for lithium-ion battery anodes, *ACS Applied Materials & Interfaces*, **2017**, **9** (14), 12680-12686;
10. Kusumaningrum, H.P., Zainuri, M., Marhaendrajaya, I.: Production of silver nanoparticle from *Chlorella vulgaris* and *Dunaliella salina*, *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry*, **2018**, **19** (4), 385-394;
11. Ahmed, S., Chaudhry, S.A., Ikram, S.: A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: a prospect towards green chemistry, *Journal of Photochemistry and Photobiology B: Biology*, **2017**, **166**, 272-284;
12. Hulkoti, N.I., Taranath, T.: Biosynthesis of nanoparticles using microbes—a review, *Colloids and Surfaces B: Biointerfaces*, **2014**, **121**, 474-483;
13. Kharissova, O.V., Dias, H.R., Kharisov, B.I., Pérez, B.O., Pérez, V.M.J.: The greener synthesis of nanoparticles, *Trends in Biotechnology*, **2013**, **31** (4), 240-248;
14. Yilmaz, M., Turkdemir, H., Kilic, M.A., Bayram, E., Cicek, A., Mete, A., Ulug, B.: Biosynthesis of silver nanoparticles using leaves of *Stevia rebaudiana*, *Materials Chemistry and Physics*, **2011**, **130** (3), 1195-1202;
15. Korbekandi, H., Iravani, S., Abbasi, S.: Optimization of biological synthesis of silver nanoparticles using *Lactobacillus casei* subsp. casei, *Journal of Chemical Technology & Biotechnology*, **2012**, **87** (7), 932-937;
16. Chauhan, R., Reddy, A., Abraham, J.: Biosynthesis of silver and zinc oxide nanoparticles using Pichia fermentans JA2 and their antimicrobial property, *Applied Nanoscience*, **2015**, **5** (1), 63-71;
17. Jha, A.K., Prasad, K., Prasad, K.: A green low-cost biosynthesis of Sb_2O_3 nanoparticles, *Biochemical Engineering Journal*, **2009**, **43** (3), 303-306;
18. Korbekandi, H., Mohseni, S., Mardani Jouneghani, R., Pourhossein, M., Iravani, S.: Biosynthesis of silver nanoparticles using *Saccharomyces cerevisiae*, *Artificial Cells, Nanomedicine, and Biotechnology*, **2016**, **44** (1), 235-239;
19. Lim, H.-A., Mishra, A., Yun, S.-I.: Effect of pH on the extra cellular synthesis of gold and silver nanoparticles by *Saccharomyces cerevisae*, *Journal of Nanoscience and Nanotechnology*, **2011**, **11** (1), 518-522;
20. Salunke, B.K., Sawant, S.S., Lee, S.-I., Kim, B.S.: Comparative study of MnO_2 nanoparticle synthesis by marine bacterium *Saccharophagus degradans* and yeast *Saccharomyces cerevisiae*, *Applied Microbiology and Biotechnology*, **2015**, **99** (13), 5419-5427;
21. Jayaseelan, C., Rahuman, A.A., Kirthi, A.V., Marimuthu, S., Santhoshkumar, T., Bagavan, A., Gaurav, K., Karthik, L., Rao, K.B.: Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2012**, **90**, 78-84;
22. Selvarajan, E., Mohanasrinivasan, V.: Biosynthesis and characterization of ZnO nanoparticles using Lactobacillus plantarum VITES07, *Materials Letters*, **2013**, **112**, 180-182;

23. Gahlawat, G., Choudhury, A.R.: A review on the biosynthesis of metal and metal salt nanoparticles by microbes, *RSC Advances*, **2019**, **9** (23), 12944-12967;
24. Ishmayana, S., Kennedy, U.J., Learmonth, R.P.: Further investigation of relationships between membrane fluidity and ethanol tolerance in *Saccharomyces cerevisiae*, *World Journal of Microbiology and Biotechnology*, **2017**, **33** (12), 218;
25. Badhusha, M.S.M., Mohideen, M.M.A.K.: Biosynthesis of silver nanoparticles using *Saccharomyces cerevisiae* with different pH and study of antimicrobial activity against bacterial pathogens, *Chemical Science Transactions*, **2016**, **5** (4), 906-911;
26. Dhanasekaran, D., Latha, S., Saha, S., Thajuddin, N., Panneerselvam, A.: Extracellular biosynthesis, characterisation and in-vitro antibacterial potential of silver nanoparticles using *Agaricus bisporus*, *Journal of Experimental Nanoscience*, **2013**, **8** (4), 579-588;
27. Saravanan, M., Amelash, T., Negash, L., Gebreyesus, A., Selvaraj, A., Rayar, V., Deekonda, K.: Extracellular biosynthesis and biomedical application of silver nanoparticles synthesized from baker's yeast, *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2013**, **4** (3), 822-828;
28. Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M.I., Kumar, R., Sastry, M.: Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, *Colloids and Surfaces B: Biointerfaces*, **2003**, **28** (4), 313-318;
29. Dong, Y., Zhu, H., Shen, Y., Zhang, W., Zhang, L.: Antibacterial activity of silver nanoparticles of different particle size against *Vibrio natriegens*, *PloS one*, **2019**, **14** (9), e0222322;
30. Jeong, Y., Lim, D.W., Choi, J.: Assessment of size-dependent antimicrobial and cytotoxic properties of silver nanoparticles, *Advances in Materials Science and Engineering*, **2014**, **2014**, Article ID 763807, https://doi.org/10.1155/2014/763807;
31. Raffi, M., Hussain, F., Bhatti, T., Akhter, J., Hameed, A., Hasan, M.: Antibacterial characterization of silver nanoparticles against *E. coli* ATCC-15224, *Journal of Materials Science & Technology*, **2008**, **24** (2), 192-196.