# Catalase inhibition by metal ions for stabilizing hydrogen peroxide to improve disinfection efficiency in fresh-cut lettuce wash water

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## Introduction

The presence of catalase in (waste)water of biological origin can decrease the performance of disinfection or oxidation processes with  $H_2O_2$ . Studies show that certain metals (including Cu, Zn, Ag) can inhibit catalase in plants and animals, a process which depends on the metal, the metal concentration, the tissue and species [1]. Furthermore, Ag, Cu, and to lesser extent Zn have antimicrobial properties [2-4]. In this study, the use of Bacsan® (containing a.o. Cu, Ag, and Zn ions) to inhibit catalase was assessed in order to improve the stability of  $H_2O_2$ , and as such improve the disinfection efficiency.

### Materials & methods

The content of the Bacsan® solution (Labola, Norway) was analyzed with inductive coupled plasma emission spectrometry to determine the actual metal ion concentrations.  $H_2O_2$  was measured with a test kit. Bacsan®, at concentrations 0, 2 and 10 mg/L Bacsan-Cu (10 mg/L Cu, 0.9 µg/L Ag, 2.8 mg/L Zn), and  $H_2O_2$  at 500 mg/L were used. Artificial lettuce wash water was made by homogenizing iceberg lettuce in tap water to obtain a desired level of chemical oxygen demand (COD). All experiments were executed at 4°C. Total psychrotrophic plate count and inoculated *Escherichia coli* were measured in the experiments. Tests with catalase were executed in 0.05 M phosphate buffer with catalase from bovine liver.

#### Results

The Bacsan® significantly reduced the catalase activity, and lowering the pH enhanced the inhibition of catalase. Bacsan® improved the stability of  $H_2O_2$  in artificial lettuce wash water and increased the disinfection efficiency of total psychrotrophic plate count and *Escherichia coli*. COD concentration had detrimental effect on the  $H_2O_2$  stability and disinfection efficiency.

The Cu alone does not explain the greater stability of  $H_2O_2$  as the combination of 10 mg/L Cu<sup>2+</sup> (from CuSO<sub>4</sub>) with  $H_2O_2$  gave a lesser inhibition of catalase and a more rapid decomposition of  $H_2O_2$  in artificial lettuce wash water than Bacsan® (10 mg/L Cu ions) with  $H_2O_2$ , yet significant inhibition of catalase and increased  $H_2O_2$  stability compared to solely adding  $H_2O_2$ . As such, the inhibition was most likely due to the combination of metal ions present in the Bacsan®.

#### Discussion

The results in this study showed i) catalase can be inhibited by a metal ion mixture (10 mg/L Cu, 0.9  $\mu$ g/L Ag, 2.8 mg/L Zn), ii) lowering the pH reduces the catalase activity, iii) the use of Bacsan® improves the stability of H<sub>2</sub>O<sub>2</sub> and the bacterial disinfection efficiency of H<sub>2</sub>O<sub>2</sub>, iv) H<sub>2</sub>O<sub>2</sub> (combined or not with Bacsan®) is a slow acting water disinfectant and as such not suited for use in a vegetable washing process to avoid cross-contamination of bacteria. However, it has potential for offline disinfection (disinfection before reuse) of fresh-cut lettuce wash water if it can be shown that Cu is not transferred to the lettuce in quantities that hold a risk towards the consumer.

## References

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