# Response of Anaerobic Granular Sludge to a Shock Load of Zinc Oxide Nanoparticles during Biological Wastewater Treatment

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**Synthetic Wastewater.** The synthetic wastewater contained carbon source (glucose and sodium acetate (pH 6.9 ± 0.1) with a chemical oxygen demand (COD) ratio of 1:1) and inorganic nutrients. The inorganic nutrients consisted of (mg/L of tap water) 1000 $NH_4Cl$, 500 $KH_2PO_4$, 200 $CaCl_2$, 200 $MgCl_2 \cdot 6H_2O$, 50 $FeCl_3$, 0.5 $H_3BO_3$, 0.5 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.5 $ZnSO_4 \cdot 7H_2O$, 0.5 $CuSO_4 \cdot 5H_2O$, 0.5 $CoCl_2 \cdot 6H_2O$, 0.5 $AlCl_3 \cdot 6H_2O$, 4 EDTA, 1 $MnCl_2 \cdot 4H_2O$, 1 $NiCl_2$ $6H_2O$, and 34.8 SDBS (as dispersing reagent). The calculated ionic strength of the synthetic wastewater is 32.4 mM.

**Detailed Operation of Parent UASB Reactor.** In the initial 15 d, the UASB reactor was operated at an organic loading rate (OLR) of 2.0 kg-COD/$m^3$/day and a substrate COD concentration of 2500 mg/L with a hydraulic retention time (HRT) of 30 h. Then the influent OLR was gradually increased to 5, 8, 12 and 16 kg-COD/$m^3$/day，and the corresponding influent COD was 2500, 3200, 4000 and 4000 mg/L, respectively, which resulted in the HRT decreased to 12, 9.6, 8 and 6 h. In each stage the acclimation was not finished until the COD removal efficiency reached relatively stable.

**Table S1. Excitation and Emission Wavelengths for Dyes and Associated Targets.**

| Dye | Excitation (nm) | Emission (nm) | Targets |
|---|---|---|---|
| FITC | 488 | 500-550 | Proteins |
| Concanavalin A | 561 | 570-590 | α-D-glucopyranose polysaccharides |
| Calcofluor white | 400 | 410-480 | β-D-glucopyranose polysaccharides |
| Nile red | 514 | 625-700 | Lipids |
| SYTO 63 | 633 | 650-670 | Total cells |
| SYTOX Blue | 458 | 460-480 | Dead cells |

**Table S2. The Statistical Analysis Results of Different Dosages of ZnO NPs and Their Released $Zn^{2+}$ Affecting Individual Compositions of EPS (Compared with the Control).**

| | | | Proteins | Polysaccharides | DNA | Lipids | Humic acids |
|---|---|---|---|---|---|---|---|
| ZnO NPs (mg/g-TSS) | 10 | $F_{observed}$ | 2.66 | 1.00 | 1.95 | 4.59 | 2.09 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | 0.18 | 0.37 | 0.24 | 0.10 | 0.22 |
| | 50 | $F_{observed}$ | 3.99 | 5.54 | 0.48 | 6.97 | 0.97 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | 0.12 | 0.08 | 0.53 | 0.06 | 0.38 |
| | 100 | $F_{observed}$ | 685.60 | 7.61 | 73.65 | 33.83 | 92.03 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | $1.26\times10^{-5}$ | 0.05 | 0.00 | $4.35\times10^{-3}$ | $6.60\times10^{-4}$ |
| | 200 | $F_{observed}$ | 981.92 | 6.42 | 259.88 | 189.05 | 163.05 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | $6.18\times10^{-6}$ | 0.06 | $8.66\times10^{-5}$ | $1.62\times10^{-4}$ | $2.17\times10^{-4}$ |
| $Zn^{2+}$ (mg/L) | 7.8 | $F_{observed}$ | 0.00 | 0.76 | 0.25 | 0.44 | 1.05 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | 0.99 | 0.43 | 0.65 | 0.54 | 0.36 |
| | 19.2 | $F_{observed}$ | 1.27 | 2.85 | 0.74 | 0.12 | 0.77 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | 0.32 | 0.17 | 0.44 | 0.75 | 0.43 |
| | 30.5 | $F_{observed}$ | 409.94 | 3.19 | 44.16 | 34.20 | 35.67 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | $3.51\times10^{-5}$ | 0.15 | $2.66\times10^{-3}$ | $4.26\times10^{-3}$ | $3.95\times10^{-4}$ |
| | 40.9 | $F_{observed}$ | 959.48 | 5.20 | 113.81 | 83.55 | 83.03 |
| | | $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| | | $P_{(0.05)}$ | $6.47\times10^{-6}$ | 0.08 | $4.37\times10^{-4}$ | $7.95\times10^{-4}$ | $8.05\times10^{-4}$ |

**Table S3. Main Functional Groups Observed from FTIR Spectra of EPS Studied.[1, 2]**

| Wave number ($cm^{-1}$) | Vibration type | Functional type |
|---|---|---|
| 3200–3400 | Stretching vibration of OH | OH into polymeric compounds |
| 2960–2930 | Asymmetric stretching vibration of CH2 | |
| 2860 | Symmetric stretching vibration of CH2 | |
| 1740 | Stretching vibration of C=O | Carboxylic acids |
| 1635 | Stretching vibration of C=O and C–N (Amide I) | Proteins (peptidic bond) |
| 1540 | Stretching vibration of C–N and deformation vibration of N–H (Amide II) | Proteins (peptidic bond) |
| 1455 | Deformation vibration of CH2 | |
| 1400 | Stretching vibration of C=O | Carboxylates |
| 1240 | Deformation vibration of C=O | Carboxylic acids |
| 1100 | Stretching vibration C–O–C | Polysaccharides |
| 1055 | Stretching vibration of O-H | |

**Table S4. The Statistical Analysis Results of Different Dosages of ZnO NPs and Their Released $Zn^{2+}$ Affecting Methane Production (Compared with the Control).**

| | ZnO NPs (mg/g-TSS) | | | | $Zn^{2+}$ (mg/L) | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 | 50 | 100 | 200 | 7.8 | 19.2 | 30.5 | 40.9 |
| $F_{observed}$ | 0.02 | 7.05 | 327.75 | 771.53 | 1.01 | 0.32 | 104.63 | 438.23 |
| $F_{significance}$ | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 | 7.71 |
| $P_{(0.05)}$ | 0.90 | 0.06 | $15.47\times10^{-5}$ | $9.99\times10^{-6}$ | 0.37 | 0.60 | $5.15\times10^{-4}$ | $3.08\times10^{-5}$ |

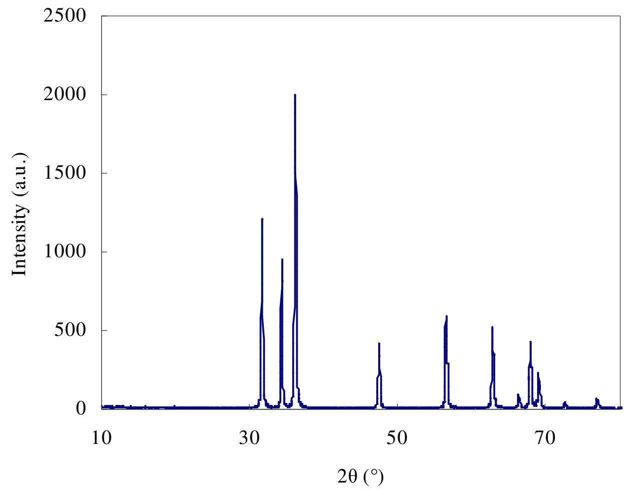


**Figure S1.** **X-ray diffraction (XRD) pattern of ZnO NPs**

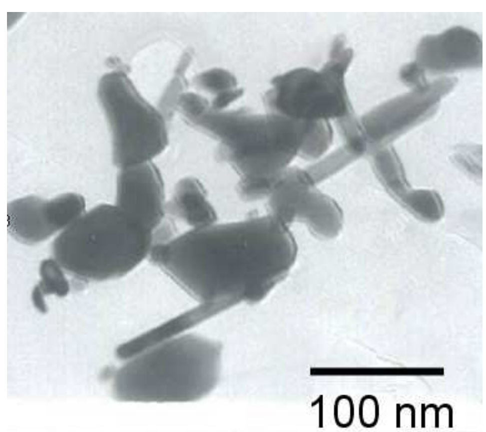


**Figure S2.** **Transmission electron microscopy (TEM) micrograph of ZnO NPs suspension.**

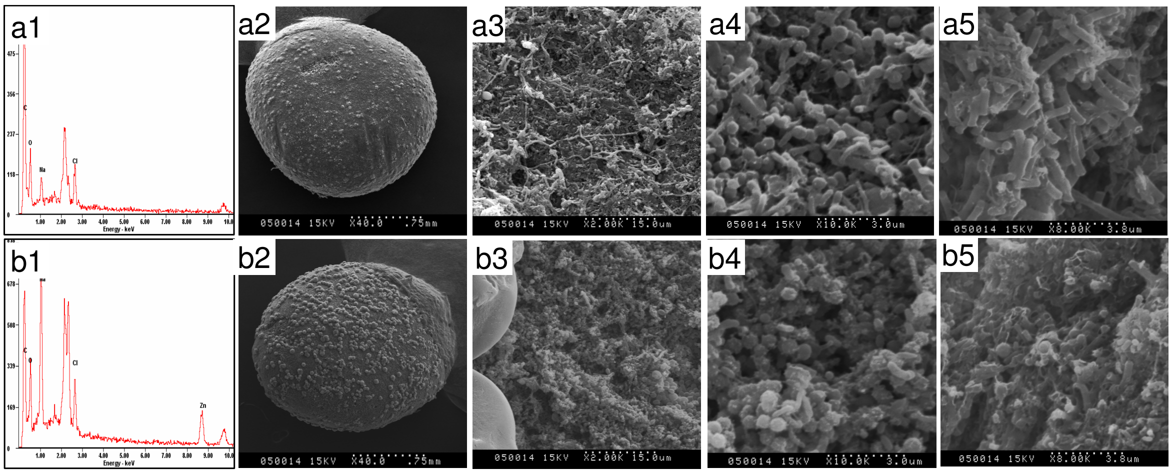


**Figure S3. The EDX (a1 and b1) and SEM (a2-a5 and b2-b5) analyses of AGS in the absence (a1-a5) and presence (b1-b5) of 200 mg/g-TSS of ZnO NPs. a2 and b2, a3 and b3, a4 and b4, and a5 and b5 represent the morphology, surface at magnification of 2000 and 10000, and interior of AGS, respectively.**

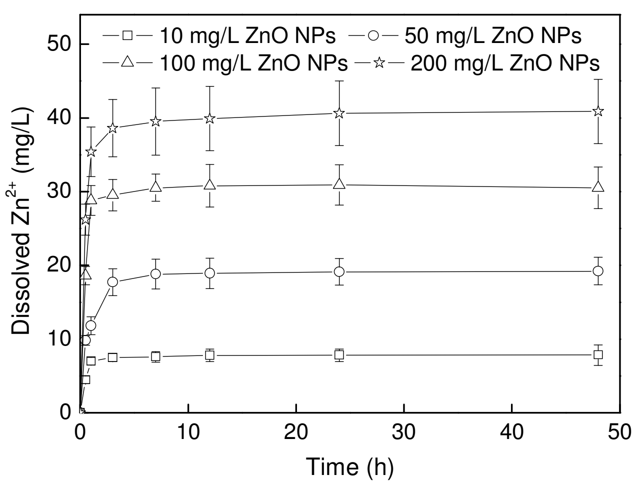


**Figure S4. Dissolution dynamic curves of ZnO NPs in synthetic wastewater containing 0.1 mM SDBS (pH=6.90 ± 0.10). Error bars represent standard deviations of triplicate tests.**

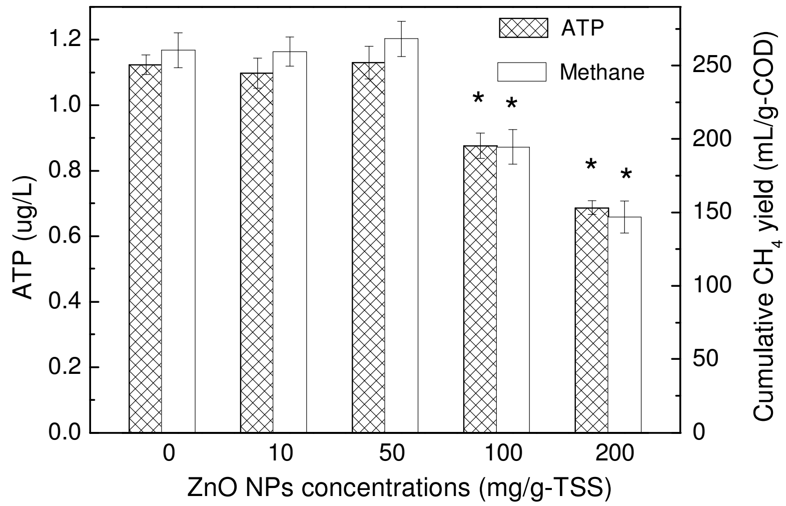


**Figure S5. Effect of ZnO NPs on ATP content of AGS. For direct comparison, the total methane productions in the presence of different dosages of ZnO NPs are also included. Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of duplicate tests.**

## REFERENCES

(1) Chen, J. P.; Lie, D.; Wang, L.; Wu, S.; Zhang, B. Dried waste activated sludge as biosorbents for metal removal: adsorptive characterization and prevention of organic leaching. *J. Chem. Technol. Biotechnol.* 2002, *77* (6), 657-662.

(2) Xu, H.; Liu, Y. Mechanisms of $Cd^{2+}$, $Cu^{2+}$ and $Ni^{2+}$ biosorption by aerobic granules. *Sep. Purif. Technol.* 2008, *58* (3), 400-411.