



Wetlands for Wastewater: A Visual Approach to Microbial Dynamics

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Abstract:

The complex character of distillery wastewater comprises high concentrations of sugars, hemicelluloses, lignin, dextrans, resins, polyphenols and organic acids which are recalcitrant to biodegradation. Microorganisms play a key role in the production and degradation of organic matter, environmental pollutants, and cycling of nutrients and metals. Due to their short life cycles microbes respond rapidly to external nutrient loading, with major consequences for the stability of biological systems. Microbial biodiversity may consequently be considered as predictive and management tool in wetland ecosystems.

We evaluated the feasibility of wetlands to treat winery and distillery effluents in experimental systems based on constructed wetlands, including down-scaled on-site distillery wetlands, small-scale controlled greenhouse systems, and bench-scale mesocosms. Chemical, visual and molecular fingerprinting (t-RFLP) techniques were applied to study the dynamics of planktonic and attached (biofilm) communities at various points in wetlands of different size, retention time and geological substrate, and under influence of shock nutrient loadings. Variable-Pressure Scanning Electron Microscopy (VP-SEM) was applied to hydrated samples from experimental wetlands to visualize microbial colonization, morphotype diversity and distribution, and 3D architecture facilitating interaction of microbial taxa.

Methods:

Experimental Wetlands varying in size (two of 45x6m, three of 6x3m), geological substrate (gravel, sand), and retention time (14d for 45m [1 sand, 1 gravel], and 4.5d, 9d and 18d for 6m [all gravel]) respectively) were constructed onsite at a distillery, planted with *Typha* and *Phragmites* spp, and fed with distillery wastewater (COD < 12,000 mg/l).

Mesocosms (20cm x 15cm x 15cm perspex) allowing control of environmental conditions, were filled with gravel similar to the natural down-scaled wetlands. A COD shock load of 24,000 mg/l, as well as variation in light and darkness, were applied to evaluate the responsiveness of biofilm communities to environmental fluctuations.

Molecular, Visual and Chemical characterization: Planktonic and biofilm communities were characterized molecularly with t-RFLP, using restriction enzymes *Alu1* and *Rsa1* (Du Plessis 2006, PhD dissertation). Visualization with VP-SEM was applied in Variable Pressure mode at 50-60Pa, using BSE detection at 15 kV. COD was determined with the Reflux Titrimetric method (Amer Public Health Association 1998).

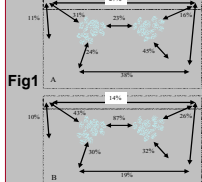


Figure 1 illustrates the correspondence in t-RFLP profiles between microbial communities in different regions of the 45m experimental wetlands. Schematic diagrams of A (gravel support) and B (soil support) show the percentage similarity based on the number of corresponding tRFs between microbial communities present in the influent (top left corner) and effluent (top right corner), biofilms on roots at inflow and outflow, as well as on support geological material. Similar high variability occurred between sampling dates while overall community function (COD removal) remained stable. Variability was similar for both restriction enzymes (*Alu1* and *Rsa1*)

	Effluent 4.5 d	Effluent 9 d	Effluent 18 d
In	21%	26%	8%

Tabulated (left) is the percentage similarity between microbial communities present in the influent and effluent of the three 6m experimental gravel wetlands with RT of 4.5d, 9d and 18 days respectively.

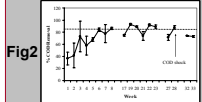
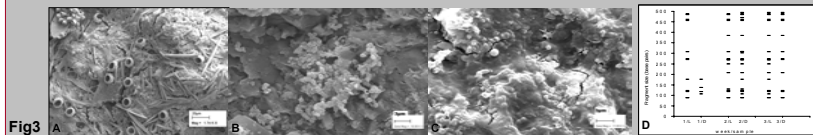


Figure 2. Mesocosms were used to replicate the experimental wetland systems, and to allow for controlled variation of environmental conditions – in this case a COD shock load of 24,000 mg/l. In this and similar experiments COD removal efficiency was restored within 3 weeks. The shaded areas indicate periods when fresh water was passed through each system. For comparison, the dotted line represents the average COD removal efficiency of the 45 m gravel wetland after 12 months of operation



Light/Dark: Influence of primary producers on community form and function

Figure 3. SEM micrographs of microbial communities cultivated in the light versus dark show conspicuous algae and diatoms in biofilms growing on the support gravel substrate exposed to light (A). Early biofilm development was slower in the absence of light (B), while biofilms developing in the deeper layers and shaded sides of the gravel in the system exposed to light formed confluent structures with more EPS production (C). The presence of algae and their exudates resulted in higher COD removal efficiency, although the difference was not significant. t-RFLP fingerprint patterns of microbial communities cultivated in the light (L) and dark (D) followed by digestion with *Alu1*, show shifts in community composition over a 3 week period, and the effect of light (algae) on community profiles

Microbial Community development over Time

Figure 4. SEM micrographs illustrate the rapid microbial colonization of gravel in wetland systems, and morphotype diversity in the resultant biofilms. Initial sparse colonization by pioneering species (Wk1) is rapidly followed by biofilm proliferation and EPS development (Wk3+) over the ensuing weeks, with the stratified appearance of the biofilm architecture visible in cases where cracks in the biofilm appear. Microcolonies of yeast (Y) and bacteria (B) are closely associated, with algae (A), fungi, protists (P) and diatoms (D) forming superficial layers covering EPS-enveloped microbial layers. This study paves the way for application of SEM-ISH (*In Situ* Hybridization) with nanogold-labelled oligonucleotide probes enabling potential identification of microbial taxa. t-RFLP fingerprinting of dominant restriction fragments (applying restriction enzyme *Alu1*) from biofilms in the mesocosm gravel during the early stages of development and in response to a COD shock at Wk 8, show the sequential nature of community development and the notable change in community structure that persisted beyond 3 weeks after the shock. In contrast to restoring function over the period (as indicated by stable COD removal, Fig 2)

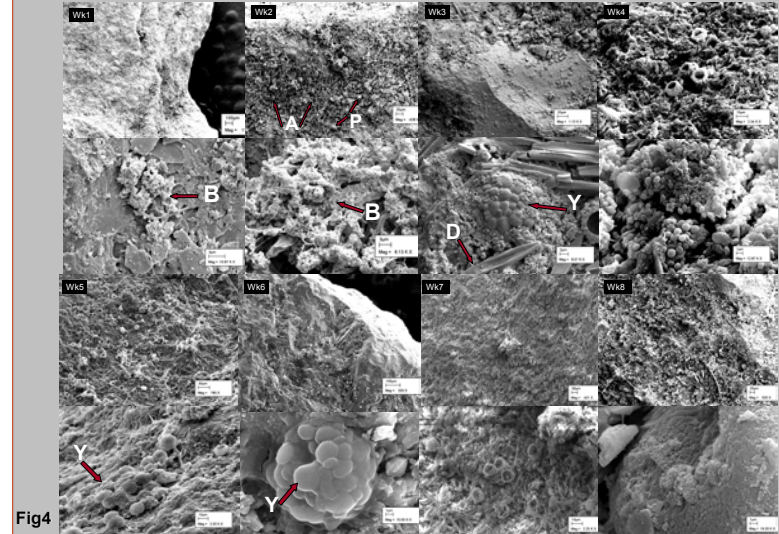
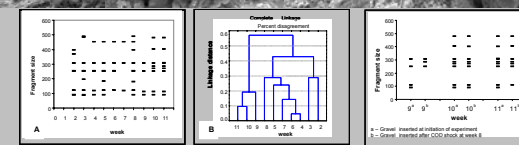


Fig4



Integration COD removal varied with geological substrate, and was positively correlated with retention time in gravel wetlands. The presence of algae affected microbial community composition and biofilm structure. Planktonic and biofilm communities varied markedly in different regions of the wetland and over time, as indicated by whole-community t-RFLP and VP-SEM. The change in distillery effluent composition may selectively stimulate and suppress growth of different microbial populations. Various biotic and abiotic factors influence microbial community composition in wetlands, and unique micro-environments may be established in response to factors that facilitate microbial degradative processes.

Conclusions

The high microbial diversity along spatial and temporal gradients, as well as the responsiveness to fluctuations in the physico-chemical environment, indicate a highly adaptable wetland ecosystem. This adaptability suggests that microbial communities maintain metabolic function by modifying species composition in response to fluctuations in their environment. It seems apparent that microbial variation and community plasticity may indeed be the distinguishing characteristics of a successful wetland system.