*Pakistan Journal of Science (Vol. 72 No. 4 December, 2020)*

# FORMULATION OF A COST-EFFECTIVE MEDIUM TO SUPPORT HIGH BIOMASS PRODUCTION AND IMPROVED AMINO ACID PROFILE OF ARTHROSPIRA PLATENSIS

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**ABSTRACT:** *Arthrospiraplatensis* is a WHO recognized super food. However, it is not extensively commercialized due to the high cost associated with its cultivation media. This study was designed to devise a cost effective media for the culturing of *A. platensis*. Standard Zarouk's media (ZK) was used a reference media for nutrient composition to formulate six cost effective cultivation media (XR-1-XR-6). Growth of *A. platensis* was analyzed on the basis of cell count, growth percentages and biomass production. Amino acid contents were determined through Amino Acid Analyzer. Growth of *A. platensis* cultivated in XR-1 and XR-3 media was observed comparable with ZK in terms of cell count and spiral structure The XR-3 medium formulated in this study was found to produce improved percent growth rate and amino acid content when compared with *A. platensis* cells grown in conventionally used Zarouk's medium.

**Key words:** *Arthrospiraplatensis,* Semi-Defined Medium, Microalgae, Superfood, Malnutrition, Zarrouk medium.

*(Received 09.09.2020 Accepted 22.11.2020)*

## INTRODUCTION

Today's world has reached new heights of technological advancements especially in the field of food and agriculture but an immense part of the developing world yet facing big challenges of hunger and malnutrition. World Health Organization (WHO) defines malnutrition as conditions with either deficiencies or excesses in nutrient intake, imbalance of essential nutrients or metabolic impairment for nutrient utilization. It includes under-nutrition, inadequate intake of vitamins or minerals, underweight, overweight, obesity, and resulting diet-related non-communicable diseases (World Health Organization, 2020). Malnutrition is still considered the top most reasons of children's death over the globe (Tette*et al*., 2015). In emerging countries around 45% of the deaths among children younger than 5 years of age are linked to under nutrition whereas 39% of the grown-ups are fronting obesity (World Health Organization, 2020). Besides linking with the high morbidity and mortality, malnutrition poses huge health and financial burdens to the individuals and their families, on communities and the economies of poor countries (Kramer & Allen, 2015).

Initially inappropriate production and distribution of the food was thought to be the key causes of malnutrition as declared by WHO (Pee *et al*., 2015). However certain related concerns such as social status, environment, economy, gender roles, behavior, and education found enormously impacted on this global disease (Sharma *et al*., 2016).Safeguarding adequate food supply is an important development towards eradicating hunger though equal availability and affordibity of food enriched with macro and micro nutrients to the growing population are the ultimate solutions of malnutrition (Adesogan *et al*., 2020).

Eradicating global disease like malnutrition multiple measures need to be taken. High population growth rate, lessening of food resources and recommendations of balanced diet warrants considering alternative resources for reducing malnutrition all over the world with natural and affordable substitutes. Researchers of the filed investigate diverse supplements mostly originate from plant extracts for growth performance, enhancing immunity, antioxidant function, reproduction, and metabolism. Scientists have achieved many accomplishments in the domain of neutraceuticals and recognizing *Arthrospiraplatensis* as a super food is among one of them (Soni *et al*., 2017).

*A. platensis* is a unicellular, filamentous blue-green cyanobacteria found in freshwater and lakes (Soni *et al*., 2017).This microalgae also referred as single cell protein because of its distinctive nutritional enriched profile with a balanced amount of all vital nutrients (Seyidoglu *et al.,* 2017). *A. platensis* contains a high protein content (up to 70%) which is higher than present in our routine diet components such as soybean, chicken and beef (Seyidoglu *et al*., 2017). The microalgae specie is enriched with essential fatty acids, essential amino acids, and multiple vitamins such as vitamin A, B1, B2, B6, E, K. It also contains important minerals such as calcium, phosphorus, sodium, potassium (Seyidoglu *et al*., 2017). Besides having ironic nutritional worth, its therapeutic potentials have also been explored. *A. platensis* has found to boost the immune system by increasing the production of the cytokines and antibodies and influences the phagocytic activity of macrophages (Banakar *et al*., 2020). Reports are available those showed that *A. platensis*boosts up body metabolism, having potent antioxidant properties and lowers blood sugar and cholesterol levels. All these properties are linked with healthier and a longer life span (Farag*et al*., 2016). Alongside biorefineries utilize *A. platensis* biomass to convert in to high added value product, to produce biofuels and bioenergy (Behera *et al*., 2015).

Growing use of *A. platensis* for eliminating malnutrition, good health and wellness its massive production is warranted to serve health-food industries as food for human, & animals, feed additive and pharmaceutical products. In spite of an enormous demand of its commercial production, high cost of the standard growth medium for *A. platensis* cultivation confines it large scale production (Madkour *et al*., 2012).The standard Zarrouk's medium is highly overpriced due to expensive analytical grade components needed for concocting it. Numerous attempts have been made to develop a cost-effective growth media for cultivation and production of *A. platensis* comparable to the Zarrouk's medium in terms of Biomass production and nutritive quality (Soni *et al*., 2019; Michael *et al*., 2019; Danishkumar *et al*., 2016).

Amino acid composition of *A. platensis* varies greatly according to the availability of nitrogen source in the growth media (Kim *et al*., 2016). In present study different nitrogen sources were assessed in line to derive a new growth medium to decrease the cost of *A. platensis* production and to evaluate and compare the growth characteristics and amino acid analysis of the produced organism with that cultivated in Zarrouk's medium.

## MATERIALS AND METHODS

**Microorganism:** *Arthrospira platensis* used in this study was purchased from Algae Research Supply USA, and maintained on Zarrouk's media.

**Growth media and experimental design:** Zarrouk's medium (ZK) was considered as standard reference medium to study the growth and amino acid content of A. *platensis* (Zarrouk, 1966). A series of new and more cost effective growth media were formulated in present study by substituting nitrogen sources present in ZK with low cost and locally available commercial chemicals and omitting all micronutrients mineral salts such as $K_2S0_4$, $MgHSO_4.7H_2O$, $FeSO_4.7H_2O$, $CaCL_2.2H_20$ and EDTA. The first medium designed was XR-1 comprising of ammonium nitrate and urea as alternative nitrogen source compare to ZK that contain sodium nitrate as a sole nitrogen sources. XR-2, contains double the amount of Urea ($CO(NH_2)_2$) compare to XR-1. In the next articulated medium was XR-3, where ammonium nitrate ($NH_4NO_3$) was omitted and used double amount of urea. In XR-4, Urea was not added instead double amount of ammonium nitrate was used. In XR-5, ammonium nitrate was replaced with the same amount of sodium nitrate ($NaNO_3$). In XR-6, no urea was added. Composition of standard and modified growth media (ZK; XR-1 to XR-6) are mentioned in Table-1

Sterilized Erlenmeyer flasks (500ml) containing 100 ml of growth medium was used for each experimental set up. Growth experiments were initiated with 10% (v/v) of inoculum consists of 30000-35000 cells of *A. platensis*. After inoculation, flasks were kept at 25°C and 150 rpm in a photosynthetic incubation shaker. Light was provided with a cool white fluorescent lights (1500-3000 Lux), with a photocyle of 16/8 hours. A constant aeration was maintained by bubbling using an aeration pump. All tests were performed in duplicates for 8-15 days.

**Growth and amino acid evaluation:** Cell count was done by using hemocytometer, and optical density was recorded at λ 720nm by spectrophotometer. The data was taken on daily basis and average values were presented in the form of graphs and tables. Cell biomass was evaluated at stationary phase of *A. platensis* growth cycle. This was achieved by centrifuging the growth media at 10,000 rpm for 10 minutes. Cell pallet was oven dried for 3 days at 37°C.Amino acid analysis of the dried cell mass of *A. platensis* in standard and the growth medium with comparable growth rate to the ZK was carried out on the Shimadzu Amino Acid Analyzer with Shim-Pack Amino-Na column containing styrene divinyl benzene copolymer resin with sulphonic group at Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratory, Karachi according to the method described by Haider *et al*., 2015.

## RESULTS

**Effect of culture medium on growth of A. platensis:** In present study six cost effective media were formulated namely XR-1 to XR-6 and evaluatedfor growth and amino acid content of *A. platensis*in comparison with standard ZK medium. The time course experiments were performed for 12 days and the growth of *A. platensis* in standard and formulated media was recorded by estimating cell population as shown in Figure-1.

All growth curves were performed either in formulated growth media or standard ZK medium, lack an initial lag phase and started with log growth phase. A rapid increase in the cell count was observed in all formulated growth media except XR-6. The growth in reduce cost formulated media (XR-1—XR-5) was comparable to the ZK. At day 9, XR-1 and XR-3 approximately similar cell count to that of standard ZK media (1060 cells/ml in ZK and 920 and 1040 cells ml in XR-1 and XR-3 medium respectively). These media were selected further to study for dry cell biomass, optical density, changes in pH during cultivation and specific growth characteristics of cell morphology including number of spiral and their length.

The two selected media XR-1 and XR-3 showed higher cell count compare ZK medium (Figure-2A). However in long time course experiment standard media had a relatively higher optical density at $10^{th}$ till $28^{th}$ day of experiment (Figure-2B). All tested media including ZK, XR-1 and XR-3 showed similar pH drift throughout the cultivation cycle of *A. platensis* (Figure-2C). Biomass dry weight of *A. platensis* obtained from ZK medium (ZK) and cost effective media formulated in this study (XR-1 and XR-3) is demonstrated in Figure-2D.

The morphological features of *A. platensis* grown in standard and formulated media were compared under microscope with 40X magnification. Numbers of spirals found to be higher in formulated media in comparison with the ZK. In XR-1 medium, *A. platensis* was appeared in longer chains compared to XR-3 (Figure 3).

***Effect of culture medium on amino acid content:*** Amino acid profile of the dried cells of *A. platensis* is summarized in Table-2. *A. platensis* contains both essential and non-essential amino acids and composition of cultivation media essentially influences their content in the cells. In total eighteen amino acids were detected in the analysis among these eight were non-essential amino acids including Aspartic acid, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystein and Tyrosine. Remaining ten amino acids were essential amino acids (Threonine, Valine, Metheionine, Isoleucine, Leucine, Phenyl alanine, Histidine, Tryptophan, Lysine and Arginine. Among the three media, *A. platensis* dry cells grown in XR-3 showed a highest content of both essential and non-essential amino acids. The quantity of both essential and non-essential amino acids in cells grown in XR-3 were found to be two to three folds higher than those obtained from cell grown in ZK and XR-1.

The amount of non-essential amino acids in dry cells of *A. platensis* cultivated in XR-1 was fairly comparable with the cells grown in ZK whereas the content of essential amino acid was found lower in *A. platensis* grown in XR-1 medium compared to that in ZK.

**Table-1: Table-1: Composition of standard Zarrouk's medium (ZK) and the formulated reduced cost media (XR-1 – XR-6).**

| Constituents | Composition $gL^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | **ZM** | **XR-1** | **XR-2** | **XR-3** | **XR-4** | **XR-5** | **XR-6** |
| **$NH_4NO_3$** | - | 0.353 | 0.353 | - | 0.706 | - | 0.353 |
| **UREA** | - | 0.088 | 0.176 | 0.176 | - | 0.088 | - |
| **$NaNO_3$** | 2.5 | - | - | - | - | 0.353 | - |
| **$K_2HPO_4$** | 0.5 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| **KCL:NaCl** | - : 1.0 | 0.8531 : 0.0449 | 0.8531 : 0.0449 | 0.8531 : 0.0449 | 0.8531 : 0.0449 | 0.8531: 0.0449 | 0.8531 : 0.0449 |
| **SEA SALT** | - | 1 | 1 | 1 | 1 | 1 | 1 |
| **$NaHCO_3$** | 16.8 | 16.8 | 16.8 | 16.8 | 16.8 | 16.8 | 16.8 |
| **$K_2S0_4$** | 1 | - | - | - | - | - | - |
| **$MgHSO_4.7H_2O$** | 0.2 | - | - | - | - | - | - |
| **$FeSO_4.7H_2O$** | 0.01 | - | - | - | - | - | - |
| **$CaCl_2.2H_20$** | 0.04 | - | - | - | - | - | - |
| **EDTA** | 0.08 | - | - | - | - | - | - |
| **pH** | 8.9 | 8.9 | 8.9 | 8.9 | 8.9 | 8.9 | 8.9 |

**Table-2: Amino acid content of the dried cells of *Spirulinaplatensis*in standard Zarrouk's medium (SCM0 and formulated media XR-1 and XR-3**

| | **Amino acid content gms/100 gm of dry cell mass of *Spirulinaplatensis*** | | |
|---|---|---|---|
| **Amino acids** | **SCM** | **XR-1** | **XR-3** |
| ***Non-essential amino acids*** | | | |
| **Aspartic acid** | 0.961 | 0.989 | 2.539 |
| **Serine** | 0.495 | 0.305 | 1.101 |

| | | | |
|---|---|---|---|
| **Glutamic acid** | 1.509 | 1.577 | 4.636 |
| **Proline** | 0.409 | 0.387 | 0.008 |
| **Glycine** | 0.360 | 0.331 | 0.789 |
| **Alanine** | 0.778 | 0.613 | 2.026 |
| **Cystein** | 0.000 | 0.000 | 0.000 |
| **Tyrosine** | 0.593 | 0.691 | 0.980 |
| ***Essential amino acids*** | | | |
| **Threonine** | 0.261 | 0.000 | 0.000 |
| **Valine** | 0.367 | 0.293 | 1.067 |
| **Metheionine** | 0.000 | 0.000 | 0.172 |
| **Isoleucine** | 0.323 | 0.208 | 0.433 |
| **Leucine** | 1.727 | 0.998 | 1.717 |
| **Phenyl alanine** | 0.753 | 0.530 | 0.940 |
| **Histidine** | 0.031 | 0.026 | 0.033 |
| **Tryptophan** | 0.086 | 0.142 | 0.048 |
| **Lysine** | 2.750 | 4.669 | 5.408 |
| **Arginine** | 3.840 | 0.000 | 0.000 |

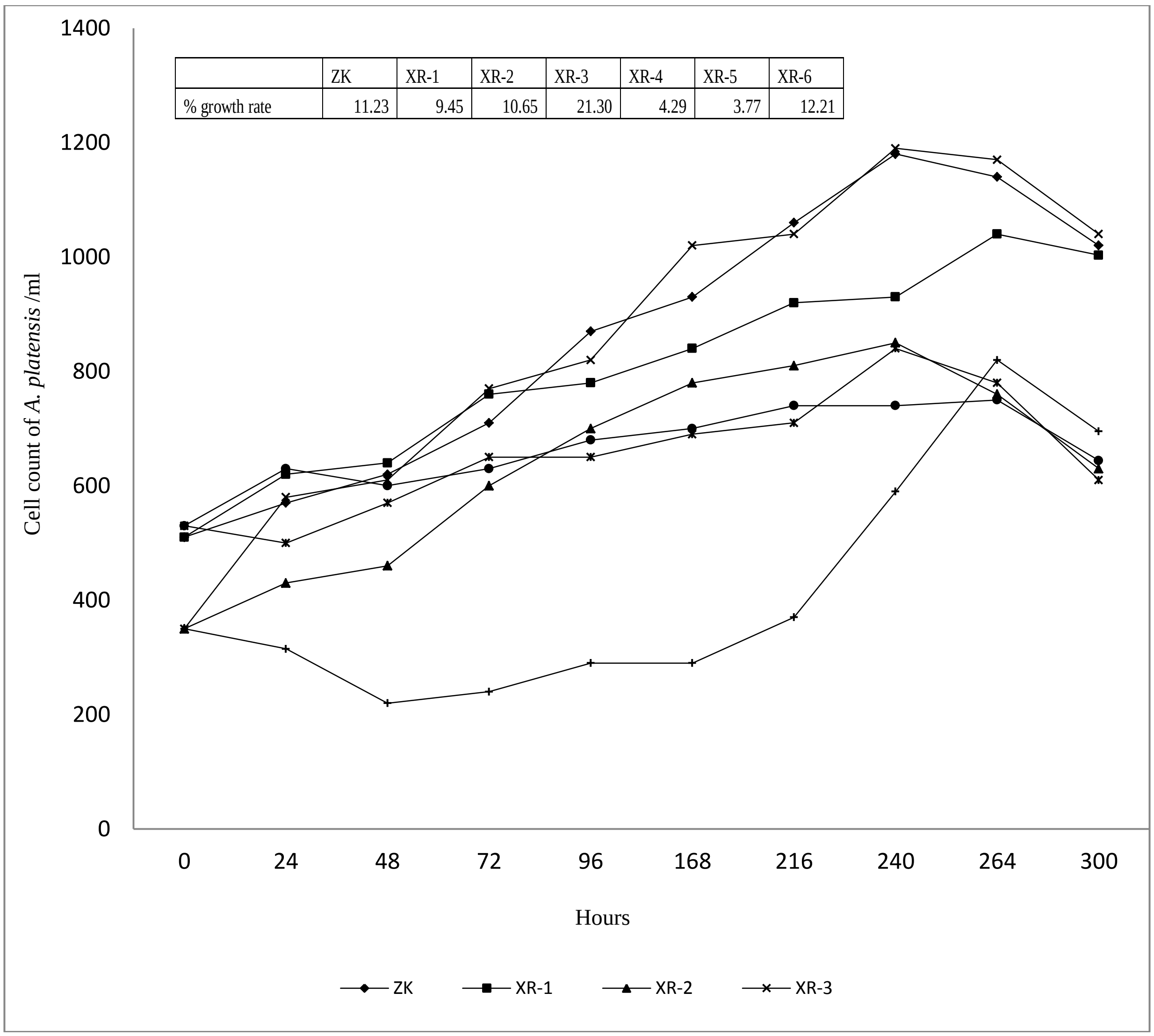


**Figure-1: Cell count of *A. platensis*grown in ZK medium and reduced cost media (XR-1 – XR-6). Each value is an average of two samples. Upper Left panel shows the percent growth rate.**

**Figure-2A**

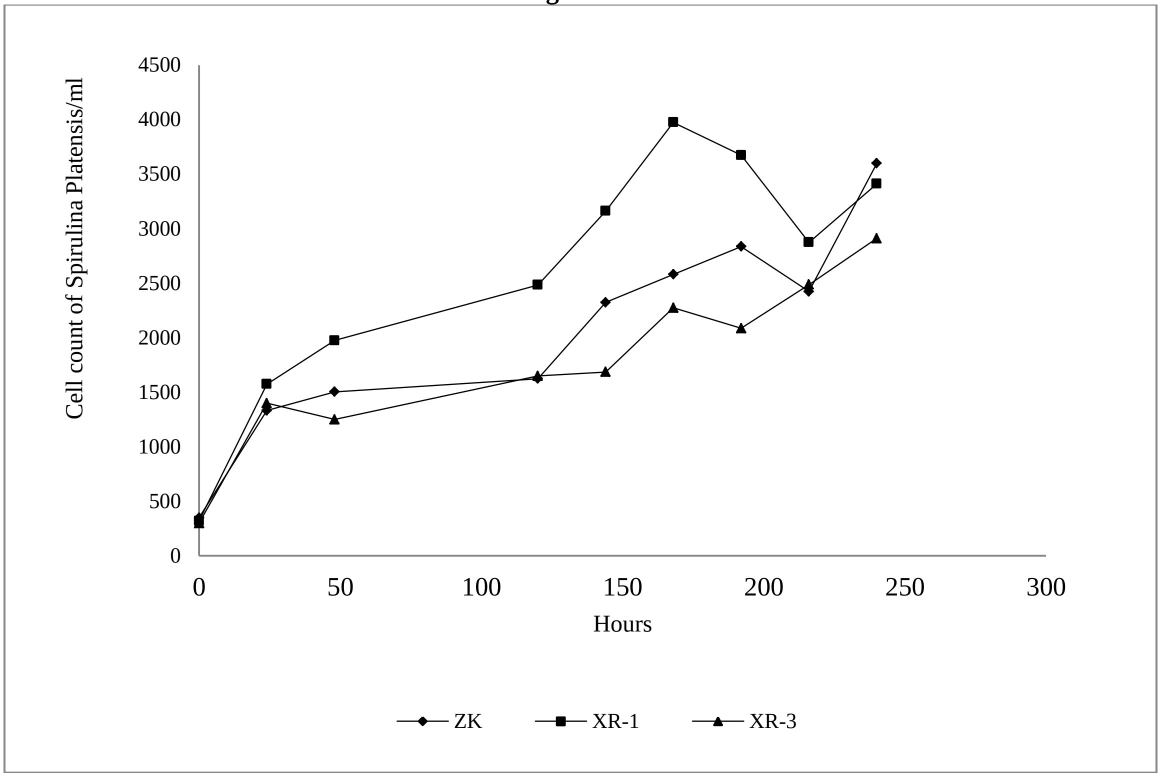


**Figure-2B**

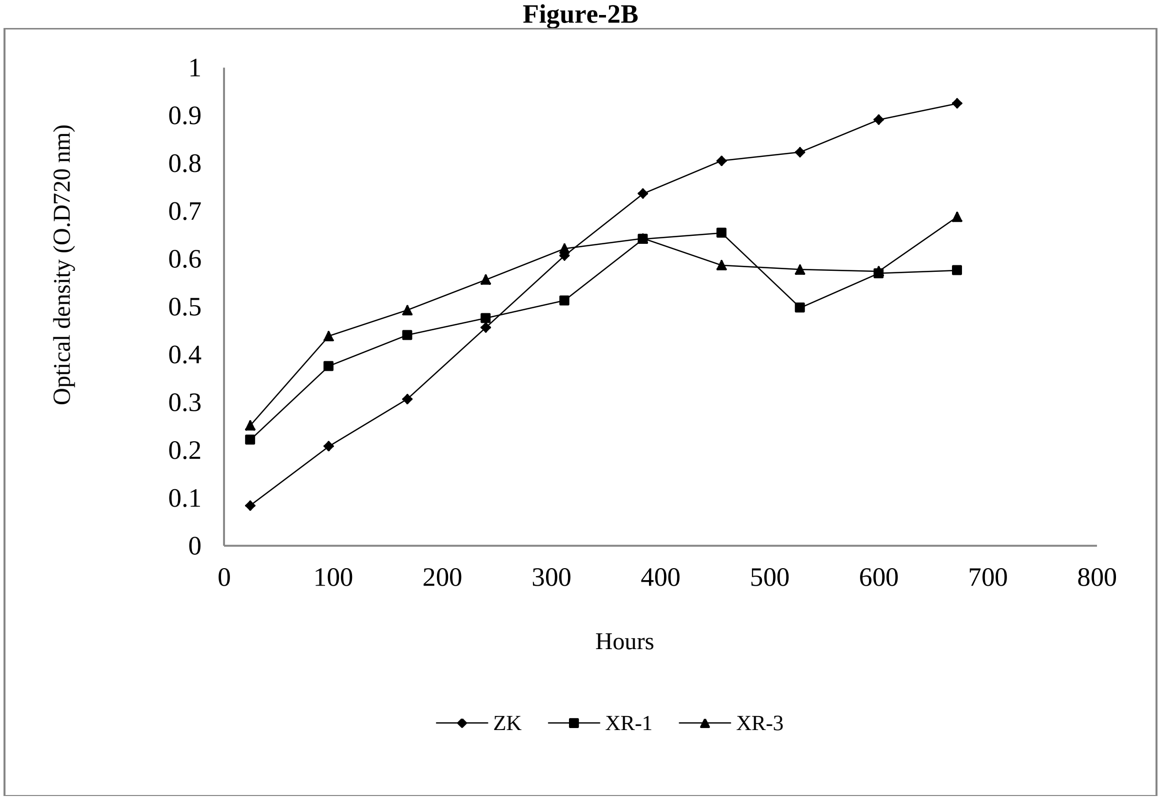

**Figure-2C**

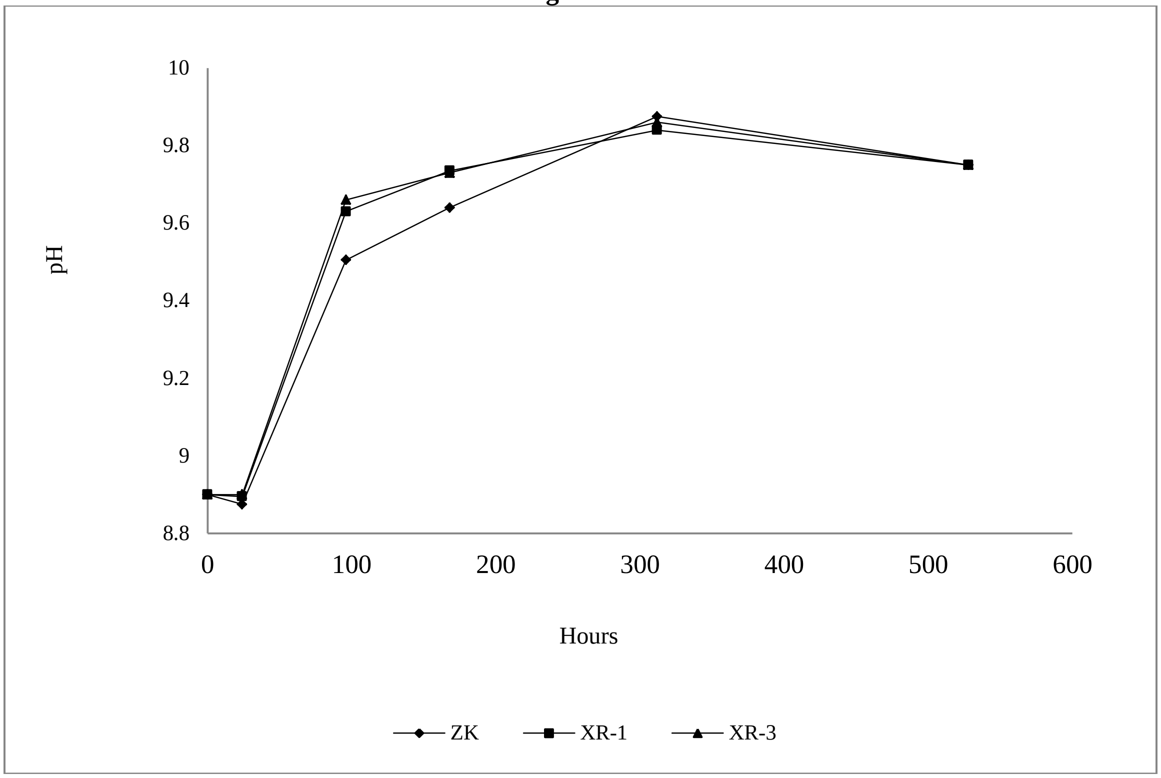


**Figure-2D**

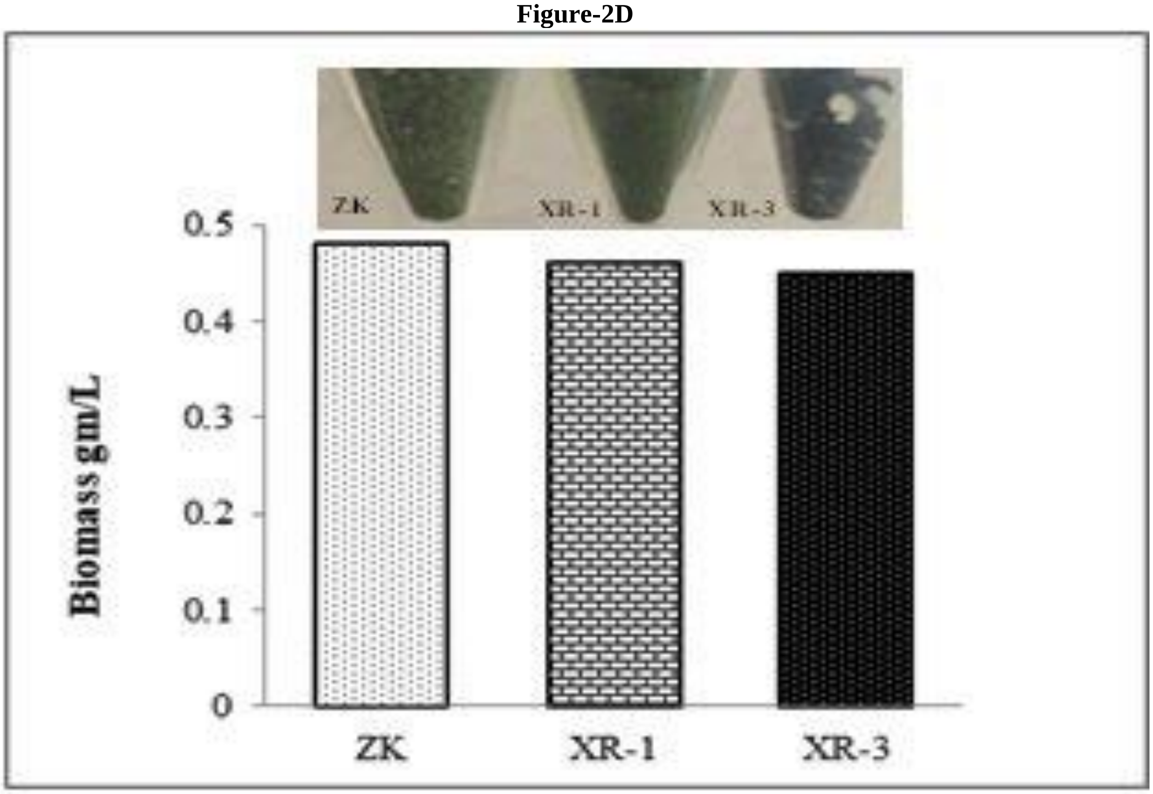


**Figure-2: Cell count/ml (A), Optical density (B), changes in pH during cultivation (C) and dry cell mass g/L (D) of *A. platensis* in standard Zarrouk's medium (SCM) and reduced cost media XR-1 and XR-3.**

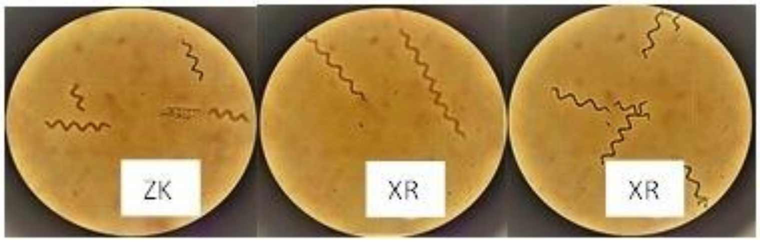


**Figure3: Spiral character of *A. platensis* grown in standard Zarrouk's medium (ZK) and formulated XR-1 and XR-3 media.**

## DISCUSSION

Undernourishment and acute hunger are yet the most imperative cause of disease and death globally (Troeger *et al*., 2018). Adequate production of food for all hungers, accessibility at economical cost and readiness & absorption of quality nutritions from food to human body are the bottleneck in coping up the subject. Microalgal biomass of *Arthrospiraplatensis* is an FDA approved dietary supplement having preventive as well as curative features to treat malnutrition (Azabji-Kenfack *et al*., 2011). *A. platensis* is blue green algae abundant in essential proteins, minerals and vitamins those readily absorb and bioavailable in the body (Nurdinov *et al*., 2015). *A. platensis* contains good quality protein that is approximately as high as 50-70% of its dry cell weight with a highly balance proportion of essential amino acids (Radhakrishnan *et al*., 2017).

Despite the reiterated significance of algal biomass, its bulk production is limited by a range of constraints including the availability of essential nutrients during algal growth and the financial impact associated with its mass production. Cultivation cost accounts for approximately 25% of total expenses of biomass production (Sukumaran *et al*., 2018). Nutritional composition of the cultivation media plays an important role in the quantity and quality of algal biomass production. Zarrouk media (ZK) considered one of the best growth media that support high cell mass production of *A. platensis* however high cost of ZK limits mass production of microalgae (Micheal *et al*., 2019). In developing countries such as Pakistan, the bulk production is much restricted due to cost. In view of this gap, present study was designed to devise a suitable and cost effective semi-synthetic media for the growth of *A. platensis* that provide a comparable biomass yield and protein quality.

Several studies on devising of cost effective media for optimal cultivation of *A. platensis* have been published (Vankataman *et al*., 1995, Raoof *et al*., 2006; Singh, 2006; Madkour *et al*., 2012; Sukumaran *et al*., 2018). Many of these were designed by varying the concentrations of phosphate and nitrogen sources of the nutrients used in ZK media (Zarrouk, 1966). In this study six cost effective growth media are formulated by varying the sources and lowering the concentrations of nitrogen, taking ZK as a reference media for comparing growth and amino acid contents. Ammonium nitrate ($NH_4NO_3$) and urea were selected as alternate sources of nitrogen in present study in place of sodium nitrate that is the sole nitrogen source present in ZK. Selected alternate nitrogen sources, ammonium nitrate and urea are comparatively cheaper sources and deliver two nitrogen atoms in the growth media compare to sodium nitrate that serves with only one nitrogen atom (Madkour *et al*., 2012). Ammonium nitrate and urea contributes 35 % and 46% respectively in the nitrogen contents to the growth media whereas sodium nitrate provided 14% nitrogen to the media (Madkour *et al*., 2012). Considering this, lower concentrations of ammonium nitrate and urea were used in present study.

Growth of *A. platensis* in six formulated growth media (XR-1-XR-6) was compared with reference ZK media. Experiments were carried out in duplicates for 12 days and results were recorded in cell count/ml and percent growth rate. Highest cell count in almost all tested media was observed at 8th day during the course of 12 days growth curve. Similar observations are reported by Raoof *et al*., 2006 with maximum percent growth rate at day six to nine days of cultivation. Out of the six formulated media, two urea containing media XR-1 and XR-3 were found most suitable for the growth of *A. platensis* and cell growth was found comparable to the conventionally used ZK medium. The two least successful media (XR-4 and XR-6) assessed in terms of growth lacked urea as nitrogen source. Highest percentage growth of *A. platensis* was observed in XR-3 medium where lowest concentration of urea was used as sole source of nitrogen (0.176 g/l, 5.88mM) compared to other variable media mentioned in Table 1. Similar observations were reported independently by Sukumaran and Soni where an improved algal growth and higher biomass yield was observed using urea as nitrogen source (Sukumaran *et al*., 2018 and Soni *et al*., 2019). Urea assimilation pathway is reported to be less energy consuming under alkaline conditions and thus yields an increased algal biomass (Danesi *et al*., 2002; Bezerra *et al*., 2012).Urea has been suggested as a preferred nitrogen source for algae but only under conditions when use in lower concentrations since higher concentration of urea has been found associated with cell toxicity resulting in lower biomass (Borowitzka, 1988; Richmond *et al*., 2013).

Composition of cultivation media not only impact on cell biomass but also on quality of protein and content of amino acid in the cells. Basis on our results, formulated media XR-1 and XR-3 were selected for further analysis. Amino acid profile was done as a measure of varying nitrogen sources. The amino acid content of *A. platensis* contain all nutritionally important essential and non-essential amino acids, comparable to other dietary sources (Bashir *et al*., 2016; Radhakrishnan *et al*., 2017). Eighteen amino acids including eight non-essential amino acids and ten essential amino acids were analyzed as a part of current study. The present data suggests that *A. platensis* grown on XR-3 comprises of lowest urea concentration (5.88mM), have the highest levels of six out of eight non-essential amino acids and six out of Ten non-essential amino acids. Dry cells of *A. platensis* grown in XR-3 display the highest level of Glutamic acid followed by aspartic acid and alanine as compared to cultures grown on XR-1 and ZK. In the profile of essential amino acids, lysine is approximately two fold higher in concentration followed by leucine, valine and phenylalanine. Similar results were reported by studies conducted previously where highest levels of glutamic acid were reported in cultures grown on urea (Kim *et al*., 2007; Bashir *et al*., 2016). Furthermore, the amino acid profile of *A. platensis* found in this study was similar to one suggested by Food and Agriculture Organization (FAO) (Bashir*et al*., 2016; WHO, 2007).

The study was aimed to devise a simple and cost effective media for the bulk production of *A. platensis* with comparable biomass production. Our results clearly indicated that XR-3 media formulated as a part of present study is a successful medium for the cultivation of *A. platensis*. In terms of cost wise calculation also, XR-3 media was found most financially viable as compared to the rest of the media tested during the study. It was found that preparation of 1000 L of XR3 would cost 5300Pkr (32 USD) as compared to the cost of XR-1 and ZK (6500 Pkr (39 USD) and 66000 Pkr (499 USD) respectively. This cost calculation indicates that noticeable financial impact of the media suggested in this study. It further emphasizes the use of optimum concentration of urea as an alternate nitrogen source which can be used successfully and economically for the large scale production of protein rich *A. platensis*.

**Conclusion:** A low cost cultivation media (XR-3) comprised of low levels of urea as a sole source of nitrogen was formulated in this study and was found to have comparable biomass to *A. platensis* grown on conventionally used Zarrouk medium. In addition, the percent growth rate of cells grown in the XR-3 medium was almost double withtwo to three fold higher amino acid contents in comparison to *A. platensis* grown in conventionally used ZK. . The study emphasizes the use of suggested media XR-3 for the bulk cultivation of *A. platensis.*

**Competing Interests:** The authors declare no conflict of interest.

**Acknowledgement:** The authors would like to express their gratitude to Pak Ethanol Pvt. Limited for providing with initial funding to the study. Authors also appreciate the part played by Pakistan Council of Scientific and Industrial Research for their help with amino acid profiling.

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