Received: 6 April 2019 | Revised: 3 July 2019 | Accepted: 5 July 2019

DOI: 10.1002/mbo3.913

ORIGINAL ARTICLE

MicrobiologyOpen Open Access WILEY

# Production of single cell protein from manure as animal feed by using photosynthetic bacteria

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**Funding information**
Thailand Research Fund, Grant/Award Number: MRG6280016

## Abstract

To reduce the cost of protein feedstock for animal feed, the use of single cell protein (SCP) produced from waste of animal agriculture is an interesting choice. This study reveals that chicken manure was the best substrate for SCP production by submerged fermentation using photosynthetic bacteria compared to swine, cow, and buffalo manure. Regression analysis showed that the productions were found to be significantly influenced by chicken manure content, inoculum size, and cultivation time. Response surface methodology based on central composite design generated the optimal condition (15% chicken manure, 30% inoculum size and cultivation time for 14 days) at which biomass, protein, and carotenoid productions were increased by 92.3%, 21.6%, and 18.2%, respectively. The percentage of error between the predicted and actual values for biomass, protein, and carotenoid productions were 1.56%, 2.64%, and 2.09%, respectively, which indicates the precision of the model. To verify the quality of SCP, the bacterium was cultured in a photobioreactor to investigate amino acid composition, protein, and nucleic acid contents. The SCP yielded 62.7% protein with essential amino acids including lysine, methionine, threonine, phenylalanine, leucine, isoleucine, valine, histidine, and low nucleic acid content of 4.52%. This study suggests an alternative SCP production for animal feed as well as the strategy for animal waste management.

**KEYWORDS**
central composite design, chicken manure, response surface methodology, *Rhodopseudomonas faecalis*, single cell protein

## 1 | INTRODUCTION

There have been significant changes in the global animal feed price which will impact the price of animal agriculture (Einstein-Curtis, 2018; Thornton, 2010). The feed cost accounts for 60%–70% of total livestock production costs, and there is an increasing reliance on soybean meal and fish meal for protein supply in feeds (Khatoon et al., 2016). To date, the rising costs of protein feed ingredients have largely been experienced by livestock, poultry, and aquaculture producers, often with significant financial loss (Bandara, 2018; Batal, 2009; Manceron, Ben-Ari, & Dumas, 2014; Singh, Paul, & Giri, 2018; Tona, 2018). However, higher costs of production have to be reflected in higher prices for meat and eggs. Consequently, the issues of alternative protein feed sources have generated as public concern.

Single cell protein (SCP) is one of the materials for appropriate protein source and feed formulation because it can be used as protein supplement to replace the costly protein materials and massive quantities of SCP can be produced in a short time (Chee,

Lakshmanan, Jeepery, Hairudin, & Sudesh, 2019; Ritala, Hakkinen, Toivari, & Wiebe, 2017). It is considered as a promising product to solve the problem of the high price of protein feed (Golaghaiee, Ardestani, & Ghorbani, 2017; Reihani & Khosravi-Darani, 2018). Alloul et al. (2018) have reported that SCP can be produced on industrial wastewater to produce the protein feed and simultaneously treat wastewater in environmental management. Currently, there is an increasing demand for carotenoids for poultry, farmed fish, and crustaceans. Since these animals cannot produce carotenoids by their own, the inclusion of carotenoids in their diets is needed. Carotenoids enhance immune system and scavenge harmful reactive oxygen species, resulting in utilizing them as feed supplement (Anbazahan et al., 2014). The color of egg yolks, fish meat, and shrimp meat depend on carotenoid combinations in the diets (Das & Biswas, 2016; Garcia-Chavarría & Lara-Flores, 2013; Nimalaratne, Wu, & Schieber, 2013). Natural carotenoids supplementing in the diet help animal producers to attain the color that are preferred by their customers.

Photosynthetic bacteria are the known microorganisms utilized as SCP for decades. They provide high amount of protein content with all essential amino acids, carotenoids, vitamins, and other high-value products (Wang et al., 2017, 2016). *Rhodopseudomonas faecalis* PA2 has been reported as the potential photosynthetic bacterium utilized as SCP. The dried biomass of this bacterium contained not only large amount of protein content but also carotenoids, lipid, and polyunsaturated fatty acids including omega-3 and omega-6 fatty acids (Patthawaro, Lomthaisong, & Saejung, 2019; Saejung & Ampornpat, 2019; Saejung & Apaiwong, 2015; Saejung & Puensungnern, 2018; Saejung & Thammaratana, 2016). Previous study has reported that this bacterium can be utilized as diet in the freshwater fairy shrimp *Streptocephalus sirindhornae*. Not only did it enhance survival rate and growth performance of the fairy shrimp but it also reduced ammonia, nitrite, and nitrate concentrations of the water compared to those fed with the alga *Chlorella vulgaris* and the yeast *Saccharomyces cerevisiae* (Saejung, Chaiyarat, & Sa-no-amuang, 2018). These findings suggest that this strain is a good candidate as SCP for animal feed.

Animal wastes predominantly include manure from cow, buffalo, swine, and poultry. There could be a serious disposal problem about animal manure because they have the potential to contaminate both surface and groundwater (Borowski et al., 2017; Lin et al., 2017; Wu et al., 2017). Therefore, manure management is the important issue for environmental policies in order to minimize the waste and to reduce pollution. Recently, the bioconversion of wastes to produce SCP has gained particular interest as an alternative protein feed due to inexpensive cost. Animal manure is considered as the potential sources of recyclable N, P, K, micronutrients, and organic matter (Gaind, 2014). However, few research of SCP produced from animal manure has been done in the literatures (Garcia et al., 2019; Vrati, 1984). Most studies have focused on the production of SCP from poultry manure by using yeast such as *Candida* sp., *Saccharomyces* sp., and *Rhodotorula* sp. (El-Deek et al., 2009; Jalasutram, Kataram, Gandu, & Anupoju, 2013). Hence, the objectives of the present study were to produce SCP by the photosynthetic bacterium *R. faecalis* PA2. The cow manure, buffalo manure, swine manure, and chicken manure were used as sole substrates to produce biomass, protein, and carotenoid productions. The response surface methodology (RSM) based on a central composite design (CCD) was used to determine the optimal culture condition (animal manure content, inoculum size, and cultivation time). Amino acid composition, protein content, and nucleic acid content of SCP produced from animal manure were also investigated.

## 2 | MATERIALS AND METHODS

### 2.1 | Microorganism and culture condition

Carotenoid-producing photosynthetic bacterium *R. faecalis* PA2 was used. This bacterium was cultivated in glutamate–malate (GM) medium pH 6.8. The temperature was controlled at 30 ± 2°C using a Lauda Alpha A immersion thermostat (LAUDA-Brinkmann, LP.). Incubation was carried out under anaerobic/phototrophic condition at light intensity of 4,000 lux for 72 hr (Saejung & Apaiwong, 2015).

### 2.2 | Preparation of animal manure media

Swine manure, chicken manure, cow manure, and buffalo manure were collected from agricultural farms located in Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. After drying and crushing, 10% (w/v) of each animal manure was mixed with reverse osmosis (RO) water and incubated at 45°C for 7 days to dissolve the organic compounds and minerals in the manure. The manure suspension was centrifuged at 7,168 *g* 4°C for 20 min to separate the sediment. The pH was adjusted to 6.8, and sterilization was carried out at 121°C for 20 min. Swine manure medium, chicken manure medium, cow manure medium, and buffalo manure medium were used as sole substrates without additional nutrients in the experiment.

### 2.3 | Optimization of animal manure media for biomass, protein, and carotenoid productions

The experiments were carried out in a 250-mL glass bottles filled completely with animal manure media and 20% inoculum (initial biomass concentration of 0.21 g dry biomass/L). Nitrogen gas was flushed into the bottles to remove oxygen. The cultures were incubated for 10 days under light intensity at 4,000 lux. Temperature was controlled at 30 ± 2°C by using immersion thermostat. Biomass production, protein production, carotenoid production, ammonia concentration, and pH were analyzed at intervals of 48 hr. The experiments were done in triplicate.

### 2.4 | Analytical methods

Biomass concentration was determined by stoichiometry calculation (Rumana, 2013), and the relationship between the optical

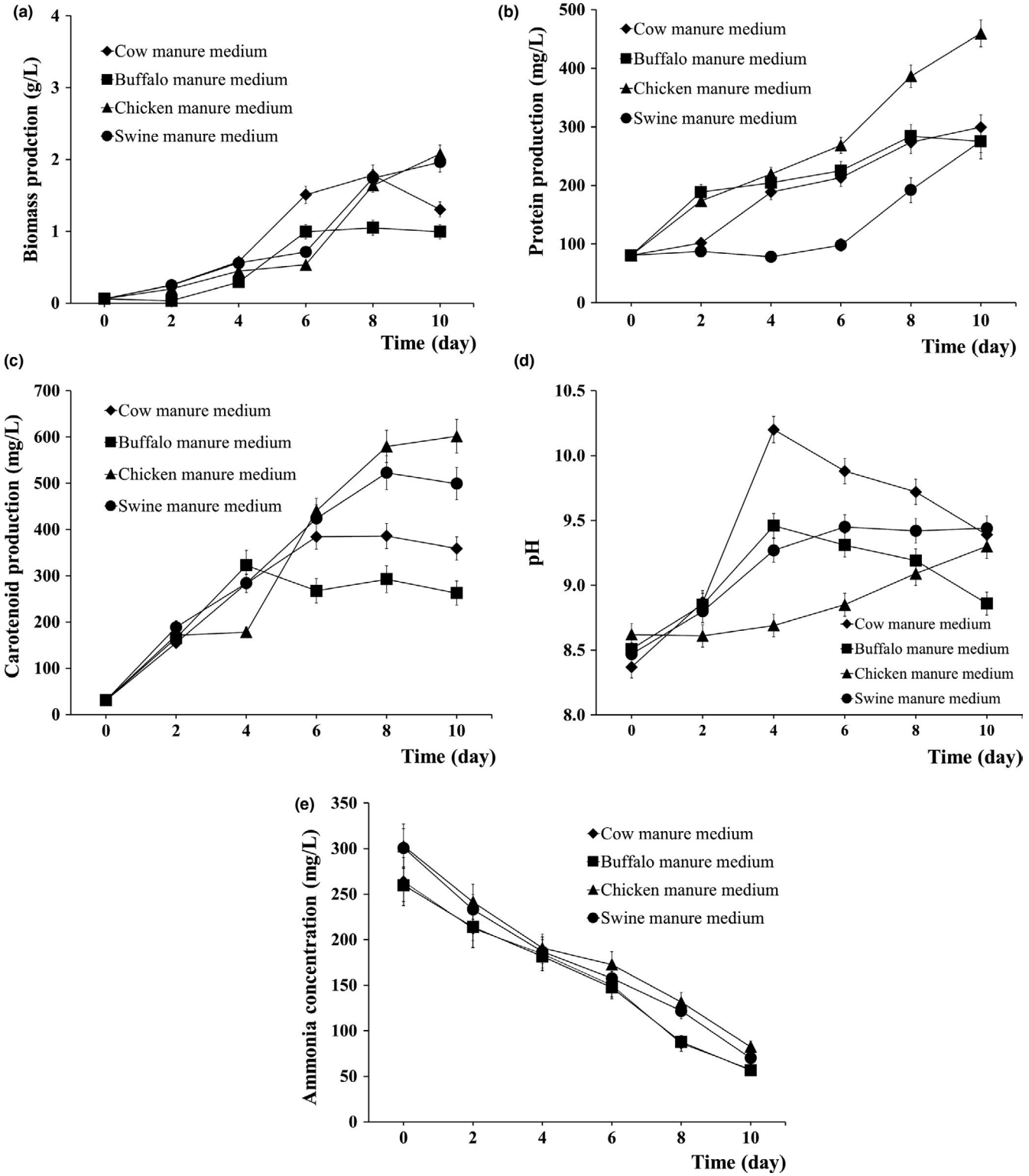


**FIGURE 1** Biomass productions (a), protein productions (b), carotenoid productions (c), pH profiles (d) and ammonia concentrations (d) of *Rhodopseudomonas faecalis* PA2 grown in animal manure media

density (OD) and biomass is presented in Equation 1. Carotenoids were extracted according to Hirayama (1968). Briefly, the cell pellets were separated from culture broth by centrifuging at 4,032 *g* 4°C for 20 min (Himac CR20B2; Hitachi, Tokyo, Japan) and washed twice with 0.9% saline solution. Carotenoid extraction was performed by using methanol–acetone (2:3 v/v) solvent. The extraction process was repeated until the colorless residues were obtained. To analyze total protein content, protein extraction was

carried out as described by Sheng, Yu, and Yu (2005). The protein content was measured by spectrophotometric protein assay (Warburg & Christian, 1941). Ammonia concentration was conducted by centrifuging 50 ml of culture broth at 4,032 *g* 4°C for 15 min. The cell pellets were discarded. The 1 ml of K-Na tartrate was added into the supernatant followed by the addition of 2 ml Nessler reagent. The absorbance was read at 425 nm (Golterman, 1991). The pH was measured by pH meter (PCTestr 35, Eutech Instruments Pte Ltd).

$$y = 0.4674x + 0.0721 \quad (1)$$

where *y* represents the OD value at 660 nm and *x* represents the biomass concentration (g/L).

## 2.5 | Optimization of culture condition for biomass, protein, and carotenoid productions by conventional approach

To study the optimal culture condition, the suitable animal manure medium mentioned in previous section was chosen as the basal medium. The parameters including animal manure content, inoculum size, and cultivation time were optimized by one-variable-at-a-time (OVAT). The animal manure content was between 10% and 60% (w/v). The effect of inoculum concentration was evaluated by adjusting inoculum size ranging from 10% to 30% with an interval of 5%. Inoculum preparation was done by cultivating the bacterium to reach the $OD_{660}$ at 0.5, initial biomass of 0.21 g dry cell/L. The cultivation time was 22 days. The cultures were incubated at 30 ± 2°C under light intensity at 4,000 lux. Biomass production, protein production, and carotenoid production were analyzed at intervals of 48 hr. The experiments were done in triplicate.

## 2.6 | Optimization of culture condition for biomass, protein, and carotenoid productions by statistical approach

In this experiment, the optimal ranges for animal manure content, inoculum size, and cultivation time were chosen based on the OVAT method. To further improve biomass, protein, and carotenoid productions, the RSM based on a CCD was employed to determine the optimal condition. Animal manure content (A), inoculum size (B), and cultivation time (C) were defined as the independent variables. The optimization studies were designed using the statistical Design-Expert software version 7.0.0 (Stat-Ease Inc.). All the variables were taken at a central coded value considered as zero. The minimum and maximum ranges of each variable and the full experimental plan were evaluated and represented by +1, 0, and −1 for the maximum, central, and minimum values, respectively. The CCD experimental design consisting of $2^3$ factorial points, 6 axial points and 6 replicates at the central point, leading to 20 runs were performed. To predict

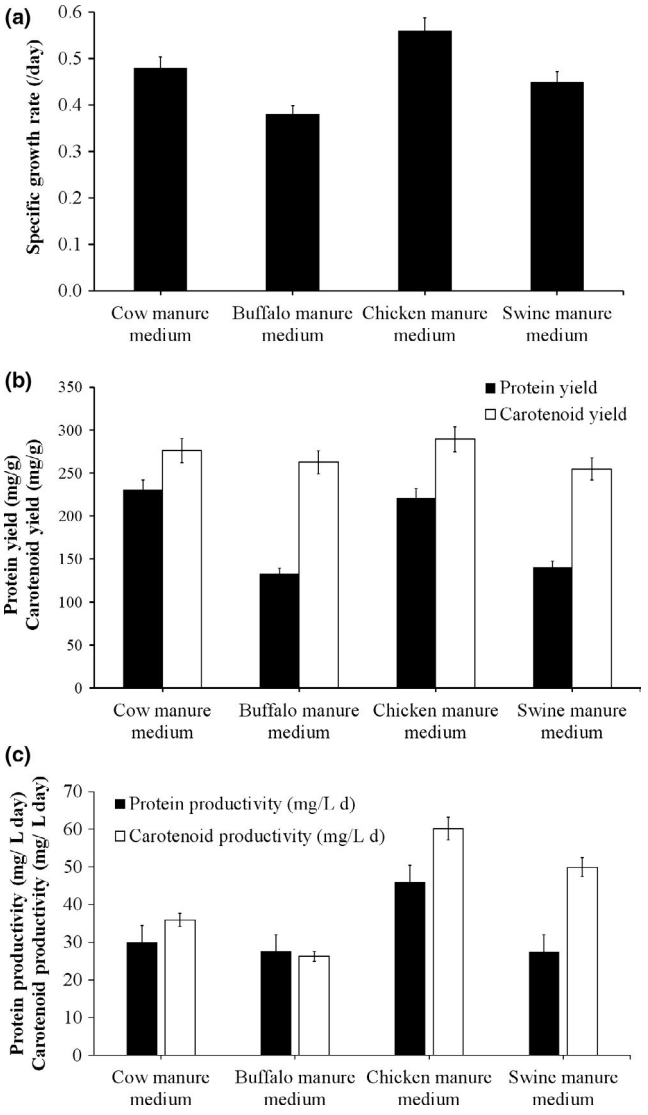


**FIGURE 2** Maximum specific growth rate (a), protein yield and carotenoid yield (b), protein productivity and carotenoid productivity (c) of *Rhodopseudomonas faecalis* PA2 grown in animal manure media

the optimal point of the three responses including biomass production ($Y_1$), protein production ($Y_2$), and carotenoid production ($Y_3$), the second-order polynomial equation was selected and expressed as Equation 2.

$$Y_N = b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2 \quad (2)$$

where $Y_N$ represents the predicted response, $b_0$ is the intercept coefficient, $b_1$, $b_2$, and $b_3$ are the linear coefficients, $b_4$, $b_5$, and $b_6$ are the interactive coefficients, and $b_7$, $b_8$, and $b_9$ are the quadratic coefficients. A, B, and C are the independent variables (animal manure content, inoculum size, and cultivation time).

To confirm the maximum biomass, protein, and carotenoid productions predicted by the model, the new set of these productions

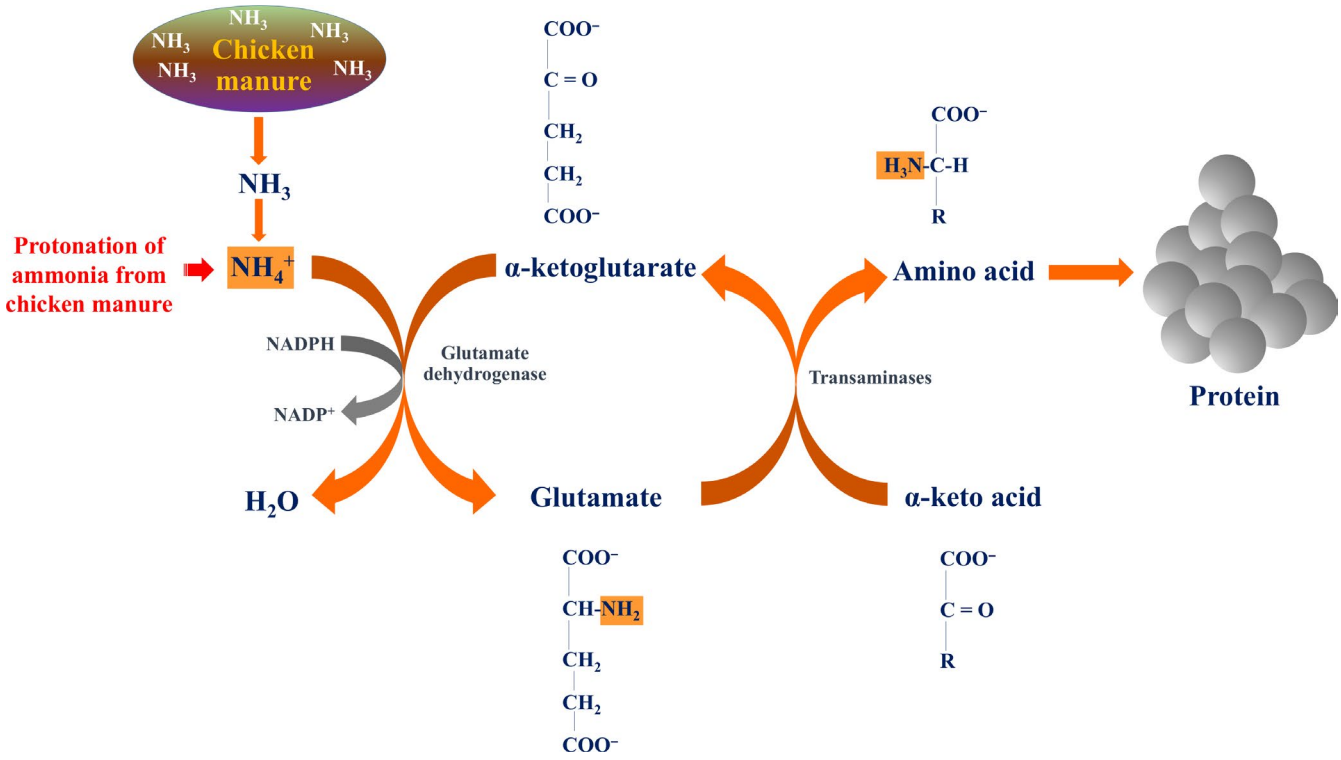


**FIGURE 3** The conceptual pathway represents protein biosynthesis using ammonia from chicken manure as the nitrogen source and stoichiometry of the pathway

were performed. The experiments were carried out in triplicate. The significance of the regression coefficient was determined. Statistical analysis of the model was performed by the analysis of variance (ANOVA). *p* value, *F* value, $R^2$, adjusted $R^2$ (adj $R^2$), coefficient of variation (C.V.), lack of fit, and adequate precision were also determined. The response surface plots and contour plots were drawn to illustrate the interaction of variables and to indicate their optimal levels. Moreover, validation of the model was performed and % error was calculated according to Equation 3.

$$\%\,\text{Error} = \frac{(\text{Observed value} - \text{Predicted value})}{\text{Predicted value}} \times 100 \qquad (3)$$

## 2.7 | Batch cultivation in a photobioreactor

The optimal condition (animal manure content, inoculum size, and cultivation time) obtained from statistical analysis was used to cultivate *R. faecalis* PA2 in a 5-L photobioreactor. Anaerobic/phototrophic condition was provided by flushing nitrogen gas into the reactor, and light intensity was controlled at 4,000 lux. The cultures were incubated under agitation speed at 150 rpm. The experiment was done in triplicate. Kinetic parameters for biomass, protein, and carotenoid productions were analyzed at intervals of 48 hr. At the end of the cultivation, the cell pellets were separated from supernatant and dried at −80°C in a freeze dryer (FreeZone 2.5L; LABCONCO).

## 2.8 | Determination of protein content, amino acid composition, and total nucleic acid content in SCP

The freeze-dried biomass was used to determine the protein content by the Kjeldahl method. Amino acid composition was investigated by an in-house method with detection by gas chromatography–mass spectrometry (GC–MS; GC Model 6890/MS Model 5973; Agilent Technologies, California, United States; AOAC, 2000). Total nucleic acid content was analyzed as previously described by Karklinya, Birska, and Limarenko (1989).

# 3 | RESULTS AND DISCUSSION

## 3.1 | Optimization of animal manure media

Figure 1a plots the growth of *R. faecalis* PA2 in the animal manure media. Animal manure is rich in carbon and nitrogen sources which can be readily assimilated by bacteria for growth and metabolism (Gaind, 2014). The logarithmic phase of the strain grown in cow manure medium appeared between 2 and 8 days, and then the growth was decreased. The growth of this strain grown in buffalo manure medium was increased between 2 and 6 days and then stabilized. On the other hand, the logarithmic phase of the bacterium grown in chicken and swine manure media appeared after 6 days of cultivation. At the end of the experiment, the highest biomass production was given by this bacterium grown in chicken manure medium. Similarly, the highest protein and carotenoid productions were observed in *R. faecalis* PA2 grown in chicken

manure medium (Figure 1b,c). The pH changes during bacterial growth are shown in Figure 1d. The pH fluctuated during bacterial growth in the manure media, possibly because the bacterium produced various organic acids and other compounds through nutrient assimilation (Ma et al., 2018). The pH was increased with a pH between 8.37 and 10.20. The increase is probably due to the consumption of volatile fatty acids in the substrate (Alloul, Wuyts, Lebeer, & Vlaeminck, 2019) and the presence of ammonia in the media. Ammonia is a by-product of animal waste due to the partial conversion of feed nitrogen into animal product (Rostami, Monaco, Sacco, Grignani, & Dinuccio, 2015). Ammonia actually exerts a toxic and inhibitory effects on microbial growth (Rajinikanth, Daniel, & Gursharan, 2013). This study showed that *R. faecalis* PA2 was able to utilize ammonia as a nitrogen source. Figure 1e indicates a significant decrease of ammonia concentration in the animal manure media, suggesting the uptake efficiency of ammonia by this bacterium.

As shown in Figure 2a, the maximum specific growth rate of *R. faecalis* PA2 grown in chicken manure medium was the highest. Similar results were also found in the protein yield in term of biomass (protein production/biomass concentration), protein productivity, carotenoid yield, and carotenoid productivity (Figure 2b,c). It has been suggested that chicken manure contains high nitrogen content (Callaghan, Wase, Thayanithy, & Foster, 2002) and the concentrations of free ammonia presenting in the medium was up to 300 mg/L (Figure 1e). Ammonia is an important nitrogen source for amino acid synthesis in bacteria. Other nitrogen sources such as nitrate and $N_2$ must be reduced to ammonia before use in protein synthesis. Ammonia in the manure is more reduced than other forms of inorganic nitrogen that can be incorporated into organic material directly for protein synthesis. In the presence of high ammonia concentration such as chicken manure, bacteria assimilated ammonia by reductive amination pathway (Willey, Sherwood, & Woolverton, 2013). Ammonia is incorporated into α-ketoglutarate to form glutamate, catalyzed by glutamate dehydrogenase. Once incorporated, the nitrogen in glutamate can be transferred to other carbon skeletons by transaminase enzymes to form other amino acids for protein biosynthesis as shown in Figure 3. This phenomenon might explain the protein production by *R. faecalis* PA2 grown in chicken manure medium. Previous research has shown that chicken manure is rich in various nutrients, including ammonia and potassium, and contains amount of trace elements such as Al, Ca, Fe, Mg, P, B, Mn, and Zn which are essential for microbial growth (Han, Rusconi, Ali, Pagkatipunan, & Chen, 2017). According to the productivities, chicken manure medium was chosen in the subsequent experiments. Although chicken manure can be traditionally used as organic fertilizer, its application in agriculture leads to environmental problems such as eutrophication and pollution of surface and ground water, production of pathogens, greenhouse gas emission, and odor (Bayrakdar, Molaey, Surmeli, Sahinkaya, & Çalli, 2017; Borowski et al., 2017). Therefore, utilization of chicken manure for SCP production is an interesting aspect.

## 3.2 | Optimization of culture condition by conventional approach

### 3.2.1 | Effect of chicken manure content

The previous experiment showed that chicken manure medium was the effective substrate. The content of chicken manure is important; thus, it was optimized by OVAT to define the optimal range. In this study, the chicken manure contents were evaluated ranging from 10% to 60%. The biomass, protein, and carotenoid concentrations of *R. faecalis* PA2 grown in chicken manure medium containing different chicken manure contents were continuously increased, and they were maximized on day 10 (Appendix Figure A1). Therefore, the biomass, protein, and carotenoid productions on day 10 are summarized as illustrated in Figure 4. The biomass, protein, and carotenoids showed the highest concentrations at 10% chicken manure, followed by 20%. Further increase in chicken manure contents decreased biomass, protein, and carotenoid productions. This incidence might associate with substrate inhibition occurring at high substrate concentration, leading to descending the curves as the chicken manure contents increased (Reed, Lieb, & Nijhout, 2010). The high concentration of ammonia has two detrimental effects on microorganisms by inhibiting the enzyme or by diffusing into the cells and leading to a proton imbalance (Kayhanian, 1999). When chicken manure was present in the media higher than 10%, the concentration of free ammonia was higher than 300 mg/L as presented in Figure 4, implying that the excessive ammonia caused growth inhibition. Previous study has shown that the yeast *Candida utilis* could not grow in the digested and undigested poultry litter without pretreatment for SCP production which might be due to the presence of complex sugars and proteins. Acid hydrolysis must be employed to hydrolyze the complex nutrients (Jalasutram et al., 2013). Therefore, this work revealed that *R. faecalis* PA2 was able to utilize nutrients in the chicken waste without acid hydrolysis which is a cost- and time-effective method for SCP production. From the results, chicken manure contents ranging from 10% to 20% were chosen.

### 3.2.2 | Effect of inoculum size

Inoculum size is an important factor for SCP production. Recent research has reported that the excessive inoculum concentration caused decomposition of microbial cell, whereas insufficient inoculum size could lead to a slow growth (Jalasutram et al., 2013). The levels of inoculum were between 10% and 30% (v/v) as described by Azad, Vikineswary, Chong, and Ramachandran (2003). Figure 5 depicts the effect of inoculum size on biomass, protein, and carotenoid productions. Biomass, protein, and carotenoid productions by *R. faecalis* PA2 were increased with the increase of inoculum levels. The optimal inoculum size ranged from 20% to 30%. The maximum protein production of 475.7 mg/L was noticed at 30% inoculum which could be due to the highest dilution of chicken manure medium, leading to the lowest ammonia and nitrogen source.

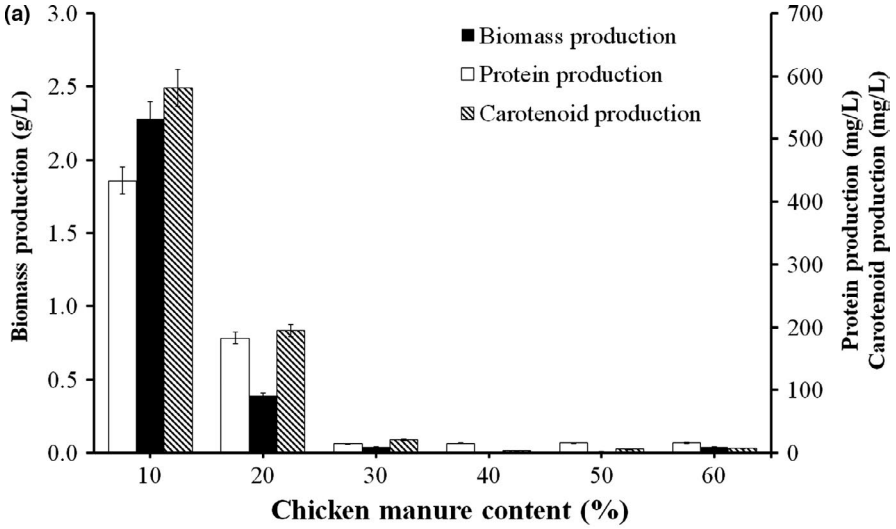


**FIGURE 4** Effect of chicken manure contents on biomass, protein, and carotenoid productions and initial ammonia concentration investigated by conventional approach

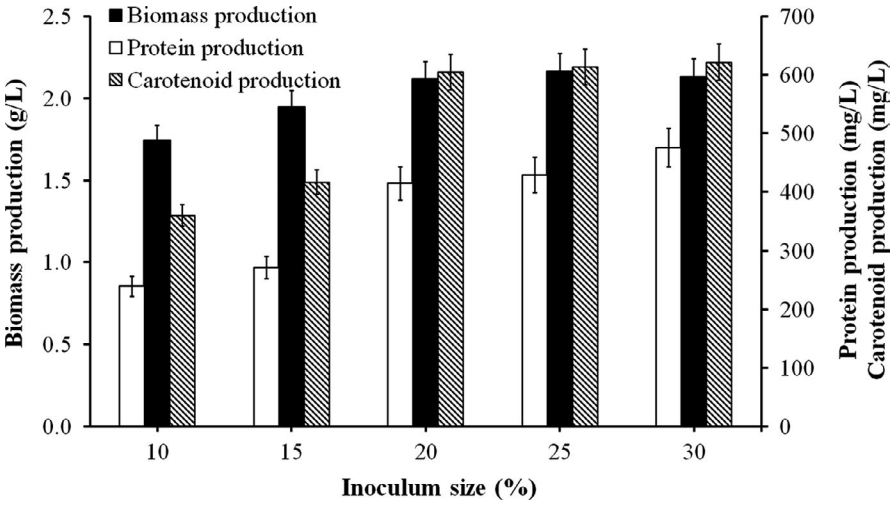


**FIGURE 5** Effect of inoculum size on biomass, protein, and carotenoid productions investigated by conventional approach

Carotenoid accumulation in photosynthetic microorganisms could be triggered at low proportion of nitrogen (Bonnefond et al., 2017; Pirastru et al., 2012). The phenomenon in this study is consistent with Saejung and Salasook (2018).

### 3.2.3 | Effect of cultivation time

The effect of cultivation time was observed for 22 days. The profile of biomass, protein, and carotenoid productions at different time intervals

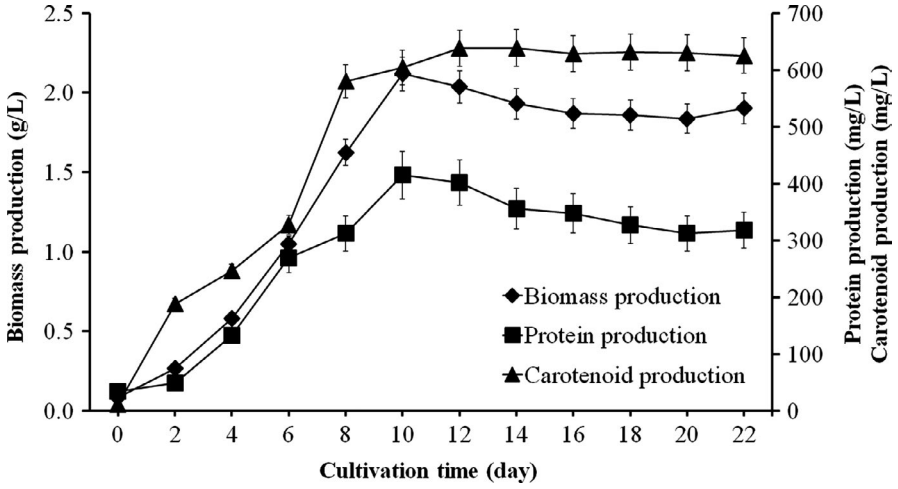


**FIGURE 6** Effect of cultivation time on biomass, protein, and carotenoid productions investigated by conventional approach

**TABLE 1** Experimental range of the independent variables

| | | | Range and levels | | | | |
|---|---|---|---|---|---|---|---|
| Variables | Symbol | Unit | $-\alpha$ | −1 | 0 | +1 | $+\alpha$ |
| Animal manure content | A | % (w/v) | 7 | 10 | 15 | 20 | 23 |
| Inoculum size | B | % (v/v) | 17 | 20 | 25 | 30 | 33 |
| Cultivation time | C | Days | 6 | 8 | 11 | 14 | 16 |

*Note:* $\alpha$ = 1.682.

is presented in Figure 6. Results showed that there was an increase in biomass and protein productions up to 10 days of cultivation and thereafter it decreased. A decline in the growth rate and protein production after 10 days could be due to the decrease in nutrient availability in the chicken manure medium. The long cultivation time caused depletion of nutrients (Liu, Zhang, Zhang, Li, & Li, 2016). These results were consistent with the previous reports investigating microbial growth and protein production in poultry manure and poultry litter (El-Deek et al., 2009; Jalasutram et al., 2013). The change in protein concentrations corresponded perfectly to the cell mass because protein is a major constituent of bacterial cell and it is positively related to the growth rate (Saejung & Salasook, 2018). As shown in Figure 6, carotenoid production was increased with the increase of cultivation time with the maximum at 14 days after which the concentration of carotenoids stabilized. It can be summarized that the high carotenoid production was found during stationary phase of growth. This was because carotenoids are secondary metabolites and the phenomenon in this study was consistent with Saejung and Apaiwong (2015). Extended period of incubation might lead to increasing chances of oxidation of the dissolved carotenoids in bacterial cells (Chen & Djuric, 2001; Limbo, Torri, & Piergiovanni, 2007). Based on the results, extended cultivation time was not suitable for practical application and productivity. Therefore, the cultivation time ranging from 8 to 14 days was chosen for statistical approach.

### 3.3 | Optimization of culture condition by statistical approach

Central composite design was employed to study the effects of animal manure content (A), inoculum size (B), and cultivation time (C) on biomass production, protein production, and carotenoid production. From the optimization studies by OVAT, the selected range of the independent variables is given in Table 1. The results of CCD experimental design are shown in Table 2. The 20 runs were chosen to demonstrate the optimal condition of three responses including biomass production, protein production, and carotenoid production. The maximum response was obtained in run 12 (2.28 g/L biomass, 487.20 mg/L protein, and 595.60 mg/L carotenoids). It is suggested that biomass, protein, and carotenoid productions were increased at moderate chicken manure content, high inoculum size, and moderate cultivation time. Runs at the central points (runs 15–20) showed the high biomass, protein, and carotenoid productions compared to the other combinations. The lowest biomass, protein, and carotenoid productions were observed in run 10 consisting of 23% chicken manure content, 25% inoculum size and cultivation time for 11 days. These might cause by the excessive chicken manure content since high chicken manure content resulted in high ammonia concentration as previously discussed.

The results of ANOVA are shown in Table 3. Results showed that the models of biomass, protein, and carotenoid productions were the suitable models for the optimization experiment within the tested range. The corresponding $p$ value of biomass production, protein production, and carotenoid production was 2.114E−08, 1.542E−08, and 1.628E−09, respectively, indicating the significance of the model ($p < 0.05$). Moreover, adequate precision of biomass, protein, and carotenoid productions was very high, implying these models could be used. The models showed that the values of lack of fit were not significant which could be used to confirm the validity of the models (Gahruie, Moosavi, & Ziaee, 2015).

**TABLE 2** CCD experimental design with three independent variables and the actual and predicted results for biomass, protein, and carotenoid productions

| | | | | Biomass production (g/L) | | Protein production (mg/L) | | Carotenoid production (mg/L) | |
|---|---|---|---|---|---|---|---|---|---|
| Runs | A | B | C | Actual | Predicted | Actual | Predicted | Actual | Predicted |
| 1 | 10 | 20 | 8 | 1.52 | 1.52 | 357.45 | 357.09 | 411.21 | 416.86 |
| 2 | 20 | 20 | 8 | 1.28 | 1.30 | 280.39 | 264.85 | 358.68 | 356.96 |
| 3 | 10 | 30 | 8 | 1.38 | 1.37 | 307.51 | 307.90 | 378.56 | 389.86 |
| 4 | 20 | 30 | 8 | 1.29 | 1.29 | 276.75 | 274.77 | 357.28 | 357.48 |
| 5 | 10 | 20 | 14 | 1.59 | 1.59 | 375.52 | 368.69 | 418.80 | 416.27 |
| 6 | 20 | 20 | 14 | 1.42 | 1.43 | 314.47 | 305.27 | 402.80 | 389.17 |
| 7 | 10 | 30 | 14 | 2.23 | 2.22 | 432.47 | 439.20 | 556.50 | 555.89 |
| 8 | 20 | 30 | 14 | 2.20 | 2.20 | 443.33 | 434.88 | 564.29 | 556.31 |
| 9 | 7 | 25 | 11 | 1.29 | 1.30 | 276.25 | 272.05 | 360.53 | 351.19 |
| 10 | 23 | 25 | 11 | 1.10 | 1.10 | 174.19 | 190.86 | 288.54 | 301.17 |
| 11 | 15 | 17 | 11 | 1.78 | 1.77 | 402.54 | 417.28 | 468.74 | 474.89 |
| 12 | 15 | 33 | 11 | 2.28 | 2.29 | 487.20 | 484.92 | 595.60 | 592.74 |
| 13 | 15 | 25 | 6 | 1.31 | 1.31 | 289.91 | 296.06 | 374.93 | 364.63 |
| 14 | 15 | 25 | 16 | 2.14 | 2.14 | 434.14 | 440.45 | 517.74 | 531.33 |
| 15 | 15 | 25 | 11 | 2.22 | 2.22 | 460.49 | 452.31 | 521.29 | 537.25 |
| 16 | 15 | 25 | 11 | 2.23 | 2.22 | 458.57 | 452.31 | 543.84 | 537.25 |
| 17 | 15 | 25 | 11 | 2.22 | 2.22 | 428.95 | 452.31 | 534.52 | 537.25 |
| 18 | 15 | 25 | 11 | 2.21 | 2.22 | 449.34 | 452.31 | 531.84 | 537.25 |
| 19 | 15 | 25 | 11 | 2.22 | 2.22 | 462.39 | 452.31 | 547.77 | 537.25 |
| 20 | 15 | 25 | 11 | 2.23 | 2.22 | 456.25 | 452.31 | 544.80 | 537.25 |

*Note:* A, chicken manure content (%); B, inoculum size (%); and C, cultivation time (day).

All the linear, interactive, and quadratic terms of the three parameters had the effects ($p < 0.05$) on biomass production. Cultivation time was shown to be the most significant factor with a $p$ value of 3.432E−10. The $R^2$ of biomass production was found to be 0.9998, indicating that 99.98% of the variability in the response can be explained by the model. The greater the $R^2$ is to 1.0, the better precision of the model (Endut et al., 2017). In this study, the $R^2$ of biomass production was in reasonable agreement with the adj $R^2$ (0.9996). The very low value of C.V. (0.50) denotes that the models are highly reliable. For protein production, all linear terms, interactions of AB and BC, and quadratic terms of $A^2$ and $C^2$ of the model were significant. The high significance of cultivation time (1.577E−09) indicates that it was a limiting factor and changing cultivation time influenced the protein production. According to the results, cultivation time was the most significant factor for both biomass and protein productions. This was because protein is one of the major cellular constituents of bacteria which is positively related to biomass produced (Caufield, Abreu, Wimble, & Uetz, 2015). The linear terms, the interactions between BC and the second-order of $A^2$ and $C^2$ had a considerable effect on carotenoid production. The high significance of chicken manure content (1.458E−09) on second-order model suggested that carotenoid production was dependent on the content of substrate. The $R^2$ of carotenoid production was 0.9906 which was in agreement with the adj $R^2$ (0.9821), indicating that the model was fit. The polynomial equations derived from regression analysis are presented in Equations 4, 5, and 6.

$$\text{Biomass production: } Y_1 = 9.600.26A + 0.66B + 1.06C + 0.15AB + 0.068AC + 0.85BC - 1.56A^2 - 0.29B^2 - 0.76C^2 \quad (4)$$

$$\text{Protein production: } Y_2 = 452.31 - 24.14A + 20.11B + 42.93C + 14.78AB + 7.20AC + 29.92BC - 78.08A^2 - 0.43B^2 - 29.72C^2 \quad (5)$$

$$\text{Carotenoid production: } Y_3 = 537.25 - 14.87A + 35.04B + 49.56C + 6.88AB + 8.20AC + 41.66BC - 74.62A^2 - 1.21B^2 - 31.56C^2 \quad (6)$$

where $Y_1$, $Y_2$, and $Y_3$ are the biomass production, protein production, and carotenoid production, respectively. A, B, and C are the animal manure content, inoculum size, and cultivation time, respectively.

**TABLE 3** Analysis of variance (ANOVA) for the quadratic model

| Source | Sum of squares | Degrees of freedom | Mean squares | *F* value | *p* value |
|---|---|---|---|---|---|
| Model (biomass production) | 6.890E+01 | 9 | 7.655E+00 | 4.897E+03 | 2.114E−08 |
| A: chicken manure content | 9.346E−01 | 1 | 9.3464−01 | 5.979E+02 | 8.537E−06 |
| B: inoculum size | 5.965E+00 | 1 | 5.965E+00 | 3.816E+03 | 5.364E−08 |
| C: cultivation time | 1.534E+01 | 1 | 1.534E+01 | 9.814E+03 | 3.432E−10 |
| AB | 1.741E−01 | 1 | 1.741E−01 | 1.113E+02 | 7.584E−07 |
| AC | 3.645E−01 | 1 | 3.645E−02 | 2.332E+01 | 6.929E−06 |
| BC | 5.780E+00 | 1 | 5.780E+00 | 3.698E+03 | 1.754E−09 |
| $A^2$ | 3.5274+01 | 1 | 3.527E+01 | 2.257E+04 | 1.674E−09 |
| $B^2$ | 1.226E+00 | 1 | 1.226E+00 | 7.845E+02 | 1.275E−08 |
| $C^2$ | 8.288E+00 | 1 | 8.289E+00 | 5.303E+03 | 1.863E−09 |
| Residual | 1.563E−02 | 10 | 1.563E−03 | | |
| Lack of fit | 1.175E−02 | 5 | 2.350E−03 | 3.025E+00 | 1.249E−01 |
| Pure error | 3.883E−03 | 5 | 7.767E−04 | | |
| Core total | 6.891E+01 | 19 | | | |
| C.V. (%) = 0.5058; $R^2$ = 0.9998; adj $R^2$ = 0.9996; adequate precision = 184.26 | | | | | |
| Model (protein production) | 1.441E+05 | 9 | 1.602E+04 | 8.577E+01 | 1.542E−08 |
| A: chicken manure content | 7.957E+03 | 1 | 7.957E+04 | 4.262E+01 | 2.035E−07 |
| B: inoculum size | 5.522E+03 | 1 | 5.522E+03 | 2.957E+01 | 2.856E−04 |
| C: cultivation time | 2.517E+04 | 1 | 2.517E+04 | 1.348E+02 | 1.577E−09 |
| AB | 1.747E+03 | 1 | 1.747E+03 | 9.355E+00 | 1.207E−02 |
| AC | 4.152E+02 | 1 | 4.152E+02 | 2.223E+00 | 1.668E−01 |
| BC | 7.163E+03 | 1 | 7.163E+03 | 3.837E+01 | 1.023E−04 |
| $A^2$ | 8.787E+04 | 1 | 8.787E+04 | 4.706E+02 | 1.338E−08 |
| $B^2$ | 2.626E+00 | 1 | 2.623E+00 | 1.406E−02 | 9.090E−01 |
| $C^2$ | 1.273E+04 | 1 | 1.273E+04 | 6.816E+01 | 1.005E−08 |
| Residual | 1.867E+03 | 10 | 1.867E+02 | | |
| Lack of fit | 1.090E+03 | 5 | 2.180E+02 | 1.403E+00 | 3.596E−01 |
| Pure error | 7.770E+02 | 5 | 1.554E+02 | | |
| Core total | 1.460E+05 | 19 | | | |
| C.V. (%) = 3.611; $R^2$ = 0.9872; adj $R^2$ = 0.9757; adequate precision = 30.43 | | | | | |
| Model (carotenoid production) | 1.579E+05 | 9 | 1.754E+04 | 1.166E+02 | 1.628E−09 |
| A: chicken manure content | 3.020E+03 | 1 | 3.020E+03 | 2.007E+01 | 1.178E−03 |
| B: inoculum size | 1.677E+04 | 1 | 1.677E+04 | 1.114E+02 | 1.253E−05 |
| C: cultivation time | 3.354E+04 | 1 | 3.354E+04 | 2.229E+02 | 1.746E−06 |
| AB | 3.787E+02 | 1 | 3.787E+02 | 2.516E+00 | 1.438E−01 |
| AC | 5.379E+02 | 1 | 5.379E+02 | 3.574E+00 | 8.799E−02 |
| BC | 1.388E+04 | 1 | 1.388E+04 | 9.223E+01 | 1.762E−06 |
| $A^2$ | 8.025E+04 | 1 | 8.025E+04 | 5.332E+02 | 1.458E−09 |
| $B^2$ | 2.123E+01 | 1 | 2.123E+01 | 1.410E−01 | 7.151E−01 |
| $C^2$ | 1.436E+04 | 1 | 1.436E+04 | 9.538E+01 | 1.095E−08 |
| Residual | 1.505E+03 | 10 | 1.505E+02 | | |
| Lack of fit | 1.003E+03 | 5 | 2.005E+02 | 1.995E+00 | 2.333E−01 |
| Pure error | 5.025E+02 | 5 | 1.005E+02 | | |
| Core total | 1.594E+05 | 19 | | | |
| C.V. (%) = 2.645; $R^2$ = 0.9906; adj $R^2$ = 0.9821; adequate Precision = 33.61 | | | | | |

*Note*: $p < 0.05$ indicate model terms are significant.

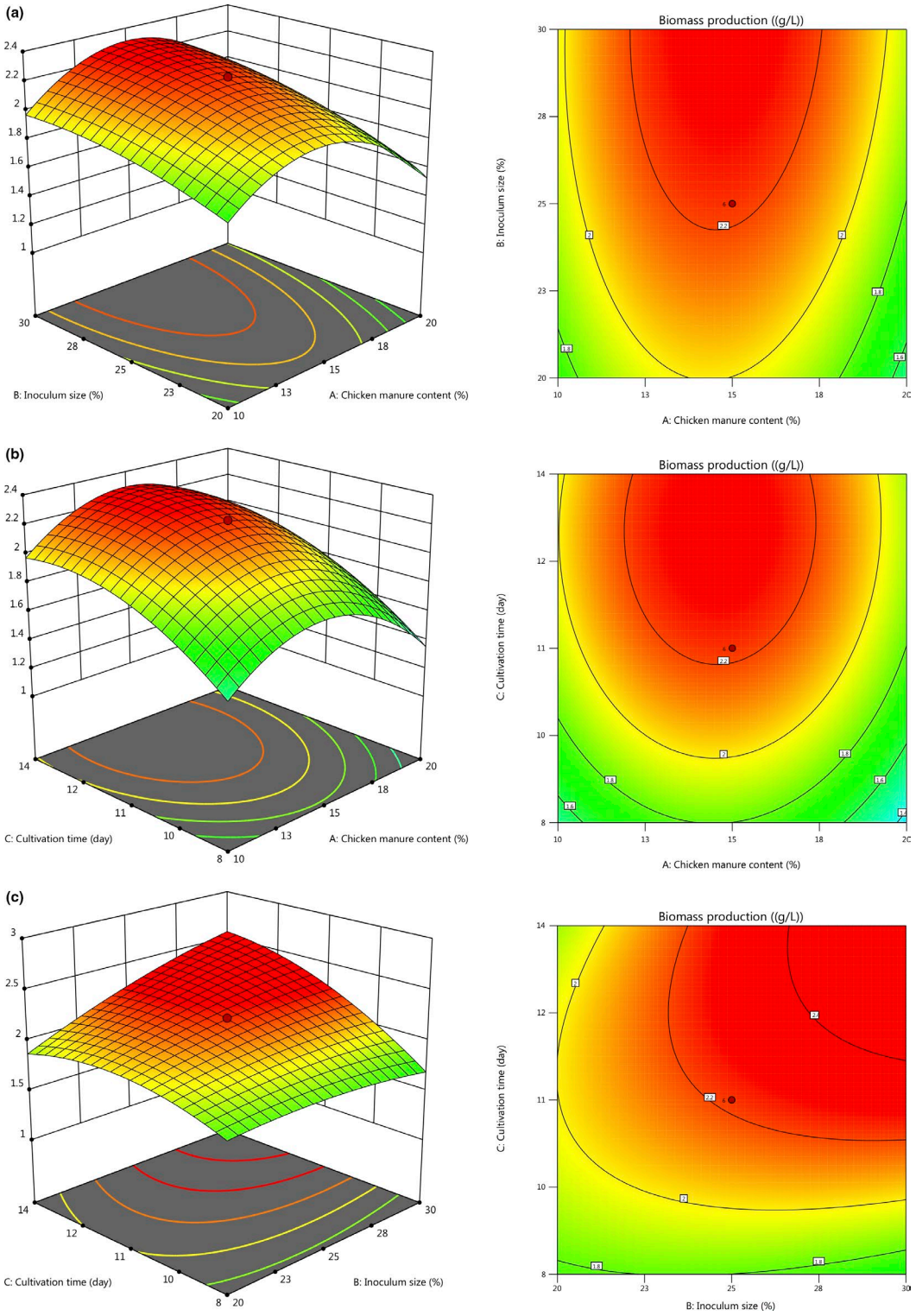


**FIGURE 7** Response surface plots and contour plots showing the effects of chicken manure content and inoculum size (a), chicken manure content and cultivation time (b), and inoculum size and cultivation time (c) on biomass production

The response surface plots and contour plots were used to illustrate the effects of the two variables and to indicate their optimal points. Figure 7a represents the interaction between chicken manure content and inoculum size on biomass production. Results revealed that biomass production was increased when inoculum size was higher than 25% and chicken manure content ranged from 13% to 18%. Similarly, the high biomass production was found when chicken manure content was between 13% and 18% and cultivation time was increased (Figure 7b). Lower and higher levels of chicken manure did not result in higher biomass production. In Figure 7c, the

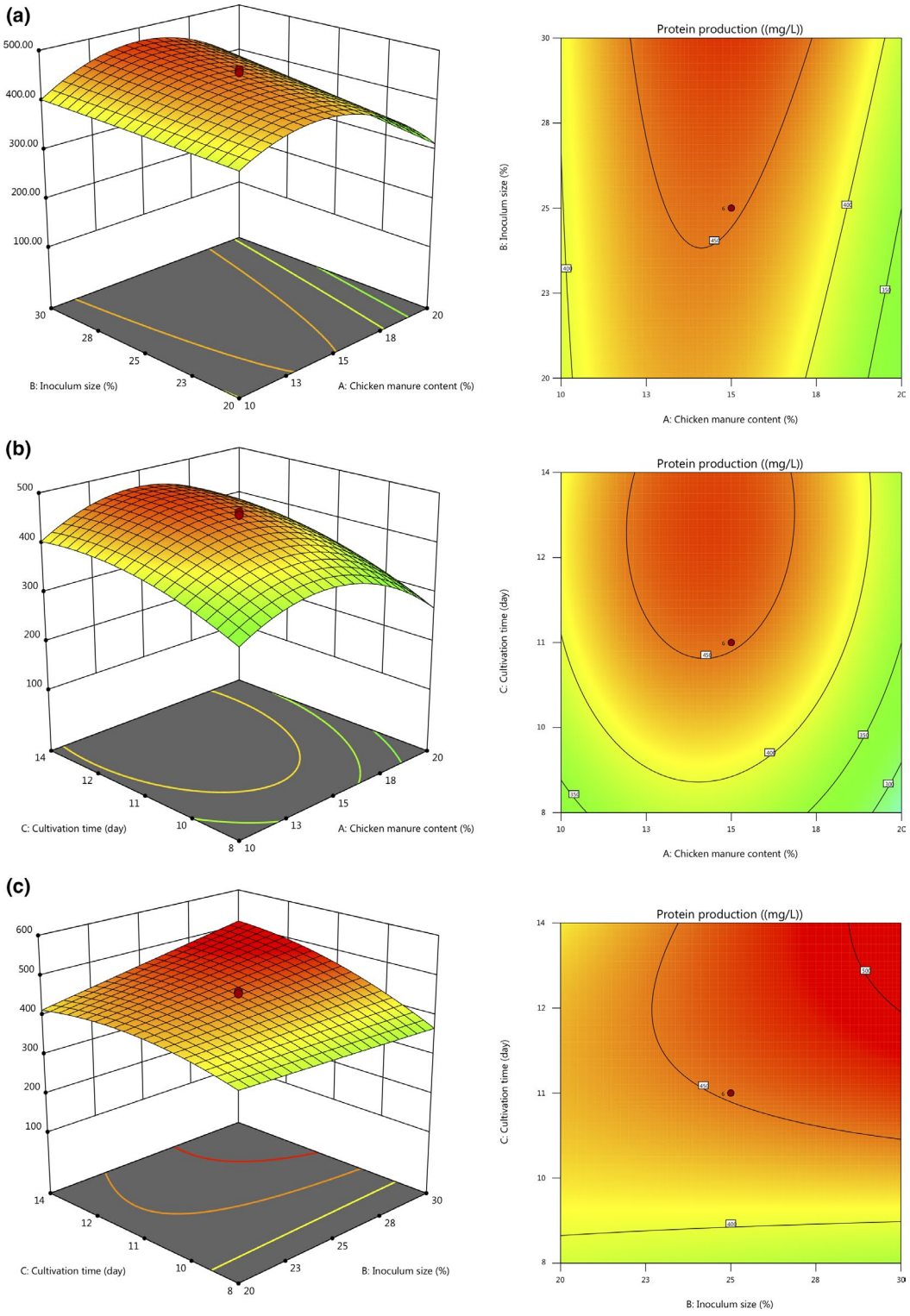


**FIGURE 8** Response surface plots and contour plots showing the effects of chicken manure content and inoculum size (a), chicken manure content and cultivation time (b), and inoculum size and cultivation time (c) on protein production

shape of the curve shows a positive interaction between the two variables. Biomass production was found to increase with simultaneous increase in both inoculum size and cultivation time. Therefore, these two factors were significant on biomass production. The increase in inoculum concentration enhanced the viable cells, and the increase in cultivation time resulted in biomass accumulation (Wardani, Cahyanto, Rahayu, & Utami, 2017). Similar results were found in the protein production. The optimal chicken manure content ranged from 13% to 18% for protein production (Figure 8a,b). A positive interaction between inoculum size and cultivation time was

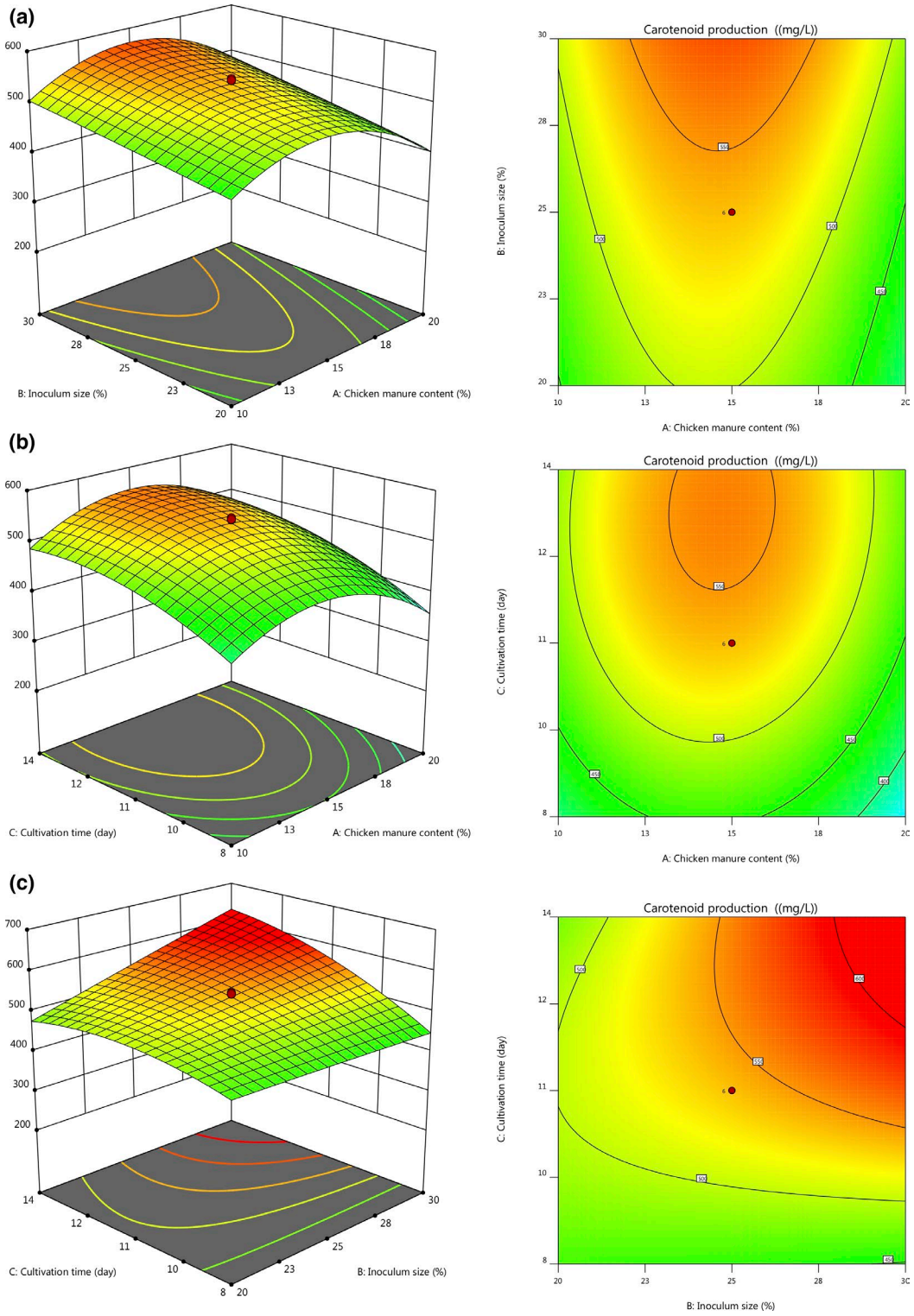


**FIGURE 9** Response surface plots and contour plots showing the effects of chicken manure content and inoculum size (a), chicken manure content and cultivation time (b), and inoculum size and cultivation time (c) on carotenoid production

also observed on protein production. It was significantly enhanced with increasing inoculum size and cultivation time (Figure 8c). The response surface plots and contour plots of carotenoid production are illustrated on Figure 9. Results showed that inoculum size and cultivation time were the significant factors for carotenoid production.

### 3.4 | Validation of the model

Based on the overlay plot (Appendix Figure A2), the optimal condition for biomass, protein, and carotenoid productions were as follows: 15% chicken manure content, 30% inoculum size and

**TABLE 4** Validation of the model

| Chicken manure content (%) | Inoculum size (%) | Cultivation time (day) | Biomass production (g/L) | | | Protein production (mg/L) | | | Carotenoid production (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Predicted | Actual | Error (%) | Predicted | Actual | Error (%) | Predicted | Actual | Error (%) |
| 15 | 30 | 14 | 2.56 | 2.60 | 1.56 | 512.31 | 525.85 | 2.64 | 626.36 | 639.45 | 2.09 |

**TABLE 5** Kinetic parameters of *Rhodopseudomonas faecalis* PA2 cultivated in chicken manure medium under the optimal condition using a 5-L photobioreactor

| Kinetic parameters | Value | Unit |
|---|---|---|
| Specific growth rate | 0.25 ± 0 | /day |
| Biomass production | 4.0 ± 0.29 | g/L |
| Protein production | 558.4 ± 64.98 | mg/L |
| Carotenoid production | 710.9 ± 29.87 | mg/L |
| Biomass productivity | 1.2 ± 0.02[a] | g/L day |
| Protein productivity | 39.9 ± 4.64[a] | mg/L day |
| Carotenoid productivity | 50.8 ± 2.13[a] | mg/L day |
| Protein yield | 139.6 ± 3.24 | mg/g |
| Carotenoid yield | 177.7 ± 1.21 | mg/g |

*Calculated from 14 days.

cultivation time for 14 days. To verify the RSM model, the experimental (actual) results were compared with the predicted results and % error was acceptable as shown in Table 4. Results showed that the actual values of biomass, protein, and carotenoid productions were in close agreement with the predicted ones. After validation test, the biomass, protein, and carotenoid productions were increased by 25.3%, 14.5%, and 6.3%, respectively, compared to the original condition. Therefore, the optimization using RSM based on CCD was the effective method for biomass, protein, and carotenoid productions by *R. faecalis* PA2 grown in chicken manure medium.

### 3.5 | Batch cultivation in a photobioreactor

A photobioreactor provides controlled environments and enables high productivity of bacteria for SCP production. The kinetic parameters of *R. faecalis* PA2 cultivated in a 5-L photobioreactor using the condition obtained from RSM are presented in Table 5. The results were greatly appreciated, suggesting that the aforementioned condition can be further utilized for SCP production. Biomass, protein, and carotenoid productions by *R. faecalis* PA2 cultivated in the original condition, RSM condition in a 250-mL bottle, and RSM mass production in a 5-L photobioreactor are presented in Figure 10. The biomass, protein, and carotenoid productions in a photobioreactor were increased by 92.3%, 21.6%, and 18.2%, respectively, compared to the original condition. Moreover, the mass production of this strain in a photobioreactor optimized by RSM yielded the highest productivity (Figure 10), suggesting that the recommended condition had the virtue of being practical.

### 3.6 | Protein content, amino acid composition, and total nucleic acid content in SCP

The aim of this work was to enhance biomass production, protein production, and carotenoids in SCP produced by *R. faecalis* PA2. Therefore, amino acid composition and protein content in the dry biomass are the crucial information. The freeze-dried biomass cultivated in a photobioreactor contained 62.7% protein which was relatively high for SCP production by photosynthetic bacteria (Table 6). Single cell protein can be used as an ingredient or a substitute for protein-rich foods in animal feeds, and photosynthetic bacteria are considered as

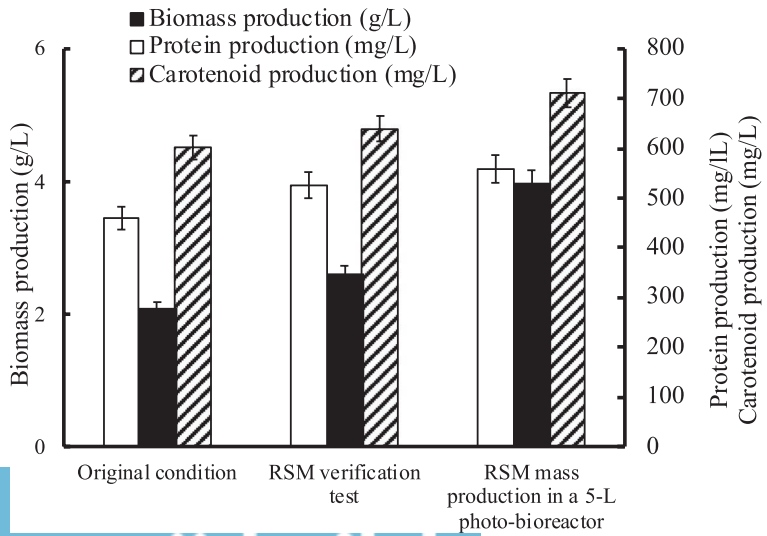


**FIGURE 10** Biomass production, protein production, and carotenoid production by *Rhodopseudomonas faecalis* PA2 cultivated in the original condition (10% chicken manure content, 20% inoculum size and cultivation time for 10 days), RSM validation test (15% chicken manure content, 30% inoculum size and cultivation time for 14 days in a 250-ml bottle), and RSM mass production in a 5-L photobioreactor (15% chicken manure content, 30% inoculum size and cultivation time for 14 days)

**TABLE 6** Protein content in SCP produced by photosynthetic bacteria grown in different substrates

| Photosynthetic bacteria | Protein content (%) | Substrate | Reference |
|---|---|---|---|
| *Rhodopseudomonas faecalis* PA2 | 62.7 | Chicken manure medium | Present study |
| *Rhodopseudomonas* sp. CSK01 | 60.1 | Municipal wastewater | Saejung and Thammaratana (2016) |
| *Rhodopseudomonas palustris* P1 | 64.7 | Latex rubber sheet wastewater supplemented with fermented pineapple extract | Kornochalert, Kantachote, Chaiprapat, and Techkarnjanaruk (2014) |
| *Rhodobacter sphaeroides* SS15 | 54.0 | Basic isolation medium | Chumpol, Kantachote, Nitoda, and Kanzaki (2018) |
| *R. sphaeroides* Z08 | 52.0 | Artificial soybean wastewater | He, Zhang, and Lu (2010) |
| *R. sphaeroides* D-8 | 58.2 | Medium with poultry dung | Paronyan and Gasparyan (2009) |
| *Afifella marina* STW181 | 46.4 | Glutamate acetate medium | Chumpol et al. (2018) |

**TABLE 7** Amino acid compositions of *Rhodopseudomonas faecalis* PA2 grown in chicken manure medium under the optimal condition from RSM compared to the reported SCP and quantitative estimates for key limiting essential amino acids in protein-rich foods and dietary amino acid requirement for fish and shrimp species

| | | SCP (% dry weight) | | Protein-rich foods (% dry matter) | | | Dietary amino acid requirement of some representative aquatic species (% dry diet) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid | *R. faecalis* PA2 (% dry weight) | *Candida utilis*[a] | *Saccharomyces cerevisiae*[b] | Maize meal[c] | Soybean meal[c] | Fish meal[c] | Common carp[d] | Catla[e] | Channel catfish[e] | Nile tilapia[f] | Penaeid shrimp[g] | Black tiger shrimp[f] |
| Lysine[h] | 3.36 | 1.24 | 3.04 | 0.17 | 2.79 | 5.05 | 2.20 | 2.50 | 1.5 | 1.4 | 1.80 | 2.1 |
| Threonine[h] | 1.40 | 0.60 | – | 0.20 | 1.68 | 3.32 | 1.50 | 2.0 | 0.5 | 1.1 | 1.81 | 1.4 |
| Methionine[h] | 0.25 | 0.44 | 0.58 | 0.28 (Met + Cys) | 1.08 (Met + Cys) | 2.31 (Met + Cys) | 0.80 | 1.40 | 0.6 | 0.8 (with 0.2% Cys) | 0.66 | 0.9 (with 0.4% Cys) |
| Phenylalanine[h] | 1.85 | 0.98 | 2.38 | 0.58 | 1.83 | 2.73 | 1.30 | 1.50 | – | – | 0.94 | – |
| Leucine[h] | 2.05 | 1.44 | 2.89 | 0.80 | 3.53 | 4.62 | 1.30 | 1.50 | – | – | 1.71 | – |
| Isoleucine[h] | 1.00 | 0.81 | 2.22 | 0.25 | 1.61 | 2.11 | 0.90 | 0.90 | – | – | 0.83 | – |
| Valine[h] | 1.16 | 0.54 | 5.39 | 0.30 | 1.41 | 3.91 | 1.40 | 1.40 | – | – | 1.04 | – |
| Histidine[h] | 1.15 | 0.19 | 1.88 | 0.17 | 0.98 | 1.48 | 0.80 | 1.00 | – | – | 0.54 | – |
| Arginine | 1.30 | 0.82 | 0.68 | 0.30 | 3.48 | 3.15 | 1.60 | 1.90 | 1.0 | 1.2 | – | 1.9 |
| Alanine | 1.44 | 1.18 | 1.33 | – | – | – | – | – | – | – | – | – |
| Glycine | 1.42 | 0.75 | 0.96 | – | – | – | – | – | – | – | – | – |
| Proline | 1.54 | 0.74 | – | – | – | – | – | – | – | – | – | – |
| Glutamic acid | 2.14 | 3.20 | 3.79 | – | – | – | – | – | – | – | – | – |
| Serine | 0.47 | 0.64 | 1.12 | – | – | – | – | – | – | – | – | – |
| Tyrosine | 0.87 | 0.86 | 5.37 | – | – | – | – | – | – | – | – | – |
| Aspartic acid | 3.54 | 1.32 | 5.08 | – | – | – | – | – | – | – | – | – |

[a]Data from Rajoka, Kiani, Khan, Awan, and Hashmi (2004); SCP produced from defatted rice polishing.
[b]Data from Samadi, Mohammadi, and Najafpour (2016); SCP produced from sugarcane bagasse.
[c]Data fromAzaza et al. (2008).
[d]Data fromOkino (1980).
[e]Data fromRavi and Devaraj (1991).
[f]Data fromNunes, Sa, Browdy, and Vazquez-Anon (2014).
[g]Data from Oura (1983). The data presented are the minimum quantity.
[h]Essential amino acids.

one of the suitable protein sources for aquatic animals (Azaza et al., 2008; Saejung et al., 2018). Therefore, amino acid composition of *R. faecalis* PA2 cultivated in the optimal condition from RSM was compared with those of the reported SCP, protein-rich foods used in aquaculture and dietary amino acid requirements of some fish and shrimp species as shown in Table 7. Obviously, SCP obtained in this work can be utilized as protein feedstock for aquatic animal diets.

One of the problems of SCP is the high nucleic acid content because the breakdown of purine increases uric acid in plasma (Ritala et al., 2017). Therefore, SCP with high nucleic acid content intended for animal feed is restrained in animals with short life spans (Strong, Xie, & Clarke, 2015). Nasseri, Rasoul-Amini, Morowvat, and Ghasemi (2011) have reported that the low nucleic acid content of SCP ranged from 3% to 8%. Previous research has also reported that nucleic acid content in SCP produced by the photosynthetic bacterium *Rhodopseudomonas gelatinosus* and the yeast *Candida tropicalis* CGMCC 2.587, *Dipodascus capitatus*, and *Dipodascus* sp. ACM 4780 was 5.10%, 5.28%, 4.65%, and 6.85%, respectively (Brown et al., 1996; Gao, Li, & Liu, 2012; Shipman, Kao, & Fan, 1975). In this study, nucleic acid content of SCP produced by *R. faecalis* PA2 grown in chicken manure medium was 4.52%, suggesting the relatively low nucleic acid content. Therefore, the results of protein content, amino acid composition, and nucleic acid content suggest that *R. faecalis* PA2 is suitable for the production of high-quality SCP using chicken manure medium as a low-cost substrate.

## 4 | CONCLUSIONS

The statistical approach showed significant results for improving biomass, protein, and carotenoids in SCP produced from chicken manure by using *R. faecalis* PA2. The high percentage of the enhanced biomass, protein, and carotenoid productions compared to the nonoptimized condition as well as the low percentage of error of the predicted and the actual values indicates the precision of the model. The results also represent the use of animal manure as sole substrate for SCP production, thus contributing to the reduction in the cost of production medium as well as minimizing the pollution and contamination of animal waste in the environment.

## ACKNOWLEDGMENTS

This work was supported by the Thailand Research Fund (TRF) and the Office of the Higher Education Commission (Grant No. MRG6280016) and Khon Kaen University.

## CONFLICT OF INTERESTS

The authors declare no conflict of interests.

## AUTHOR CONTRIBUTIONS

CS conceived the study and contributed to funding for financial support and scholarship. CS and SP conducted the experiments and analyzed the data. SP performed the statistical software. CS interpreted the results and drafted the manuscript. All authors approved the final version of manuscript.

## ETHICS STATEMENT

None required.

## DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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## REFERENCES

Alloul, A., Ganigue, R., Spiller, M., Meerburg, F., Cagnetta, C., Rabaey, K., & Vlaeminck, S. E. (2018). Capture-Ferment-Upgrade: A three-step approach for the valorization of sewage organics as commodities. *Environmental Science and Technology*, *52*, 6729–6742. https://doi.org/10.1021/acs.est.7b05712

Alloul, A., Wuyts, S., Lebeer, S., & Vlaeminck, S. E. (2019). Volatile fatty acids impacting phototrophic growth kinetics of purple bacteria: Paving the way for protein production on fermented wastewater. *Water Research*, *152*, 138–147. https://doi.org/10.1016/j.watres.2018.12.025

Anbazahan, S. M., Mari, L. S., Yogeshwari, G., Jagruthi, C., Thirumurugan, R., Arockiaraj, J., … Harikrishnan, R. (2014). Immune response and disease resistance of carotenoids supplementation diet in *Cyprinus carpio* against *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, *40*, 9–13. https://doi.org/10.1016/j.fsi.2014.06.011

Association of Official Analytical Chemists (AOAC) (2000). *Official Methods of Analysis*. Arlington, Virginia: AOAC International.

Azad, S. A., Vikineswary, S., Chong, V. C., & Ramachandran, K. B. (2003). *Rhodovulum sulfidophilum* in the treatment and utilization of sardine processing wastewater. *Letters in Applied Microbiology*, *38*, 13–18. https://doi.org/10.1046/j.1472-765X.2003.01435.x

Azaza, M. S., Mensi, F., Ksouri, J., Dhraief, M. N., Brini, B., Abdelmouleh, A., & Kraiem, M. M. (2008). Growth of Nile tilapia (*Oreochromis niloticus* L.) fed with diets containing graded levels of green ulva meal (*Ulva rigida*) reared in geothermal waters of southern Tunisia. *Journal of Applied Ichthyology*, *24*, 202–207.

Bandara, T. (2018). Alternative feed ingredients in aquaculture: Opportunities and challenges. *Journal of Entomology and Zoology Studies*, *6*, 3087–3094.

Batal, A. B. (2009). How much DDGS for poultry? *Feed International*, *30*, 18–19.

Bayrakdar, A., Molaey, R., Surmeli, R. O., Sahinkaya, E., & Çalli, B. (2017). Biogas production from chicken manure: Co-digestion with spent poppy straw. *International Biodeterioration and Biodegradation*, *119*, 205–210. https://doi.org/10.1016/j.ibiod.2016.10.058

Bonnefond, H., Moelants, N., Talec, N., Mayzaud, P., Bernard, O., & Sciandra, A. (2017). Coupling and uncoupling of triglyceride and beta-carotene production by *Dunaliella salina* under nitrogen limitation and starvation. *Biotechnology for Biofuels*, *10*, 25. https://doi.org/10.1186/s13068-017-0713-4

Borowski, S., Matusiak, K., Powałowski, S., Pielech-Przybylska, K., Makowski, K., Nowak, A., … Gutarowska, B. (2017). A novel

microbial-mineral preparation for the removal of offensive odors from poultry manure. *International Biodeterioration and Biodegradation*, *119*, 299–308. https://doi.org/10.1016/j.ibiod.2016.10.042

Brown, M. R., Barrett, S. M., Volkman, J. K., Nearhos, S. P., Nell, J. A., & Allan, G. L. (1996). Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. *Aquaculture*, *143*, 341–360. https://doi.org/10.1016/0044-8486(96)01286-0

Callaghan, D. A., Wase, D. A. J., Thayanithy, K., & Foster, C. F. (2002). Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and Bioenergy*, *22*, 71–77. https://doi.org/10.1016/S0961-9534(01)00057-5

Caufield, J. H., Abreu, M., Wimble, C., & Uetz, P. (2015). Protein complexes in bacteria. *PLOS Computational Biology*, *11*(2), e1004107. https://doi.org/10.1371/journal.pcbi.1004107

Chee, J. Y., Lakshmanan, M., Jeepery, I. F., Hairudin, N. H. M., & Sudesh, K. (2019). The potential application of *Cupriavidus necator* as polyhydroxyalkanoates producer and single cell protein: A review on scientific, cultural and religious perspectives. *Applied Food Biotechnology*, *6*, 19–34.

Chen, G., & Djuric, Z. (2001). Carotenoids are degraded by free radicals but do not affect lipid peroxidation in unilamellar liposomes under different oxygen tensions. *FEBS Letters*, *505*, 151–154. https://doi.org/10.1016/S0014-5793(01)02671-0

Chumpol, S., Kantachote, D., Nitoda, T., & Kanzaki, H. (2018). Administration of purple nonsulfur bacteria as single cell protein by mixing with shrimp feed to enhance growth, immune response and survival in white shrimp (*Litopenaeus vannamei*) cultivation. *Aquaculture*, *489*, 85–95. https://doi.org/10.1016/j.aquaculture.2018.02.009

Das, A. P., & Biswas, S. P. (2016). Carotenoids and pigmentation in ornamental fish. *Journal of Aquaculture and Marine Biology*, *4*(5), 1–3. https://doi.org/10.15406/jamb.2016.04.00093

Einstein-Curtis, A. (2018). *Feed prices forecast to rise*. William Reed Business Media Ltd. Retrieved from https://www.feednavigator.com/Article/2018/03/29/Feed-prices-forecast-to-rise

El-Deek, A. A., Ghonem, K. M., Saffa, M. H., Aser, M. A., Fahad, M. A., & Osman, M. M. (2009). Producing single cell protein from poultry manure and evaluation for broiler chickens diets. *International Journal of Poultry Science*, *8*, 1062–1077. https://doi.org/10.3923/ijps.2009.1062.1077

Endut, A., Abdullah, S. H. Y. S., Hanapi, N. H. M., Hamid, S. H. A., Lananan, F., Kamarudin, M. K. A., … Khatoon, H. (2017). Optimization of biodiesel production by solid acid catalyst derived from coconut shell via response surface methodology. *International Biodeterioration and Biodegradation*, *124*, 250–257. https://doi.org/10.1016/j.ibiod.2017.06.008

Gahruie, H. H., Moosavi, M. N., & Ziaee, E. (2015). Application of response surface methodology for xanthan gum and biomass production using *Xanthomonas campestris*. *Journal of Chemical Health Risks*, *5*, 167–177.

Gaind, S. (2014). Effect of fungal consortium and animal manure amendments on phosphorus fractions of paddy-straw compost. *International Biodeterioration and Biodegradation*, *94*, 90–97. https://doi.org/10.1016/j.ibiod.2014.06.023

Gao, Y., Li, D., & Liu, Y. (2012). Production of single cell protein from soy molasses using *Candida tropicalis*. *Annals of Microbiology*, *62*, 1165–1172. https://doi.org/10.1007/s13213-011-0356-9

Garcia, D., de Godos, I., Dominguez, C., Turiel, S., Bolado, S., & Munoz, R. (2019). A systematic comparison of the potential of microalgae-bacteria and purple phototrophic bacteria consortia for the treatment of piggery wastewater. *Bioresource Technology*, *276*, 18–27. https://doi.org/10.1016/j.biortech.2018.12.095

Garcia-Chavarría, M., & Lara-Flores, M. (2013). The use of carotenoid in aquaculture. *Research Journal of Fisheries and Hydrobiology*, *8*, 38–49.

Golaghaiee, S., Ardestani, F., & Ghorbani, H. R. (2017). Microbial protein production from *Candida tropicalis* ATCC13803 in a submerged batch fermentation process. *Applied Food Biotechnology*, *4*, 35–42.

Golterman, H. L. (1991). Direct nesslerization of ammonia and nitrate in fresh-water. *Annales De Limnologie – International Journal of Limnology*, *27*, 99–101. https://doi.org/10.1051/limn/1991007

Han, X., Rusconi, N., Ali, P., Pagkatipunan, K., & Chen, F. (2017). Nutrients extracted from chicken manure accelerate growth of microalga *Scenedesmus obliquus* HTB1. *Green and Sustainable Chemistry*, *7*, 101–113.

He, J., Zhang, G., & Lu, H. (2010). Treatment of soybean wastewater by a wild strain *Rhodobacter sphaeroides* and to produce protein under natural conditions. *Frontiers of Environmental Science and Engineering in China*, *4*, 334–339. https://doi.org/10.1007/s11783-010-0239-5

Hirayama, O. (1968). Lipids and lipoprotein complex in photosynthetic tissue: 4 lipid and pigments of photosynthetic bacteria. *Agricultural and Biological Chemistry*, *32*, 34–41.

Jalasutram, V., Kataram, S., Gandu, B., & Anupoju, G. R. (2013). Single cell protein production from digested and undigested poultry litter by *Candida utilis*: Optimization of process parameters using response surface methodology. *Clean Technologies and Environmental Policy*, *15*, 265–273.

Karklinya, V. A., Birska, I. A., & Limarenko, Y. A. (1989). Quantitative determination of nucleic acids in salmonidae milt by various methods. *Chemistry of Natural Compounds*, *25*, 109–122. https://doi.org/10.1007/BF00596713

Kayhanian, M. (1999). Ammonia inhibition in high-solids biogasification: An overview and practical solutions. *Environmental Technology*, *20*, 355–365. https://doi.org/10.1080/09593332008616828

Khatoon, H., Banerjee, S., Guan Yuan, G. T., Haris, N., Ikhwanuddin, M., Ambak, M. A., & Endut, A. (2016). Biofloc as a potential natural feed for shrimp postlarvae. *International Biodeterioration and Biodegradation*, *113*, 304–309. https://doi.org/10.1016/j.ibiod.2016.04.006

Kornochalert, N., Kantachote, D., Chaiprapat, S., & Techkarnjanaruk, S. (2014). Use of *Rhodopseudomonas palustris* P1 stimulated growth by fermented pineapple extract to treat latex rubber sheet wastewater to obtain single cell protein. *Annals of Microbiology*, *64*, 1021–1032. https://doi.org/10.1007/s13213-013-0739-1

Limbo, S., Torri, L., & Piergiovanni, L. (2007). Light-induced changes in an aqueous β-carotene system stored under halogen and fluorescent lamps, affected by two oxygen partial pressures. *Journal of Agriculture and Food Chemistry*, *55*, 5238–5245. https://doi.org/10.1021/jf0634348

Lin, Y., Chen, A., Wu, G., Peng, L., Xu, Z., & Shao, J. (2017). Growth, microcystins synthesis, and cell viability of *Microcystis aeruginosa* FACHB905 to dissolved organic matter originated from cattle manure. *International Biodeterioration and Biodegradation*, *118*, 126–133.

Liu, S., Zhang, G., Zhang, J., Li, X., & Li, J. (2016). Performance, carotenoids yield and microbial population dynamics in a photobioreactor system treating acidic wastewater: Effect of hydraulic retention time (HRT) and organic loading rate (OLR). *Bioresource Technology*, *200*, 245–252. https://doi.org/10.1016/j.biortech.2015.10.044

Ma, B., Zhang, G., Song, H., Zhang, Y., Zhiguo, Z., & Peng, M. (2018). A new photosynthetic bacteria consortium and treatment of low-COD wastewaters. In: Y. Han (Ed.), *Advances in energy and environmental materials. CMC 2017*. Springer proceedings in energy (pp. 913–919). Singapore City, Singapore: Springer.

Manceron, S., Ben-Ari, T., & Dumas, P. (2014). Feeding proteins to livestock: Global land use and food *vs*. feed competition. *OCL*, *21*, D408.

Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H., & Ghasemi, Y. (2011). Single cell protein: Production and process. *American Journal of Food Technology*, *6*, 103–116. https://doi.org/10.3923/ajft.2011.103.116

Nimalaratne, C., Wu, J., & Schieber, A. (2013). Egg yolk carotenoids: Composition, analysis, and effects of processing on their stability. In P. Winterhalter, & S. E. Ebeler (Eds.), *Carotenoid cleavage products* (pp. 219–225). Washington, DC: American Chemical Society.

Nunes, A. J. P., Sa, M. V. C., Browdy, C. L., & Vazquez-Anon, M. (2014). Practical supplementation of shrimp and fish feeds with crystalline amino acids. *Aquaculture*, *431*, 20–27. https://doi.org/10.1016/j.aquaculture.2014.04.003

Okino, C. (1980). Requirements of carp and rainbow trout for essential amino acids. *Bulletin of the Japanese Society of Scientific Fisheries*, *46*, 171–174.

Oura, E. (1983). Biomass from carbohydrates. In H. Dellweg (Ed.), *Biotechnology* (pp. 3–42). Weinheim, Germany: Verlag Chemie GmbH.

Paronyan, A. K., & Gasparyan, A. V. (2009). Production of biomass of photosynthetic bacteria on base of stock-breeding and poultry wastes. *Biological Journal of Armenia*, *1*, 5–10.

Patthawaro, S., Lomthaisong, K., & Saejung, C. (2019). Bioconversion of agro-industrial waste to value-added product lycopene by photosynthetic bacterium *Rhodopseudomonas faecalis* and its carotenoid composition. *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-018-00571-z

Pirastru, L., Darwish, M., Chu, F. L., Perreault, F., Sirois, L., Sleno, L., & Popovic, R. (2012). Carotenoid production and change of photosynthetic functions in *Scenedesmus* sp. exposed to nitrogen limitation and acetate treatment. *Journal of Applied Phycology*, *24*, 117–124.

Rajinikanth, R., Daniel, I. M., & Gursharan, S. (2013). A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresource Technology*, *143*, 632–641. https://doi.org/10.1016/j.biortech.2013.06.030

Rajoka, M. I., Kiani, T. M. A., Khan, S. H., Awan, M. S., & Hashmi, A. S. (2004). Production of single cell protein from rice polishings using *Candida utilis*. *World Journal of Microbiology and Biotechnology*, *20*, 297–301. https://doi.org/10.1023/B:WIBI.0000023845.96123.dd

Ravi, J., & Devaraj, K. V. (1991). Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). *Aquaculture*, *96*, 281–291. https://doi.org/10.1016/0044-8486(91)90158-4

Reed, M. C., Lieb, A., & Nijhout, F. H. (2010). The biological significance of substrate inhibition: A mechanism with diverse function. *BioEssays*, *32*, 422–429.

Reihani, S. F. S., & Khosravi-Darani, K. (2018). Mycoprotein production from date waste using *Fusarium venenatum* in a submerged culture. *Applied Food Biotechnology*, *5*, 243–252.

Ritala, A., Hakkinen, S. T., Toivari, M., & Wiebe, M. G. (2017). Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. *Frontiers in Microbiology*, *8*, 2009. https://doi.org/10.3389/fmicb.2017.02009

Rostami, M., Monaco, S., Sacco, D., Grignani, C., & Dinuccio, E. (2015). Comparison of ammonia emissions from animal wastes and chemical fertilizers after application in the soil. *International Journal of Recycling of Organic Waste in Agriculture*, *4*, 127–134. https://doi.org/10.1007/s40093-015-0092-4

Rumana, R. (2013). *Fundamentals of wastewater treatment and engineering* (p. 132). London, UK: CRC Press.

Saejung, C., & Ampornpat, W. (2019). Production and nutritional performance of carotenoid-producing photosynthetic bacterium *Rhodopseudomonas feacalis* PA2 grown in domestic wastewater intended for animal feed production. *Waste and Biomass Valorization*, *10*, 299–310.

Saejung, C., & Apaiwong, P. (2015). Enhancement of carotenoid production in the new carotenoid-producing photosynthetic bacterium *Rhodopseudomonas faecalis* PA2. *Biotechnology and Bioprocess Engineering*, *20*, 701–707. https://doi.org/10.1007/s12257-015-0015-2

Saejung, C., Chaiyarat, A., & Sa-noamuang, L. (2018). Effects of algae, yeast and photosynthetic bacteria diets on survival and growth performance in the fairy shrimp, *Streptocephalus sirindhornae* (Branchiopoda, Anostraca). *Crustaceana*, *91*, 1505–1522. https://doi.org/10.1163/15685403-00003847

Saejung, C., & Puensungnern, L. (2018). Evaluation of molasses-based medium as a low cost medium for carotenoids and fatty acid production by photosynthetic bacteria. *Waste and Biomass Valorization*. https://doi.org/10.1007/s12649-018-0379-6

Saejung, C., & Salasook, P. (2018). Recycling of sugar industry wastewater for single-cell protein production with supplemental carotenoids. *Environmental Technology*, 1–12. https://doi.org/10.1080/09593330.2018.1491633

Saejung, C., & Thammaratana, T. (2016). Biomass recovery during municipal wastewater treatment using photosynthetic bacteria and prospect of production of single cell protein for feedstuff. *Environmental Technology*, *37*, 3055–3061. https://doi.org/10.1080/09593330.2016.1175512

Samadi, S., Mohammadi, M., & Najafpour, G. D. (2016). Production of single cell protein from sugarcane bagasse by *Saccharomyces cerevisiae* in tray bioreactor. *IJE*, *29*, 1029–1036.

Sheng, G. P., Yu, H. Q., & Yu, Z. (2005). Extraction of extracellular polymeric substances from the photosynthetic bacterium *Rhodopseudomonas acidophila*. *Applied Microbiology and Biotechnology*, *67*, 125–130. https://doi.org/10.1007/s00253-004-1704-5

Shipman, R. H., Kao, I. C., & Fan, L. T. (1975). Single cell protein production by photosynthetic bacteria cultivation in agricultural byproducts. *Biotechnology and Bioengineering*, *17*, 1561–1570. https://doi.org/10.1002/bit.260171102

Singh, P., Paul, B. N., & Giri, S. S. (2018). Potentiality of new feed ingredients for aquaculture: A review. *Agricultural Reviews*, *39*, 282–291.

Strong, P. J., Xie, S., & Clarke, W. P. (2015). Methane as a resource: Can the methanotrophs add value? *Environmental Science and Technology*, *49*, 4001–4018. https://doi.org/10.1021/es504242n

Thornton, P. K. (2010). Livestock production: Recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 2853–2867. https://doi.org/10.1098/rstb.2010.0134

Tona, G. O. (2018). Current and future improvements in livestock nutrition and feed resources. In B. Yucel (Ed.), *Animal Husbandry and Nutrition* (pp. 147–169). London, UK: IntechOpen.

Vrati, S. (1984). Single cell protein production by photosynthetic bacteria grown on the clarified effluents of biogas plant. *Applied Microbiology and Biotechnology*, *19*, 199–202. https://doi.org/10.1007/BF00256454

Wang, H., Yang, A., Zhang, G., Ma, B., Meng, F., Peng, M., & Wang, H. (2017). Enhancement of carotenoid and bacteriochlorophyll by high salinity stress in photosynthetic bacteria. *International Biodeterioration and Biodegradation*, *121*, 91–96. https://doi.org/10.1016/j.ibiod.2017.03.028

Wang, H., Zhang, G., Peng, M., Zhou, Q., Li, J., Xu, H., & Meng, F. (2016). Synthetic white spirit wastewater treatment and biomass recovery by photosynthetic bacteria: Feasibility and process influence factors. *International Biodeterioration and Biodegradation*, *113*, 134–138. https://doi.org/10.1016/j.ibiod.2016.01.001

Warburg, O., & Christian, W. (1941). Isolation and crystallization of enolase. *Biochemische Zeitschrift*, *310*, 384–421.

Wardani, S. K., Cahyanto, M. N., Rahayu, E. S., & Utami, T. (2017). The effect of inoculum size and incubation temperature on cell growth, acid production and curd formation during milk fermentation by *Lactobacillus plantarum* Dad 13. *International Food Research Journal*, *24*, 921–926.

Willey, J., Sherwood, L., & Woolverton, C. (2013). *Prescott's Microbiology* (9th ed.). New York, NY: The McGraw-Hill Companies.

Wu, S., Shen, Z., Yang, C., Zhou, Y., Li, X., Zeng, G., … He, H. (2017). Effects of C/N ratio and bulking agent on speciation of Zn and Cu and enzymatic activity during pig manure composting. *International Biodeterioration and Biodegradation*, *119*, 429–436. https://doi.org/10.1016/j.ibiod.2016.09.016

**How to cite this article:** Patthawaro S, Saejung C. Production of single cell protein from manure as animal feed by using photosynthetic bacteria. *MicrobiologyOpen*. 2019;e913. https://doi.org/10.1002/mbo3.913

## APPENDIX A

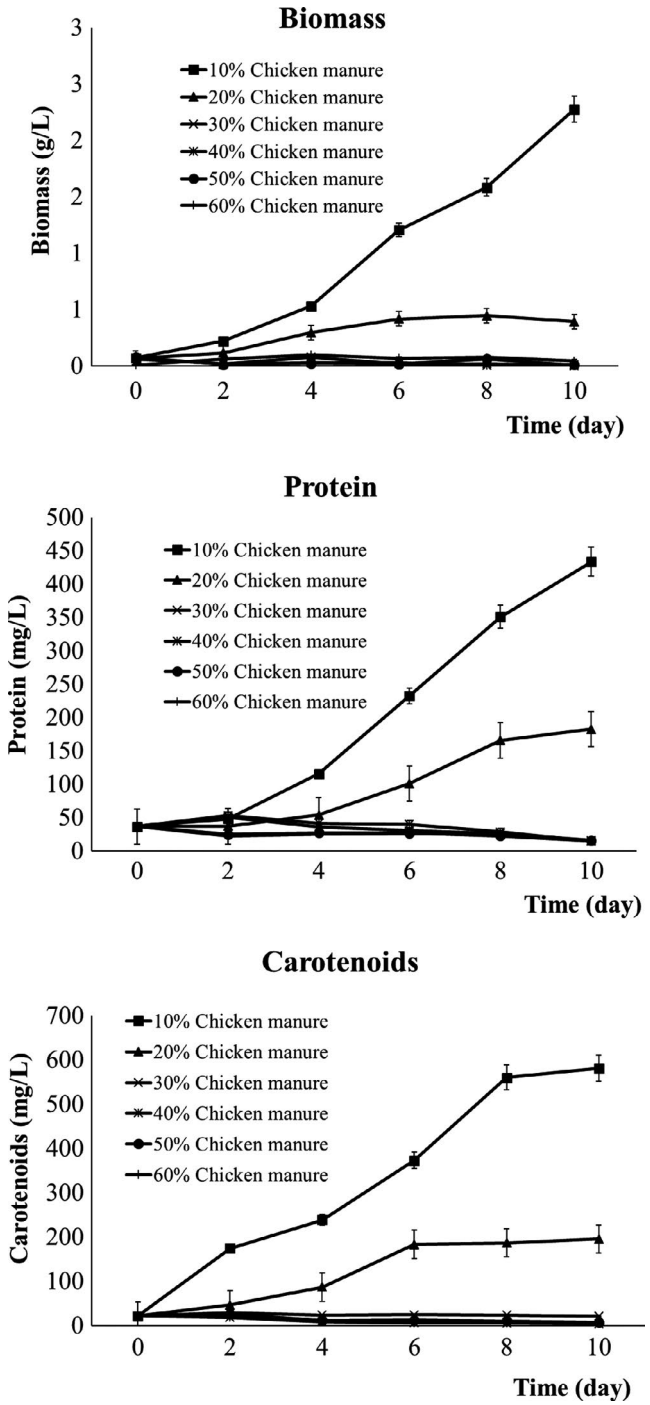


**FIGURE A1** The change over time in biomass, protein, and carotenoid concentrations of *Rhodopseudomonas faecalis* PA2 grown in chicken manure medium containing different chicken manure contents

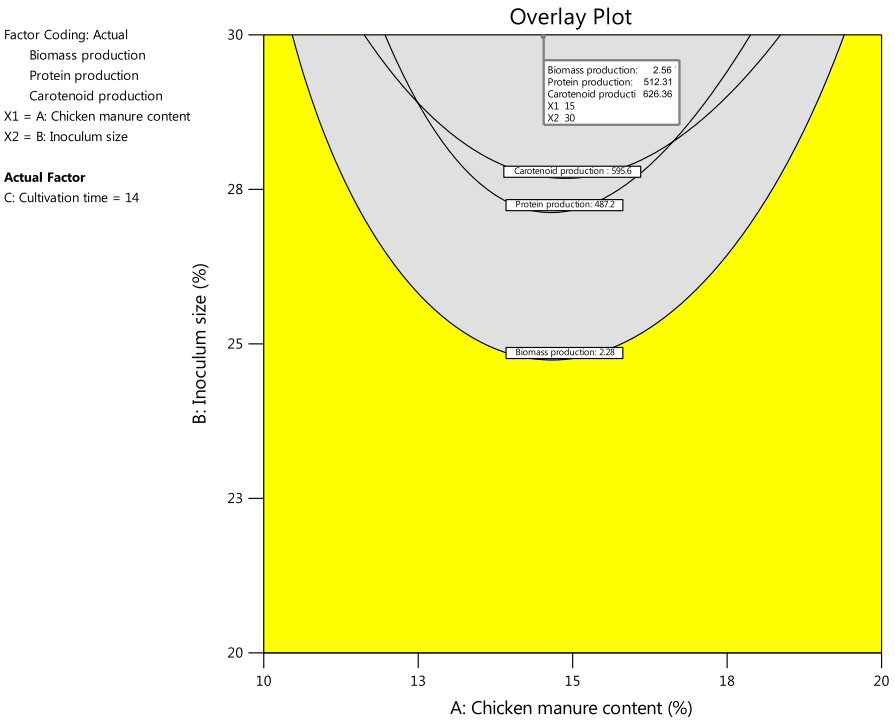


**FIGURE A2** The overlay plot representing the optimal condition for biomass, protein, and carotenoid productions by *Rhodopseudomonas faecalis* PA2