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Artificial intelligence model for monitoring biomass growth in semi-batch *Chlorella vulgaris* cultivation

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ABSTRACT

There is a great demand for a clean, economical, and long-term energy source, due to the depletion of fossil fuels. Large-scale production of microalgae biomass for biofuel production is likely attributable to several challenges, including the high cost of photobioreactors, the need for a sustainable medium for optimum development, and time-consuming algal growth monitoring techniques. Firstly, the research novelty aims at improving the strategy of recycling culture media for semi-batch cultivation of *Chlorella vulgaris*. Two cycles were performed with varying amounts of recycled medium replacement to evaluate algal growth and biochemical content. As compared to all other culture ratio combinations, the mixing ratio of recycled medium to fresh medium is at 40 % (40RB) combination yielded the greatest biomass growth (4.52 g/L), lipid (317.40 mg/g), protein (280.57 mg/g), and carbohydrate (451.37 mg/g) content. Next, custom vision was applied to *Chlorella vulgaris* maturing stages, and a unique digital architecture framework was developed. The iteration model delivers result interpretation with an accuracy of more than 92 % of every data set based on the trained Model Performance.

1. Introduction

Microalgae biomass are emerged as a novel, sustainable, and clean energy source for the synthesis of third-generation biofuels [1]. According to the US Energy Information Administration [2], non-renewable energy resources such as petroleum are expected to reach maximum output in 2019, followed by a downward trend, particularly for crude oil supply. Gielsen et al. (2019) reported that the energy consumption has risen dramatically, with global energy demand expected to rise by more than 85 % by 2040 [3]. Since biodiesel been established as one of the most promising alternative fuels, alternative energy sources were continuously researched depending on the current environment, as the expanding global population, industrialization, and increasing demand for transportation developed. Biodiesel was described as mono-alkyl esters produced from vegetable oils, animal fats, or waste cooking oil that have a long chain of fatty acids [4]. As

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compared to conventional petroleum-based diesel, biodiesels are characterized as biodegradability with higher Cetane number, higher flash point, and lower exhaust pollutants [5]. However, the production of biodiesel from crops has a major controversy due to the food vs fuel competition.

Microalgae-based biomass is proposed as a potential feedstock for the production of sustainable and renewable biodiesel as an approach to these issues. Omar et al. (2020) discovered that microalgae with high biomass productivity and lipid content, are favorable for biodiesel generation [6]. Environmentally, the development of the culture system into a competitive green energy generator without competing food crops for arable land and water depends on the growth of microalgae paired with CO_2 fixation and wastewater bio-treatment [7]. Based on areal productivity, microalgae species such as *Botryococcus*, *Chlamydomonas*, and *Chlorella* could generate 15–300 times more oil for biodiesel generation than conventional crops [8]. Microalgae also have short harvesting cycle (ranging from 1 to 10 days depending on the culture condition), allowing continuous harvests with higher biomass yield as compared to traditional crops that are harvested once or twice a year [9]. Hence, microalgae cultivation is recognized as a modern biotechnologist advancement and it is projected that by 2036, the global market for microalgae-based biomass would have the capacity to produce 5000 tons of dry biomass per year with a revenue of USD 1250 million [10].

For large scale microalgae cultivation process, photo-bioreactor is the most preferred system as it saves land area and allow the culture to grow in a closed environment with less exposure to external contamination [11]. Nevertheless, the capital and operational costs for photobioreactors are significantly high due to the fabrication of high-volume tank and control system for nutrient medium, carbon supply, and light source [12]. Hence, growing microalgae in a semi-continuous culture mode is the most cost-effective cultivation system compared to batch mode for large biomass output within a short period of time. The semi - batch culture mode is preferred as it prevents the downtime at the end of the culture and minimizes the limitation in terms light penetration during stationary phase as compared to batch cultivation. However, excessive addition of fresh culture medium may result in a rise of osmotic pressure, which may damage the photosynthetic mechanism of microalgae [13]. Thus, in this study *Chlorella vulgaris* cultivation was cultivated in semi-batch mode with nutrient recycling and the fresh medium supply ratio was optimized.

Moreover, industrial revolution 4.0 has the ability to break down traditional barriers and allow for sustainable growing conditions with quick testing on biomass growth. In previous years, photo-bioreactors are widely investigated on its design structure, in terms of sustainable and economical aspects [4]. With the use of technology, photo-bioreactors would often provide a consistent optimum biomass production by monitoring microalgae development with a variety of linked sensors, altering conditions as necessary, and using machine learning to discover the perfect growth parameters [14]. However, implementation of artificial intelligence able to overcome the tough challenge, by identifying the best time to harvest the algae before it degraded. The current UV– Vis's methods are too tedious, costly and time consuming which prevents stake holders in venturing into large scale algae growth. Hence, this study also includes the application of custom vision service in monitoring the algae growth via image classifiers.

2. Procedure

2.1. Culture medium preparation

The *Chlorella vulgaris* species was obtained from National Cheng Kung University (NCKU). The fresh medium and photobioreactor, PBR setup was created as described in a recent work [15]. Following the initial batch culture of 7 days, the succeeding cycles were done immediately using the recycled medium with comparable PBR operating settings. Once the harvesting day reached, the stirring is stopped, and a portion of the culture medium was extracted out for centrifugation process. Meanwhile, the remaining culture medium (in this case 20 %, 40 %, 60 %, and 80 %) was recycled back into a fresh culture medium, making it back to a total of 1L nutrient medium. The photobioreactors and fresh media were placed in a horizontal laminar flow cabinet equipped with ultraviolet (UV) light (AHC-5A1, Esco Micro Pte. ltd., Singapore) during the transformation of medium to avoid any environmental contamination into the culture. The harvesting day was fixed to be after 7th day of the culture and each round was considered as a cycle [15]. The biomass growth was compared with mixing ratio of 20 %, 40 %, 60 %, and 80 % as 20RB, 40RB, 60RB, and 80RB respectively.

2.2. Azure bot architecture

Windows Azure compute service runs application on a Windows Server foundation. The Azure platform has the following components such as Azure Compute, Azure Storage, Fabric Controller, SQL Azure, Azure Platform AppFabric, Content Delivery Network, and Connect. Azure IoT portal natively supports virtualized containers that can hold firmware to applications which allows better associated hardware dependent IoT device. Fig. 1 depicts the general workflow, prerequisites, service configuration, and settings utilized in this experimental scheme. The workflow includes three primary steps: (1) Image classification model on Microsoft powered Custom Vision; (2) Azure IoT hub driven by Edge Virtual Machines; (3) Deployment application to be distributed to Edge gateway and IoT devices.

2.3. Azure custom vision services

Azure custom vision is an Artificial Intelligence service that allows computers to mimic capabilities of the human brain such as learning, understanding, and recognizing patterns without needing to be explicitly coded for that as we generally do with algorithms. Machine learning is a subset of AI that train a computer system to make predictions based on the available data. In this study, 1000 images were used per tag in the initial training set with 300 pixels on the shortest edge. Three tags were created classified as initial phase (0.41 g/L–1.59 g/L); growth phase (1.60 g/L–2.50 g/L); and harvest phase (more than 2.5 g/L) based on previous study [15]. Thus, a total of 3000 images were uploaded and tagged to allow the custom vision service to train the images. Then the classifier uses all the current images to create a model that identifies the visual qualities of each tag. The training process would take about 30 min where simultaneously the performance about the training process is displayed. Lastly, the Custom Vision Service uses the images that were submitted for training to calculate precision and recall, using a process called k-fold cross validation [16]. Precision, recall, and average precision are measurements that displays the effectiveness of a classifier model [17]. The model was trained using images obtained in previous study [15].

2.4. Determination of microalgae cell growth via UV–vis

The dry cell weight, DCW of the *Chlorella vulgaris* was measure as described in a recent work [15] using a UV–vis's spectrophotometer (UV-1800, Shimadzu) at wavelength of 680 nm. Equations (1) and (2) indicate the cell growth rate, P_b (mg/L/d), and the growth performance, respectively μ (d^{-1}):

$$P_b = \frac{X_2 - X_1}{t_2 - t_1} \tag{1}$$

$$\mu = \frac{ln\frac{X_2}{X_1}}{t_2 - t_1} \tag{2}$$

Where X_1 and X_2 are dry cell weight (g/L) at time t_1 (initial) and t_2 (final).

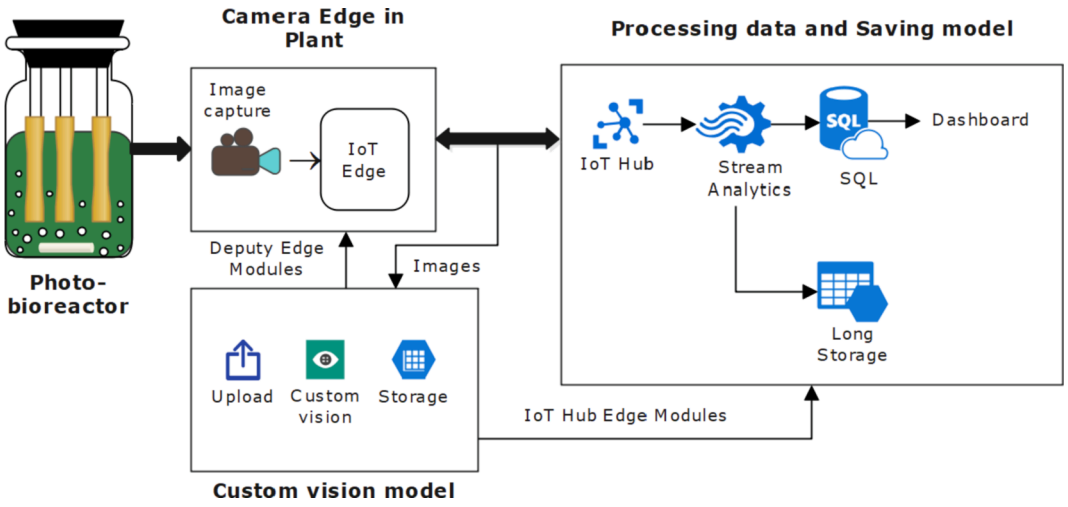

Fig. 1. Digital architecture system of Algae growth monitoring.

2.5. Bio-composition content assessment

The microalgae samples were analyzed every day for the bio-composition measurement. UV–vis spectrophotometer at 220 nm optical density was used for nitrate detection while The AAS spectrophotometer was adjusted to 213.6 nm for phosphorus removal detection as shown in the previous study [15]. The concentrations of lipids, proteins, and carbohydrates were measured as described in a previous works [15]. The lipid content is expressed as a percentage difference (wt %) of DCW in Equation (3). Furthermore, the protein concentration of the extracted microalgae biomass was determined using the Bradford method [18]. A standard correlation between absorbance (OD_{595}) and protein concentrations was established using the bovine serum albumin analysis. The carbohydrate content of the transparent supernatant was determined using the phenol–sulphuric method [11]. The conventional relationship between OD_{490} and total carbohydrates was observed using starch extracts of various concentrations.

$$\text{Lipid content (wt\%)} = \frac{\text{Final weight} - \text{Initial weight}}{50\,\text{mg}} \times 100\% \quad (3)$$

2.6. Statistical data analysis

The one-way ANOVA with two-tailed test in IBM SPSS Statistics version 26 was used to interpret data on total protein, lipid, and carbohydrate, biomass development rate, nitrate, and phosphorus level. In this study, descriptive statistical analysis is provided as mean ± standard deviation. The data given in this investigation are the mean of three runs. The summation of variances was assessed using a significance level of $P \leq 0.05$ in all statistical analyses of the data. Microsoft Azure IoT portal (custom vision), was designed to satisfy the initial experiment setup demands.

3. Findings and discussions

3.1. Effect of recycled supernatant culture medium on microalgae biomass yield

Chlorella vulgaris was cultivated in a closed photobioreactor to determine the best medium replacement ratio for semi-batch operation. There will be a little variation in the nutrient delivery since the general culture medium characteristics change depending on the previous culture mixing ratio. The biomass growth was compared with mixing ratio of 20RB, 40RB, 60RB, and 80RB. In comparison to all the other media mixing combinations, Fig. 2 shows that 40RB yielded the highest

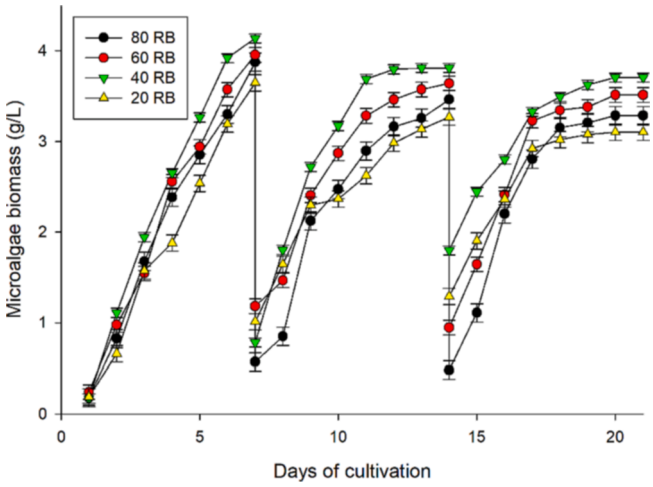

Fig. 2. Semi-batch Experimental *Chlorella vulgaris* Development.

biomass development. Optimum biomass content was recorded for 40RB at 4.52 g/L on the seventh day, followed by 60RB, 20RB, and 80RB at 3.45 g/L, 3.15 g/L, and 2.77 g/L respectively. The first cycle of culture growth was observed to be near to each other since its initial growth condition was already optimized in previous study [15]. There was a noticeable difference in the microalgae development after combining new medium with part of the existing culture from day seven. Fig. 2 indicates that combining new media with previous media takes just three days to display significant growth and reach its stationary growth phase on the fifth day of the second cycle, whereas fresh medium alone continues to grow on the sixth day of the first cycle. While cultivated in its optimum culture medium combinations, Peter et al. (2021) revealed that *Chlorella vulgaris* growth can take up to 7–10 days to reach its stationary growth phase [19], hence the microalgae growth cycle was only observed for 7 days in this study. Up-scaling of microalgae production can be made economically achievable, if the growth method was advanced from batch to semi-batch. Thus, determining the ideal culture medium recycling volume that increases total microalgae biomass production for a longer length of time is critical.

Another round of cultivation was carried out using the same mixing ratio combination to validate the growth pattern of cycle 2 (C2). As for the third cycle (C3), the optimum biomass concentration attained was at 2.11 g/L for 40RB, followed by 2.05 g/L, 1.85 g/L, and 1.32 g/L for 60RB, 20RB, and 80RB respectively. Based on Fig. 2, the optimal

biomass growth was found to be slightly decreasing as the number of cycles increased as the medium's colour changes to dark green more rapidly due to the presence of re-cycled culture as compared to the first cycle. Imran et al. (2018) also reveals that microalgae require light energy to convert carbon dioxide, CO_2 and water into glucose and oxygen during photosynthesis [20]. Even though both sides of the PBR were exposed to 24 h of LED fluorescent light, the culture's initial colour thickness differed. The colour medium for 80RB recycled medium was reported to be the darkest, followed by 60RB, 40RB, and 20RB. This is due to the fact that a large volume of recycled culture media results in a high concentration of green biomass in the culture. However, the microalgae development is not only influenced by its light distribution intensity but also the nutrient content [21].

Table 1 reports the cell growth rate and productivity of *Chlorella vulgaris* at day 7 of all three of its cultivation cycles. The 40RB medium had the highest biomass productivity for all three cycles at 165.50 mg/L/d (first cycle, C1), 118.45 mg/L/d (second cycle, C2), and 75.71 mg/L/d (third cycle, C3) respectively. Furthermore, it has been observed that as the number of cycles advances, the biomass productivity decreases. This is due to a rapid depletion of the recycled medium's nutrition supply and a restriction in light distribution. An accurate combination of nutrition supplies, as well as adequate light penetration, is required to carry out an effective growing process. Thus, full replacement of the inorganic medium is not recommended. In this study the light distribution was kept constant throughout the monitoring period, since controlling the light parameters for large scale microalgae production would be more expensive and time consuming due to additional labor force requirement [20]. In accordance with Table 1, integrating 40RB from a previous culture cycle promotes microalgae growth for at least 21 days continuously and without interruption. Previous study reported that a batch culture (fresh culture) yields 174.99 mg/L in about 10 days, however in this study with recycled medium, roughly about 4.52 mg/L was produced on the 7th day [15]. In summary, semi-batch culture is preferred for large-scale microalgae production as it is more cost-effective and energy-efficient, with optimal biomass output [4].

3.2. Image classifier model performance metrics

Conventionally, a UV–vis spectrophotometer was used to track biomass increase daily. This method necessitates the use of additional energy and labor on a regular basis for surveillance. Thus, the image classifier model was examined to develop a digital platform for microalgae growth monitoring. The accuracy and performance metrics of the model was observed and one such records from the runs are presented in Fig. 2. For all the inputs, images of algae growth phases were fed to the classifier model. The Custom Vision service calculates three metrics (1) Precision indicates the percentage of the class predictions that were correct; (2) Recall indicates the percentage of class predictions that were correctly identified; (3) Average precision (AP) measures model performance by computing the precision and recall at different thresholds. The model predicted a precision and recall accuracy of 92.5 % followed by an average precision of 97.1 %. It is however very important to publish the model as this information were uploaded to build the Python application for future studies.

In this study, the probability threshold was adjusted by adding more images at 90 %, as it represents the accuracy of the image tagging. It is noticed that, as the probability threshold increases, the model will tend to be more accurate as it will only classify when the model is more confident in the tag. As a result, the precision of the model will be higher. However, 100 % probability threshold is not advisable in this case, as the recall begins to decrease. The recall of the model will decrease because the model is strictly tagging photos it is more confident in, which makes plenty of other images go undetected. On the other hand, decreasing the probability threshold will increase the decrease the precision of your model, but the model will have a much higher recall as it classifies more photos. Hence, the optimized probability threshold discovered in this study was at 90 % and kept constant throughout. Quick tests were carried out daily with an image of the algae growth and the comparisons of experimental algae growth monitoring. It is discovered that custom vision service was able to replace the traditional method of UV–vis based algae monitoring to a single click, one iteration model training by producing an accuracy of more than 92 % of every data set. Fig. 3 illustrated an example of a quick test conducted on the 5th day of the cultivation process.

Table 1
Characteristics of *Chlorella vulgaris* grown in IBG and industrial dairy waste culture mixtures.

Culture mixtures	20RB	40RB	60RB	80RB
Cycle 1, C1				
DCW (g/L)	3.15 ± 0.05	4.52 ± 0.08	3.45 ± 0.05	2.77 ± 0.04
P_b (mg/L/d)	139.56 ± 4.30	165.50 ± 2.85	151.12 ± 5.41	134.03 ± 2.11
μ (d^{-1})	0.34 ± 0.01	0.56 ± 0.03	0.52 ± 0.06	0.44 ± 0.01
Cycle 2, C2				
DCW (g/L)	2.55 ± 0.05	3.91 ± 0.01	2.10 ± 0.15	2.52 ± 0.02
P_b (mg/L/d)	92.11 ± 4.10	118.45 ± 2.25	107.67 ± 5.40	95.99 ± 2.11
μ (d^{-1})	0.54 ± 0.04	0.65 ± 0.05	0.50 ± 0.01	0.48 ± 0.02
Cycle 3, C3				
DCW (g/L)	2.05 ± 0.05	2.11 ± 0.01	1.85 ± 0.05	1.32 ± 0.04
P_b (mg/L/d)	68.90 ± 4.30	75.71 ± 2.11	64.67 ± 5.21	54.34 ± 2.11
μ (d^{-1})	0.45 ± 0.05	0.66 ± 0.03	0.68 ± 0.02	0.41 ± 0.01

3.3. Nutrient reductions

Microalgae require nitrogen and phosphate as key nutrients for the metabolite cycle and biogenesis during cell growth [22]. The 40RB combination of fresh industrial biscuit waste have been proven to provide an adequate nutrient that improvise the microalgae growth in batch scale [15]. Panahi et al. (2015) proves that the nitrate content declines when it is absorbed by the microalgae cells throughout the cultivation phase [23]. However, this study discovered that when a fresh medium is added, the nitrate content will increase again. This means that the respective culture combination can be utilize for a semi-batch cultivation system to boost microalgae growth while lowering overall processing costs.

Fig. 5 illustrates that the first cycle's initial nitrate concentration is more persistent than the other two cycles. Since the initial cycle uses only fresh medium; however, when recycled and fresh medium are combined, the initial nutritional content changes. Moreover, whenever a sufficient organic medium is substituted with BG-11, the pH value is also affected by the fluctuations in nutrient content [24]. Nonetheless, the nitrate compound is homogenized after 2 days, and each recycled medium combination demonstrates a significant difference. Despite having varied fresh medium volumes in the culture, it has been reported that nitrate concentration for the growth phase decreases as the number of cycles increases. As a result, it has been established that a high nutrient concentration does not assure optimal microalgae growth. However, the perfect combination of nutrients and medium is very important. Additionally, it has been discovered that as the number of cycles rises, the *Chlorella vulgaris* species absorbs more nitrate content to achieve optimal growth. This is a result of excessive nitrogen starvation caused by the PBR's frequent usage of recycled medium, which caused the algal species to consume all the nitrate supply throughout the culture phase.

Fig. 5 also indicated that the nitrate concentration was still reducing although the biomass development had reached a saturation point on the 12th day (cycle 2). his suggests that the medium is prompting *Chlorella vulgaris* cells to synthesis optimum bio-chemical composition in

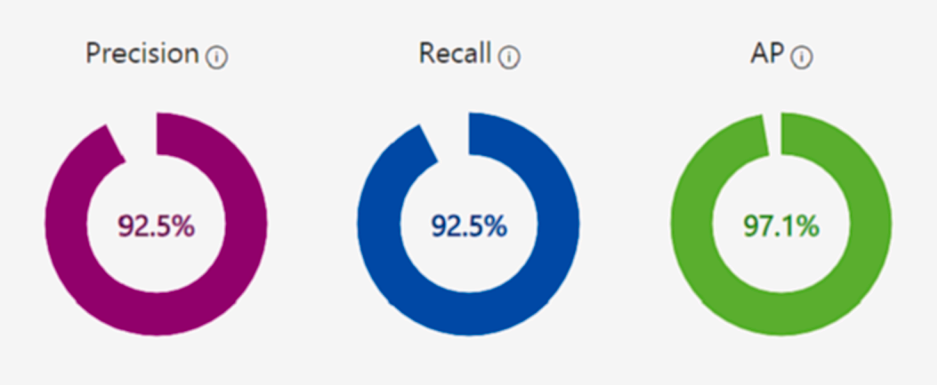

Fig. 3. Overall trained Model Performance using Custom Vision Service.

Fig. 4. Model Prediction of a quick test conducted on the 5th day of the cultivation process.

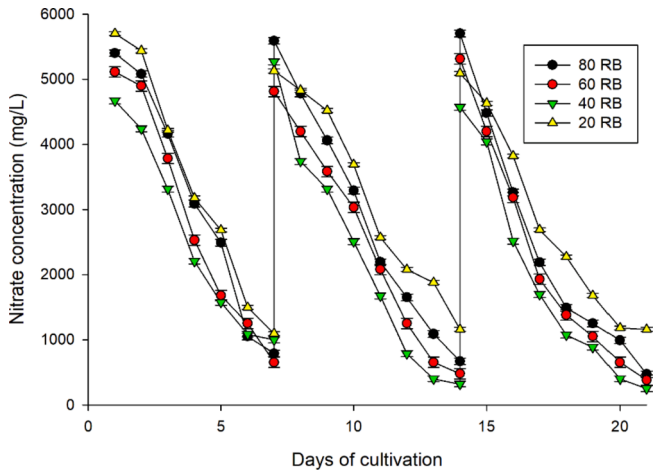

Fig. 5. Nitrate concentration for the semi-batch *Chlorella vulgaris* cultivation.

a nitrate-depleted environment. A previous study found a similar trend, with cells able to produce the maximum carbohydrate, protein, and lipid productivities whenever nitrate concentrations are low while cell maturation is constant [25]. However, on the 21st day (cycle 3), the nitrate content in the 40RB mixing ratio medium remained consistent, which correlates with its cell culture growth. This is quite significant because, despite the abundance of nutrients, the microalgal biomass in 40RB has reached its maximal growth productivity. According to Fig. 5, 60RB has the greatest nitrate removal at (87 %), followed by 80RB at (85 %), 20RB at (81 %), and 40RB at (79 %) respectively for the first batch cycle.

However, upon the second cycle the nitrate reduction rate changes where 40RB has the greatest nitrate reduction removal at 94 %, followed by 60RB at (90 %), 80RB at (88 %), and 20RB at (77 %) respectively. The variation in nutritional concentration between a fresh media and a recycled medium causes this shift. However, Loftus et al. (2019) have shown that, though including industrial biscuit medium boost the development phase in the early stages, other aspects like as light dispersion are equally important [26]. In this study, as compared to the second cycle with recycled medium integration, the first cycle has a more continuous light source onto the culture during its initial phase. Based on Fig. 5, it also proven that 40RB have the best nitrate concentration in the culture medium to generate an optimum semi-batch biomass growth for 21 days.

Polyphosphate is the most abundant speciation in microalgae, making phosphorus (P) an important resource for growth [27]. The major mineral, which is mostly consist in biscuit processing powders are phosphorus and previous research has found that biscuit manufacturing waste powders can be drawn as a feed source for microalgae development [19]. However, while using industrial biscuits manufacturing waste as a recycled medium, the cultures potentially possess different levels of phosphorus, as shown in Fig. 6. Hence, the phosphate analysis assists in identifying the appropriate recycled medium for *Chlorella vulgaris* growth in a semi batch mode. The phosphate content was measured every 7 days in this study to see if there were any variations between the initial fresh medium cycle and the recycled medium cycle. Day 7 (C1) is measured at the end of the cycle 1 (before adding fresh medium), meanwhile day 7 (*C*2) is measured in the beginning of cycle 2 (after adding fresh medium + recycled medium).

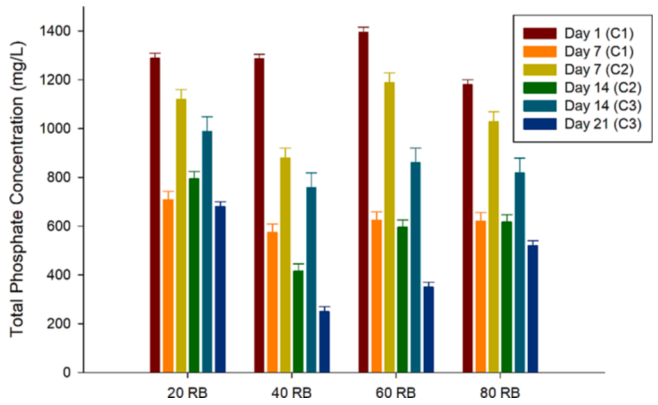

Fig. 6. Phosphate variations for the semi-batch *Chlorella vulgaris* cultivation.

The highest initial phosphorus concentration for the first batch cycle was at 1395 mg/L in 60RB, followed by 1288 mg/L, 1285 mg/L, and 1180 mg/L in 20RB, 40RB, and 80RB, respectively. A high phosphorus concentration at the beginning does not guarantee optimal microalgae production. In this study, it is observed that the rise in phosphorus during the initial phase reduces as the number of cycle increases. This is because the integrated of recycled medium consists lower phosphate content, compared to the fresh medium. Thus, determining the optimal recycled medium to fresh modified industrial biscuit waste medium ratio is essential. The consumption of phosphate was highest for 40RB during the first and second cycles, at 55 % removal, and at 67 % removal during the third cycle. On the other hand, the phosphate removal reduction for the other recycled medium combinations is in the range of 25 % to 45 %, which has an influence on biomass growth. Based on Fig. 6, 60RB had the greatest beginning phosphorus content and had 48 % of the phosphorus removed, the algal cells did not respond well to biomass development. This is because when there is an excess of phosphorus in the water, the microalgae cells to acquire minerals for effective microalgae development is diminished [28]. In additional, during stationary phase, both phosphate and nitrate limitations would create an optimal surrounding for *Chlorella vulgaris* to generate lipid, protein, and carbohydrate.

4. Bio-compositions analysis

4.1. Micrsoalgae-based lipid production

Microalgae biomass has drawn global interest due to its potential as a biofuel source. Findings reveal that using industrial biscuit manufacturing waste powder for culture promotes both microalgae biomass production and lipid content at the same time [15]. This study, on the other hand, presented a semi-batch cultivation approach based on recycled culture medium, which resulted in a more frequent high yield lipid synthesis. According to Table 2, the highest lipid content was found in 40RB for the first cycle, at 317.40 mg/g, followed by 60RB, 20RB, and 80RB, at 313.60 mg/g, 310.20 mg/g, and 308.00 mg/g indicating that the 40RB recycled medium provides optimum stress that induces lipid production. As for the following cycles, it was observed that the lipid generation reduces. This signifies that lipid deposition is influenced by the nutrients ingested throughout the culture medium's development phase as well as the nutrient deficiency stage [29]. Nutrient restriction is the major factors for developing and promoting lipid production. In this investigation, nitrate insufficiency develops on the sixth day of culture and continues through the second and third cycles. Since the recycle medium is already lacking in nitrate and phosphate, and only a portion of the new medium is consumed, the nutritional deficiency persists.

Previous research found that nutrient restriction for two days enhanced lipid generation to 260.44 mg/g without reducing biomass

Table 2
Bio-composition analysis for the semi-batch *Chlorella vulgaris* cultivation.

Culture combination	Lipid (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)
Cycle 1, (C1)			
20C1	310.20 ± 5.5	207.30 ± 2.1	326.61 ± 2.3
40C1	317.40 ± 3.2	280.57 ± 1.5	451.37 ± 0.1
60C1	313.60 ± 2.2	230.41 ± 4.2	365.39 ± 3.1
80C1	308.00 ± 4.5	205.25 ± 5.1	302.25 ± 1.8
Cycle 2, (C2)			
20C2	229.19 ± 3.1	201.98 ± 1.5	224.18 ± 1.8
40C2	286.36 ± 1.3	266.56 ± 2.3	388.70 ± 0.1
60C2	272.22 ± 2.2	213.88 ± 0.6	229.31 ± 1.3
80C2	208.44 ± 4.2	202.56 ± 2.5	218.05 ± 2.0
Cycle 3, (C3)			
20C3	117.95 ± 3.5	199.63 ± 0.8	179.96 ± 3.3
40C3	132.82 ± 2.6	254.35 ± 1.1	279.09 ± 2.1
60C3	114.06 ± 2.2	204.40 ± 2.2	231.38 ± 0.3
80C3	121.99 ± 1.3	194.92 ± 1.3	170.27 ± 0.4

*Note: Data present are the mean of three runs ± SD.

growth, indicating that it might produce biofuel at a low cost [15]. However, the highest lipid concentration in this study was 317.40 mg/g for a 40C1 ratio with less than 24 h of nutritional deprivation. Light penetration impacts lipid production because microalgae collect radiance for photosynthesis and capture bio-compound such as lipids. Microalgal biomass concentration drops when light intensity is low, leading to poor growth and a negative impact on lipid accumulation. Meanwhile, too much light induces photoinhibition, which harms microalgal photosystems and reduces lipid production [30]. As a result, adequate light distribution must be provided during culture to encourage microalgae growth. However, when recycling media is utilized, the light dispersion changes, notably in cycles 2 and 3. Thus, it is observed that 40RB ratio mixing accumulated under 6000 lx (LED light) yielded the most lipid synthesis. In summary, a culture media deficient in phosphate and nitrate with enough light distribution would develop an efficient lipid from the algae cells. It is observed that 40RB medium had the highest lipid for all three cycles at 317.40 mg/L/d (first cycle, C1), 286.36 mg/L/d (second cycle, *C*2), and 132.82 mg/L/d (third cycle, C3) respectively.

4.2. Microalgae protein extraction

Protein and polysaccharide formation is prominent in dairy industry due to their molecular content in the process cycle [31]. Nevertheless, protein has a high amino acid content and is suitable for meals, pharmaceutical products, and livestock feed [32]. As a result, the efficiency of protein production for the recycling ratio of industrial biscuit manufacturing waste coupled with BG-11 was studied. Based on Table 2, the optimal protein concentration in 40RB at 286.36 mg/g followed by 60RB, 20RB, and 80RB with protein value of 272.22 mg/g, 229.19 mg/g, and 208.44 mg/g. The proportion of nitrate uptake in *Chlorella vulgaris* cells is the primary source of protein production. Nitrates is discovered in cellular macromolecules such as proteins, polysaccharides, enzymes, and algae, and it is required for the absorption mechanism that changes nitrates to its basic formula [33]. According to Fig. 4, the algae cells absorb 94 % of the nitrate throughout culture. Table 2 lists the quantity of protein generated by *Chlorella vulgaris* grown in standard and nitrogen deficiency cultures.

Light conditions during the microalgae growing phase are also important for protein development in microalgae, as prior study has indicated that greater light source and extended photoperiod leads in higher protein synthesis [34]. In this study, light penetration varied throughout the first two days of cycles 2 and 3, depending on the integration ratio, even though the LED light distribution remained constant throughout the culture period. However, for the first batch cycle all the

PBR has a constant light distribution. Microalgae consumes light for metabolism, cell division, and nutritive value buildup, protein content fluctuates [35]. In comparison to a prior study, *Chlorella vulgaris* generated 263.93 mg/g protein concentration after 24 h of light exposure [15]. Meanwhile, this study shows that with 40RB of recycled culture media, the protein content may be kept at an optimal level (280.57–254.35 mg/g of protein content).

4.3. Evaluation of microalgae carbohydrate content

In photosynthesis, carbohydrate is the primary product *via* carbon fixation metabolism [36]. These starches are either kept in the plastids (starch) as backup supplies of the cell membranes. Nonetheless, the distribution and synthesis of carbohydrates in microalgae can vary dramatically relying on culture circumstances such as light, nitrate exhaustion, temperature shift, pH shift, and CO_2 supply [37]. In this work, nitrate restriction and light distribution were two crucial criteria that led to the formation of carbohydrate in *Chlorella vulgaris* cells. It was observed that, 40RB (451.37 mg/g) had the greatest carbohydrate value, followed by 60RB (365.39 mg/g), 20RB (326.61 mg/g), and 80RB (302.25 mg/g). Moreover, it is observed that the carbohydrate content reduces rapidly when there is a combination of recycled medium. This is due to the extremely low nitrate content at the end of the culture cycles. When microalgal cells are subjected to nitrogen-depleted growth medium, it typically exhibits biomass yield, that restricts their biochemical profile [38].

Meanwhile, the *Chlorella vulgaris* strain was able to boost lipid, protein, and carbohydrate content while maintaining microalgae development. Although the recycled medium has a lower carbohydrate content than the first batch cycle, it is still suitable for large-scale production due to the expense of the re-starting batch cycle cultivation approach. Furthermore, appropriate lighting is required for microalgal growth since light sources can generate energy that is subsequently captured in the algal cells as starches [39]. Light distribution was discovered to produce starch accumulation in algal cells, and this investigation makes use of a 6000 Lux LED light supply for 24 h throughout the cultivation period. Previous studies have showed that light intensities ranging from 40 to 400 mol/m^2s^1 enhance carbohydrate accumulation with the range of 269.62 mg/g to 343.84 mg/g [40]. Overall, 40RB recycle medium combination generates the optimum surroundings for lipid, protein, and carbohydrate concentration for a semi-batch cultivation system. Utilizing recycled media with smart algae growth monitoring device, can reduce the cost of *Chlorella vulgaris* cultivation system especially for large scale production.

5. Conclusion

There is a great demand for a clean, economical, and long-term energy source, due to the depletion of fossil fuels. This gives the algal production-based biofuel a wider energy source business standpoint, as Industry 4.0 is widely being explored. The conventional UV–vis Spectrophotometry approaches are excessively time consuming, costly, and inconvenient, preventing stakeholders from pursuing large-scale algae cultivation. As a result, modern digital technologies, and Internet of Things (IoT) were implemented by creating a mobile/digital app [FindAlgae]. This study also shows that semi-batch cultivation with recycling culture medium of 40RB ratio could yield the maximum biomass growth (4.52 g/L), lipid yield (317.40 mg/g), protein content (280.57 mg/g), and carbohydrate concentration (451.37 mg/g). Ultimately, this study proposes that 40RB of industrial biscuit manufacturing waste fresh medium combined with 40RB recycled media can be applied for semi-batch biomass production, and that a custom vision service is best used to detect *Chlorella vulgaris* development for smart data microalgae growth surveillance. These models can be further developed for downstream monitoring in near future.

CRediT authorship contribution statement

Angela Paul Peter: Investigation, Methodology, Writing – original draft. **Kit Wayne Chew:** Project administration, Supervision, Writing – review & editing. **Ashok Pandey:** Conceptualization. **Sie Yon Lau:** Resources, Software. **Saravanan Rajendran:** Resources, Software. **Huong Yong Ting:** Resources, Software. **Heli Siti Halimatul Munawaroh:** Formal analysis, Methodology. **Nguyen Van Phuong:** Data curation, Validation, Visualization. **Pau Loke Show:** Funding acquisition, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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