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*Article*

# Effect of Pretreatment and Fruit Waste Medium Concentration on Biomass Concentration and Nutrient Removal of *Chlorella* sp. Microalgae

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**Abstract:** Fruit waste (FW) is a nutrient-rich but underutilized substrate with potential for sustainable microalgae cultivation. This study evaluated the effects of FW concentration (10–100%) and pretreatment on the growth, nutrient removal, and biomass composition of four *Chlorella microalgal* strains namely *Chlorella vulgaris* FSP-E, *Chlorella vulgaris* ESP-31, *Chlorella sorokiniana* CY1, and Chlorella vulgaris Beijerinck. Pretreatment strategies, including acidification, autoclaving, and non-thermal approaches, were assessed for their ability to improve nutrient availability and biomass productivity. Results showed that moderate FW concentrations (10–50%) significantly enhanced biomass accumulation and nutrient removal, whereas excessive FW (≥75%) inhibited growth and delayed nitrogen and phosphorus uptake. *C. vulgaris* ESP-31 exhibited the highest biomass at 0.4626 g/L (50% FW), while COD, TN, and TP removal reached up to 99% under optimal conditions. Comparison with literature confirmed that FW-based media can produce comparable biomass productivity. These findings underscore the feasibility of using FW for microalgae-based biorefinery applications, enabling simultaneous waste valorization, nutrient recovery, and production of value-added compounds.

**Keywords:** fruit waste; *Chlorella* sp. microalgae; biomass productivity; nutrient removal; pretreatment; biorefinery

## 1. Introduction

Waste management has become one of the most pressing global challenges driven by rapid population growth, urbanization, industrial expansion, and changing of consumption patterns. As societies continue to generate increasing volumes of waste, conventional waste handling strategies primarily disposal-oriented have proven insufficient to mitigate environmental degradation. In 2023, global waste generation reached approximately 2.1 billion tons per year, and projections indicate a continued upward trend if current practices persist [1]. Among the diverse categories of waste, fruit waste represents a substantial yet underutilized fraction, arising from agricultural production, food processing industries, wholesale markets, and household consumption [2].

Alarmingly, nearly 33% of global waste is inadequately managed, resulting in severe environmental impacts such as greenhouse gas emissions, soil contamination, leachate generation, and ecosystem disruption.

Fruit waste is particularly problematic due to its high moisture content, rapid biodegradability, and rich organic composition. When disposed of in landfills, fruit waste undergoes anaerobic decomposition, leading to the release of methane ($CH_4$) and greenhouse gas with a global warming potential significantly higher than carbon dioxide ($CO_2$). In addition, leachate produced from decomposing fruit waste can contaminate soil and groundwater, posing risks to human health and biodiversity. Despite its environmental burden, fruit waste is rich in carbohydrates, organic acids, nitrogen, phosphorus, and micronutrients, making it a promising feedstock for resource recovery. However, existing waste management systems often fail to exploit this biochemical potential, highlighting a critical disconnect between waste generation and sustainable resource utilization.

Beyond solid waste challenges, liquid waste and wastewater management further compound global environmental pressures. The continuous growth in global water demand has led to a substantial increase in wastewater generation and pollution load, with over 80% of wastewater globally, and more than 95% in some least developed countries, being released into the environment without treatment [3]. This uncontrolled release may negatively introduces excessive nutrients, pathogens, and toxic compounds into aquatic ecosystems, leading to eutrophication, oxygen depletion, and waterborne diseases. Although wastewater treatment technologies are well established, their widespread implementation remains constrained by high capital investment, operational costs, and energy demand [4]. The need for complex infrastructure, stringent regulatory compliance, and skilled labor limits access to effective treatment systems, particularly in developing regions [5]. Consequently, only a limited number of organizations can effectively manage wastewater, with the financial barrier posing a substantial impediment to more widespread adoption [6].

Within this global panorama of waste management, our research places fruit waste in the spotlight. Fruit waste forms a significant portion of the global waste stream, contributing to the underutilization of valuable organic resources. The challenge lies in curtailing the generation of fruit waste and also in harnessing it residual for beneficial purposes. Fruit waste often meets its end in landfills, thus contributing to a litany of environmental predicaments, including greenhouse gas emissions and soil contamination. The path to genuine sustainability necessitates approaches that reduce fruit waste and also align with the ambitious objectives of achieving zero waste emissions [7]. Our research paper introduces a pioneering and innovative concept in which microalgae biorefinery utilizing fruit waste as an alternative culture medium for its biomass production. This concept represents a groundbreaking approach aimed at realizing net-zero waste emission through the deployment of microalgae technology. Previous studies de Medeiros et al. [8] have shown that freshwater microalgae such as *Lagerheimia longiseta*, *Monoraphidium contortum*, and *Scenedesmus quadricauda* cultivated in low-cost fruit–vegetable biocompost media exhibited enhanced growth, antioxidant activity, and improved fatty acid and protein profiles compared to synthetic media, demonstrating their potential for value-added applications. In addition, Awathare et al. [9] have demonstrated that microalgae cultivated in fruit waste and wastewater media can achieve biomass productivity up to 117.14 mg/L/d, lipid content of 22%, and biodiesel yield of 91.50%, highlighting their strong potential as sustainable bioresource platforms.

To maximize the efficiency of microalgae bioconversion, pretreatment of fruit waste is often necessary. Raw fruit waste may contain complex polymers, inhibitory compounds, and variable nutrient composition that can limit microbial accessibility and reduce assimilation efficiency. Pretreatment strategies, such as physical, chemical, or enzymatic methods can enhance nutrient availability, reduce microbial inhibitors, and improve the overall digestibility of the substrate, thereby promoting faster growth, higher biomass accumulation, and improved production of valuable compounds. For instance, Awathare et al. [9] reported that autoclave hydrolysis of wastewater and fruit waste significantly increased the release of nutrient composition such as $NO_3^-$-N, $PO_4^{3-}$-P, and COD resulting 20.03 ± 0.05, 71.22 ± 0.19, and 528 ± 22.62 mg/L, respectively. However, sonication pretreatment is more effective to increase the COD of 664 ± 11.31 mg/L, $NO_3^-$-N of 21.50 ± 0.15 mg/L, and $PO_4^{3-}$-P of 84.94 ± 0.40 mg/L [9]. Despite these advances, systematic studies comparing pretreatment strategies and their concentration-dependent effects on different microalgae strains remain limited.

In this context, we study four types of *Chlorella* strain including *Chlorella vulgaris* FSP-E, *Chlorella vulgaris* ESP-31, *Chlorella sorokiniana* CY1, and *Chlorella vulgaris* Beijerinck microalgae due to its robustness, high growth rate, and adaptability to mixotrophic cultivation. Various pretreatment strategies, including acidification, autoclaving, and non-thermal approaches, were carried out to investigate it ability to improve nutrient availability and biomass productivity. Previous research has highlighted the ability of *Chlorella* species to efficiently assimilate organic carbon and nutrients from waste-derived media, making them suitable candidates for waste-based biorefinery applications. To-date, the systematic investigations into the use of fruit waste as a nutrient source remains limited, particularly with respect to concentration-dependent effects on the growth of *Chlorella* microalgae strain.

## 2. Material and Method

### *2.1. Microalgae Strains*

Four microalgae strains were used in this study: *Chlorella vulgaris* FSP-E, *Chlorella vulgaris* ESP-31, *Chlorella sorokiniana* CY1, and *Chlorella vulgaris* Beijerinck. All strains were isolated from freshwater sources located in southern Taiwan by Professor Dr. Jo-Shu Chang from National Cheng Kung University, Taiwan and maintained under laboratory conditions prior to experimentation.

### *2.2. Fruit Waste and Conventional Medium Preparation*

Microalgae were cultivated in a modified BG-11 medium, which contained the following components per liter: 1.5 g $NaNO_3$, 0.03 g $K_2HPO_4$, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.006 g citric acid anhydrous, and 10 mL of each of four stock solutions.

- Stock solution 1 (10 mL/L): 2 g $Na_2CO_3$ dissolved in 250 mL distilled water.
- Stock solution 2 (10 mL/L): 3.6 g $CaCl_2 \cdot 2H_2O$ dissolved in 250 mL distilled water.
- Stock solution 3 (10 mL/L): 0.6 g ferric ammonium citrate and 0.1 g EDTA dissolved in 250 mL distilled water.
- Stock solution 4 (1 mL/L): 2.86 g $H_3BO_3$, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.3 g $Na_2MoO_4 \cdot 2H_2O$, 0.07 g $CuSO_4 \cdot 5H_2O$, and 0.04 g $Co(NO_3)_2 \cdot 6H_2O$ dissolved in 250 mL distilled water.

The modified BG-11 medium served as the conventional nutrient source (i.e., control) for comparison with fruit waste-based media in all cultivation experiments. After preparation of main chemicals in 900 mL distilled water for autoclave. Add Stock solution 1, 2, 3 & 4 and top up with autoclaved distilled water to 1 L. Tropical organic fruits consist of watermelon, pineapple, orange, and mango were collected from the fruit grocery store nearby Yuan Ze University (Taiwan). The fruits were cut, blended using a kitchen sieve and blender. The composition of fruit waste of watermelon, pineapple, orange, and mango are 13%, 23%, 20%, and 44%, respectively. The ratio of distilled water was 3 L:1 kg fruit waste. Then, the Duran bottles contained filtered fruit waste solution via 150–100 mm sieve. The bottles were left for sedimentation of excess solid residual in the refrigerator at 4 °C for 3 days. After that, the bottle was filtered with vacuum filtration in 70 mm. The bottles were kept in the refrigerator at 4 °C to avoid nutrient decomposition. To prepare 250 mL of different concentrations (5%, 10%, 20% and 25%) of watermelon, pineapple, orange, and mango fruit waste mediums, each fruit waste was diluted with deionized water.

### *2.3. Cultivation Setup*

Pre-culturing was done in an in Duran bottles which working vessel containing 80% of medium, aerated with compressed air and equipped with external LED light sources on both sides of the vessel. The PBR was operated at room temperature with agitation. The light intensity on the vessel wall of the PBR was adjusted to 200 μmol/$m^2$/s using a digital lux meter (TES 1332N, Taiwan). Air was filtered (0.45 μm) and mixed with $CO_2$ to give a concentration of 2.0%. The culture broth was aerated continuously at a rate of 200 mL/min (0.2 vvm, volume gas per volume media per minute). Similar PBR operating conditions were used for the subsequent batch cultures in 1 L of medium. The experiments were conducted as single runs under controlled and consistent operational conditions. Due to experimental and resource limitations, biological replicate experiments were not performed. Therefore, statistical analysis using ANOVA was not conducted and equipment is set up as seen in Figure 1.

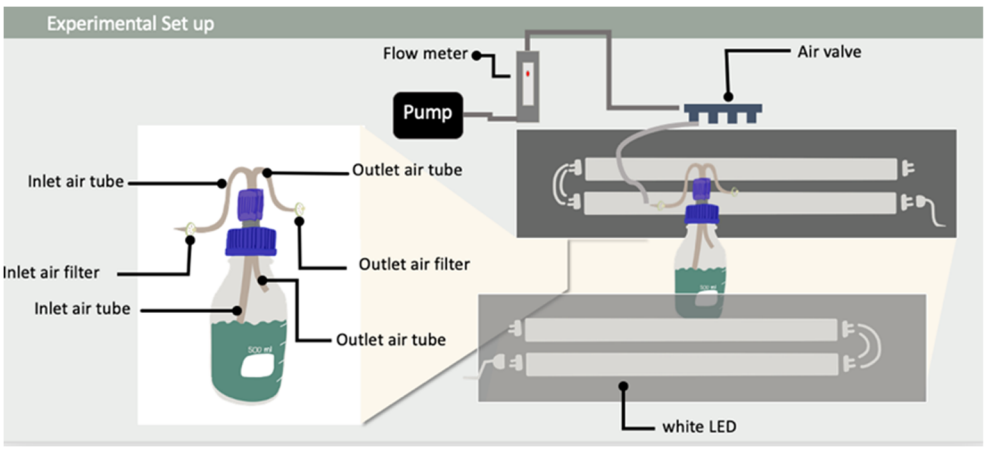


**Figure 1.** A schematic of the photobioreactor system for the cultivation of microalgae.

### *2.4. Microalgae Species and Pretreatment Selection*

Microalgae biomass was cultivated and harvested prior to the experiments. Fruit waste, at a fixed concentration of 10% (*w*/*v*), was used as the substrate as a baseline condition for initial screening of microalgae strain and pretreatment effects to maintain consistent organic load.

Two pretreatment methods were applied to fruit waste (FW):

(1) Autoclave (A): Samples were autoclaved at 121 °C for 15 min to induce thermal hydrolysis.
(2) Non-autoclave (NA): Samples were used without thermal treatment to evaluate the effect of untreated FW.

The pretreated fruit waste media were then inoculated with the microalgae, as described in Section 2.5. Biomass concentration and chemical oxygen demand (COD) removal were monitored to determine the optimal condition combination of microalgae strain and pretreatment method for microalgae performance and substrate utilization efficiency.

### *2.5. Fruit Waste Concentration*

After selecting the suitable microalgae strain and pretreatment condition (Section 2.4), this experiment was conducted to investigate the effect of fruit waste concentration on fermentation performance. To investigate the effect of substrate concentration on fermentation performance, fruit waste was prepared at different concentrations (i.e., 10%, 25%, 50%, 75%, and 100% *v*/*v*). For each test, the selected microalgae strain and pretreatment condition (determined from Section 2.3) were combined with the respective fruit waste concentration. During cultivation, parameters including biomass concentration, COD, TN, TP, and biochemical composition were monitored over time. This approach enabled assessment of how substrate availability influences microalgae growth, nutrient uptake, and biochemical composition.

### *2.6. Analysis Method*

Microalgae biomass, nutrient removal, and biochemical composition were analyzed to evaluate cultivation performance. Biomass concentration of *C. vulgaris* FSP-E, *C. vulgaris* ESP-31, *C. sorokinana* CY1, *C. vulgaris* Beijerinck were determined gravimetrically after centrifugation, washing, and drying at 105 °C, as shown in Equations (1)–(4), respectively.

$$y = 2120.2x + 0.6002, R^2 = 0.9898 \quad (1)$$

$$y = 2124x + 0.0186, R^2 = 0.9974 \quad (2)$$

$$y = 2231.5x + 0.2553, R^2 = 0.9947 \quad (3)$$

$$y = 2544.1x - 0.1147, R^2 = 0.9946 \quad (4)$$

where $y$ is absorbance and $x$ is biomass concentration (g/L). These calibration equations were then used to convert measured absorbance values into biomass concentrations for each microalgae strain.

#### 2.6.1. Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is performed to ascertain the chemical oxygen demand in a sample. Depending on the sample's characteristics, an appropriate reagent concentration is selected. In a vial, 2 mL of pure water is added at a 45-degree angle to serve as the blank sample (or 0.2 mL when employing a 15,000 ppm COD vial). Another vial receives 2 mL of the sample (or 0.2 mL for a 15,000 ppm COD vial) at the same angle. The vials are then tightly capped and gently shaken to ensure even mixing, resulting in a rise in temperature. Subsequently, the vials are placed into the COD reactor (CR 25), and the COD program is initiated, heating the vials to 150 °C for a duration of 2 h. After digestion, the reactor is switched off, and the vials are allowed to cool to room temperature. They are then removed, cleaned, and examined to ensure proper mixing and precipitation. The WD 100 colorimeter is powered on, and reagent brand and concentration range are selected. The blank sample vial is inserted for zero calibration, followed by the test sample vial for analysis, resulting in the determination of the COD concentration.

#### 2.6.2. Total Nitrogen

Total Nitrogen (TN) Test *i* to determine the concentration of total nitrogen in a given sample. To begin, the CR 25 reactor is activated and the TN program initiated to preheat to 105 °C. An appropriate reagent concentration

is selected according to the nitrogen content of the sample. Each vial is loaded with a Total Nitrogen Persulfate powder pillow, and pure water is added to one vial to serve as the blank sample, with specific volumes varying depending on the level of precision required. The test sample is added to another reagent vial, again with volumes adjusted as per precision requirements. After closing and vigorously shaking the vials for at least 30 s, they are placed into the CR 25 reactor for precisely 30 min at 105 °C. Following this step, Total Nitrogen Reagent A power pillow is introduced into each vial, followed by a second round of vigorous shaking and a subsequent 3-min wait. Total Nitrogen Reagent B power pillow is then added, with further shaking and a 2-min stand. A 2 mL portion of the prepared sample is transferred to a new Total N-LR or Total N-HR vial using a pipette, inverted ten times, and allowed to stand for 5 min. The sample is then cleaned, and the WD 100 colorimeter is powered on to select the test item and concentration range. A zero calibration is performed with the blank sample vial before analyzing the test sample vial to obtain TN concentration.

#### 2.6.3. Total Phosphorus

Total Phosphorus (TP) Test aims to determine the concentration of total phosphorus in a given sample. The CR 25 reactor is activated and the TP program started to preheat to 150 °C. An appropriate reagent concentration is chosen based on the sample's phosphate content. For the blank sample, 5 mL of pure water is added to a reagent vial, while the test sample receives 5 mL of the sample itself. Each vial is then furnished with a Potassium Powder pillow and thoroughly shaken to dissolve the powder. After this, the vials are placed into the CR 25 reactor, where they remain for exactly 30 min at 150 °C. Upon removal, 2 mL of 1.5 N Sodium Hydroxide Standard Solution is introduced to each vial, followed by 0.5 mL of Molybdovanadate reagent. A 7-min reaction period ensues, during which the sample is measured between the 7th and 9th min after adding the Molybdovanadate reagent. The vials are then cleaned, and the WD 100 colorimeter is powered on, with reagent brand and concentration range selection. A zero calibration is conducted using the blank sample vial, followed by analysis of the test sample vial to determine the TP concentration.

## 3. Result and Discussion

### *3.1. Effect of Pretreatment and Selection of Microalgae Species on 10% Fruit Waste as Nutrient Source*

In this study, the effects of pretreatment and species selection on carbohydrate release varied markedly among the tested microalgae, as shown in Figure 2. The biomass concentration of the tested microalgae varied significantly depending on the strain and pretreatment applied. *C. vulgaris* ESP-31 consistently showed the highest biomass accumulation under control conditions, reaching 0.88 g/L, suggesting strong inherent growth and solubilization without pretreatment. *C. vulgaris* FSP-E and *C. vulgaris* Beijerinck exhibited moderate biomass accumulation under control (0.75 and 0.35 g/L, respectively), but their biomass was enhanced by pretreatment, such as non-autoclave (NA) treatment increased *C. vulgaris* FSP-E to 0.57 g/L, and *C. vulgaris* Beijerinck reached 0.70 g/L, while autoclave (A) treatment showed a slight improvement for *C. vulgaris* Beijerinck (0.62 g/L) but reduced biomass for *C. vulgaris* FSP-E (0.30 g/L). In contrast, *C. sorokiniana* CY-1 showed limited biomass increase under all pretreatments, with maximum concentrations 0.72 g/L, indicating that this strain is less responsive to thermal or chemical pretreatments. These trends highlight that biomass accumulation is strain-dependent and that pretreatment effectiveness varies, *C. vulgaris* ESP-31 maintains high growth without pretreatment, while *C. vulgaris* FSP-E and *C. vulgaris* Beijerinck benefit from tailored treatments to enhance substrate availability and fermentable biomass, which are crucial for downstream biohydrogen production. These findings are consistent with previous studies reporting that thermal or chemical pretreatments can improve biomass solubilization and carbohydrate availability, enhancing fermentable substrate yield [10].

The COD removal efficiency varied slightly among the tested microalgae and pretreatment methods. *C. vulgaris* ESP-31 exhibited the highest COD removal, reaching 82.6% under non-autoclave treatment and 82.1% under autoclave, indicating its strong capacity for organic matter degradation regardless of thermal treatment. *C. vulgaris* FSP-E and *C. sorokiniana* CY-1 showed moderate COD removal, with FSP-E achieving 71.7% (autoclave) and 74.1% (non-autoclave), while CY-1 reached 79.6% (autoclave) and 82.8% (non-autoclave). *C. vulgaris* Beijerinck maintained high COD removal (81.5% for autoclave and 81.5% for non-autoclave), suggesting that this strain efficiently utilizes organic substrates even without thermal pretreatment. Overall, non-autoclave pretreatment slightly improved COD removal for most strains, especially FSP-E and CY-1, likely due to enhanced substrate accessibility.

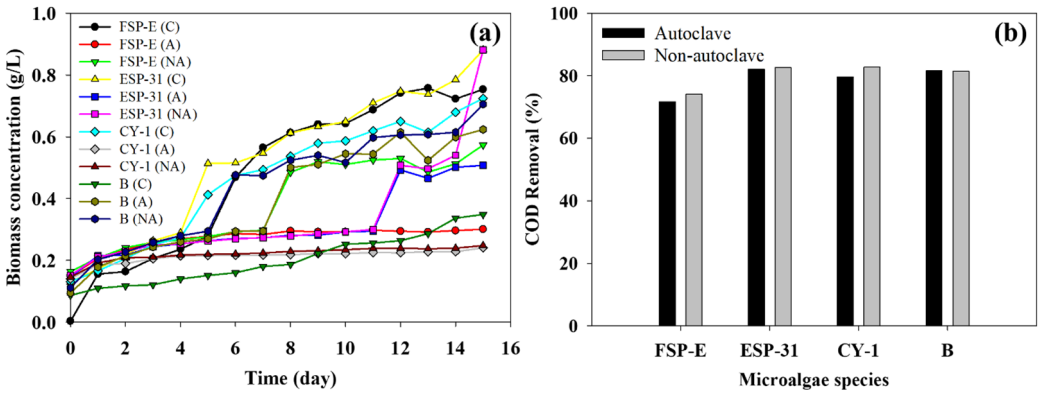


**Figure 2.** Growth kinetics and pollutant removal efficiency of various microalgae species with (**a**) Biomass concentration (g/L) and (**b**) Chemical Oxygen Demand (COD) removal percentage.

### *3.2. Effect of Fruit Waste Concentration as Nutrient Source for Chlorella Vulgaris ESP-31*

Figure 3 illustrates the effect of varying fruit waste concentrations on the growth performance and nutrient utilization of *C. vulgaris* ESP-31. The results clearly demonstrate that fruit waste can serve as an effective nutrient source, significantly enhancing microalgae performance compared to the control without fruit waste supplementation.

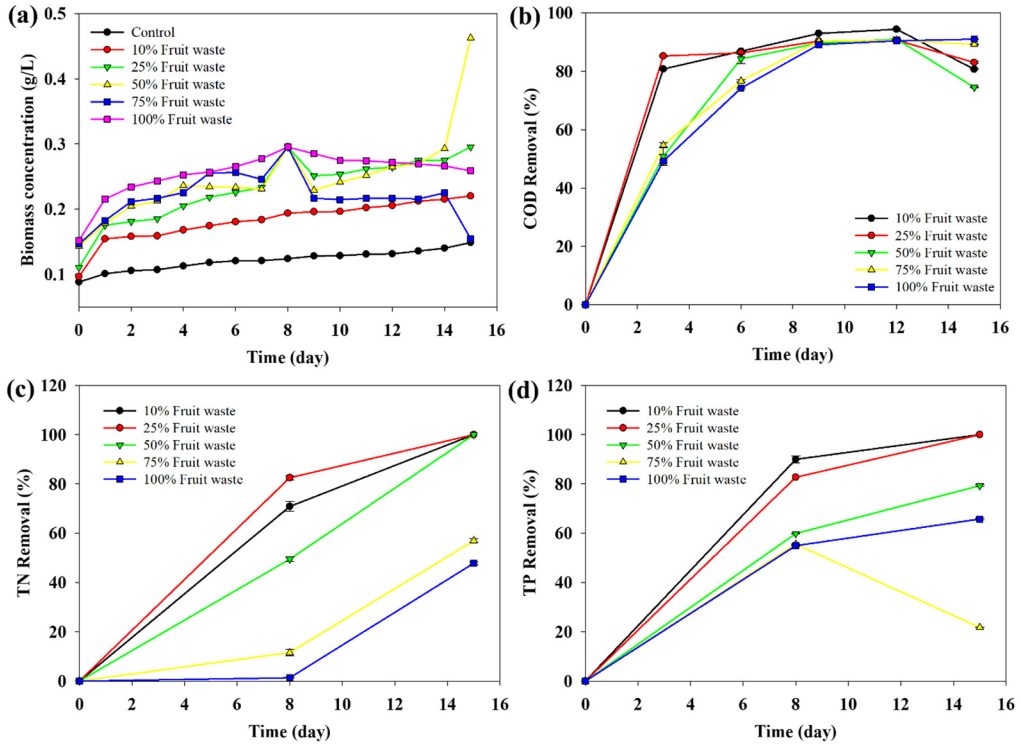


**Figure 3.** Growth kinetics of microalgae (**a**) and the removal efficiency of COD (**b**), TN (**c**), and TP (**d**) under different concentrations of fruit waste over a 15-day cultivation period.

Figure 3a–d demonstrate that fruit waste (FW) concentration strongly influenced the growth performance, nutrient removal, substrate utilization, and biomass conversion efficiency of *C. vulgaris* ESP-31. According to Figure 3a, moderate FW supplementation (10–50%) significantly enhanced biomass accumulation compared to the control, with the highest biomass observed at 50% FW reaching 0.4626 g/L. Lower FW concentrations (10–25%) achieved moderate increases (0.2202–0.2954 g/L), while excessive FW loadings (75–100%) led to growth inhibition, with biomass decreasing to 0.1545 g/L (75% FW) and 0.2588 g/L (100% FW) by day 15. This pattern suggests that moderate FW provides optimal nutrient availability for mixotrophic growth, whereas high organic loading induces light limitation, metabolic imbalance, and competition with heterotrophic microorganisms. These findings are consistent with previous studies by Yadav et al. [11] reported that *Chlorella sorokiniana* achieved a high biomass concentration of 3.16 g/L under optimal FW composition, specifically 2.8 mg/L for orange peels, 35.5 mg/L for papaya peels, and 35.5 mg/L for pumpkin peels, highlighting the critical role of substrate concentration

in maximizing microalgae growth. Similarly, other studies have shown that cultivation of *C. vulgaris* using a single type of fruit waste, such as digested rotten potato supernatant, reached a cell density of $192.83 \pm 1.75 \times 10^5$ cells on 20% fruit waste medium [12]. However, the protein content in this case was only 39.43 ± 1.67%, which is considerably lower compared to cultures grown in standard Bold's Basal Medium (BBM), suggesting that while fruit waste can effectively support biomass growth, nutrient composition may be insufficient for optimizing biochemical quality.

In addition, COD removal (Figure 3b) was most efficient at 25% FW, reaching 92% at the end of cultivation (Day 12), while the lowest removal occurred at 75% FW during the early stage (Day 3, 49%). This indicates that moderate FW supplementation promotes rapid and stable organic carbon assimilation by *Chlorella vulgaris* ESP-31, whereas excessive FW can hinder efficient substrate utilization. Similar trends have been observed in other studies, Condori et al. [13] reported that *Chlorella* sp. achieved the highest removal efficiencies for COD (89.07 ± 0.31%), $PO_4^{3-}$ (93.82 ± 0.61%), and $SO_4^{2-}$ (76.01 ± 0.68%), with significant differences depending on the culture medium, such as CFR–10% $H_2O$ or CFR–10% MBM. These findings collectively emphasize that careful optimization of substrate concentration is crucial for maximizing both biomass growth and nutrient removal efficiency.

For TN removal as shown in Figure 3c, the highest efficiency was achieved at 10–50% FW by the end of cultivation (Day 12, 100%), while the lowest mid-stage removal (Day 8) occurred at 100% FW (1.33%), reflecting delayed nutrient uptake under excessive organic loading. Similarly, TP removal as shown in Figure 3d reached the highest efficiency at 10–25% FW (100%) and was lowest at 75% FW (21.76%), suggesting that excessive FW disrupts phosphorus assimilation and metabolic balance. Overall, these results indicate that moderate FW supplementation (10–50%) maximizes biomass productivity, COD, TN, and TP removal efficiencies, whereas high FW concentrations (≥75%) induce growth inhibition, delayed nutrient uptake, and incomplete substrate conversion, emphasizing the importance of optimizing FW concentration for efficient microalgae cultivation. The growth inhibition at high FW concentrations may be attributed to excessive organic loading leading to light limitation, metabolic imbalance, or competition with heterotrophic microorganisms, although detailed mechanistic studies are limited and require further investigation. Strain-dependent responses suggest that inherent metabolic capabilities and substrate assimilation efficiencies differ among microalgae species.

### *3.3. Comparison Study*

These studies collectively indicate that fruit and vegetable wastes are versatile nutrient sources for microalgae, although biomass yield, productivity, and biochemical composition vary depending on the substrate type, pretreatment, and microalgal strain. In general, hydrolysate or juice-based substrates tend to enhance nutrient availability, supporting higher growth and metabolite accumulation compared to raw or minimally processed wastes. This comparison highlights the importance of optimizing both the waste substrate and cultivation conditions to achieve maximum biomass and bioactive compound production. The results of the present study can be compared with previous reports on microalgal cultivation using fruit and vegetable waste as nutrient sources as shown in Table 1.

**Table 1.** Comparison of microalgae cultivation on various fruit and vegetable waste substrates.

| Nutrient Source | Microalgae Species | Biomass Concentration and Productivity | Specific Growth Rate ($Day^{-1}$) | Removal Efficiency | Remarks | References |
|---|---|---|---|---|---|---|
| Orange peel | *E. gracilis* LIMS-1351 | 12.0 g/L and 3.3 g/L/day | | - | Highest beta-glucan production of 56% | [14] |
| 1 % potato juice | *Chlorella* sp. | - | 0.45 | - | Algal biomass maintains amino-acid, pigment, and fatty-acid profiles | [15] |
| Waste onion peels | *Chlorella vulgaris* (USM 101) | 1.76 g/L | - | - | Biomass achieved with low inoculum concentration | [16] |
| Tomato waste hydrolysate | *I. galbana* | 2.13 g/L biomass | - | - | Enhances fucoxanthin content up to 21.02 mg/g | [17] |
| Potato peel waste hydrolysate | *Spirulina* sp. | 1506 mg/L and 59.84 mg/L/d | - | - | Supports rapid growth and pigment accumulation | [18] |
| 10% Fruit waste | *C. vulgaris* FSP-E | 0.30 g/L | - | 71.7% | Moderate biomass yield; suitable for low-concentration FW | Present study |
| 50% Fruit waste | *C. vulgaris* ESP-31 | 0.4626 g/L | - | 74.49% | Highest biomass in FW series; optimal nutrient assimilation | Present study |
| 10% Fruit waste | *C. sorokinana* CY1, | 0.72 g/L | - | 79.6% | Enhanced growth and nutrient removal at low FW concentration | Present study |
| 10% Fruit waste | *C. vulgaris* Beijerinck | 0.62 g/L | - | 81.5% | High nutrient removal efficiency; stable biomass production | Present study |

Note: "-" indicates data not reported (NR) in the original study.

## 4. Conclusions

This study demonstrates the potential of fruit waste as an effective and sustainable nutrient source for microalgae cultivation. Moderate fruit waste concentrations (10–50%) were found to maximize biomass production and nutrient removal, while excessive FW (≥75%) resulted in growth inhibition and delayed nitrogen and phosphorus uptake. Among the strains tested, *C. vulgaris* ESP-31 exhibited the highest biomass concentration (0.4626 g/L at 50% FW), whereas *C. sorokiniana* CY1 and *C. vulgaris* Beijerinck showed strong nutrient removal efficiency at lower FW concentrations (10%). Pretreatment strategies, particularly acidification and non-thermal methods, improved substrate accessibility and reduced microbial contamination, contributing to enhanced biomass accumulation and biochemical quality. When compared with other fruit and vegetable wastes reported in the literature, fruit waste-supported cultivation achieved comparable or superior biomass and metabolite yields, highlighting its versatility and practical applicability. These findings underscore the feasibility of fruit waste and microalgae biorefineries as a circular bioeconomy strategy, enabling simultaneous waste valorization, nutrient recovery, and production of high-value biomass and bioactive compounds, with promising potential for scale-up and industrial implementation.

## Author Contributions

C.S.: writing—original draft preparation, investigation, visualization, methodology, formal analysis, validation; A.A.R.: writing—reviewing and editing, investigation, visualization, methodology, formal analysis, validation; J.C.-W.L.: writing—reviewing and editing, investigation, validation; S.S.: writing—reviewing and editing, investigation, validation; K.S.K.: conceptualization, writing—reviewing and editing, validation, investigation, project administration, funding acquisition, supervision. All authors have read and agreed to the published version of the manuscript.

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## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

Not applicable.

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We would also like to acknowledge and attribute the graphic elements and design resources used for the Figures, which were created using Canva (https://www.canva.com/).

## Conflicts of Interest

The authors declare no conflict of interest.

## Use of AI and AI-Assisted Technologies

During the preparation of this manuscript, ChatGPT (OpenAI) was used solely for language editing and proofreading to improve clarity and grammar. The authors reviewed and verified all content and take full responsibility for the accuracy and integrity of the manuscript.

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