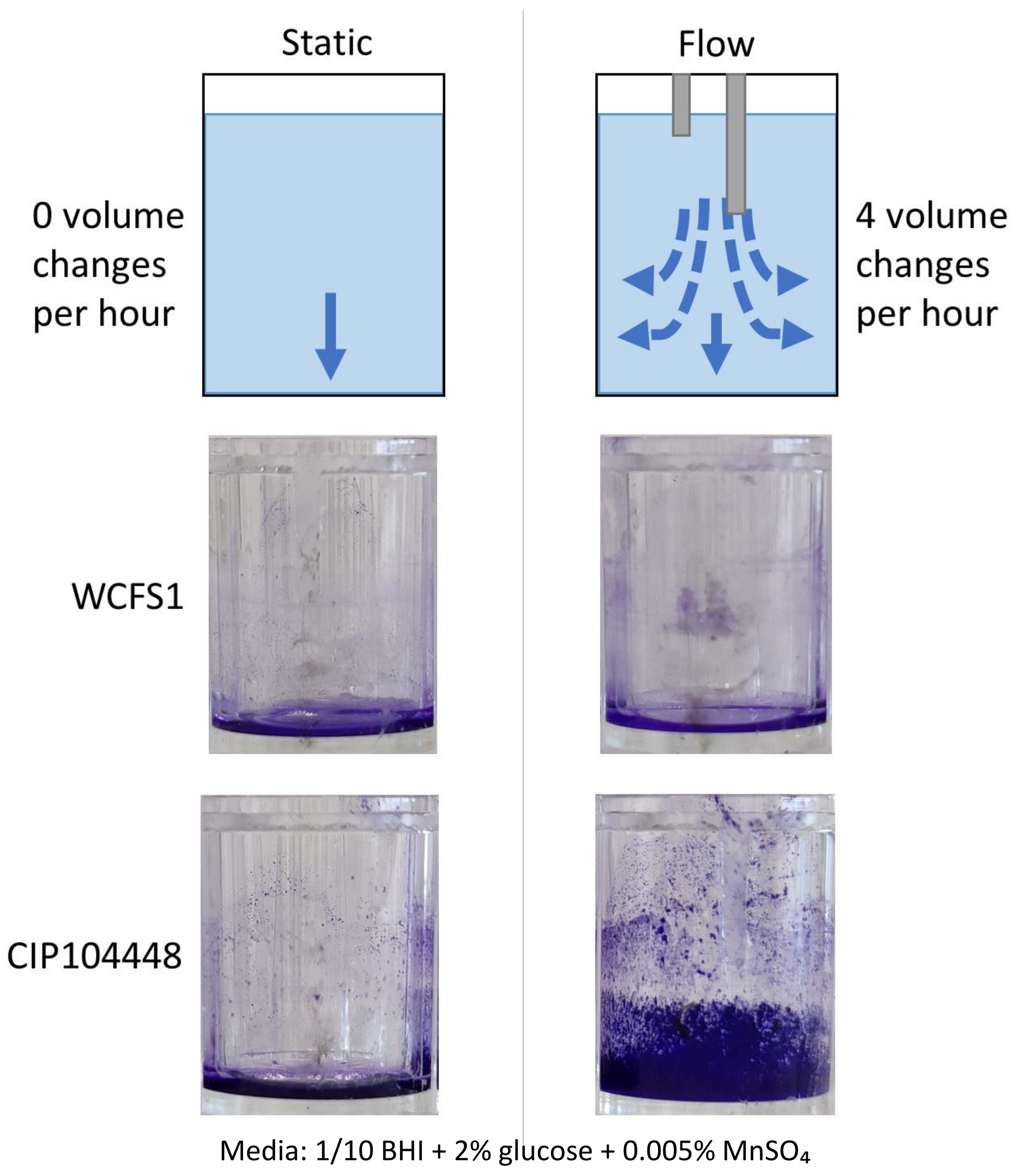
Phenotypic and proteomic differences in biofilm formation of two *Lactiplantibacillus plantarum* strains in static and dynamic flow environments

Linda Huijboom, Parisa Rashtchi, Marcel Tempelaars, Mehdi Habibi, Erik van der Linden, Tjakko Abee

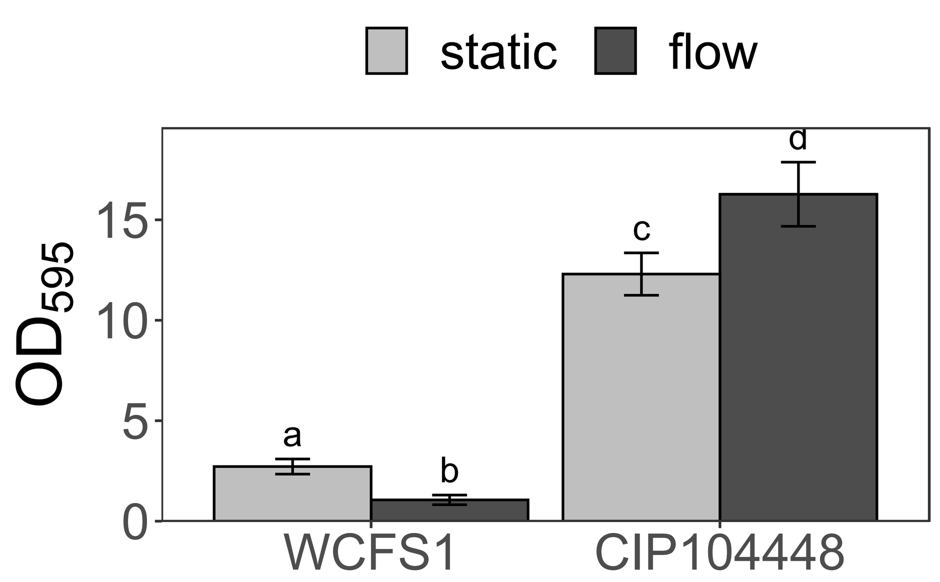
Besides the beneficial properties of *Lactiplantibacillus plantarum*, this organism can also cause problems in the food industry as a **spoilage** organism. One way that this spoilage organism can contaminate food products is via a **biofilm**. Biofilms are layers of cells attached to a surface and surrounded by an extracellular matrix that offers increased protection to the cells against external stresses, such as cleaning agents. In our study, we aimed to further investigate the biofilm formation of two strains (model strain WCFS1 and isolate CIP104448) of *L. plantarum,* both in the commonly studied **static environment**, as well as the less studied **dynamic 'flow' environment**. Using proteomics and various techniques to study the phenotypical differences, we've gained valuable insights between the phenotypical differences and protein expression patterns of the two studied strains in biofilm formation under static and flow conditions.

Schematic model and crystal violet visualization of 24h biofilms (30°C)



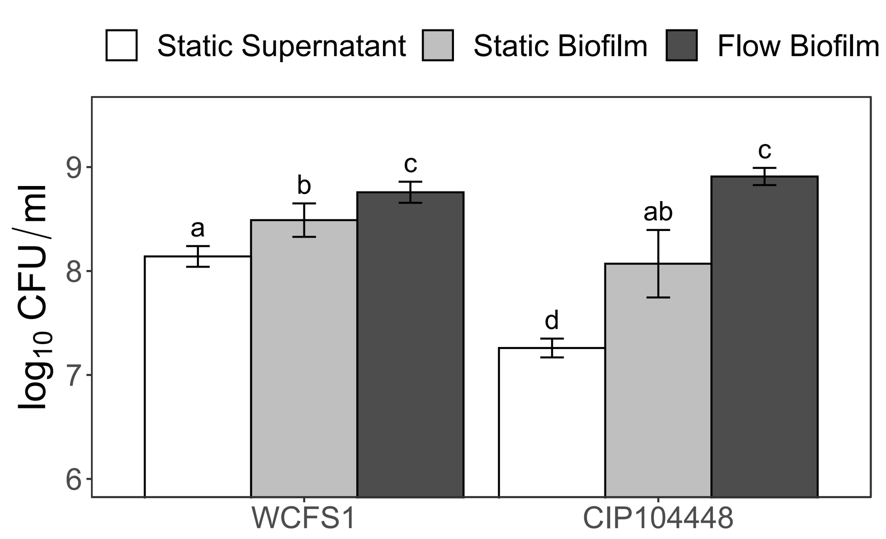
Media: 1/10 BHI + 2% glucose + 0.005% $MnSO_4$

Quantification of the crystal violet



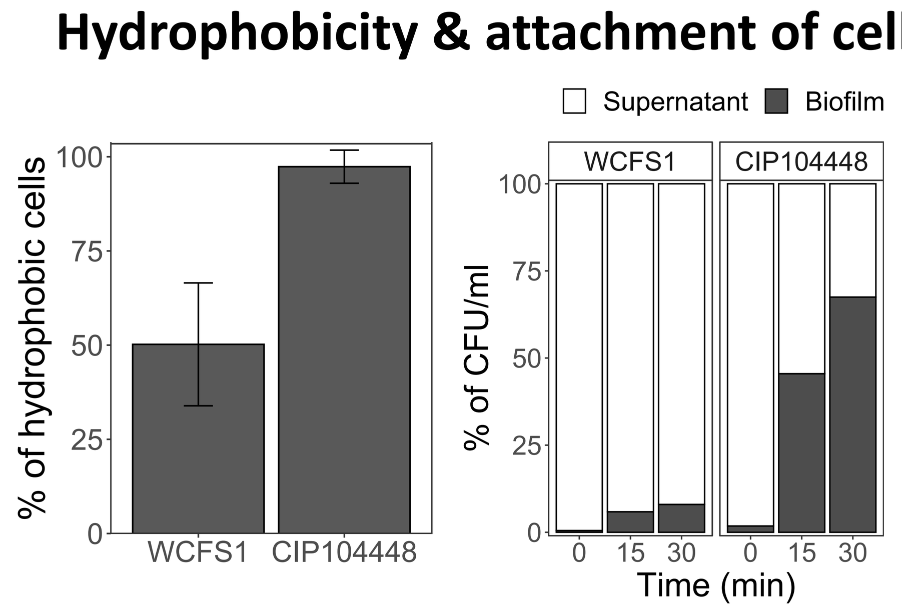
The influence of flow on biofilm formation is strain dependent

Total viable counts



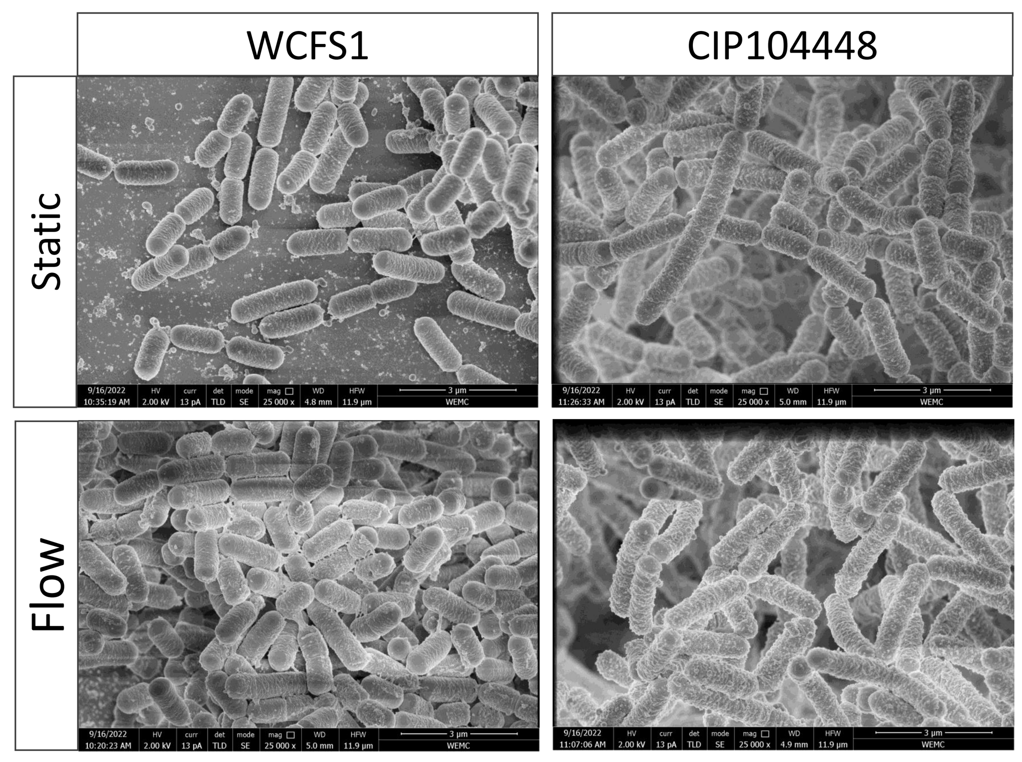
The total number of cells increases with flow, regardless of the total biofilm amount

Hydrophobicity & attachment of cells



CIP104448 is highly hydrophobic, likely resulting in the increased attachment

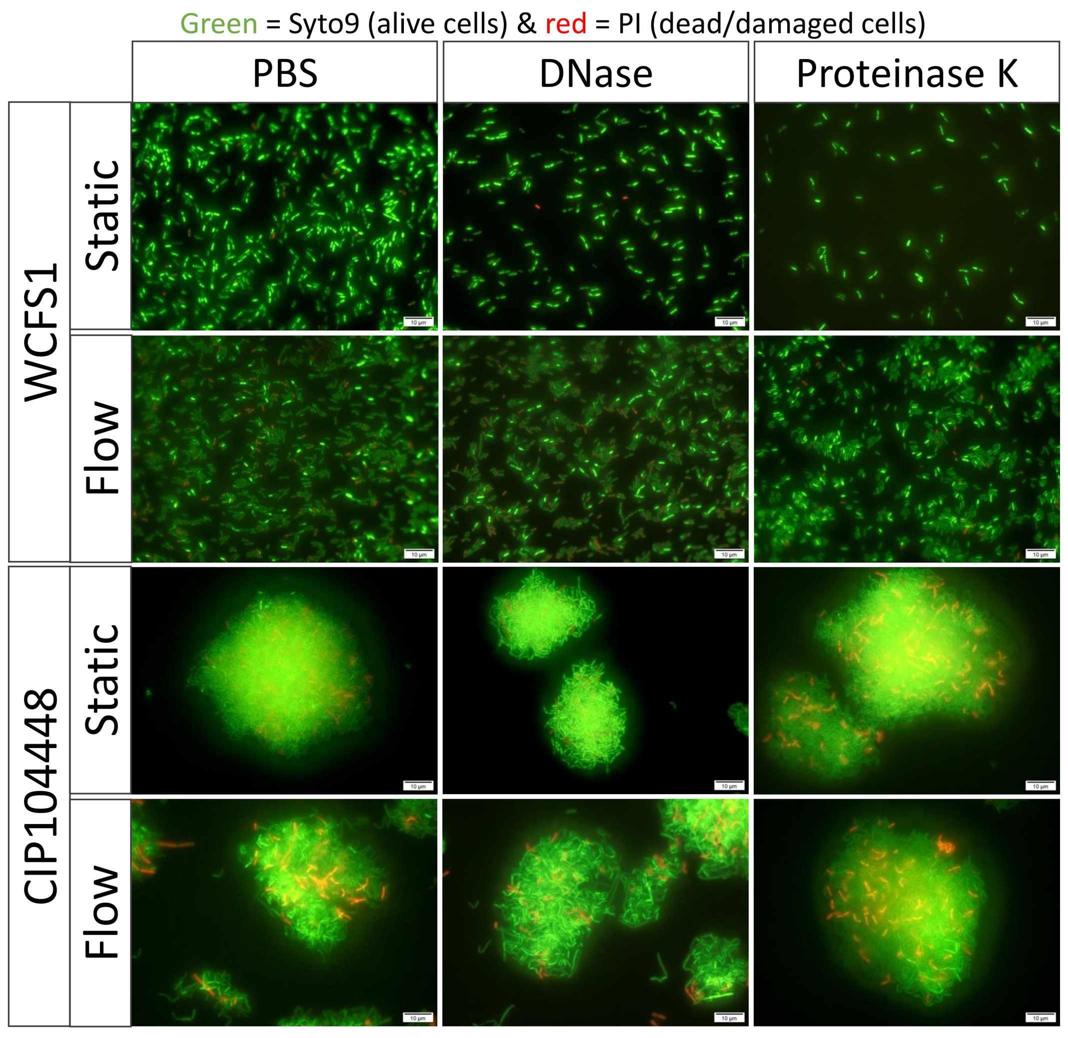
Scanning Electron Microscopy images of the biofilms



WCFS1 cells are closely packed together whereas CIP104448 has open '3D' structures

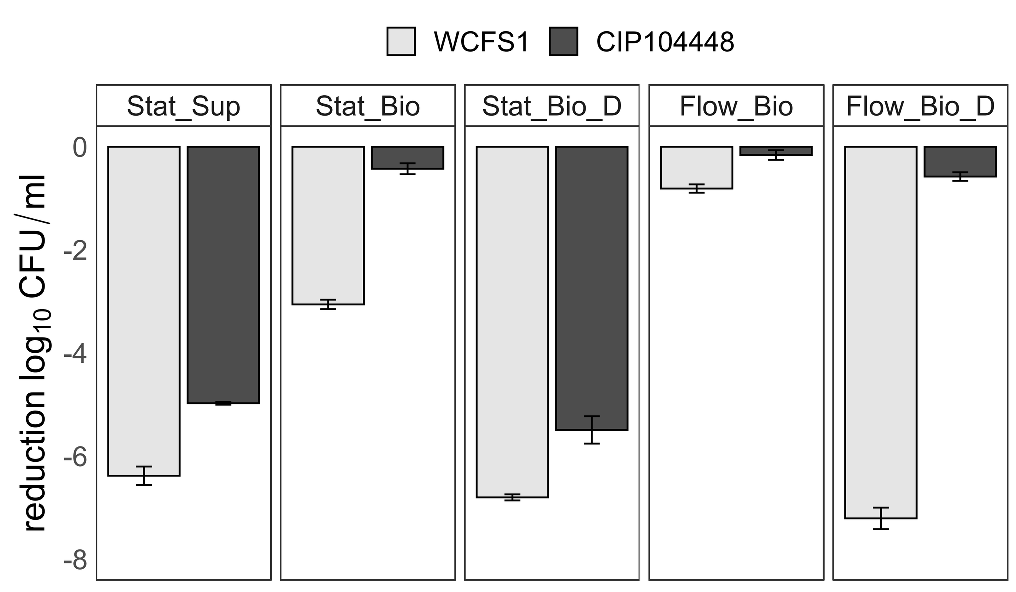
Influence of enzymatic treatments on the biofilms

Green = Syto9 (alive cells) & red = PI (dead/damaged cells)



Only WCFS1 static biofilm cells are influenced by enzymatic treatments indicating a role for eDNA and protein in this type of biofilm

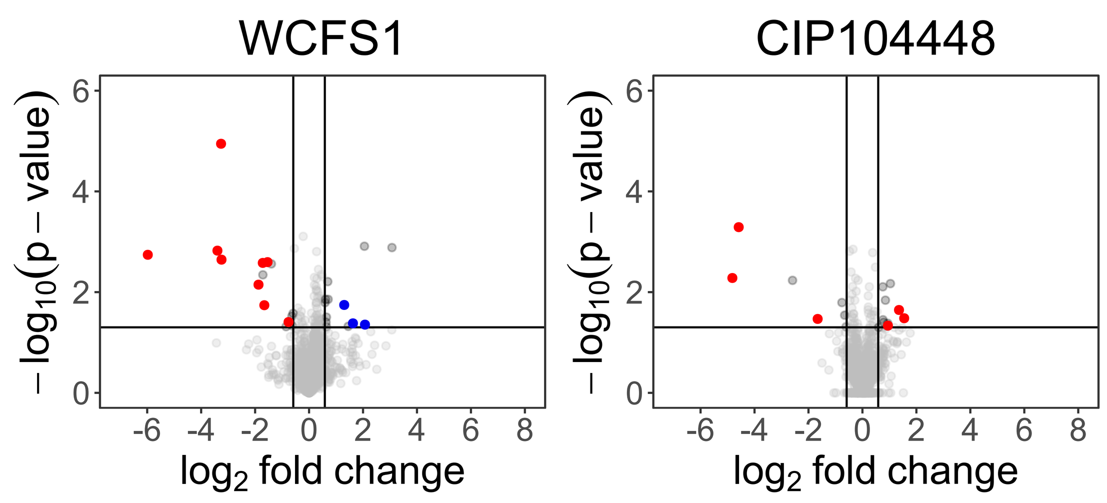
Disinfectant resistance



Stat_Sup= static supernatant cells
Stat_Bio= static biofilm cells
Stat_Bio_D= static biofilm cells dispersed
Flow_Bio= flow biofilm cells
Flow_Bio_D= flow biofilm cells dispersed

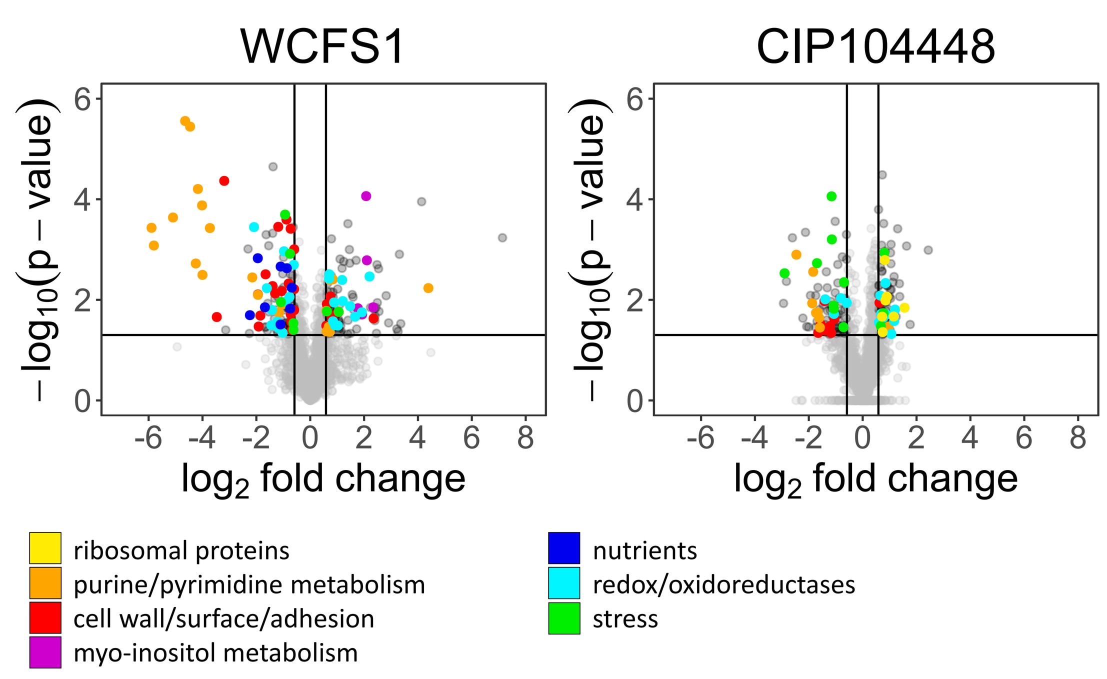
Exposure to the disinfectant peracetic acid (20 µg/ml) is effective against supernatant cells, but static and flow biofilm cells show a limited reduction. The protective properties of the biofilm are lost for WCFS1 when static or flow biofilm cells are dispersed. Interestingly, CIP104448 flow biofilm cells remain resistant, even when dispersed!

Protein expression static biofilm over static supernatant



Biofilm formation is mostly a passive process with limited protein changes for both strains. Most protein changes found are related to the cell wall.

Protein expression flow biofilm over static biofilm



For both strains, most protein changes in flow biofilm cells are related to metabolic pathways, fitting with the increased availability of nutrients and higher cell counts.
With flow, the cell wall related proteins continue to be lower expressed, indicating a general biofilm trend.
Interestingly, most stress proteins are lower expressed in flow. However CIP104448 also has a universal stress protein higher expressed, possibly contributing to the increased stress resistance against the disinfectant for CIP104448 flow biofilm cells.

Key Points

1. For both strains, the formation of a biofilm in a static environment is mostly a passive process.

2. The response on biofilm formation with flow is strain dependent and has a strong correlation to the hydrophobicity of the cells.

3. Cells within an intact biofilm are overall more resistant to peracetic acid. Additionally, flow biofilm cells are even more resistant than static biofilm cells.

WAGENINGEN UNIVERSITY & RESEARCH

Add me on LinkedIn!

Linda Huijboom
PhD Candidate
Linda.Huijboom@wur.nl

14th International Symposium on Lactic Acid Bacteria
August 2023, Egmond aan Zee, The Netherlands