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Research article

Recycling air conditioner-generated condensate water for microalgal biomass production and carbon dioxide sequestration☆

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ABSTRACT

Air conditioners alleviate the discomfort of human beings from heat waves that are consequences of climate change caused by anthropogenic activities. With each passing year, the effects of global warming worsen, increasing the growth of air conditioning industry. Air conditioning units produce substantial amounts of non-nutritive and (generally) neglected condensate water and greenhouse gases. Considering this, the study explored the potential of using air conditioner condensate water (ACW) to cultivate *Chlorella sorokiniana*, producing biomass, and sequestering carbon dioxide (CO_2). The maximum biomass production was obtained in the BG11 medium (1.45 g L^{-1}), followed by ACW-50 (1.3 g L^{-1}). Similarly, the highest chlorophyll-*a* content was observed in the BG11 medium (11 μg mL^{-1}), followed by ACW-50 (9.11 μg mL^{-1}). The ACW-50 cultures proved to be better adapted to physiological stress ($F_v/F_m > 0.5$) and can be suitable for achieving maximum biomass with adequate lipid, protein, and carbohydrate production. Moreover, *C. sorokiniana* demonstrated higher lipid and carbohydrate yields in the ACW-50 medium, while biomass production and protein yields were comparable to the BG11 medium. The lipid, protein, and carbohydrate productivity were 23.43, 32.9, and 23.19 mg L^{-1} d^{-1}, respectively for ACW-50. Estimation of carbon capture potential through this approach equals to 9.5% of the total emissions which is an added advantage The results indicated that ACW could be effectively utilized for microalgae cultivation, reducing the reliance on freshwater for large-scale microalgal biomass production and reduce the carbon footprints of the air conditioning industry.

1. Introduction

The use of air conditioning systems have increased worldwide mainly because of the intense heat due to the effects of global warming/climate change. Other reasons include an increase in disposable income and individuals' desire for comfort (Biardeau et al., 2020; Muzhanje et al., 2022). Whilst air conditioners ease societies' living and working space conditions, they are a source of greenhouse gas (GHG) emissions, producing various gases such as carbon dioxide (CO_2), nitrous oxide, methane, fluorinated GHGs, etc. Air conditioning units also consume approximately 20 times more electricity than ceiling fans on average, accounting for ~10–20% of the world's energy demand (D'Agostino et al., 2020; Myat, 2022; Sovacool et al., 2021; Castro et al., 2021).

Condensate water is produced as a byproduct of air conditioners. Recovered condensate water has a neutral pH range, low salinity and conductivity, and it contains trace amounts of nutrients In general, the quantity of condensate water amounts to approximately 3.5–4.5 L h^{-1} in a 2-ton air conditioner, equating to around 25 L Day^{-1} (Algarni et al., 2018; Abdullah and Mursalin, 2021). The water is usually considered negligible and is disregarded, often left to be released into the surrounding environment. The condensate water is subsequently evaporated and lost to the atmosphere. Occasionally, the water is repurposed for non-potable applications, such as industrial and domestic use, irrigation, toilet flushing, etc. (Moosa et al., 2020; Hosseinkhani and Kargari, 2022; Algarni et al., 2018). Hence, the condensate water may be recycled for microalgal cultivation instead of being left unattended.

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Microalgae are a diverse group of mostly aquatic microorganisms. They are photoautotrophic, utilizing freely available sunlight and atmospheric CO_2 to grow, while releasing oxygen (Costa et al., 2019; Molinuevo-Salces et al., 2019). In addition, microalgae have short generation times, have high photosynthetic efficiency, and require simple, inorganic, macro- and micronutrients for their growth (Costa et al., 2018; Zhou et al., 2022). These benefits of microalgae make it a suitable biomass for sustainable, renewable and eco-friendly usage.

Microalgae can also grow in various water types, including freshwater, seawater, brackish water and wastewater. Microalgal cultivation in wastewater streams is of special interest as it not only contributes to bioremediation, but also reduces the requirement for valuable freshwater reserves (Chew et al., 2018; Do et al., 2019; Koyande et al., 2019; Srimongkol et al., 2022). Algal cultivation have a large requirement for water using 4.59–6.39 m^3 of water per year for every 1 m^2 of cultivation land (Bhandari and Prajapati, 2022), and 2.4–6.8 m^3 of water is used to produce 1 kg of algal biomass using a photobioreactor (Martins et al., 2018).

Microalgae sequestrate an estimated 1.83 tons of CO_2 from the environment for every ton of biomass produced (Chisti, 2007). This supports efforts to achieve sustainable development goals (SDGs) 11 and 13, namely 'Sustainable cities and communities' and 'Climate action' respectively, through water sustainability promotion as well as CO_2 mitigation. The total carbon (C) produced from a 1.5-ton air conditioner is about 5.7 kg day^{-1} (Kumar et al., 2013). Therefore, recovering water generated by air conditioning for microalgal cultivation can lead to a circular and integrated process for C capture, where the CO_2 emissions from the air conditioning unit are offset by the CO_2 consumed during algal cultivation. This approach ensures a partial 'C neutral' process that effectively captures C. Moreover, utilizing air condition-generated condensate water for microalgal cultivation capitalizes on conserving depleting freshwater resources (Algarni et al., 2018; de Mendonça et al., 2021). The produced biomass can be further used (either in part or whole) for several applications in the production of food, feed, biofuels, high-value compounds, biofertilizers, nutraceuticals, pharmaceuticals, cosmetics and other bio-based products (Garrido-Cardenas et al., 2018; Zhou et al., 2022; de Mendonça et al., 2021).

To establish an economically viable and environmentally sustainable method, this study examined the effectiveness of using air condition condensate water (ACW) in conjunction with BG11 growth media at different ratios to cultivate C. *sorokiniana*. The algal growth kinetics, physiology, biochemical composition, pigment contents, and maximum CO_2 sequestration ability were analyzed. The feasibility of using air conditioning-generated water for microalgae cultivation and subsequent CO_2 sequestration, and the potential applications of the resultant biomass were also assessed to verify the practicality and acceptability of ACW. To the best of the author's knowledge, there have been no previous reports on the using of air condition-generated condensate water for microalgal biomass production and CO_2 sequestration.

2. Materials and methods

2.1. Microalgae cultivation

The microalgae strain, *Chlorella sorokiniana* was isolated from the Durban region of KwaZulu Natal, South Africa, and purified by subsequent sub-culturing using the streak plate method (Ramanna et al., 2014). Seed culture was prepared by inoculating BG11 medium with *C. sorokiniana* in 1 L flasks. Cultures were maintained at 22 ± 2 °C under 80 μmol photons m^{-2} s^{-1} irradiance using Sylvania Gro-Lux lamps (Gupta et al., 2016), with a 16/08 h light/dark cycle. The cultures were routinely shaken to avoid algal clumping and for circulation of nutrients. The pH of the culture medium was adjusted to 7 using either H_2SO_4 or NaOH.

2.2. Air conditioning water collection and physiochemical characterization

The ACW was collected in 25 L containers from the residential areas located in the city of Durban, South Africa. The pH, temperature, DO, salinity, and conductivity was recorded using a YSI 556 MPS (Yellow Spring Instruments Co.; USA), (Table 1). Nitrate, nitrite, and phosphate concentrations were determined based on standard methods using a Gallery™ Automated Photometric Autoanalyzer (Thermo Scientific™, Germany). Total dissolved solids were also determined using the standard method (APHA, 2005).

2.3. Experimental details

Different concentrations of ACW (*v/v*) were blended with BG11 medium for the cultivation of *C. sorokiniana i.e.*, ACW-25 (ACW-25% + BG11-75%), ACW-50 (ACW-50% + BG11-50%), ACW-75 (ACW-75% + BG11-25%), ACW-100 (ACW-100% + BG11-0 %). Cultures grown in BG11 (ACW-0% + BG11-100%) were used as a positive control. All experiments were performed in 1L flasks with a 500 mL working volume. Freshly grown *C. sorokiniana* with an optical density of 2.5 at 680 nm was used to inoculate the flasks. The study was conducted for 15 days, during which 5 mL of the culture from each flask was collected every other day for analysis. After 15 days of the cultivation period, biomass was harvested by centrifuging at 3000 rpm for 10 min. The wet algae biomass was dried at 60 °C for 5 h in a hot air oven and powdered using a mortal pestle. The dried powdered biomass was stored in a desiccator for further analysis. Experimentation was carried out in triplicate and represented by the mean value with the standard deviation.

2.4. C. sorokiniana growth, biomass, and CHNS analyses

The biomass concentrations were determined spectrophotometrically (Spectroquant Pharo 300, Merck) at a wavelength of 680 nm. Dry cell weight (DCW) was determined by filtering the algal samples using a pre-dried and pre-weighed 25 mm Whatman filter paper and drying them in the oven at 105 °C overnight. The dried samples were placed in a desiccator and weighed to determine DCW. The biomass productivity was calculated using equation (1).

$$\text{Biomass productivity } \left(\text{mg L}^{-1}\text{d}^{-1}\right) = \frac{\textit{Biomass yield } \left(\text{mg L}^{-1}\right)}{\textit{Number of days}} \quad (1)$$

The carbon (C), hydrogen (H), and nitrogen (N) contents of microalgal biomass were quantified using a CHNS analyzer (PerkinElmer 2400, USA).

2.5. Physiological analysis of C. sorokiniana

Non-invasive Pulse Amplitude Modulated (PAM) fluorescence measurements were conducted to determine the overall photosynthetic

Table 1
Physiochemical characteristics of ACW.

Parameters	Value
pH	7.40 ± 0.35
Color	Clear
Odor	Odorless
Temperature (°C)	22.91 ± 1.2
Electrical conductivity (μS·cm^{-1})	34.00 ± 2.13
Salinity (mg·L^{-1})	0.01 ± 0
Total dissolved solid (mg·L^{-1})	10.21 ± 1. 31
Dissolved oxygen (mg L^{-1})	2.34 ± 0.21
NO_3^- (mg·L^{-1})	6.76 ± 1.21
NO_2^- (mg·L^{-1})	0
PO_4^{3-} (mg·L^{-1})	5.64 ± 0.43

performance of microalgae grown in ACW-25, ACW-50, ACW-75, ACW-100, and BG11 medium. The PHYTO-PAM fluorometer was used to detect changes in the P680 (photosystem II [PSII]) chlorophyll fluorescence (PAM-II/ED Compact Version, Heinz Walz Gmbh, Effeltrich, Germany). The PHYTO-PAM-II, with integrated emitter-detector, features five wavelengths of fluorescence excitation and actinic illumination (440, 480, 540, 590, and 625 nm) and four algal groups. The obtained fluorescence signals were de-convoluted from the five diode signals based on the microalgal-specific reference spectra (Chlorophyte).

Briefly, 2 mL samples were removed and diluted until constant turbidity was reached (0.1 at 750 nm). These were allowed to dark acclimate for 20–30 min. Samples were transferred to a quartz cuvette, and rapid light curves (RLCs) were generated by measuring the relative electron transport responses to eleven irradiances (PAR 103, 86, 93, 103, 113, 123, 140, 160, 184, 211, and 247 μmol photons m^{-2} s^{-1}) for 20 s using the Saturating Pulse (SP) method. Based on the RLCs, data was plotted at 160 μmol photons $m^{-2}s^{-1}$. Fluorescence parameters were calculated automatically by the PhytoWin-3 Software (Windows 7/8/10) (Heinz Walz Gmbh, Effeltrich, Germany). The quantum efficiency (F_v/F_m) was calculated using equation (2).

$$F_v \Big/ F_m = \frac{(F_m - F_0)}{F_m} \tag{2}$$

Where F_v, F_m, and F_0 represent variable, maximum, and minimum fluorescence after dark adaptation, respectively. The SP induced the Fm value. This ensured multiple turnovers with a transient closure of the photosynthetic reaction centers (RCs). Consequently, F_m is related to a state of fully closed PSII RCs (Nama et al., 2018; Yadav et al., 2020).

2.6. Chlorophyll-a analysis

For the extraction and estimation of chlorophyll, the standard procedure as described by Bhandari and Prajapati (2022). In brief, algal samples consisting of 1 mL were centrifuged at 4436×*g* for 5 min before the pellets were retained. Next, the pellet was resuspended in 1 mL methanol and then incubated in a water bath at 60 °C for 30 min. After that, the sample was left standing to gradually cool to room temperature. Lastly, optical density measurements were recorded at 652, 665.2, 670, and 750 nm for the samples. The total Chlorophyll-*a* content was calculated using equation (3).

$$\text{Chlorophyll} - \text{a}\ \left(\mu\text{g mL}^{-1}\right) = 16.29\ (A_{665.2} - A_{750}) - 8.54(A_{652} - A_{670}) \tag{3}$$

Where, A_{652}, $A_{665.2}$, and A_{670}, A_{750} represented the absorbance at 652, 665.2, 670 and 750 nm, respectively.

2.7. Biochemical analyses

The microalgal cultures were centrifugated to obtain concentrated biomass after 15 days of cultivation. The biomass was then dried in an oven and ground into a powder using a mortar and pestle for lipid extraction. The extraction was carried out using a microwave-assisted technique. Briefly, 0.02 g of dried biomass was mixed with 20 mL of chloroform and methanol mixture (2:1 ratio *v/v*) and heated at 100 °C for 10 min at 1000 W in a microwave digester. The lipid yields were determined gravimetrically (Guldhe et al., 2017), while the lipid productivity was calculated according to equation (4).

$$\text{Lipid productivity}\ \left(\text{mg L}^{-1}\ \text{d}^{-1}\right) = \text{Biomass productivity}\ \left(\text{mg L}^{-1}\ \text{d}^{-1}\right) \times \frac{\textit{Lipid content}\ (\%)}{100} \tag{4}$$

The total carbohydrates of algae biomass were quantified using phenol-sulfuric acid (Dubois et al., 1956). In brief dried algae biomass was mixed with sulfuric acid (2% *v/v*) and autoclaved for 30 min at 121 °C (Ansari et al., 2017). The absorbance of this solution was measured at 490 nm using a spectrophotometer. Total carbohydrates were quantified using a calibration curve prepared by using glucose as a standard. Carbohydrate productivity was determined by equation (5).

$$\text{Carbohydrate productivity}\ \left(\text{mg L}^{-1}\ \text{d}^{-1}\right) = \text{Biomass productivity}\ \left(\text{mg L}^{-1}\ \text{d}^{-1}\right) \times \frac{\textit{Carbohydrate}\ (\%)}{100} \tag{5}$$

Protein analysis in microalgal biomass was done following the protocol of Ansari et al. (2015), and the percentage yield of proteins was determined as per Lowry's method. The spectrophotometer was used to measure the absorbance at 750 nm. Bovine serum albumin (BSA) was used to prepare the calibration and protein quantification standards. The protein productivity was calculated using equation (6).

$$\text{Protein productivity}\ \left(\text{mg L}^{-1}\ \text{d}^{-1}\right) = \text{Biomass productivity}\ \left(\text{mg L}^{-1}\ \text{d}^{-1}\right) \times \frac{\textit{Protein}\ (\%)}{100} \tag{6}$$

2.8. Statistical analysis

The experiment was carried out in triplicate, and the data are reported as mean values with standard deviations (mean ± SD, n ≥ 3) or with error bars in all graphs.

3. Results and discussion

3.1. C. sorokiniana cultivation in ACW and BG11 added media and effect on chlorophyll-a

The results for biomass production in ACW and BG11 supplemented medium are depicted in Fig. 1. The cultivation of *C. sorokiniana* in BG11 medium (control) showed a biomass production of 1.45 g L^{-1}. In contrast, the biomass production in ACW-25, ACW-50, ACW-75, and ACW-100 media were 1.15, 1.3, 0.54, and 0.43 g L^{-1}, respectively. The highest biomass production in BG11 was due to its optimal nutrient concentrations (Guldhe et al., 2017), which were lacking in ACW. Matos et al. (2021) found similar results when *Spirulina platensis* was cultivated in inland desalination concentrate (DC), and they also found the lowest biomass production in 100% DC. Table 1 shows that ACW only contained trace amounts of N and P which resulted in the lowest biomass

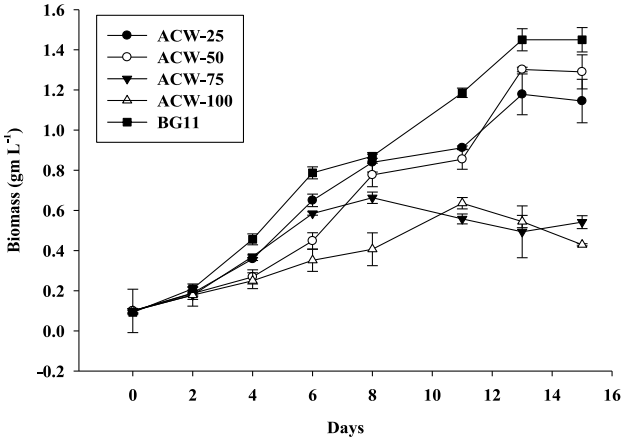

Fig. 1. Biomass concentration (g L^{-1}) of *C. sorokiniana* grown in ACW-25, ACW-50, ACW-75, ACW-100 and BG11 media. Each data point is represented by the mean value (n ≥ 3) with the standard deviation as error bars.

production in ACW-100 among all tested sets. However, ACW-50 produced a biomass yield (1.3 g L^{-1}) was comparable to that of BG11 (1.45 g L^{-1}) as shown in Fig. 1. Similarly, Bhandari and Prajapati (2022), observed that 1.25 g L^{-1} of biomass production in *C. pyrenoidosa* when cultivated in 50% RORI which was slightly higher than BG11 growth medium (1.29 gL^{-1}). The findings demonstrate that the best mixing combination for higher microalgal biomass production is 50% inclusion of BG11 medium in ACW. In terms of biomass productivity *C. sorokiniana* showed the highest biomass productivity of 96.67 mg L^{-1} d^{-1} when cultivated in BG11 medium. This was followed by ACW-50, ACW-25, ACW-75, and ACW-100 which were 86.0, 76.33, 36.11 and 28.6 mg L^{-1} d^{-1}, respectively). The results showed that ACW-50 as suitable growth medium combination, beyond which the biomass productivity decreases (Fig. 5). Gao et al. (2016), observed 42.6 mg L^{-1} d^{-1} of biomass productivity when cultivating *C. vulgaris* in aquaculture wastewater which was lower than the BG11, ACW-25, and ACW-50 growth media. Hence, the current results reinforce the idea that the growth and productivity of microalgal biomass are reliant on the growth conditions and nutrient availability supplied.

Fig. 2 depicts the variation of chlorophyll-*a* content over the 15-day cultivation period. The chlorophyll-*a* content aligned with biomass production in ACW-25, -50, −75, −100, and BG11 media (Fig. 1). The highest chlorophyll-*a* content of 10.97 ± 0.28 μg mL^{-1} was obtained in BG11 medium, followed by ACW-50 (9.11 ± 0.21 μg mL^{-1}), (day 11). Cultures grown in ACW-100 medium exhibited the lowest chlorophyll-*a* content (1.45 ± 0.25 μg mL^{-1}). As the experiment reached the late log phase, the chlorophyll-*a* content decreased in all growth media. Chlorophyll-*a* relies on N and P availability, and a higher concentration of N promotes biomass production through photosynthesis. Thus, a higher N medium results in increased chlorophyll-*a* content. Conversely, media deficient in N and P can cause a reduction in chlorophyll pigments, which leading to a decrease in photosynthesis efficiency (Bhandari and Prajapati, 2022; Huang et al., 2021). The decrease in chlorophyll-*a* observed in this study was likely due to the lower concentration of N and P in the growth medium.

3.2. Microalgal physiology

At the start of experimentation, F_v/F_m values for all cultures were high (±0.6), (Fig. 3). The highest F_v/F_m ratios were ±0.8 estimated on day 2. All algal cultures showed a similar trend in quantum efficiencies. Increased F_v/F_m values from approximately 0.6 to 0.8 between day 0–8,

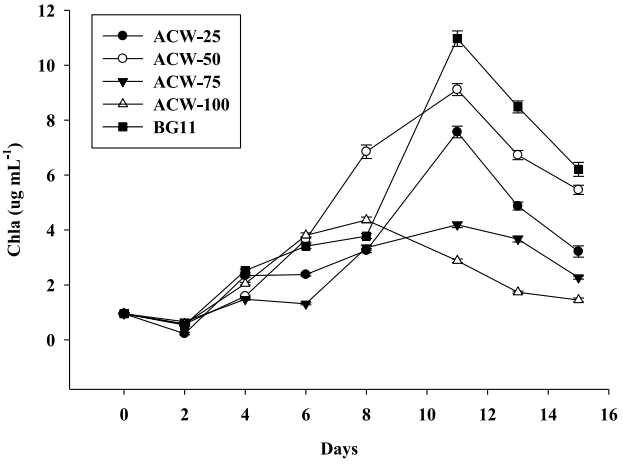

Fig. 2. Chlorophyll-*a* concentration (μg mL^{-1}) of *C. sorokiniana* grown in ACW-25, ACW-50, ACW-75, ACW-100, and BG11 media. Each data point is represented by the mean value (n ≥ 3) with the standard deviation as error bars.

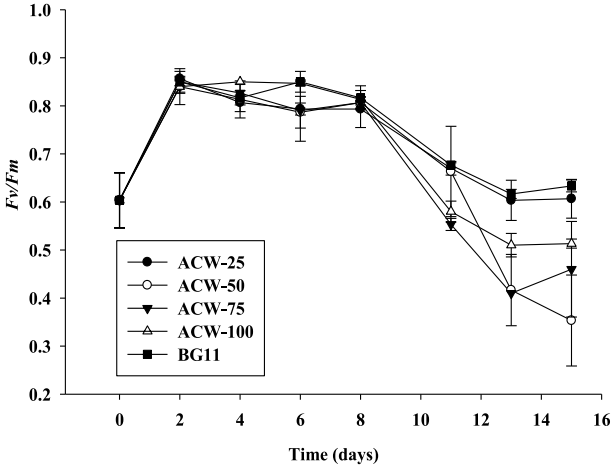

Fig. 3. Physiology (F_v/F_m) of *C. sorokiniana* grown in ACW-25, ACW-50, ACW-75, ACW-100 and BG11 media. Each data point is represented by the mean value (n ≥ 3) with the standard deviation as error bars.

indicated enhanced PSII function. Increased F_v/F_m ratios also indicate active photosynthesis and are correlated with increased cell densities and growth rates (Sung et al., 2018). This could explain the observed increases in biomass levels (Fig. 1). Additionally, the increased quantum efficiencies were correlated with increased chlorophyll-*a* concentration (Fig. 2). Active growth and cellular division lead to increased biomass, stimulating pigment biosynthesis for light capture. Similar findings have been reported by Sung et al. (2018), whereby increases in chlorophyll production led to increases in the F_v/F_m ratios in *Nannochloropsis gaditana*, implying that the increased portion of chlorophyll was related to elevated photochemical efficiency. After day 8, the quantum efficiency of all cultures was reduced by between 20 and 50%. The minimum F_v/F_m ratios were exhibited on day 15, *i.e.*, 0.63, 0.61, 0.51, 0.46, and 0.35 for the cultures grown in BG11, ACW-25, ACW-100, ACW-75, and ACW-50, respectively. Decreased chlorophyll-*a* levels coincided with decreased F_v/F_m ratios (Fig. 2), whereas there was no reduction in the biomass levels (Fig. 1), indicating that *C. sorokiniana* grown in ACW retained the physiological means to regulate the growth.

3.3. Biochemical and elemental composition of C. sorokiniana

The biochemical composition of algal biomass primarily consists of lipids, proteins, and carbohydrates. The proportions of these constituents in microalgal biomass vary depending on the species, cultivation conditions, and stage of cell growth at harvesting (Kebelmann et al., 2013). Fig. 4 shows the biochemical composition of *C. sorokiniana* cultivated using ACW-25, ACW-50, ACW-75, ACW-100, and BG11 media. *C. sorokiniana* grown in BG11 medium yielded 16.09% lipids, while those grown in ACW-25, ACW-50, ACW-75, and ACW-100 showed increases in lipid yields of 23.41, 27.24, 26.05, and 24.94%, respectively (Fig. 4a).

Interestingly, *C. sorokiniana* cultivated in ACW-50 produced higher lipid yield than ACW-25, ACW-75, ACW-100 and BG11 growth medium. The microalgae *C. sorokiniana* produced a maximum lipid of 27.24% in ACW-50 medium which was 41% higher than BG11 followed by 26.05% in ACW-75. A similar trend in lipid yields was observed by Bhandari et al. (2023), with 28.85% in *C. sorokiniana* and 24.57% in *Scenedesmus* sp when cultivated in 50% RO reject water. Bhandari and Prajapati (2022), obtained 28.85% of lipid content when *C. pyrenoidosa* cultivated in 50% RORI (reverse osmosis reject) medium. Ansari et al. (2019) observed a 26.5% of lipid yield in *Scenedesmus obliquus* cultivated in wastewater. Nitrogen-stressed conditions slow microalgae growth,

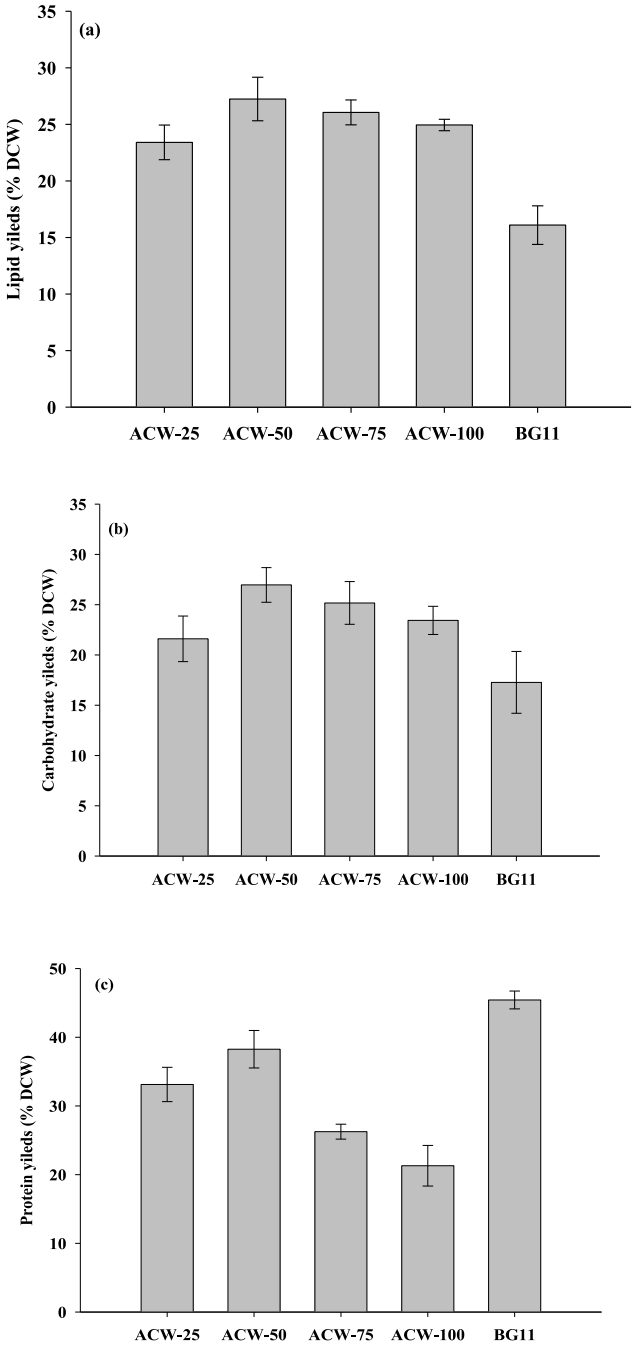

Fig. 4. (a) Lipid, (b) carbohydrate, (c) protein yield (% DCW) of *C. sorokiniana* grown in ACW-25, ACW-50, ACW-75, ACW-100, and BG11 media. Each data point is represented by the mean value (n ≥ 3) with the standard deviation as error bars.

however, it facilitates to accumulate of lipid and carbohydrates (Bhandari et al., 2023). For example, Singh et al. (2015) reported a high lipid yield (59.6%) and productivity (74.07 mg L-1 d-1) under nutrient stress with 750 mg L-1 of Nitrogen and 0 mg L-1 phosphorus and 9 mg L-1 of iron. Similarly, Danesh et al. (2017) observed that a 33.9% improvement in lipid accumulation. Thus, low nutrient in higher ACW inclusion is a favorable growth medium for the enhancement of lipid yield in microalgae.

Similarly, carbohydrate yields of *C. sorokiniana* were 17.27 % (DCW) when cultivated using BG11 medium. In this study, an increased yield of carbohydrates was observed for ACW-25 (21.60%), ACW-100

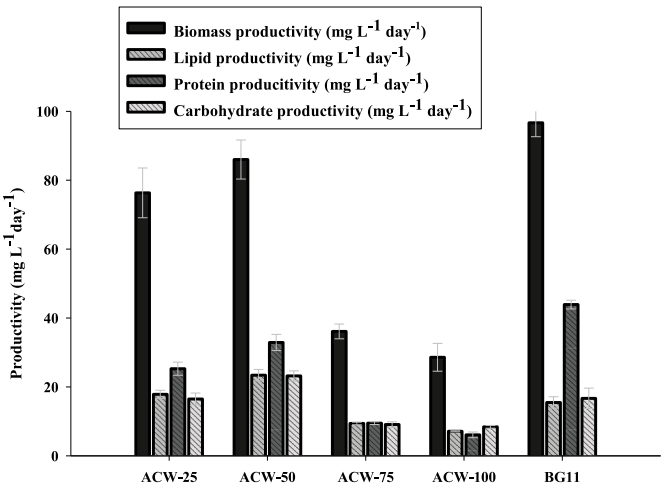

Fig. 5. Biomass, lipid, protein, and carbohydrate productivity in *C. sorokiniana* grown in different ratios of ACW and BG11 media. Each data point is represented by the mean value (n ≥ 3) with the standard deviation.

(23.435%), ACW-75 (25.17%), and ACW-50 (26.96%) growth media (Fig. 4b). The highest carbohydrate was obtained in ACW-50 (26.96%) followed by ACW-75 (25.17%) growth medium. The lowest carbohydrate yield (17.27%) was obtained in BG11. The results revealed that microalgae accumulate higher lipid and carbohydrate in nutrient stress conditions as a defense mechanism (Ansari et al., 2019). This observation was in agreement with the current study, in which enriched ACW contained lower nutrient concentrations compared with BG11 medium, indicating a relatively stressed condition.

A similar carbohydrate yield was observed by Renuka et al. (2018) in *A. obliquus* (17.95%) grown on BG11 medium. Dean et al. (2010), also reported that freshwater algae grown in limited nitrogen stressed condition tended to alter the metabolic pathway for survival. In comparison to microalgae grown in BG11 or domestic wastewater, nutrient enriched ACW proved a superior growth medium for accumulating high carbohydrate output. Microalgae tend to accumulate more lipids and carbohydrates in nitrogen-stressed conditions (Singh et al., 2016). It is known that microalgae under nutrient-stressed conditions reduce biomass production while increasing lipids and carbohydrates in order to survive in these unfavorable conditions (Danesh et al., 2017). The low concentration of macronutrients, particularly N and P, in ACW media compared to the BG11 medium, may have contributed to the stress experienced by the growth of *C. sorokiniana*. This stress may have resulted in lower growth and consequently, lower protein yield (Fig. 4c).

The protein yields of *C. sorokiniana* in BG11 enriched ACW depicted in Fig. 4 (c). The protein yield (% DCW) of microalgae grown in BG11 medium was 45.42%, whereas the protein yields for ACW-25, ACW-50, ACW-75, and ACW-100 growth media were 33.13%, 38.25%, 26.25%, and 21.29%, respectively, (Fig. 4c). The higher protein yields were observed for *C. sorokiniana* grown in BG11 medium (45.42%) followed by ACW-50. (38.25%), ACW-25 (33.13%, ACW-75 (26.25%) and ACW-100 (21.29%). There was 53.12% reduction of protein yield in ACW-100 compared to the BG11 medium which resembled the variation in nutrients in the growth medium. For instance, Bhandari and Prajapati (2022) observed 34.0.38% of protein yields when *C. pyrenoidosa* was cultivated in 50% RORI. Protein, lipids, and carbohydrates are the three primary metabolites of microalgae, and their yield depends on the nutrient concentration in the medium and cultivation conditions (light, temperature, photoperiod etc.) provided (Bhandari and Prajapati, 2022). A low N:P ratio enhances the protein synthesis while a high N:P ratio increases the lipid (Mao et al., 2023). Particularly, ACW-50 growth media has demonstrated a protein yield of 38.25%, allowing the biomass to be used as feed. Research has shown that the overall amount of essential amino acids (EAA) in microalgae is comparable to reference

protein sources such as eggs and soybeans (Fatima et al., 2023). The optimal concentration of nitrogen in nutrient rich medium is vital for growth, protein, genetic material synthesis and other material such as chlorophyll (Guldhe et al., 2017).

Regarding productivities, ACW-100 showed lower protein, lipid, and carbohydrate productivities than BG11 medium due to lower biomass production (Fig. 5). Algae grown in ACW-50 medium exhibited maximum lipid productivity (23.42 mg L^{-1} d^{-1}) due to high biomass production and lipid yields, while the ACW-25 and BG11 medium showed comparable values. In terms of protein productivity, BG11-grown algae showed the highest values (43.97 mg L^{-1} d^{-1}), followed by ACW-50 (32.9 mg L^{-1} d^{-1}), ACW-25 (25.28 mg L^{-1} d^{-1}) while ACW-75 (9.48 mg L^{-1} d^{-1}) and ACW-100 (7.13 mg L^{-1} d^{-1}) exhibited lower protein yields. Nitrogen concentration in the nutrient medium is positively correlated with biomass production and protein yields (Guldhe et al., 2017). ACW-50 exhibited the maximum carbohydrate productivity (23.91 mg L^{-1} d^{-1}), while ACW-25 and BG11 media-grown algae showed comparable carbohydrate productivities. There is trade-off between biomass and lipid productivity with nitrogen addition in growth medium. For example, Ansari et al. (2017), cultivated *C. sorokiniana* in aquaculture wastewater and observed that lipid productivity increased up to 600 mg L^{-1} N (sodium nitrate) supplementation whilst biomass productivities constantly increase with an increase in N. Overall among tested combination of ACW and BG11 medium for *C. sorokiniana* cultivation, ACW-50 was found the suitable combination for biomass production, lipid, protein, carbohydrate yield and productivity.

Table 2 shows the elemental composition (CHNOS) of microalgal biomass and falls in the anticipated range (Kebelmann et al., 2013). The C content (%) of microalgal biomass was higher in BG11 (47.11%) than in ACW-25, ACW-70, and ACW-100 medium, whereas ACW-50 (48.18%) showed comparable values to BG11-grown biomass. The H content was similar across all growth conditions. The N content depended on nutrient availability in the growth medium, and BG11-grown algal biomass showed higher N content (6.55%) than ACW-100, ACW-75, ACW-50, and ACW-25, (Table 2). For example, the reduction in N content in ACW-25 was found 36.45%. compared to BG11. However, more than a 50% reduction (53.44%) in N content was in ACW-100. The results have also shown that the N content in biomass decreases with an increasing level of ACW (e.g., A CW-75 & ACW-100), as N concentration has a significant impact on algal cellular N (Bhandari and Prajapati, 2022; Singh et al., 2016). The optimal level of N concentration in BG11 medium led to high N (%) and protein yields.

The HVV (kJ·g $^{-1}$) defines the total heat energy that can be produced by the unit mass of microalgae on complete combustion (Ansari et al., 2019). The HHV of ACW-25, ACW-50, ACW-75, ACW-100 and BG11 were similar with 18.22–19.89 kJ g $^{-1}$. Our finding was consistent with Illman et al. (2000). Their study found that *C. vulgaris* had HVV of 18 kJ g^{-1} when grown in nutrient rich medium. The HHV (kJ·g $^{-1}$) value is strongly dependent on the composition of microalgal biomass primarily on oxygen to carbon (O/C) and on hydrogen to carbon (H/C) ratios which increased with decreasing O/C or increasing H/C ratio (Francavilla et al., 2015).

Table 2
Ultimate analysis and calorific values for *C. sorokiniana* biomass grown in different ratios of ACW and BG11 media. Each data point is represented by the mean value (n ≥ 3) with the standard deviation.

Growth media	C (%)	H (%)	N (%)	S (%)	O[a] (%)	C/N ratio	HHV (kJ·g $^{-1}$)
ACW-25	44.57	6.67	4.16	0.43	44.6	10.71	18.22
ACW-50	48.18	6.79	5.04	0.51	39.99	9.56	19.89
ACW-75	46.54	7.2	4.13	0.42	42.13	11.27	19.11
ACW-100	45.11	7.21	3.05	0.35	44.63	14.79	18.35
BG11	47.11	5.87	6.55	0.73	4.47	6.89	19.46

[a] Calculated by differences,[b] Calculated by HHV (high heating value) = $5.22C^2$– 319C–1647H + 38.6C × H + 133N + 21,028 (Friedl et al., 2005).

3.4. Comparison with previous studies

The study demonstrated the potential of using ACW as a water source for cultivating *C. sorokiniana* (Table 1). Results of the study support previous studies to reduce freshwater usage for microalgae cultivation and to investigate alternate sources. Bhandari et al. (2023) evaluated the potential of reverse osmosis (RO) reject water for cultivating *C. sorokiniana* and found that algae exhibited comparable biomass, lipid, protein, and carbohydrate values in ROR-50 as in our study (ACW-50). The biomass, lipid, protein, and carbohydrate values reported were 1.05 g L^{-1}, 28.5, 22, and 19.5%, respectively. Similarly, Bhandari and Prajapati (2022) observed the high biomass, lipid, and protein yield of *C. pyrenoidosa* in RORI-50 of 1.37 g L^{-1}, 28.85%, and 34%, respectively. Do et al. (2021) also investigated the potential of reverse osmosis concentrate (ROC) and primary settled wastewater (PS) for cultivating *C. sorokiniana* KNUA071, and the results indicated the potential of using these water sources for microalgal cultivation (Table 3). Although slight variations in the nutrient levels of different water sources affected the metabolite content, the findings of the study suggest that ACW could be a potential alternative water source for microalgal cultivation, especially with the combination of ACW-50 and BG11-50, which showed promising results. Table 3 also represents the biochemical composition compared with those reported in the literature using different microalgae species and growth medium.

3.5. Potential for CO_2 assimilation

Reducing the CO_2 footprint of air conditioning is crucial for mitigating the effects of climate change that are indirectly caused by the consumption of thermal based electrical energy in air conditioning. One of the mitigation approaches can be the production of algal biomass from the ACW and using the biomass to produce energy, thereby reducing the C emissions that are produced by using thermal-based electricity in the operation of air conditioners. Assuming the large-scale production scenario using algal raceway ponds for biomass cultivation from ACW, the estimation of carbon capture is shown in Table 4. The estimations of carbon capture are based on the fact that atmospheric CO_2 is consumed by the algal biomass for photosynthesis and the produced biomass can act as a clean energy source that can proportionally reduce the dependency of thermal based electricity. After deducting the energy consumed in the operation of biomass production, the estimated results showed that using condensate water from air conditioners for algal production can sequestrate 0.36 kg day^{-1} CO_2 (Table 4) that equates to 9.5% of the CO_2, produced by operating an air conditioner on a thermal power plant-based electricity. This amount of sequestrated CO_2 equates to 9.5% of the CO_2, produced by operating a one-ton air conditioner per day on a thermal power plant-based electricity. The contribution in this total CO_2 reduction of 9.5% constituted 3.7% as consumption of atmospheric CO_2 in algal photosynthesis and the remaining sequestration of 5.8% will be from the bioenergy production out of biomass. This estimate can vary depending on the amount of ACW generated from the air conditioning.

However, more ACW will increase biomass production and, thereby, returns in terms of CO_2 sequestration. The United Nations has set ambitious targets to reduce CO_2 footprints and mitigate the effects of climate change (Leaf et al., 2003; United Nations, 2023). The Paris Agreement, signed by nearly 200 countries in 2015, aims to limit global warming to well below 2 °C which requires reducing global CO_2 emissions significantly (Glanemann et al., 2020). The estimations justify that using ACW for biomass production can bring additional advantages such as to help achieve the C reduction target in the air conditioning process. Additionally, reducing the CO_2 footprint of air conditioning technology can help companies to meet their sustainability goals and create a more sustainable future for generations to come.

Table 3
Comparison of the biomass and biochemical composition of *C. sorokiniana* grown in different ratios of ACW and BG11 media.

Microalgae	Media	Biomass ($g{\cdot}L^{-1}$)	Lipid (%)	Protein (%)	Carb. (%)	References
C. sorokiniana	WW-25		27.68	–	–	Gupta et al. (2016)
C. vulgaris UTEX 395	WW		9.81	35.13	16.84	Zhao et al. (2014)
Chlorella spp.	PW		1.8–3	56–59	25–34	Michelon et al. (2016)
S. obliquus	WW-25		28.36	–	–	Gupta et al. (2016)
C. vulgaris	ADMW		23	41.8	6.3	Tao et al. (2017)
	ADPP		21.7	30.3	6.8	
C. vulgaris	BBM		12.1	43.98	18.32	Daneshvar et al. (2018)
C. sorokiniana KNUA071	ROC	0.85*	13.31	23.06	23.02	Do et al. (2021)
	PS	0.32*	28.18	28.18	41.55	
	BG11	0.95*	–	–	–	
Mixed algae	ST-BG11		25.6	29	18.4	Ansari et al. (2021)
	ST-N		18.3	22	16	
	ST-N&P		24.5	28	20	
C. pyrenoidosa	ROR1-25	1.27	23.81	38.22	31.53	Bhandari and Prajapati (2022)
	ROR1-50	1.37	28.85	34	–	
	ROR1-75		24.76	32.38	–	
	ROR1-100		10	11.69	28.59	
C. sorokiniana	BG11	1.3	16.5*	32*	26*	Bhandari et al. (2023)
	ROR-50	1.05	28.5*	22*	19.5*	
	ROR-75	0.82	19.5*	17*	18*	
	ROR	0.6	16.5*	12.5*	16*	
C. sorokiniana	BG11	1.45	16.09	45.42	17.26	The current study
	ACW-25	1.15	23.41	33.13	21.6	
	ACW-50	1.3	27.24	38.25	26.96	
	ACW-75	0.54	26.05	26.25	25.17	
	ACW-100	0.43	24.94	21.29	29.44	

WW-25: Wastewater 25% + 75 secondary treated wastewater; PW: Piggery wastewater; WW-25: Wastewater 25% + 75 secondary treated wastewater; ADMW: digestates originating from wastewater treatment plant; ADPP: digestates originating from pulp and paper wastewater treatment plant; BBM: Bold's Basal Medium; ROC: Reverse osmosis concentrate; PS: primary settled wastewater; BG11: Blue-Green Medium; ST-BG11: Blue-Green Medium added to secondary treated wastewater; ST-N: Nitrogen added to secondary treated wastewater; ST-N&P: Nitrogen and phosphorus added to secondary treated wastewater; ROR1-25: Reverse osmosis reject water 25% + Synthetic medium; ROR1-50: Reverse osmosis reject water 50% + Synthetic medium; ROR1-75: Reverse osmosis reject water 75% + Synthetic medium; ROR1-100: Reverse osmosis reject water 100%; BG11: Blue-Green Medium; ROR-50: Reverse osmosis reject water 50% + BG11 Medium; ROR-75: Reverse osmosis reject water 75% + BG11 Medium; ROR: Reverse osmosis reject water 100%; ACW-25: 25% air-conditioning water +75 BG11 Medium; ACW-50: 50% air-conditioning water +50% BG11 Medium; ACW-75: 75% air-conditioning water +25% BG11 Medium; ACW-100: 100% air-conditioning water +0% BG11 Medium; - not measured; * extrapolated from graph.

Table 4
Estimations of the CO_2 reduction using condensate water for the algal biomass production.

Description	Value	Reference
Total CO_2 input by AC per day out of electricity consumption (**kg CO_2 d^{-1}**)	3.80	Kumar et al. (2013)
Water condensate produced during the operation of 1-ton AC per day (m^3 d^{-1})	0.026	Weynand (2009)
Dilution factor for mineral medium	2.00	
Algal biomass production from condensate water ($kg{\cdot}m^{-3}$)	1.40	The current study
Algal biomass production from condensate water from 1-ton AC unit per day (kg d^{-1})	0.074	Estimated
CO_2 sequestration capacity by Algae (kg CO_2 kg^{-1} dry biomass)	1.88	Iglina et al. (2022)
CO_2 reduction as photosynthesis by Algae from AC condensate (kg CO_2 d^{-1})	0.138	Estimated
Net carbon credit from photosynthesis %	3.66	Estimated
Energy consumed during microalgae cultivation in raceway pond (Kw h kg^{-1} biomass)	1.05	Jorquera et al. (2010)
Energy content in biomass (Kw h kg^{-1} biomass)	8.76	Jorquera et al. (2010)
Net energy produced (Kw h kg^{-1} biomass)	7.71	Estimated
CO2 saved by energy production from Algae (kg CO_2 kg^{-1} biomass)	3.00	Estimated
CO_2 reduction as energy production from Algae grown from AC condensate (kg $CO_2 d^{-1}$)	0.222	Estimated
Total CO_2 reduction by Algae production from AC condensate (kg CO_2)	0.361	Estimated
Total carbon credit (photosynthesis + Energy production) %	9.50	Estimated

3.6. Potential application of biomass

Microalgae biomass has tremendous potential as a third-generation biofuel feedstock, offering a myriad of benefits over traditional fossil fuels. With a high heating value of 24 MJ kg^{-1} and oil content of 20–50% on a dry weight basis, microalgae are an ideal candidate for producing biofuel oil and synthesis gas (Guldhe et al., 2017; Laurens and Wolfrum, 2011). Despite the high energy requirements for cultivation, harvesting, and lipid extraction, microalgal biodiesel production has a lower environmental impact than the production of fossil-derived diesel (Caporgno et al., 2015). Moreover, microalgal cells contain a range of bioactive compounds such as lipids, proteins, carbohydrates, fatty acids, pigments, vitamins, and mineral salts (Ansari et al., 2015; Dolganyuk et al., 2020; Singh et al., 2023). After lipid extraction, the lipid-extracted residual algal biomass can also be used as animal feed or bio-fertilizers due to its protein and carbohydrate content (Maurya et al., 2016; Zhao et al., 2014; Singh et al., 2023). However, the economic feasibility of microalgal biomass depends upon the production costs as well as the unit operations for the assorted products resulting from the biomass. Valorization of one microalga biomass using a biorefinery approach or integrated approach are economically feasible ways. Microalgae cultivation in wastewater has shown immense potential, *e.g.*, removing nutrients from wastewater and producing valuable biomass that can be used further for different applications (Fig. 6), (Gupta et al., 2016; Olabi et al., 2022).

However, the fundamental issue in growing microalgae in wastewater is the limited application of algal biomass. Using wastewater-grown algae is limited to specific applications such as energy (biodiesel, biogas, biohydrogen etc.) or fertilizer (Renuka et al., 2018). Because of toxic constituents in wastewater, the grown algal biomass cannot be used for food/feed, nutraceutical, or pharmaceutical purposes

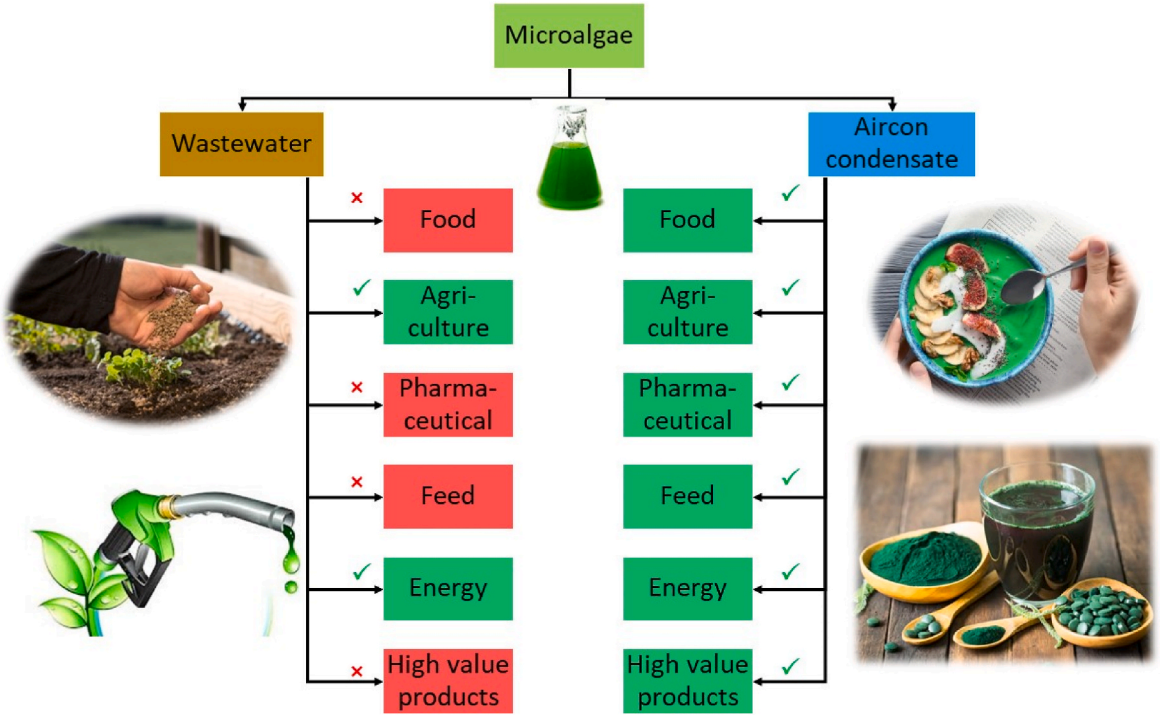

Fig. 6. Potential applications of microalgal biomass cultivated in wastewater and ACW.

(Fig. 6).

The economic viability of microalgae production in synthetic media using freshwater is limited. Multiple products derived from a single microalgal biomass can be manufactured via several different unit operations to produce lucrative, in-demand primary and secondary bioproducts, providing financial and environmental advantages (Ansari et al., 2021; Daneshvar et al., 2018). Using a combination of unit operations in an algal biorefinery strategy improves process performance, resource recovery, cost-effectiveness and aids in transitioning from a non-renewable energy based linear economy to a circular, green economy. An important global sustainability requirement is transitioning from a linear economy based on fossil fuels to a circular economy, and the combination of microalgal biorefinery in a circular operation promotes autonomy/self-support and zero waste discharge into the natural environment (Ansari et al., 2017, 2021; Daneshvar et al., 2018).

4. Conclusions

The study demonstrated that *C. sorokiniana* can be grown successfully in ACW, with the combination of ACW-50 and BG11-50 being the most suitable combination for maximizing biomass production and productivity. Different combinations of the media resulted in varying yields for lipids and carbohydrates, while BG11 medium showed the highest protein yields and biomass followed by ACW-50. Carbon sequestration capacity assessment revealed partial CO_2 assimilation, contributing to a circular economy. These findings showed that the use of ACW not only reduces the *C. sorokiniana* cultivation cost but also produced rich biomass. The integration of *C. sorokiniana* cultivation using ACW is a promising way of generating biomass, which could be supplemented into fish feed or animal feed. Overall, the findings indicate that using ACW for microalgae cultivation is an economically viable and feasible approach for generating rich algal biomass.

CRediT authorship contribution statement

F.A. Ansari: Conceptualization, Data curation, Methodology, Writing - original draft. **H. Hassan:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **L. Ramanna:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **K.M. Gani:** Writing - original draft, Writing - review & editing. **K. Singh:** Writing - original draft, Writing - review & editing. **I. Rawat:** Writing - original draft, Writing - review & editing. **S.K. Gupta:** Writing - original draft, Writing - review & editing. **S. Kumari:** Writing - original draft, Writing - review & editing. **F. Bux:** Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare no conflict of competing or financial interests.

Data availability

Data will be made available on request.

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