

Anaerobic membrane bioreactor and its efficacy in removing microbial contaminants

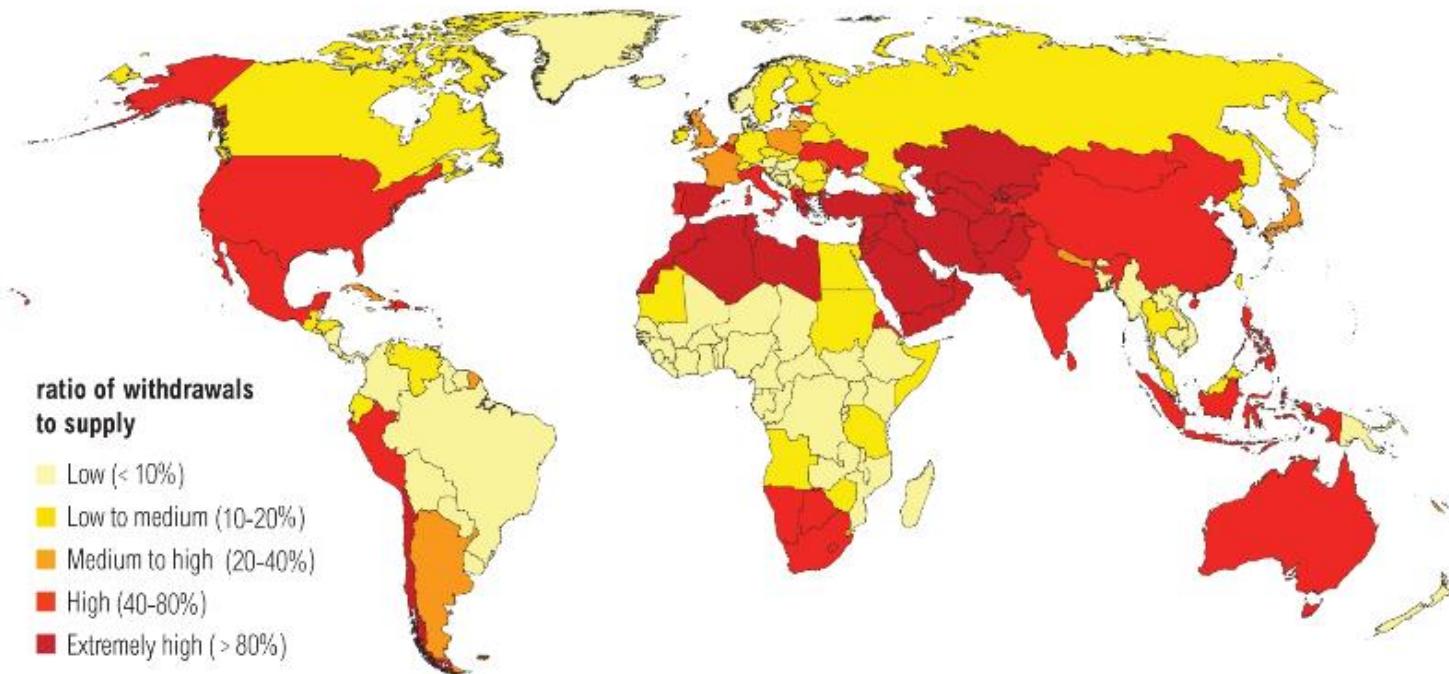
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Water scarcity is a global concern

Water Stress by Country: 2040



NOTE: Projections are based on a business-as-usual scenario using SSP2 and RCP8.5.

For more: ow.ly/RiWop

Water scarcity in Saudi Arabia and possible alternatives

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Amount of water used for food production was reported to be **57.1 million m³** per day*.

Amount of wastewater flows was estimated to be **4.23 million m³** per day.



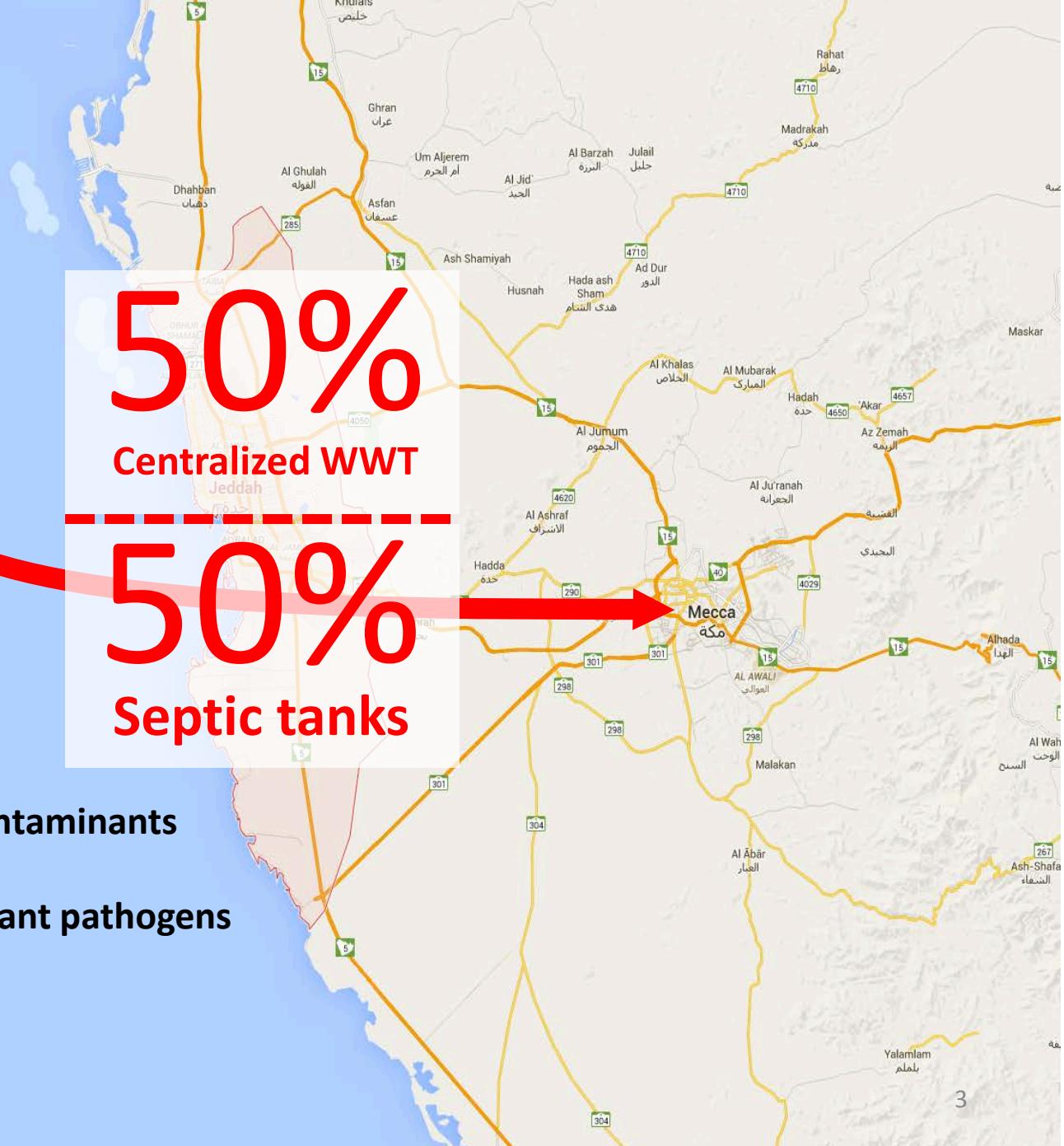
If all treated water were being used at full capacity, this would have met ca. **10%** of the needs required by the agricultural sector.



Hajj
2.5 M

50%
Centralized WWT
50%
Septic tanks

Emerging contaminants
↓
Antibiotic-resistant pathogens

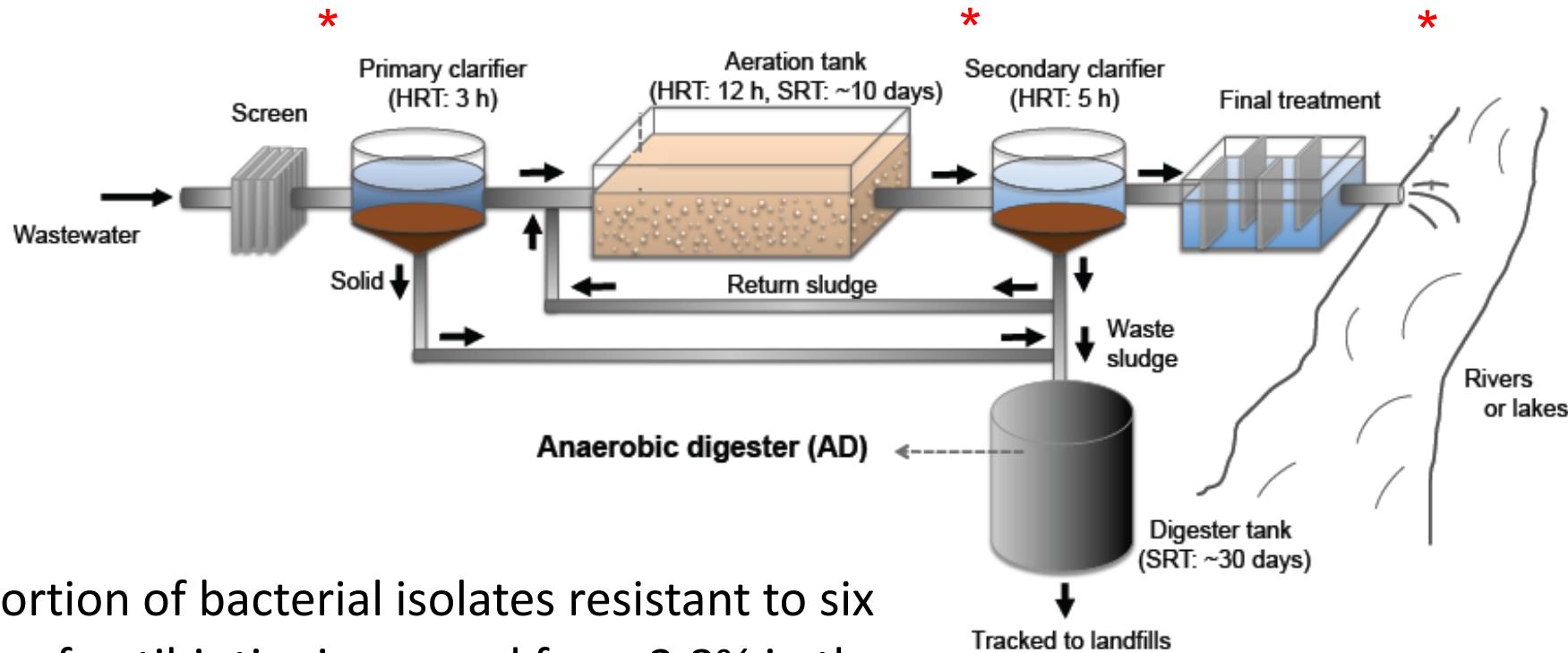


Conventional WWTP enrich antibiotic-resistant bacteria

Primary treatment

Secondary treatment

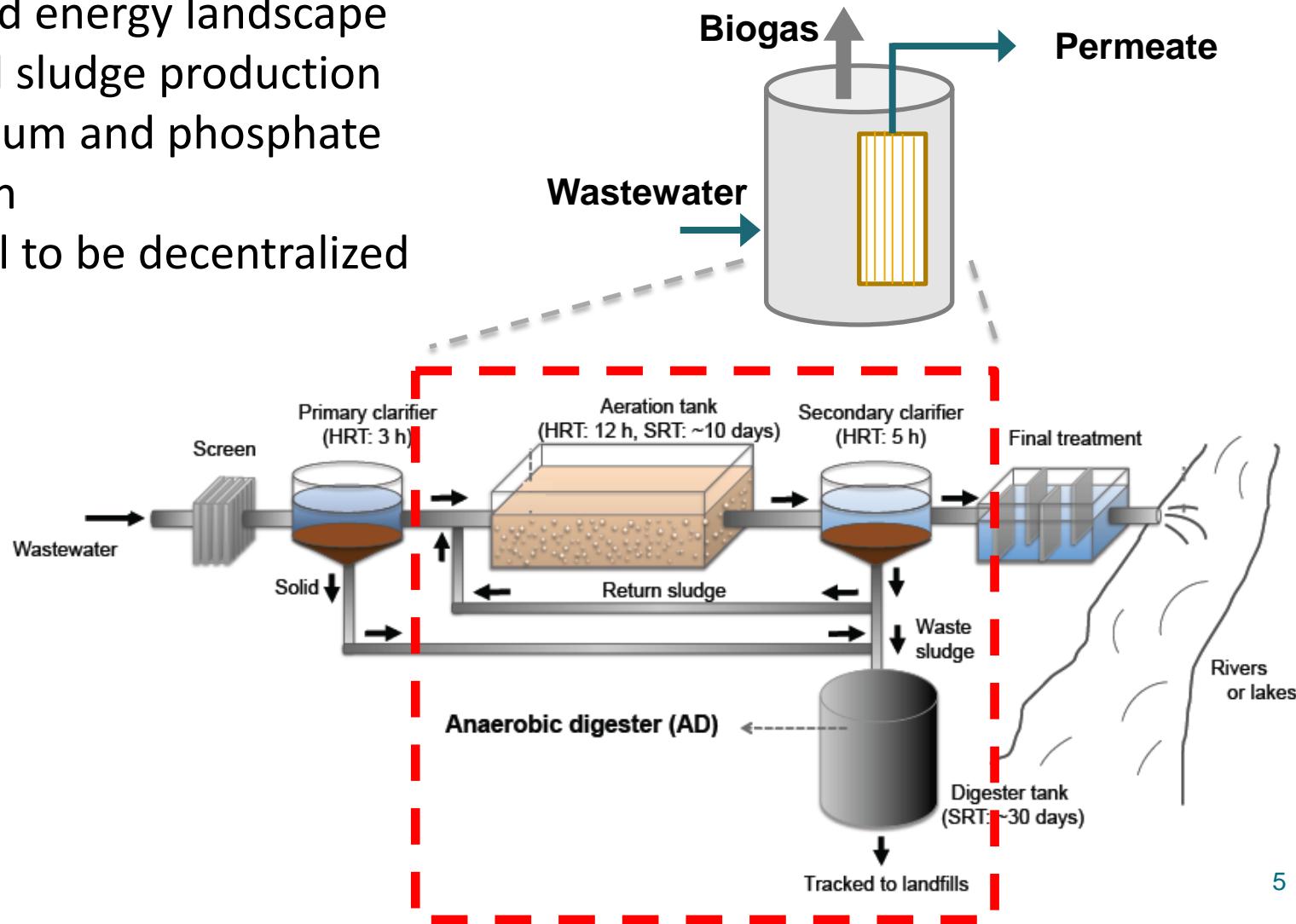
Tertiary treatment



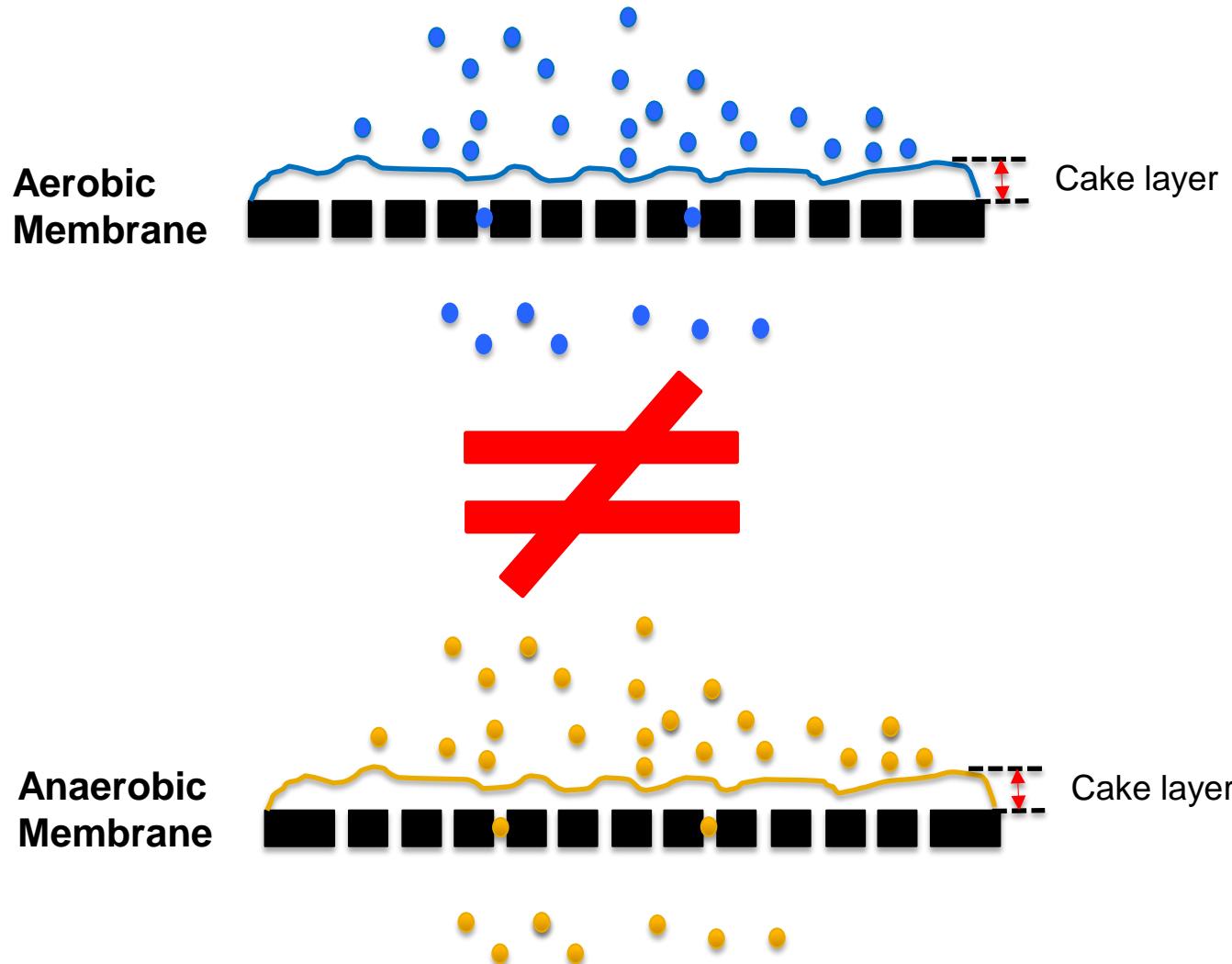
Proportion of bacterial isolates resistant to six types of antibiotics increased from 3.8% in the influent to 6.9% in the chlorinated effluent.

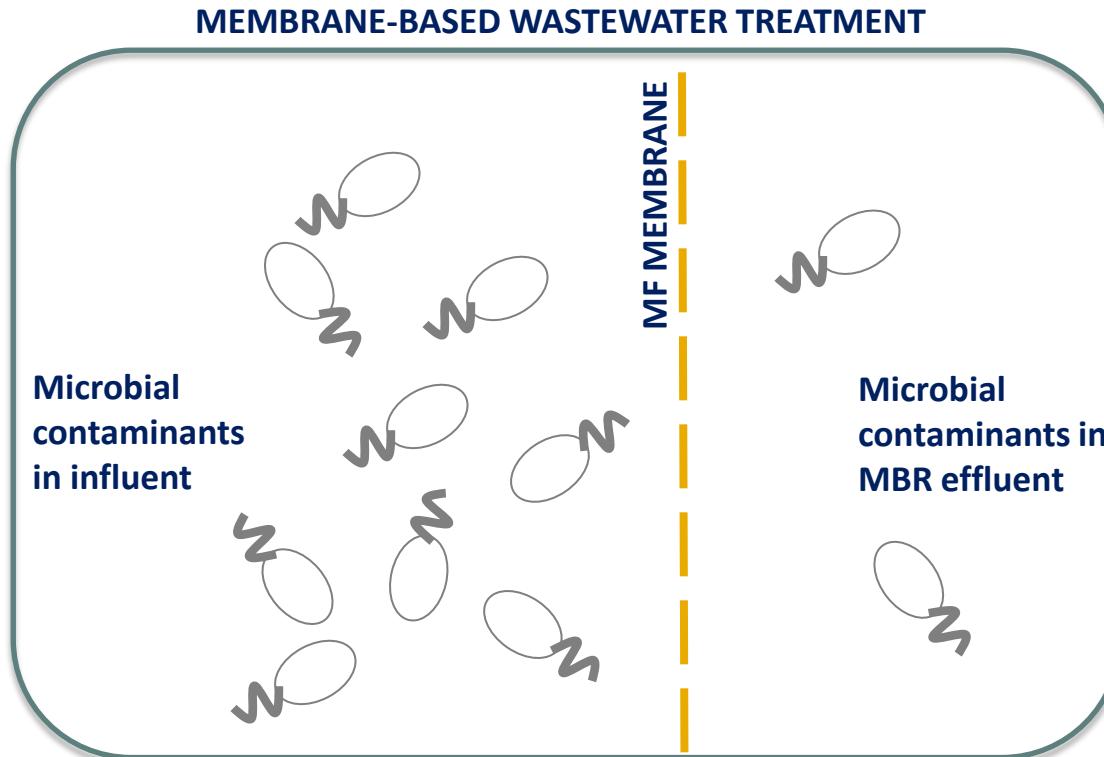
Advantages of anaerobic membrane bioreactor (anMBR)

- Improved energy landscape
- Reduced sludge production
- Ammonium and phosphate retention
- Potential to be decentralized



Aerobic vs. anaerobic cake layers

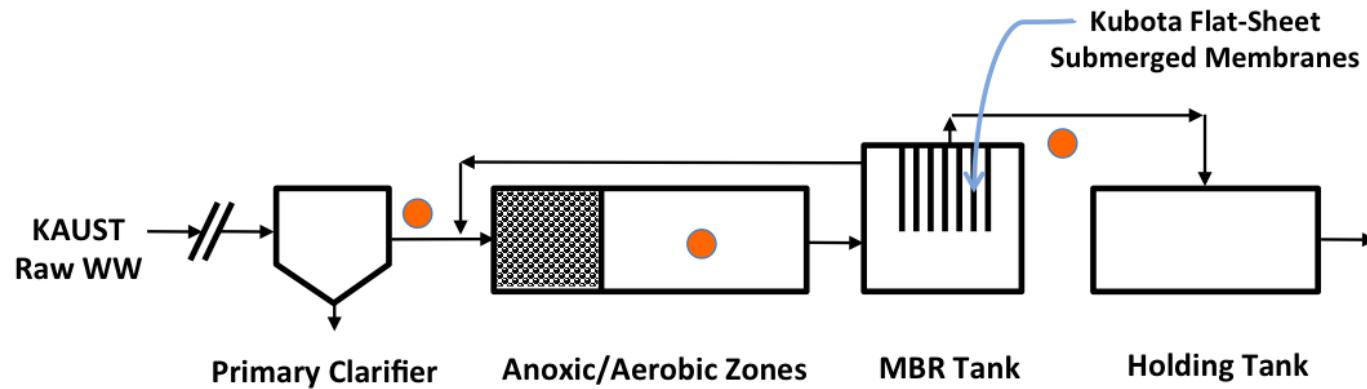




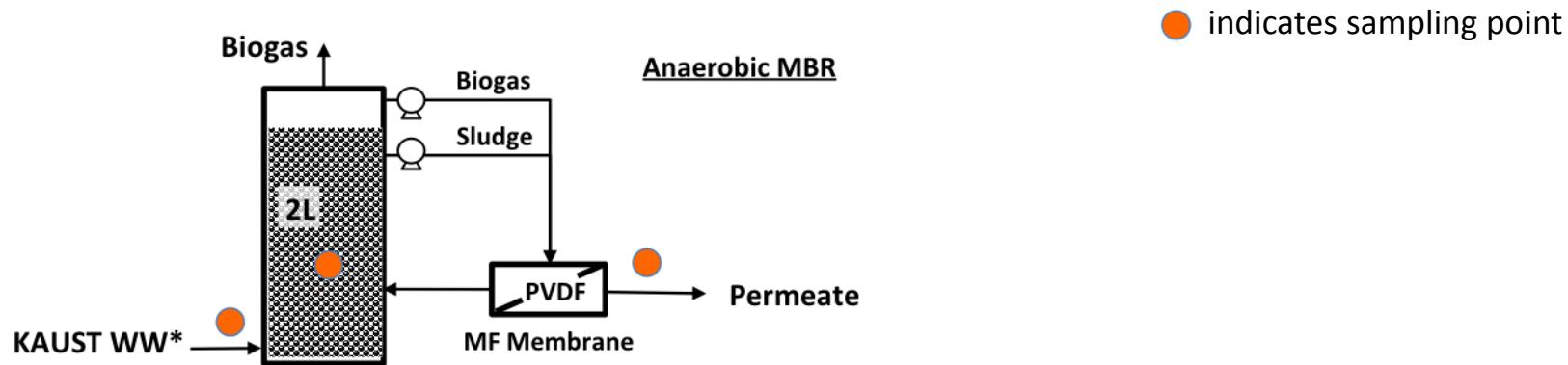
- **Case study 1:** Molecular-based detection of opportunistic pathogens: removal achieved by aerobic vs. anaerobic MF-based MBR
- **Case study 2:** Removal of antibiotic-resistant bacteria and antibiotic resistance genes by varying degrees of fouling on anaerobic microfiltration membranes

Case study 1: Sampling

1. KAUST Wastewater Treatment Plant (Full-scale MBR)



2. Lab-scale anaerobic MBR



Molecular-based measurements and analyses

- Microbial Community Analysis
 - 16S rRNA gene-based Illumina MiSeq Sequencing
 - Influent, Aerobic reactor Sludge and effluents
- Pathogen Detection
 - Quantitative digital PCR (dPCR) targeting pathogenic species

Occurrence of pathogenic genera and their log removal values

Genera

Mycobacterium

Treponema

Arcobacter

Neisseria

Acinetobacter *

Pseudomonas *

Legionella

Unclassified

Enterobacteriaceae *

Escherichia

Stenotrophomonas

Aeromonas

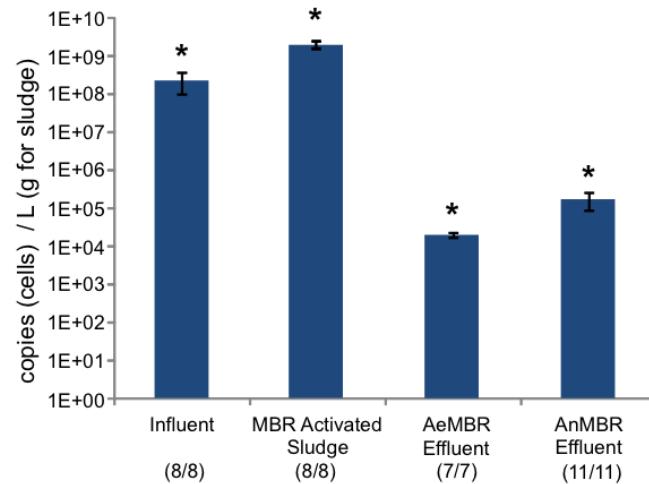
Streptococcus

Enterococcus

Dialister

ND denotes not detected

Abundance of specific pathogenic species



What're the risks?

- Quantitative microbial risk assessment
- Estimate risk of exposure by specific pathogen detected by dPCR
- Event 1: using the effluent for agricultural irrigation
- Event 2: using the sludge for land application
- Bacterial concentrations from dPCR results
- Exposure assessment, infection probability obtained from qmrawiki.canr.msu.edu
- Annual risk

Exposure dosage	Influent WW irrigation exposure	AeMBR effluent irrigation exposure	AnMBR effluent irrigation exposure	AeMBR sludge land application dermal exposure	AeMBR sludge land application ingestion exposure
Annual risk					
<i>Acinetobacter baumannii</i>	1.0×10^0	6.0×10^{-3}	4.3×10^{-5}	5.0×10^{-3}	2.6×10^{-2}
<i>Pseudomonas aeruginosa</i>	1.0×10^0	3.2×10^{-1}	6.3×10^{-2}	-	-
<i>Klebsiella pneumoniae</i>	1.0×10^0	4.2×10^{-3}	7.3×10^{-4}	4.9×10^{-3}	2.6×10^{-2}

- Acceptable microbial risk $\leq 1 \times 10^{-4}$



high risk



moderate risk

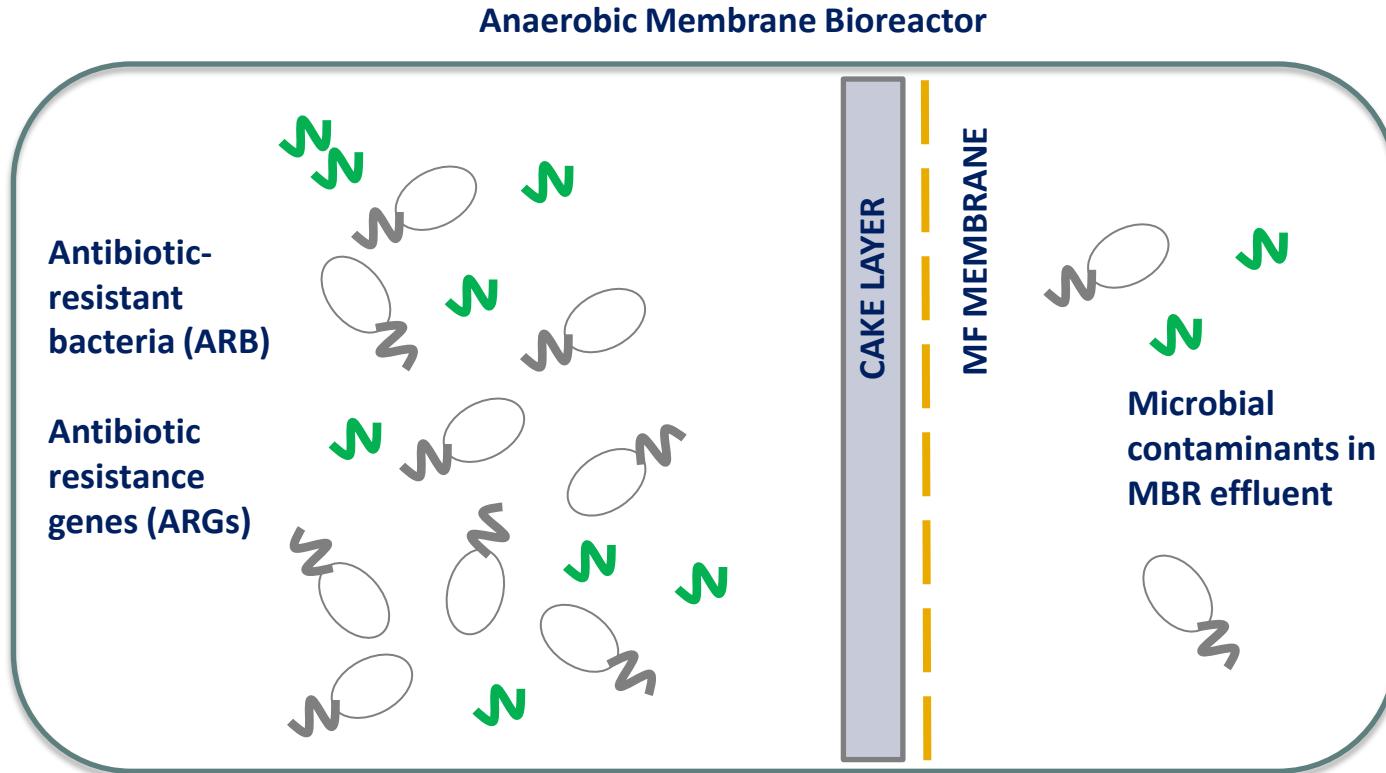


low risk

Case study 1: conclusions

- Targeting of specific pathogens by both high-throughput sequencing and dPCR showed higher removal rates by the lab-scale anaerobic MBR.
- Compared to aeMBR, anMBR treatment of municipal wastewater could be a viable means for improving effluent reuse and sludge disposal pathogen risks.

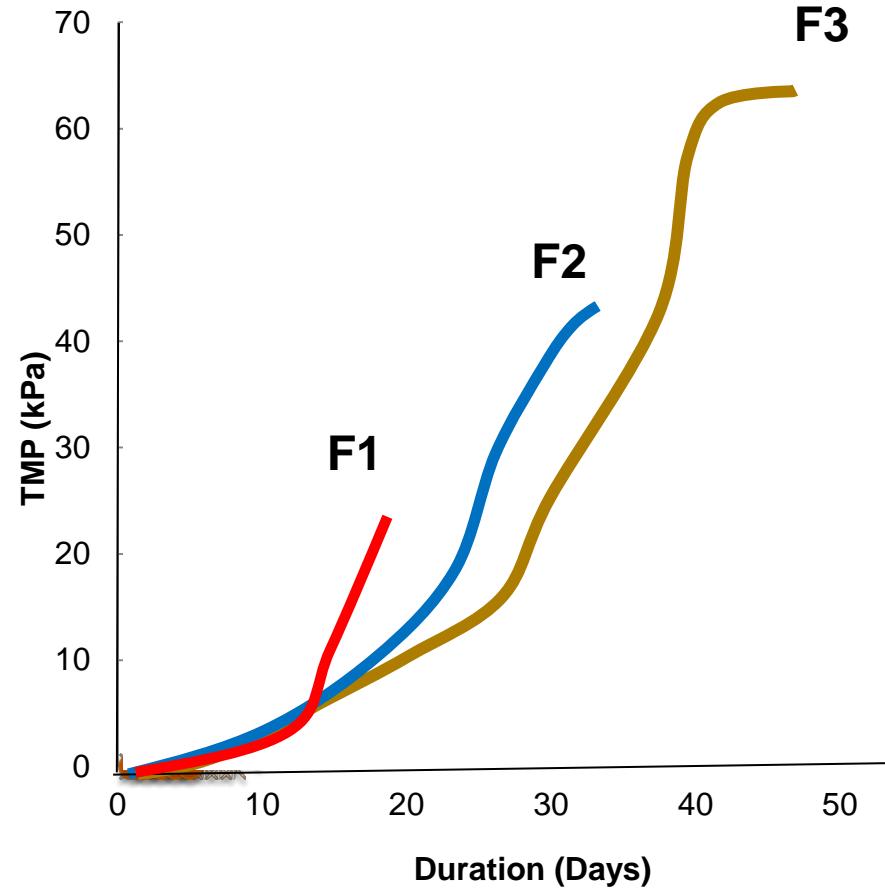
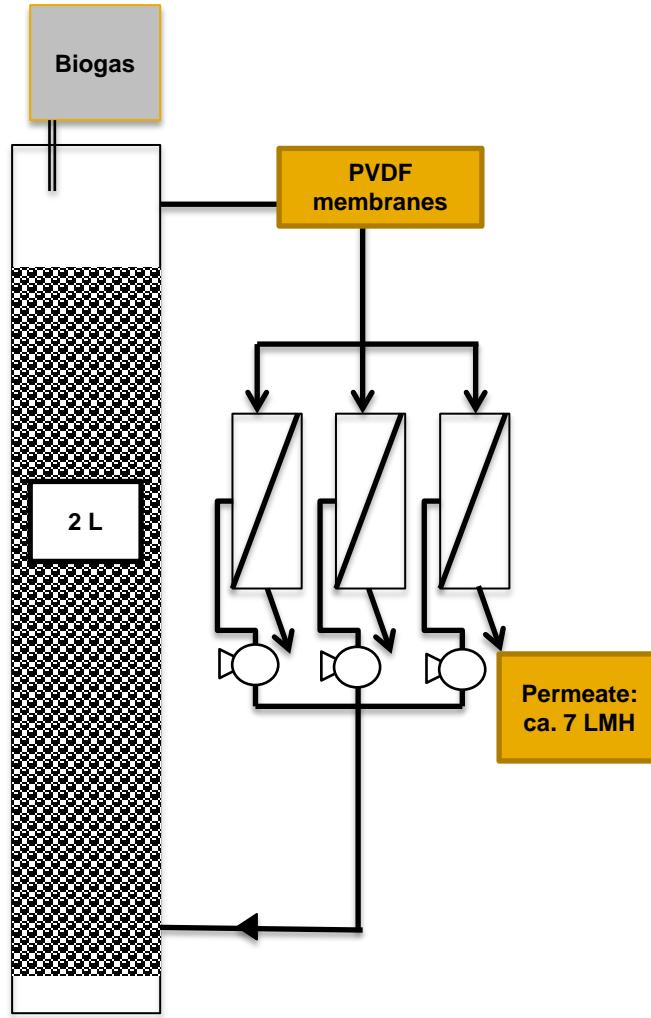
Case study 2



Hypothesis:

Removal of ARB and ARGs affected by varying degrees of fouling on anaerobic microfiltration membrane

Experimental design 1

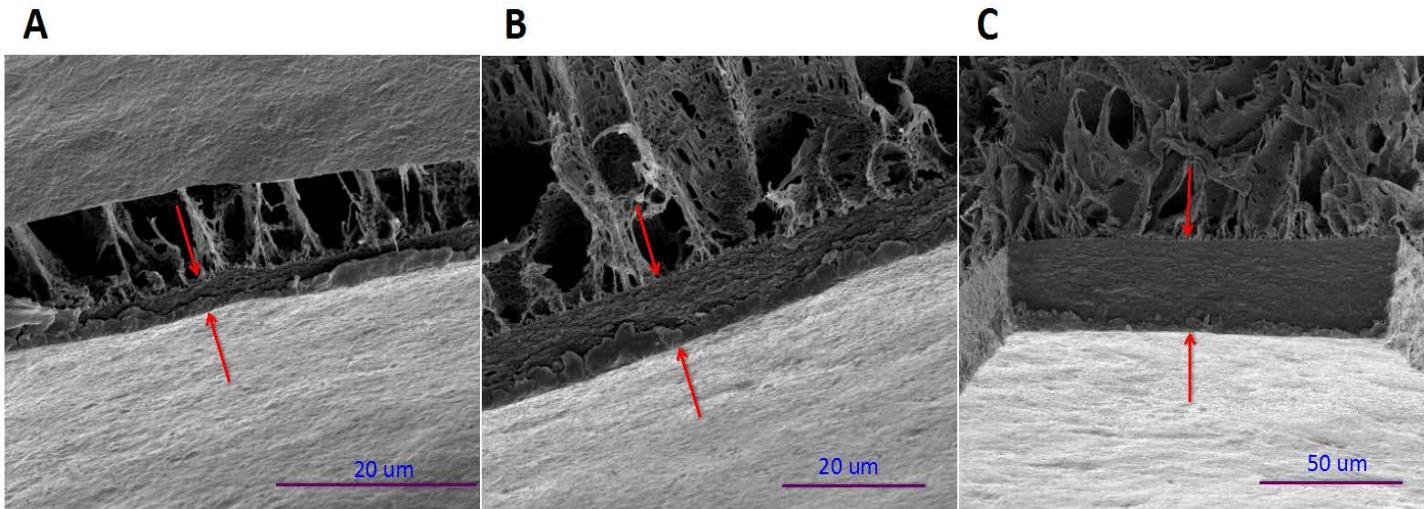


SEM images of fouled membranes

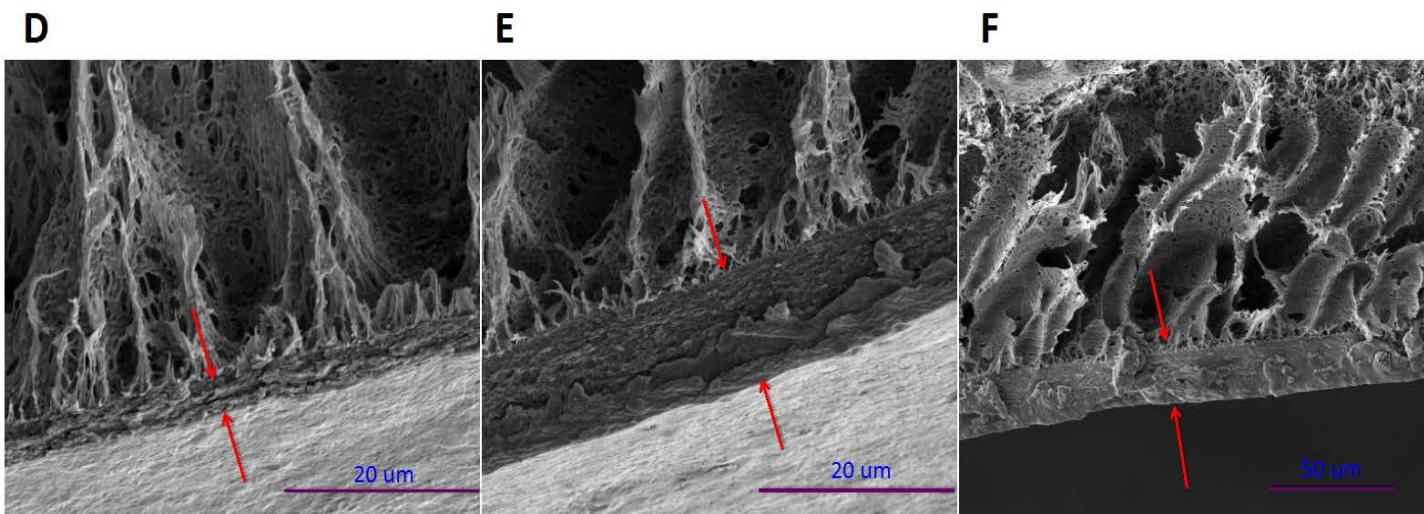
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Run 1



Run 2



Physical parameters of fouled membranes

Membrane	Thickness (μm)		Estimated dried biovolume (mm ³) ^a		Roughness		Hydrophilicity	Surface zeta potential
	Run 1	Run 2	Run 1	Run 2	R _a (nm) ^b	R _q (nm) ^c		
N0	N.A.	N.A.	N.A.	N.A.	118.3 ± 27.0	144.0 ± 36.0	78.7 ± 3.8	-44.3 ± 4.4
F1	2.99 ± 0.17	3.96 ± 0.26	15.0	19.8	79.2 ± 10.4	102.5 ± 17.5	90.2 ± 2.3	-17.3 ± 0.3
F2	8.80 ± 0.13	10.4 ± 0.48	44.0	52.2	61.7 ± 6.5	76.1 ± 7.6	95.5 ± 1.9	-19.2 ± 1.9
F3	26.9 ± 3.24	20.3 ± 0.43	134	102	51.1 ± 6.5	65.2 ± 8.6	107.4 ± 1.6	-25.8 ± 2.3

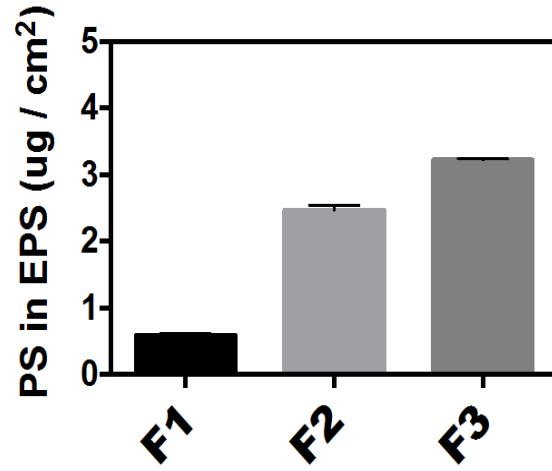
^aEstimated dried biovolume was determined by multiplying the average thickness by the membrane surface area.

^bR_a is the arithmetic average of the absolute values of the surface height deviations measured from the mean plane.

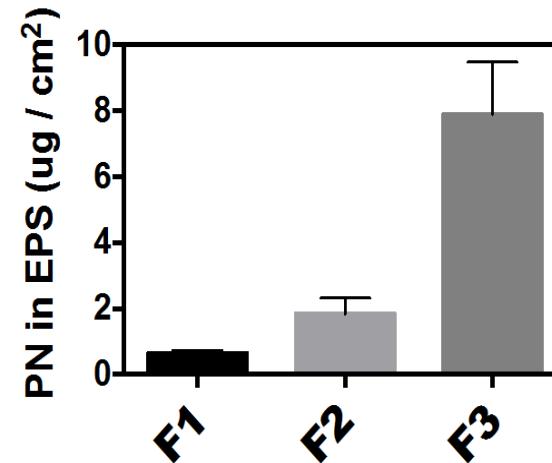
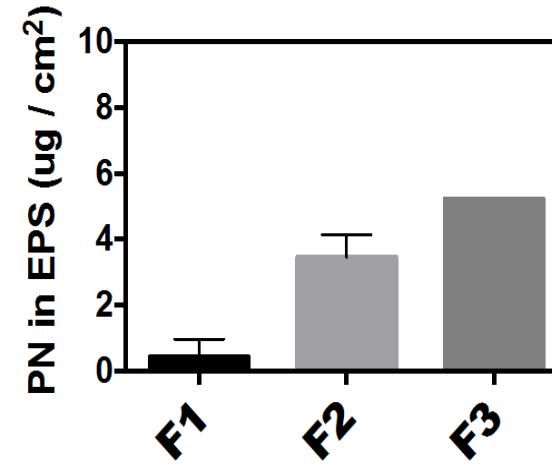
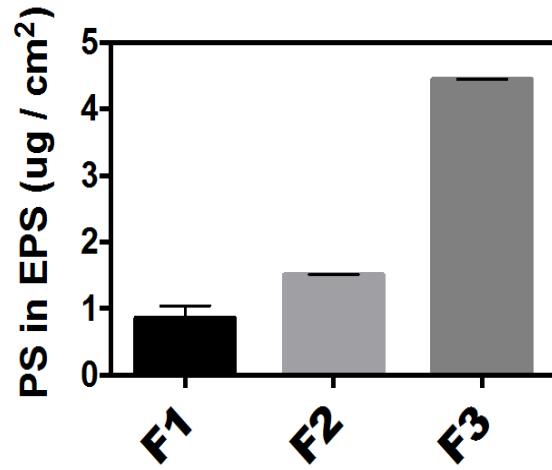
^cR_q is the root mean square average for height deviation taken from the mean image data plane.

Chemical parameters of fouled membranes

Run 1

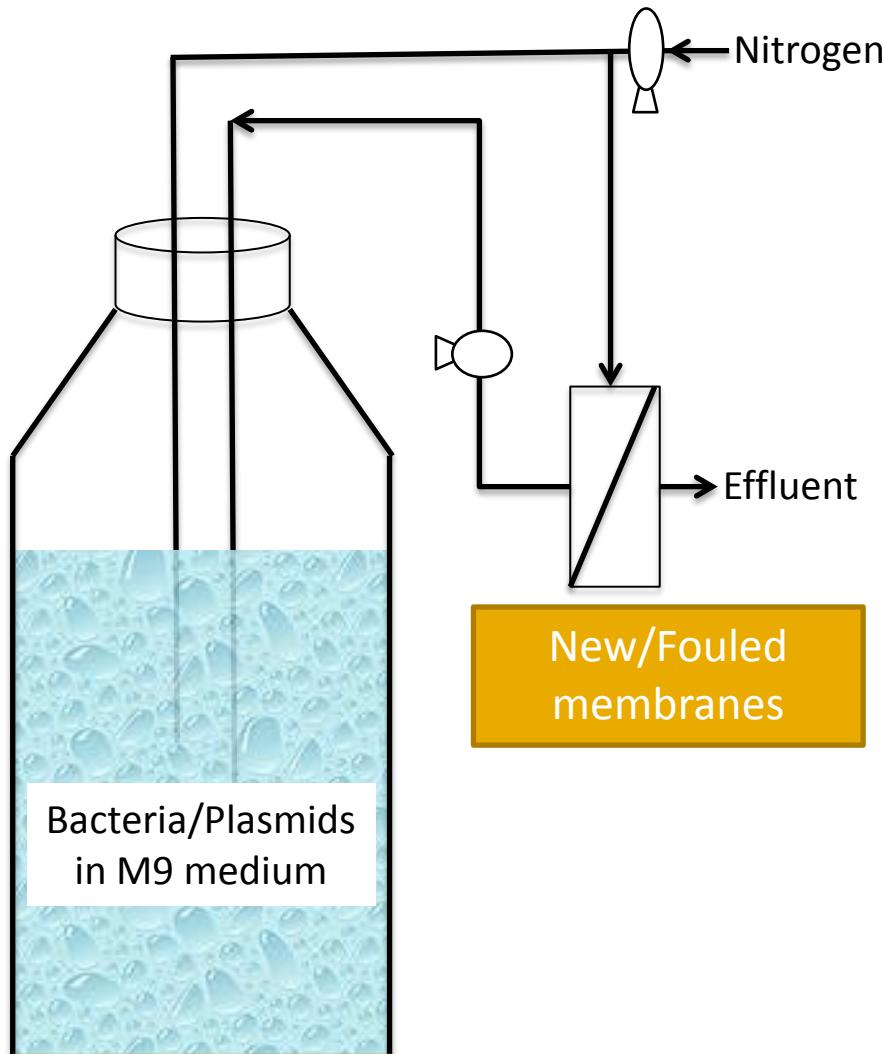


Run 2



- Surface characteristics of anaerobic membranes change with increasing fouling: more hydrophobic, lower surface roughness and lower negative charge
- Biofilm thickness increase with increase protein and polysaccharide contents
- Would these parameters result in differences when removing ARB and ARGs?

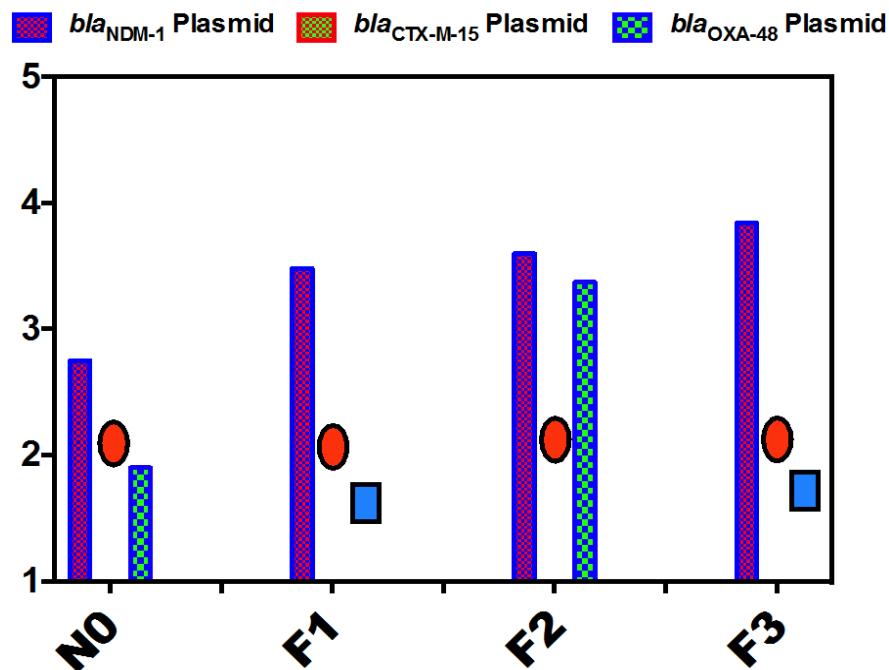
Experiment design 2



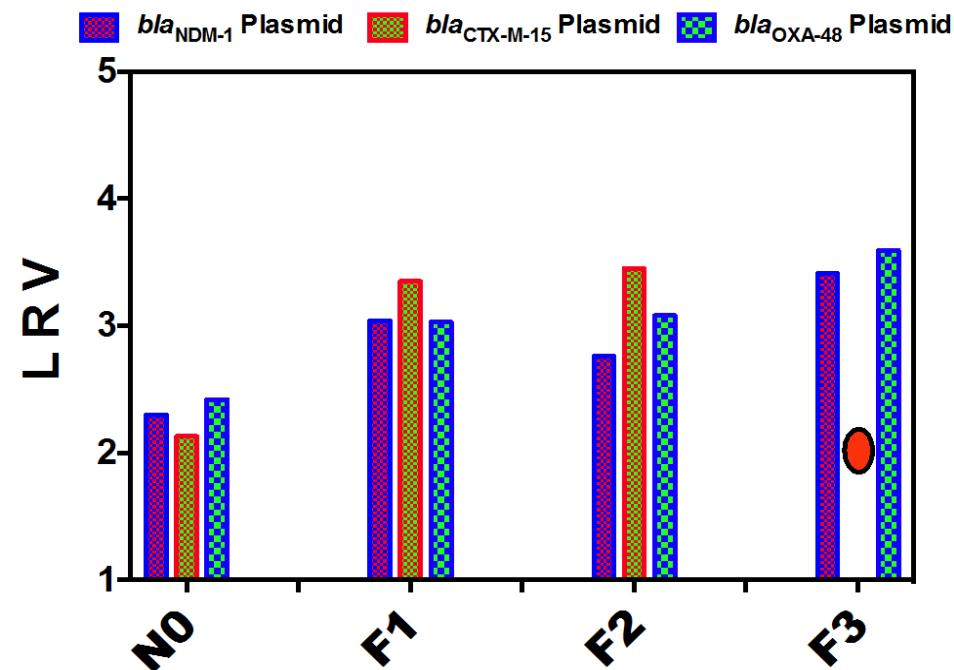
- F 1-1, F 1-2, F 1-3 and F 1-4: Fouled 1 membrane (20 kPa) sampled at **5, 10, 15 and 20 kPa**;
- F 2-1, F 2-2, F 2-3 and F 2-4: Fouled 2 membrane (40 kPa) sampled at **10, 20, 30 and 40 kPa**;
- F 3-1, F 3-2, F 3-3 and F 3-4: Fouled 3 membrane (60 kPa) sampled at **10, 20, 40 and 60 kPa**;
- N 0-1, N 0-2, N 0-3, N 0-4: New membranes sampled at 0 kPa but different time.

Foulant enhance removal of ARGs

Run 1



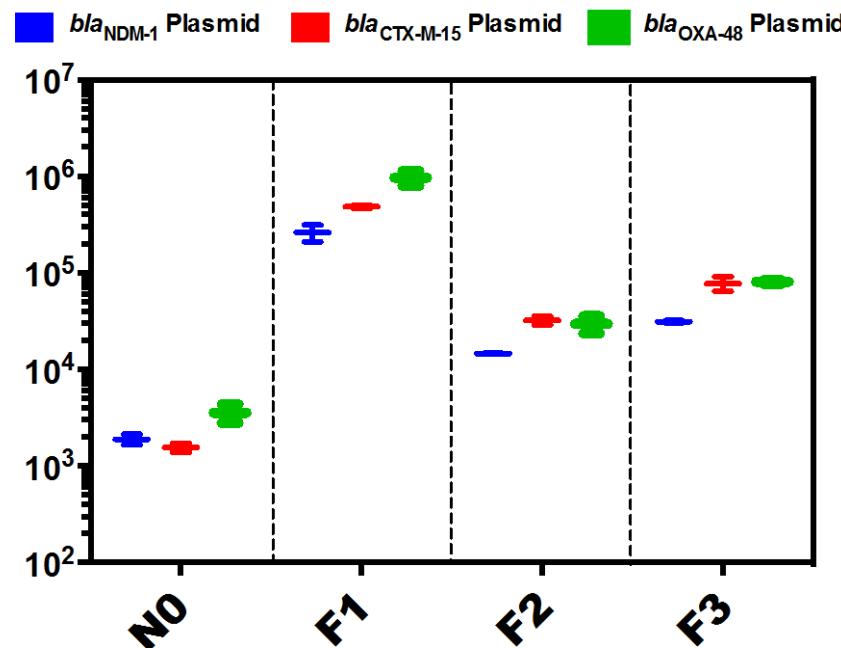
Run 2



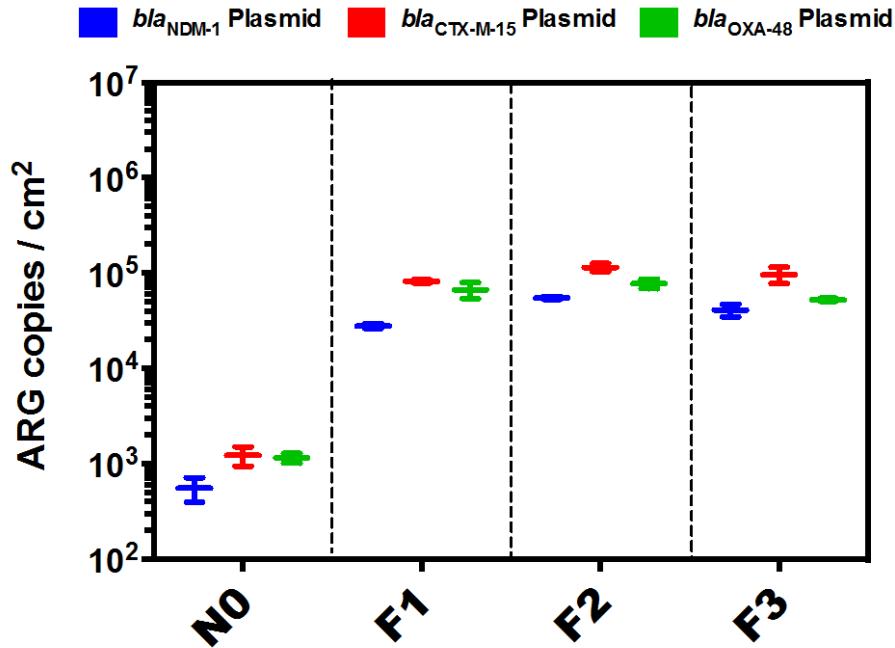
○ ■ denote gene target cannot be detected in permeate and thus no LRV was determined

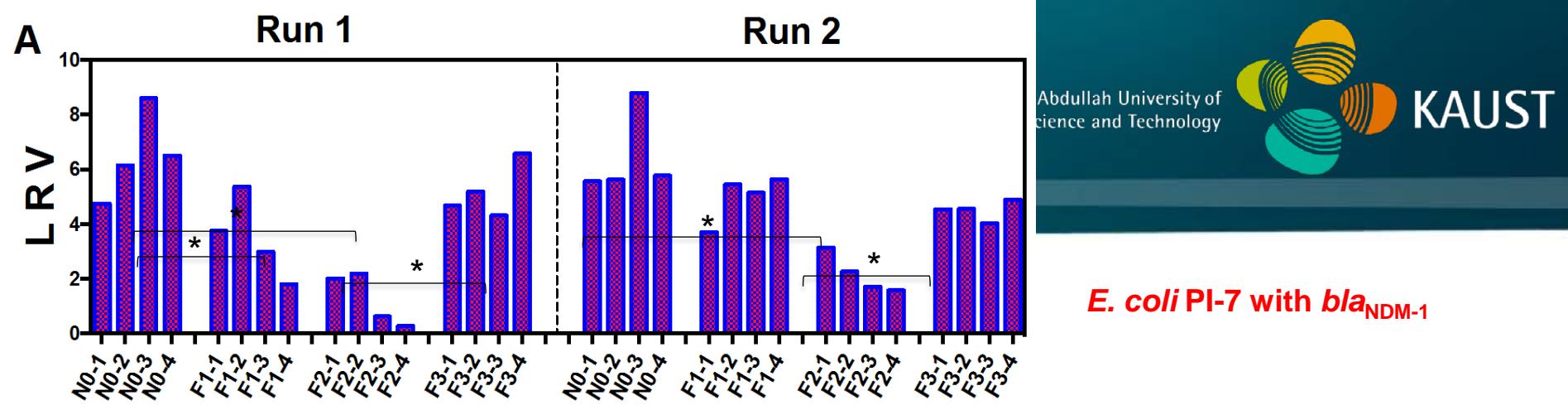
Higher adsorption of ARGs on fouled membranes

Run 1



Run 2

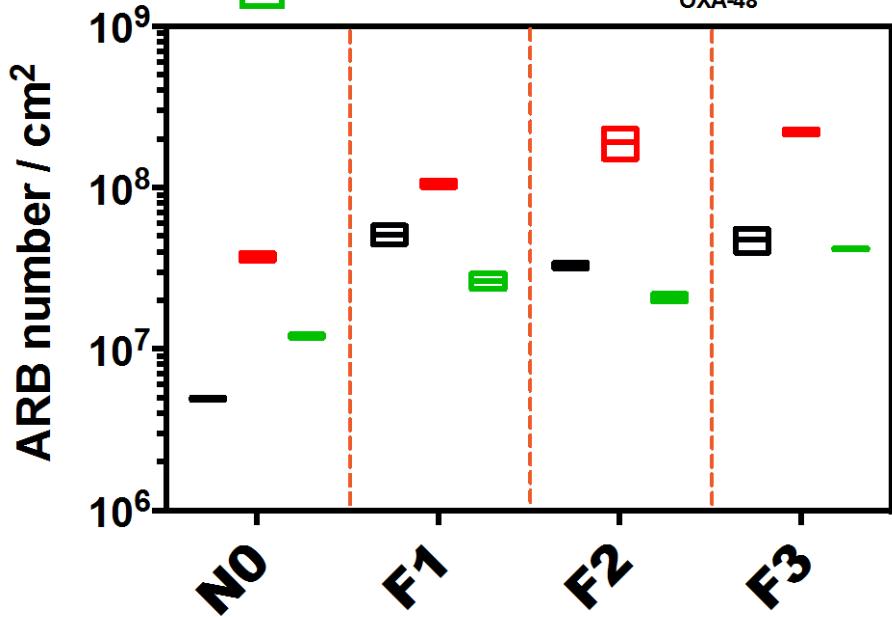




Higher adsorption of ARB on fouled membranes

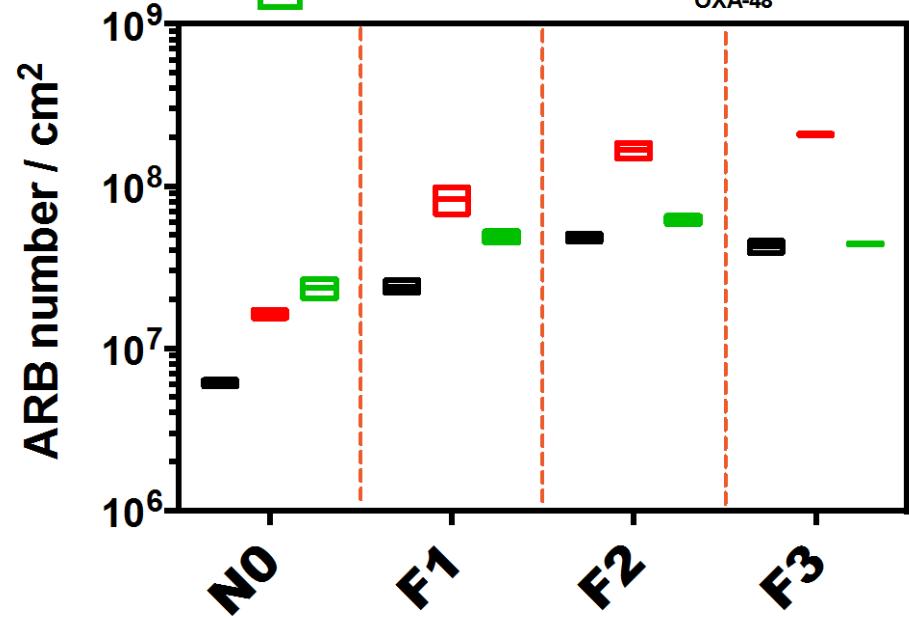
Run 1

- $E. coli$ with bla_{NDM-1}
- $K. pneumoniae$ L7 with $bla_{CTX-M-15}$
- $E. coli$ UPEC-RIY-4 with bla_{OXA-48}



Run 2

- $E. coli$ with bla_{NDM-1}
- $K. pneumoniae$ L7 with $bla_{CTX-M-15}$
- $E. coli$ UPEC-RIY-4 with bla_{OXA-48}



- Total LRV of ARGs increased with the extent of fouling
- LRV of ARB decreased initially before stabilizing at a LRV similar to that of new microfiltration membranes
- Specifically, LRV of ARB decreased when membranes were sub-critically fouled
- Removal mechanisms in an anMBR is a combination of size exclusion and adsorption, as well as the filtration pressure/force
- Long term operation of anaerobic MBR favors the removal of emerging contaminants like ARB and ARGs

Conclusions

- The advantages of anMBRs towards aeMBRs are
 - (i) a lower energy and costs required to operate;
 - (ii) the production of methane that can be harvested to become an energy source; and
 - (iii) less volume of antibiotic-laden sludge that needs to be disposed adequately (Shoener et al., 2016)
 - (iv) anMBR may provide treated effluent that is safer than that produced by aeMBR (Harb et al., 2016; Cheng and Hong, 2017)

Acknowledgements

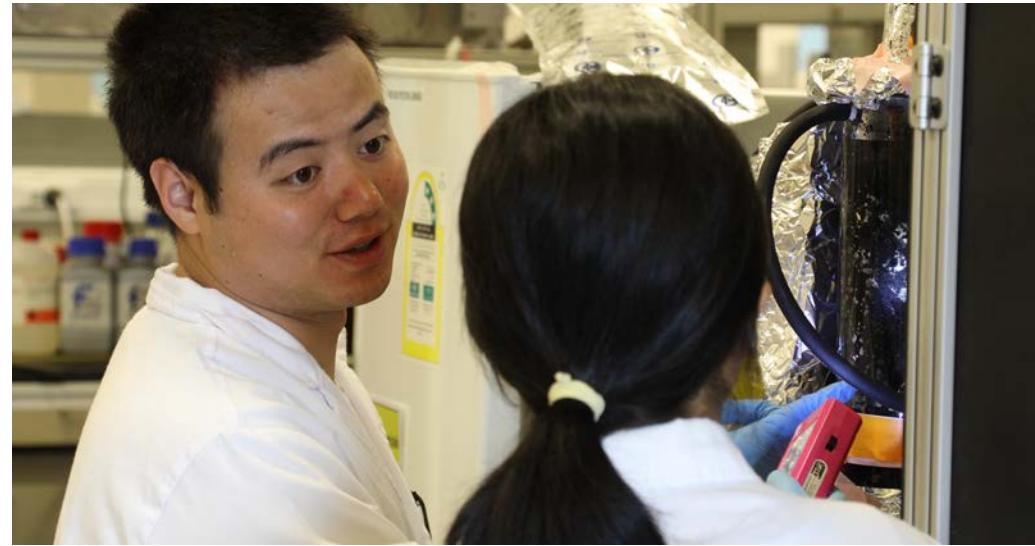
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