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#### **USING FLUORESCEIN AS A FLUOROPHORE TO TEST UV AND LIGHT PENETRATION OF FLOCCULATED PARTICLES**



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## Water treatment – raw water



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## Pathogen log removal







## **Challenging situations**



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The New Zealand Water & Wastes Association Wainra Antea

#### **Particle count going through UV treatment**



■2-5 um ■5-10 um ■10-15 um ■15-25 um ■> 25 um ■





### **Challenges for UV treatment**

Particles containing/shielding pathogens

Cryptosporidium 4-6 µm – chlorine resistant - 12 mJ/cm2 Giardia 10-14 µm - 11 mJ/cm2 Entamoeba histolytica 10-20 µm Coliforms 1-2 µm Viruses 143 mJ/cm2 Particulate material can be strongly UV absorbing e.g. humic substances, organic substances Particulate material can be UV reflecting e.g. silicate material





## **Examples of shielding in UV treatment**

UV disinfection effectiveness is reduced with organic particle sizes 2  $\mu m$  and smaller

[Templeton et al. (2005)]

UV light incapable of inactivating coliform bacteria protected by particles as small as 11  $\mu\text{m}$ 

[Cantwell and Hofmann (2008)]

Aggregated *Escherichia coli* (*E. coli*) and particle sizes larger than 25  $\mu$ m reduces the inactivation of *E. coli* 

[Kollu and Örmeci (2012)]





How to measure UV penetration in floc?

Can any information about the floc and what is in it be obtained?

One approach to measuring light penetration is using fluorescence





#### Want I need

Need a fluorescent probe

e.g. quantum dots or fluorescent dyes that can easily incorporated into floc

Ideally something that emits light at a higher wavelength that the wavelength used to induce fluorescence

Ideally something whose fluorescence lifetime changes depending on what it is attached to

Need a set-up that can easily measure fluorescence

Need a set-up that can measure fluorescence lifetime to give information about the floc



#### Fluorescein as a fluorescence probe



[Le Guern et al, 2020]





#### **Fluorescein emission**





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## Using fluorescence to measure light penetration



Light source



\* \* \*

Detector

- Fluorescence Intensity base
- Fluorescence Lifetime based







## **Examples of using fluorophores**

Fluorescent probes to detect different pathogens in environmental and medical studies.

[Key et al., 2009; Singh et al., 2016]

Fluorescent microparticles were substituted as Cryptosporidium parvum to test the efficiency of a metallic membrane in drinking water treatment.

[Li et al., 2019]

Modified microspheres to study pathogen transport in ground water.



## My prototype for measuring fluorescence and lifetime









#### **Data obtained**





Need to get the fluorescein into floc, ideally without changing floc properties

Made humic acid and kaolin floc using alum as a flocculant

Samples flocculated in a boltac jar tester

Examined the effect of humic acid, kaolin, alum and fluorescence concentrations at native pH and pH 7 on:

Turbidities, particle sizes, floc morphology and fluorescence



### **Particle size of the floc**

Unbuffered flocculated solutions at different concentrations of humic acid and kaolin and 0.48 mg/L fluorescein and alum dose adjusted according to solution concentration and zeta potential measurements.







### Particle size of the floc

Buffered flocculated solutions 60 mg/L humic acid and kaolin solutions at different fluorescein concentrations and 20 mL 1g/L alum to 400 mL humic solution and 14 mL for kaolin solution.





## **Floc morphology**

Microscope images (20x magnification)





Kaolin floc particles 20 mg/L unbuffered with 0.48mg/L fluorescein.

Humic acid floc particles (a) 20 mg/L, (b) 40 mg/L, (c) 60 mg/L and (d) 80 mg/L, unbuffered with 0.48mg/L fluorescein.



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#### pH measurements different fluorescein concentrations



Fluorescein concentration (mg/L)



## Turbidity of 60 mg/L humic acid and kaolin solutions at different fluorescein concentrations



# Fluorescence emission data for flocculated solutions containing different concentrations of fluorescein in 60 mg/L of humic acid







# Fluorescence emission data for flocculated solutions containing different concentrations of fluorescein in 60 mg/L of kaolin





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#### **Fluorescein adsorption**



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#### CONCLUSION

We have an experimental set up to measure the fluorescence lifetime and intensity from floc

But fluorescein changed floc morphology from a dense spherical floc to a flat non-spherical shape and also did not like to stick to the floc

Need an alternative fluorophores or modify the fluorophore to measure floc penetration

e.g. by chemically grafting it to a particle





## **THANK YOU**





#### **EEM measure for different concentration of humic acid**





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#### EEM measure for different concentration of Kaolin

Material concentration (mg/L)







# Fluorescence emission data for flocculated solutions containing different concentrations of fluorescein in 60 mg/L of humic acid







# Fluorescence emission data for flocculated solutions containing different concentrations of fluorescein in 60 mg/L of kaolin





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#### EEM MEASURE FOR DIFFERENT CONCENTRATIONS





