Spring 6-11-2014

Wastewater disinfection with UV or PAA: Are those surviving microbes really benign?

Ronald Gehr
McGill University

Wastewater disinfection with UV or PAA: Are those surviving microbes really benign?

Ronald Gehr

Department of Civil Engineering and Applied Mechanics,

McGill University, Montréal,

Québec, H3A 0C3, Canada

Wastewater and Biosolids Treatment and Reuse: *Bridging* Modeling and Experimental Studies
"Wastewater Treatment, Disinfection and Reuse" (Theme A)
Otranto (Lecce), ITALY
June 11, 2014

Introduction and context (1)

- Wastewater (WW) disinfection does not aim to inactivate ALL microorganisms
  - typical indicator target levels:

    200 – 1,000 CFU/100 mL (after dilution)
- No information on
  - differential inactivation or selection of pathogenic/non-pathogenic microorganisms during WW disinfection, or
  - the effect of disinfection on antibiotic resistance

Introduction and context (2)

- Given the large number of pathogens, we use
  - **indicator organisms** (for convenience of testing) or
  - **model organisms** (as representative of pathogens)
- Fortunately, *E. coli* fit into both categories for bacteria:
  - easily isolated and cultured
  - large body of research on pathogenesis and genomics
  - includes pathogenic and non-pathogenic strains

Objective of the study

Elucidate the dynamics of:

A) pathogenic and non-pathogenic strains of *E. coli*, and

B) their development or loss of antimicrobial resistance,

following disinfection by PAA or UV

We will base our results on information obtained using microbial methods, i.e. at the genetic level.

Examples of legislation and guidelines for municipal effluent discharges affecting bathing waters

Health Canada Santé Canada

Your health and safety… our priority. *Votre santé et votre sécurité… notre priorité.*

Guidelines for Canadian Recreational Water Quality

Third Edition

April, 2012

4.1 Indicator organisms for primary contact recreation

4.1.1 Fresh waters: Escherichia coli (E. coli)

Guideline values

For fresh recreational waters used for primary contact activities, the guideline values are as follows:

Geometric mean concentration (minimum of five samples): ≤ 200 *E. coli*/100 mL Single-sample maximum concentration: ≤ 400 *E. coli*/100 mL

EEA Report | No 1/2014

European bathing water quality in 2013

ISSN 1725-9177

European bathing water quality in 2013

Box 2.1 Assessment methodology for bathing water quality in the 2013 season

Assessment during the transition period

Assessing bathing water quality under the new Bathing Water Directive requires a data set spanning four consecutive years. While those data are being compiled, the rul

This means that the classification of bathing waters is defined o
enterococci and *Escherichia coli* reported under Directive 2006/
intestinal enterococci is evaluated according to the guide value
rating that would classify a water body as having 'excellent' qua
given in Directive 76/160/EEC. The parameter *Escherichia coli* i
guide values for the parameter faecal coliforms given in Directiv
in the following three categories: compliant with the mandatory
values; or not compliant with the mandatory value of the Direct

Assessment under the new Bathing Water Directive (200

When four consecutive years of samples of intestinal enterococ
are available, the assessment is done according to assessment
The directive requires a sample to be taken shortly before the s
that the minimum number of samples taken per bathing season
eight weeks long, then three samples are sufficient). Sampling
bathing season, with the interval between sampling dates never
tolerated.

Map 4.2 Bathing water areas with short-term pollution events in 2013

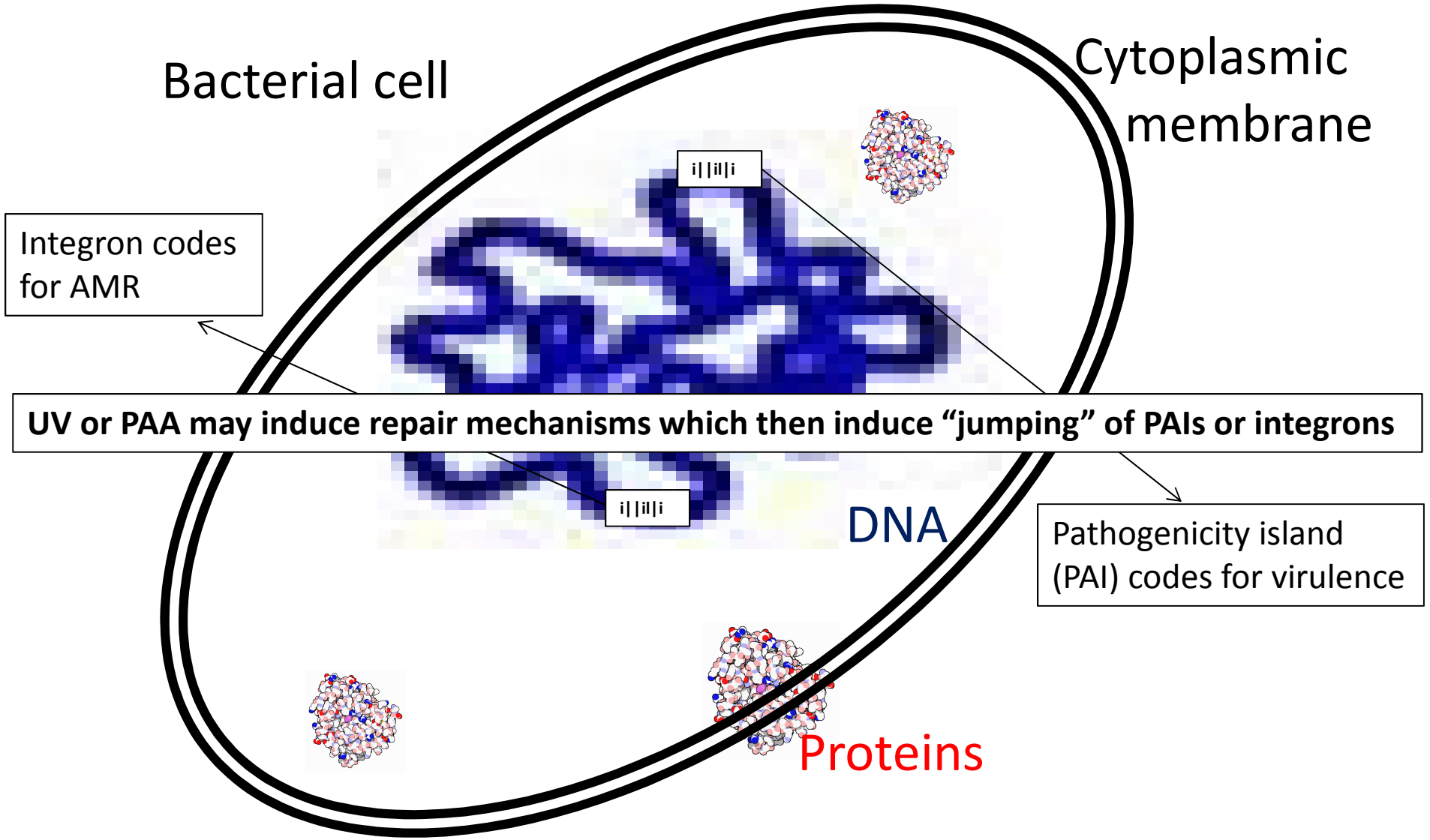
Which strains of *E. coli* should we use as model organisms?

We based the selection on key pathotypes and virulence genes

What cell characteristics create a particular pathotype?

- Pathogenesis based on:
  - suitable number of virulence genes (VGs)
  - suitable combination of VGs
  - all encoding one or multiple virulence factors (VFs)

Review of virulence, pathogenicity islands (PAIs) and integrons (coding for AMR)



Classification of *E. coli* on the basis of clinical symptoms and phylogenetic groups.

Pathotype classification based on clinical symptoms and phylogroups		
Pathotype	Clinical symptoms	Phylogroups
Non-pathogenic *E. coli*	Commensal	A or B1
Intestinal pathogenic *E. coli* (IPEC)		
Enterotoxigenic *E. coli* (ETEC)	Diarrhea	A and B1
Shiga toxin-producing *E. coli* (STEC)	Bloody diarrhea, hemolytic uremic syndrome (HUS)	
Enterohemorrhagic *E. coli* (EHEC)	Haemorrhagic colitis, HUS, diarrhea	
Enteroinvasive *E. coli* (EIEC)	Dysentery	
Enteropathogenic *E. coli* (EPEC)	Diarrhea, vomiting	A, B1, B2 and D
Enteroaggregative *E. coli* (EAEC)	Diarrhea with mucous	
Diffusely adherent *E. coli* (DAEC)	Diarrhea	
Extraintestinal pathogenic *E. coli* (ExPEC)		
Uropathogenic *E. coli* (UPEC)	Cystitis, pyelonephritis	B2 and D
Septicemia-causing pathogenic *E. coli* (SEPEC)	Septicaemia, bacteraemia	
Meningitis-associated *E. coli* (MNEC)	Acute meningitis	

What cell characteristics create a particular pathotype?

- Pathogenesis based on:
  - suitable number of virulence genes (VGs)
  - suitable combination of VGs
  - all encoding one or multiple virulence factors (VFs)
- Our previous work: UPECs are the predominant pathotypes in WWTP effluents
- A majority of UPEC virulence genes are clustered on pathogenicity islands (PAIs)

Frigon, F., et al."Biological and Physicochemical Wastewater Treatment Processes Reduce the Prevalence of Virulent Escherichia coli". AEM 79, 3, 835- 844 (2013).

Classification of *E. coli* on the basis of clinical symptoms and phylogenetic groups.

Pathotype classification based on clinical symptoms and phylogroups		
Pathotype	Clinical symptoms	Phylogroups
Non-pathogenic *E. coli*	Commensal	A or B1
Intestinal pathogenic *E. coli* (IPEC)		
Enterotoxigenic *E. coli* (ETEC)	Diarrhea	A and B1
Shiga toxin-producing *E. coli* (STEC)	Bloody diarrhea, hemolytic uremic syndrome (HUS)	
Enterohemorrhagic *E. coli* (EHEC)	Haemorrhagic colitis, HUS, diarrhea	
Enteroinvasive *E. coli* (EIEC)	Dysentery	
Enteropathogenic *E. coli* (EPEC)	Diarrhea, vomiting	A, B1, B2 and D
Enteroaggregative *E. coli* (EAEC)	Diarrhea with mucous	
Diffusely adherent *E. coli* (DAEC)	Diarrhea	
Extraintestinal pathogenic *E. coli* (ExPEC)		
Uropathogenic *E. coli* (UPEC)	Cystitis, pyelonephritis	B2 and D
Septicemia-causing pathogenic *E. coli* (SEPEC)	Septicaemia, bacteraemia	
Meningitis-associated *E. coli* (MNEC)	Acute meningitis	

Pathotyping rule for UPECs

Our study: Need at least 5 of 19 virulence genes below for an isolate to be a UPEC. *(Rule from Frigon et al, AEM, 2013)*

Virulence factors	Virulence genes	No. of genes required
Adhesins	P-fimbriae: *pap*A, *pap*C, *pap*G, *pix*A	2
Capsules	*kps*M(II) , *kps*M(III)	1
Iron uptake systems	*E. coli* siderophore: *iro*N Yersiniabactin: *fyu*A, *irp*(1), *irp*(2) Aerobactin: *iuc*D, *iut*A ABC Fe^{2+} transporter: *sit*A, *sit*D	1
Toxins	Heamolysins: *hly*A, *vat* Cytotoxins/transporter: *cnf*(1), *cnf*(2), *sat*	1

Refining the questions regarding **virulence**, corresponding to our objectives

- Are there changes in the proportions of UPEC *E. coli* when disinfecting with UV or PAA?
- Do UV and PAA produce similar effects?
- Will free-swimming populations (i.e. following filtration) respond differently than particle-associated populations?

Antimicrobials

Mode of action and resistance mechanism of various antimicrobials

Antimicrobial	Mode of action	Resistance mechanism
Aminoglycoside	Inhibit protein synthesis	Enzymatic modification of antimicrobial
β-lactams	Inhibit cell wall synthesis	β-lactamases, alteration of penicillin-binding proteins (PBPs)
Chloramphenicol	Inhibit protein synthesis	Decreased antimicrobial permeability
Macrolides	Inhibit protein synthesis	Alteration of ribosomal RNA, drug efflux
Quinolones	Inhibit DNA synthesis	Mutation of DNA gyrase
Rifamycins	Inhibit RNA synthesis	Mutation of RNA polymerase
Sulfonamides	Inhibit metabolic pathway	Production of drug-insensitive enzymes
Tetracyclines	Inhibit protein synthesis	Active efflux followed by chemical modification

Link between virulence and antimicrobial resistance (AMR)

- *E. coli* can serve as vectors for dissemination of AMR genes
- Positive co-occurrence of virulence and AMR genes has been demonstrated in UPECs

Frigon, D.,et al. Impact of Wastewater Treatment Processes on Antimicrobial Resistance Genes and their Co-occurrence with Virulence Genes in *Escherichia coli*. Water Research, 50, 245-253 (2014)..

Questions regarding antimicrobial resistance (AMR) genes

- Are there changes in the number and classes of AMR genes in UPECs when disinfecting with UV or PAA?
- Do UV and PAA produce similar effects?
- Will free-swimming populations respond differently than particle-associated populations?

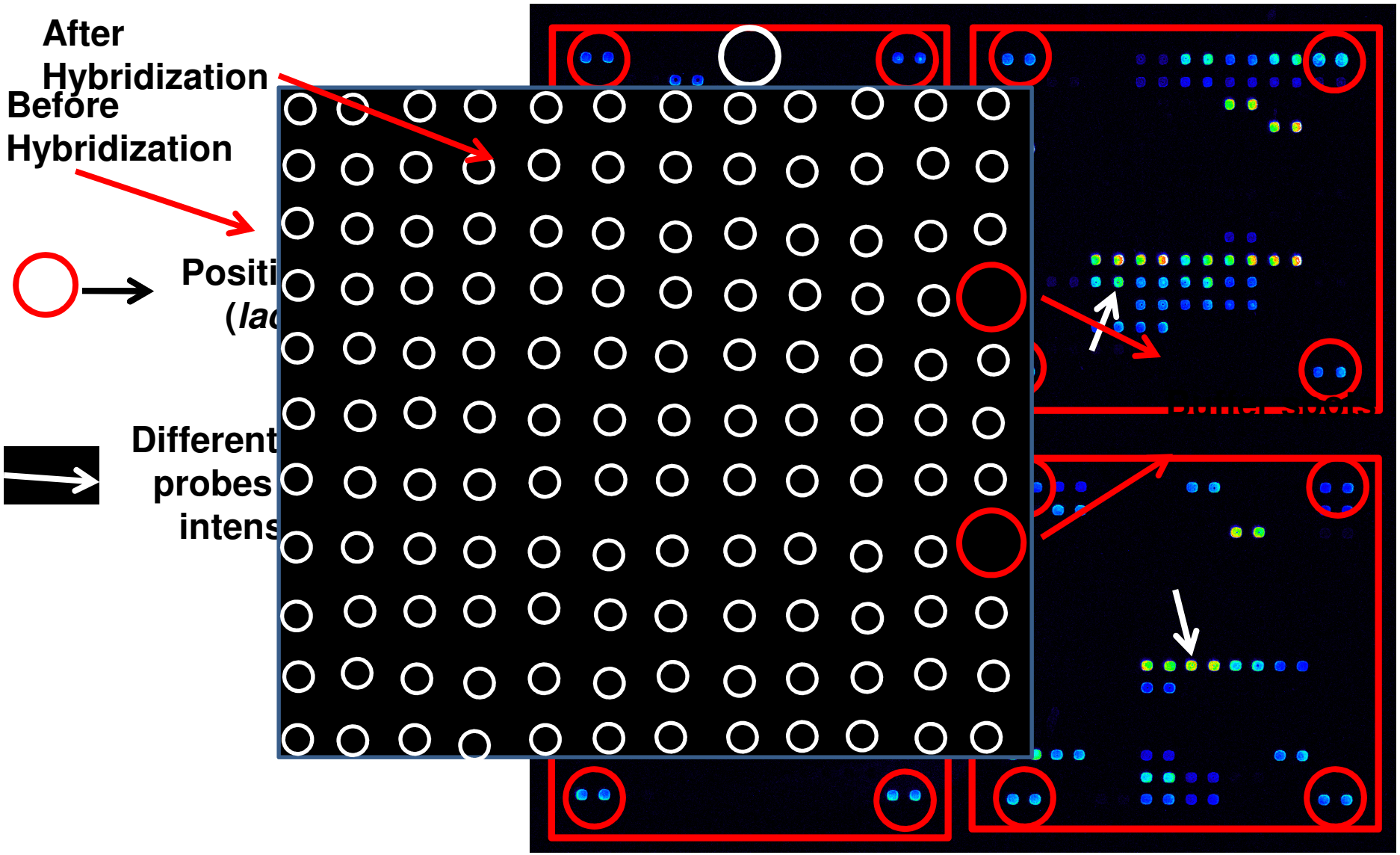
Key methods - Virulence

- Effluent samples from activated sludge (AS), biofilter (BF), and physicochemical (PC) plants
- For some samples, particles removed by centrifugation and 20 µm filter
- UV disinfection – collimated beam
- PAA disinfection – 12% PAA, 30 or 60 min contact time; residuals by DPD
- **Target *E. coli* level 200 CFU/100 mL**
- Initial screening for 3 UPEC genes using Bioplex PCR
- **Major data source: microarray**

Key methods - AMR

- Same effluent samples and isolates as for virulence testing; same disinfection
- For some samples, particles removed by centrifugation and 20 μm filter
- The screen-positive isolates for the AS, BF1, BF2, and PC1 samples and all the isolates from the PC2 samples were genotyped by microarray
- The microarray probed 70 AMR genes of 11 classes, and 8 mobile genetic element sequences

DNA microarray image of an *E. coli* isolate

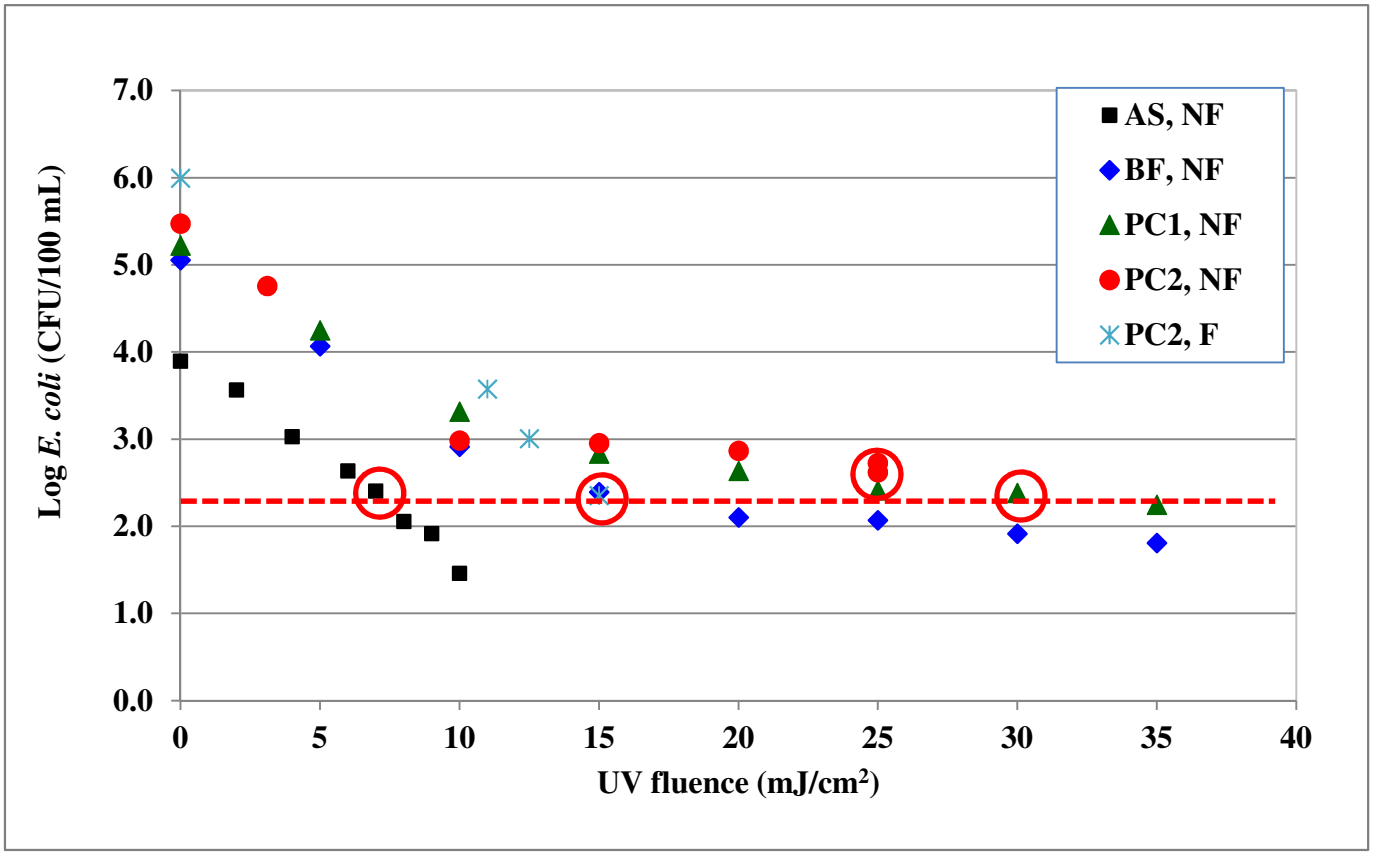


Results

Wastewater characteristics

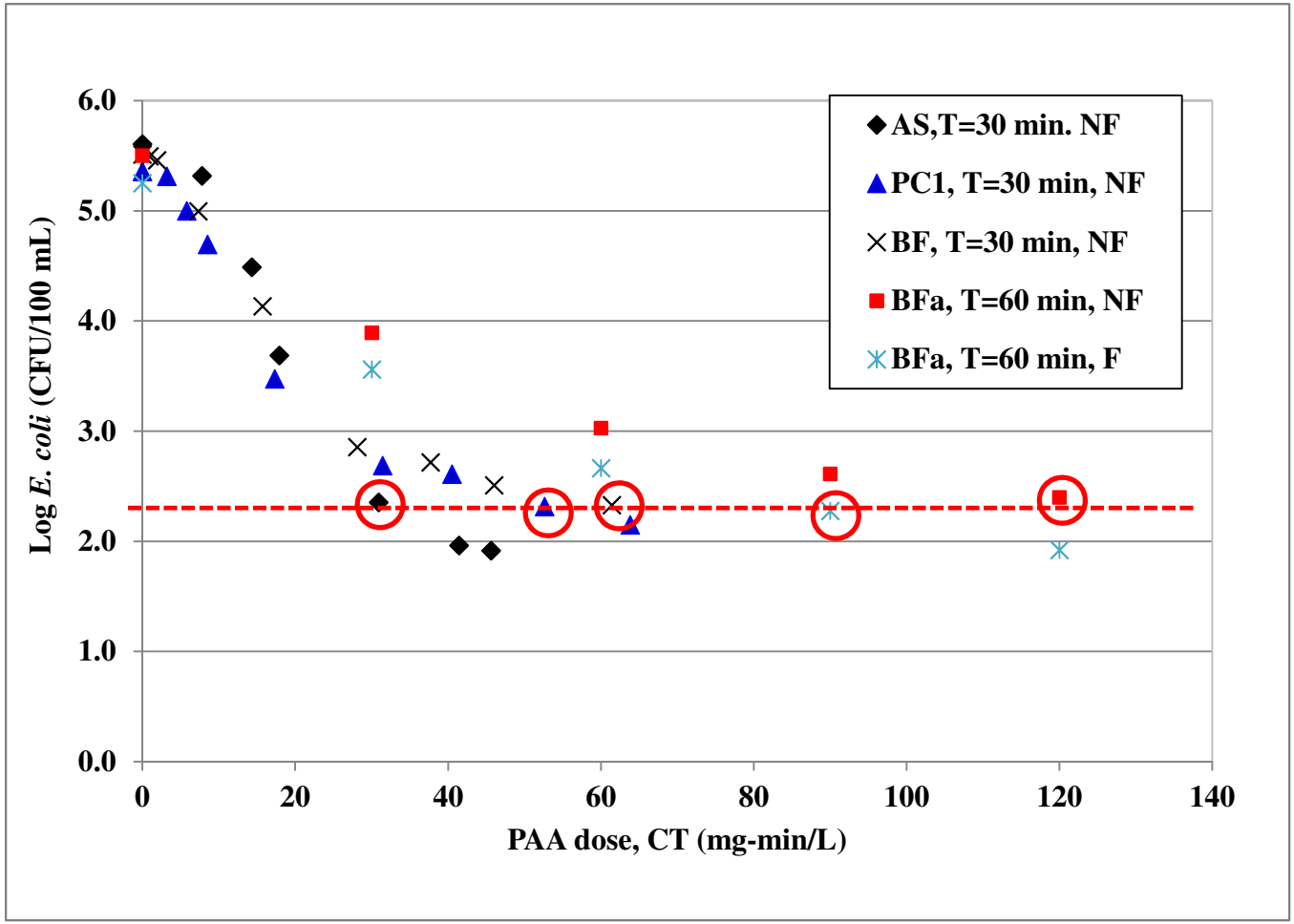
Parameter (unit)	Wastewater treatment plants				
	AS	BF	BFa	PC1	PC2
Treatment processes	Conventional activated sludge	Biological filtration		Physico-chemical	Physico-chemical
pH	7.1	7.7	-	7.2	7.1
UV T (%)	67.2	63.6	-	54.5	42.6
SS (mg/L)	10	5.0	14	15	18
COD (mg/L)	38	46	62	45	92
E. coli (CFU/100 mL)	7.8×10^{3}	1.1×10^{5}	3.0×10^{5}	1.6×10^{5}	9.9×10^{5}

UV inactivation curves



Plant	UV fluence to reach 200 CFU/100 mL
AS NF	~7
BF NF	~15
PC1 NF	~30
PC2 NF	~30
PC2 F	~15

PAA inactivation curves



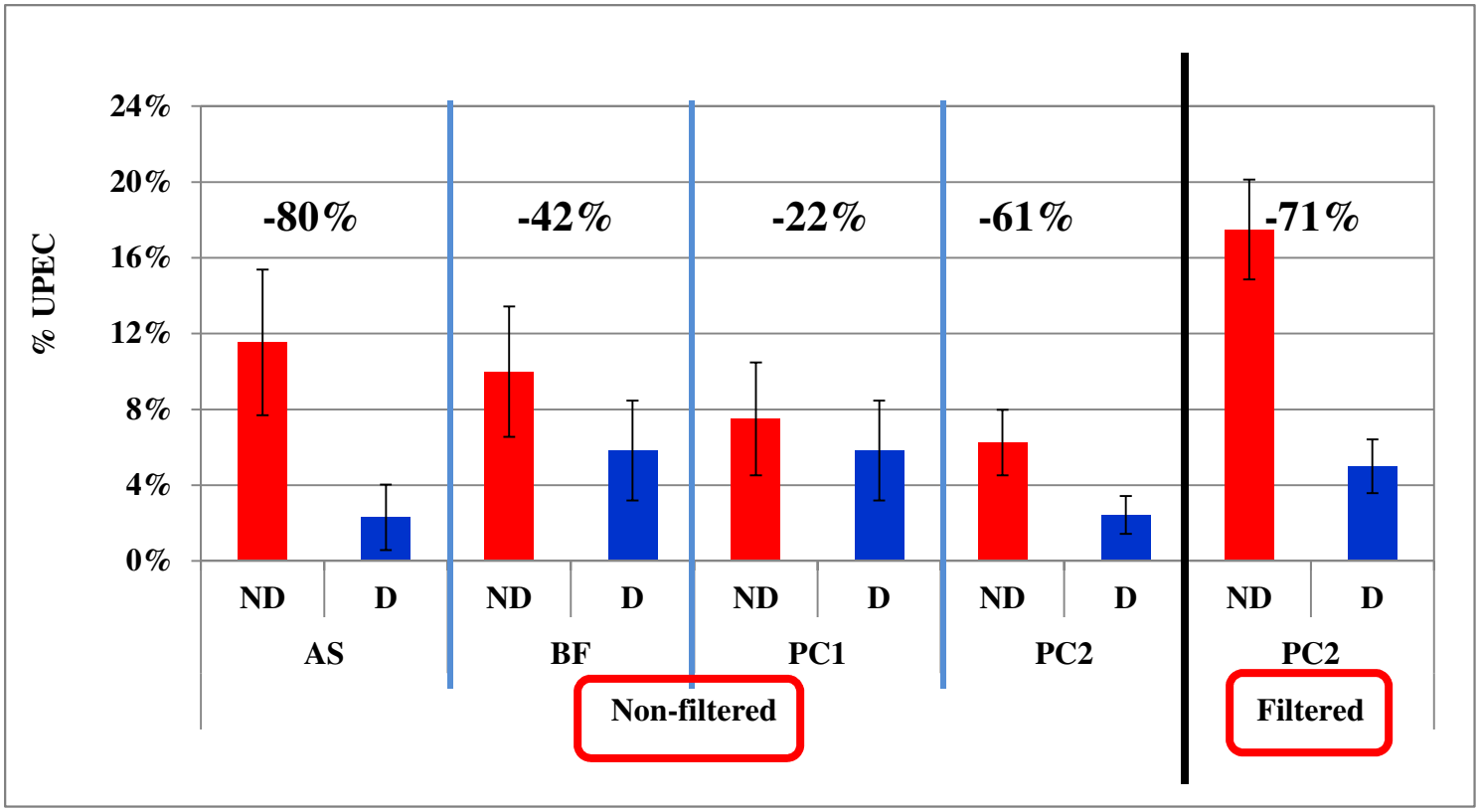
Plant	PAA CT (mg/l-min) to reach 200 CFU/100 mL
AS NF	~30
BF NF	60 - 120
BF F	~90
PC1 NF	~55

In the following slides:

ND = non-disinfected

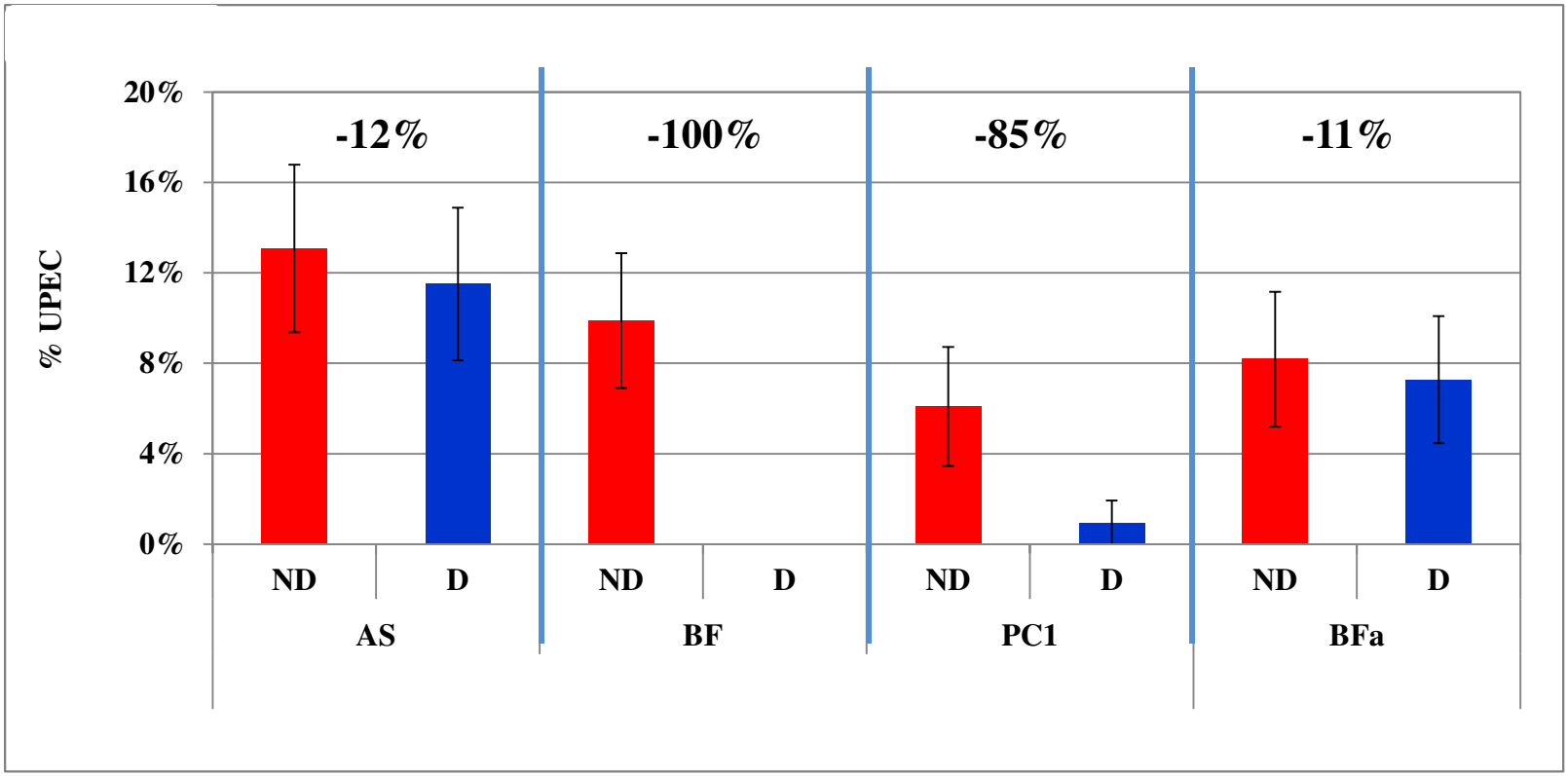
D = disinfected

Impact of UV on UPEC fractions



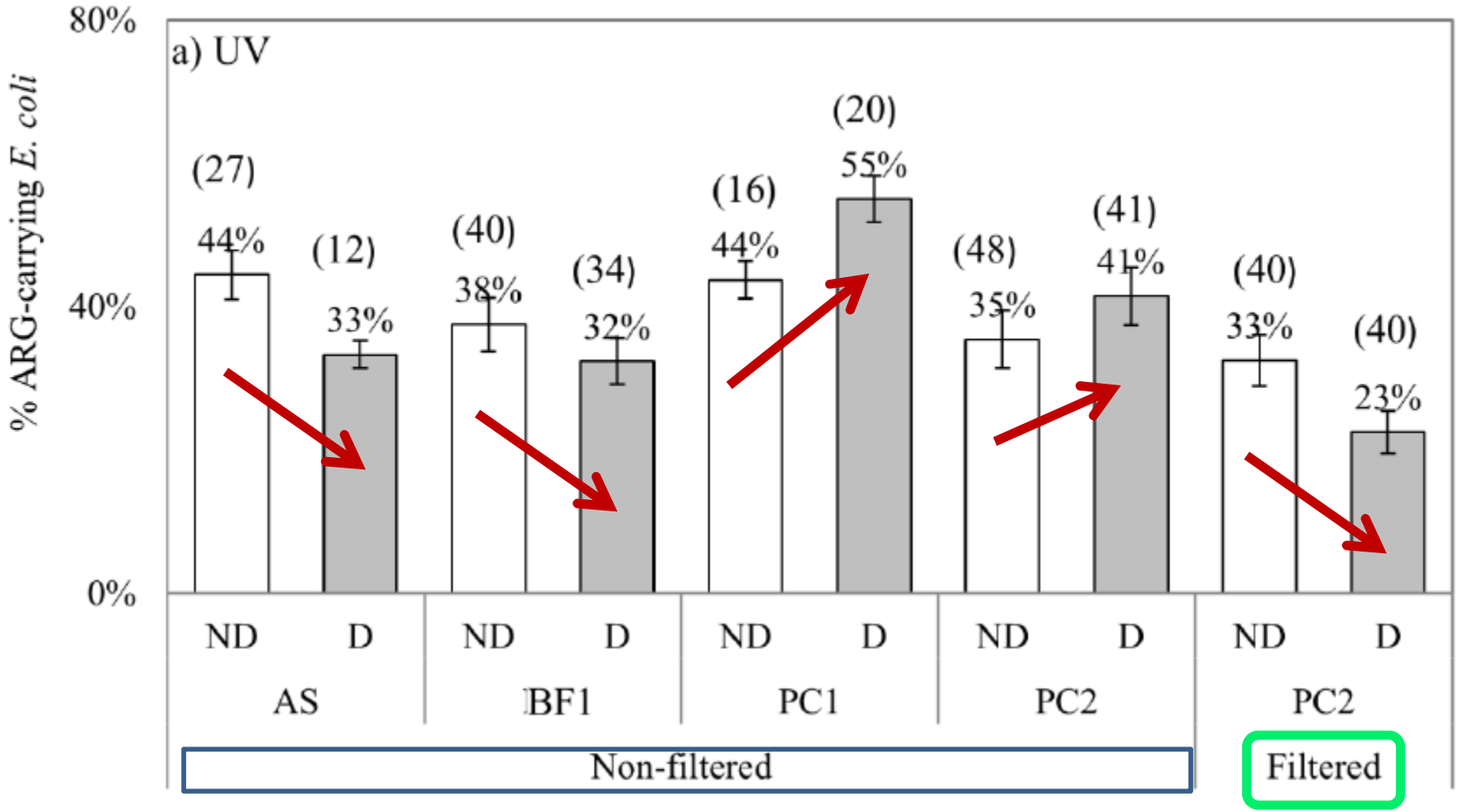
Average reduction of UPEC fractions: 55%
For the PC plants, greater reduction in the free-swimming UPECs

Impact of PAA on UPEC fractions



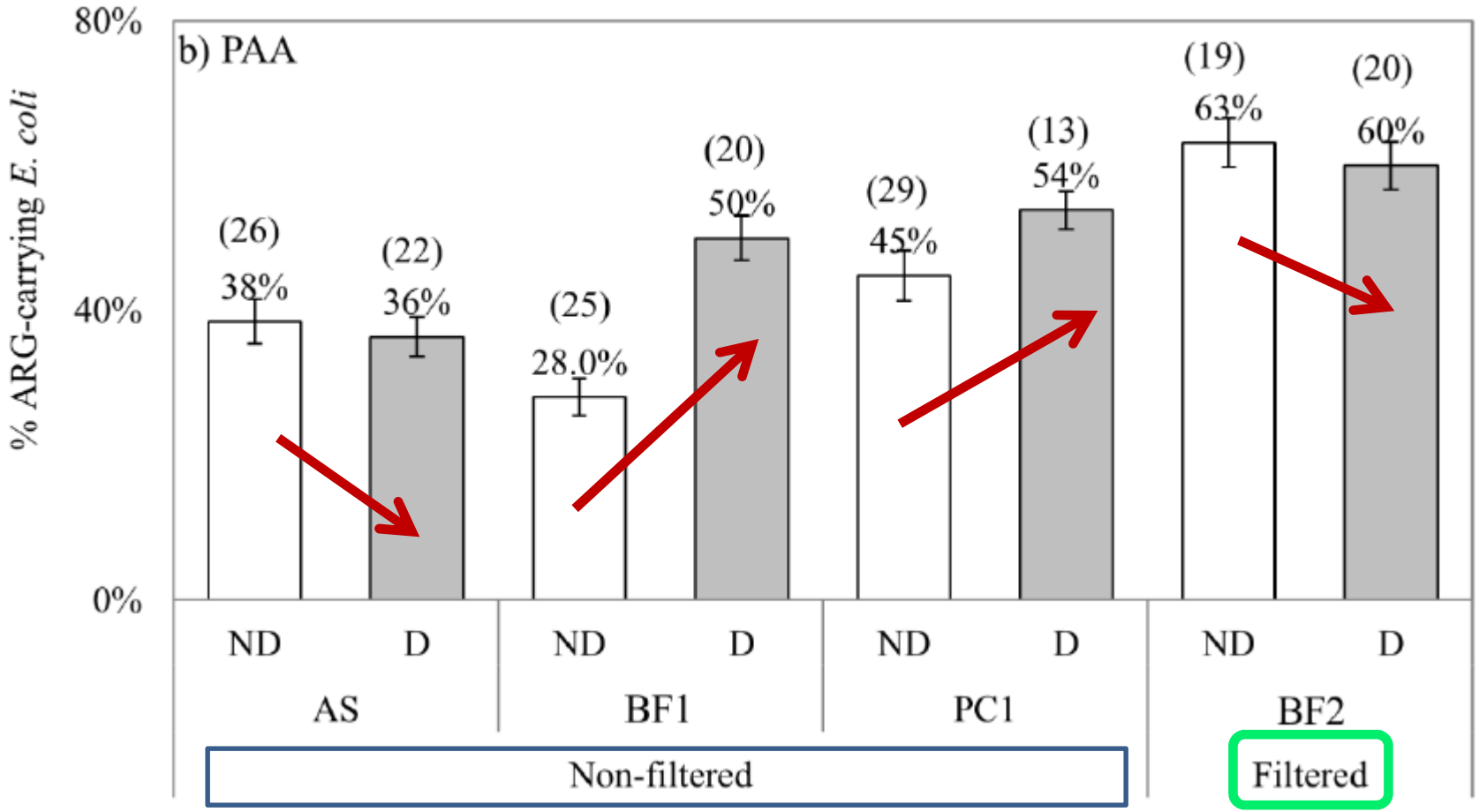
Average reduction of UPEC fractions: 52%

Impact of UV on prevalence of antimicrobial resistance gene (ARG)-carrying *E. coli*



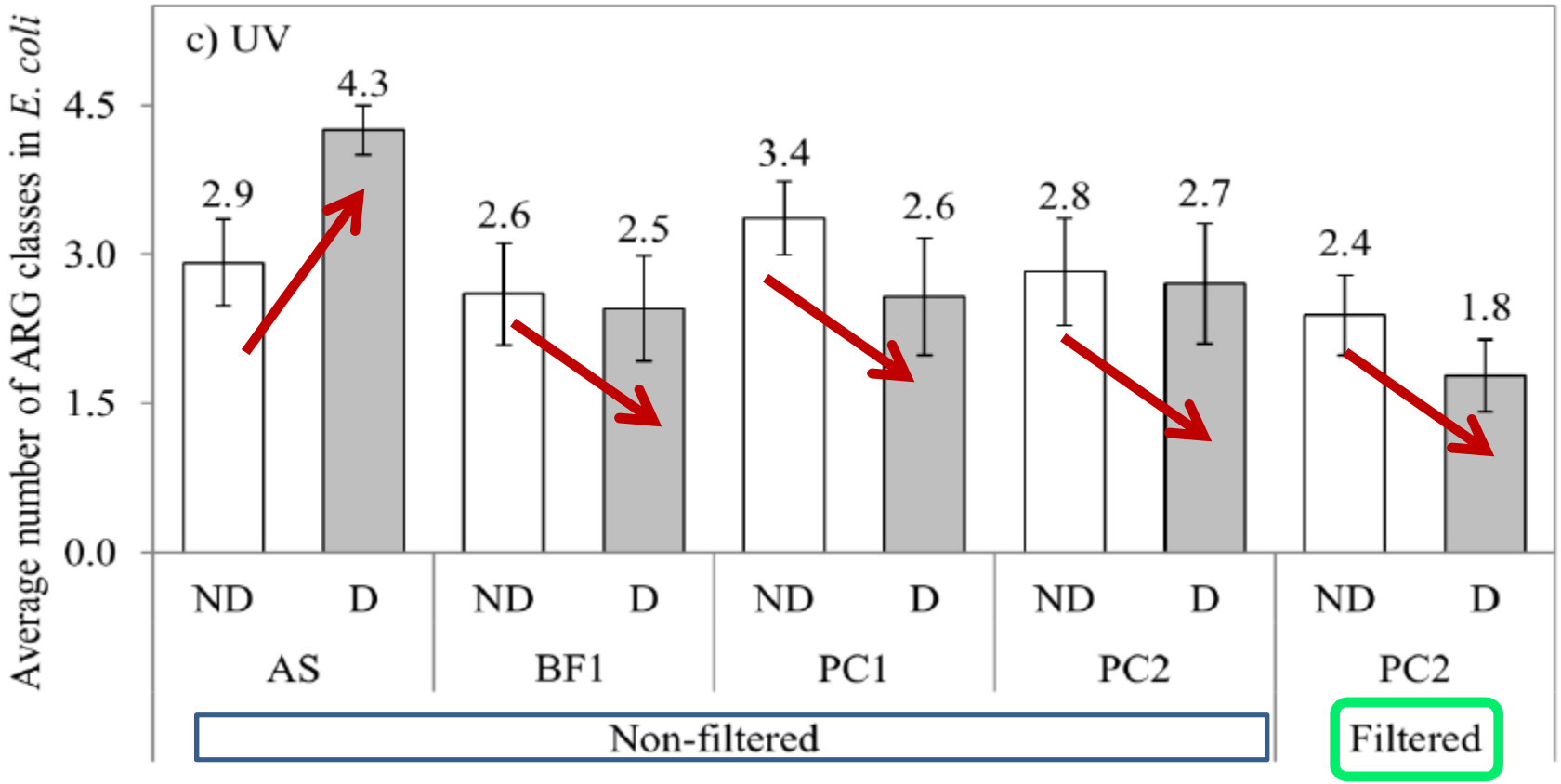
→ **inconsistent**

Impact of PAA on prevalence of antimicrobial resistance gene (ARG)-carrying *E. coli*



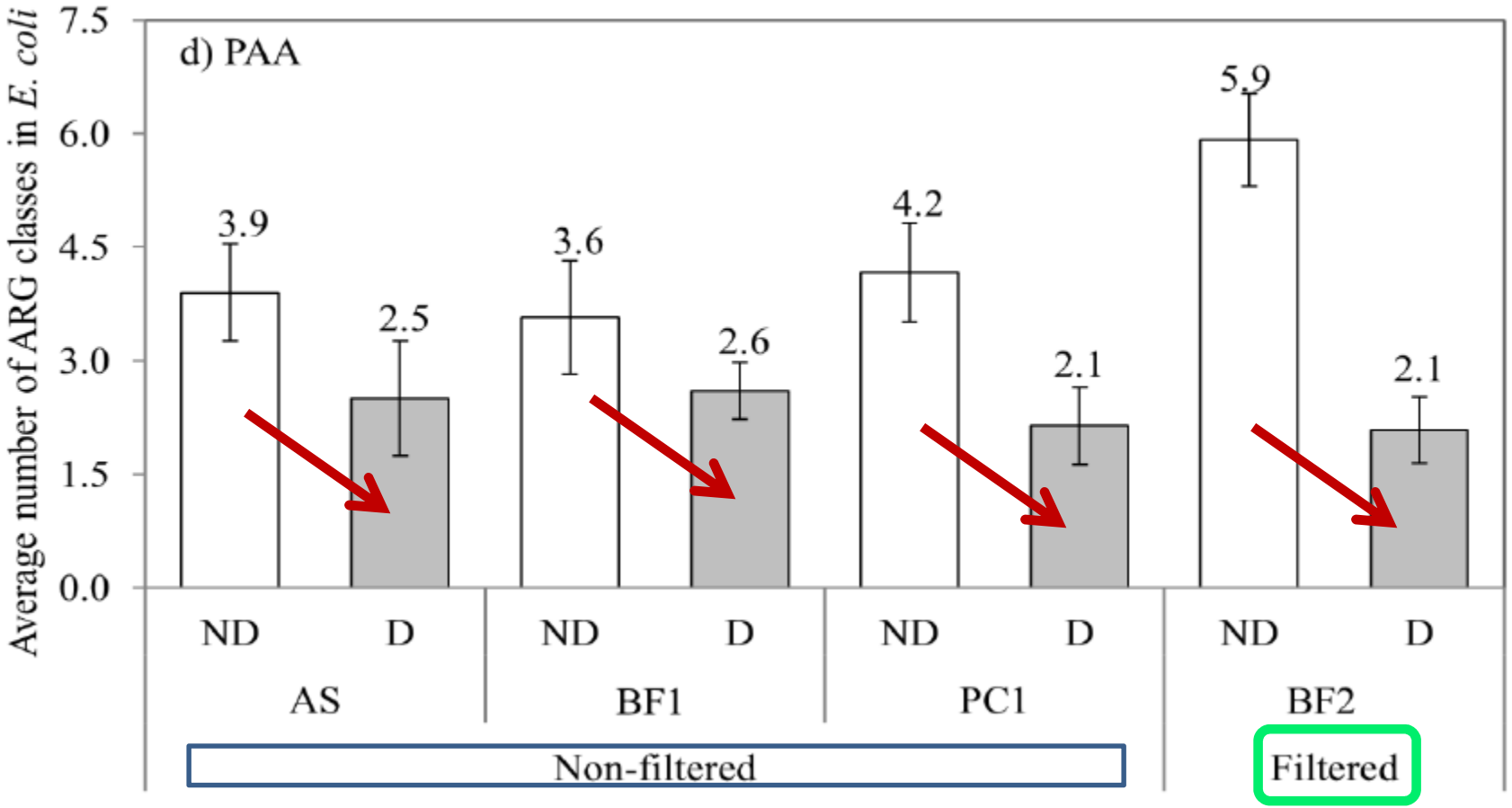
→ inconsistent

Impact of UV on occurrence of the mean number of antimicrobial resistance gene **classes**



→ mainly down

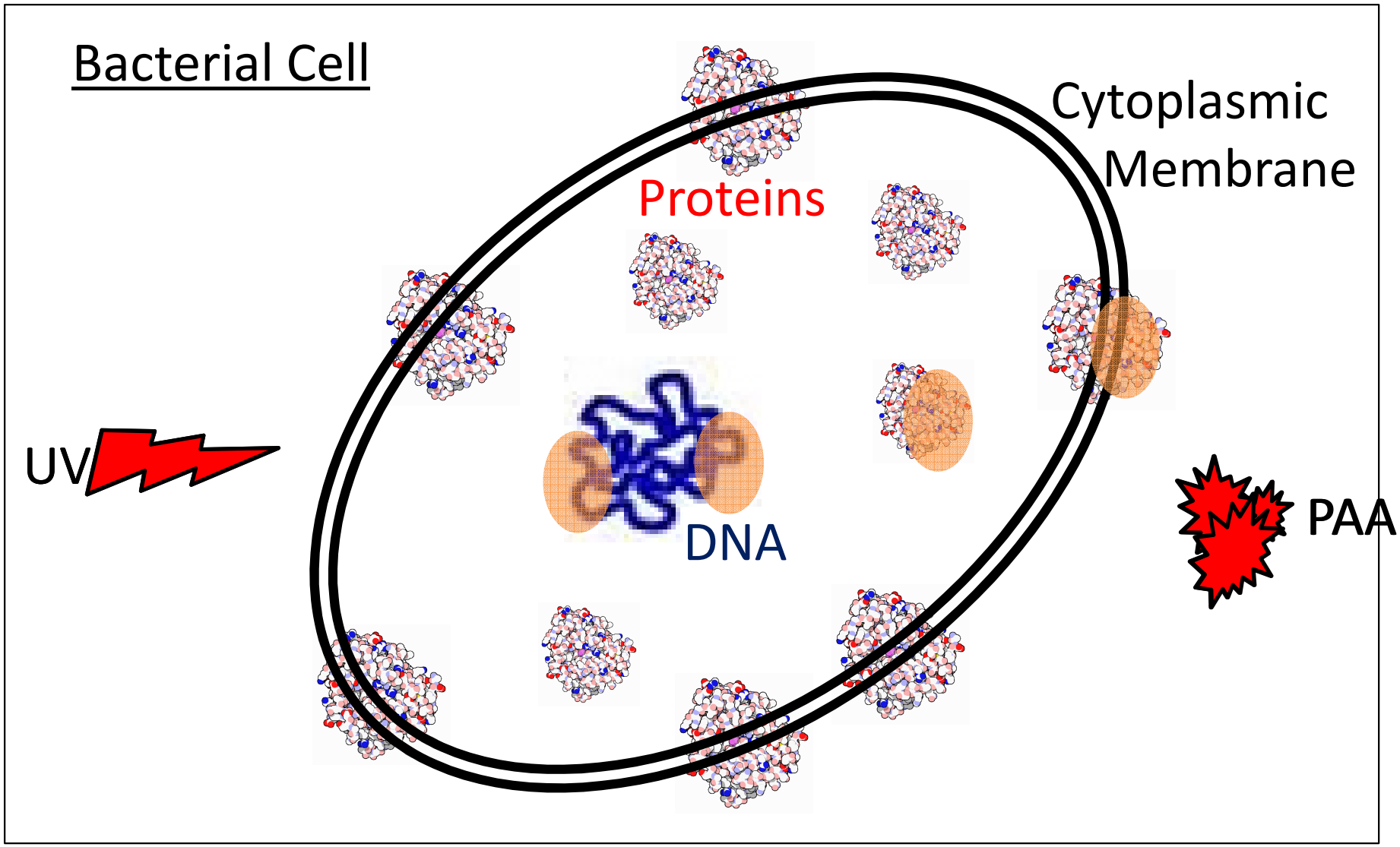
Impact of PAA on occurrence of the mean number of antimicrobial resistance gene **classes**



→ all down

Mechanisms? Reasons for different behaviour for UV and PAA?

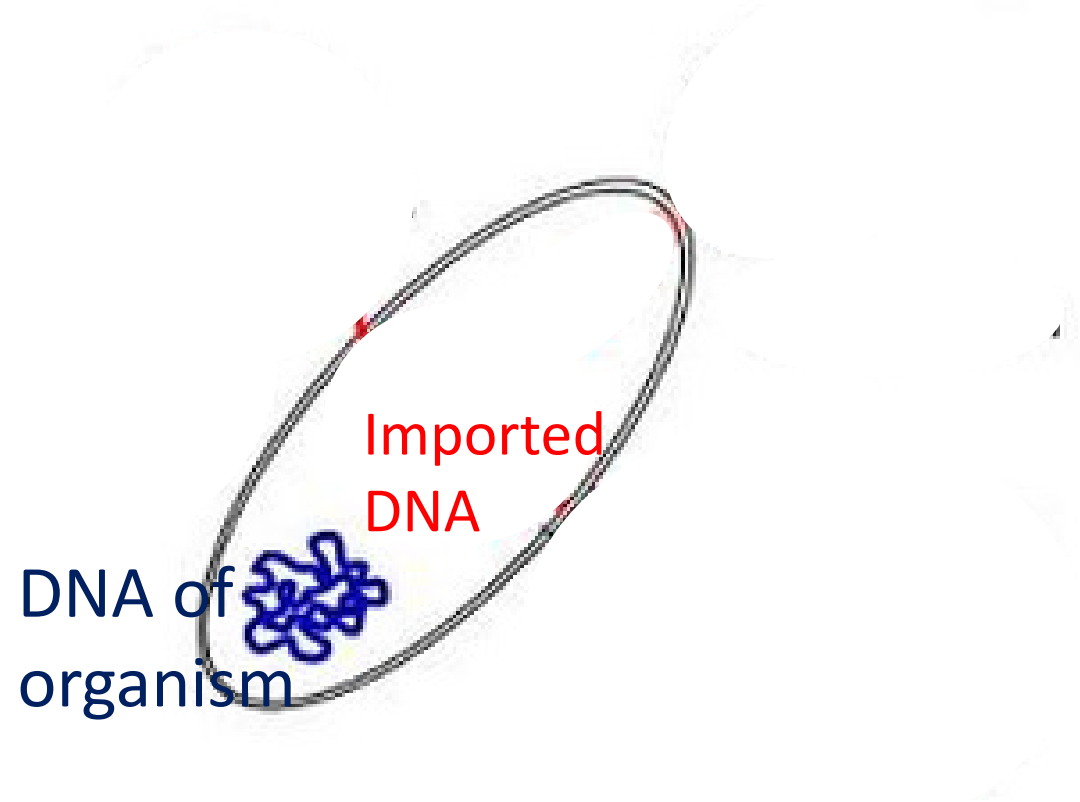
Different disinfection mechanisms for UV and PAA



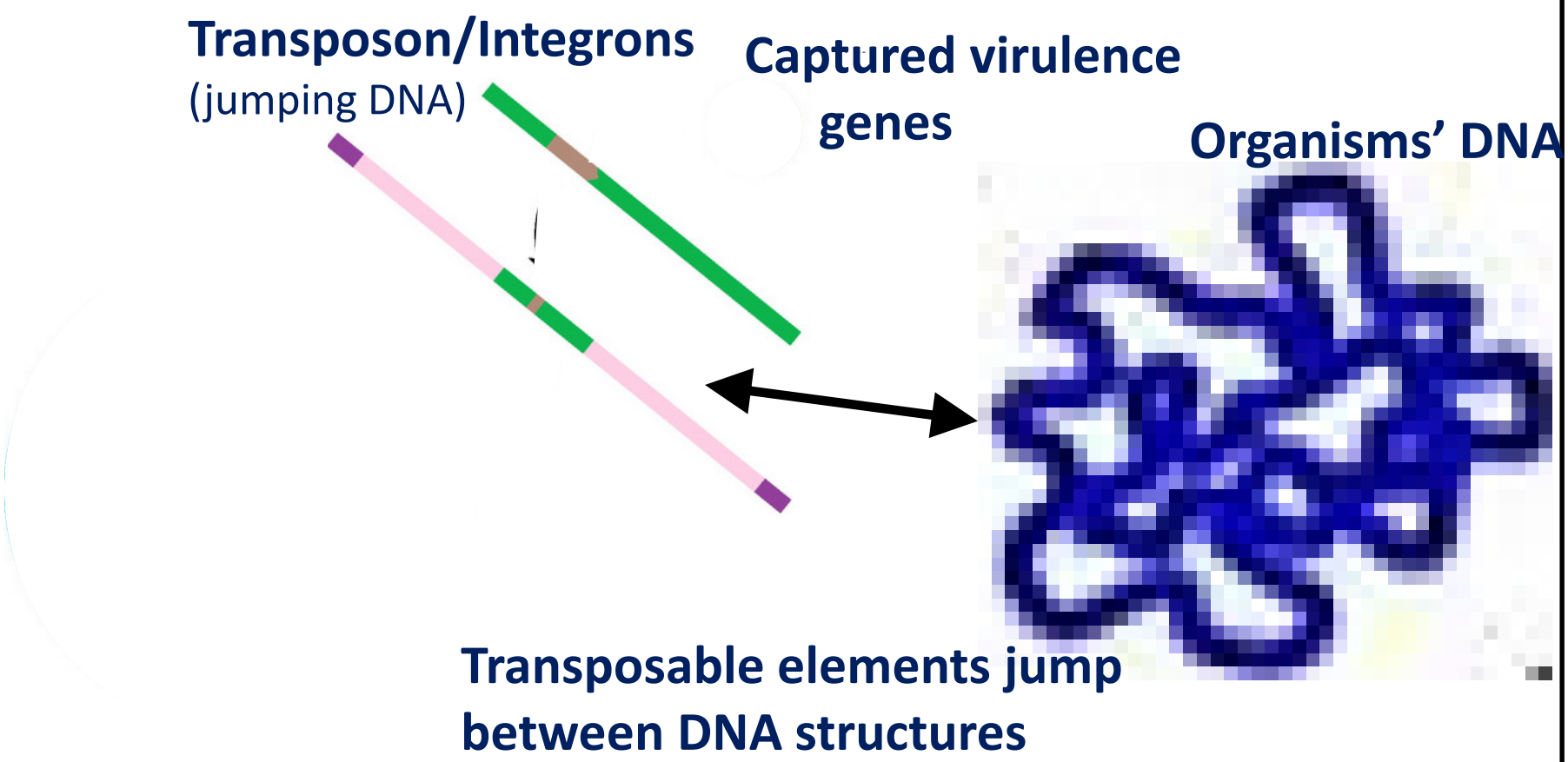
Different disinfection mechanisms for UV and PAA

	Ask your favourite expert	
	The engineer	**How does the surviving cell respond? → (up-regulated genes)**
UV	• Readily penetrates cells • Reacts mainly with DNA	• Mainly DNA repair • Some protein expression • Some nucleotide metabolism
PAA	• Diffuses/reacts: outside → inside • Forms OH-radicals which react with proteins (oxidation of sulfur groups) and DNA	• Oxidative stress response • Processing of sulfur amino acids • DNA repair

Non-pathogenic strains can become pathogenic by inter-cellular mechanisms of horizontal gene exchange:



Virulence genes can be assembled inside the cell in units by transposable elements



Result: assembly of **Pathogenicity Islands (PAIs)**

→All genes for a pathotype can be transferred at once

Summary – impact of disinfection

- Proportion of UPEC isolates relative to non-pathogenic isolates decreased by ~ 55% for UV and PAA
- Although UV and PAA interact differently with cells, impact on virulence factors is similar
- Inconsistent effects on prevalence of ARGs, but except for UV on AS effluents, mean number of ARG **classes** decreased
- Filtration:
  - reduces UV fluence requirements, as expected
  - had little effect on PAA requirements:

    wastewater COD more important
  - impact on virulence: apparent reduction for UV
  - impact on AMR: reduction in all cases (PAA & UV; genes & classes)

Explanations

- Both UV and PAA disrupt DNA and genetic elements, but may also stimulate repair mechanisms, including gene transfer
- “Importing” gene mechanisms can also function as “exporting” mechanisms. If PAIs are exported out of the cell, they will not be detected and the cell will not be virulent or have AMR

Consequences and future work

- Virulence is rare, and the genetic requirements complex, hence loss of virulence is a reasonable first consequence of disinfection
- Public health aspects: surviving microbes less likely to be virulent → standards may be conservative (good news!!)
- Disinfection does not, in general, increase AMR (also good news!!)
- Must examine repair in stressed and non-stressed environments

Acknowledgements

- Basanta Kumar Biswal, Dominic Frigon, Ramzi Khairallah, Luke Masson, Alberto Mazza
- Funding from NSERC Strategic Grant # STPGP 35117-07 and Trojan Technologies Inc.
- Bill Cairns for UV
- Solvay/Interox for PAA
- Plant operators at four WTPs

Thanks for listeniNg.

And don't worry, we'll survive this one too!

Any questions?