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Formulation of Cost-effective Medium Using Urea as a Nitrogen Source for *Arthrospira platensis* Cultivation under Real Environment

Puganeswary Sukumaran¹, Rosimah Nulit¹, Normala Halimoon²,
Sanimah Simoh³, Hishamuddin Omar¹ and Ahmad Ismail^{1*}

¹Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

²Department of Environmental Sciences, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

³Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia.

Authors' contributions

This work was carried out in collaboration between all authors. Author PS designed the study, managed the algal cultivation and analyses of the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Authors HO, RN and NH revised the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Even though *Arthrospira* is a well-known superfood, it is not extensively commercialized in developing countries like Malaysia due to the high production cost with low biomass yield. Based on literature, the algal production cost can be discounted by reducing the cost of medium utilizing cheaper and readily available chemicals. Therefore, the present study was conducted to

*Corresponding author: E-mail: aismail@upm.edu.my;
E-mail: puganeswarysukumaran@gmail.com;

experiment the prospect of *Arthrospira platensis* cultivation in newly designed medium with commercial or industrial grade fertilizers under real environment. Consequently, growth and yield of *A. platensis* was investigated under outdoor condition using modified Kosaric medium (MKM) which was designed by substituting the major laboratory chemicals in standard Kosaric medium (SKM) with commercial grade baking soda, sea salt, urea, phosphoric acid, potassium hydroxide and Epsom salt. Urea as an alternative nitrogen resource to sodium nitrate was pulse-fed throughout the cultivation period. The algal growth was measured through optical density, biomass dry weight and chlorophyll *a* content. The algal yield was determined by calculating its productivity and specific growth rate. The growth and yield of *A. platensis* was significantly higher ($p < 0.05$) in MKM in terms of optical density with 2.541 ABS, biomass dry weight with 1.30 g L⁻¹, chlorophyll *a* content with 12.96 mg L⁻¹, productivity with 0.141 g L⁻¹ d⁻¹ and specific growth rate with 0.253 μ d⁻¹ compared to SKM in eight days of cultivation period. The present finding showed the potential of MKM in lowering the medium cost up to 97% compared to SKM without compromising the algal yield under natural condition with proper cultivation techniques such as preadaptation and fed-batch addition of urea in the late evening.

Keywords: *Arthrospira platensis*; cost-effective medium; urea; productivity; outdoor conditions.

1. INTRODUCTION

Arthrospira is a photosynthetic, blue-green, spiral-shaped and multicellular cyanobacterium, which is being consumed as food for thousands of years [1]. Currently, this microorganism is recognized as a potential dietary supplement due to its rich nutrition value such as abundant protein content (50 - 70% dry weight), low nucleic acid content, vitamins, pigments, minerals, polyunsaturated fatty acids (PUFAs) and so on [2]. Beside this, NASA acknowledged *Arthrospira* as 'food of future' and proposed this microalga as an ideal food for astronauts [3]. However, *Arthrospira* cultivation is not widely practiced especially in developing countries like Malaysia beyond the laboratory scales, due to the high production cost with a lower yield. Thus, cost-effective microalgal cultivation techniques should be enhanced through outdoor mass production [4,5] under natural climate using available resources. Since the temperature tolerance level of *Arthrospira* ranges from 20°C to 40°C [6], Malaysian climate is suggested to be favorable condition for the good growth of this microalga. Besides that, utilization of cost-effective cultivation medium with optimum nutritional support should be considered in the development of low-cost *Arthrospira* production.

Nutrition condition is one of the important aspects in determining microalgal productivity and its biochemical composition [7,8]. When considering large-scale *Arthrospira* cultivation, the growth medium is the second major factor affecting production cost, accounted for 25% of the total expenses [9]. Hence, utilization of

cheaper medium is essential towards the effective commercialization of this microalga. One way to alleviate the nutrient cost is by reducing the concentration of the generally used standard medium without affecting algal growth [10]. In addition, incorporation of commercial fertilizers and industrial grade chemicals in growth medium is an important cost-saving factor in large-scale cultivation of *Arthrospira* [11,12]. Previously, different media have been successfully designed for the optimum growth of *Arthrospira* such as revised medium (RM₆) [3], OFERR medium [13], CFTIR medium [14] and Bangladesh medium [15]. Accordingly, the present investigation was intended to formulate modified Kosaric medium (MKM) by substituting certain major substances in the half concentration of standard Kosaric medium (SKM) with locally available industrial grade chemicals.

Among the major nutrient constituents of growth medium, nitrogen is the second most abundant, representing about 10% of the total content [16]. Although nitrates are the commonly used nitrogen source, former studies demonstrated the benefit of using alternative commercial nitrogen sources such as ammonium nitrate, urea, ammonium sulphate and ammonium chloride in economical cultivation of *Arthrospira* with high biomass yield [17,18,19,20]. Among them, urea (CO(NH₂)₂) appeared to be more attractive as it is cheaper and contains two nitrogen atoms in a single molecule with 46% of nitrogen [21] thus, required in smaller amount compared to ammonium and nitrate salts to supply similar quantity of nitrogen to the algal culture. Nevertheless, some research findings showed a

marked decrease in microalgal growth using urea due to its toxic effect at high concentration as well as volatilization under high pH and temperature, which could collapse the algal culture [12,22,23]. Accordingly, the growth inhibitory effect of urea has been successfully reduced through the fed-batch process, which resulted in better or at par growth rates of *Arthrospira* as compared to other nitrate salts [21,24]. Whereas, most of these findings are from laboratory practice and the feasibility of fed-batch cultivation of urea never been experimented before in outdoor conditions under the hot tropical climate of Malaysia.

Therefore, the aim of present study was to design and to evaluate the efficiency of MKM with the fed-batch addition of urea over SKM in outdoor conditions under Malaysian climate without adversely affecting the growth and yield of *A. platensis*.

2. MATERIALS AND METHODS

2.1 *A. platensis* Culture

An axenic culture of *A. platensis* was obtained from The Culture Collection of Algae at The University of Texas, Austin (UTEX). This microalgal species was cultured and pre-adapted in outdoor conditions under varying Malaysian tropical climate using SKM and MKM through batch and fed-batch cultivation respectively for two months to facilitate *A. platensis* adaptation to the new environmental conditions and media.

2.2 Preparation of the Experimental Growth Medium

SKM with half concentration was used as reference culture medium and prepared based on [25] with slight modification as followed (g L^{-1}): 4.500 NaHCO_3 , 0.250 NaCl , 0.010 CaCl_2 , 0.050 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.625 NaNO_3 , 0.125 K_2HPO_4 , 0.250 K_2SO_4 , 0.025 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.5 mL L^{-1} of trace metals solution composed of the following elements (g L^{-1}): 2.86 H_3BO_3 , 1.81 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.22 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.08 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.01 MoO_3 , and 0.01 $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$ following Table 1.

Meanwhile, MKM was modified from SKM by replacing the expensive laboratory chemicals

that cover a large portion of the growth medium and supplement major nutrients for *A. platensis* growth, with industrial grade chemicals. Accordingly, MKM was formulated with commercially available industrial grade chemicals as followed (g L^{-1}): 4.500 baking soda, 0.250 sea salt, 0.010 CaCl_2 , 0.050 Epsom salt, 0.221 urea, 0.070 phosphoric acid, 0.242 potassium hydroxide, 0.025 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.5 mL L^{-1} of trace metals solution composed of following components (g L^{-1}): 2.86 H_3BO_3 , 1.81 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.22 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.08 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.01 MoO_3 and 0.01 $\text{COCl}_2 \cdot 6\text{H}_2\text{O}$. The newly designed medium was prepared based on Table 1. Addition of nutrients was done following the sequence and mixed properly before adding another to avoid formation of precipitation and cloudiness in the growth medium.

2.3 Fed-batch Addition of Urea

Urea was supplemented to *A. platensis* culture during sunset through fed-batch method with exponentially increasing mass flow rate following [21]. Prepared urea stock solution (100 g L^{-1}) was added intermittently every 48 hours for 6 days of cultivation period based on the following equation:

$$\text{Mu} = 0.8e^{kt} \quad (1)$$

where, "Mu" represents the volume of added urea stock solution (mL/L algal culture) from the beginning of the cultivation process until the present t and "Mu _{t} " is "Mu" when t is the total feeding time. Total feeding time used in the present study was 6 days. When $t = 6$, $\text{Mu}_t = 2.21 \text{ mL urea stock solution/L algal culture}$, which supplying 221 mg urea/L algal culture and $k = 0.1694$. The initial volume of added urea stock solution was fixed at 0.8 mL urea stock solution/L algal culture, which supplying 80 mg urea/L algal culture [26].

2.4 Quality Control of Growth Medium

Physicochemical properties (pH and salinity) and nutrient content (N, P, K and Mg) of MKM were analyzed before being used in *A. platensis* cultivation to ensure the nutrient composition of the medium in acceptable proportion as in the standard medium (SKM) and meet the standard

Table 1. Ingredients of standard Kosaric medium (SKM) and modified Kosaric medium (MKM)

Components	Stock solution (g L ⁻¹ DDW)	Quantity added in 1L SKM	Quantity added in 1L MKM
NaHCO ₃	-	4.50 g	-
Baking soda	-	-	4.50 g
NaCl	-	0.25 g	-
Sea salt	-	-	0.25 g
CaCl ₂	10.00	1.00 mL	1.00 mL
MgSO ₄ ·7H ₂ O	50.00	1.00 mL	-
Epsom salt	50.00	-	1.00 mL
NaNO ₃	62.50	10.00 mL	-
Urea	100.00	-	*
K ₂ HPO ₄	12.50	10.00 mL	-
Phosphoric acid	70.00	-	1.00 mL
K ₂ SO ₄	25.00	10.00 mL	-
Potassium hydroxide	242.00	-	1.00 mL
FeSO ₄ ·7H ₂ O	25.00	1.00 mL	1.00 mL
Trace metals solution		0.50 mL	0.50 mL
H ₃ BO ₃	2.86		
MnCl ₂ ·4H ₂ O	1.81		
ZnSO ₄ ·7H ₂ O	0.22		
CuSO ₄ ·5H ₂ O	0.08		
MoO ₃	0.01		
CoCl ₂ ·6H ₂ O	0.01		

* Urea was pulse fed into culture throughout the cultivation period; DDW: Double distilled water

cultivation condition of this cyanobacterium. Each analysis was replicated into five to maintain the precision of results.

The pH which is contributed by bicarbonates as well as hydroxides and the salinity which is contributed by salts in the medium were measured using portable waterproof multi-parameter (PCS Testr™ 35, Oakton® Instruments). Total nitrogen (TN) in medium was analysed through oxidation with alkaline persulfate digestion to nitrate and followed by colorimetric technique based on hydrazine-copper reduction as described by [27]. Total phosphorus (TP) was determined as orthophosphate (PO₄³⁻) through persulfate oxidation followed by colorimetric determination based on molybdate-ascorbic acid reaction according to [28]. Next, the growth medium was filtered through pre-washed filter paper (qualitative filter paper, grade 1, 150 mm Ø, Whatman®) and properly diluted in 2% (v/v) nitric acid (Merck Millipore) [29] for the determination of K and Mg using Inductive Couple Plasma-Mass Spectrometry (ICP-MS, Elan 6000, Perkin-Elmer SCIEX) following [30]. Certified reference materials (Merck Millipore) were used for the preparation of standard solutions for each element following manufacturer's recommendation to calibrate the instrument before using it on real samples.

2.5 Experimental Design

A. platensis cultured in SKM was considered as control to study the growth and yield of *A. platensis* cultured in MKM under outdoor condition (treatment, T). Control and treatment were cultured in 5 replicates in 5 L polyethylene bottles containing 4 L working volume. Consequently, 400 mL of the pre-adapted algal culture, which is 10% (v/v) of the working volume, was transferred into 3.6 L cultivation medium of control and treatment respectively. Two holes were designed on cap of the bottle to hold tubing for aeration and gas exchange respectively. The algal culture was aerated through standard 3/16-inch diameter airline tubing with an air stone suspended in the middle of the bottle to provide continuous mixing and agitation. The cultivation of *A. platensis* was conducted in a randomized complete block design (RCBD) where the experimental units were grouped into five blocks with 20 cm distance and each block contains control (SKM) and treatment (MKM). The cultivation period was fixed at eight days based on preliminary observation where the algal culture started to experience stationary phase on day nine and onwards. This study was replicated for two individual cycles to ensure feasibility of the cultivation procedure and to find the average algal yield throughout the different cultivation

cycles. This experiment was conducted mostly at sunny, clear sky with minimum rainfall weather conditions.

2.6 Measurement of Environmental Factors during *A. platensis* Cultivation

Throughout the *A. platensis* cultivation period, the environmental parameters including light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and air temperature ($^{\circ}\text{C}$) were recorded daily from 7 am to 7 pm with 2 hours gap using a light meter (Li-250, LI-COR[®] Biosciences) and thermometer (Traceable[®] Big-Digit Four-Alert Alarm, Fisher Scientific) respectively.

2.7 Measurement of *A. platensis* Growth

The growth of *A. platensis* cultured in control and treatment was measured using three growth parameters, which were optical density, biomass dry weight and chlorophyll *a* content to precisely determine the growth pattern of microalgae. The algal sample was homogenized each time before being analysed to avoid sedimentation of cells, which could adversely affect the precision of measurements. The optical density of *A. platensis* culture was measured daily using spectrophotometer (Hitachi U-1900) at 620 nm [31]. Biomass dry weight of *A. platensis* was determined gravimetrically every alternate day following [32].

Chlorophyll *a* was determined spectrophotometrically on day eight after extraction with 95% ethanol for 5 min in a water bath at 70°C [33] and subsequent refrigeration at 4°C for 24 hours under dark conditions for maximum chlorophyll extraction. The absorbance was measured at 664 and 649 nm through spectrophotometer (U-1900, Hitachi) against prepared blank and the Chlorophyll *a* concentration was computed following [34].

2.8 Productivity of *A. platensis*

Productivity of *A. platensis* was calculated according to [35] using the following equation:

$$P_x = (X_m - X_i) (T_c)^{-1} \quad (2)$$

where,

$$P_x = \text{productivity (g L}^{-1} \text{ day}^{-1}),$$

$$X_i = \text{initial biomass concentration (g L}^{-1}),$$

X_m = maximum biomass concentration (g L^{-1}),
and

T_c = cultivation time related to the maximum biomass concentration (days).

2.9 Specific Growth Rate of *A. platensis*

Specific growth rate (μ) of *A. platensis* was calculated according to [12] using the following equation:

$$\mu = (\ln X_m - \ln X_i) (T_c)^{-1} \quad (3)$$

where,

X_i = initial biomass concentration (g L^{-1}),

X_m = maximum biomass concentration (g L^{-1}),
and

T_c = cultivation time related to the maximum biomass concentration (days).

2.10 Data Analysis

Maximum optical density, maximum biomass dry weight, chlorophyll *a* content, productivity and specific growth rate of *A. platensis* cultured in control and the treatment (T) were compared using SPSS software (version 21) through independent-samples t-test.

3. RESULTS

Physicochemical properties and nutrients content of SKM and MKM were depicted respectively in Table 2. The pH and salinity of MKM were slightly higher compared to pH and salinity of SKM. Nutrient content in both the cultivation media was relatively comparable (Table 2).

Table 2. Physicochemical properties and nutrient content of standard Kosaric medium (SKM) and modified Kosaric medium (MKM)

Properties	SKM	MKM
pH	8.61 ± 0.029	9.30 ± 0.032
Salinity (ppt)	2.53 ± 0.009	2.78 ± 0.017
Nutrients content (ppm)		
Nitrogen (N)	104.8 ± 1.55	105.1 ± 1.23
Phosphorus (P)	22.8 ± 0.65	23.7 ± 0.82
Potassium (K)	170.8 ± 2.29	171.0 ± 1.75
Magnesium (Mg)	4.8 ± 0.17	5.0 ± 0.12

Values are presented as mean ± SE (n = 5)

Average light intensity throughout the cultivation period for cycle 1 and 2 was as shown in Fig. 1. The light intensity was fluctuated between 2.0 - 400.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the morning, 890.1 - 1,307.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the midday and 42.9 -

762.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the late afternoon. Meanwhile, the ambient temperature was fluctuated between 26.0 - 30.1°C in the morning, 34.3 - 40.4°C during the midday and 32.8 - 37.4°C in the late afternoon.

The growth curve based on optical density and biomass dry weight of *A. platensis* culture from cycle 1 and 2 was as demonstrated in Fig. 2. The microalga cultured in control and treatment respectively showed relatively similar growth pattern during both cultivation cycles. Based on the plotted growth curves, there was no presence of lag phase due to the pre-acclimation process. The growth curves also indicate better growth of *A. platensis* in MKM compared to SKM.

Table 3 shows the maximum growth, productivity and specific growth rate of *A. platensis*.

Microalga cultured in MKM had significantly higher ($p < 0.05$) growth in terms of maximum optical density, maximum biomass dry weight, chlorophyll a, productivity and specific growth rate during both cycle 1 and cycle 2.

4. DISCUSSION

Zarrouk's medium is the first synthetic medium formulated for *Arthrospira* cultivation [36], which is still being used in many studies as a standard medium. Kosaric medium (KM) is a simplified and modified form of half concentration Zarrouk's medium [37]. SKM, which served as the reference medium in the present investigation, was basically half concentration of the original KM and quarter concentration of Zarrouk's medium as shown in Table 4. Under such reduced amount of nutrients, *A. platensis*

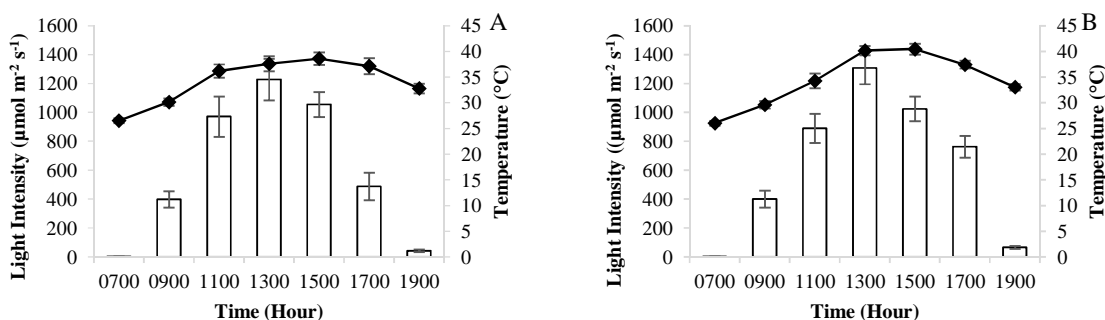


Fig. 1. Average light intensity (\square) and temperature (\blacklozenge) throughout the cultivation duration during cycle 1 (A) and cycle 2 (B)
 Values are presented as mean \pm SE ($n = 8$)

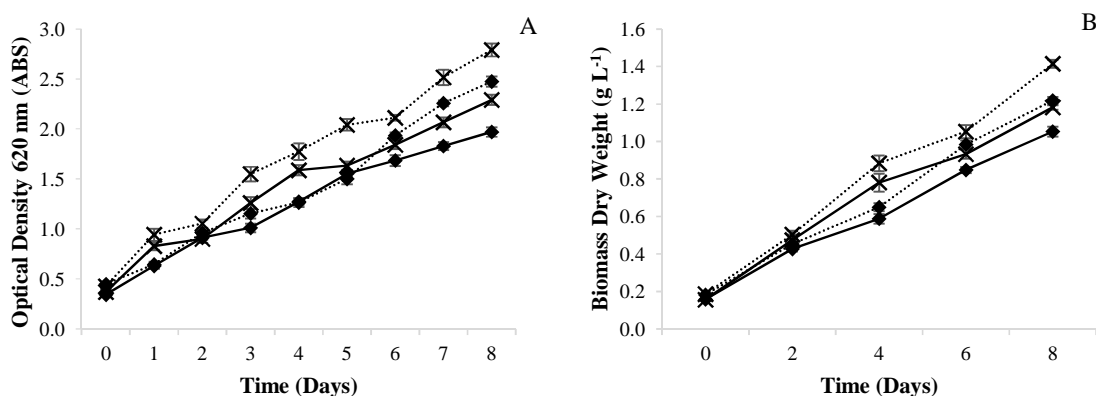


Fig. 2. Optical density (A) and biomass dry weight (B) of *A. platensis* cultured in SKM (\blacklozenge) and MKM (\times) during cycle 1 (—) and cycle 2 (....)
 Values are presented as mean \pm SE ($n = 5$)

Table 3. Growth of *A. platensis* cultured in SKM and MKM

Medium	Maximum optical density (ABS)	Maximum biomass dry weight (g L ⁻¹)	Maximum chlorophyll <i>a</i> (mg L ⁻¹)	Productivity (g L ⁻¹ d ⁻¹)	Specific growth rate (μ d ⁻¹)
Control (SKM) – C1	1.97 ± 0.047	1.05 ± 0.026	10.40 ± 0.263	0.112 ± 0.0029	0.237 ± 0.0017
Treatment (MKM) – C1	2.29 ± 0.051	1.18 ± 0.011	11.70 ± 0.283	0.128 ± 0.0011	0.253 ± 0.0018
Control (SKM) – C2	2.48 ± 0.052	1.22 ± 0.020	12.12 ± 0.273	0.130 ± 0.0022	0.238 ± 0.0022
Treatment (MKM) – C2	2.79 ± 0.065	1.42 ± 0.021	14.21 ± 0.219	0.154 ± 0.0025	0.254 ± 0.0015

SKM: Standard Kosaric medium; MKM: Modified Kosaric medium; C1: Cycle 1; C2: Cycle 2.

Values are presented as mean ± SE (n = 5)

cultured using SKM grew satisfactorily in outdoor conditions and achieved maximum growth (on average) in terms of optical density with 2.222 ABS, biomass dry weight with 1.14 g L⁻¹, chlorophyll *a* content with 11.26 mg L⁻¹, productivity with 0.121 g L⁻¹ d⁻¹ and specific growth rate with 0.237 μ d⁻¹ in eight days of cultivation period. Likewise, [10] demonstrated that the concentration of certain component in Zarrouk's medium can be saved up to 75% without significant loss of the algal biomass production.

The result from present study also showed better yield compared to the outdoor cultivation of *A. platensis* in open bioreactor by [38] under UV protected greenhouse in the extreme south of Brazil with 1.33 ± 0.006 g L⁻¹ of maximum biomass dry weight, 0.050 ± 0.007 g L⁻¹ d⁻¹ of productivity and 0.160 ± 0.046 μ d⁻¹ of specific growth rate using 20% Zarrouk's medium in 25 days of cultivation period. Compared to most of the previous studies using standard medium such as Zarrouk's, Kosaric, Paoletti, Schlosser and Spirulina medium with higher amount nutrients ranged from 6.80 – 18.60 g L⁻¹ NaHCO₃, 1.25 – 5.00 g L⁻¹ NaNO₃, 0.25 – 1.00 g L⁻¹ K₂HPO₄ and 0.50 – 2.00 g L⁻¹ K₂SO₄, SKM in present study with curtailed amount of nutrients containing 4.500 g L⁻¹ NaHCO₃, 0.625 g L⁻¹ NaNO₃, 0.125 g L⁻¹ K₂HPO₄ and 0.250 g L⁻¹ K₂SO₄ which are below the standard range of nutrients mentioned above, produced satisfactory algal yield within shorter cultivation period under natural environment (Table 4).

Next, MKM was successfully formulated by substituting the costly laboratory grade chemicals of the major portion of SKM with inexpensive and readily available commercial grade baking soda, sea salt, urea, phosphoric acid, potassium hydroxide and Epsom salt. Previously, [3] and [12] used both baking soda and sea salt in revised medium (RM₆) and reduced cost medium respectively. The benefit of using sea salt was that the macro and micro minerals required for *A.*

platensis growth are partly supplied by this type of low-cost raw material [12]. Besides, phosphoric acid has been used as an alternative phosphorus source in Bangladesh medium [15] and NRC medium [11]. [39] also suggested baking soda, sea salt, urea, phosphoric acid, and Epsom salt as low cost ingredients in the preparation of Spirulina growth medium. However, potassium hydroxide never been used or suggested before by any researcher as an ingredient in *Arthrospira* growth medium. Utilization of this alkaline potassium source is something new which advantageously increased the pH of MKM to 9.3 compared to SKM with pH 8.6 as shown in Table 2 to provide favourable growth condition for this alkalophilic microalga where, pH 9.5 to 10.5 [40] is considered optimal for *Arthrospira* cultivation.

The substitution of these commercial grade fertilizers in MKM did not deviate much the physicochemical properties and nutrient content as in the standard medium, SKM as illustrated in Table 2. In addition, the newly designed cost-effective medium, MKM increased the growth of *A. platensis* in terms of optical density, biomass dry weight, chlorophyll *a* content, productivity and specific growth rate about 12.6%, 12.5%, 13.1%, 14.3% and 6.3% respectively compared to SKM. The result showed the potential of MKM which mostly containing industrial grade chemicals, in providing optimum culture condition for maximum algal growth. Besides, substitution of urea as nitrogen source in MKM advantageously enhanced the algal yield by providing an energetic gain due to its unprompted hydrolysis to ammonia under alkaline condition, which is readily assimilated by *Arthrospira* without further energy consumption [21,41]. On the other hand, utilization of NO₃ as nitrogen source in SKM require a high energy consuming enzyme-catalysed process as NO₃ reduced to NO₂ by nitrate reductase and followed by NO₂ reduction to ammonia by nitrite reductase [21,41] which leads to energy loss and reduced algal yield.

Table 4. Growth of *A. platensis* in present study compared to previous investigations

Medium	Major components in medium (g L ⁻¹)	M _{NPK} (g L ⁻¹)	X _{max} (g L ⁻¹)	P _x (g L ⁻¹ d ⁻¹)	μ (d ⁻¹)	Chla _{max} (mg L ⁻¹)	Duration (days)	Medium cost (US\$ ton ⁻¹)	References
Zarrouk's	NaHCO ₃ (18.00); NaNO ₃ (3.00); K ₂ HPO ₄ (0.50); K ₂ SO ₄ (1.00)	N (0.494); P (0.090); K (0.677)	3.11	0.114	0.101	≈ 5.700	25	NI	[42]
Half strength Zarrouk's	NaHCO ₃ (9.00); NaNO ₃ (1.25); K ₂ HPO ₄ (0.25); K ₂ SO ₄ (0.50)	N (0.206); P (0.045); K (0.339)	0.99	0.044	0.106	5.710	20	NI	[43]
Modified Zarrouk's	NaHCO ₃ (16.80); KNO ₃ (2.50); K ₂ HPO ₄ (0.50); K ₂ SO ₄ (1.00)	N (0.346); P (0.090); K (1.644)	5.10	-	-	0.078	40	NI	[44]
Modified Zarrouk's	NaHCO ₃ (16.80); NaNO ₃ (1.50); K ₂ HPO ₄ (0.50); K ₂ SO ₄ (1.00)	N (0.247); P (0.090); K (0.677)	3.27	0.153	0.199	-	21	NI	[45]
Kosaric	NaHCO ₃ (9.00); NaNO ₃ (1.25); K ₂ HPO ₄ (0.25); K ₂ SO ₄ (0.50)	N (0.206); P (0.045); K (0.563)	0.91	0.091	0.503	8.150	10	NI	[37]
Paoletti	NaHCO ₃ (15.15); KNO ₃ (2.57); K ₂ HPO ₄ (0.50)	N (0.356); P (0.090); K (1.222)	1.16	0.079	0.224	-	14	NI	[21]
Half strength Schlosser BG-11	NaHCO ₃ (6.80); NaNO ₃ (1.25); K ₂ HPO ₄ (0.25); K ₂ SO ₄ (0.50)	N (0.206); P (0.045); K (0.339)	2.10	0.076	0.094	-	25	NI	[46]
	NaNO ₃ (1.50); K ₂ HPO ₄ (0.04)	N (0.247); P (0.007); K (0.018)	1.45	0.053	0.096	-	30	NI	[47]
Spirulina medium	NaHCO ₃ (18.60); NaNO ₃ (5.00); K ₂ HPO ₄ (1.00); K ₂ SO ₄ (2.00)	N (0.824); P (0.181); K (1.354)	1.12	0.022	0.031	-	31	NI	[48]
NCL	NaHCO ₃ (10.00); NaNO ₃ (2.50); K ₂ HPO ₄ (0.50); K ₂ SO ₄ (1.00)	N (0.412); P (0.090); K (0.677)	1.08	0.069	0.095	8.500	15	NI	[49]
CFTRI	NaHCO ₃ (4.50); NaNO ₃ (1.50); K ₂ HPO ₄ (0.50); K ₂ SO ₄ (1.00)	N (0.247); P (0.090); K (0.677)	0.80	0.050	0.083	6.880	15	NI	[49]
RM ₆	NaHCO ₃ (8.00); NaNO ₃ (2.50); SSP (1.25); MOP (0.90)	N (0.412); P (0.087); K (0.477)	0.57	0.030	0.173	23.220	18	16.00	[3]
Reduced cost medium NPK (P2C3)	NaHCO ₃ (16.80); NH ₄ NO ₃ (0.35); SSP (1.25); MOP (0.90)	N (0.124); P (0.087); K (0.477)	8.13x10 ⁻⁰⁴	2.789x10 ⁻⁰⁵	0.233	6.850 x10 ⁻⁰⁵	27	13.00	[12]
	NaHCO ₃ (10.00); NPK-10:26:26 (0.76)	N (0.076); P (0.198); K (0.198)	1.22	0.077	0.110	8.920	15	86.62	[49]
CMU02	NaHCO ₃ (8.50); NaNO ₃ (1.50); K ₂ HPO ₄ (0.50); NPK-16:16:16 (0.60)	N (0.343); P (0.186); K (0.324)	0.53	0.037	0.169	-	14	13.14	[50]
SKM	NaHCO ₃ (4.50); NaNO ₃ (0.63); K ₂ HPO ₄ (0.13); K ₂ SO ₄ (0.25)	N (0.103); P (0.022); K (0.168)	1.14	0.121	0.237	11.262	8	180.80	Present study
MKM	NaHCO ₃ (4.50); CO(NH ₂) ₂ (0.22); H ₃ PO ₄ (0.07); KOH (0.24)	N (0.103); P (0.022); K (0.168)	1.30	0.141	0.253	12.958	8	5.47	Present study

M_{NPK}: Concentration of NPK in medium; X_{max}: Maximum biomass dry weight; P_x: Productivity; μ: Specific growth rate; Chla_{max}: Maximum chlorophyll a; NI: Not indicated

Moreover, fed-batch addition of urea in MKM efficiently reduced the growth-limiting effects of urea, which is toxic to algal cells at the high concentration above 100 mg L^{-1} and off-gas under high temperature and pH [21]. Urea spontaneously hydrolysed into ammonia due to urease action and/or high alkaline condition [16]. At pH 10 and above, ammonia is capable of diffusing across the microorganism's cell membrane, which is primarily driven by pH gradient and being stored as ammonium in the cell [51]. Accumulation of ammonium at a high concentration above 2 mM causes a detrimental effect on microalgae by uncoupling photosynthesis, which in some cases even to cause cell death [16]. Previously, some researchers used a lower concentration of urea in *A. platensis* batch cultivation to avoid the toxic effect of this fertilizer [12,52] but, this would lead to nitrogen limitation after some days due to volatilization of this compound at high temperature and pH.

Therefore, in present study, urea was intermittently pulse-fed every 48 hours at an exponentially increasing rate to maintain the culture below the toxic threshold level of ammonia and to avoid nitrogen deficiency in the algal culture. Consequently, urea in MKM favoured significantly higher ($p < 0.05$) growth of *A. platensis* (on average) under Malaysian outdoor conditions in terms of optical density with 2.541 ABS, biomass dry weight with 1.30 g L^{-1} , chlorophyll a content with 12.96 mg L^{-1} , productivity with $0.141 \text{ g L}^{-1} \text{ d}^{-1}$ and specific growth rate with $0.253 \mu \text{ d}^{-1}$ compared to NaNO_3 in SKM. Similar results were observed in previous investigations where, the fed-batch addition of urea aided in better growth of *Arthrospira* compared to nitrate nitrogen sources such as KNO_3 and NaNO_3 [26,35,53].

Furthermore, numbers of cultivation techniques were implemented in present study aiming to maximize the productivity of this cyanobacterium. Initially, *A. platensis* was preadapted to the new medium and environmental condition which produced exponential algal growth without initial lag phase (Fig. 2). Besides, pulse-feeding of urea was done during sunset throughout the cultivation period, to enable higher nitrogen assimilation rate by *A. platensis* under cool and dark night conditions as well as to reduce the loss of ammonia through off-gassing especially during the hot midday. Based on the cost-wise calculation, preparation of 1,000 L of SKM medium costs about RM 770.65 (US\$ 180.80)

meanwhile, RM 23.33 (US\$ 5.47) for MKM. Therefore, utilization of MKM which composed of cheap and locally available commercial chemicals, beneficially reduced the cost of growth medium up to 97%. Moreover, the cost of MKM is also notably lower compared to previous studies as shown in Table 4.

5. CONCLUSION

An alternative low-cost growth medium (MKM) was successfully formulated for *A. platensis* cultivation by substituting the main chemical components of SKM with cheap and readily available industrial grade chemicals in the reduced amount without compromising the algal growth and yield. Beside this, urea in MKM was proven to be a suitable nitrogen source for this cyanobacterium growth through an appropriate fed-batch method with pulse-feeding of urea at late evening. This study accentuates the advantages of the new modified medium, not only as a highly productive input but also as a low-cost alternative, which can be used profitably for large-scale *Arthrospira* biomass production under the real environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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