Chinese Chemical Letters 34 (2023) 107721

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Integrated microalgae culture with food processing waste for wastewater remediation and enhanced biomass productivity

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ARTICLE INFO

Article history:
Received 22 March 2022
Revised 19 July 2022
Accepted 2 August 2022
Available online 3 August 2022

Keywords:
Medium contamination
Food processing
Waste powder
Organic medium
Waste remediation

ABSTRACT

Waste generation from food manufacturing facilities poses a serious hazard like environmental degradation, water pollution, and land pollution due to its high nutrient composition. Specifically, solid waste (powder) disposal requires additional energy sources in terms of scientific treatment, structured collection, and disposal packaging according to the safety regulation. Thus, this research discusses the viewpoint of integrating food processing waste as an organic carbon source with BG-11 medium for *Chlorella vulgaris* (FSP-E) growth. The food processing waste powders investigated in this study were obtained from milk, and biscuit manufacturing facilities. The culture medium was modified by combining both BG-11 and food processing waste powders to identify the optimal algal growth and biochemical content. Compared to the microalgae grown in BG-11 alone (IBG), the combination of biscuit waste and IBG produced higher biomass concentration (44%), with increased lipid (11%), protein (20%), and carbohydrate (57%) contents. *Chlorella vulgaris* was able to uptake nutrients from the culture medium with combination of food processing waste and IBG thus enhancing its growth. The results obtained also indicate that an integrated culture system using food processing waste and synthetic sources can generate energy out of waste by improving the bio-composition of the microalgae biomass.

The global demand for dairy food products is increasing, and as a result, dairy industries produce variety of products while generating both solid and liquid waste [1]. A standard dairy factory produces approximately 4–11 million tons of waste annually, with a high concentration of organic matter (fat, protein, and carbohydrates) and nutrients (primarily nitrate and phosphorous) derived from milk based items [2]. All liquid waste sources are usually combined to create a drainage reservoir, where wastewater is treated before being discharged into the surrounding water system. Kolev *et al.* reported that industrial dairy wastewater consists of three major sources such as processing cooling waters, pipeline and tankers cleaning waters and sanitary waste (by-products of process) [3]. Although this type of generated effluent is highly unstable in nature, there are various advanced technologies for industrial dairy wastewater treatment such as physio-chemical process (electrocoagulation, adsorption, membrane treatment), aerobic process (sequencing batch reactor), anaerobic process (up flow anaerobic sludge blanket, hybrid anaerobic digesters, anaerobic sequencing batch reactors) [4].

Apart from wastewater, dairy food processes also generate solid waste such as leftover dust powders after packaging, waste powder recovery from dust collector following the spray-drying pro-

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https://doi.org/10.1016/j.cclet.2022.08.001
1001-8417/

cess and damage stocks [5]. Mostly, dust powders produced by dairy industry will be further processed to livestock feed or disposed by mixing it with the wastewater pond [1]. The disposal of dairy food processing waste is more challenging than managing dairy wastewater treatment. This is because powdered dairy waste tends to clog water streams and has the ability to clump together to form a hard rock texture when exposed to air. Moreover, dairy dust powders are a significant threat to the environment since milk-based dust suspensions with density of 75–1000 g/m^3 in air will combust or self-ignite upon contact with hot surfaces [6]. Hence, most industrial corporations prefer disposing out their powder waste to livestock farmers. On the other hand, researchers have ventured in utilizing the milk-based dust powders for fermentation process which enhances acetone-butanol-ethanol production by *Clostridium acetobutylicum* and *Clostridium beijerinckii* [7].

Microalgae can contribute to various sectors such as biofuels, biopharmaceuticals, and food supplementation industry. High value-added products in microalgae extracts are used for health supplements, bio-fuels, pharmaceuticals and also in cosmetics [8]. In depth, microalgae consist of high health promoting properties such as anticoagulant, antioxidants, antitumor, anticancer, and antihypertensive which widen the research scope in microalgae based processes [9]. Therefore, studies related to enhancing the cultivation of microalgae are important to achieve a promising biomass feedstock. For scaling up and commercialization of microalgae production, large quantity of cost-saving culture medium is required without compromising the nutrients content. There have been many interventions in using solid waste (Palm Oil Mill Effluent, POME waste, fertilizers) as organic culture medium for the production of microalgal biomass [10]. This strategy can be used to repurpose industrial dairy food processing waste for the cultivation of *Chlorella vulgaris*, a microalgae species that captures nitrate, phosphorus, carbon, and sulfur from wastewater [11]. Hence, this research investigates various sources of industrial dairy waste as a potential source of microalgae growth medium.

Integrated solid waste management (ISWM) is an advance and productive approach that implies various technology to reduce, reuse, recycle, and manage waste which can benefits human and natural environment. ISWM strategic varies based on the solid waste properties and mixing conditions. Hence, the integration of industrial dairy food processing waste and BG-11 medium (IBG) was proposed to determine its efficiency in enhancing microalgae productivity than IBG culture alone. The integration of mediums has high probability of increasing the carbon source which enhances the cultivation process and its biochemical properties. This would result in a more efficient microalgae growth of biorefineries in near future. Thus, the focus of this study was to examine the prospective of integrating BG-11 medium (IBG), with industrial dairy powder waste: CW, MW, BW and FW as nutrient medium to produce *Chlorella vulgaris* microalgae species. The possibility of integrating industrial dairy food processing waste for microalgae production was investigated by assessing the accumulation of algae biomass and biochemical content using four different types of organic mediums. The biomass growth efficiency was investigated by altering the industrial waste medium configurations and its impact on both biomass production and biochemical content.

Chlorella vulgaris (FSP-E) was collected from a water field in southern Taiwan, China. The preparation of inorganic medium (BG-11) was prepared as describe in previous study [12]. In this study, the batch culture of microalgae was performed for 14 days in 1 L of photo-bioreactor (PBR) glass vessel consisting of 3000 K warm white LED light intensity, maintained at 27 °C temperature and mixed air rate of 200 mL/min with 1.5% of compressed industrial grade CO_2. All the runs were performed in triplicates and its average readings were taken. Dairy companies supplied dried industrial dairy food processing waste. About 2 g of each type of industrial dairy food processing waste was mixed with 200 mL distilled water in a 250 mL beaker. The vials were rotated for 30 min at 150 rpm at room temperature. Specifically, industrial dairy powder waste: cheese waste CW, MW, BW and FW were evaluated in this study with IBG. The culture medium comprised of 200 mL industrial dairy food processing waste solution and the remaining 800 mL with IBG medium in a 1 L PBR set up. Throughout the study, all mixture combinations had a pH range of 6–7.5 which was measured using Mettler Toledo SevenCompact™ pH Meter S210, Brand (Merck).

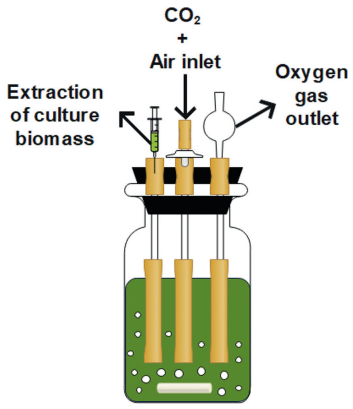

Fig. 1. Daily microalgal cell growth monitoring configuration.

The microalgal cell growth concentrations were measured using a UV–vis spectrophotometer (UV-1800, Shimadzu) at 680 nm. 5 mL of culture was filtered *via* a filter membrane, the dry cell weight (DCW) of microalgae biomass was measured. The DCW of microalgae was identified by deducting the mass of the loaded filter to blank filter (0.45 μm pore size) as shown in Fig. 1. The absorbance value obtained from the UV–vis analysis was converted to cell growth after calibration. A diluted solution of each culture mixture was utilized to prevent the impact of color shade in the absorbance measurements. For each mixture change, the clear top layer was extracted from the microalgae culture after agitation at 6000 rpm for 15 min. The biomass yield, P_b (mg L^{-1} d^{-1}), is represented by Eq. 1, and the specific growth rate, μ (d^{-1}) is represented by Eq. 2.

$$P_b = \frac{X_2 - X_1}{d_2 - d_1} \quad (1)$$

$$\mu = \frac{ln\ \frac{X_2}{X_1}}{d_2 - d_1} \quad (2)$$

where X_1 and X_2 are DCW (g/L) at d_1 (day 1) and d_2 (day 14), respectively.

On the 14th day, the entire culture biomass was centrifuged at 6000 rpm and freeze dried (−25 °C at 0.0021 mbar) to attain the biomass for further bio-composition analysis.

Nitrate concentrations were measured *via* the spectrophotometric method [13]. Samples were taken from the PBR every 24 h and diluted with distilled water before being measured at 220 nm. Standard calibration was developed by varying the concentrations of pure sodium nitrate ($NaNO_3$). Phosphorus concentration was determined by the atomic absorption spectroscopy (AAS, PerkinElmer A Analyst 400) [14]. The relationship between absorbance and phosphorus content was derived using a known concentration of dibasic ammonium phosphate, $(NH_4)_2HPO_4$. The AAS spectrophotometer detection wavelength was set at 213.6 nm to detect phosphorus. The content difference over the baseline was utilized to

evaluate the total phosphorus concentration reduction (%) as Eq. 3.

$$\text{Phosphorus removal } (\%) = \frac{C_2 - C_1}{C_1} \times 100\% \quad (3)$$

where C_1 and C_2 represent initial and final phosphorus concentration, respectively.

The Bligh and Dryer method was employed to extract the lipid content from microalgae cells [15]. The lipid, protein, and carbohydrate concentrations were measured as describe in previous study [12]. Eq. 4 describes the lipid concentration as a percentage difference (wt%) of DCW. Moreover, Bradford technique was used to determine the protein content of the isolated microalgae biomass [16]. Bovine serum albumin assay was used to establish a standard relationship between absorbance (OD_{595}) and protein concentrations. The phenol-sulphuric technique was used to quantify the carbohydrate content of the clear supernatant [17]. Using starch extracts of varying concentrations, the usual correlation between OD_{490} and carbohydrate content was discovered (Eq. 4).

$$\text{Lipid content } (\text{wt}\%) = \frac{\text{Final weight} - \text{Initial weight}}{50 \text{ mg}} \times 100\% \quad (4)$$

The active compound present in industrial dairy powder waste: cheese waste (CW), milk waste (MW), biscuit waste (BW), and animal feed waste (FW) were identified using Fourier transform infrared (FTIR) spectroscopy where the types of functional groups and its concentrations were analysed and evaluated. Four different waste samples from dairy production facilities were analysed as cheese waste (CW), milk waste (MW), biscuit waste (BW), and animal feed waste (FW), respectively. The industrial dairy waste powder was scanned using an FTIR model (Perkin Elmer Frontier, USA) with a default range of 400 cm^{-1} to 4000 cm^{-1} that allows the discovery of chemical bonds (functional groups).

The elemental composition of industrial dairy food processing waste (CW, MW, BW, and FW) was determined using energy dispersive X-Ray spectroscopy, energy dispersive X-ray spectroscopy (EDX) (FEI Quanta 400F FESEM). To detect a more precise weight percentage, all the samples were scanned at four different locations with magnification times of 200×, 1200×, 2500×, 5000×, and 6000×. The average weight percentage of each scanning are reported. The results were used to evaluate the comparison of microalgae biomass as a potential energy source. The study was carried out using an Oxford Instrument INCA 400, which offers material characterization that collects precise data at the micro-nano scales. EDX processing was used to obtain results that consists of spectra with peaks that corresponded to the actual substance properties.

The high-performance liquid chromatography analysis was conducted to identify the concentration of cobalamin in the waste powders sample. Chromatograms were recorded using a liquid chromatograph (Shimadzu, Kyoto), a UV–vis spectrophotometer (SPD 6A), and a C-R6A Chromatopac processor. The injection valve was a Rheodyne 7725i with a 100 or 20-mL loop. A reversed-phase Nova-Pack C (150 mm × 3.9 mm × 4 μm) from Waters 18 (Milford, MA, USA) type of HPLC column was used in this study. The industrial dairy food processing waste (CW, MW, BW, and FW) were diluted with water at 1:10 ratio and injected with 1 mg/L of B_{12} stock solution for solid phase extraction [18]. The specimen was then added to the column in a 200-mL aliquot. Using a reference sample provided by Fort Dodge, this technique was verified in terms of repeatability, linearity, and accuracy.

A one-way ANOVA with a two-tailed test was used in IBM SPSS Statistics version 26 for data analysis. Descriptive statistical analysis was provided as mean standard variation. The values presented are the mean of three replicates. The data was statistically analysed to determine the total of differences with a significance level of $P \leq 0.05$.

FTIR can classify chemical bonds in a molecule by generating an infrared absorption spectrum. The peaks observed in the spectrum (Fig. S1 in Supporting information), represents the existence of specific functional groups of each industrial dairy waste powder. Not all peaks are considered, as there will be some external disturbance such as frequency of the vibration and interference in light absorption through the sample which leads to the tiny peaks. The binding sites of the powders reported are based on the sample peaks detected within the absorption range [19].

All the waste powders tested for this study observed carbo sources group, as C–O and C=O stretch vibrations, suggesting the presence of both carbon and oxygen in the culture. However, nitrate and phosphate are the most essential nutrients needed in order to enhance the growth of microalgae [20]. From Fig. S1 (Supporting information), it was observed that BW and FW both had double bond nitro groups (N=O) bend as the spectrum detects peaks at 1400−1300 cm^{-1}. This gives an early indication that these two wastes will be appropriate to be replaced with IBG medium as the double bond nitro groups shares more nitrate electrons among them which act as nutrients as compared to other powders waste for the growth of microalgae. On the other hand, the spectrum detected a peak at 3252.39 cm^{-1} in MW where it can be amines – primary and secondary. As nitrate breaks loose from amines, hydrogen atom will be released. These hydrogen atoms have a high risk of forming bonds with oxygen, that further dilutes the culture medium [21]. Lack of nitro bonds peaks also predicts that MW may not be the best option to enhance microalgae growth. As for CW, it is observed that there is a mixture of both double layer nitro groups (N=O) at 1386.31 cm^{-1} and single layer amines bonds (N–H) at 1661.02 cm^{-1} [22]. Furthermore, detailed elemental analysis was carried to identify the weight percentage of each element present in the industrial dairy waste powders individually which could be able to predict the best option for replacing IBG medium for microalgae growth medium.

The industrial dairy food processing waste was used in this study are well-recognised as supply source of protein, carbohydrate, dietary fiber and minerals for human/animal diet [23]. In general, dairy-based minerals are a combination of chemically complex compounds with wide range of stability and resistant to physical and chemical assault. The mineral consistency of each waste powder varies depending on variety of factors. Physical and chemical factors such as temperature, water content, pH, preservative additives, flavours, inhibitors influence the durability and properties of dairy waste materials [24]. Therefore, elemental analysis was recommended to analyze the specific element (nutrient) present in each of the industrial powder waste sample which plays an important factor for microalgal biomass productivity.

Table 1 lists the weight percentages of carbon, oxygen, and nitrate are substantially higher than the weight percentage of other elements. The EDX spectrum peaks are shown in Fig. S2 (Supporting information). Nitrate is a fundamental component of amino acids, which is the molecular building blocks of protein [25]. Nitrate, phosphorus, and carbon forms the backbone of microalgae ($CH_{1.7}$, $O_{0.4}$, $N_{0.15}$, $P_{0.0094}$) [26]. Nitrate and phosphorus limitation in microalgae culture, can reduce the growth and biomass productivity. Based on the EDX analysis conducted, BW carries the highest nitrate content (25.56 wt%) while MW detects the highest phosphorus content (0.27 wt%). Although the elemental distributions in the powder dairy waste vary due to the sources of their origins, they still consist of both nitrate and phosphorus, which is needed for microalgae cultivation. Additional micronutrients are only necessary in minimal levels since they regulate various enzymatic activity in algal cells [27]. All four types of dairy waste contain nitrate and phosphorus with less toxic heavy metals that can replace

Table 1
Average weight percentage of elements and concentration of vitamin B_{12} (cobalamin) in industrial dairy food processing waste CW, MW, BW, FW.

Element	Average weight (%)			
	CW	MW	BW	FW
Al	0.42 ± 0.13	–	–	1.50 ± 0.39
C	56.20 ± 4.88	28.60 ± 1.18	52.35 ± 4.15	52.76 ± 4.45
Cl	0.07 ± 0.21	0.29 ± 0.02	0.25 ± 0.05	–
N	14.46 ± 0.39	2.53 ± 1.18	25.56 ± 0.11	13.66 ± 2.84
O	25.65 ± 5.97	56.90 ± 6.92	32.08 ± 3.13	29.71 ± 5.41
P	0.15 ± 0.01	0.27 ± 0.06	0.07 ± 0.01	0.01 ± 0.01
S	1.04 ± 0.10	11.41 ± 5.81	0.22 ± 0.10	1.28 ± 0.66
Si	–	–	–	1.08 ± 0.02
Zn	1.31 ± 0.67	–	–	–
Vitamin	Concentration of vitamin B_{12} (μg/100 g)			
B_{12}	46.9	47.5	46.0	41.8

the IBG medium's nutrient source. Thus, further study on the contribution of each dairy waste on the biomass productivity and production of carbohydrates, lipid and protein was carried out.

Dairy products usually have high bioavailability of vitamin B_{12} (cobalamin) as it is more easily absorbed *via* gastrointestinal tract than any other synthetic vitamins [28]. Thus, presence of vitamin B_{12} from industrial dairy waste powders used for this study was also tested. Vitamin B_{12} refers to a group of compounds known as cobalamins, all of which contain a cobalt complex with a corrin system [29]. Table 1 represents the concentration of vitamin B_{12} that was found in all the industrial dairy food processing waste used in combination with BG-11 combinations.

Considering the results shown in Table 1, MW consists of the highest concentration of vitamin B_{12} at 47.5 μg/100 g followed by CW, BW, and FW at 46.90 μg/100 g, 46.00 μg/100 g, and 41.80 μg/100 g. According to Dalto *et al.*, vitamin B_{12} concentrations fluctuate depending on the temperature at which dairy goods are stored, and external contamination exposure that could range from 25 μg/100 g to 250 μg/100 g [28]. However, the industrial dairy food processing waste used in this study was collected from left-over dust powders, and waste powder recovery from dust collector which is also extremely vulnerable to external contamination and storage temperature. On the other hand, microalgae can also absorb vitamin B_{12} as growth factor [30]. Micronutrient absorption by microalgal groups is tough since they are available in extremely low quantities in sea and fresh water. The concentration of vitamin B_{12} in seawater ranges from 0 to 70 ng/L which is still quite low to sustain vitamin B_{12} dependent microalgae development [31]. Grant *et al.* reported that microalgae require a minimum concentration of cobalamin in the range of 20–50 ng/L to thrive [32]. This implies that water bodies cannot be the only source of algal growth media, although they have the capability to influence algae growth.

Nutrients' availability, carbon dioxide and light are the main contributors for the growth of microalgae. Nitrate is the most essential source of nutrient for the enhanced microalgal biomass production [8]. Microalgae can utilize wide range of nitrate sources including ammonium, organic nitrogen, and nitrate. The productivity of cell growth and nitrate intake are proportional to each other, as the cells of microalgae aggressively consume nitrate to facilitate growth [11]. Although the BW indicates the highest average weight percentage of nitrate, it is also important to observe the growth efficiency of *Chlorella vulgaris* FSP-E with all other medium combinations. Fig. 2 demonstrates the cultivation growth of *Chlorella vulgaris* using industrial dairy food processing waste as its source of nutrients. The BW medium mix achieved the highest microalgae biomass concentration at 3.61 g/L, followed by 3.22 g/L, 3.13 g/L, and 2.78 g/L, obtained for FW, MW, and CW mixtures, respectively, while IBG medium source alone achieved a biomass production of 2.67 g/L after 14 days. Usually, the cultivation of microalgae can

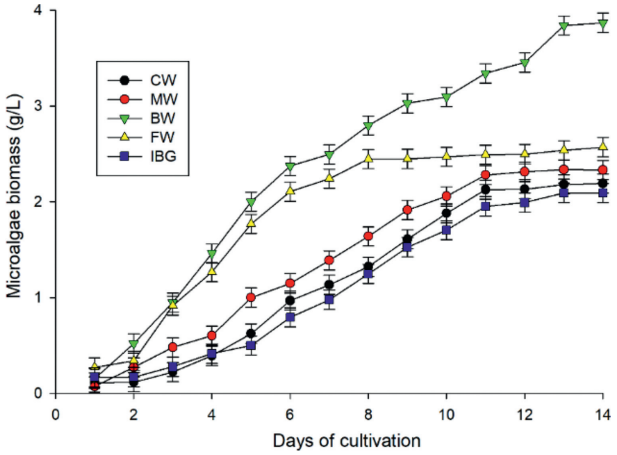

Fig. 2. Cultivation curve of *Chlorella vulgaris* grown in IBG and industrial dairy food processing waste culture mixtures.

take up to 16 to 21 days to approach the stationary growth phase [33]. However, with industrial dairy food processing waste as nutrient source, the cultivation days could be minimized to 12–14 days. This enhances the overall microalgae biomass productivity. Within 14 days, the microalgae biomass productivity growth in all types of industrial dairy food processing waste medium was observed to be higher than the inorganic medium, IBG. The growth pattern of microalgae consists of four-phases: lag, exponential, stationary, and death phase [9]. Main function of the growth media is to trigger the initial phase till the microalgae adapt to the surrounding environment and to sustain its stationary phase [34]. The presence of lipid and carbohydrate will be initiated and developed during the stationary phase [35]. Thus, the behavior of microalgae during the stationary phase decides on quality of biomass and its capability for bioenergy production. Based on Fig. 2, it was observed that FW combinations grew rapidly in the first four days and remained stationary over the next ten days, whereas BW took around 13 days to reach its stationary phase. The growth of microalgae in FW is noticed to be higher than the control inorganic IBG medium till 8^{th} day of the culture period, and eventually started to be stationary from 9^{th} day onwards. This is due to the very low amount of nitrate content left in FW medium mixture. On the other hand, the algal growth in MW, CW, and IBG was rapid for the first 11 days, and remained stationary till the 14^{th} day. This shows that the microalgae have reached it maximum productivity and may start to degrade if harvesting process does not take place. It is also not advisable to prolong the stationary growth as apoptosis of the algae may occur due to insufficient carbon and nitrate source [36]. The IBG medium source was found to be the lowest compared with all the other medium mixture combination. Apart from increasing the biomass productivity of microalgae, using the industrial dairy food processing waste would save cost and energy for the cultivation of microalgae. This is because there is no additional pre-treatment and filtration technology required for the medium preparation.

The average specific growth rates (μ) measured throughout the 14 days culture is shown in Table 2. The daily average growth rate was also reported to be highest for BW followed by MW, CW and FW as compared to the control IBG medium. Addition of industrial dairy food processing waste is likely to increase the micronutrient percentage in the culture medium which enhanced the amount of biomass produced. Hence, the combination of industrial dairy waste with BG-11 intensifies the culture mediums' nutrient content. Microalgae biomass yield (P_b) can be increased by adding a significant volume of micronutrients resulting in a higher biomass yield. It is reported that neither too high nor too low concentra-

Table 2
Characteristics of *Chlorella vulgaris* grown in IBG and industrial dairy waste culture mixtures.

Culture mixtures	DCW (g/L)	P_b (mg $L^{-1}d^{-1}$)	μ (d^{-1})
CW	3.13 ± 0.05	86.68 ± 4.23	0.25 ± 0.01
MW	2.78 ± 0.08	101.09 ± 2.89	0.29 ± 0.03
BW	3.61 ± 0.05	170.20 ± 5.41	0.37 ± 0.06
FW	3.22 ± 0.04	134.44± 2.15	0.19 ± 0.01
IBG	2.67 ± 0.05	81.08 ± 2.33	0.13 ± 0.01

Table 3
Comparisons of *Chlorella vulgaris*, biomass productivity cultivated using various organic nutrient integrated sources.

Organic nutrition	Integrated sources	Culture conditions	Biomass productivity (mg L^{-1} d^{-1})	Reference
Organic fertilizer	Bold's Basal medium	A: without CO_2, pH 6.8, B:80%	30.84 ± 8.15	[38]
Palm oil mill effluent, POME	Urea	A: without CO_2, pH 6.8–7.2, B:50%	58.40 ± 5.26	[39]
Industrial waste	Bold's Basal medium	A: with CO_2, pH 6.8, B:1%	120.01 ± 0.01	[40]
Food waste compost	BG-11	A: with CO_2, pH 7.2, B:25%	120.31 ± 7.84	[20]
Biscuit processing waste (BW)	BG-11	A: with CO_2, pH 7.4, B:20%	170.20 ± 5.41	This study

*Note: A, Carbon dioxide, CO_2 supply; B, Integration percentage.

tion of industrial dairy waste will be appropriate for microalgae biomass since residues in the medium may obstruct the microalgae's growth and biochemical composition. Hence, a pre-trial study was carried out and it was identified that 2 g of each industrial dairy waste provides the best growth for microalgae biomass for 1 L of culture medium. Moreover, the industrial dairy food processing waste has no significant influence on the color intensity of its culture medium. Unlike commercial industrial wastewater leachate, food waste compost may contribute to darken the culture medium [20]. Dark mediums prevent light from reaching microalgae, inhibiting their growth as light is an essential factor in microalgae growth quality [37]. Table 3 [20,38-40] compares the biomass growth of *Chlorella vulgaris* using other organic sources combinations with inorganic nutrients alongside with discoveries of this study. According to the culture condition comparison in Table 3, it can be concluded that the supply of CO_2 gas (50 ppm) and pH range (7–8) generates greater microalgae biomass productivity. Besides, the integration of organic culture with BG-11 is proven to be the most influential factor that influences the biomass growth and cell composition. Thus, further analysis into the biochemical composition was conducted to investigate whether the integration of expired dairy premixes can be combined with IBG to produce high quality bio-composition microalgae biomass.

During the cultivation phase, microalgae can accumulate nourishment while still removing organic matter from the medium. Nitrate and phosphorus are the most important sources for microalgae growth and these nutrients will be absorbed from its culture medium to enhance the cell formation which leads to greater biomass productivity [40]. As per the FTIR analysis (Fig. S1 in Supporting information), the composition of industrial dairy food processing waste is primarily composed of carboxylic acid, nitrate and amides functional group, which provide an adequate amount of nutrients to boost the growth microalgae biomass more than the

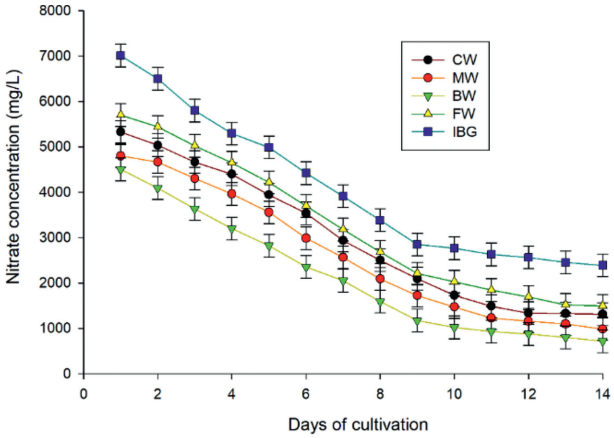

Fig. 3. Nitrate content throughout the growth of *Chlorella vulgaris* grown in IBG and industrial dairy food processing waste medium.

chemically modified nutrients sources, BG-11 [41]. Although, the industrial dairy food processing waste still has high nutrient values based on the elemental analysis, it is not appropriate for re-cycling back into dairy food manufacturing processes as these powders are exposed to open air and may lead to product quality deficiency. The potential of microalgae to effectively consume nitrate, phosphorus and B_{12} vitamin provides an advantage for dairy product manufacturers to recycle their powder waste which can save disposal cost and venture into a more sustainable production. Thus, the integration of industrial dairy food processing waste with BG-11 can improve the growth rate of microalgae and recovery of bioproducts.

Throughout 14 days of cultivation process of this analysis, the nitrate content decreased. It is observed that the nitrate reduction rate at the end was more stable compared to the initial stage. This is because, microalgae tend to consume large amount of nitrate in its initial growth phase, where the ability of phosphorus is also active [42]. The highest nitrate removal percentage within the first 8 days occurred for BW of 74% followed by MW, FW, and CW of 64%, 61%, and 60% respectively. However, for IBG medium, the nitrate reduction rate was 59%, which is the lowest compared to all the other premix combination. Although the initial nitrate content was high for inorganic medium, the growth of microalgae is slightly slower in IBG as compared with the mixture of industrial dairy food processing waste with BG-11 medium. This is because, the integration of BG-11 accelerates the growth process resulting in higher nitrate reduction percentage. Besides, the medium color has no influence on the light penetration for all culture combinations, and an equivalent volume of CO_2 is supplied to all mediums throughout the 14-day cycle. BW are observed to uptake 84% of nitrate from its medium which is the highest compared to MW (79%), FW (75%), CW (74%) and IBG (67%) combinations in Fig. 3. Previously, a study of microalgae cultivation using paper industry waste as an organic medium was conducted and it was reported that a maximum of 57% removal of nitrate was achieved at the end of a 10 days′ cultivation period [43]. Additionally, the use of food waste compost as organic cultivation medium achieves a maximum of 12% of nitrate reduction [20]. This signifies that industrial dairy food processing waste for a segment of a chemical medium could generate a comparable and perhaps greater amount of biomass as the availability of nitrate is adequate.

Inorganic culture media such as BG-11, Basal, and Zarrouk have high phosphorus content, which is necessary for microalgae production [44]. Hence, it is important that the organic medium would be able to provide sufficient phosphorus source for the growth of microalgae. According to the elemental analysis, MW

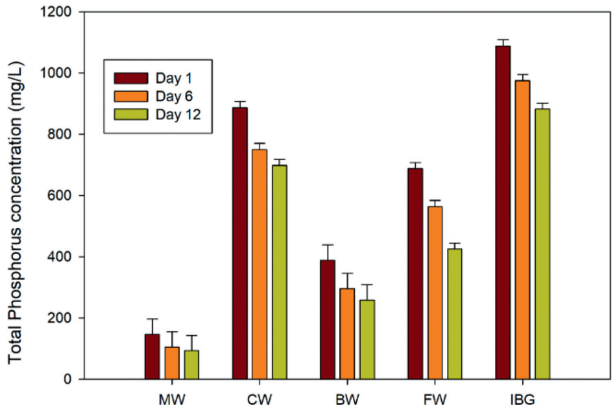

Fig. 4. Reduction trend of phosphorus content in each combination of industrial dairy food processing waste with BG-11 medium.

had the highest phosphorus content, whereas all the other industrial dairy food processing waste had about 30% lower phosphorus content. Phosphorus is the element that provides flavouring and moistness particularly for frozen food, hence MW had the most phosphorus content [45]. It was observed that the mixing of industrial dairy food processing waste with BG-11 did not extremely increase the mediums' initial phosphorus content but instead the combination modified the overall culture medium to have slightly lower phosphorus concentration as compared to IBG alone. Fu *et al.* concluded that excess amount of phosphorus could result in producing low quality microalgae biomass as it has the possibility to effects the genetic materials and energy for cell division [46]. Thus, neither too little nor too much of phosphorus content would inhibit the microalgae growth phase. In this study, the industrial dairy food processing waste were combined with IBG (ratio of 1:4) of 1 L culture, and CW measures the highest initial phosphorus concentration in comparison with MW, BW and FW. Although CW has highest phosphorus content, the biomass productivity of BW is 50% more than CW. Although the culture medium consists of high initial phosphorus content as per Fig. 4, microalgae only consume adequate amount of nutrients that are needed for its growth as the requirement of phosphorus differs in different microalgal species [47]. IBG consists highest phosphorus concentration with lowest phosphorus removal percentage of 18.93%. However, MW indicates the highest removal at 36.30%, followed by BW, FW and CW at 33.68%, 31.23% and 21.31% phosphorus removal, respectively. MW uptakes more phosphorus than other industrial dairy food processing waste due to its lowest initial phosphorus content. This is because, under phosphorus limited conditions, microalgae species tend to uptake all the available phosphorus in the medium for its cell survival as well as enhance its lipid accumulation [46]. Hence, it is predicted that MW and BW have possibilities to be the best combination of industrial dairy waste and IBG for microalgae cultivation. Detailed analysis on lipid, protein and carbohydrate were conducted to identify the best industrial dairy waste culture combination.

Lipid content produced from microalgal biomass varies according to the types of culture medium used for growth as listed in Table 4. The lipid concentration for MW was the highest at 239.69 mg/g, followed by BW, FW, and CW, at 238.20 mg/g, 220.95 mg/g, and 220.15 mg/g, respectively. In comparison with the IBG medium, the biomass had the lowest lipid production all the medium combinations at 211.99 mg/g. The proportion of phosphorus in the culture medium affects lipid accumulation, and it is well known that lipid production is optimum if phosphorus is lacking [47]. The high initial phosphorus content in IBG does not provide the cells a stress condition during its growth phase which

Table 4
Bio-chemical concentration of *Chlorella vulgaris* in IBG and industrial dairy waste mediums.

Culture mixtures	Lipid (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)
CW	220.15 ± 5.50	192.99 ± 4.10	293.09 ± 8.00
MW	239.69 ± 2.10	189.99 ± 3.90	314.01± 1.90
BW	238.20 ± 5.50	227.45 ± 0.90	425.89 ± 0.40
FW	220.95 ± 4.10	225.69 ± 3.00	235.05 ± 4.10
IBG	211.99 ± 5.60	182.77 ± 0.20	134.58 ± 4.40

also leads to a lower lipid accumulation and MW based biomass generates the highest lipid content due to the phosphorus starvation condition. Similar previous studies have been conducted on *Chlorella* species algae, and it was reported that culture mediums with optimum temperature, light intensity and nitrates stress conditions can also activate the lipid synthesis [48]. In this study, the temperature, agitation, duration, and light intensity were kept constants for all medium combinations to avoid any additional energy input to the overall cultivation process.

IBG had the highest initial nitrate content followed by FW, CW, MW and BW. Growing microalgae in a nitrate-restricted setting could influence and prompt the reduction of biomass growth to occur which also consequently effect the overall lipid productivity. Previous study reported that lack of nitrate availability in the culture media, not only increases the lipid concentration but also cause a decrease in carbohydrate [49]. This is because, nitrate starvation blocks the starch synthesis as lipid and carbohydrate paths compete for a common carbon precursor. Hence, in this study one stage cultivation was carried out in which the medium contained the desired nitrate concentration, and as the culture grew, its nutrients started depleting, gradually leading to starvation. Nitrate levels must be regulated during the culture process to retain the nutrient content of a medium and avoid disruption to the cell membrane, biomass degradation, and other changes in cell structure [50]. Development of lipids and biomass is species-dependent in each of these techniques. In short, all the industrial dairy food processing waste combinations generate higher lipid content than IBG due to greater phosphorus starvation culture conditions which enhanced efficient lipid accumulation in the biomass. The result obtained indicates that BW attain an optimum lipid production among all the other culture medium combinations. A previous research also supports the use of industrial biscuit processing waste in conjunction with BG-11 for microalgae cultivation [12].

Protein can be characterized as an extremely long chain polyamide. The amides contain nitrate, which comprises about 16% of protein content [51]. During the elemental analysis, almost all of the industrial dairy food processing waste contain amide. Even so, after its combination with BG-11, the overall nitrate content must be considered, particularly for microalgae cultivation. Rahpeyma *et al.* reported that microalgae has the ability to store protein in different parts of the cell like cytoplasm, organelles, plastids, cell wall in the cultivation phase and protein extraction occurs by disrupting microalgae cell walls to extract the protein which can be processed into useful products [52]. The protein content was derived from the biomass of microalgae and the highest protein content was identified in BW at 227.45 mg/g, followed by FW at 225.69 mg/g, and CW at 192.99 mg/g. The protein concentration for MW and IBG are less compared with the other culture combinations, containing 189.99 mg/g and 182.77 mg/g, respectively as displayed in Table 4.

Xie *et al.* indicated that there will be slight decline in the protein content if the culture medium consists of excessive nitrate concentration. Nitrate in the medium will gradually deplete causing nitrate starvation while the biomass growth occurs. Protein content proportionally improved with increase in starvation time,

but excessive extension of starvation time results in lower protein content [53]. As per Fig. 3, the consumption of nitrate was more than 50% for all the organic and inorganic combinations in the first 7 days and from the 8^{th} day onward, starvation time begins. There was no excess nitrate supply to the *Chlorella vulgaris* cells in between the cultivation process, therefore protein accumulation was not inhibited. The starvation time was considered for all industrial dairy waste combination, it was observed that the nitrate concentration of BW was the lowest at 420 mg/L which resulted in highest protein content. The improvement of protein content in BW was found to be 24% more as compared to the inorganic medium. Hence, the integration of industrial dairy food processing waste with BG-11 provided adequate nitrate for microalgae growth as well as effective protein accumulation.

In majority of a biomass conversion technologies, carbohydrates are the main substrate for production of biofuels such as bioethanol, bio-hydrogen, and biogas. The production of carbohydrate serves two main purposes for microalgae; they act as structural components in the cell walls, and as storage component inside the cell [54]. Carbohydrate as storage compound provide the energy needed for the metabolic processes which allows microalgae to survive in harsh conditions such as temporary survival in dark conditions. The carbohydrate content of biomass depends on the microalgal species and on the conditions of cultivation. Ravindran *et al.* reported that microalgae species such as *Porphyridium cruentum* and *Spirogyra* have the ability to generate 40%–57% and 33%–64% of carbohydrate content, respectively [55]. Nevertheless, for the biofuel production to be maximized, high content of carbohydrate has to be combined with the capability of algae species to produce high biomass productivity. Since the combination of industrial dairy waste with inorganic medium enhance the biomass production, its carbohydrate content is taken into consideration for the selection of optimal combination. The carbohydrate concentration of *Chlorella vulgaris* biomass developed in multiple culture integration is shown in Table 4. The highest carbohydrate content was observed in MW, (425.89 mg/g) followed by BW (314.01 mg/g), CW (293.09 mg/g), FW (235.05 mg/g), and IBG (134.58 mg/g).

As compared to the combination of food compost using BG-11, it is measured that BW has 57% more carbohydrate content [20]. This can be due to the high carbon content in the industrial dairy food processing waste as shown in Fig. S1. CO_2 gas is also the major carbon source that promotes the growth of microalgae photosynthesis cells and allows carbon fixation metabolism. In addition to the presence of organic carbon in industrial dairy food processing waste, the addition of industrial CO_2 boosts carbohydrate bioaccumulation in microalgae cells [56]. Besides, nitrate starvation was used to monitor the partitioning of biomass, while the stress conditions coupled with high light intensity, sufficient CO_2 concentration results in high total carbohydrate content. Whereas, for the generation of lipid and protein an environmental stress due to nitrate starvation is adequate. Hence, in this study, the carbohydrate content is observed to be higher than the lipid and protein composition from the cultivated *Chlorella vulgaris* biomass. Overall, this study validates that BW attained an optimum combination of carbohydrate, lipid, and protein production among all the other culture medium combinations.

In conclusion, integration of the industrial dairy food processing waste with IBG culture for the growth of microalgae provides numerous economic and environmental advantages. In comparison to the non-integrated medium, the mixture of BW as growth media offers the greatest microalgae production (146.63 mg L^{-1} d^{-1}), lipid yield (238.20 mg/g), protein (227.45 mg/g), and carbohydrate concentration (314.01 mg/g). As compared to the BG-11 culture media (IBG), the integration of organic medium (BW) significantly increases biomass production, lipid, protein, and carbohydrate content by 44%, 11%, 20%, and 57% respectively. Overall, this study proves that industrial dairy food processing waste can be integrated with BG-11 medium for microalgae cultivation process with further investigation on modern digital technologies for large scale smart production plant in near future.

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.

Acknowledgments

This work was supported by the Fundamental Research Grant Scheme, Malaysia (No. FRGS/1/2019/STG05/UNIM/02/2) and MyPAIR-PHCHibiscus Grant (No. MyPAIR/1/2020/STG05/UNIM/1). This work was also supported by the National Natural Science Foundation of China (No. 41876124).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.08.001.

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