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Reverse Salt Flux Effect on Dewatering *Chlorella vulgaris* in a Forward Osmosis System

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Abstract: Microalgae shows a high potential to produce biofuel and forward osmosis (FO) has been proposed as a promising dewatering process for algal biomass separation from water. However, the effect of reverse salt flux (RSF) on algal biomass during the dewatering process using FO has not been completely explored. This study was to investigate the effect of different types of salt and their concentrations on algal biomass in terms of conductivity, settling velocity, and lipid contents in FS during a simulated FO-driven dewatering of *Chlorella vulgaris* microalgae. Three draw solution (DS) salts (NaCl, KCl and NH_4Cl) were evaluated in RSF-simulating batch tests. The salt diffusion from the DS to the algal feed solution (FS) caused a static growth of algal biomass while increasing lipid content up to 14.8% at 8 mM NH_4Cl. With the addition of the different salts, pH was maintained to the optimal algal thriving range (7.2–10.6), but the presence of salt stressed the algal cells and inhibited photosynthesis and algal growth within the experimental conditions. The settling velocity of the algal cells improved with the increase of salt content from 8 to 80 mM of each DS. It seemed that cell division could be accelerated in the presence of NH_4Cl, and microscopic images showed a change in the algal cell size distribution, which may negatively affect algal settleability. DS salt in an FO-algal harvesting system should be selected based on the final algal properties and constituents required.

Keywords: *Chlorella vulgaris*; draw solution; feed solution; forward osmosis; microalgae; reverse salt flux

1. Introduction

Microalgae have a high potential for the production of biofuel, biodiesel, supplemental nutrient and feedstock. Depending on the conditions of cultivation, microalgae can vary in the constituent ratios of carbohydrates, lipids, and proteins [1,2]. The cultivation of microalgae can be achieved with a low impact on natural resources. For example, algae can grow using secondary treated wastewater as a nutrient source which can serve as a polishing stage in wastewater treatment. In the presence of sunlight, algae can utilize carbon dioxide (CO_2) in exhaust gas from power plants [3,4].

The use of forward osmosis (FO) membranes as an attempt to harvest grown algae in a sustainable way has been studied for the past decade. FO has the capacity to preserve energy by utilizing osmotic pressure to concentrate and harvest microalgae while producing clean water. FO requires two salt concentration gradient solutions to interact on the opposite sides of the membrane surface: draw solution (DS) and feed solution (FS). The DSs used in FO systems are preferred to be economical, abundant, non-toxic and easy to regenerate. In best practice, the diluted DS would be directly utilized in other applications, such as fertigation or as a feed solution in desalination plants [5,6]. Using the DS, the FO system can reduce the volume of the algal FS to approximately 20% [7]. However, during this process, salts can flow backward from the DS to the algal FS by diffusion, which is called reverse salt flux (RSF) (Figure 1). The accumulation of salts in a feed solution due to

RSF can decrease water flux and inhibit the biological activities of the microalgae [8]. The hypothesis taken into account is that the RSF can enhance the growth rate of algal during the algal dewatering, as it can be considered as an additional nutrient source in the case of NH_4Cl and KCl. Moreover, specific salt concentrations can cause algal toxicity, which may lead to algal degradation. The low RSF concentrations may enhance algal growth and lipid content for some salts, while the salts might create a growth inhibition with the high concentration [1,9–11]. pH can change with each salt, which can also affect algal characteristics. The added salts can also affect the algal excretion (dissolved organic content) [12], which can adversely impact the process of algal separation via membranes or settling.
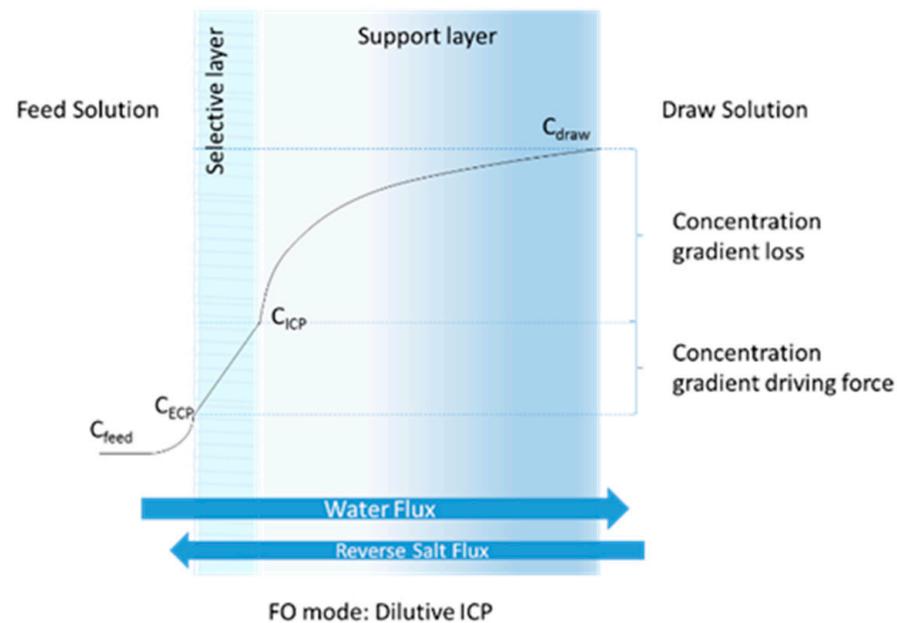

Figure 1. Permeate flux and reverse salt flux (RSF) on a FO membrane configuration. Internal and external concentration polarization (ICP and ECP) in a FO membrane selective layer FO.

The concentration of the salts transferring from the DS to the algal FS varies depending on the concentration gradient between DS and FS, the type of salt, and the membrane configuration. In our previous study with three different types of DS (NaCl, KCl and NH_4Cl) [7], it was found that at a DS concentration between 0.5–1.0 M, the RSF can vary with 0.7–1.7 g m^{-2} h^{-1}, resulting in different salt concentrations between 8 and 80 mM after running for 1–2 days under the cross-flow velocities (CFVs) at 5 cm s^{-1}. The concentration and type of salt can affect algal growth, composition, metabolism, photosynthesis and morphology [2,13]. RSF in FO has been widely studied in terms of membrane fouling and permeate flux loss [14]. However, the effect of RSF on algal biomass during the dewatering process using FO is not completely understood.

The objective of this study was to investigate the effect of different types of salt and their concentrations on algal biomass in terms of conductivity, settling velocity, and lipid contents in FS during a simulated FO-driven dewatering of *C. vulgaris*. Specific salts can have different interactions with enzyme channel use, and the ratio between potassium and sodium can stress the bioenergetic processes of photosynthesis [13]. NaCl, KCl and NH_4Cl were selected to represent salts for RSF in a FO system [7]. For practical applications, NaCl simulates seawater or brine effluent from desalination plants, while KCl and NH_4Cl represent fertilizer salts that can be used as DS to extract water and later be used in fertigation. Three different DS concentrations were applied: 8, 32, and 80 mM [7]. pH and conductivity were continuously monitored during the algal dewatering FO process. Lipid contents in microalgal biomass were measured to investigate the effect of salt on algal biomass in terms

of potential bioenergy production (e.g., biodiesel). Settleability and morphology changes of microalgae were also investigated overtime during the FO operation.

2. Materials and Methods

2.1. FO System

A custom-made bench-scale FO system was used to investigate water flux, RSF, and fouling of a new flat sheet PES FO membrane (Aquaporin–Sterlitech, Kent, WA, USA) with an active surface area of 12.5 cm^2. The system consisted of an FS tank (0.5 L) and DS tank (15 L) [7]. In the previous study [7], the salts used in the FO operation showed effects on water flux and the final algal biomass properties, including color. In this study, the RSF was considered to be a potential contributor to stressing algal biomass in FS and possibly altering algal metabolism in FS. Upon that, the experiments were designed to investigate the effect of the NaCl, KCl and NH$_4$Cl salts on the algal culture. The RSF concentrations used in the experiments were determined in the previous FO operation with 2 days of hydraulic retention time (HRT) [7].

2.2. Algal Species and Cultivation

Chlorella vulgaris microalgae (UTEX 2714, Austin, TX, USA) was cultivated in a 4 L photo-bioreactor at room temperature (23.0 $\pm$ 0.2 °C). The system was aerated with filtered ambient air to supply CO$_2$ (0.04%) under continuous white fluorescent light illumination (light intensity: 159–189 µmol m^{-2} s^{-1} PAR) (06-662-63, Fisher). Modified Bold's Basal Medium (BBM) was used as growth media which consisted of (mg per liter): 175 KH$_2$PO$_4$; 25 CaCl$_2$·2H$_2$O; 75 MgSO$_4$·7H$_2$O; 250 NaNO$_3$; 75 Na$_2$HPO$_4$; 25 NaCl; 10 Na$_2$EDTA·2H$_2$O; 6.2 NaOH; 4.98 FeSO$_4$·7H$_2$O; 0.001 mL H$_2$SO$_4$ (concentrated); 11.5 H$_3$BO$_3$, and a Trace Metal Solution of 2.86 H$_3$BO$_3$; 1.81 MnCl$_2$·4H$_2$O; 0.222 ZnSO$_4$·7H$_2$O; 0.079 CuSO$_4$·5H$_2$O; 0.0494 Co(NO$_3$)$_2$·6H$_2$O [15,16]. The algal concentration was monitored to be maintained in the exponential growth phase at a concentration of about 0.37 g L^{-1} dry algal biomass. The pH and conductivity of the algal solution were 10.0 $\pm$ 0.5 and 700–800 µS cm^{-1}, respectively.

2.3. Analytical Methods

Conductivity was measured to monitor salt depletion or uptake (Hach HQ40d Portable multi-parameter meter) in FS. pH change was monitored over time (Hach HQ40d). pH values of healthy *C. vulgaris* solutions range from 8.0–11.0. Total suspended solids (TSS) (standard methods 2540) and optical density (OD) (DR5000 portable spectrophotometer, Hach, CO, USA) were measured to determine the dry biomass of the algal solution. Microscopic and visual observations of the bulk algal solution were carried out to identify the color change and morphology of the microalgae using microscopy (Omax 40-2500X LED digital trinocular lab microscope, AmScope, CA, USA). Settling velocity was measured using graduated cylinders (100 mL Borosilicate, Karter Scientific, LA, USA), which was monitored using a time-lapse image recorder. Lipid cell content was determined at the end of the experiments by using a modified gravimetric determination of the total lipids [17].

2.4. Experimental Protocol

Several batch experiments were conducted to investigate the salt effects on algal biomass due to RSF during the FO operation. For each batch test, salts were added to a 500 mL algal FS. The algae used in the experiments were in the exponential growth phase (OD = 3 abs @ wavelength of 685 nm) with a concentration of 0.37 g L^{-1}. The experiment duration was 2 days to simulate the HRT used in the previous FO system [7], where the RSF in the FO system was found for different CFVs and DS concentrations. The salt concentrations due to RSF in this study were 8, 32, and 80 mM for each salt, covering the spectrum of the salt concentrations found in the FS of the previous study when 80% of dewatering was completed [7]. The associated algal morphological changes from the RSF in FS were determined with three DSs. Table 1 displays the matrix of conditions with different applied salt dosing. All experiments were conducted at room temperature (23 $\pm$ 0.2 °C).

**Table 1.** Experimental conditions to test the effect of DS salts on algae.

Draw Solution (mM)	Draw Solution (mg L^{-1})			Feed Solution (g L^{-1})
	NaCl	KCl	NH$_4$Cl	
8	467.5	596.4	427.9	
32	1870.1	2385.6	1711.7	0.37 dry *C. vulgaris* biomass
80	4675.2	5964.1	4279.3	

3. Results

RSF is the reverse permeation of a DS across an asymmetric membrane in a FO operation. There are a few studies on RSF in FO operations. For example, Ferby et al. (2020) demonstrated that RSF from DS into an FS could result in several negative effects, such as decreased water flux and inhibition of biological activities, and proposed a strategy to reduce solute buildup [8]. Phillip et al. (2010) conducted model predictions based on experiments related to the reverse permeation in the FO operation, showing that the reverse flux selectivity, the ratio of the forward water flux to the reverse solute flux, is an important parameter in FO design [14]. This study investigated the effects of types and concentrations of DS on algal cells in terms of lipid production and settling velocity, as well as general water quality, such as pH and conductivity.

3.1. pH

pH was found to decrease slightly with time. The initial pH of the cultivated algal solution was around 10.0 without salt. When NaCl was introduced to the algal solution, there was a 0.3 increase in pH and for KCl pH increase was about 0.2 to 0.6, while NH$_4$Cl immediately decreased pH to a pH drop to 7.2–7.9 with the different concentrations (8, 32, and 80 mM). Figure 2 displays the pH changes in FS over time with the addition of NaCl, KCl, or NH$_4$Cl of different concentrations.
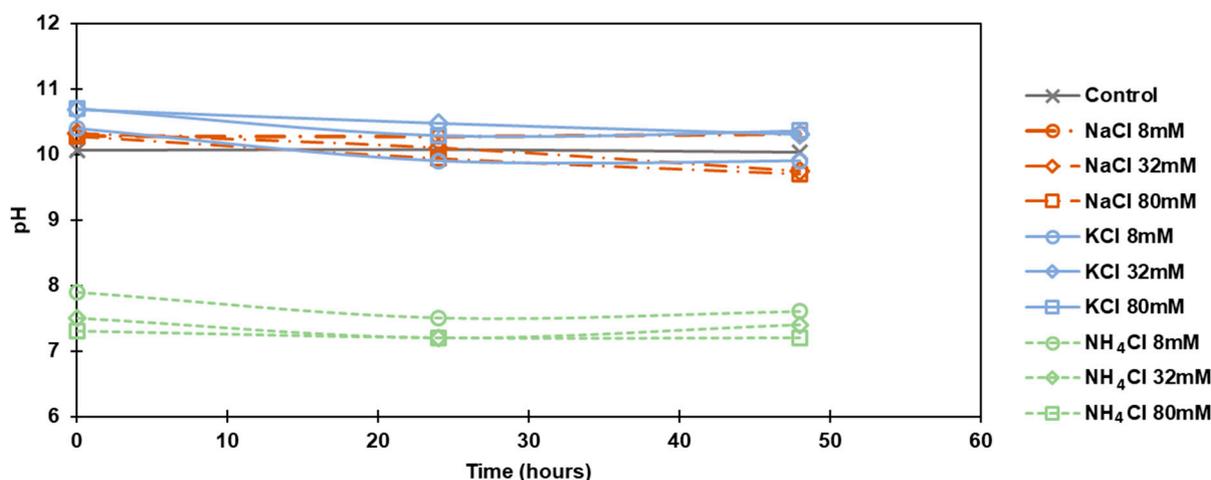
Figure 2. pH changes in FS over time with the addition of NaCl, KCl and NH$_4$Cl of different salt concentrations.

Visibly, the addition of salt changed the color of the algal solutions in FS (Figure 3). In particular, the addition of NH$_4$Cl significantly affected algal growth, making the solution dark green, probably due to the mitosis [18]. The growth of *C. vulgaris* containing NH$_4$Cl was different compared to NaCl and KCl. The addition of NH$_4$Cl altered pH from the initial pH of 10 to the range of 7.2–7.9. The drop in pH to a neutral level showed no negative impact on the growth nor algal viability, as algae can thrive in even lower pH changes [19,20].
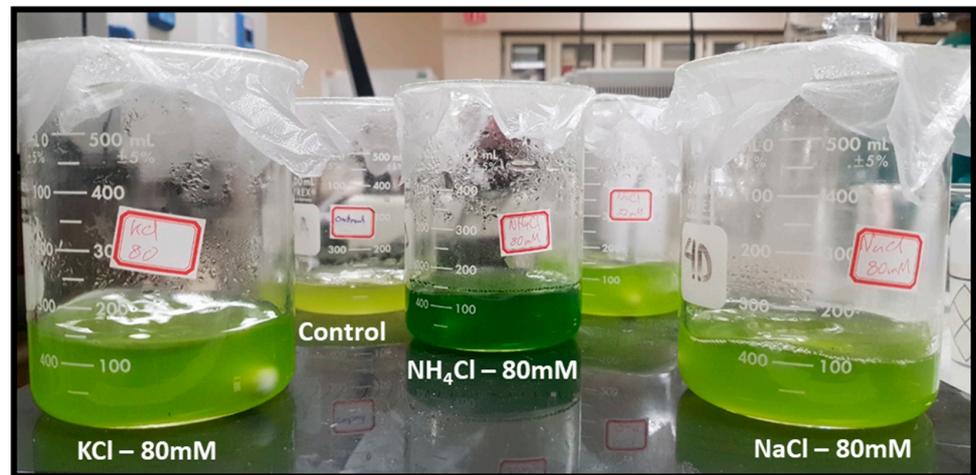
Figure 3. The visual effects of different salts (simulating RSF in a FO operation) on color changes of the *C. vulgaris* solutions. The picture was taken after 2 days of the addition of salts.

3.2. Conductivity

It was observed that the addition of salt as a simulated RSF affected the conductivity of algal FS, increasing the conductivity with the salt concentrations (Figure 4). Over time, the conductivity changes were also measured. At 8, 32 and 80 mM of KCl addition, the conductivity decreased 10, 160, and 300 $\mu S\,cm^{-1}$, respectively, which is equivalent to about 0.4, 70 and 155 $mg\,L^{-1}$ of KCl $mg\,L^{-1}$ being removed. The conductivity in the FS decreased as the KCl dose increased, and this would be from the salt assimilation by algae. On the other hand, NH_4Cl showed an increase in conductivity around 160–250 $\mu S\,cm^{-1}$. For the conductivity increase with the addition of NH_4Cl, it seems that the algal cells may release organic matters, which contributes to an increase in conductivity (equivalent to 20 $mg\,L^{-1}$ of NaCl). It was also reported that electrolyte leakage from damaged cells could increase conductivity in the presence of an NH_4Cl [21]. Another explanation can be that the addition of NH_4Cl had accelerated the cell division [18]; this would have led to micro-releases of electrolytes adding to conductivity. NaCl showed no significant changes in conductivity over time.
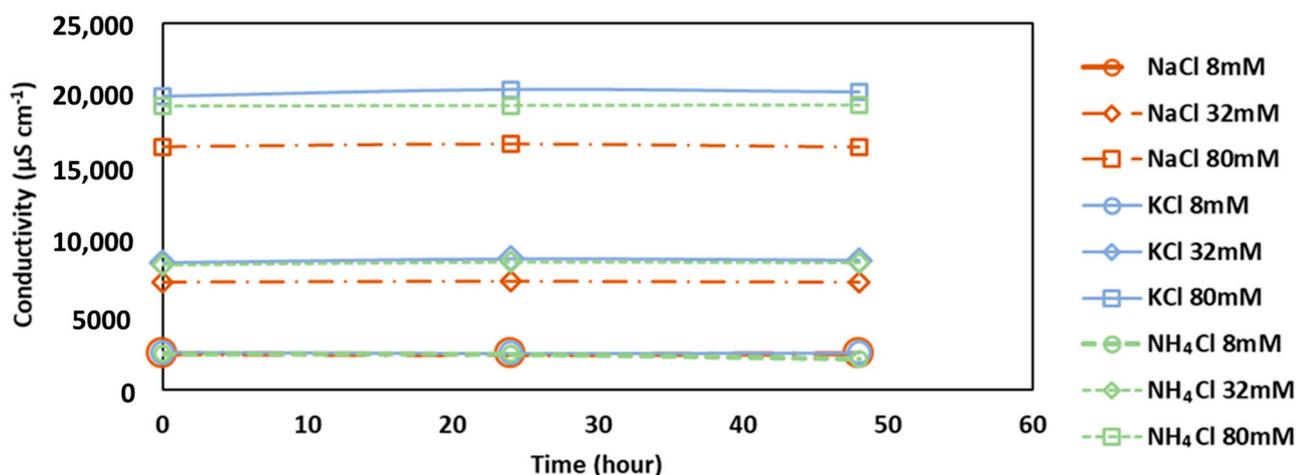

Figure 4. Conductivity changes of FS over time for the addition of NaCl, KCl and NH_4Cl under different salt concentrations. Original algae conductivity was 750 ± 50 $\mu S\,cm^{-1}$.

3.3. Lipid Content

Lipid analysis was conducted at the end of the experiment (i.e., after 48 h) to investigate how RSF can affect the biofuel productivity of *C. vulgaris* in FS (Figure 5). For the initial concentration of 0.37 $g\,L^{-1}$ of dry algal biomass (OD_{685}: 3 abs), the lipid content was about

9.47% (*w/w*) of the algal biomass. Each salt showed a different effect on the *C. vulgaris* cells' lipid accumulation. The lipid contents (%) after 48 h of the addition of KCl showed an adverse effect on lipid accumulation except for 32 mM. It is known that nitrate reductase inhibition is triggered by the presence of KCl [22], limiting the formation of protein in the algal cells and later stressing the algal to promote lipid accumulation. However, the two-day duration was not sufficient for KCl to show a clear trend in lipid cell generation. NaCl showed an increase in lipid content ranging from 0.2 to 49% with different salt concentrations between 8, 32 and 80 mM. In general, it is known that the increase in NaCl causes growth loss while enhancing the lipid accumulation [9,10,23]. This study showed that a low NaCl concentration of 8 mM for 2 days could improve the lipid accumulation of the algal cell from 9.47 to 14.8%. For ammonia, it was reported that the presence of ammonia (e.g., 2.0–2.5 mM) could function as a growth and photosynthesis inhibitor for various species of algal [24]. In this study, the concentrations of ammonia were much higher (e.g., 8, 32 and 80 mM) compared to the previous work [24], and similar to NaCl, NH_4Cl showed an enhanced lipid accumulation within the two days ranging from 8 to 56%. 8 mM of NH_4Cl showed the highest lipid content of 14.8%.

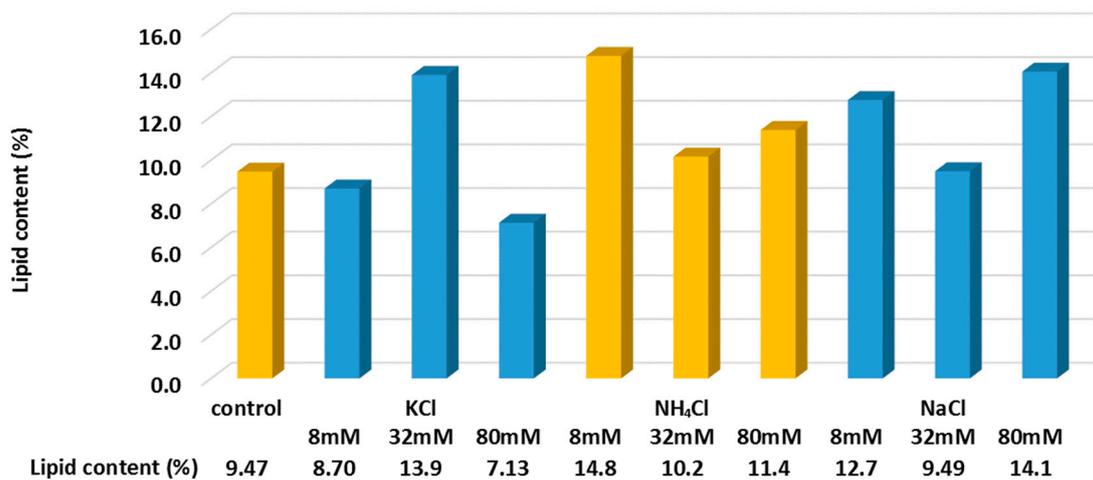| Lipid content (%)	9.47	8.70	13.9	7.13	14.8	10.2	11.4	12.7	9.49	14.1
	control	8mM	32mM	80mM	8mM	32mM	80mM	8mM	32mM	80mM
			KCl			NH₄Cl			NaCl	

Figure 5. Lipid content (%) change in algal biomass in FS after 2 days of the addition of NaCl, KCl, and NH_4Cl under different salt concentrations.

3.4. Settling Velocity

The overall trend of settling velocity showed that with the addition of KCl and NaCl increased the settling velocity (Figure 6). The increased settling velocity for KCl and NaCl may be possible, probably due to the ability of the salt to strengthen specific attributes of water, such as the density and surface tension [25], causing more resistance on the *C. vulgaris*. In the case of NH_4Cl, the low concentration of 8 mM showed an adverse effect on the settling ability, but with the increased NH_4Cl concentrations, the settling velocity was increased, but up to a similar level of the initial settling velocity.

In general, it was found that when the salt concentration increased, the settling velocity was enhanced. Even in very high concentrations (e.g., 15, 30, and 45% of NaCl), it was reported that the settling velocity was also found to increase [10]. The increase in the ionic strength seemed to enhance the settling velocity.
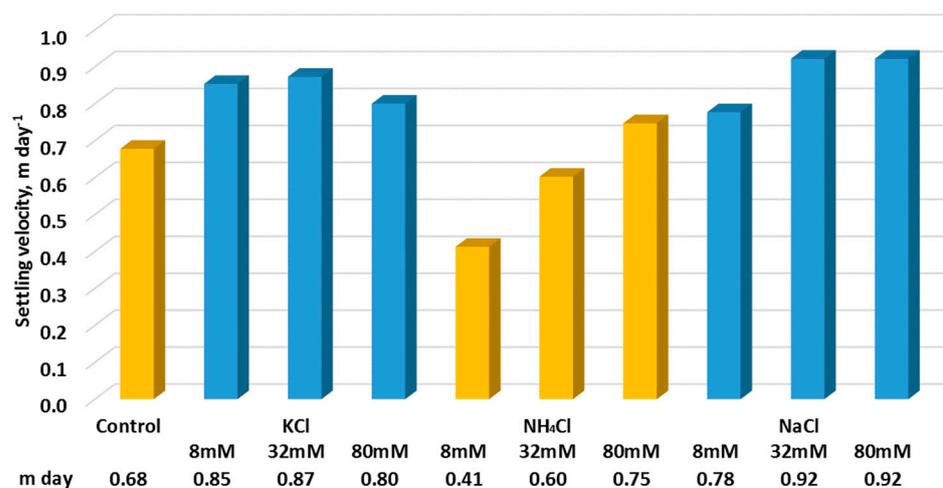
Figure 6. Settling velocity of *C. vulgaris* subjected to different salt concentrations of NaCl, KCl and NH₄Cl.

3.5. Algal Biomass

In the FO simulated conditions, the effect of the added salts was minimal on the algal biomass changes (Table 2). The algal cells seemed to be delayed growth by the salt effect. In the presence of KCl, there was a fluctuating change in the dry algal biomass. Compared to the initial concentration of 0.370 g L^{-1}, the addition of 8 mM KCl showed the highest increase to 0.379 ± 0.023 g L^{-1}. The addition of 32 mM and 80 mM KCl showed a decrease in the algal concentration to 0.377 ± 0.015 and 0.366 ± 0.012 g L^{-1}, respectively. When NH₄Cl was added to algal FS, the concentration of the algal biomass decreased to 0.358 ± 0.014 g L^{-1} from the initial concentration of 0.370 g L^{-1}. NaCl had an increase in dry biomass concentration to 0.382 ± 0.009, 0.382 ± 0.009, and 0.385 ± 0.013 g L^{-1}, respectively, after the addition of 8, 32 and 80 mM NaCl. It was found that there was a correlation between the density of the biomass (g L^{-1}) and the settling velocity (m day^{-1}). Lower algal biomass in FS after the addition of NH₄Cl resulted in lower settling velocity.

Table 2. Algal biomass (dry biomass, g L^{-1}) changes with different salt concentrations. The initial concentration was 0.370 g L^{-1}.

	KCl	NH₄Cl	NaCl
8 mM	0.379 ± 0.023	0.358 ± 0.014	0.382 ± 0.009
32 mM	0.377 ± 0.015	0.361 ± 0.007	0.382 ± 0.009
80 mM	0.366 ± 0.012	0.358 ± 0.014	0.385 ± 0.013

3.6. Microscopic Investigation

Compared to the microscopic image of algal cells before the salt application (Figure 7), microscopic images after the RSF applications showed minimal variations in the algal morphology and cell colony formation (Figure 8). The average size of *C. vulgaris* was 3–5 μm. The microscopic images were collected with a magnification of 100 and 1000 times. The images with 100 times magnification show an overall picture of algal cell distribution (e.g., aggregates or scattering). The microscopic images with 1000 times magnification provided details of the algal cell morphology. The control algal sample had scattered algal cells throughout the 2-day period. Duplicate samples were examined at times 0, 24 and 48 h for each salt concentration. Long-term exposure is expected to further affect growth, morphology and aggregate formation. However, based on the relatively short retention time in a FO system (e.g., a few days), it was sufficient to monitor the reactions for 48 h.
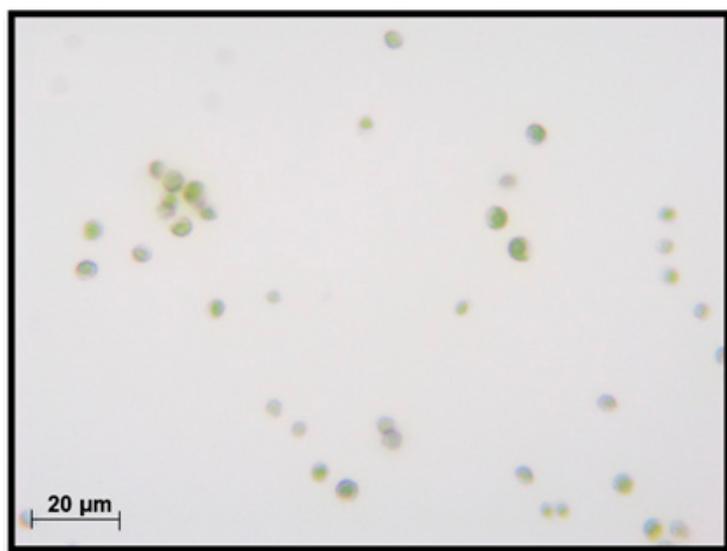
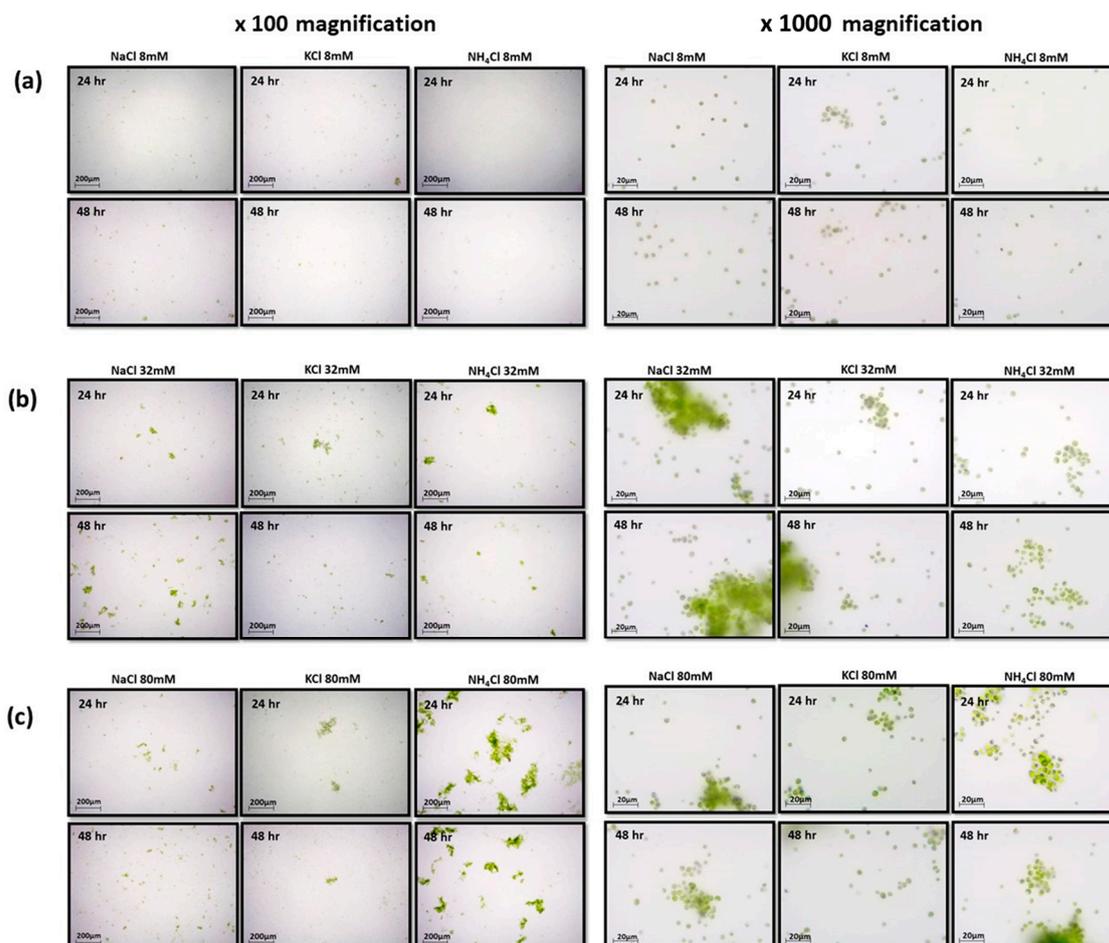**Figure 7.** Microscopic images (×1000 time magnification) of the control algal FS.

Figure 8. Microscopic images (×100 and ×1000 time magnification) of the algal FS after 48 h of exposure to different salt concentrations (NaCl, KCl and NH₄Cl): (**a**) 8 mM, (**b**) 32 mM, and (**c**) 80 mM.

For the initial concentration of 8 mM, all samples had similar algal distribution to the control with well-distributed cells (Figure 8a). The addition of low concentrations (i.e., 8 mM) for the three salts showed no clear formation of coagulation or aggregates of

cells, and no clear morphological variation was noticed for all salts. The increase in the salt concentration (e.g., 32 mM) developed more groupings of algal cells (Figure 8b). This can be an indicator of the destabilization of the algal cells' surface charge by the addition of salts which lead to the coagulation and agglomeration of algal cells. The algal aggregate formation was observed in the solutions dosed with NaCl and less in KCl and NH_4Cl, while KCl had the least grouping formation compared to NH_4Cl (Figure 8b). At the high concentrations of 80 mM, NH_4Cl showed large algal aggregate formation (Figure 8c). For NH_4Cl, it appears that there was an accelerated cell division, and this phenomenon could have caused algal cells to develop settling resistance in addition to algal cell excretions (e.g., dissolved organic matter). NaCl and KCl also showed an increase in the formation of algal aggregates compared to concentrations of 8 and 32 mM. The microscopic images with a magnification of 1000 times showed that the increase of dose to 80 mM showed a more aggregate of algal cells (Figure 8c).

4. Conclusions

During the operation of FO, *C. vulgaris* microalgae, with its high potential for biofuel production, can develop morphological and compositional alteration when subjected to RSF within a short time frame. This study simulated the RSF with the addition of different types of salt and concentrations to the algal FS. Overall, *C. vulgaris* showed a static growth phase while increasing lipid contents in algal biomass up to 14.8% in two days. The settling velocity of the algal cells was found to improve with the increase of salt content from 8 to 80 mM up to 38%. It was found that NH_4Cl can accelerate *C. vulgaris* cell division, altering the cell size distribution and reducing the settleability of the algal particles in the solution. pH was found to be within the optimal algal thriving pH (7.2–10.6), but the presence of salt stressed the microalgae, which inhibited growth and photosynthesis. Such a study can contribute to determining the future treatment of the algal product and its suitable usage. The RSF caused by DS in an FO was found to modify algal cell characteristics; future work should monitor algal cell components (e.g., protein, carbohydrates and lipid content) in FO operations, with microbial fatty acid profiles and amino acid composition analyses to determine the optimal algal use as a final product.

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Data Availability Statement: All data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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