Determination of amino acid and carbohydrate content of the PhyCoVi medium

In order to determine the carbohydrate and amino acid composition of the algae extract (our developed medium termed “PhyCoVi”), several high-pressure liquid chromatographys (HPLC)were performed.

Carbohydrate HPLC: The same algae extract concentration as used in our experiments testing bacterial growth on our medium (batch PCV#2) was used for a carbohydrate high-pressure liquid chromatography (HPLC). 50 µL carbohydrate standard was added to 950 µL of diluted samples (1:10 and 1:20 diluted in HPLC water) of our PhyCoVi medium from batch PCV#2 solved in distilled water (concentration of 30 g/L). We measured 6 technical replicates. For calibration and concentration determination 5 standards with a known concentration of glucose, fructose, saccharose and raffinose was used (0, 5, 20, 50, 100). The samples were put in the HPLC-machine from DIONEX, consisting of ED50 Electrochemical Detector and GP50 Gradient Pump, and an already created program for carbohydrate concentration measurement was loaded und used for measurement. Each dilution was measured separately three times. The CarboPac PA1 4 X 250 mm HPLC Column was used in this experiment. The CarboPac PA1 4 X 50 mm HPLC Column served as a precolumn.

Amino Acid HPLC:

The two batches of PhyCoVi medium (PCV#2 and PCV#3) were solved in water to reach a concentration of 30 g/L and 60 g/L. The amino acid content of the medium was measured in triplicates using a high-pressure liquid chromatography (HPLC; Agilent 1200 Series HPLC System). The ZORBAX Eclipse Plus C18 Analytical 4.6 X 250 mm column was used in this experiment. For calibration , an amino acid mix was set up harboring all 20 essential amino acids (5, 10, 20, 50, 100, 200 µM). To normalize the obtained values 50 µL of internal-GABA-standard was added to 950 µL of PhyCoVi medium. For analysis, n the peaks of the amino acid mix were assigned according to the retention time and the integration area was checked for each peak for each sample.