Disinfection of Selected Finfish Pathogens Important in Irish Freshwater Aquaculture using Pulsed Ultraviolet Light Technology

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Abstract

The aquaculture sector is becoming the fastest growing food sector in the world. The freshwater aquaculture sector in Ireland is worth an estimated €115 million, providing employment to 1,936 in rural inland communities. Stringent limits on water abstraction rates necessitate recirculation of processed water. Traditional methods struggle to cope with the increasing demands on recycled water to meet food safety and environmental regulations. These methods of production and the increased bio-load in recirculated water may hinder the potential development in aquaculture by promoting harmful finfish pathogens causing detrimental effects on fish stock. The introduction of advanced technology including novel non-chemical disinfection systems and aeration processes seek to improve the energy efficiency, productivity and overall sustainability of the freshwater farming industry. This study investigates the use of pulsed ultraviolet light to control selected problematic finfish pathogens in Ireland. Pulsed ultraviolet light has been validated using *E.coli* as a model organism. A bacterial population of $1x10^6$ CFU/ml of *E.coli* following treatment with this disinfection technology was reduced to a non-detectable number. The fish pathogen *Aeromonas salmonicida* was subjected to this disinfection regime and the results indicate a decrease in the bacterial load to a non-detectable number following treatment.

Introduction

Justification:

- Intensification of aquaculture practices has become a necessity due to lower fishing capacity worldwide and higher fish demands due to increased consumption rates per capita (Donnelly, 2011).
- Disease is a major factor in the decrease in value of the Irish Aquaculture industry which, according to BIM declined from €133m in 2012 to €115m in 2014, and so lies the need for a novel efficient disinfection regime.
- Infectivity due to the worldwide pathogen *Aeromonas salmonicid*a results in the development furunculosis, a disease characterized by abscess formation on fish flanks; salmonids being the main hosts affected.
- *Aeromonas salmonicida* is a gram negative, rod-shaped bacteria which targets the gills, anus and mouth (Austin, 1997).

Figure 1. Rainbow trout with furunculosis disease (Nilsen, 2012)

Pulsed UV Light (PUV):

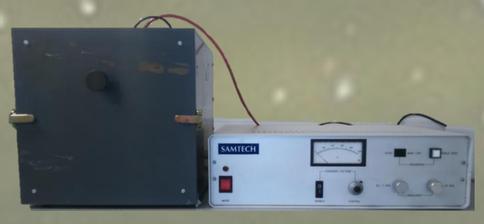

Figure 2. Pulsed UV Light Apparatus

- Ultraviolet light prevents DNA replication of microbes due to alteration of pyrimidine bases of nucleic acids resulting in thymine dimers (Lamont *et al.,* 2004).
- The PUV system produces an intense UV flash with a high peak power and high current (Sharifi-Yazdi & Darghahi, 2006) resulting in irreversible DNA alteration and rapid inactivation (Hosseini, *et al*., 2011). This offers an advantage over conventional UV due to a more energy efficient system (Krishnamurthy, *et al.,* 2007).

Aim: This project aims to develop a novel disinfection regime for the treatment of problematic finfish pathogens in Irish freshwater.

Methods

E. coli was used as a bacterial surrogate to validate the PUV process as per Garvey *et al.,* (2011). Bacteria cultured to a density of $1x10^6$ CFU/ml were subjected to static UV light and pulsed UV light for a range of seconds and pulses, respectively. The samples were grown on nutrient agar plates and colonies enumerated following appropriate incubation periods. Important parameters for the PUV system are voltage and pulses; for static UV the most important parameter is time. Death (D) values, the length of time or the number of pulses required to reduce the bacterial count in CFU/ml by 1 log, were calculated for both systems. Turbidity studies were carried out using YSI turbidity standards to determine the effects of turbidity on PUV penetrance, measured in Nephelometric Turbidity Units (NTUs). *A. salmonicida* was exposed to PUV light in diluents of differing turbidity; the control was Ringer's solution.

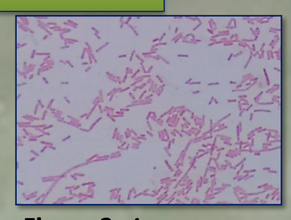

Figure 3. *Aeromonas salmonicida* under oil immersion (100X magnification)

Results & Conclusions

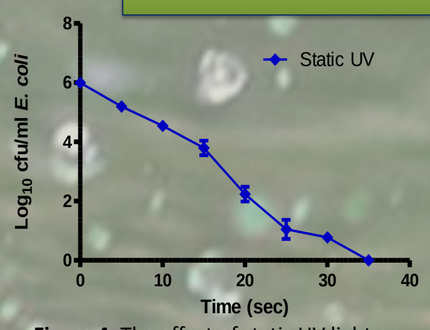

Figure 4. The effect of static UV light on *E. coli* (D Value = 4.80)

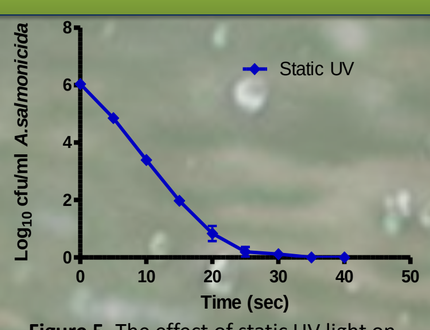

Figure 5. The effect of static UV light on *Aeromonas salmonicida* (D Value = 4.27)

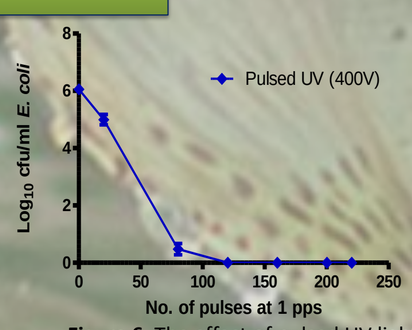

Figure 6. The effect of pulsed UV light on *E. coli* (D Value = 17.40)

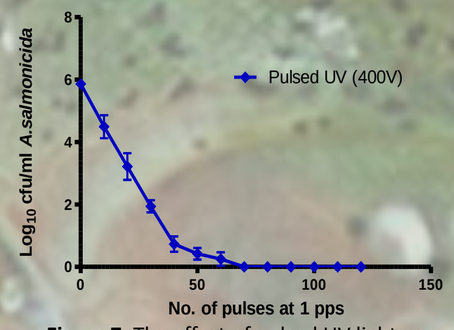

Figure 7. The effect of pulsed UV light on *Aeromonas salmonicida* (D Value = 9.66)

- *E. coli* and *A. salmonicida* were inactivated following 35 seconds of static UV light exposure displaying a 6 $\log_{10}$ CFU/ml reduction.
- *A. salmonicida* demonstrated greater susceptibility to PUV treatment over *E. coli,* with a 6 $\log_{10}$ CFU/ml reduction after 70 pulses compared to 120 pulses for *E.coli.*

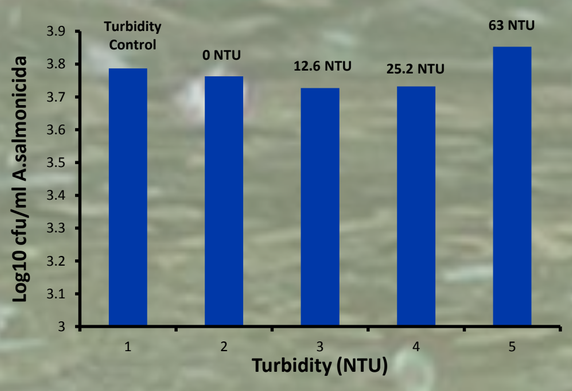

Figure 8. The effect of turbidity on PUV inactivation of *Aeromonas salmonicida* at 400V & 20 pulses (1pps).

- The initial cell density for both organisms was $1x10^6$cfu/ml; 1,000,000 times greater than the pathogenic load recorded from a collaborating fish farm.
- As the turbidity increases, the ability of PUV light to inactivate microorganisms decreases, however as the turbidity of water in a fish farm varies from 0 to 15 NTUs. PUV penetrance is sufficient to kill the pathogen in aquaculture.

Conclusions: This initial research demonstrates the ability of the pulsed ultraviolet light system to control the finfish pathogen *Aeromonas salmonicida* and indicates its potential in the aquaculture industry.

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