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# Understanding the mechanisms involved in *Escherichia coli* decay during wastewater treatment in High Rate Algal Ponds

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# Abstract

Little is known about the mechanisms and magnitude of pathogen disinfection in High Rate Algal Ponds (HRAPs). However, maturation ponds are used worldwide for wastewater disinfection, and pathogens can experience similar environmental conditions in maturation ponds and HRAPs. The literature suggests that pathogen removal in maturation ponds is primarily supported by sunlight-mediated mechanisms (direct DNA damage, endogenous photo-oxidation, and exogenous photo-oxidation), and a range of poorly characterized "dark" mechanisms. Based on this evidence, and knowing HRAPs are specifically designed to optimize light supply into the broth, there is reason to believe sunlight mediated disinfection mechanisms should be significant in HRAPs. This thesis therefore aimed at identifying and quantifying the mechanisms responsible for *Escherichia coli* (*E. coli*) decay in HRAPs under the hypothesis that understanding the mechanisms involved in disinfection during wastewater treatment in HRAPs can provide the scientific foundation needed to optimize the design and operation for this critical wastewater treatment service. *E. coli* was selected for being an established indicator of the removal of faecal contamination during wastewater treatment.

Two pilot scale HRAPs (0.88 $m^3$) were commissioned and monitored over 1-2 years, showing a mean *E. coli* decay coefficient of 11.90 $d^{-1}$ (std = 24.05 $d^{-1}$, N = 128), equivalent to a mean *E. coli* log removal of 1.77 (std = 0.538, N = 128) when operated at a hydraulic retention time (HRT) of 10.3 d (std = 2.01 d, N = 139). Hourly monitoring showed high daily variations of *E. coli* log removal (up to 2.6 $\log_{10}$ amplitude) during the warmest summer days, with the lowest *E. coli* cell counts observed in the late afternoon, when the broth pH, dissolved oxygen concentration, and temperature typically reached peak values in the HRAP. No mechanisms driving *E. coli* removal in HRAP could be identified during the monitoring of pilot scale HRAPs so a mechanistic study of *E. coli* decay was performed at laboratory and bench scale to individually quantify potential mechanisms.

At laboratory scale under various conditions (e.g. darkness vs sunlight exposure, neutral pH vs alkaline pH, RO water vs filtered HRAP broth), direct DNA damage, endogenous photo-oxidation, and high-pH toxicity were identified as the main mechanisms contributing to *E. coli* decay. Exposure to potentially toxic algal metabolites and exogenous photo-oxidation were not found to be significant under the conditions tested. Natural decay (i.e. decay in conditions identified not to be detrimental to *E. coli* survival) was never significant. The impact of predation could not be investigated due to technical challenges although pilot scale observations suggested this mechanism may be significant in certain conditions.

Subsequent bench-scale tests conducted in HRAP broth indicated that temperature-dependent uncharacterized dark decay (i.e. decay in conditions not known to be detrimental to *E. coli* survival) was likely to be the dominant mechanism of *E. coli* removal under conditions relevant to full-scale operation. Temperature-dependent high-pH toxicity was confirmed to further increase *E. coli* decay at pH levels commonly reached in HRAPs. The contribution of sunlight mediated mechanisms was however not significant. Exposure to toxic algal metabolites was suspected to cause significant *E. coli* decay at times of extreme photosynthetic activity, but more research is needed to confirm this mechanism and its true significance.

Results from laboratory scale and bench scale experiments enabled the development of a model capable of predicting *E. coli* decay in HRAP broth according to pH, temperature, and sunlight intensity distribution. A model predicting HRAP broth temperature and pH according to design and weather data was also developed and validated against data from the pilot scale HRAPs monitored during this study for temperature (average absolute error of predictions 1.35°C, N = 25,906) and pH (average absolute error of predictions 0.501 pH unit, N = 23,817). Coupling the *E. coli* decay model with the environmental model enabled long term predictions of *E. coli* removal performances in HRAP for various weather conditions, design, and operational regimes. Simulations predicted that a 3-HRAPs series would sustain average yearly *E. coli* log-removal of 3.1 in Palmerston North, New Zealand when operated in conditions similar to the pilot scale HRAPs used in the present study. Such performance would deliver year round compliance with local microbial quality guidelines. Disinfection performance could be further improved by increasing the hydraulic retention time, lowering the depth, or collecting the effluent once daily in the late afternoon while letting HRAP depth fluctuate.

Overall, this research challenges the common belief that sunlight mediated disinfection mechanisms contribute the most to pathogen removal in HRAPs. Instead, uncharacterized dark decay was predicted to cause 87% of the total *E. coli* decay over one year simulation. High-pH toxicity may significantly contribute to overall *E. coli* decay in specific conditions (e.g. low depth where high-pH toxicity was predicted to account for 33% of total yearly *E. coli* decay), while sunlight mediated disinfection was limited under all simulated designs and operations (highest contribution predicted being 16% of total yearly *E. coli* decay). Because this study also confirmed the potential of HRAP to achieve sustained wastewater disinfection, further research is needed to better characterize dark decay mechanisms (for *E. coli* and other key indicators) as this knowledge has the potential to further improve HRAP design and operations for wastewater disinfection.

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