Oestrogen Analysis by ELISA and Removal by Trickling Filters

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INTRODUCTION

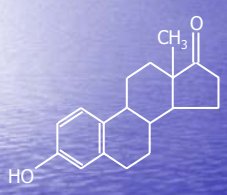
- Background
- Properties of oestrogens
- Estimation model
- Analytical method

Background

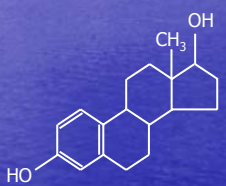
- Oestrogens are to be regulated by Environmental Quality Standards.
- Uncertainties still remain in terms of the ability of wastewater treatment processes to remove oestrogens at the ng/L level.
- A national demonstration programme has been started to evaluate the performance and efficacy of current treatment technologies at reducing oestrogen concentration and oestrogenic activity in STW effluents.
- In the UK, 70% STWs are using trickling filters, but results from them are quite few.

Properties of oestrogens

Natural oestrogen

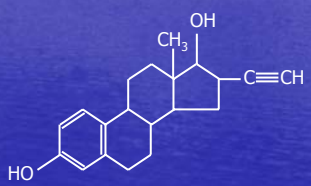


E1— Oestrone



E2 — 17β Oestradiol

Synthetic oestrogen



EE2 — 17α Ethinyl oestradiol

Oestrogen	Aqueous Solubility (mg/L)	Log K_{ow}	H_c (atm m^3/mol)
E1	13	**2.81**	2×10^{-11}
E2	12.96	**3.94**	6.3×10^{-7}
EE2	4.83	**4.15**	3.8×10^{-7}

- Oestrogenic activity: EE2>>E2>E1. (*total oestrogen concentration=10*[EE2]+[E2]+[E1]/3 — E2 equivalent*)
- Biodegrability: E2>E1>EE2

Fate of oestrogens when spiked into activated sludge

Oestrogen	Spiked conc.	Rate of loss	Products
E2	1 µg/L 1mg/L	>90% in 30 mins, 95% in 3 hrs 95% in 3 hrs	E1
E1	1mg/L	50% in 24 hrs	Unknown
EE2	1 µg/L 1mg/L	20% in 24 hrs 0% in 24 hrs	Unknown

(Ternes, T.A., et al, 1999)

Estimation model

- As the primary source of steroid oestrogens, STW influent concentration of oestrogens could be estimated by defining human population in a catchment. (*Johnson, A. et al, 2004*)
- 1) pregnant females (0.88%)
  2) menstrual females (30%)
  3) menopausal females (11%)
  4) females taking HRT (2%)
  5) males (50%)
- Normalised excretion value of E1 and E2 are 13.7 and 3.3 µg/d per person, and EE2 is 10.5 µg/d per woman.

Analytical method

- There is no standard method available for oestrogen quantification in water samples at ng/L level so far.
- Chemical methods (GC-MS/MS,HPLC-MS/MS): highest detection certainty, lowest detection limits, highest cost.
- Biological methods (ELISA, RIA, YES): high sensitivity, suitable detection limits, acceptable cost, less detection certainty.
- Whatever methods, a time consuming sample pretreatment process is needed because of ubiquitous interference and the low concentration.

ELISA- enzyme-linked immunosorbent assay; RIA-radio immunosorbent assay; YES-yeast estrogen screen

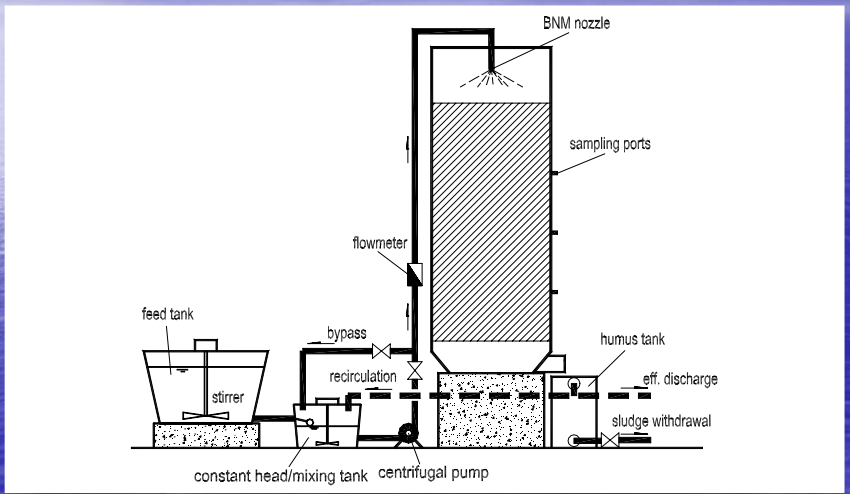
OBJECTIVES

- Assess the use of ELISA for oestrogen analysis in sewage treatment process
- Investigate the performance of trickling filters both in well controlled conditions of the lab and the STW.
- Investigate the behaviour of oestrogens in various biofiltration steps of the STW.

MATERIALS & METHODS

- The pilot trickling filter
- The STW
- Sample collection and preparation
- ELISA analysis
- Comparisons with chemical analysis

The pilot trickling filter



- UU standard design
- 50mm blast-furnace slag, depth of 1.8 m
- Single pass for complete nitrification
- Height: 2.6 m, diameter: 0.9 m
- Surface area: 0.64m^2
- Bed volume: 1.14m^3

The synthetic sewage

- Enable changes in organic and SS loading
- Enable changes in oestrogen dosing

The synthetic sewage recipe

Ingredients	Conc. (mg/L)	Ingredients	Conc. (mg/L)
Dextrin	150	Detergent	50
NH_4Cl	85	NaH_2PO_4	20
Yeast extract	120	K_2SO_4	8.3
Glucose	100	Maize starch	100

The pilot trickling filter operation

Phase	Recirculation	Oestrogen dosing (ng/L)		
		E1	E2	EE2
1	NO	50	50	50
2	NO	50	15	5
3	1:1	50	15	5

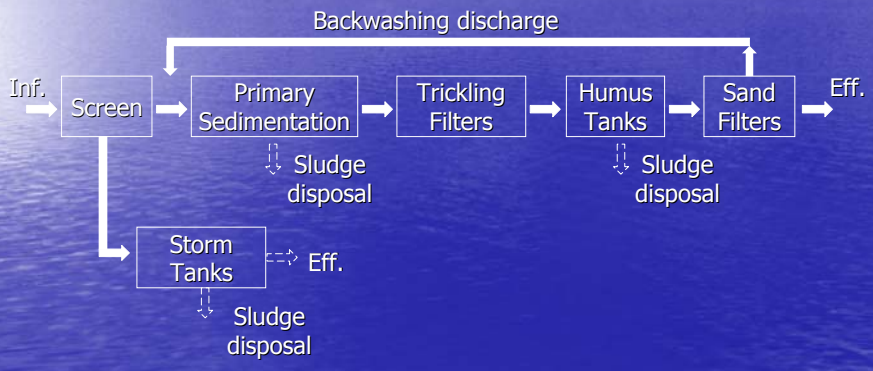
Operational parameters of the pilot trickling filter

Flow rate (m^3/d)	Loading			Wetting rate (m^3/m^2d)
	BOD_5 (Kg/m^3d)	Hydraulic (Kg/m^3d)	NH_3-N (Kg/m^3d)	
1.2	0.09	1.053	0.014	1.875
2.4	0.09	2.105	0.013	3.750

The STW

- Located in North Midland
- Catchment population: 13,794
- Single filtration
- Combined sewage
- Flow rate (m^3/d)

DWF	3427
Average	4284
Maximum	9550



Schematic diagram of STW treatment process

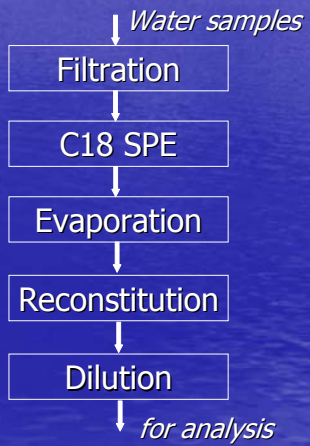
Sample collection & preparation

- 1 L grab sample from the STW at 5 different points
- 100 mL grab samples from the pilot trickling filter (influent and effluent)
- The pretreatment is to enrich samples and avoid interference from the matrix
- Sample Pretreatment Protocol for Female Steroid Hormones (Japan EnviroChemicals, Ltd. 2005)

Sample pretreatment process

Dynamic range of JEC ELISA kits

Kit	Range (ng/L)
E1	50-5000
E2	50-1000
EE2	50-3000



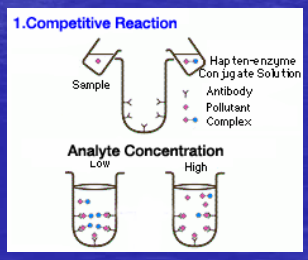
Special treatment for E1 in the influent

- To avoid the interference from E1-3-sulphate and E1-3-glucuronide.
- Aminopropyl SPE after reconstitution.
- Collect the filtrate, then repeat evaporation, reconstitution and dilution steps.

ELISA analysis

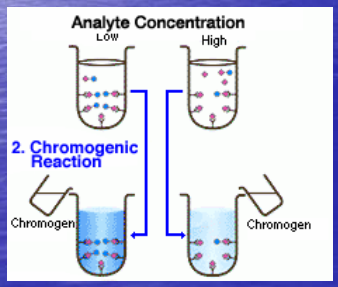
Competitive reaction

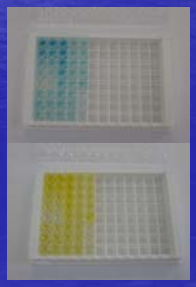
- Oestrogen and oestrogen-enzyme conjugate compete for antibody binding sites on the microplate walls



Chromogenic reaction:

- Enzyme conjugates catalyze colour solution to a blue colour product, and it will turn yellow after adding the stop solution



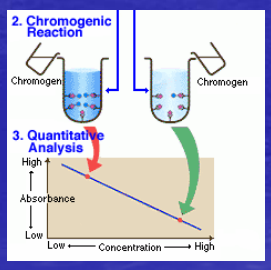


After adding colour solution

After adding stop solution

Quantitative analysis:

- Measure the absorbance by a microplate reader at 450 nm
- The absorbance signal and the oestrogen concentration are inversely related.
- Calculate using a 4-parameter logistic fitting software



RESULTS & DISCUSSION

Pilot scale

Oestrogen removal of the pilot trickling filter

oestrogens	Influent Conc. (ng/L)	Effluent Conc. (ng/L)	Removal rate	Sample Recovery rate	Sample number
Phase 1					
E1	47.20±1.15	20.8±2.31	55.93±5.32%	85.7±0.8%	5
EE2	46.42±0.96	23.54±1.21	49.40±7.67%	86.5±1.2%	5
Phase 2					
E1	47.0±0.81	23.55±3.83	49.93±7.67%	83.1±1.1%	5
EE2	4.74±0.15	4.42±0.34	45.36±6.57%	77.4±0.9%	5

* Tabulated values are: mean ± standard deviation

oestrogens	Influent Conc. (ng/L)	Effluent Conc. (ng/L)	Removal rate	Sample Recovery rate	Sample number
Phase 3 (09/2005)					
E1	47.72±0.52	15.66±4.06	67±8%	83.1±1.1%	3
E2	13.58±1.20	3.87± 0.92	72±1%	86.1±1.4%	1
EE2	4.86±0.08	1.73±0.24	64±5%	77.4±0.9%	3
Phase 3 (10/2005-12/2005)					
E1	45.13±2.91	6.13±1.81	86±4%	85.7±0.7%	3
E2	12.90±0.92	2.30±0.10	82±1%	86.1±1.4%	3
EE2	4.93±0.81	0.87±0.06	82±2%	79.2±1.8%	3
Phase 3 (01/2006-03/2006)					
E1	45.72±3.91	22.07±4.81	51±4%	76.9±2.1%	3
E2	12.07±1.86	6.29±0.10	48±1%	79.1±1.4%	3
EE2	4.59±1.13	2.08±0.88	55±2%	81.2±1.7%	3

Other water quality parameters of the pilot trickling filter

Phase		TOC (mg/L)	SS (mg/L)	NH_3-N (mg/L)	Temp (°C)	pH	Date
1	Inf.	144.3±33.2	133±17	26.0±5.9	11.2±4.8	6.9±0.2	03/2005 ~ 04/2005
	Eff.	18.37±12.0	15±10	8.5± 2.8	9.7±4.6	7.7±0.4	
2	Inf.	147.9±37.4	139±18	23.9±6.4	19.2±4.7	6.7±0.2	05/2005 ~ 08/2005
	Inf.	17.56 ±8.9	14±6	6.6±1.9	15.3±5.2	7.2±0.3	
3	Inf.	146.4±41.5	129±13	27.4±6.1	9.1±5.2	7.1±0.1	09/2005 ~ 12/2005
	Eff.	15.01± 7.4	13±10	6.7±2.1	6.6±5.7	7.2±0.2	
	Inf.	143.1±23.2	131±21	28.27±4.5	4.6±1.1	7.1±0.2	01/2006 ~ 03/2006
	Eff.	67.21±13.9	22±7	19.76±3.7	2.9± 0.9	7.1±0.3	

Discussion points

- The pilot trickling filter removed 45-56% oestrogens without recirculation.
- The 1:1 recirculation improved the oestrogen removal to 82-86%.
- 48-55% oestrogens removed when bioactivity of the filter was limited at freezing time.
- Similar results to literatures

Full Scale

Measured oestrogen concentrations of the STW

	CS (ng/L)	SS (ng/L)	AF (ng/L)	HE (ng/L)	FE (ng/L)	N	RR(%)
19/09/2005, dry							
E1	74.25±3.56	71.12±3.78 (4.22%)	39.34±2.85 (47.02%)	35.61±3.05 (52.04%)	18.39±1.91 (75.23%)	1	83.1±1.1
EE2	3.61±0.97	3.38±1.05 (6.37%)	2.12±0.84 (41.27%)	1.64±0.87 (54.57%)	1.06±0.74 (70.64%)	1	77.4±0.9
02/12/2005, wet							
E1	28.12±2.61	12.80±2.17 (54.48%)	10.76±1.33 (61.74%)	5.96±1.66 (78.81%)	3.32±0.82 (88.19%)	1	81.9±1.7
E2	6.42±1.14	5.53±1.32 (11.54%)	2.04±0.52 (67.31%)	1.68±0.97 (73.08%)	0.72±0.24 (88.46%)	1	86.1±1.4
EE2	0.20±0.13	0.20±0.11 (0.00%)	ND	ND	ND	1	79.2±1.8

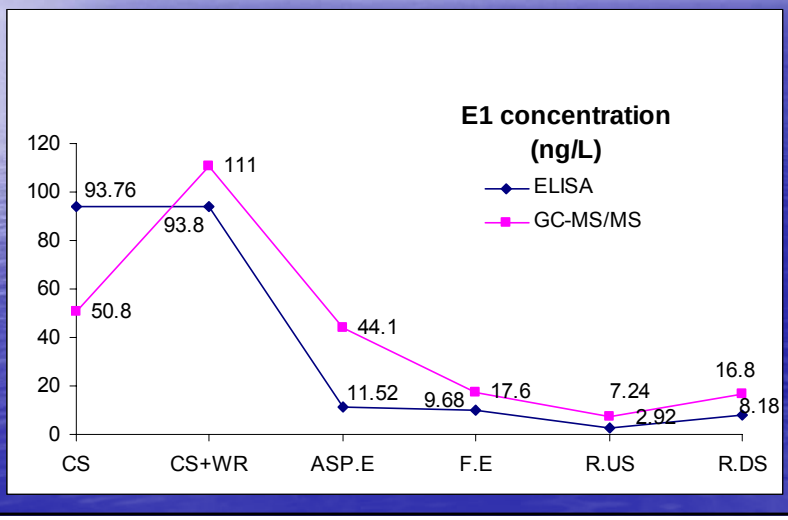
* Tabulated values are: mean± standard deviation (removal rate%);
** CS-crude sewage, SS-settled sewage, AF-after filtration, HE-humus tank effluent, FE-final effluent., N-sample numbers(duplictes), RR- recovery rate, ND-not detected

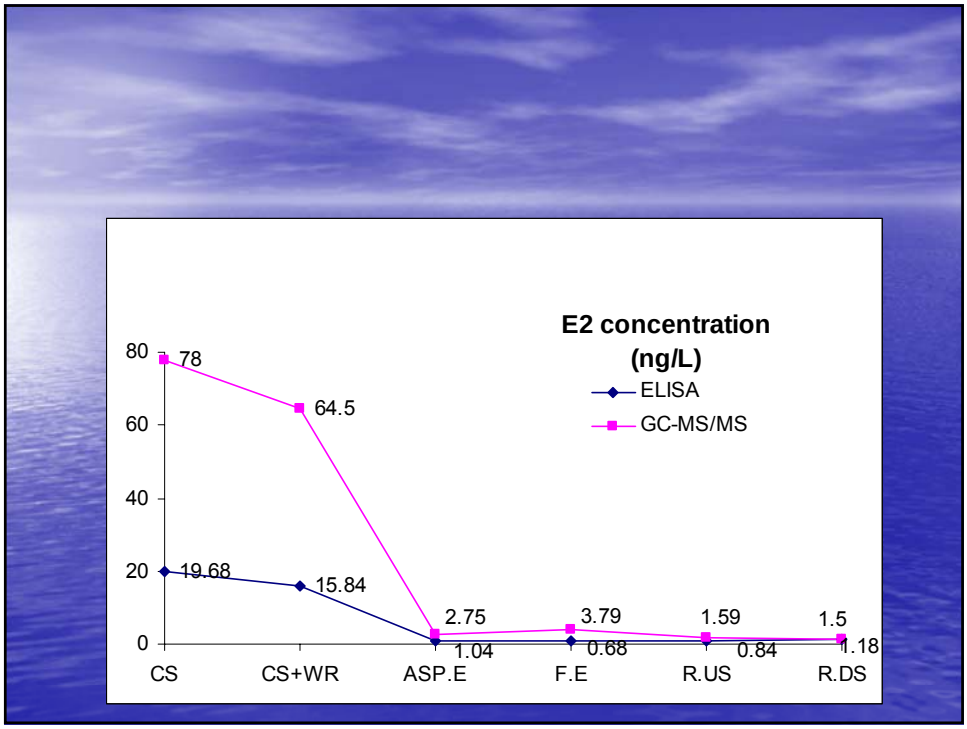
Discussion points

- Oestrogen concentrations dropped with the treatment train.
- Single filtration removed 41-67% oestrogens.
- Humus settlement improved removal rate to 52-79%.
- The tertiary treatment increased the removal rate to 70-88%.

Analysis Comparison

- 6 samples from an ASP STW: crude sewage, influent of primary tanks (with backflow from sludge treatment), ASP effluent, final effluent, river water upstream, river water downstream.
- Analyzed by GC-MS/MS and ELISA.
- E1 and E2 concentrations were compared.





Discussion points

- High discrepancy between two sets of results.
- ELISA analysis gave lower results, while literature reported biological analysis for complicated samples always overestimated.
- One of the possible reason is samples for GC-MS/MS analysis were added with Cu^{2+}/HCl, while those for ELISA were preserved only under 4 °C and pretreated within 2 days after collection.
- More comparisons are now carrying out.

CONCLUSIONS

- ELISA kits could quantify the ng/L level oestrogens in sewage after a time-consuming sample pretreatment, but validation by instrumental methods would still be necessary.
- Single filtration of trickling filters could remove 40-67% oestrogens, and humus settlement could slightly increase the rate.
- Recirculation could improve oestrogen removal, it is suspected that bioadsorption played an important role.
- Tertiary or multistage treatment is a good design option

Big Thanks to UU!

Many thanks for listening!

Questions & Help Please