# Employing microbes and earthworms to treat winery and brewery wastewaters



Waters Collaborative **Research Grant** 

# Introduction

Finding means to recycle wastewater for other uses is necessary due the limited access of freshwater. The most energy-intensive portion of the treatment process is aerating the wastewater to facilitate microbial oxidation of compounds. Since 2015, we have partnered with D'Argenzio Winery in Santa Rosa, to test a system for onsite treatment of wine wastewater that does not require aeration. Wastewater (WW) from a pH neutralization tank is pumped into two tubular microbial fuel cells (MFCs), one of which flows through a earthworm-containing filter to irrigate landscaping, while other recycles back to the tank (Figure 1). Here we report on the capacity of natural bed media filters, with and without earthworms, to remove organic compounds from the winery MFC effluent as well as from full strength brewery WW.

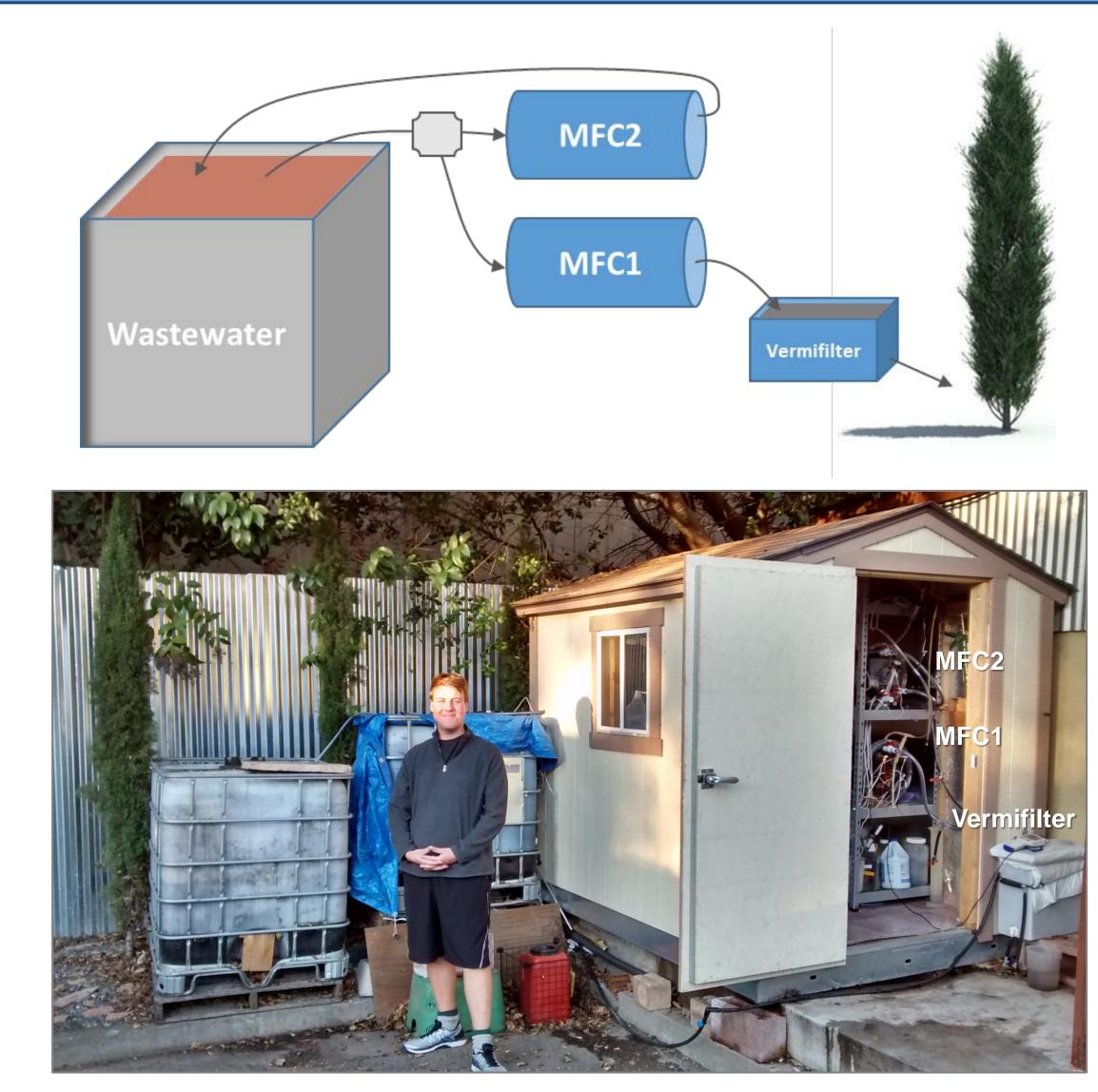


Fig. 1. Top: A flow schematic of the treatment system. Bottom: The field site located at Vintners Square in Santa Rosa, CA.

# Summary of previous findings

- □ 75.2% of the organics in the WW were removed by passage through MFC1 at a 6-day hydraulic retention time.
- □ Most of the WW organics consumed in MFC1 are converted by the internal microbes to methane-rich biogas, while the production of electrical power, though small, is a useful indicator of MFC health status.
- Organics removal activity correlated positively with higher minimum daily outdoor air temperature.
- Consumption of acids increased the pH of the water across MFC1 by 0.67.
- □ The vermifilter removed 35.2% of the residual organics from the MFC1 effluent, which is substantially better than the 8.7% removal performance of a sand filter that preceded it.
- □ Plant growth assays demonstrated that, although the quality of the water for irrigation improves across the system, growth-inhibitory components still remain at the end of the treatment process. We hypothesize that these may be phenolic compounds.

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# Study aims

- Test of organic filtration beds as a final polishing step for MFC-treated winery wastewater for primary treatment of brewery WW.
- Measure removal of organics from WW (as chemical oxygen demand; COD) and phenolics (from wine WW only).
- Determine the impact of earthworms (*Eisenia fetida*) on the removal activities of the filter beds.

# Polishing of MFC-treated winery wastewater

- Degradation of tannins and polymeric anthocyanins occurred across the winery vermifilter but not within the MFCs (Fig. 2), but levels of total phenolics did not decrease appreciably across the system (data not shown).
- □ Filters with peat: paper bed medium did not remove organics from MFC1 effluent (data not shown) but did support the growth of earthworms (Fig. 3)
- □ From the bed medium of a vermicompost fed with MFC1 effluent a *Pseudomonas aeruginosa* bacterium and Penicillium fungus were co-isolated on an agar medium with hydroquinone as the sole carbon source. The bacterium showed a positive tropism for the fungus upon co-reintroduction on an agar medium (Fig. 4).

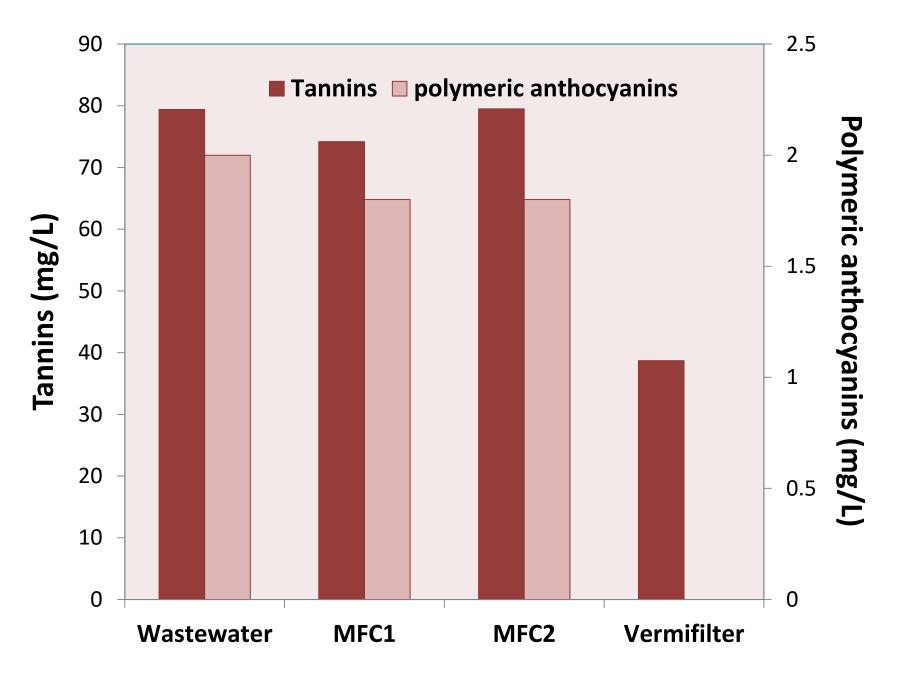


Fig. 2. Concentrations of phenolics in water flowing through the Vintners Square treatment system. Left: Tannins and polymeric anthocyanins; detection limit, 0.2 mg/L (Sample date, 5/14/2018). *Right:* Relative clarity of MFC1 effluent compared to effluent from the vermifilter.

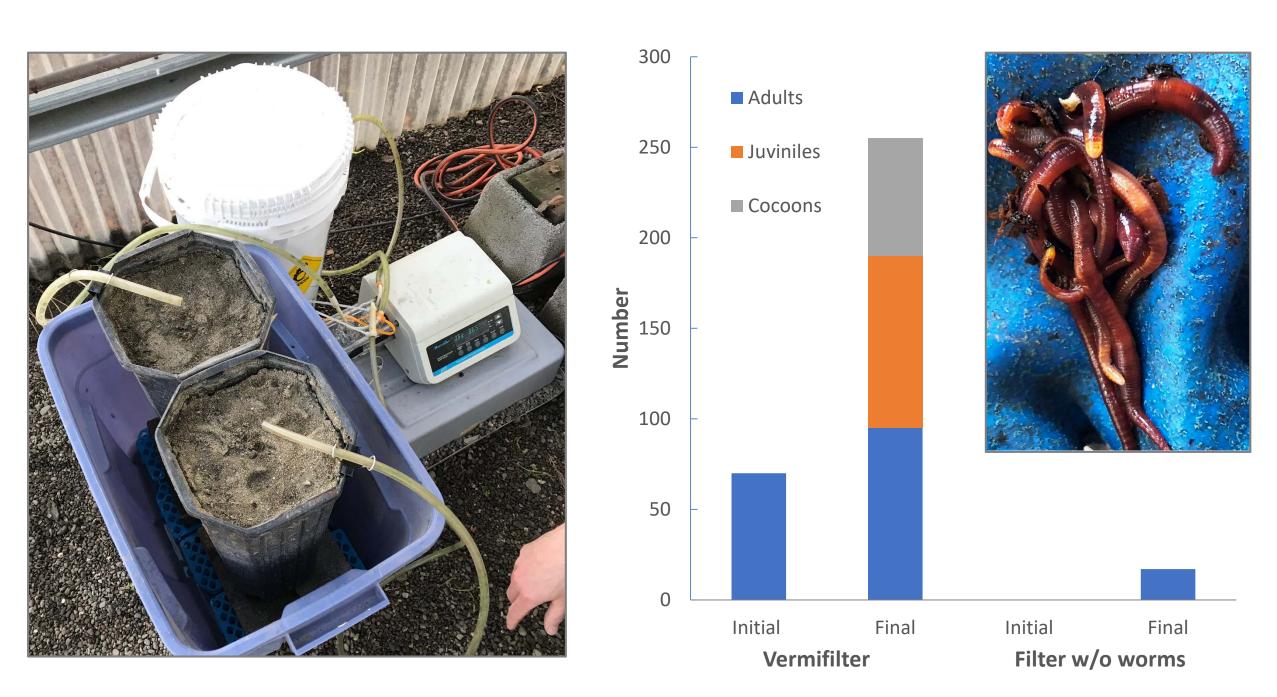


Fig. 3. Experimental 7-L filters containing a 1:1 peat: paper bed medium with sand cover (left). Worm counts and life stage upon inoculation and after two months of operation (*right*). Note that 17 adult worms had migrated to the uninoculated filter by the end of the experiment. *Photo inset: Eisenia fetida*.

### Conclusions and future prospects:

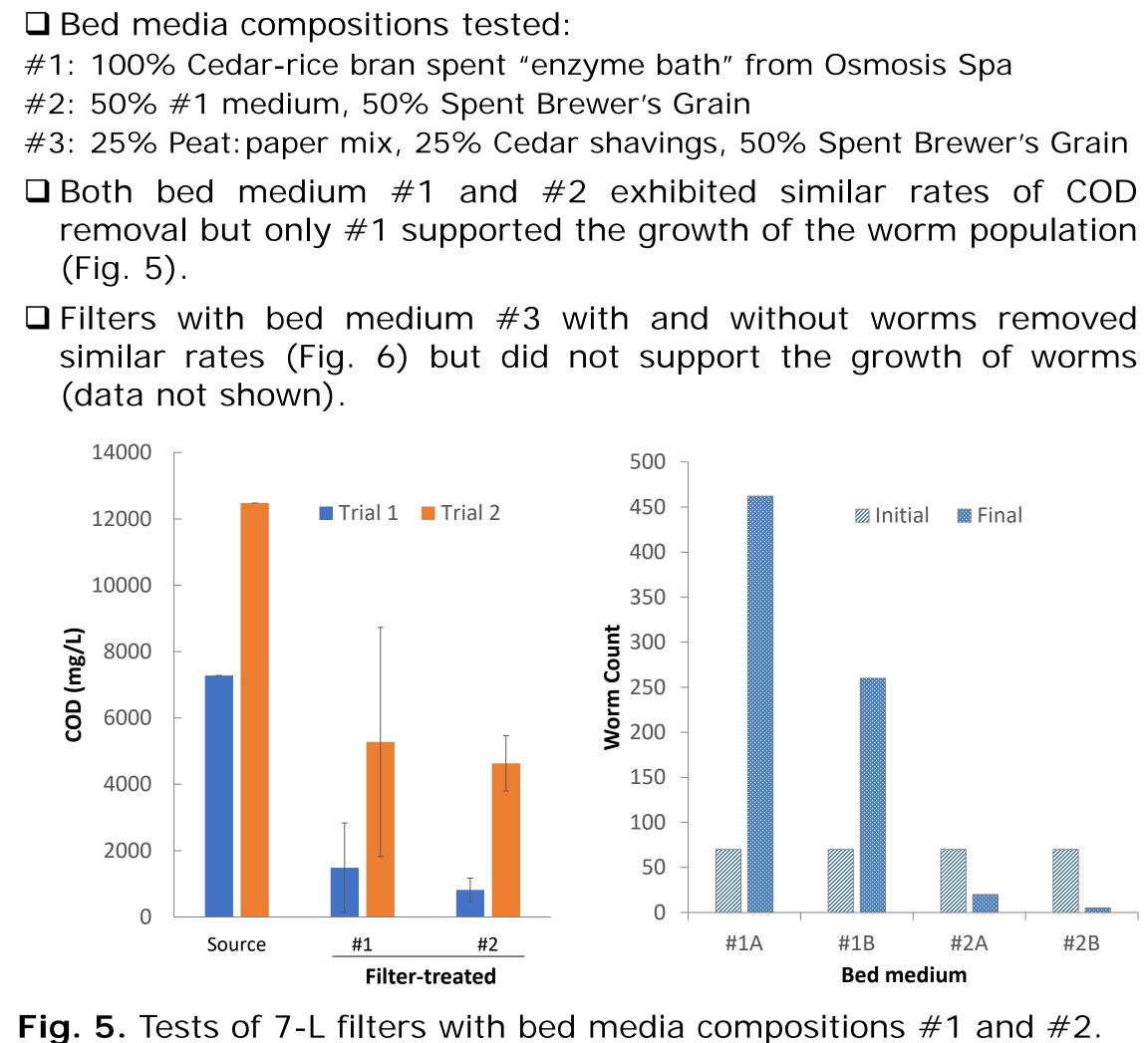
- The bed medium in the Vintners Square vermifilter (see medium #1, next section) is superior to peat: paper bed medium for removal of COD from MFC-treated winery WW effluent. Reduced coloration conferred by the Vintners Square medium may be due to its removal of polymerized phenolics from the water.
- Depending microbes will be investigated for potential use in inoculating bed media to further increase the removal rate of phenolics by the filters.



Vermifilter MFC1



Fig. 4. Positive tropism of the *P*. aeruginosa vermicompost isolate (1<sup>st</sup> from the right) to a perpendicular streak colony of the Penicillium vermicompost isolate.



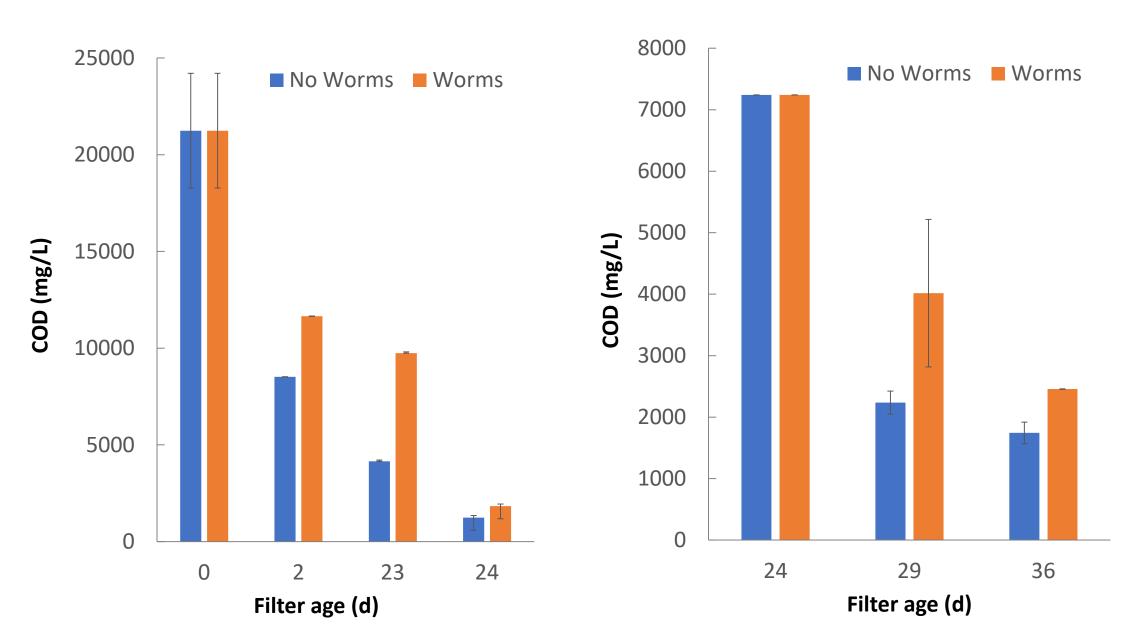


Fig. 6. COD removal from brewery wastewater by 7-L filters containing bed medium formulation #3. First trial (top left). Debris was removed from the sand layer on day 23. To initiate the second trial new wastewater was added on day 24 (top right).

### **Conclusions:**

□ Filters efficiently remove organics from brewery wastewater irrespective of the presence of earthworms. □ Spent Brewer's grain appears to not be conducive to the growth of earthworms.

# Acknowledgements

We are grateful to the owner Ray D'Argenzio and staff of D'Argenzio Winery for hosting our research project, to Alameda Island Brewing Company for providing wastewater, and to Osmosis Spa for the spent enzyme bath medium. Many Sonoma State students have contributed to the development and testing of the treatment system.

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## **Treatment of brewery wastewater**

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Averaged COD concentrations of source WW and 1-week filter-cycled WW (*left*) and individual filter worm counts at 3 months (*right*).

