WEBINAIRE
29 - 30 septembre 2022

SOLUTIONS BASÉES SUR LA NATURE – NBS – POUR LE TRAITEMENT ET LA RÉUTILISATION DES EAUX USÉES ET DES BOUES D’ÉPURATION EN MÉDITERRANÉE

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Constructed wetlands systems and emerging contaminants
### Pollutants

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional pollutants</td>
<td>SS, BOD, COD, COT, ammonia, nitrates, nitrites, total N, TKN, organic N, phosphorus, bacteria, viruses</td>
</tr>
<tr>
<td>Non conventional pollutants</td>
<td>recalcitrant organic substances, VOCs, surfactants, heavy metals, total dissolved solids</td>
</tr>
<tr>
<td>Emerging contaminants (Ecs)</td>
<td>??? Known or unknown??</td>
</tr>
<tr>
<td>Contaminants of emerging concern (CECs)</td>
<td></td>
</tr>
</tbody>
</table>

**ECs** are in general *unregulated* compounds, which may be candidate for future regulation depending on research on their potential health effects and monitoring data regarding their occurrence.
A few words to clarify...

• **Contaminants** are defined as inputs of alien and potentially toxic substances into the environment; not all contaminants cause pollution, as their concentrations may be too low.

• ‘**Pollutants**’ are defined as anthropogenically-introduced substances that have harmful effects on the environment.

Sometimes the distinction between *contaminants* and *pollutants* is not simple:
• concentrations at which contaminants become pollutants cannot always be defined;
• long-term damage to organisms or systems may occur that is not evident initially.

In the following we will assume that all the micropollutants may pose negative effects to the environment and thus

**microcontaminants = micropollutants**
What is «emergent»?

What is an emerging contaminant?

in a broad sense any synthetic or naturally-occurring chemical or any microorganism that is not commonly monitored or regulated in the environment with potentially known or suspected adverse ecological and human health effects.

Their names...

Chemicals found in pharmaceuticals, personal care products, pesticides, industrial and household products, metals, surfactants, industrial additives and solvents.

Their quantities...

Many of them are used and released continuously into the environment even in very low quantities and some may cause chronic toxicity, endocrine disruption in humans and aquatic wildlife and the development of bacterial pathogen resistance.
Emerging contaminants: Sources, Pathways, Receptors

Rasheed et al., 2019
https://doi.org/10.1016/j.envint.2018.11.038
Common classes of emerging contaminants

Different types of compounds...

Vasilachi et al., 2020 Water
Examples of emerging contaminants and their structure

**Personal care products**
- Triclosan
- Triclocarban

**Pharmaceuticals**
- Erythromycin

**DBPs**
- Bromodichloromethane

**Gasoline additives**
- Methil tert-butyl ether

**Flame retardants**
- Pentabromo chloro cyclohexane
- Tetrabromo phthalic anhydride
- Hexabromo benzene

**Perfluoroctanoic acid (PFOA)**

**Perfluorooctane sulfonate (PFOS)**

**Generalized structure of PBDE**

**2,2',4,4',5,5'-Hexabromodiphenyl ether (PBDE-153)**
Recently added among the emerging contaminants

• **Microplastics** (particles whose size is in the range 0,1 - 5.000 µm) and **nanoplastiche** (particles whose size is in the range 0,001 a 0,1 µm)
• **Antibiotic resistant bacteria**, ARB
• **Antibiotic resistant genes**, ARG

**Most studied in the water compartment:**
**Pharmaceutical compounds**
(still unregulated)
Regulated micropollutants at EU level

- Pesticidi polari, Erbicidi a base di fenilurea Triazina (e.g. atrazina)
- Agenti complessanti, tensioattivi
- Alchilfenoli
- Benzeni clorurati
- Tin organics
- DDT, Lindano
- IPA
- PCB
- Diossine policlorurate, furani

Ternes and Joss, 2006
Others emerging contaminants

*Ternes and Joss, 2006*

Focus on PHARMACEUTICAL compounds
Pharmaceutical administration → Excretion

% excretion: 2-95%

Ternes and Joss, 2006
Pharmaceutical compounds differ for:

- Dimension and molecular weight
- Percentage of excretion
- Biodegradability
- Tendency to adsorb onto a solid phase
- Volatility
- Photodegradability, persistence in the environment, stability
\[ \frac{dc_A}{dt} = k_{biol} c_A^n \]

- \( c_A \) = concentration of compound A,
- \( t \) = time,
- \( k_{biol} \) = degradation constant,
- \( n \) = reaction order

**Biodegradability**

- **Very good biodegradability**
- **Quite good biodegradability**
- **Poor biodegradability**

_Ternes and Joss, 2006_
Sorption onto a solid phase (sludge, activated carbons, particles...)

$K_{ow}=$ water octanol partition, $k_d$ sorption coefficient

$\log k_d = 1.14 + 0.58 \log K_{ow}$

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Use</th>
<th>MW (g/mol)</th>
<th>$\log K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemfibrozil</td>
<td>Anti-cholesterol</td>
<td>250.2</td>
<td>4.77</td>
</tr>
<tr>
<td>Triclosan</td>
<td>Antibiotic</td>
<td>289.6</td>
<td>4.76</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Steroid</td>
<td>272.2</td>
<td>4.01</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Pain reliever</td>
<td>206.1</td>
<td>3.97</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Steroid</td>
<td>314.2</td>
<td>3.87</td>
</tr>
<tr>
<td>Oxybenzone</td>
<td>Sunscreen</td>
<td>228.1</td>
<td>3.79</td>
</tr>
<tr>
<td>Ethynylestradiol</td>
<td>Birth control</td>
<td>296.2</td>
<td>3.67</td>
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<tr>
<td>Testosterone</td>
<td>Steroid</td>
<td>288.2</td>
<td>3.32</td>
</tr>
<tr>
<td>Naproxen</td>
<td>Analgesic</td>
<td>230.1</td>
<td>3.18</td>
</tr>
<tr>
<td>Estrone</td>
<td>Steroid</td>
<td>270.4</td>
<td>3.13</td>
</tr>
<tr>
<td>Erythromycin-H₂O</td>
<td>Antibiotic</td>
<td>733.9</td>
<td>3.06</td>
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<td>Diazepam</td>
<td>Anti-anxiety</td>
<td>284.8</td>
<td>2.82</td>
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<td>Androstenedione</td>
<td>Steroid</td>
<td>286.2</td>
<td>2.75</td>
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<td>Atrazine</td>
<td>Herbicide</td>
<td>215.1</td>
<td>2.61</td>
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<td>Dilantin</td>
<td>Anti-convulsant</td>
<td>252.3</td>
<td>2.47</td>
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<td>Carbamazepine</td>
<td>Analgesic</td>
<td>236.3</td>
<td>2.45</td>
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<tr>
<td>Estriol</td>
<td>Steroid</td>
<td>288.4</td>
<td>2.45</td>
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<td>DEET</td>
<td>Insect repellent</td>
<td>191.3</td>
<td>2.18</td>
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<td>TCEP</td>
<td>Fire retardant</td>
<td>285.5</td>
<td>1.44</td>
</tr>
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<td>Trimethoprim</td>
<td>Antibiotic</td>
<td>290.1</td>
<td>0.91</td>
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<tr>
<td>Sulfamethoxazole</td>
<td>Antibiotic</td>
<td>253.1</td>
<td>0.89</td>
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<tr>
<td>Diclofenac</td>
<td>Arthritis</td>
<td>318.1</td>
<td>0.70</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>Anti-anxiety</td>
<td>218.3</td>
<td>0.70</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>Analgesic</td>
<td>151.2</td>
<td>0.46</td>
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<tr>
<td>Pentoxifylline</td>
<td>Blood viscosity control</td>
<td>278.1</td>
<td>0.29</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Stimulant</td>
<td>194.2</td>
<td>−0.07</td>
</tr>
<tr>
<td>Iopromide</td>
<td>X-ray contrast media</td>
<td>790.9</td>
<td>−2.1</td>
</tr>
</tbody>
</table>

Excellent adsorption: $\log K_{ow} = 4$

Good adsorption: $\log K_{ow} = 2.5$

Low adsorption: $\log K_{ow} = 2.5$

Ternes and Joss, 2006
Stripping/volatilization

Ternes and Joss, 2006
CONTRIBUTION TO REMOVAL DUE TO SOLAR EXPOSURE


CECs are classified into three classes:

- **FAST-PHOTODEGRADABLE** (half-life < 8h)
- **MEDIUM-PHOTODEGRADABLE** (8h < half-life < 168h = 7 days)
- **SLOW-PHOTODEGRADABLE** (half-life > 168h)

Influence of the functional groups on the photodegradability

*Effect on photodegradability*
How can we apply these considerations to CW systems?

To understand/predict EC pathways in the bed or basin

- Foliar uptake
- CEC and plant interaction
- Run off
- Wind erosion
- Volatilization and photodegradation
- Plant uptake and traslocation
- Sedimentation, precipitation
- Microbial degradation

CECs

Pullagurala et al., 2018
Different types of Constructed wetlands

**Surface** flow system

**Horizontal** subsurface flow system

**Vertical** subsurface flow system

**Hydroponic** gravel bed

Different operational conditions...
How do they affect the removal mechanisms?

Verlicchi and Zambello, 2014
Different *role* in the Treatment = different STEP

**Primary step**
- Raw influent → CW → Effluent

**Secondary step**
- Raw influent → Prel. Treat., Primary Treat. (Imhoff, septic tank, HUSB, primary clarifier,...) → CW → Effluent

**Tertiary step**
- Raw influent → Prel. Treat., Primary Treat. (Imhoff, septic tank, HUSB, primary clarifier,...) → Secondary Treat (CAS, MBR) → CW → Effluent

**Restoration Wetland**
- WWTP1 effluent → CW → WWTP2 effluent → CW → WWTP3 effluent

**Multistage steps**
- Sampling point

How do they affect the removal mechanisms?

Different influent concentrations
Different matrix effects..

Verlicchi and Zambello, 2014
Treatment stages

Hybrid systems

Different influent concentrations
Different matrix effects..

How do they affect the removal mechanisms?
Lessons learned from the literature
CW acting as primary step: influent and effluent

Verlicchi and Zambello, 2014
CW acting as a primary step: average removal efficiencies

Verlicchi and Zambello, 2014
CW acting as a secondary step: influent concentrations

Verlicchi and Zambello, 2014
CW acting as a secondary step: removal efficiencies

Verlicchi and Zambello, 2014
CW acting as a tertiary step: influent concentrations

Verlicchi and Zambello, 2014
CW acting as a tertiary step: removal efficiencies

Verlicchi and Zambello, 2014
CW acting as a tertiary step: removal efficiencies

Verlicchi and Zambello, 2014
Hybrid CWs: removal efficiencies

Verlicchi and Zambello, 2014
HSSF acting as a polishing step

Raw influent → Pre-Treatment → Secondary Treat Conventional activated sludge system AS → H-SSF → Effluent

Verlicchi et al., 2013
HSSF acting as a polishing step

AS= conventional activated sludge  Verlicchi et al., 2013
HSSF acting as a polishing step

AS= conventional activated sludge

Verlicchi et al., 2013
Warning: Micropollutants occurrence: high variability over time (day, week...)

![Graphs showing fluctuation of mass flux for selected antibiotics in wastewater.](image)

*Fig. 1 – Fluctuations of mass flux for selected antibiotics in wastewater throughout a one day period (a) and during the course of a year (b) – adapted from Coutu et al. (2013).*

Important to adopt the most pertinent sampling mode and frequency and to clearly report them. Composite samples...flow proportional... sufficient number of samples.... removal evaluated on the influent and effluent load... → **check the quality and assure data reliability**
Free water surface systems

main PhC removal mechanism is photodegradation, while microbial degradation and plant uptake also contribute to some extent in the removal of diclofenac, naproxen, and clarithromycin. Diclofenac, naproxen, and clarithromycin are well removed.

Ilyas and Van Hullebush (2020)
Horizontal subsurface flow systems H-SSF

Anaerobic biodegradation is an important removal mechanism of PhCs besides their removal by the filter media (through sedimentation, adsorption, and precipitation) and plant uptake.

Anaerobic biodegradation is slower than aerobic one, thus longer HRT are necessary to achieve the removal efficiencies in aerobic conditions.

Good removal for naproxen, sulphamethoxazole, sulfapyridine, trimethoprim, atenolol, bezafibrate

Ilyas and Van Hullebush (2020)
Vertical subsurface flow systems (V-SSF)

The **aerobic biodegradation** is responsible for the removal of PhCs by V-SSF among other dominant processes (e.g., sedimentation, adsorption, and plant uptake).

Ilyas and Van Hullebush (2020) Good removal for ibuprofen, salicylic acid, acetaminophen, codeine, caffeine, gemfibrozil, metoprolol
Sometimes, the combination of different conditions may enhance the removal of many PhCs. This may occur in the hybrid systems HCWs (violet in the pictures).
Hydraulic retention time (HRT) — This is an important parameter for the empirical design and operation of H-SSF beds. According to Zhang et al. (2012), the removal efficiencies for salicylic acid, ketoprofen, clofibric acid were linearly proportional to the influent mass loading rate at HRTs ranging between 2 and 6 days, making it possible to describe the removal of these substances by a constant first-order kinetic decay.

Temperature — High temperatures improve the removal of some compounds. Zhang et al. (2012) found that at tropical temperatures, ketoprofen was better removed than in temperate climates; Similarly, Hijosa-Valsero et al. (2010b) found higher removal efficiencies in the summer than in the winter.

Redox potential (RP) — It seems that anoxic (−100 mV < RP < 100 mV) and aerobic (RP > 100 mV) conditions favour the biodegradation of organic micropollutants through the promotion of biogeochemical reactions (Matamoros et al., 2008a).
Attempts to predict the behavior

Fig. 9. Percentage average removal efficiency profiles and hydrophobicity (log $K_{ow}$) of the selected compounds.

Verlicchi et al., 2013
Focus on the antibiotics

**Sulfonamide Antibiotics**

**FLUOROQUINOLONES**

**Tetracyclines**

End in "cycline"

Doxycycline  Tetracycline

**Antibacterials:**
A. Natural Penicillins
B. Antistaphylococcal Penicillins
C. Extended spectrum Penicillins
D. Antipseudomonal Penicillins
Removal of the most prescribed classes of antibiotics in different types of CWs

**Mechanisms**
- Volatilization
- Photodegradation
- Precipitation
- Substrate adsorption
- Plant uptake and accumulation
- Microbial degradation

**Efficiencies**
- SAs: sulfonamides; TCs: tetracyclines; FQs: fluoroquinolones; MLs: macrolides; BLAs: β-lactams

**Design and Configuration**
- Operational conditions
- Plant species
- Hydraulic load

*Lv et al., Stoten 2022*

*(FTW= floating treatment wetlands)*
Removal pathways

**H bondings** among ionic functional groups (*aniline and amide*) and polar groups (i.e., hydroxyl and phenolic hydroxyl) present in organic materials;

**Electrostatic interactions** between the negatively charged soil, clay or other filling material and the positively charged antibiotics

**Cation bridging** responsible for the sorption of anions by natural sorbents in wetland systems

**Cation exchange** between the cationic amine group and the negatively charged surface sites

**Complex formation** with metal ions on the substrate surface, such as Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, Al$^{3+}$, for antibiotic molecules containing polar/ion functional groups

**Most antibiotics are ionizable molecules.** At pH= 6-8 macrolides are cationic and are efficiently adsorbed, sulfonomides are in anionic form and their removal is mainly due to degradation.

**Different responses to HLR changes:** they may favor/disturb sorption process
Role of macrophytes

Higher removal in presence of macrophytes

**Root uptake**: difficult to evaluate and predict. It is widely accepted that for organic compounds with moderate hydrophobicity (1.0 < log $K_{ow}$ < 3.5) and/or with low MW (MW < 500) can easily penetrate cell membranes and be taken up by plant roots ([Le-Minh et al., 2010; Yan et al., 2016]) through different mechanisms, such as passive uptake via protein channels, protein-mediated energy-dependent active uptake, etc. By contrast, antibiotics with strong hydrophobicity (log $K_{ow}$ > 3.5) and/or with high MW (>500) tend to bind tightly to the substrate and/or root surface, and be precluded by cell membranes of plant root. Therefore, **plant uptake may play a minor role** in their removal and other mechanisms, such as plant stabilization and/or rhizosphere bioremediation, might be involved in the remediation.

**Translocation**: physiochemical properties of antibiotics, particularly ionization behavior and hydrophobicity, can also significantly influence the translocation in plants. Hydrophobic compounds tended to remain in the roots with limited in-plant redistribution, while hydrophilic compounds were susceptible to moving toward leaves in the direction of the transpiration stream. Neutral compounds with log $K_{ow}$ values between -1 and 5 are considered mobile in the transpiration stream.
Role of macrophytes

Higher removal in presence of macrophytes

**microbial and enzymatic activities** through the secretion of exudates, thus enhancing the removal of antibiotics

Evidence that **antibiotics** caused a significant **increase in the ARGs in wetlands**, which were harbored in biofilms on plant rhizosphere and substrate, due to selection pressure on rhizospheric and microbes. In addition to causing the proliferation of their corresponding ARGs, antibiotics can cause the **spread of diverse ARGs**.
Role of microorganisms

Microorganisms play an essential role in biosorption and biodegradation of antibiotic in CWs.

Irrespective of the different removal mechanisms, antibiotics may undergo hydrolysis, oxidation, side chain breakdown, acetylation, hydroxylation, ring cleavage, demethylation, decarboxylation, and dihydroxylation.

Due to the complexity of microbial communities in CW systems and the differences in the chemical structure of antibiotics, the biodegradation mechanism and functional microorganisms for specific antibiotics removal remain ambiguous. Therefore, the contribution of different biodegradation pathways to the removal of specific antibiotics needs to be fully elucidated to optimize the design criteria of CW for removal enhancement.
Enhancement in the removal

**Artificial aeration:** the presence of oxygen is beneficial for most of the investigated compounds. Why don’t increase the content of oxygen in the bed?

**Tidal flow CW** characterized by regular cycle of a “filled/wet” phase or a “drained/dry” phase promoting the aeration of the system: air is drawn into the soil pores and the bio-waterfilms is rapidly oxygenated.

**Innovative filling materials**

**Bioaugmentation:** this is an effective process for intensifying the degradation of organic pollutants and optimizing the optional conditions in CW systems (Tara et al., 2019). By introducing **bacterial strains** with the ability to degrade specific contaminants into CWs, the population, density, diversity and activity of functional microbes could be reinforced (Wang et al., 2018; Zhao et al., 2019), leading to the acceleration of the pollutant biodegradation.

**Towards engineered CW...** providing artificial electron acceptors...
Attempts to enhance the removal: *ARTIFICIAL AERATION*

Advantages of enhancing oxygen availability in CWs
(1) improved biodegradation;
(2) reduced clogging;
(3) enhanced removal of organic matter, nitrogen, and phosphorous; and
(4) reduced land area requirement

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>DO (mg L(^{-1}))</td>
<td>6.0/na</td>
<td>1.6 ± 1.3/8.9 ± 0.7</td>
<td>4.5 ± 0.7/7.5 ± 1.1</td>
<td>2.3 ± 2.4/11</td>
<td>Biodegradation (aerobic/anaerobic)</td>
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<tr>
<td>Diclofenac</td>
<td>na</td>
<td>21 ± 12/48 ± 22</td>
<td>56 ± 7/68 ± 9</td>
<td>56 ± 32/99</td>
<td>Biodegradation (aerobic)</td>
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<tr>
<td>Ibuprofen</td>
<td>na</td>
<td>23 ± 8/99</td>
<td>96 ± 2/99 ± 1</td>
<td>na</td>
<td>Biodegradation (aerobic/anaerobic)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>na</td>
<td>28 ± 6/99 ± 1</td>
<td>89 ± 2/94 ± 1</td>
<td>na</td>
<td>Biodegradation (aerobic/anaerobic)</td>
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<tr>
<td>Acetaminophen</td>
<td>99/97</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>Biodegradation (aerobic/anaerobic)</td>
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<td>Tramadol</td>
<td>na</td>
<td>6.8/1.6</td>
<td>na</td>
<td>na/99.9</td>
<td>Biological transformation</td>
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<tr>
<td>Caffeine</td>
<td>82/94</td>
<td>89 ± 8/99.5 ± 0.7</td>
<td>97 ± 1/99</td>
<td>na</td>
<td>Biodegradation (aerobic/anaerobic)</td>
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<td>Carbamazepine</td>
<td>na</td>
<td>12 ± 9/11 ± 11</td>
<td>− 8.5 ± 0.7/− 2.5 ± 2.1</td>
<td>27 ± 20/94</td>
<td>Adsorption/sorption</td>
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<td>Atenolol</td>
<td>na</td>
<td>na/96 ± 1</td>
<td>na</td>
<td>73 ± 37/98</td>
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<td>Metoprolol</td>
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<td>na</td>
<td>na</td>
<td>99/98</td>
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<td>Sotalol</td>
<td>na</td>
<td>24 ± 6/32 ± 13</td>
<td>na</td>
<td>82/99</td>
<td>Biodegradation (aerobic)</td>
</tr>
</tbody>
</table>
The oxygen supply routes and consumption processes in CWs

Ilyas and Van Hullebush (2020)
Another direction of the research for enhancing ECs removal: “engineered CW”

Major limitations of CWs = slow reaction kinetics, mainly due to the limited electron acceptor and slow microbial metabolism in presence of massive anaerobic conditions in the CW systems.

Incorporation of bio-electrochemical systems (BESs) into the CWs, such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) (Ilyas and Hullebusch, 2020; Zhang et al., 2020b), has gained a considerably increasing attention.

CW-BESs can provide artificial electron acceptors in the anaerobic regions of CW, and electrical stimulation in CW-BESs may enhance the variation in cell membrane structures and enzyme activities of some microorganisms,

→ thus, promoting the utilization of mineral elements and carbon sources by microorganisms,

→ and thus, improving degradation performance to antibiotics.

Lv et al., Stoten 2022
Enhancement in the removal: CW Microbial fuel cell

The application CW-MEC for contaminant removal is based on the same principle as CW MFCs, with a slight difference at configuration.

**CW-MFC can transform chemical energy into electricity by oxidizing organic matter and produce bioelectricity**

**CW-MEC requires additional electron donors for the treatment (see the principle in the picture)**

*Zhao et al., 2013*

*Ramirez-Vargas et al. 2018*
Considerations and hints for discussion - Conclusions

Different mechanisms affect the removal of these micropollutants presenting a variety of chemical and physical properties.

Researches are dealing with:

• **Key metabolic and co-metabilic removal pathways** for the different compounds

• **Transformation products** (usually unknown compounds, and the relative research is currently viewed as a challenge)

• **Bioaugmentation** (some key issues remain unresolved in the bioaugmentation process, such as how the succession of microbial community affects the performance of bioaugmentation system and how the added strains interact with local microbial community). The integration of **microalgae into CWs** can enhance biodegradation performance by compensating for microbial activity at low temperature.

• **Artificial aeration**
• However, the **feasible applications** are still scarce and future research on the integrated systems is essential for improving a low-cost and environmentally sustainable alternative for micropollutants removal.

• Great efforts have been made to develop **intensified CW systems for enhancing the antibiotics removal ability in CWs**.

• However, most of the trials are limited to **lab-scale applications** without field validation. Consequently, the resilience and sustainability of these new approaches have to be thoroughly evaluated via pilot scale investigation to establish field-based data on accumulation and biodegradation of antibiotics in soil-plant system.

• It is clear that the research requires the synergies among different researchers: biologists, chemists, environmental engineers, chemical engineers, geologists, biochemists, biotechnologists, ... in order to match the different experiences and provide robust, reliable and “resilient” solutions to enhance the removal of target ECs and to reduce the environmental impact of their presence in the aquatic environment!
Thanks for your kind attention!

And now time for questions or discussion

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References