



## LJMU Research Online

**Bergman, RC, Wright, DA and Ralebitso-Senior, TK**

**Ecological Media Reveal Community Structure Shifts in a Municipal Wastewater Treatment Train**

<http://researchonline.ljmu.ac.uk/id/eprint/8208/>

### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Bergman, RC, Wright, DA and Ralebitso-Senior, TK (2015) Ecological Media Reveal Community Structure Shifts in a Municipal Wastewater Treatment Train. Journal of Bioremediation & Biodegradation, 06 (03). ISSN 2155-6199**

LJMU has developed [LJMU Research Online](#) for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact [researchonline@ljmu.ac.uk](mailto:researchonline@ljmu.ac.uk)

<http://researchonline.ljmu.ac.uk/>

# Ecological Media Reveal Community Structure Shifts in a Municipal Wastewater Treatment Train

Bergmann RC, Wright DA and Ralebitso-Senior TK\*

School of Science and Engineering, Teesside University, Borough Road, Middlesbrough, Teesside, TS1 3BA, UK

## Abstract

Unique ecological/habitat media derived from four phases of a municipal wastewater treatment plant revealed the highest diversity (2.55-2.86) and evenness (0.79-0.87) for the raw sewage (R) medium. Richness was, however, inoculum- and media-dependent hence inocula R and P recorded the highest counts on media A and F, respectively.

**Keywords:** Municipal wastewater; Habitat media; Ecological index; Community structure

## Introduction

The continuing need for robust, sustainable and reliable (bio) technologies that are also characterized by minimal carbon emissions/footprints must be matched by an equally growing and sound knowledge base of the underpinning microbial communities. Despite their analytical power/capacity, ecogenomic tools do not provide phenotypic or physiological characterization of novel and previously uncultivated microbial strains [1]. A discrepancy that limits a more complete understanding of microbial and process dynamics. Although research continues on culturing strains that were considered unculturable, particular focus is on soil ecosystems [2-4]. A limited understanding exists, in particular, of the phenotypes underpinning a key biotechnology, wastewater treatment.

As a result, directed research must address the well-documented limitations of molecular-based methods [5] so that seemingly important community members or representatives of functionally significant groups can be cultivated for further analyses. Despite the debate for/against cultivation, with/without molecular analyses [6,7], the approach has been exemplified by many researchers [1,4,8-11] who used different approaches, and remains essential to elucidate the physiological responses of process microbial communities for subsequent exploitation.

## Material and Methods

### Habitat media preparation and inoculation

Samples (1 L) were collected from four stages of a municipal wastewater treatment plant - raw sewage (R), primary settlement tank (P), aeration tank (mixed liquor) (A) and final effluent (pre-UV treatment; F). Following thorough gentle mixing, 50 mL aliquots were centrifuged (15,000 rpm x g; 15 minutes, 4°C) and the supernatants series filtered (Whatman: Grade 2; Nalgene: 0.45 µm; 0.2 µm) and autoclaved (121°C; 20 minutes; 15 lb psi). Each sterile filtrate was mixed 1:1 (v/v) with autoclaved 2X technical agar (Oxoid, U.K.) and allowed to set at room temperature for 48 hours prior to duplicate inoculations with 100 µL of 10-fold dilution of the original wastewaters.

### Colony DNA extraction and PCR

Following incubation at 25°C for 72 hours, total colony scrapes were re-suspended in 200 µL sterile saline (0.9% w/v NaCl) for DNA extraction (FastDNA™ SPIN Kit, MP Biomedicals, U.K.). The 16S rRNA gene (V3 region) was amplified with the F357GC/R518 primer set [12] with 1 µL DNA templates. The PCR was made [13] with 25 µL reaction

volumes on a Primus 96 Plus (MWG Biotech, Ebersberg, Germany) and amplicons (5 µL) visualised on 1.5% (w/v) agarose gels stained with SYBR Safe (Molecular Probes, Eugene, U.S.A.).

### DGGE community profiling

DGGE profiling [12] used an Ingeny Phor U system (Ingeny, Leiden, The Netherlands). Amplicons (20 µL) were loaded onto a 10% (w/v) polyacrylamide gel with a 30-65% denaturing gradient and electrophoresed (18 h; 60°C; 100V) in 0.5× TAE buffer. The gels were stained for 20 minutes with SYBR Gold (Molecular Probes, Eugene, U.S.A.) and digitised (Alpha Imager Gel Documentation System; ProteinSimple, Santa Clara, U.S.A.). Bands per lane were quantified (Phoretix 1D Pro gel analysis software; TotalLab, Newcastle, U.K.) and cluster analysed by the unweighted pair group method with arithmetic averages (UPGMA) [14,15].

### Ecological index and statistical analyses

Bacterial community diversity, evenness and similarity were assessed by the Shannon-Wiener diversity ( $H'$ ), Shannon evenness (E) [16] and Sørensen indices [17], respectively. Inoculum- and media-dependent differences in community structure, taxa richness and community similarity were evaluated by two-way ANOVA tests (Microsoft Office Excel 2007; Microsoft, Redmond, U.S.A.)

## Results and Discussion

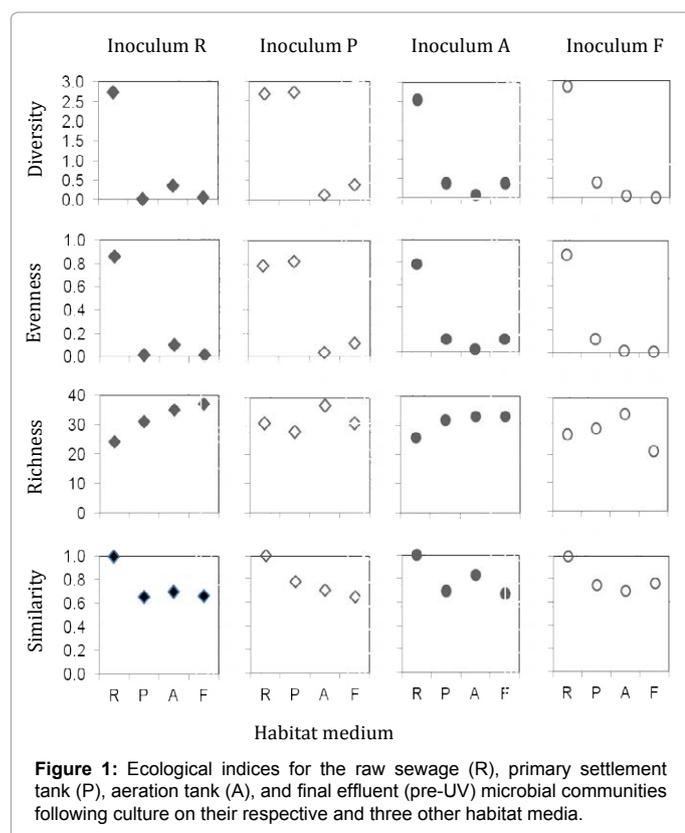
For all inocula, the Shannon-Wiener diversity ( $2.7 \pm 0.06$ ) and evenness ( $0.8 \pm 0.02$ ) were highest after cultivation on the raw sewage medium with statistically significant ( $p=0.0009$ ) decreases recorded (0.4,  $H'$ ; 0.12, E) for the other three media (Figure 1). These findings probably related to the high nutrient availability in the raw sewage tank and decreased types/concentrations of the subsequent [18]. Only the community from the primary settlement tank inoculated on its respective medium showed similarly high diversity and evenness values as the raw sewage culture, indicating a marked medium change.

\*Corresponding author: Ralebitso-Senior TK, School of Science and Engineering, Teesside University, Borough Road, Middlesbrough, Teesside, TS1 3BA, UK, Tel: +44 1642 342525; Fax: +44 1642 342401; E-mail: [K.Ralebitso-Senior@tees.ac.uk](mailto:K.Ralebitso-Senior@tees.ac.uk)

Received April 04, 2015; Accepted April 28, 2015; Published April 30, 2015

Citation: Bergmann RC, Wright DA, Ralebitso-Senior TK (2015) Ecological Media Reveal Community Structure Shifts in a Municipal Wastewater Treatment Train. J Bioremed Biodeg 6: 293. doi:10.4172/2155-6199.1000293

Copyright: © 2015 Bergmann RC, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



In general, the average taxa richness of all inocula increased ( $27 \pm 1.5$  to  $34 \pm 1$ ) through the treatment process indicating a change in the selection pressure [18,19]. The one exception was for the P-inoculum cultured on its respective medium, which decreased from 31 to 28. The Sørensen index of similarity indicated comparable community composition throughout the treatment process and suggested that species abundance only varied at different stages.

Emergent research has been made to culture previously uncultured microbial strains from soil ecosystems with only limited investigations for wastewater management. This is in direct contrast to the multiple molecular-based analyses that have been applied, developed and optimised to characterise the complex microbial communities in different wastewater biotechnologies. Matching and complementing genotypic tools with culture-based (phenotypic) analyses will facilitate: (i) identification of novel strains; (ii) quantification of their upper and lower physiological limits and function characterisation; (iii) culture maintenance of important monocultures/ communities [20]; and (iv) more informed exploitation in wastewater treatment plants for increased efficiency/stability/reliability.

## Conclusion

This study exemplified the use of wastewater-based media to culture microbial communities that characterized specific phases of a continuous treatment train. DGGE-based analysis then facilitated measurements of diversity, evenness, taxa richness and similarity between treatment stages. Future work should entail detailed physiological/phenotypic studies of the cultivated strains/communities and, subsequently, sequencing to allow genotypic comparisons with these and uncultured wastewater species in existing databases.

## Acknowledgements

This study was supported partly by the Teesside University SAR13 scheme.

## References

- Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, et al. (2010) Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. *Appl Environ Microbiol* 76: 2445-2450.
- Liebeke M, Brözel VS, Hecker M, Lalk M (2009) Chemical characterization of soil extract as growth media for the ecophysiological study of bacteria. *Appl Microbiol Biotechnol* 83: 161-173.
- Bastida F, Nicolás C, Moreno JL, Hernández T, García C (2010) Tracing changes in the microbial community of a hydrocarbon-polluted soil by culture-dependent proteomics. *Pedosphere* 20: 479-485.
- Bucková M, Godocíková J, Zámocký M, Polek B (2010) Screening of bacterial isolates from polluted soils exhibiting catalase and peroxidase activity and diversity of their responses to oxidative stress. *Curr Microbiol* 61: 241-247.
- Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59: 143-169.
- Tyson GW, Banfield JF (2005) Cultivating the uncultivated: a community genomics perspective. *Trends Microbiol* 13: 411-415.
- Ritz K (2007) The plate debate: cultivable communities have no utility in contemporary environmental microbial ecology. *FEMS Microbiol Ecol* 60: 358-362.
- Sait M, Hugenholtz P, Janssen PH (2002) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environ Microbiol* 4: 654-666.
- Chaer GM, Fernandes MF, Myrold DD, Bottomley PJ (2009) Shifts in microbial community composition and physiological profiles across a gradient of induced soil degradation. *Soil Sci. Soc. America J.* 73: 1327-1334.
- Bollmann A, Palumbo AV, Lewis K, Epstein SS (2010) Isolation and physiology of bacteria from contaminated subsurface sediments. *Appl Environ Microbiol* 76: 7413-7419.
- Bougnom BP, Knapp BA, Elhottová D, Koubová A, Etoa FX, et al. (2010) Designer compost with biomass ashes for ameliorating acid tropical soils: effects on the soil microbiota. *Appl. Soil Ecol.* 45: 319-324.
- Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59: 695-700.
- Manefield M, Whiteley AS, Griffiths RI, Bailey MJ (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Appl Environ Microbiol* 68: 5367-5373.
- Silva EP, Russo CAM (2000) Techniques and statistical data analysis in molecular population genetics. *Hydrobiologia* 420: 119-135.
- Fromin N, Hamelin J, Tarnawski S, Roesti D, Jourdain-Miserez K, et al. (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environ Microbiol* 4: 634-643.
- Shannon CE, Weaver W (1949) *The Mathematical Theory of Communication*. Champaign: University of Illinois Press.
- Sørensen T (1957) A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. *Kongelige Danske Videnskaberne Selskab* 5: 1-34.
- Johnson DR, Lee TK, Park J, Fenner K, Helbling DE (2014) The functional and taxonomic richness of wastewater treatment plant microbial communities are associated with each other and with ambient nitrogen and carbon availability. *Environ. Microbiol.*
- Vuono DC, Munakata-Marr J, Spear JR, Drewes JE (2015) Disturbance opens recruitment sites for bacterial colonization in activated sludge. *Environ Microbiol*.
- Goodhead AK, Head IM, Snape JR, Davenport RJ (2014) Standard inocula preparations reduce the bacterial diversity and reliability of regulatory biodegradation tests. *Environ Sci Pollut Res Int* 21: 9511-9521.