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Biomass production and growth performance of Momela Lake's spirulina (*Arthrospira fusiformis*) cultured under urea and N: P: K fertilizers as cheaper nitrogen sources

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ABSTRACT

Spirulina species are world widely known for their ability to produce high quantities of useful metabolites such as protein, fatty acids and vitamins. One of the major challenges in production of this microalgae is the cost and composition of culture medium. In the present study, effect of two culture media with agricultural fertilizers as nitrogen sources *viz.* urea containing medium (UM) and N: P: K containing medium (NPKM) on production of a locally found spirulina (*Arthrospira fusiformis*) was investigated. Zarrouk medium (ZM) was used as a standard, other culture conditions included 67.5 µmol m^{-2} s^{-1} radiation, 10 pH and 30 ºC temperature. *A. fusiformis* was isolated from Momela Lake in Arusha National Park Tanzania. There was no difference ($p > 0.05$) in mean biomass production (BM_{mean}) and doubling time (DT) between ZM (1.205 g/L, 1.437 days respectively) and UM (1.168 g/L, 1.63 days respectively). Significantly higher ($p < 0.05$) maximum biomass BM_{max} production and exponential growth rate (μ) in a sequential order was from ZM (1.844 g/L, 0.483/day respectively), UM (1.6 g/L, 0.425/day respectively) and NPKM (0.57 g/L, 0.273/day). It appeared that the wild-isolated *A. fusiformis* was able to grow well in less costly UM showing high potential for mass production.

Keywords: Momela Lake's spirulina, biomass production, agricultural fertilizers, nitrogen sources.

INTRODUCTION

Spirulina are filamentous, gram negative species of blue-green microalgae (cyanobacteria) from two separate genera *Spirulina* and *Arthrospira* which consist of about 15 species (Habib et al., 2008). Of these, *Arthrospira spp* are the most common and widely available possessing diverse biochemical compounds of biological and nutritional significance such as protein (55-70% dry weight, making it the richest plant protein of all known plant protein sources), minerals (calcium and iron) and vitamins (Habib et al., 2008). It has further gained worldwide acceptance due to its therapeutic effect on different health problems

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like cancer, diabetes, HIV, hyperlipidemia, hypertension, nephrotoxicity and obesity (Belay, 2002; Ouedraogo et al., 2013; Barry et al., 2014; Haunget et al., 2018). In addition to their potential use as a healthy food for human, spirulina is reported to be a good immunnostilmulant and growth promoter in fish, poultry and rats (Kana et al., 2016; Abdelkhalek et al., 2017; Kata et al., 2018).

Arthrospiara spp usually grows luxuriantly in warm alkaline lakes including the soda lakes of East Africa such as Nakuru in Kenya, Natron, Empakai, Elmenteita, El Kekhotoito, Manyara, Rishetani and Momela in Tanzania (Grant, 2006; Kaaya et al., 2007). Generally, it is said that in lakes where flamingos are, spirulina can be found (Mlingwa and Baker, 2006). *Arthrospira fusiformis* is the most abundant phytoplankton in Momela lakes located in Northern Tanzania (Hamisi et al., 2017).

Spirulina production has been conducted in both small and large commercial scales (Shimamatsu, 2004; Habib et al., 2008). Examples of large scale commercial production include Earthrise Nutritionals and Cynotech in the USA and DCI Microalgae in China, small scale production has been report in south East Asia particularly in India where *Arthrospira* is produced in small ponds and pots for local consumption to fight malnutrition (Jeej-Bai, 2006: Siva-Kiran et al., 2015). However, cost and composition of culture medium for spirulina has been one of the major challenges facing production of this cyanobacterium (Habib et al., 2008; Nor, 2015). Usually culture media compose of both macro-elements such as carbon, nitrogen, phosphorous, potassium, sulphur, magnesium, sodium, chlorine, calcium, iron and trace elements such as zinc, copper, nickel, cobalt and selenium (Habib et al., 2008). Zarrouk medium has been used universally and recommended in all subsequent studies with high alkalinity and salt content being the basic requirements for most of strains investigated (Pandey et al., 2010). Despite good results from Zarrouk medium, high costs of chemical ingredients make it unsuitable for economic effective mass production, prompting researchers to focus on coming up with culture media that are less costly.

The primary modification in many laboratories is the elimination of trace element solution (Jeej-Bai, 2006). Alternatively, media formulation using sea water, sewage water, kitchen waste water, waste water from factories and agricultural grade fertilizers have been proposed (Habib et al., 2008). Among the major nutrient ingredients in *Arthrospira spp* culture medium, nitrogen is the second most important in terms of quantity, representing about 10% of the total content (Soletto et al., 2005). Although sodium nitrate and potassium nitrate salts have been used as conversional source of nitrogen, previous studies indicate the possibility of using alternative nitrogen sources in low cost medium and still get high biomass production (Danes et al., 2002; Costa et al., 2004; Matsudo et al., 2009). Feasibility of replacing nitrate salts with cheaper agricultural fertilizer such urea and NPK as nitrogen sources for *Arthrospira* cultivation is documented by a number of studies (Sanchez et al., 2003; Sigh, 2006; Seth and Naik, 2007; Sukumaran, 2018).

Given the above background in addition to hot tropical climate in most of Tanzania Regions, establishing simpler and cheaper culture media for culture of a locally found *A. fusiformis* could be of great importance for future exploitation of this untapped microalga. In the present study, biomass production and growth performance of a Momela Lake's *A. fusiformis* cultured under two agricultural fertilizer nitrogen source based media; urea-nitrogen source medium and N: P: K 15:15:15-nitrogen source medium were evaluated. Nitrate-nitrogen source medium was used as a standard to compare against the above growth media.

MATERIALS AND METHODS

Sampling

Arthrospira fusiformis was sampled from one of the Momela Lakes (Big Momela Lake) which is located in Arusha national park at 3° 14" S, 36° 54" E. Momela Lakes are shallow alkaline lakes made up of several

lakes; Big Momela, Small Momela, Lekandiro, EL- kekhotoito, Reshiteni, Kusare and Tulusia. All the seven lakes are mainly fed by separate underground sources. Due to high alkalinity, the water is not preferred by animals for drinking, instead they attract a wide variety of African bird life especially lesser flamingos. After collection, water samples were preserved in the ZM (Table. 1) and maintained in dark cool environment (< 20 °C) in a cool box prior to transportation to the laboratory for isolation.

Isolation

Isolation was done using serial dilution microbiology technique at the Department of Botany, University of Dar es Salaam. Water samples were diluted with Zarrouk's medium up to 10^{-8} dilution and thereafter dilution tubes were incubated in a growth chamber at 30 °C and pH 10 illuminated with a day light fluorescent tubes supplying 67.5 µmol m^{-2} s^{-1} light intensity at the surface of vessels (Pandey et al., 2010). The purity of the culture was ensured by the repeated plating on solidified ZM for selection of pure colonies followed by inoculation in liquid media until unialgal cultures were obtained. *Arthrospira fusiformis* cells were identified according to Kaggwa et al. (2013) under the microscope at 4X to 10X magnification.

Inoculum preparation

For inoculum preparation, an aliquot from the above isolated unialgal culture was added to the sterilized 1L Erlenmeyer flasks containing synthetic ZM and then scaled up to 2L Erlenmeyer flasks under continuous illumination (67.5 µmol m^{-2} s^{-1}), 30 °C and pH10. Standardization of alga inoculum was done using spectrophotometer at 680 nm. The algal volume obtained was used as the initial inoculum to study the effect of different nitrogen sources.

Effect of culture media

In order to test the effect of different culture media with agricultural fertilizers (Urea and NPK) as nitrogen sources on *A. fusiformis* biomass production and growth performance, two culture media i.e. OFERR medium with urea as nitrogen source developed by Sigh (2006) and a simple medium with N: P: K 15:15:15 as nitrogen source suggested by Seth and Naik (2007) hereinafter referred to as UM and NPKM respectively were used in triplicate. Zarrouk medium (Zarrouk, 1996) with sodium nitrate as a nitrogen source hereinafter referred to as ZM was used as a standard. The composition of the different culture media is shown in Table1. 250 ml of experimental medium was added to 500 ml conical flasks and inoculated with 20 ml inoculum at an Optical Density (OD) of 1.0. Other growth conditions were: 30 °C maintained by using a water bath, initial pH of 10 and light intensity of 67.5 µmol m^{-2} s^{-1} continuous illumination provided by white cool florescent lamp (75 Watts, TORCH). The light intensity was measured using a light meter (VERTEX VXLM-636). The inoculated flasks were covered with cotton wool and were manually shaken three times a day (in the morning, mid-day and night).

Biomass and growth estimation

Biomass concentration (BM) as g/L was estimated by filtering homogeneous suspension of *A. fusiformis* sample through Whatman GF/C filter paper (1.2 µm, 47 mm - diameter), rinsing it with distilled water and oven drying at 60 °C overnight. The filter papers containing *A. fusiformis* biomass were cooled in a desiccator and weighed using an analytical balance (SHIMADZU AVU 220). The difference between initial and final dry weights represented the biomass. Measurements were done at 24 hours interval until decline of growth was observed. Biomass were categorized into maximum biomass (BM_{max}) and mean biomass (BM_{mean}). Exponential growth rate (μ) and doubling time (DT) were calculated using the following equation for batch microalgae culture (Guillard, 1973).

$$\mu = \left(\frac{\text{Ln N2 - Ln N1}}{\text{T2 - T1}}\right), \qquad \text{DT} = \frac{0.693}{\mu}$$

Where N2 and N1 are biomass concentrations at time T2 and T1 respectively.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences between treatment means at $p < 0.05$. Post-hoc analysis was done where significant differences were detected between treatments means using Tukey's Honest Significant Difference (THSD) Test. Analyses were performed using statistica software version 7.

Table 1: Composition of different experimental culture media.

	Conc (g/L)		
Ingredients	**ZM**	**UM**	**NPKM**
Sodium bicarbonate	18	8	4
Sodium nitrate	0.5	-	-
Di-potassium hydrogen phosphate	2.5	-	-
Potassium sulphate	1.0	0.5	-
Sodium chloride	1.0	5	-
Calcium chloride	0.04	-	-
Sodium ethylene-di-aminetetra acetate	0.08	-	0.08
Magnesium sulphate	0.2	0.16	0.2
Ferrous sulphate	0.01	0.05	-
Urea	-	0.2	-
N:P:K (15:15:15) fertilizer	-	-	1.0
Phosphoric acid	-	0.052 ml	-
TE solution	1 ml	-	-

TE solution include: boric acid (2.86), ammonium molybdate (0.02), manganese chloride (1.8), copper sulphate (0.08) and zinc sulphate (0.22).

RESULTS

Arthrospira fusiformis identification

Cultured *A. fusiformis* was identified based on microscopic characteristics. The spiral shape of filaments was solitary, free floating, displaying gliding motility and the arrangements of the multicellular cylindrical trichomes in an open left hand helix along the entire length. Most filaments were coiled or helical consisting of shorter than broader cells with clear and visible transverse cross walls (Figure 1). These characteristics were used to identify *A. fusiformis* trichomes (Kaggwa et al., 2013).

Nutrient effect on *Arthrospira fusiformis* growth

The aim of the present study was to asses two media (UM and NPKM) with Urea and NPK fertilizers respectively as nitrogen sources for Momela Lake's *A. fusiformis* growth and biomass production in comparison to when cultured in a standard Zarrouk culture media (ZM) with Sodium nitrate nitrogen

sources. Results are as presented in Table 2 and Figure 2. There were short lag phases (< 1 day) in all the three culture media, with exponential phases in the following six days and then stationary phases commenced, followed by decline phases. Lag phases were not affected by culture media. Furthermore, results showed that *A. fusiformis* cultured in ZM and UM had comparable results in terms of mean biomass production, BM_{mean} (p = 0.076) and doubling time, DT (p = 0.16). On contrary to BM_{mean} and DT in the present study, maximum biomass BM_{max} and exponential specific growth rate μ of *A. fusiformis* were found superior in ZM over UM and NPK ($p < 0.05$). On the other hand, cultivation using NPKM resulted into significantly ($p<0.05$) poorest BM_{mean}, BM_{max}, μ and DT compared with ZM and UM.

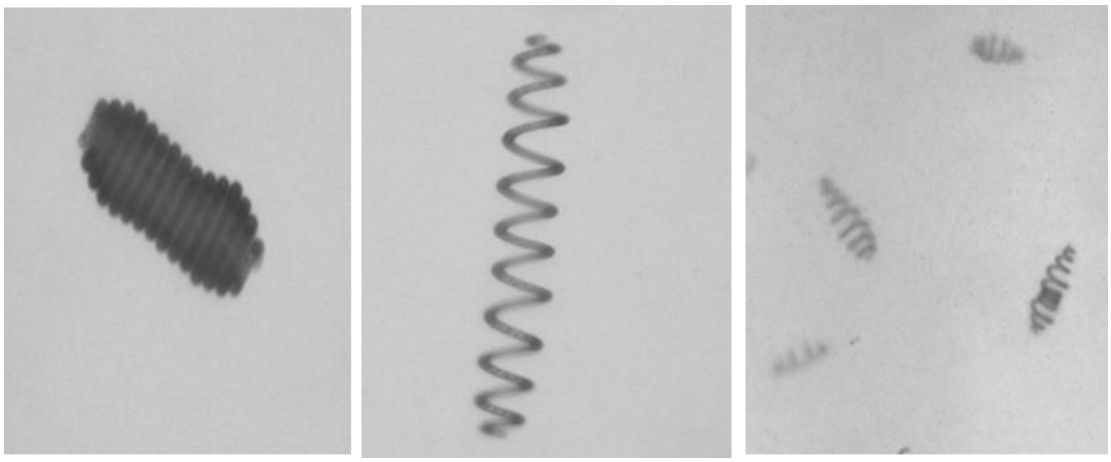

Figure 1: Different spiral morphologies of Momela Lake's *A. fusiformis* isolates.

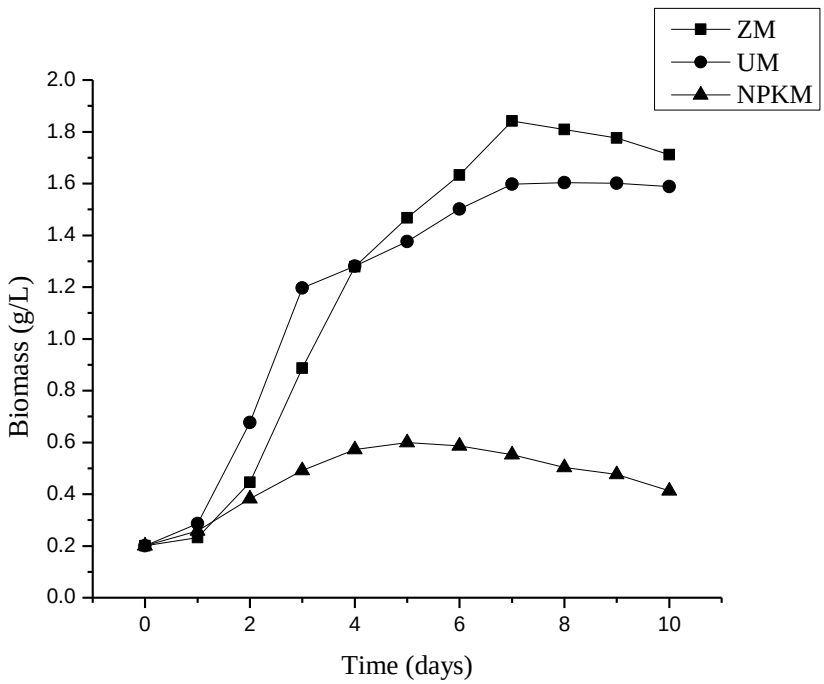

Figure 2: *A. fusiformis* growth curves under various culture media.

Table 2: Effect of the experimental culture media on biomass and growth performance of *A. fusiformis* (mean ± SE, n=3).

Parameters	Culture media		
	ZM	**UM**	**NPKM**
BM_{max} (g/L)	1.844±0.027[a]	1.6±0.001[b]	0.57±0.01[c]
BM_{mean} (g/L)	1.205±0.013[a]	1.168±0.006[a]	0.454±0.007[b]
μ (/day)	0.483±0.012[a]	0.425±0.005[b]	0.273±0.01[c]
DT (days)	1.437±0.036[a]	1.63±0.018[a]	2.546±0.103[b]

Values in the same row having different superscripts are significantly different ($p < 0.05$).

DISCUSSION

Once light intensity, temperature and pH requirements have been satisfied such that they do not limit growth, the culture media define spirulina growth and production yield (Pandey et al., 2010; Madkour et al., 2012). Lag phases were not affected by culture media indicating that the wild-isolated. *A. fusiformis* was able to adapt to the three culture media in the first few hours. The comparable BM_{mean} and DT between UM and a control media, ZM in the present study is similar to the results by Danes et al. (2011) who compared nitrate and urea as nitrogen sources for spirulina and found even higher biomass (1.648 g/L) with urea compared to (0.486 g/L) with sodium nitrate. Urea which commercially is readily available to majority of local farmers at a more than five times cheaper price as sodium nitrate could be of interest in terms of economic effectiveness point of view and chemical properties. Taking Urea in comparison to expensive nitrate nitrogen sources, each molecule of it renders two nitrogen atoms while each molecule of nitrate contains only one (Sigurdarson et al., 2018). However, excessive use of urea may lead to *Arthrospira* poor growth due to ammonia toxicity (Dai et al., 2014). According to Faucher (1979), spirulina did not grow above urea concentration of 2 g/L but instead development of strong ammonia odor was observed. At higher concentration, urea inhibits spirulina production due to the fact that in alkali condition, urea decomposes into ammonia which is a known toxin hindering photosynthesis and growth of a large number of microalgae (Torre et al., 2003; Peccia et al., 2013; Dai et al., 2014). The present results are in agreement with Rodrigues et al. (2011) and Madkour et al. (2012) who conducted studies on use of urea as cheaper nitrogen sources for cultivation of *Arthrospira spp* and found encouraging results. Superior B_{max} from ZM in the present results could be attributed by the fact that ZM has more complete and balanced macro and microelements. On the other hand, poor performance from NPKM would be probably due to insufficient nutrients to support *A. fusiformis* growth. Furthermore, this study has shown that concentration of 18 g/L $NaHCO_3$ in ZM could be reduced to 8 g/L in UM medium without much loss in productivity as an important cost saving factor in *A. fusiformis* cultivation.

Conclusion

These findings highlight the potential of using UM media with less expensive urea as alternative to sodium nitrate-nitrogen source in small and large-scale commercial cultivation of the locally found Momela Lake's *A. fusiformis* for wide range of uses such as aquaculture nutrition, household malnutrition combating and therapeutic purposes in Tanzania.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the work and to the preparation of the manuscript.

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