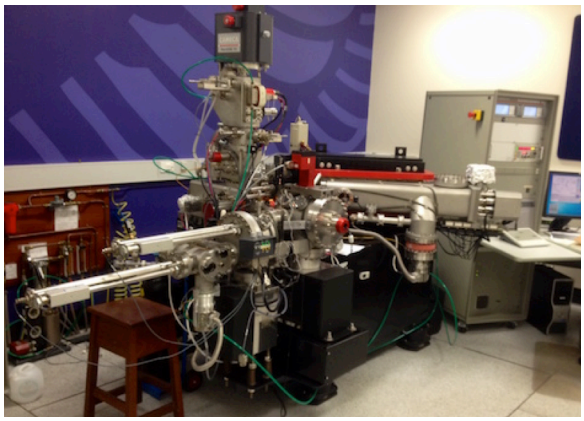
Using NanoSIMS to study Glycogen Accumulating Organisms in winery wastewater.



FINAL REPORT to
AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: AGT 1502

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Research Organisation: **THE UNIVERSITY OF ADELAIDE**

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Abstract:

Winery wastewater (WWW) samples containing Glycogen Accumulating Organisms (GAO) were analysed using NanoSIMS. The main objective of this study was to observe the effect of nitrogen addition on the carbon metabolism of WWW bacteria. Incubations with different ratios of stable isotope labelled substrates were performed prior to analyses. Images showing the intracellular 15N and 13C enrichment of GAO were obtained. Significant differences were observed in the 15N and 13C levels of GAO incubated with different carbon:nitrogen ratios.

Executive summary:

- Cristobal Onetto (PhD student- University of Adelaide) travelled to the facilities of the Centre of Microscopy (University of Western Australia) to analyse winery wastewater samples using a NanoSIMS instrument.

- Established collaborations with Australia's experts in NanoSIMS

- Two set of samples from different Australian wastewater treatment plants were analysed, one from a small winery in Yarra Valley, Victoria and another from a large winery in the Barossa Valley, South Australia.

- Basic principles in the operation of a NanoSIMS instrument were obtained.

- Skills in the use of NanoSIMS data processing software (Image J,

OpenMIMS) were obtained.

- Differences in the carbon enrichment of GAO in response to different nitrogen additions were observed.

Key activities and outcomes

Prior to the funded travel, incubations with different carbon (^{13}C acetic acid) and nitrogen (^{15}N urea) ratios were performed in winery wastewater samples that contained Glycogen Accumulating Organisms. The stable isotope labelled substrates where added to investigate the effect of different carbon:nitrogen ratios. GAO were identified as *Defluviicoccus vanus* by the application of a CY3-labelled Dflu probe. The samples were placed on silicon wafers and then mapped using confocal microscopy image stitching and fluorescent in situ hybridization. These images were essential for the selection of raster areas in the NanoSIMS instrument.

The NanoSIMS instrument includes an integrated CCD camera. By correlating the confocal images with the observations from the camera we could select and save coordinates of interest to be analysed for their isotopic enrichment.

The aim was to obtain ^{13}C, ^{15}N and ^{32}S images from GAO under different carbon to nitrogen ratios of 100:1, 60:1, and 20:1. 6 samples were analysed from both treatment plants and a set of approximately 4 to 10 cells were measured from different locations in each sample.

As this is the first application of NanoSIMS to WWW samples internationally, special consideration was required for the calibration of the instrument. The topography of

these samples was not homogeneous, and consequently imaging took longer than for other type of samples. After obtaining these isotopic images, the enrichment values for the cells were calculated by selecting the areas of interest in each image and then adding the counts of pixels for each mass.

From the results we could observe significant differences in the carbon enrichment between the different treatments, indicating that changes in the nitrogen addition have an effect on the carbon uptake by these WWW microorganisms.

Further analysis of this data needs to be performed to explain more precisely the effect of nitrogen in the GAO carbon metabolism.

Conclusion

This is the first application of NanoSIMS in WWW in Australia, and it is also the first application of this instrument to study GAO internationally.

From the results we can observe that the exposure to different nitrogen levels results in different utilisation of carbon by the GAO. This information is will play a vital role in resolving the metabolism of the GAO in WWW and gives us direction in developing targeted control strategies for these organisms in WWW treatment plants.