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WASTEWATER TREATMENT USING MIXED CONSORTIA OF MICROALGAE AND CYANOBACTERIA

Ana Valentina ARDELEAN (ZAMFIRESCU)[1, 2], Cristina MOISESCU[1], Calina Petruta CORNEA[2]
[1]Institute of Biology Bucharest, Romanian Academy, Splaiul Independentei 296, Bucharest 060031, Romania,
[2]Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăsti Blvd, District 1, Bucharest, Romania
e-mail: ana.ardelean@ibiol.ro, cristina.moisescu@ibiol.ro, pccornea@yahoo.com

Abstract. This study evaluated the ability of two mixed populations of photosynthetic microorganisms, a cyanobacteria-green microalgae consortium (C1) and a special laboratory-designed cyanobacteria-green microalgae consortium (C2), to remove organic and inorganic contaminants from various wastewaters. During these experiments, the C1 and C2 consortia were grown on both artificial and real wastewater, and their ability to remove organic and inorganic compounds was evaluated using a variety of tests. In this regard, the concentrations of biodegradable organic matter, such as BOD_5, as well as nitrate, ammonium, and inorganic phosphorus were measured both before and after wastewater treatment with photosynthetic biomass. During a five-day experiment in which the consortium was grown on artificial wastewater, the estimated BOD_5 organic load decreased from 130 mg O_2 to zero, demonstrating the effectiveness of these microorganisms in oxidizing organic compounds and treating wastewater. In addition, the measured concentrations of nitrate, ammonium, and inorganic phosphorus revealed an increase in ammonium concentrations and a decrease in nitrate concentrations, resulting in a decrease of 9% for the two nitrogen-containing compounds and a decrease of 33% for inorganic phosphorus. The increase in ammonium concentration indicates that its assimilation by the photosynthetic consortium in the presence of organic carbon sources is less intense than its synthesis by microorganisms in the reaction vessel. In contrast, the decrease in nitrate and inorganic phosphorus concentrations indicates that their assimilation is greater than their synthesis in the presence of organic carbon sources. Parallel research was conducted with real wastewater as the growth medium. Similarly, to artificial wastewater experiments, the photosynthetic consortia demonstrated their ability to oxidize and remove all organic compounds during the five-day trial. The results showed that the microbial consortium was effective in removing both organic and inorganic compounds from the wastewater, with significant reductions observed in BOD_5, nitrate, ammonium, and inorganic phosphorus concentrations. This suggests that photosynthetic microorganisms could be a promising solution for wastewater treatment and nutrient recovery. This study draws attention to an extremely vital topic: the interrelationships between photosynthetic and non-photosynthetic bacteria in wastewater treatment. In addition, the retention time must be optimized for optimal organic and inorganic compound removal.
Keywords: cyanobacteria, green microalgae, BOD_5, effluent

1 INTRODUCTION

In recent years, in addition to the rapid urbanization of the world's population, the inappropriate discharge of wastewater into aquatic environments has led to the need for more appropriate and efficient wastewater treatment methods. The occurrence of inappropriate disposal of wastewaters, which have an overabundance of nutrients, particularly nitrogen and phosphorus, is a cause for worry because of its ecological effects such as eutrophication, algal blooms, unrestrained development of some aquatic macrophytes, depletion of oxygen, loss of major species, and deterioration of freshwater ecosystems (Wang et al., 2010; Doria et al., 2012). Traditional treatment methods, such aerobic and anaerobic reactors, are associated with high costs, intricate operational procedures, significant creation of sludge byproducts, and significant energy consumption. Such treatment methods can be expensive to implement and moreover can be inefficient and non-environmentally friendly. Therefore, it is essential to build a technology that is both commercially successful and ecologically sustainable, using resources that are financially possible. In recent years wastewater treatment technology has undergone significant innovation with numerous technologies being developed to reduce pollution caused by human activity. According to Sood et al. (2011), phytoremediation, which employs plants or algae, is a feasible alternative for the management of wastewater. Due to their high requirement for nitrogen (N) and phosphorous (P) throughout their growth, microalgae, a general term which includes eukaryotic microalgae and cyanobacteria, have been used to remove nutrients from wastewater (Mata et al., 2012). The major advantage of algae-mediated wastewater bioremediation is that it can simultaneously correct the pH, reduce the total dissolved solids (TDS) and remove both chemical oxygen demand (COD) and biological oxygen demand (BOD) (Bharagava, 2019; Koul et al., 2022).

Engineered photobioreactors were developed to sustainably treat wastewater with low costs and efficient carbon, nitrogen, and phosphorus recovery. The photosynthetic microorganisms used in these photobioreactors can capture solar irradiation via photosynthesis and obtain energy, which is used for their growth and the assimilation of associated carbon and nutrients. By harnessing the power of photosynthesis, microalgae not only treat wastewater and capture carbon dioxide from the atmosphere and generate oxygen but also produce biomass that can be further used as a source of renewable energy. For example, some strains of algae can be used to produce biofuels, while others can be used to create high-value chemicals and pharmaceuticals (Rawat et al., 2011). This demonstrates a further benefit of the system. Moreover, the use of microalgae reduces the need for chemical treatments that can harm the environment. With the growing concern for sustainable development, this approach is gaining more and more popularity as it offers a viable solution to address environmental issues while also producing valuable resources. However, there are some challenges associated with this technology, such as the need for specialized equipment and expertise. Despite these challenges, the potential benefits of phytoremediation by microalgae make it a promising area for further research and development. By implementing this technology in various areas such as agriculture, wastewater treatment, and biofuel production, we can make a positive impact on the environment while also producing valuable resources for society.

Several studies have demonstrated the effectiveness of using wastewater as a growth substrate for the proliferation and biomass production of microalgae (Cho et al., 2011; Sydney et al., 2011; Zhou et al., 2012; Renuka et al., 2013). But most of these studies refer to single-strain cultures. On the other hand, there are other studies showing that microalgae/cyanobacteria and bacteria consortia are more effective in removing organic compounds and nutrients from wastewater (Cho et al., 2017; Zhang et al., 2017; Qi et al., 2021; Aditya et al., 2022; Gururani et al., 2022). This consortium of algae and bacteria can act in a synergistic way much more effectively than individual microorganisms. However, there are also disadvantages in using microalgae-bacteria consortia, with the main one being that certain bacteria can inhibit algal growth. For example, an antagonistic relationship was observed between algae and *Leptothrix ochracea* in the iron-rich streams (Sheldon and Wellnitz, 1998).

Therefore, a consortium composed exclusively of photosynthetic microorganisms would be an interesting approach. According to Subashchandrabose et al. (2011), the combined use of cyanobacteria and microalgae in consortia has demonstrated effectiveness in the detoxification of organic and inorganic pollutants, as well as in the removal of nutrients from wastewater, surpassing the capabilities of individual strains. Mixed populations (co-culture or consortia) can carry out complex procedures that are challenging or even impossible for individual strains or species (Brenner et al., 2008). Living together may give consortium members resilience to environmental changes, stability, the ability to survive food shortages, and a greater efficiency of biomass production and nutrient removal (Bhatnagar et al., 2010; Silva-Benavides and Torzillo, 2012). However, a significant impediment prevents upscaling the use of unicellular microalgae in the treatment of sewage. This barrier is their microscopic sizes (i.e., 0.5 to 30 µm) which makes the collecting of biomass labor-intensive and economically unfeasible (Molina Grima et al., 2003). Instead, the use of filamentous microalgae with length of around 200 µm gives a viable alternative. These microalgae have the potential to drastically cut harvesting costs since they are easily collected by filtering or by producing aggregates or mats (Chinnasamy et al., 2010; Chen et al., 2011; Hori et al., 2002).

Therefore, this study evaluated the ability of two mixed populations of photosynthetic microorganisms, a native (C1) and a specially created in our laboratory (C2) cyanobacteria-green microalgae consortium, to remove organic and inorganic contaminants from various wastewaters, as compared to single strain cultures.

2 MATERIALS AND METHODS

2.1 Photosynthetic consortia

Two mixed populations of photosynthetic microorganisms hereinafter referred to as Consortium 1 (C1) and Consortium 2 (C2) were used in this study. Consortium 1 (C1) was previously described (Moisescu et al., 2018) and consisted of a mixture of photosynthetic populations from the laboratory collection, from a fish farm, and from domestic aquaria. Consortium 2 (C2) was specially created for this study, by mixing equal amounts of different microalgal and cyanobacteria strains from the laboratory collection, specially selected for their water-cleaning capabilities (Ardelean et al., 2017; 2018; 2022) and allowed to acclimatize for consortia development by periodic transfers into BG11 medium (Zhang et al., 2012). Both C1 and C2 consortia were maintained in laboratory conditions in an actively growing state.

2.2 Nutrient removal

The nutrient removal efficiency was monitored for 48 h. Briefly, the photosynthetic consortia biomass initially grown in BG11 medium, was harvested by centrifugation at 7000 rpm for 5 min and the pelleted cells were washed twice with deionized water. The washed biomass was transferred and cultivated in transparent plastic boxes, each containing 500 ml of artificial wastewater (AWW) or real wastewater (RWW), at 28℃ under 16500 lx white fluorescent illumination with a light: dark cycles of 12 : 12 h, for 2 days. These conditions were kept constant for all the experiments. The AWW composition was formulated according to Takaya et al. (2003) respectively: 0.085% NaNO3, 0.06% peptone, 0.04% bouillon extract, 0.01% urea, 0.003% NaCl, 0.01% KH2PO4, 0.0014% KCl, 0.002% MgSO4 •7H2O, 0.00185% CaCl2 • 2H2O; pH 7.2-7.4. Before use, the AWW was diluted 4 times so that the content of nitrogenous and organic substances to be closer to a real aquaculture recirculating system situation. The RWW was collected in clean plastic jars from a domestic aquarium, transported to the laboratory, stored at 4℃, and used at it was for subsequent studies.

For nutrients removal monitoring, 10 ml of sample was collected from each culture box, centrifuged at 8000 rpm for 7 min, and the cell-free supernatant was used for the analysis of nitrate (NO_3), ammonium (NH_4), and phosphate (PO_4).

2.3 Biological oxygen demand (BOD_5)

The BOD_5 was determined by conventional methods (APHA, 1995, Section 5210) with a BOD Direct (Hach Lange LZQ087) according to manufacturer instructions. Briefly, the BOD_5 was measured in each water sample (i.e., AWW and RWW) before and after microbial treatment. The total amount of oxygen used by microorganisms to decompose the organic matter was recorded during 5 days of incubation in the dark, at 20℃. Each set of experiments was done in duplicate.

2.4 Analytical methods and data analysis

Time-dependent consumption of NO_3, NH_4, and PO_4 was measured spectrophotometrically on a Specord® 210 Plus (Analytik Jena) using the Spectroquant® reagent test kits (MerckMillipore).

Data analysis was performed with Microsoft Excel software. The nitrification/denitrification rate formula is $(C_0 – C_n)/h$, where C_0 is the initial concentration and C_n is the final concentration of N source (NH_4 or NO_3) at n hour. h is the time of microbial treatment. The removal efficiency percentages were calculated according to Ansari et al. (2017): Percentage removal % = (IC − FC / IC) x 100, where IC= initial concentration (mg/L) and FC= final concentration (mg/L).

3 RESULTS AND DISCUSSION

3.1. Consortia growth analysis

The wastewater treatment experiments that used AWW were carried out in parallel with those that used RWW. Fig. 1 shows the experimental setup for the cultivation conditions of photosynthetic microorganisms during wastewater treatment experiments. Both C1 and C2 consortia grew well on both types of water (Fig. 1), maximum biomass content recorded was for C2 on AWW (Table 1).

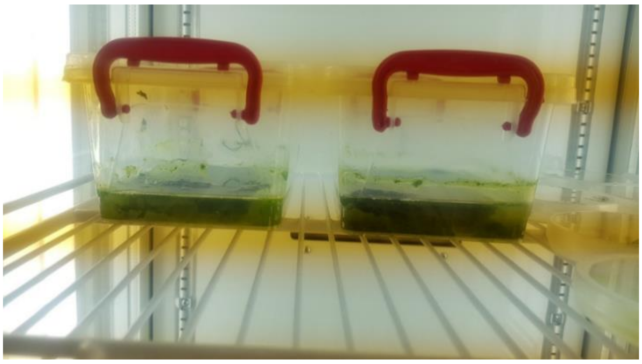


Figure 1. Experimental setup overview of cultivation conditions of photosynthetic consortia C1 (left) and C2 (right) during wastewater treatment experiments.

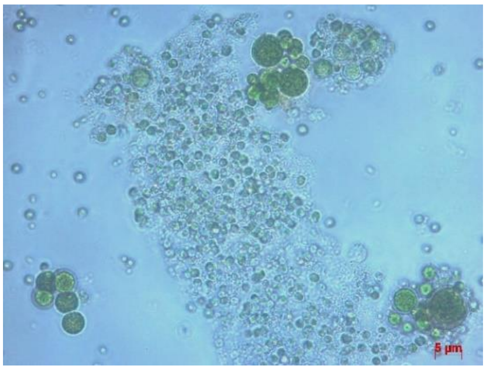
Table 1. Biomass content for C1 and C2 recorded on both types of wastewaters (RWW and AWW)

Consortium	Biomass (g/l)	
	RWW	**AWW**
C1	0.013	1.56
C2	2.73	4.2

These results suggest that both C1 and C2 consortia are suitable for use in wastewater treatment processes, regardless of wastewater composition. The experimental setup used in this study was effective in cultivating photosynthetic microorganisms, as evidenced by the successful growth of both consortia. Interestingly, the maximum biomass content was recorded for C2 on AWW, indicating that this consortium may be particularly effective in treating this type of wastewater. These findings have important implications for the development of sustainable wastewater treatment systems that can effectively remove pollutants while also producing valuable biomass. Further research is needed to fully understand the mechanisms underlying the observed differences in biomass production between the two consortia and to optimize their use in practical applications.

In our previous studies, the biomass accumulated by C1 grown on AWW was 1.56 g/l in 2 days, while on RWW it was only 0.013 g/l (Ardelean et al., 2019). For consortium C2, in the present study the biomass accumulation in 48 hours was 2.73 g/l and 4.2 g/l, on RWW respectively AWW (Table 1). These results suggest that C1 is not as well adapted to grow on RWW, while C2 is able to thrive in both AWW and RWW. This is an important finding as it indicates that C2 has the potential to be used in bioremediation processes targeting both types of wastewaters. Furthermore, the higher biomass accumulation observed for C2 on AWW compared to RWW suggests that the composition of the wastewater can have a significant impact on microbial growth. Therefore, it may be beneficial to tailor the composition of wastewater to promote the growth of specific microbial consortia for efficient bioremediation. Overall, these findings highlight the importance of understanding microbial adaptation and response to different environmental conditions to develop effective bioremediation strategies.

Microscopic analysis revealed sizable morphological differences between the two consortia (Fig. 2).



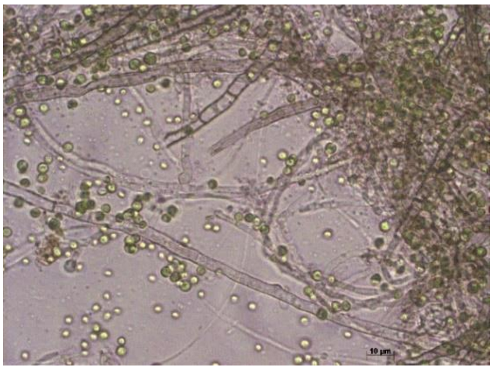


Figure 2. The optical microscope images of consortium C1 (left) and C2 (right).

Regarding their composition, consortium C1 comprises mostly unicellular microorganisms of diverse sizes ranging between 2 - 6 µm, whereas C2 exhibits a combination of size variation and the presence of filamentous structures. It is plausible that these filaments contribute to the aggregation of the culture and to the formation of macro aggregates (bioflocs), leading to a better gravitational sedimentation of the culture. The aspect of these aggregates, along with the time sequence of C2 sedimentation, is depicted in Figure 3.

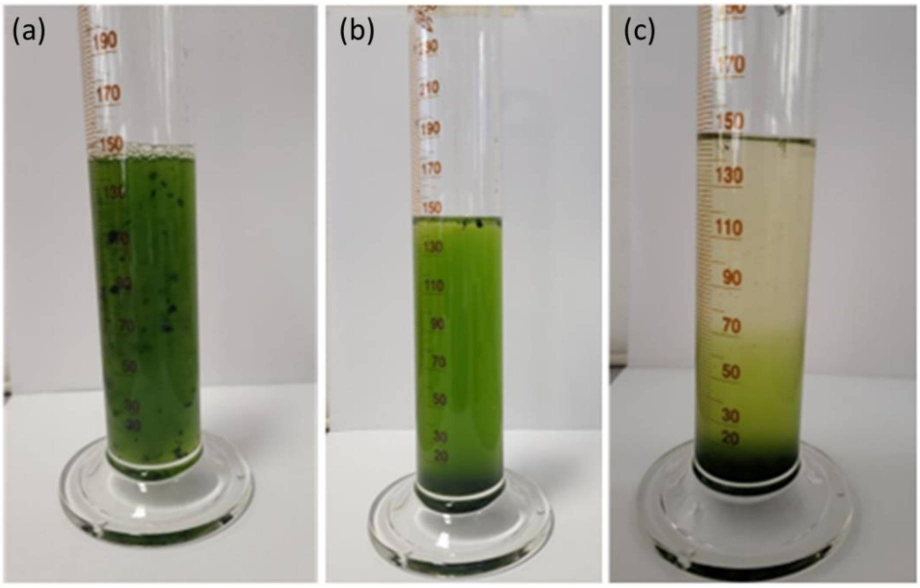


Figure 3. The natural gravitational sedimentation of C2 consortium at (a) T0 and (b) after 15 minutes, and (c) approximately 1 h.

3.2. Wastewater characterization

Both AWW and RWW were found to contain the macronutrients (nitrates, ammonia, and phosphates) necessary for microalgal growth. The initial composition and physicochemical parameters of the two types of wastewaters are depicted in Table 2. Although the AWW was diluted 4 times, it still contained much higher concentrations of macronutrients as compared with RWW. The RWW showed a concentration of only 4.56 mg/L ammonia (NH_4), 2.64 mg/L of nitrates (NO_3), 6.15 mg/L of phosphate (PO_4), and 20 mg/L BOD_5 (Table 2). Similar values of wastewater from aquaculture have been reported in the literature (Ansari et al., 2017; Kurniawan et al., 2021).

Table 2. Chemical composition of artificial (AWW) and real (RWW) wastewater

mg/L	**BOD_5**	**NO_3**	**NH_4**	**PO_4**	**pH**
AWW	130	171.79	10.50	6.99	7.2
RWW	20	2.64	4.56	6.15	7.85

3.3. Nitrogen and phosphorus removal performances of photosynthetic consortia

Due to the rapid growth of the aquaculture industry, a significant amount of wastewater is generated, leading to concerns about eutrophication in receiving waters (Kurniawan et al., 2021). To mitigate this issue, it is crucial to remove nitrogen and phosphorus from wastewater before it is discharged into natural water environments. Photosynthetic microorganisms have shown potential in nutrient removal through phytoremediation, as they can uptake nutrients and convert them into biomass.

In this study, the measurements made on the AWW in the presence of the C1 consortium revealed an increase in NH_4 concentrations and a decrease in NO_3 concentrations, resulting in a decrease of 9% for total nitrogen compounds and a decrease of 33% for inorganic phosphorus. The increase in NH_4 concentration indicates that its assimilation by the photosynthetic consortium in the presence of organic carbon sources is less intense than its synthesis by microorganisms in the reaction vessel. In contrast, the decrease in nitrate and inorganic phosphorus concentrations indicates that their assimilation is greater than their synthesis in the presence of organic carbon sources. Regarding the evolution of nitrate, ammonium, and inorganic phosphorus concentrations over time on the RWW, the results demonstrated an increase in PO_4 concentration, indicating that their assimilation by C1 microorganisms in the presence of organic carbon sources is weaker than its synthesis by all microorganisms present in the reaction vessel.

Table 3. The removal rates of NH_4, NO_3 and PO_4 from AWW and RWW by C1 and C2 consortia.

Removal rates (mg/L/h)	AWW			RWW		
	NH_4	NO_3	PO_4	NH_4	NO_3	PO_4
C1	0	0.39	0.05	0.02	0.06	0
C2	0.22	1.62	0.09	0.1	0.05	0.12

Table 3. The removal efficiencies of NH_4, NO_3 and PO_4 from AWW and RWW by C1 and C2 consortia.

Removal efficiency (%) 48 h	AWW			RWW		
	NH_4	NO_3	PO_4	NH_4	NO_3	PO_4
C1	0	9	33	14	79	0
C2	99.62	45.73	63.57	100	92.31	95.45

The measurements performed on C2 show a much higher removal efficiency of NH_4, PO_4 and NO_3 in 48 h compared to consortium C1. It can also be observed that when grown on RWW, the elimination percentages are over 90%.

Thus, the best results were obtained on the C2 consortium grown on RWW, and the decrease over time in the concentrations of NH_4, NO_3 and PO_4 is graphically represented in Fig. 4.

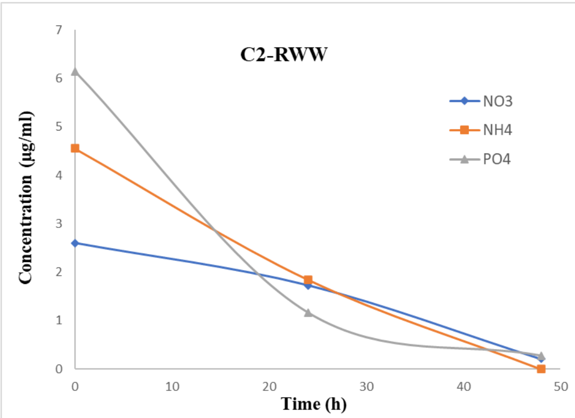


Figure 4. Nutrient removal efficiency over time (48 h) of ammonium (NH_4), nitrate (NO_3) and phosphate (PO_4) by C2 consortium in real wastewater.

Although both microalgae and cyanobacteria have demonstrated varying efficacies in terms of nutrient removal rates when grown as stand-alone cultures (Cho et al., 2011), consortia have a far greater capacity to remove nutrients than monocultures.

In a previous study (Ardelean et al., 2022), using the same type of artificial wastewater but with native stand-alone axenic microalgal strains (strains that are now part of consortium C2), the following results were obtained during a three days trial: Rd-N strain had an efficiency of 100% for NH_4 removal, 5% for $NO_{3,}$ and 92% for PO_4, Ra strain had an efficiency 90% for NH_4, 3% for NO_3, and 83% for PO_4 and the removal efficiencies by Ra-N were 0% for NH_4, 7% for $NO_{3,}$ and 80% for PO_4. By comparing the results obtained by consortium C2 in this study, it can be observed that the efficiency of NO_3 removal is greatly improved, which can be attributed to the presence of cyanobacteria in the mixture. Additionally, slight differences can be observed in the removal of PO_4, but it should be noted that the duration of the experiments in this study was 48 hours, compared to the previously presented results where the experiments spanned 72 hours.

Besides the content of NO_3, NH_4 and PO_4, the content of biodegradable organic matter such as BOD_5, was determined for the AWW and RWW before and after the 48 h contact with the photosynthetic biomass. In case of C1, the organic load of both AWW and RWW estimated as BOD_5, drops from 130 mg O_2 respectively 20 mg O_2, to zero. Following the activity of C2 consortium, the organic load drops from 130 to 15 mg O_2 in the case of AWW, and from 20 to 13 mg O_2 in the case of RWW. In the European Union, according to the Water Framework Directive (Directive 2000/60/EC), for rivers and lakes, an effluent is considered to be of good quality when the BOD5 value is below 2 mg/l. However, it is important to note that these values can vary depending on the country, region, and specific purpose of water use. In Romania, the legal standard for the BOD5 value in wastewater effluent is established by Law no. 241/2006 on wastewater treatment. According to this law, the maximum permissible value for BOD5 in effluent from treatment plants is 25 mg/l.

These results add to the results regarding the evolution over time (48 h) of the concentrations of NO_3, NH_4, and PO_4, presented in Table 3 and 4 and demonstrate the potential of photosynthetic microorganisms in oxidizing organic substances and cleaning wastewater.

4 CONCLUSIONS

In this work, the removal of nitrogen, phosphorus, and organic carbon from a growth medium with a composition that can be assimilated to the composition of real wastewaters, either domestic or from fish farms, was examined for two photosynthetic consortia (C1 and C2). During two days of experimental time, both of the studied consortia could simultaneously remove nitrogen, phosphorus, and carbon, however, C2 was shown to be more effective. The composition in microbial species of C2 consortia seems to be more equilibrate and adequate, suggesting its potential for future applications in the biological treatment of real wastewater. The retention time must be optimised for optimal organic and inorganic compound removal and the addition of nutrients may also enhance the efficiency of C2 consortia. Additionally, the use of C2 consortia in combination with other treatment technologies such as membrane filtration or electrochemical processes may further improve wastewater treatment. However, more research is needed to fully understand the mechanisms behind C2 consortium's effectiveness and to optimize its use in different wastewater treatment scenarios. Overall, C2 consortia shows promise as a sustainable and efficient solution for treating real wastewater and reducing environmental pollution caused by organic and inorganic compounds.

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