



Investigation of potential protective effects of microbial levan and pullulan for reducing copper toxicity using Micro-Oxymax respirometer



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Background

Reducing bioavailability or mobility of heavy metals ions is important for reducing hazard of their uptake and bioaccumulation [1]. Since copper and its compounds are one of the leading pollutants in water, numerous assessments of its negative effects have been evaluated [2]. Exopolysaccharides (EPS) are a term for bacterial polysaccharides that are synthesized and secreted into extracellular environment [3]. They are produced for self-defense against dewatering or toxic substances and assist as carbon end energy sources. Specific physico-chemical properties of EPS allow them to sequester metal ions, which is interesting for investigations of heavy metals contamination reduction [1]. Levan is a branched fructose based EPS, which is produced by a wide range of microorganisms and can usually be found in microbial biofilm [4]. On the contrary, pullulan is a linear EPS produced by the fungus *Aureobasidium pullulans* and consists of maltotriose repeating units. [5]. This study investigated potential effects of microbial levan and pullulan for reducing copper toxicity in model organism *Daphnia magna* using Micro-Oxymax respirometer.

Methods

Levan was produced by *Bacillus licheniformis* NS032 [6]. Pullulan was produced by *Aureobasidium pullulans* strain CH-1 (ICHM, Collection of Microorganisms). *D. magna* animals were prepared according to manufacturer instructions and tests were done in Aachener Daphnien Medium (ADaM) [7]. The cumulative production/consumption of gases (O₂ and CO₂) of copper exposed animals was monitored by Micro-Oxymax respirometer (Columbus Instruments, USA). The concentrations of gases (Figure 1) were measured in intervals of 2.5 h. The concentration of Cu²⁺ was 50 µg/L, and concentrations of glucose, levan and pullulan were 100 mg/L. Per twenty animals were exposed at a temperature of 25±2 °C and photoperiod of 16 h light/8 h dark during 45 h of exposure.

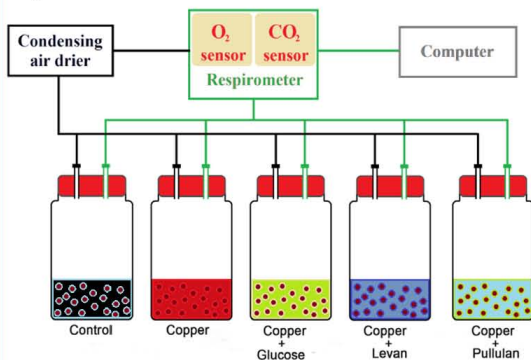


Figure 1. Scheme of experimental setup for respiration test

Results

Survival of animals and consumption/production of gases after 25 h and 45 h is showed in Table 1. It was possible to follow animal survival only at the end of an experiment since the respiration test was conducted in closed Micro-Oxymax system. There was no observed mortality in the control hence the validity criterion was fulfilled. From obtained results (Table 1, Figure 2a, 2b), it can be seen that 50 µg/L Cu²⁺ strongly inhibits the respiration, since the cumulative O₂ consumption and CO₂ production are almost twofold lower compared to control. When EPS were present, the values return to those of the unexposed animals.

Table 1. Comparison of cumulative O₂ consumption and CO₂ production after 25 h and 45 h of exposure and animal survival at the end of experiment.

| | Survival (%) | Cumulative O ₂ (µL) | | Cumulative CO ₂ (µL) | |
|-----------------------------|--------------|--------------------------------|----------|---------------------------------|--------|
| | | 25 h | 45 h | 25 h | 45 h |
| Control | 100 | -505.19 | -1023.05 | 41.63 | 61.43 |
| Cu ²⁺ | 25 | -215.16 | -501.98 | 26.94 | 39.91 |
| Cu ²⁺ + Glucose | 55 | -426.35 | -976.85 | 27.33 | 141.64 |
| Cu ²⁺ + Levan | 90 | -531.06 | -1508.93 | 44.57 | 220.44 |
| Cu ²⁺ + Pullulan | 80 | -424.42 | -1465.88 | 40.67 | 306.20 |

Differences in cumulative CO₂ production after 45 h could be enhanced by the higher mortality in sample with only Cu²⁺ and sample with combination of Cu²⁺ and glucose. When levan and pullulan were present survival of *D. magna* exposed to Cu²⁺ was higher compared animals exposed with only Cu²⁺.

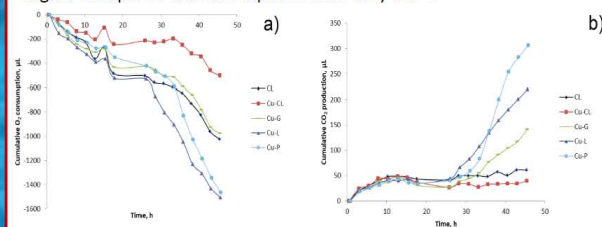


Figure 2. Cumulative production of CO₂ and O₂ of 20 *D. magna* juveniles during respiration test [8].

Conclusion

The results showed that during the acute exposure to Cu²⁺ the cumulative production/consumption of gases (O₂ and CO₂) in *D. magna* decreased, but levan and pullulan eliminated that effect and enhanced respiration. Also presence of EPS increased the survival of animals exposed to Cu²⁺. This study shows a protective effect of levan and pullulan against copper toxicity and these EPS could potentially be used as copper sorbents in the aquatic environment.

References

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Acknowledgments

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