Supplementary File S1

Culturing of algal material

For isolation, purification, and routine subculturing, algal material was transferred using a sterile needle or wire loop and rinsed thrice with sterile PBS (pH 7.5; Lonza, Switzerland) buffer containing 10 mgL-1 germanium dioxide to inhibit diatom growth. The isolated algae were cultured in 300 mL of algal culture broth (Sigma-Aldrich Chemie GmbH, Switzerland), supplemented with 10 mgL-1 germanium dioxide. Uniform inoculants for experimental work were prepared from liquid media using a Gilson Adjustable Volume Pipetman with sterilized plastic tips, dispensing a known volume of suspension. Culturing was performed at 20 °C without aeration, under a light intensity of 100 µmol/m2/s, following a circadian rhythm of 12 hours of light and 12 hours of darkness. Axenicity of the microalgae cultures was verified every three days using a compound microscope at 1250x magnification. Non-axenic cultures were subjected to repeated isolation procedures. Long-term axenic laboratory cultures of *Chlorella* were maintained through routine serial subculture over a 3-month period.

Each *Chlorella* culture was grown for 14 days in liquid algal culture broth. To assess the interaction with wastewater from maturation pond 4 of the Motetema WWTPS, total cell counts were measured using the Countess automated cell counter (Invitrogen, California, USA). The microalgae, either *C. vulgaris*, *C. protothecoides*, or a combination of both, were exposed to wastewater effluent in a ratio of 1/1000 based on total cell counts. A control sample consisted of wastewater effluent without the selected algae. The purpose was to observe the algae's competition with indigenous bacteria and algae species. Total cell counts were recorded at the outset and after 7 days. Additionally, 1 ml samples were collected on days 0, 3, and 7 for chlorophyll a and b determination as per Porra et al. (1989). Growth rates

of total algal biomass, expressed in terms of total chlorophyll, were measured before exposure and after 7 days following standard procedures of Porra et al. (1989). The dominance of algae species was examined using a compound microscope at 1250x magnification.

Testing of the exudate from ponds 6 and 7

For the evaluation of physical, chemical, and biological characteristics in the Motetema WWTPS, samples were consistently collected from the outlets of ponds 6 and 7. The sampling was conducted in the morning, specifically between 9:00 and 11:00 a.m. Two distinct time points were chosen for sample collection: prior to algae inoculation and one year after the commencement of continuous inoculation.

In situ measurements of Dissolved Oxygen (DO), temperature (°C), pH, and Electrical Conductivity (EC) were conducted in the water column using a Hach HQ 40d multiparameter instrument (Hach, Loveland, Colorado, USA). For further analysis, surface water column samples (from the top 5 cm) were collected using a grab sampler. These samples were stored in 1-liter polyethylene bottles, which had been previously rinsed with dilute sulfuric acid to reach a pH of 2.0. To preserve sample integrity, they were transported to the laboratory in a cool, dark container.

All water analyses followed standard methods as prescribed by the American Public Health Association (APHA, 1992). To assess the effectiveness of algae treatment in removing Total Nitrogen (TN) and Total Phosphorous (TP), water samples from ponds 6 and 7 were filtered through 0.22 µm pore size Whatman GF/filters. This process separated the algae from the treated water, enabling precise determination of nutrient uptake. Both filtered and unfiltered samples were used for comprehensive analysis.

Furthermore, for ongoing monitoring, a 1-liter water sample from the outlet of each pond was collected monthly over a 12-month period. Each sample was divided into two sub-samples for specific analyses: (a) identification of soft algae and (b) identification of diatoms, as outlined by Oberholster et al. (2021).

Statistical analysis of data

All variables were log-transformed prior to analysis to minimize skewed distributions. A two-way ANOVA (site and time) was employed to determine the physicochemical and biological differences among algae tests, using parameters with three replicates per sampling time. Homogeneity of variances and normality of data were verified before analysis. Significant differences ($p < 0.05$) identified by the ANOVA were further analyzed using a Tukey-b test.