Evaluating Enzyme Performance in the Face of Process Complexity

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Complexity and Enzyme Variability

<u>Aim: Understand how a complex process environment impacts on enzyme activity.</u>

- Wastewater is a highly complex process environment.
- Enzymes are critical in wastewater treatment.
- Multiple factors impact enzyme performance.
- Enzyme variability = Inconsistent output.

Inherent Enzymes
Present in the wastewater system. (Biological Performance Indicator)

Exogenous Enzymes
Added to the wastewater system. (Bioactive Chemical Removal)

Enzyme Performance

- **Process Complexity → Wastewater Matrix**
- Inherent Enzymes → Biological Performance Indicator
- Exogenous Enzymes → Bioactive Chemical Removal

Process Complexity

External Influences

- Seasonal temperatures and rainfall
- pH, COD, TSS, metals

Variability in Composition

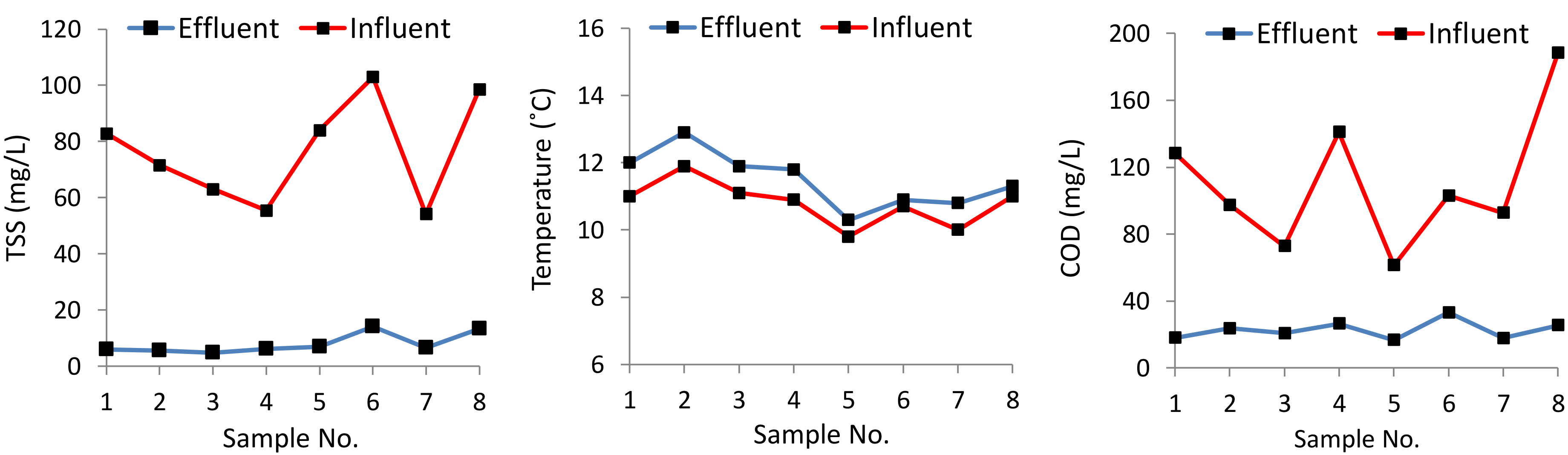
- Municipal/ industrial waste streams
- Population behaviour (e.g. seasonal antibiotic spike)
- Bioactive chemicals (natural/synthetic)

Operational Parameters

- Different process configurations
- DO, MLSS, temperature

Wastewater Characterisation

1) Water quality parameters (e.g. TSS, COD and temperature).
2) High temporal and spatial variability.
3) Correlation with enzyme performance identifies key process and environmental factors that can affect output.



Sampling between Jan 2016 to Feb 2016 → Stoke Bardolph (Nottingham) WWTP

Enzyme Performance

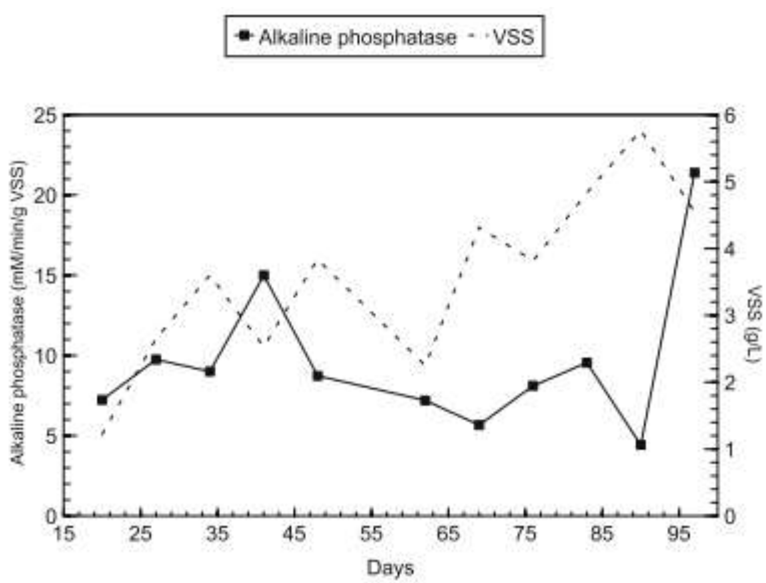
Process Complexity →
Wastewater Matrix

Inherent Enzymes →
Biological Performance Indicator

Exogenous Enzymes →
Bioactive Chemical Removal

Inherent Enzymes

- Hydrolases break down the majority of organic pollutants (e.g. polysaccharides).
- Peptidases, lipases, esterases, glucosidases and phosphatases commonly analysed.
- Enzymatic profiles inform on biological performance.
- Research mainly on activated sludge from lab-scale units.



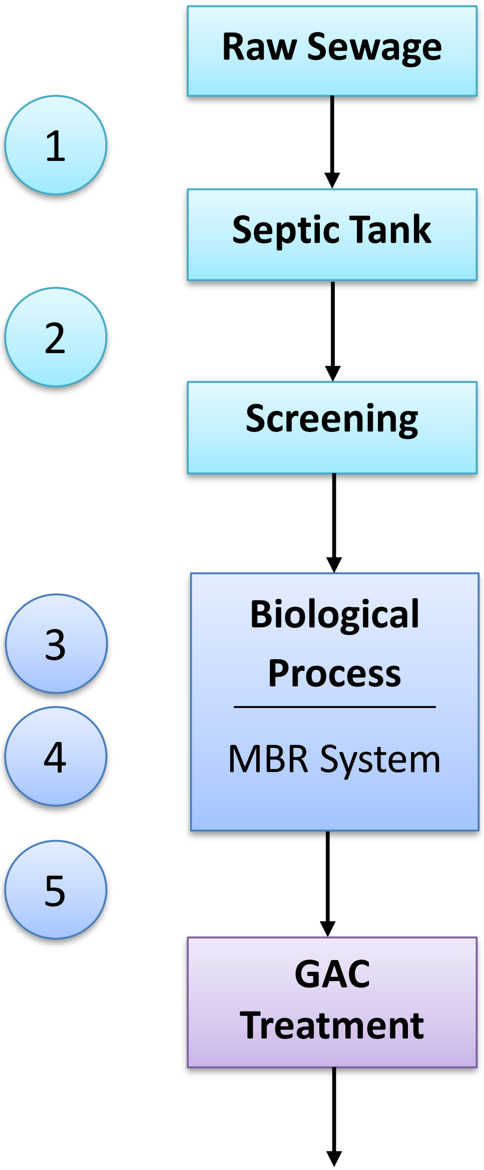
Molina-Muñoz M et al (2007)

- Volatile suspended solids (VSS) linked to biomass concentration.
- Enzyme activity was expected to increase with VSS.
- Activity tended to decrease instead.

Old Ford Water Recycling Plant

- **Direct non-potable water reuse**
- **574,000 litres daily output capacity**
- **Membrane bioreactor (MBR) system**
- **Granular activated carbon (GAC) unit**

Inherent Enzyme Analysis



Enzyme activity assayed through a fully operational WWTP

<u>Sample Locations:</u>

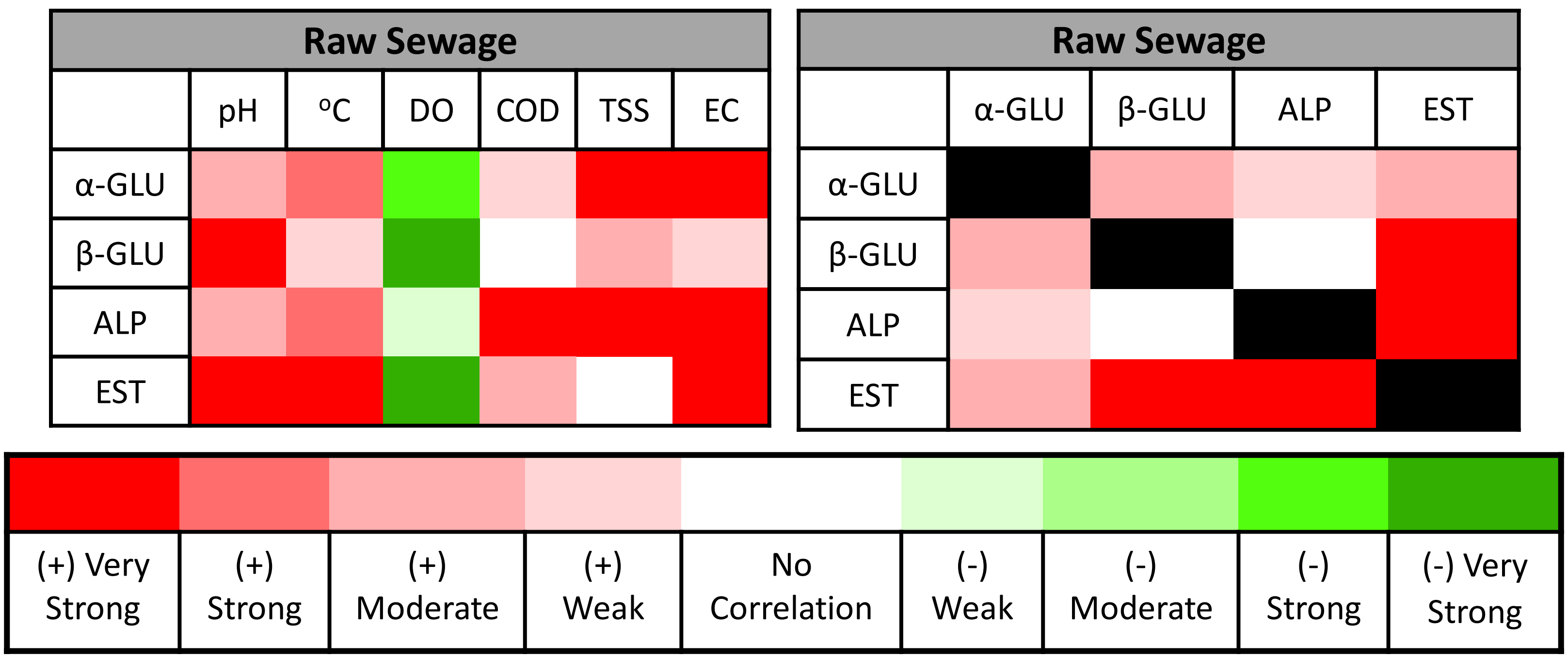
1) Raw Sewage
2) Screened Sewage
3) Mixed Liquor
4) Returned Activated Sludge
5) Post MBR

<u>Assayed Enzymes:</u>

- α-Glucosidase (α-GLU)
- β-Glucosidase (β-GLU)
- Alkaline Phosphatase (ALP)
- Esterase (EST)
- Sulfatase (SUL)

Inherent Enzyme Correlations

- 7 sampling campaigns carried out in May 2016.
- Samples characterised by multiple water quality parameters.
- Pearson's (r) used to correlate the two variables.
- (+) → both variables increase together
- (–) → inverse relationship

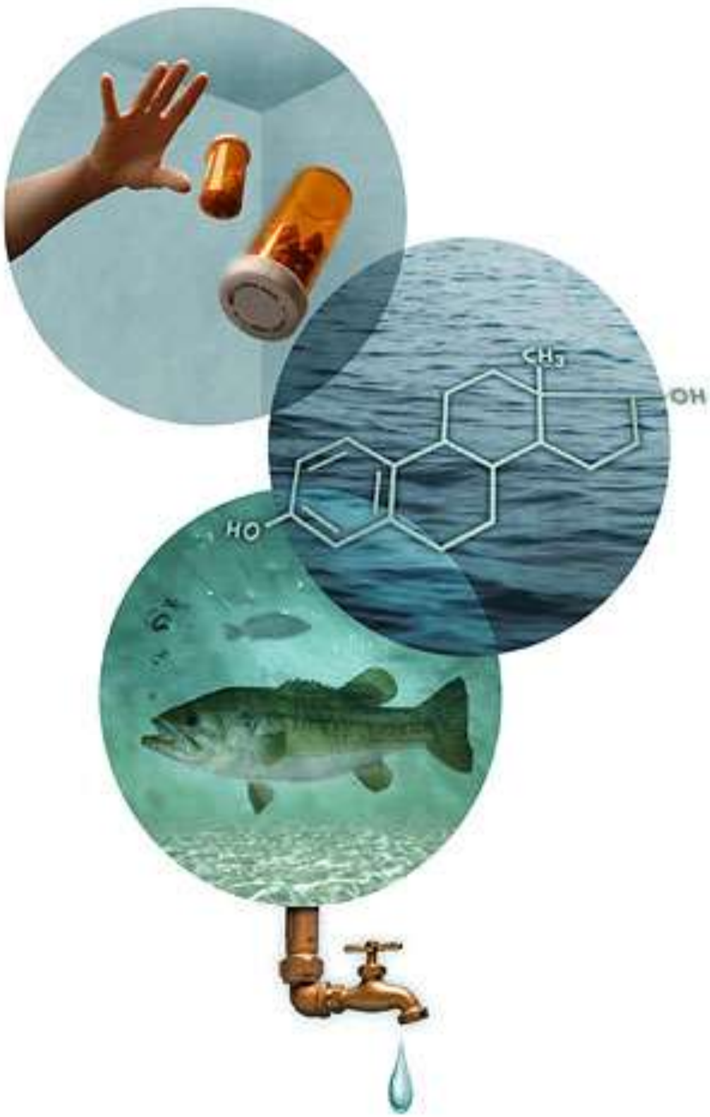


- DO = Dissolved Oxygen
- COD = Chemical Oxygen Demand
- TSS = Total Suspended Solids
- EC = Electrical Conductivity

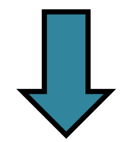
Enzyme Performance

- Process Complexity →
  Wastewater Matrix
- Inherent Enzymes →
  Biological Performance Indicator
- **Exogenous Enzymes →**
  Bioactive Chemical Removal

Bioactive Chemical Removal

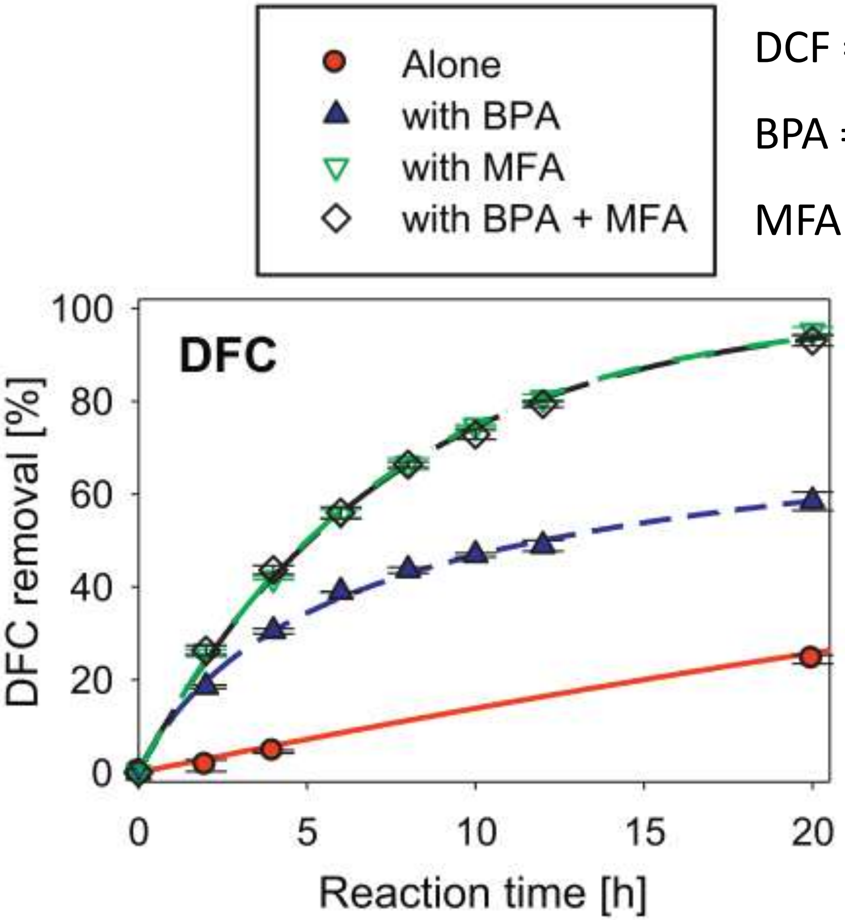


- WWTPs were not designed to tackle bioactive chemicals (BACs).
- BACs are a major concern for environmental authorities.
- The European Union has published a WatchList.



- Current enzyme technologies focus on oxidoreductases (e.g. laccase).
- Experimental conditions need to reflect the wastewater environment.
- Interactions between multiple BACs can influence enzymatic removal.

Bioactive Chemical Removal



Margot J et al (2013)

DCF = Diclofenac

BPA = Bisphenol A

MFA = Mefenamic Acid

- DCF removal by laccase increased with the addition of BPA and MFA.
- Numerous different BACs present in the wastewater matrix.

Degradation Results

Laccase (1U/ml)		
Substrate	Additive	Removal (%)
E1	-	100.0
DCF	-	100.0
SMX	-	3.2
E1	DCF + SMX	97.0 ± 0.2
DCF	E1 + SMX	100.0 ± 0.0
SMX	E1 + DCF	12.8 ± 2.7
SMX	E1	4.7
SMX	DCF	5.7

Tyrosinase (100U/ml)		
Substrate	Additive	Removal (%)
E1	DCF + SMX	100.0
DCF	E1 + SMX	0.0
SMX	E1 + DCF	5.6
SMX	E1	18.7
SMX	DCF	0.0

Experimental Conditions:

- Substrate → 5μg/ml
- Matrix → Deionised Water
- Contact Time → 21 Hours at RT
- Estrone (E1), Diclofenac (DCF), Sulfamethoxazole (SMX)

Conclusions:

- Laccase and tyrosinase are two widely applied oxidoreductases.
- SMX removal by the two enzymes increased with the addition of both E1 and DCF.
- SMX removal by tyrosinase improved when E1 was the single additive.

Wastewater Matrix

Experimental Conditions:

Laccase → 5U/ml

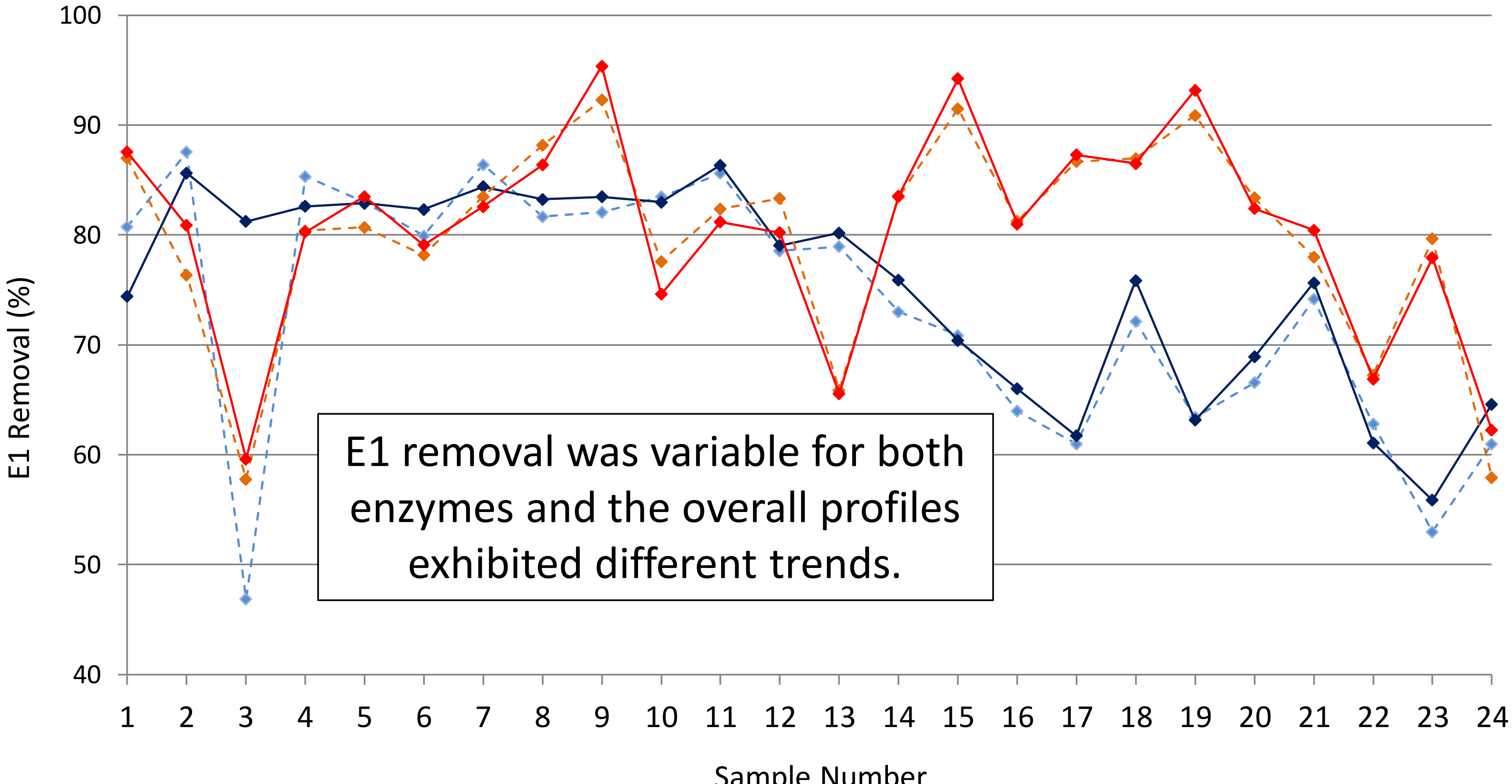
Tyrosinase → 40U/ml

Estrone (E1) → 0.5μg/ml

Matrix → Effluent (Stoke Bardolph WWTP)

Contact Time → 1 Hour

Temperature → 20°C



Conclusions

- Inherent enzyme activities vary both spatially and temporally.
- Enzyme activity and water quality correlations reveal factors that strongly influence biological performance.
- Mixed substrate matrices can enhance overall BAC degradation.
- The variable results for E1 removal in wastewater shows the difference in behaviour for two similarly classed enzymes.

Future Work

- Metals and chemicals such as EDTA are present in wastewater.
- The above can inhibit or enhance enzyme activity not considered in synthetic matrices.
- Investigate enzyme response to these wastewater constituents.
- Study process factors at ranges reflecting operational WWTPs.

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