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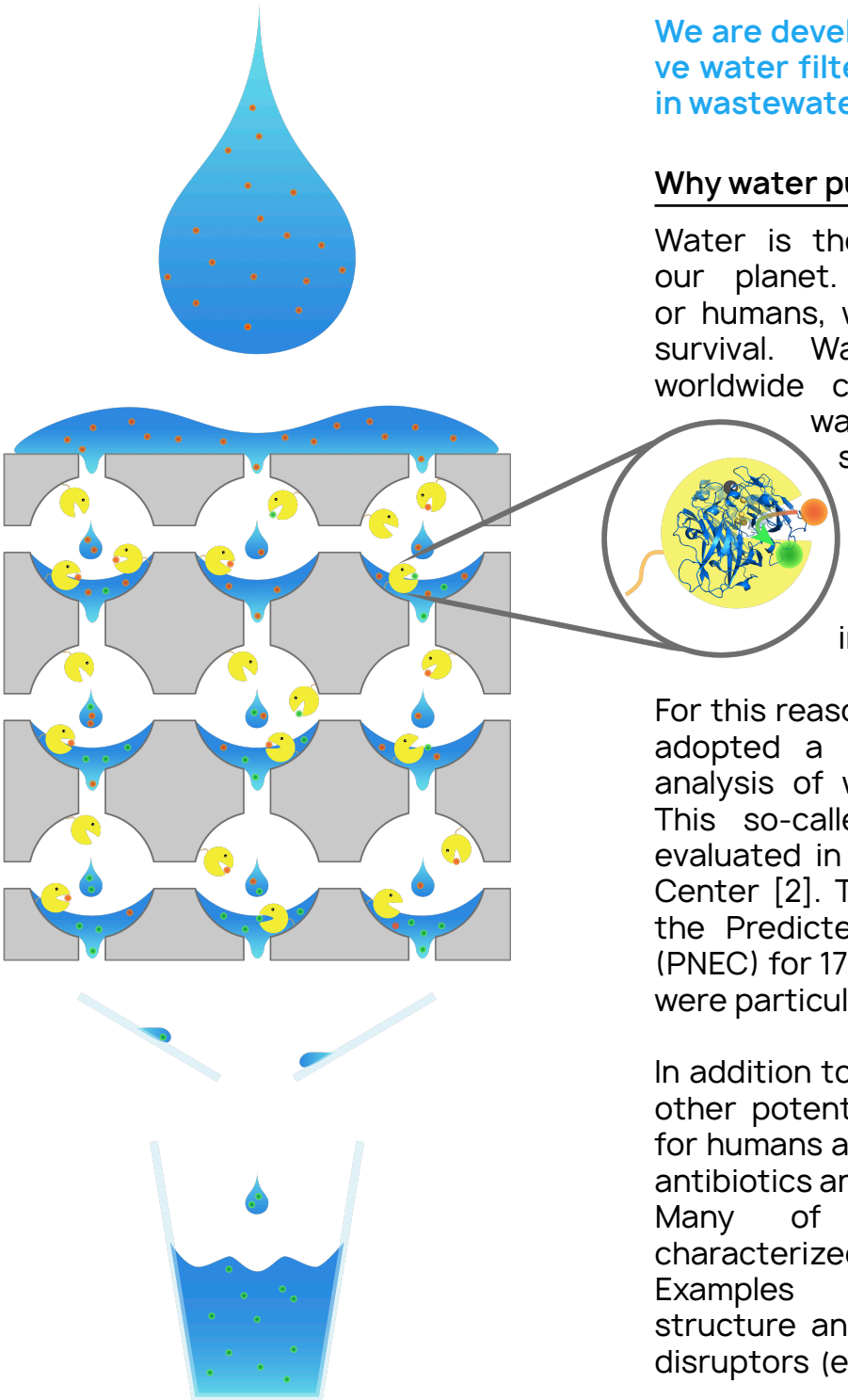
LAC-MAN

AN IGEM PROJECT AT THE UNIVERSITY OF STUTTGART



We are developing Lac-Man, the effective water filter to counter drug residues in wastewater.

Why water purification?

Water is the most basic resource on our planet. Whether plants, animals or humans, we all depend on it for our survival. Water scarcity and purity worldwide cause conflicts. Above all, water pollution from drugs, such as antibiotics or estrogen derivatives and other non-medical pollutants is proving to be an emerging issue in industrialized countries.

For this reason, in 2013 the EU Parliament adopted a directive on the constant analysis of water within the Union [1]. This so-called "watch list" was first evaluated in 2018 by the Joint Research Center [2]. The frequent exceedance of the Predicted No-Effect Concentration (PNEC) for 17-alpha-ethinylestradiol (EE2) were particularly noticeable.

In addition to EE2, the watch list includes other potentially dangerous substances for humans and the environment, such as antibiotics and diclofenac (pain relievers). Many of these molecules are characterized by common properties. Examples include aromatic ring structure and their effect as endocrine disruptors (e.g. EE2) and / or the toxicity

of these compounds (e.g. diclofenac, carbamazepine), which justify the hazard potential for humans and animals [3]. Aromatic ring structures serve as potential targets for enzymes, the so called laccases, that have been identified in various fungi and bacteria.

Why laccases?

Laccases oxidize phenol groups, which turns the substrate oxygen into a radical. At the same time, free oxygen is reduced to water. The degradation products produced here have no negative effects on people and the environment [4].

Our goal is to improve the stability of these enzymes and to select a combination of laccases, which can neutralize a large number of pollutants due to their wide range of substrates.

The laccase expressed by Trametes versicolor is to be optimized with regard to its pH and thermostability by means of codon optimization and modification of the N-terminal sequence. To be able to analyze the properties of the modified laccase in advance, protein modeling with the SWISS-MODEL offers a promising option [5].

How is laccase synthesized?

The laccases are produced using a classic biotechnological approach, in which the template DNA is digested by restriction enzymes and ligated into an expression vector. This is used to transform competent cells. This expression vector is integrated into the genome in order to generate a stable expression strain [6]. Because the above Laccase has several disulfide bridges, production in eukaryotic cells is necessary. The Pichia pastoris X33. 2010 strain has been successfully used for the synthesis of laccases.

Why immobilization?

Immobilization using a mesoporous silica foam is suitable to make the laccases sustainable on the long run [7, 8].

Silicon-based materials are well suited because they are environmentally friendly, bio-compatible and, above all, resistant to organic solvents and microbial attacks. Furthermore, the immobilization makes the laccases pH- and thermostable. In addition, immobilized enzymes have a half-life up to 18 times longer [9]. At the same time, the enzymes used are firmly bound to the matrix and therefore do not have to be filtered out of the wastewater again. This process also has the advantage that no genetically modified organisms can be released into the environment.

iGEM Team 2020

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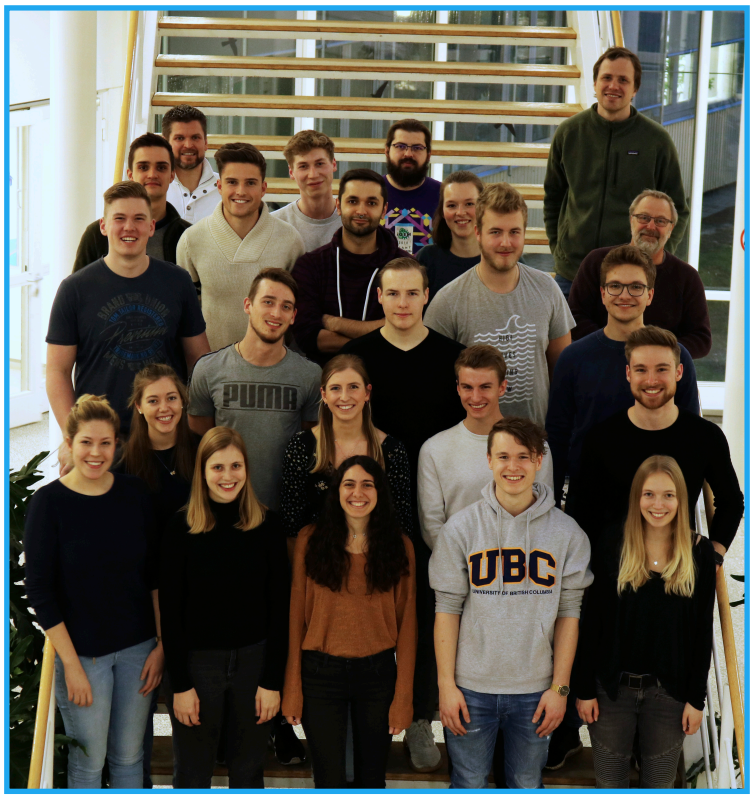

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