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Enhancing Sewage Sludge Stabilization, Pathogen Removal, and Biomass Production through Indigenous Microalgae Promoting Growth: A Sustainable Approach for Sewage Sludge Treatment

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Abstract: Sewage sludge (SS), a byproduct of wastewater treatment plants, poses significant environmental and health risks if not properly handled. Conventional approaches for SS stabilization often involve costly and energy-consuming processes. This study investigated the effect of promoting native microalgae growth in SS on its stabilization, pathogen bacteria removal, and valuable biomass production. The effect on settleability, filterability, and extracellular polymeric substances (EPSs) was examined as well. Experiments were conducted in photobioreactors (PBRs) without O_2 supply and CO_2 release under controlled parameters. The results show a significant improvement in SS stabilization, with a reduction of volatile solids (VSs) by 47.55%. Additionally, fecal coliforms and *E. coli* were efficiently removed by 2.25 log and 6.72 log, respectively. Moreover, *Salmonella* spp. was not detected after 15 days of treatment. The settleability was improved by 71.42%. However, a worsening of the sludge filterability properties was observed, likely due to a decrease in floc size following the reduction of protein content in the tightly bound EPS fraction. Microalgae biomass production was 16.56 mg/L/day, with a mean biomass of 0.35 g/L at the end of the batch treatment, representing 10.35% of the total final biomass. These findings suggest that promoting native microalgal growth in SS could be sustainable and cost-effective for SS stabilization, microalgal biomass production, and the enhancement of sludge-settling characteristics, notwithstanding potential filtration-related considerations.

Keywords: sewage sludge; stabilization; pathogen removal; microalgae–bacteria biomass; sludge properties; extracellular polymeric substances

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1. Introduction

Sewage sludge (SS) production in Europe has become a pressing issue, with approximately 10 million tons of dry solids generated annually [1]. The management and treatment of this byproduct pose significant challenges, both due to the substantial costs involved, which may account for up to 60% of wastewater treatment plant (WWTP) overall operational costs [2], and its environmental impact [3]. Therefore, the development of cost-effective and environmentally friendly approaches is imperative to alleviate the financial burden on WWTPs and promote a circular economy toward converting sludge into a valuable resource.

The solid content of sludge before dewatering typically ranges from 0.2 to 12% by weight and is primarily composed of organic matter, mineral matter, and nutrients, making it a valuable source for soil amendment [4,5]. However, it may contain harmful components that include heavy metals and organic pollutants [4,6]. Furthermore, pathogenic

microorganisms have been detected in SS, such as fecal coliforms, *Salmonella*, *Escherichia coli*, *Shigella*, *Clostridium*, *Mycobacterium*, *Campylobacter*, *Listeria*, and others [7–10]. Depending on diverse factors, including environmental conditions, some of these microorganisms may not survive when sludge is applied to the soil, while others can thrive and potentially contaminate crops, posing a potential risk to public health [11,12]. The 2011 *Escherichia coli* O104 outbreak in Germany caused by the consumption of infected beansprouts serves as a stark example [13].

In Europe, the use of SS in agriculture is regulated by Directive 86/278/EEC, which mandates an appropriate stabilization treatment to reduce its fermentability and associated health risks. This directive does not specify microbiological parameter limits, despite the potential health risks linked with its usage per various studies [14–17]. In response, the European Commission published a draft working document in 2000, which outlines a set of restriction limits. These include (i) a reduction of *E. coli* by at least 6 log to less than 5×10^2 CFU/g ww (wet weight); (ii) an absence of *Salmonella* spp. in 50 g ww; and finally, (iii) a reduction of volatile solids by 38% or a specific oxygen uptake rate below 1.5 mg/h/g TSs to ensure effective stabilization without causing odor nuisance.

Sludge stabilization is a crucial step in SS treatment as it aims to reduce organic matter, pathogens, and odors [18]. Various methods are used for this purpose, as thoroughly discussed in [19], each with its advantages and drawbacks. Anaerobic digestion is a highly effective technique that generates biogas and destroys pathogens. However, it requires a long residence time and may not ensure high degradation of volatile solids [20]. In contrast, aerobic digestion may effectively decrease volatile solids by up to 38%, as supported in [21], and generate compost suitable for agricultural use. Nevertheless, it is crucial to acknowledge that aerobic digestion may provide difficulties, especially pathogen persistence, if not conducted at high temperatures [22]. Furthermore, the process incurs costs due to the mechanical oxygen input and may result in gaseous emissions, especially ammonia, leading to unpleasant odors [20]. Lime stabilization generates pathogen- and odor-free products beneficial for agriculture [23]. However, its effectiveness depends on the pH of the mixture, the mixing duration, the type of sludge, and its water content. Additionally, odor problems may recur once the pH reaches 10.5 [20]. Finally, incineration offers the benefits of reducing sludge volume, recovering renewable energy, and destroying pathogens. However, it also entails considerations such as high heat costs, the potential emission of undesired emissions (dioxins, furans, NO_x, and N_2O), and the generation of a high ash content [3,24]. Hence, it is crucial to develop an alternative method for sludge treatment that prioritizes efficiency, cost-effectiveness, and environmental sustainability.

Microalgae have recently gained considerable attention due to their unique capabilities. They can assimilate nutrients from sludge, such as nitrogen (N) and phosphorus (P), and convert them into algal–bacterial biomass, with significant potential as a biofertilizer [25,26]. In a co-system of microalgae and bacteria, there exists a mutualistic interaction that benefits both organisms involved. Microalgae produce O_2 through photosynthesis, which can be utilized by heterotrophic bacteria for the decomposition of organic matter, potentially replacing mechanical aeration systems and reducing energy costs [27,28]. In return, bacteria supply essential metabolites and CO_2 for algae growth, thereby mitigating greenhouse gas emissions [29]. Microalgae have also been found to play a crucial role in pathogen removal. This is achieved through various mechanisms, such as the content of humic substances, the secretion of antimicrobial metabolites and toxins, and the capacity of attachment to bacteria and sedimentation [30]. Additionally, it was noted that the operational conditions associated with microalgae production, which include high pH and dissolved oxygen levels, light exposure, and hydraulic retention time, create an inhospitable environment for some pathogenic bacteria, especially *E. coli* and total coliforms [30–32]. Recently, it was reported in [30] that microalgae decrease coliforms by 93%. Moreover, in a study conducted by the authors of [33], antibiotic-multi-resistant *Salmonella enterica serovar Typhimurium* was completely eradicated within 48 h from swine wastewater in the presence of microalgae.

This study aims to investigate the potential benefits of promoting the development of native microalgae in SS for its stabilization in a closed system under no O_2 addition and CO_2 release. The main objectives are to examine the reduction of volatile solids and pathogen bacteria (*E. coli*, fecal coliforms, and *Salmonella*), as well as assess microalgae biomass generation. Furthermore, the impact of the proposed process on the extracellular polymeric substance (EPS) profile, settleability, and filterability properties of sludge are also investigated.

2. Materials and Methods

2.1. Samples

Sludge samples were collected from the secondary tank of the wastewater treatment plant Grosses Battes located in Liege, Belgium. Additionally, a treated wastewater sample was collected at the exit of the same station before its discharge into the Meuse and used as a diluent. The characteristics of the samples are presented in Table 1.

Table 1. Characterization of sewage sludge and treated wastewater.

Parameter	Raw Sludge	Treated Wastewater
pH	7.05 ± 0.03	7.7 ± 0.70
Conductivity (µs/cm)	281.33 ± 3.78	-
TS (g/L)	6.16 ± 0.55	-
VS (g/L)	3.93 ± 0.58	-
TSS (g/L)	5.54 ± 0.14	0.004 ± 0.002
VSS (g/L)	3.59 ± 0.05	-
DCO (mgO_2/L)	-	17.1 ± 4.10
Fecal coliforms (CFU/gTS)	$8.67 \times 10^7 \pm 4.01 \times 10^6$	-
E. coli (CFU/gTS)	$7.49 \times 10^6 \pm 3.83 \times 10^5$	-
Salmonella spp. (in 25 gTS)	Present	ND

2.2. Experimental Setup

The experimental setup consisted of a series of batch photobioreactors (PBRs), which were glass Erlenmeyer flasks of 2 L with a working volume of 1 L. To ensure consistent initial biomass concentrations in all reactors, Raw Sludge (RS) was first concentrated through gravity sedimentation for 12 h at 4 °C. The sediment was then centrifugated at 6000× *g* for 10 min. The resulting concentrated sludge was then resuspended in treated wastewater to obtain a Total Suspended Solid (TSS) concentration of 4.5 ± 0.2 g/L in each PBR. The experiments were conducted in a temperature-controlled room at 22 °C. In addition, the PBRs were continuously stirred at 90 rpm using an orbital table. The experimental conditions involved a varied lighting schedule, with continuous light during the first 5 days, followed by 12 h light cycles and 12 dark cycles during the following 15 days. Continuous light was chosen to ensure a high level of oxygen produced by microalgae, thereby preventing the septicity of the sewage sludge. This decision was based on preliminary experiments that showed significantly higher oxygen levels under continuous light compared to light/dark cycles. The photosynthetic active photon flux density (PPFD) at the surface of reactors was 50.25 ± 14.77 µmol PAR photon·m^{-2}·s^{-1} (KIPP & ZONEN, PQS 1 PAR Quantum Sensor, Delft, The Netherlands), provided by 5 daylight fluorescent tubes (36 W/840, Philips, Poland). In order to promote gas exchange (O_2/CO_2) between microalgae and bacteria and prevent any exchange with the ambiance, the reactors were tightly sealed. Last but not least, analysis was conducted in triplicate on fresh samples taken from reactors that were opened on the same day. The remaining content of the opened reactors was sacrificed.

2.3. Determination of Biomass Concentration

The total biomass was determined by the gravimetric method after drying. In detail, 10 mL of well-mixed samples was filtered through pre-weighed 0.45 µm membrane filters and dried at 105 °C until weight stabilization. As a co-culture system, the biomass concentration included microalgae and sludge ($C_{TB} = C_m + C_s$), where C_{TB} is the total biomass concentration (g/L), C_m is the microalgae concentration (g/L), and C_s is the sludge concentration (g/L). To determine the microalgae biomass concentration (C_m), chlorophyll-a (chl_a) was extracted using an acetone solution and measured spectrophotometrically at specific wavelengths (663, 645, 630, and 750 nm), as described in [34]. A 90% acetone solution was used as the blank.

Chl-a concentration (µg/L) was calculated according to Equation (1) (Eq A.1).

$$\text{Chl\_a} = \frac{[11.64(OD663 - OD750) - 2.16(OD645 - OD750) + 0.10(OD630 - OD750)]V1}{\text{V}.\partial} \quad (1)$$

where V and $V1$ refer to the sample volume (L) and the volume of acetone-based extract (mL), respectively. *OD* (Optical Density) is the absorbance of light at a given wavelength, and ∂ is the optical path of the cuvette (1 cm).

The Chl-a concentration was subsequently converted into dry weight by means of a calibration curve with the equation: $y = 10.622x - 0.4453$ ($R^2 = 0.9822$). This equation links the dry weight of microalgae in a sample to its Chl-a concentration. The standard curve is presented in Figure S1 in Supplementary Data.

2.4. Microbiological Analysis

Microbiological analysis was conducted on two matrices, liquid samples (LSs) and dewatered samples (DSs), obtained by centrifugation at 6000× *g* for 10 min. The quantification of fecal coliform and *E. coli* was carried out according to Belgium norms [35] using the membrane filtration method. Briefly, samples were serially diluted in autoclaved peptone water from 10^{-1} to 10^{-9} and filtrated through a 0.45 µm pore size filter. The filters were then plated on agar plates containing fecal coliform agar medium (VWR, Leuven, Belgium) for fecal coliforms and Chromocult coliform agar (Merck, Overijse, Belgium) for *E. coli*. The plates were incubated at 36 ± 2 °C for 18 to 24 h. Following incubation, the colonies were identified per the manufacturer's instructions and counted. The results are expressed as colony-forming units (CFUs) per gram of Total Solids (CFU/gTS) following Equation (2) (Eq. A.2).

$$CFU/gTS = \frac{no.of\ colonies\ x\ total\ dillution\ factor}{Volume\ of\ the\ sample\ plated\ (mL) * \%TS\ of\ the\ sample} \quad (2)$$

The elimination of *E. coli* and coliform bacteria was estimated in terms of Log Reduction Values (LRVs).

The detection of *Salmonella* spp. was carried out according to the Belgium standard method ISO 6579-1:2017 [36], which comprises four stages: (1) enrichment in buffer peptone water, (2) selective pre-enrichment in Modified Semi-Solid Rappaport-Vassiliadis (MSRV) agar for 21 to 27 h at 40.5 ± 2 °C, (3) isolation in XLT-4 agar for 18 to 24 h at 35 ± 2 °C, and (4) confirmation. Typically, *Salmonella* colonies are black with a metallic sheen.

2.5. EPS Extraction and Analysis

As described in [37], 50 mL of the sludge sample was centrifuged at 4000× *g* for 5 min to separate the supernatant, designated as a Soluble EPS (S-EPS). The residual pellets were resuspended in 50 mL of 0.05% NaCl preheated to 70 °C, vortexed for 1 min, and then centrifugated at 4000× *g* for 10 min. The supernatant was collected as a Loosely Bound EPS (LB-EPS). Subsequently, the pellets were resuspended in 50 mL of 0.05% NaCl, heated to 60 °C for 30 min, and centrifuged at 4000× *g* for 15 min, yielding a tightly bound EPS (TB-EPS). The use of a 0.05% NaCl solution during extraction is essential, as it has an

electrical conductivity similar to that of the supernatant, thereby preventing any influence on the EPS. Prior to analysis, the three EPS fractions were filtered through a 0.45 µm filter membrane and stored at 4 °C for a maximum of 48 h. Protein content was quantified using a modified Lowry assay kit (VWR, Leuven, Belgium) with bovine serum albumin as standard. Polysaccharides were determined by the phenol–sulfuric acid method [38], using glucose as standard.

2.6. Other Analytical Methods

pH and dissolved oxygen (DO) were measured using a pH meter and a dissolved oxygen probe (Hach factory) 5 to 6 h after the start of the light cycle. Total Solids (TSs), volatile solids (VSs), Total Suspended Solids (TSSs), and the SVI (Sludge Volume Index) were determined according to the Standard Methods [39]. Microscopic analysis was performed using an optical microscope (Leica model, DM LS2 DFC 280, Belgium). The floc size was determined by means of a particle size analyzer (Equipement Delsa^{TM}Nano manufactered by Beckman Coulter, USA), which uses dynamic light scattering to measure particle distribution. The samples were diluted 10 times before analysis. A filtration test was carried out using a normalized filtration–expression cell (AFNOR 1979, Belgium). The filter was a polypropylene material with a permeability of 8 L/dm^2/min and a thickness of 0.7 mm. The filtration was stopped after a predetermined time of 2.5 h for all tests with constant pressure maintained at 5 bars.

2.7. Statistical Analysis

Bacterial analysis was conducted on two types of samples: liquid and dewatered. The results were compared using a *t*-test conducted with Excel's Analysis ToolPak 2017.

3. Results and Discussion

3.1. Biomass Growth

This study delves into the process of promoting the growth of indigenous microalgae in sludge to ensure its stabilization and obtain valuable biomass. Figure 1 illustrates the variation in the total biomass and distribution of microalgae and sludge biomass. The initial biomass was determined to be 4.683 g/L. It consisted of 4.667 g/L of sludge and only 0.016 g/L of indigenous microalgae, representing 99.66% and 0.34% of the total biomass, respectively. Throughout the operation, the total biomass decreased slightly to 3.38 g/L. However, the fraction of microalgae biomass increased to 0.35 g/L, representing 10.35% of the total biomass at the end of the experiment. This suggests that the microalgae population could outcompete the sludge bacteria for nutrients and thrive in the system [25].

In addition, in photoheterotrophic conditions, microalgae could assimilate organic carbon, such as sugars and organic acids, quickly compared to heterotrophic bacteria that limit their growth [40]. It was shown in [41] that growing microalgae in a liquid digestate reduced the abundance and diversity of the bacterial community, primarily due to variations in the operational conditions, which was reported in [42]. Moreover, as we proposed a stabilization process that effectively removes pathogen bacteria (*E. coli*, fecal coliforms, and *Salmonella* spp.), according to Tables 2 and 3, the reduction of bacterial biomass may be attributed, at least partially, to the removal of these bacteria.

On the other hand, the authors of Ref. [43] demonstrated that a low COD/N ratio in wastewater favors the growth of microalgae over sludge bacteria, which is advantageous for microalgae enrichment. In this study, treated wastewater with a low COD/N ratio of 2.25 was used as a diluent. No additional feeding was introduced to the sludge since it was assumed that it already had sufficient nutrients to support the growth of indigenous microalgae in the presence of light.

Even though no feeding is added, which is cost-effective, this method efficiently converts sludge into a valuable biomass known as a microalgae–bacteria consortium. This consortium has been highlighted in several studies for its dual functionality as a slow-release fertilizer and soil conditioner [42,43].

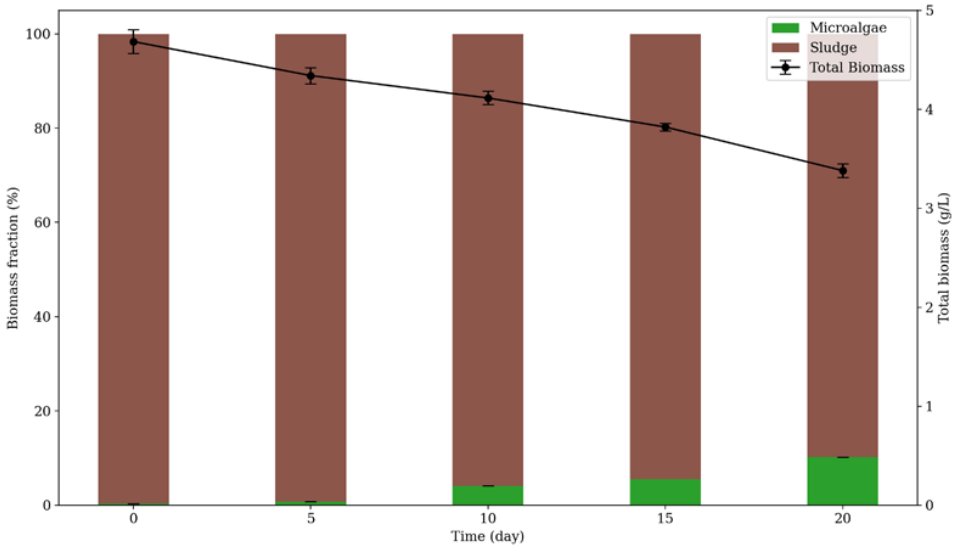

Figure 1. Evolution of the total biomass, biomass fraction of microalgae, and sludge during the treatment.

3.2. Dissolved Oxygen and pH Variation

The photosynthetic activity of microalgae was assessed by measuring Dissolved Oxygen (DO), as it serves as an indicator [44]. As shown in Figure 2, initially, the DO concentration was relatively low, with an average of 1.3 mg/L. Over time, the DO concentration gradually increased, reaching a maximum of 8.05 mg/L recorded on the final day of treatment. This significant increase indicates that microalgae were actively photosynthesizing and producing oxygen, which was sufficient for the aerobic degradation of 47.55% of VSs (organic matter), as demonstrated in Figure 3. In comparison, in a study carried out by the authors of [45], the organic matter degradation efficiency was around 40% after 20 days of digestion with continuous aeration (1.2 m^3 h^{-1} kg^{-1} dry basis). Furthermore, the CO_2 generated through oxidation is consumed by microalgae for their growth, mitigating greenhouse emissions. Likewise, a variation in pH was observed during treatment (Figure 2). The pH increased from 7.02 to 9.16. This is mainly due to the photosynthesis activity of microalgae, where they assimilate CO_2 and HCO_3^- and produce hydroxide ions (OH-), resulting in an increase in pH [44].

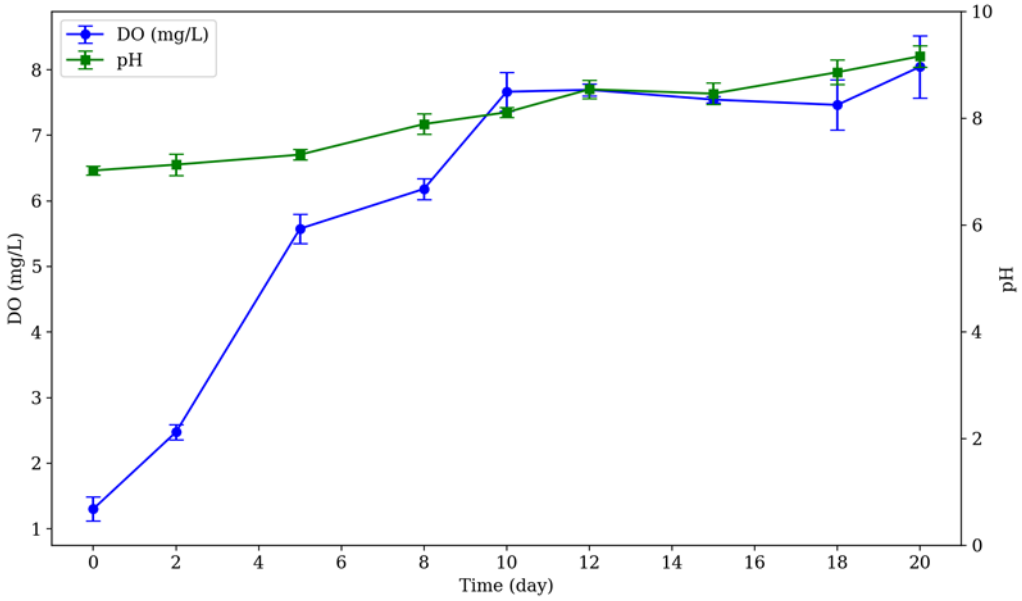

Figure 2. pH and DO change during the treatment process.

3.3. Volatile Solid and Total Solid Reduction

To evaluate the effectiveness of a stabilization process, it is essential to monitor parameters such as the volatile solid (VS) content, which should decrease by at least 38–40% after treatment to define sludge as stabilized [46]. The VS content gradually decreased from about ww 0.3% to 0.157%, representing a removal rate of 47.55%, as presented in Figure 3. This indicates that the organic matter was effectively degraded.

Researchers have found that the efficiency of aerobic digestion in VS reduction is often low. To enhance this process, various approaches have been explored. For instance, the authors of Ref. [47] achieved a 36% ± 4% reduction in VSs within 15 days by adding free ammonia (NH_3) to sludge as a pretreatment compared to a 23 ± 3% reduction in the control. Similarly, the authors of Ref. [48] induced sustained nitrite accumulation in an aerobic digester operated at a low pH (<5.5), resulting in a 35–38.4% reduction in VSs after 300 days of treatment. Our study presents even more promising results despite no external oxygen addition and specific treatment application.

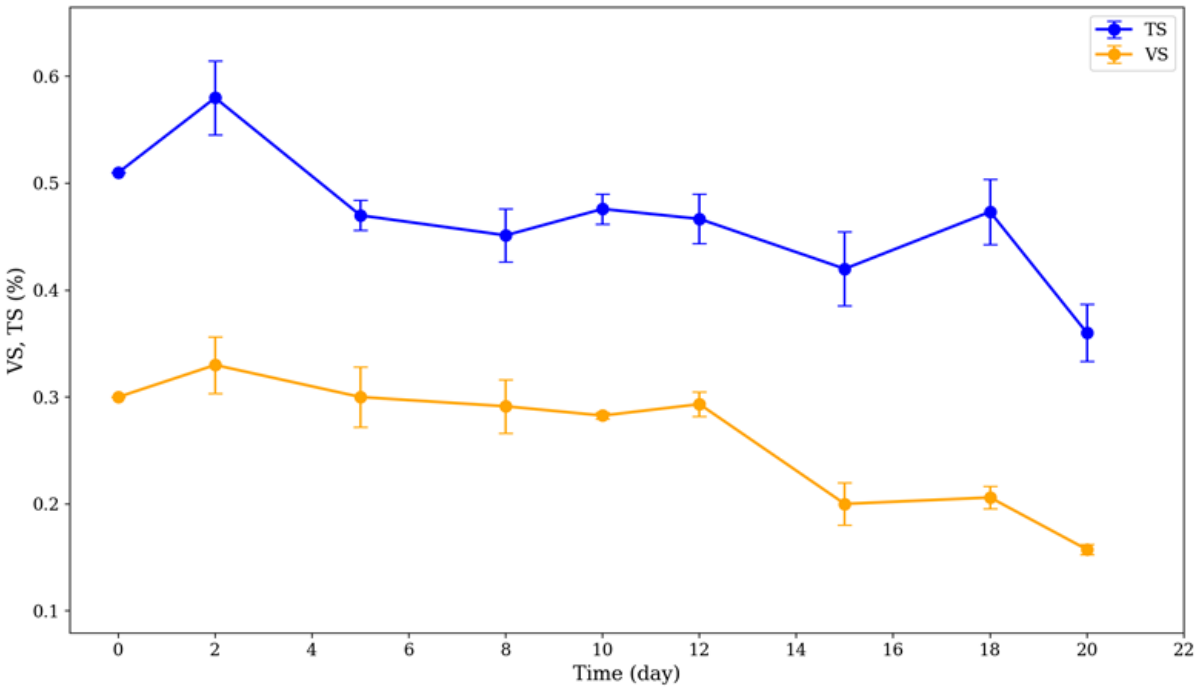

Figure 3. TS and VS reduction during treatment.

Regarding Total Solids (TSs), a slight increase was initially recorded, which can be attributed to microorganisms accessing the remaining organic substances from the wastewater treatment system for their growth [49]. This was followed by a decrease, primarily caused by organic matter degradation and latterly by endogenous respiration, as bacteria oxidize their own tissue under depleted conditions for cell reaction maintenance [50]. This decrease was observed to be 29.41%, as it reduced from 0.51% to 0.36% over the treatment. It is worth mentioning that reducing TSs or VSs is also crucial during sludge treatments since they reduce the amount of sludge that needs to be disposed of [51].

3.4. Effect of Treatment on Floc Size, Settling, and Filtration Properties

The sludge's settleability was notably improved by approximately 71,42% with this treatment, as evidenced by the decrease in the SVI from 178.04 to 50.91 mg/L (Figure 4b). This is principally due to the floc breakup. When digestion occurs, the floc size reduces due to changes in bacterial growth and their adaptation to starvation conditions, leading to a decrease in the SVI [49]. Additionally, the decrease in solids by 29.41%, as illustrated in Figure 3, contributed to the enhanced settling properties of sludge as its concentration

was reduced. This finding is similar to earlier research conducted by the authors of [50] that showed the aerobic digestion of sludge increases its settleability by 60%. The observed increase in floc size on the 10th day may be attributed to high microbial activity, particularly from microalgae, which actively contribute to floc formation, leading to a high SVI observed on the same day.

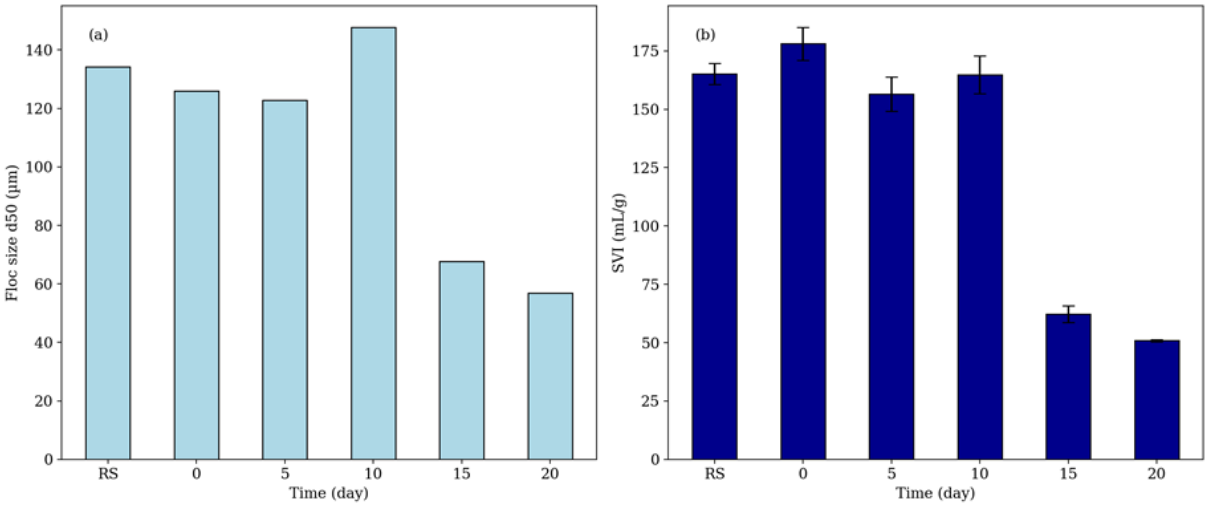

Figure 4. (**a**) Floc size and (**b**) SVI variation during treatment. RS: Raw Sludge.

To investigate the effect of the digestion process on sludge filterability, a series of filtration tests was conducted at different times. The results reveal a clear pattern in the filtration ability (Figure 5), with the order of effectiveness as follows: 5th-day digestion sludge exhibited the highest filterability, followed by 0th-day, 10th-day, 15th-day, and lastly, 20th-day. The high filterability observed on the fifth day can be attributed to the increased secretion of extracellular polymeric substances (EPSs) (Figure 7). During this day, there is a notable increase in protein content within the tightly bound (TB) and loosely bound (LB) EPS fractions. These proteins, being hydrophobic, facilitate improved water release during filtration by reducing water retention within the flocs.

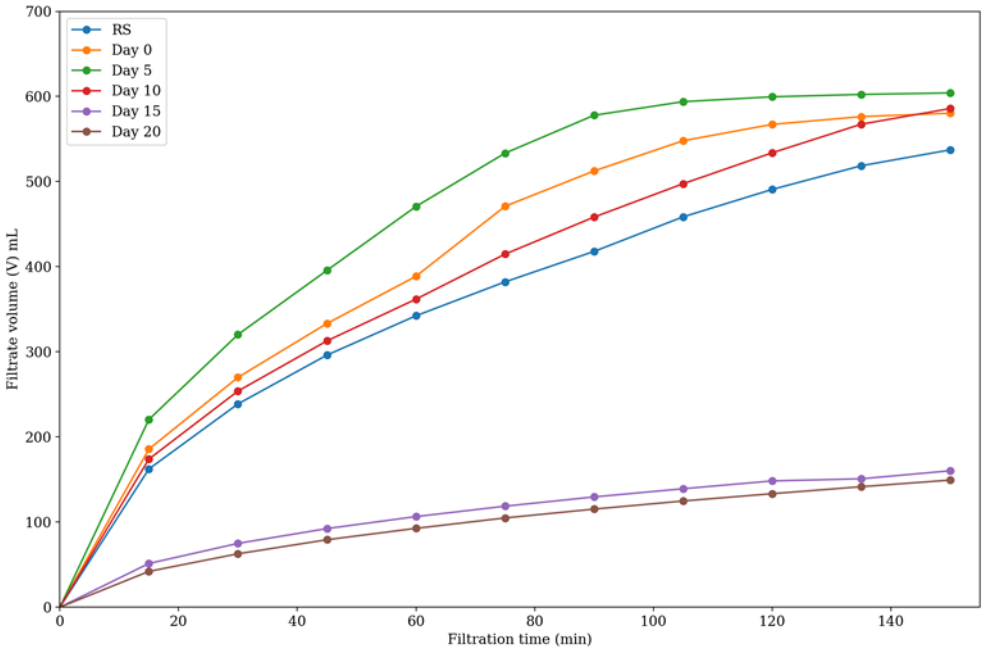

Figure 5. Filtrate volume versus filtration time for the sludge after different treatment durations. RS: Raw Sludge.

Numerous studies have demonstrated that the dewatering performance of sludge deteriorates during aerobic digestion treatment due to the significant destruction of sludge flocs [51–53].

Moreover, the microscopic observation presented in Figure 6 illustrates that, initially, sludge's flocs are characterized by their significant size and density and are primarily composed of bacteria. However, following treatment, the flocs become smaller and less dense while also embedding microalgae. Based on the morphological examination described in [54], the predominant species include Chlorella, Scenedesmus, and Ulothrix.

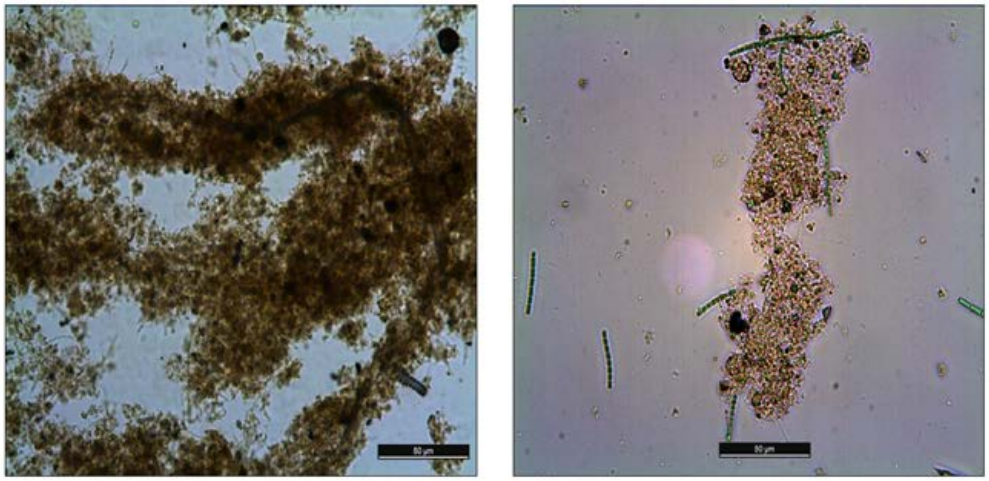

Figure 6. Microscopy images of the mixture in the PBRs (40×): on the left, from the initial day of treatment; on the right, from the end of treatment (20 days). Depicted scale bars measure 50 µm in length.

3.5. The Effect of Treatment on EPS Distribution

Extracellular polymeric substances (EPSs) are complex high-molecular-weight polymers primarily secreted by microorganisms, often resulting from cell lysis. Proteins and polysaccharides are the major components of EPSs, with proteins typically being prevalent due to the substantial presence of exoenzymes [55]. The sum of proteins and polysaccharides constitutes the total EPS concentration. Based on their proximity to the cell, EPSs can be categorized into three fractions: Soluble EPSs (S-EPSs), loosely bound EPSs (LB-EPSs), and tightly bound EPSs (TB-EPSs). The TB-EPS fraction is considered "the active fraction" as it plays a crucial role in maintaining the floc sludge structure [56,57].

In this study, we examined the variation of EPS concentration and their distribution during the sludge stabilization process. The concentration of total EPSs decreased from 34.49 to 27.14 mg/gTS over the process (Figure 7c). Only a spike to 40.10 mg/gTS was observed on the fifth day of treatment, primarily due to enhanced microbial growth that resulted in increased EPS secretion, particularly in the TB fraction. The reduction in EPS levels can be attributed to their microbial consumption under starvation conditions [58].

Focusing on each fraction, we noted a substantial increase in S-EPSs by about 215%. LB-EPSs showed minimal variation. TB-EPSs decreased by 58.75%, with corresponding reductions of 59.77% and 55% in proteins and polysaccharides, respectively. Given the critical role of TB-EPSs in maintaining the three-dimensional structure of sludge flocs, mainly thanks to proteins rather than polysaccharides, the reduction in protein content within this fraction due to its consumption by cells or its shift to other fractions significantly destabilizes the sludge flocs. This destabilization leads to floc disintegration and impairs the dewatering properties, as demonstrated in [59] and illustrated in Figure 4bandFigure 5.

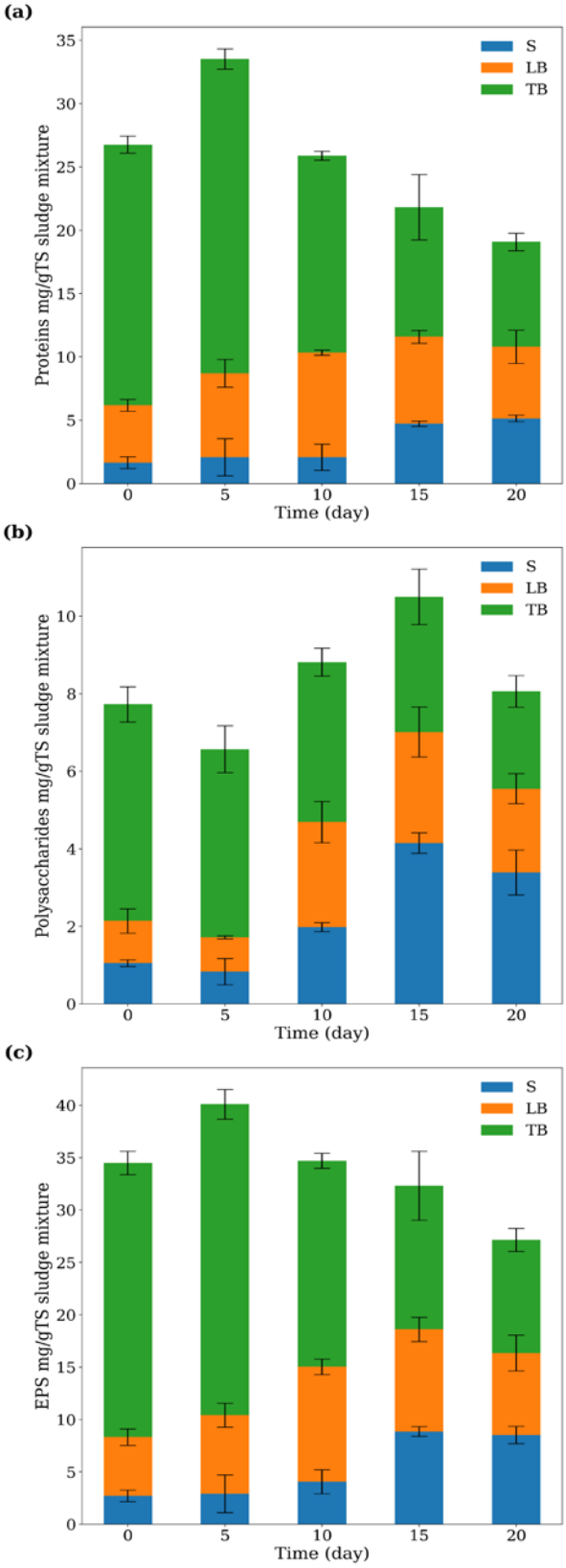

Figure 7. Effects of the process on EPS dynamics at different times. (**a**) The changes in protein content, (**b**) polysaccharide content, and (**c**) total EPS content in different fractions.

3.6. Pathogen Removal

In this study, the quantities of fecal coliforms and *E. coli* in liquid samples (LSs) and dewatered samples (DSs) are compared in Table 2 at different times of treatment. Statistical analysis reveals no significant difference in bacteria abundance between the two analyzed samples ($p > 0.05$). However, the liquid samples exhibited the highest bacterial abundance, indicating that there may be a loss of bacteria during the dewatering step.

Table 2. Reduction of *E. coli* and fecal coliforms in liquid samples (LSs) and dewatered samples (DSs) at different times of treatment.

	RS	0	5	10	15	20	LRV	% Reduction
E. coli (LS) CFU/gTS	7.49×10^6 $\pm 3.83 \times 10^5$	5.23×10^6 $\pm 9.24 \times 10^5$	9.81×10^5 $\pm 1.67 \times 10^4$	3.52×10^3 $\pm 3.32 \times 10^2$	6.46 $\pm 1.29 \times 10^1$	<10 UFC	6.72	99.999
E. coli (DS) CFU/gTS	$4.22 \times 10^6 \pm$ 5.12×10^5	3.61×10^6 $\pm 4.47 \times 10^5$	4.57×10^5 $\pm 2.73 \times 10^4$	2.04×10^3 $\pm 6.77 \times 10^2$	ND	<10 UFC	6.56	99.999
Fecal. Coliforms (LS) CFU/gTS	8.67×10^7 $\pm 4.01 \times 10^6$	2.48×10^7 $\pm 3.57 \times 10^6$	3.04×10^6 $\pm 1.70 \times 10^5$	1.63×10^5 $\pm 1.70 \times 10^4$	1.56×10^5 $\pm 2.94 \times 10^4$	1.39×10^5 $\pm 7.07 \times 10^4$	2.25	99.44
Fecal Coliforms (DS) CFU/gTS	5.33×10^7 $\pm 1.57 \times 10^6$	1.37×10^7 $\pm 1.57 \times 10^6$	2.17×10^6 $\pm 7.86 \times 10^4$	1.89×10^5 $\pm 6.29 \times 10^4$	ND	2.22×10^4 $\pm 1.11 \times 10^4$	2.79	99.84

LRV: Log Removal Value, <10 UFC: Method detection limit, and ND: Data not available.

E. coli and fecal coliforms display a substantial log reduction value of 6.72 (99.999%) and 2.25 log (99.44%) after 20 days of treatment in LSs, respectively. Various studies have shown that microalgae can effectively reduce coliform bacteria. For instance, the authors of Ref. [60] reported a 3-log removal of total coliforms from untreated wastewater using a mixed culture of microalgae–cyanobacteria. Similarly, the authors of Ref. [61] showed a 4-log removal of total coliforms and *E. coli* from effluent used as a culture medium for microalgae. Researchers concur that the decrease in coliforms is most likely due to microalgae photosynthetic activity, leading to variations in pH and OD inactivating coliforms [62]. The impact of microalgae biomass concentration on the destruction of fecal coliforms was investigated in [63]. Under light conditions, the authors showed that the reduction in fecal coliforms increases with the increase in chlorophyll-a concentration. Moreover, they suggested that light promotes the production of toxic forms of oxygen species under high pH and DO levels, damaging the cytoplasm membrane of bacteria through photo-oxidation. The decay of fecal coliforms in darkness was attributed to the toxin compound produced by microalgae, as reviewed in [64,65].

As for *Salmonella* spp., it was not detected after 15 days of treatment (Table 3). Similar findings have been reported. For instance, the authors of Ref. [66] demonstrated that *Salmonella* was removed by 1.5 to 2 log after 48 h from wastewater treated with microalgae-activated sludge. Another study [33] reported that microalgal photosynthetic activity inhibits antibiotic-resistant *Salmonella typhimurium* within 48 h.

Table 3. Detection status of *Salmonella* spp. in liquid samples (LSs) and dewatered samples (DSs) analyzed at different times of treatment.

Treatment Day	LS	DS
0	+	+
5	+	+
10	+	+
15	−	ND
20	−	−

(+): Detected, (−): Not detected, ND: Data not available.

The proposed algal system is effective in removing pathogenic bacteria from sludge. The final product meets the European standards for sewage sludge agricultural use, and as it contains microalgae, it could be a good fertilizer. The recovered water from this process is pathogen-free, so it can be recycled back into wastewater treatment plants.

4. Conclusions

In conclusion, the findings of this study highlight the promising potential of promoting indigenous microalgae growth for sewage sludge stabilization. The unique natural gas exchange process between microalgae and bacteria not only reduces the need for costly oxygen input and decreases CO_2 emissions but also effectively removes harmful pathogens, such as *E. coli*, fecal coliforms, and *Salmonella*. While the results show significant reductions in volatile solids and improvements in the settleability properties of sludge, further research is needed to address other factors, such as the removal of heavy metals and pollutants, as well as nutriment leaching, during the treatment process. The observed increase in the microalgae biomass and diverse species present suggests the possibility of utilizing this biomass as a biofertilizer, which will be further explored through agronomic assays. Overall, this study provides valuable insights into a sustainable and environmentally friendly approach to sewage sludge treatment that has the potential to benefit both the agricultural and wastewater treatment industries. The novel integration of indigenous microalgae growth not only enhances sewage sludge stabilization and pathogen removal but also reduces reliance on external oxygen sources, thus minimizing operational costs and greenhouse gas emissions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/recycling9050097/s1, Figure S1: The standard curve of chlorophyll-a content and dry weight of microalgae.

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