The effects of varying dilution levels of wastewater on the cultivation of *Spirulina sp.*

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Abstract

Spirulina sp. is a photosynthetic cyanobacteria containing excellent nutritional content to be used as food as well as source of feed supplement for animal. However, the key factor in developing a competitive process to produce *Spirulina* sp. as feed and as a source of added-value products is by using a low-cost medium to lower the cost production. Animal wastewater demonstrated to be one of the best nitrogen sources to produce a low-cost medium. The aim of this study was to determine the effects of four different types of wastewater: goat, poultry, seawater (30 ppt) and UPM farm supplied water at dilution rates of 25%, 50%, 75% and 100% on the cultivation of *Spirulina platensis TBSH1-5*. The culture was randomly arranged between the treatments and wastewater with three replicates and was maintained for 30 days under 24 hours of light, together with aeration. Cultivation of *Spirulina* sp. in different wastewater types was found to be significantly ($P<0.05$) affected by different types of wastewater with higher growth of *Spirulina* was notified in goat wastewater and farm supplied water at 25% dilution, whereas, the dry weight was found to be significantly higher in seawater compared to other wastewater types at 0.27 g (25%), 0.29 g (50%), 0.18 g (75%) and 0.08 g (100%). In conclusion, dilution of media with farm supplied water and goat wastewater may be used as alternative sources for low-cost culture media for the biomass gain of *Spirulina* sp.

Keywords: *Spirulina*, low-cost media, wastewater, dry weight

Introduction

Spirulina is an economically important blue – green algae with a thread-like structure that produces a high population of growth in high carbonate, bicarbonate and alkaline condition (Yilmaz & Sezgin, 2014). *Spirulina* can be easily harvested and consist of high macro- and micronutrient content (Habib et al., 2008). The characteristic of the genus is the helical shape of the filaments and is maintained in liquid environments or culture medium (Habib et al., 2008). It is one of the natural ingredients gaining popularity as animal feed supplements due to its high content of protein, vitamins, essential amino acids, minerals, essential fatty acids and high carotenoid content (Belay et al., 1996; Chaiklahan et al., 2010; Kharde et al., 2012).

Zarrouk is commonly accepted as the standard medium for the cultivation of *Spirulina* (Habib et al., 2008). However, the nutritional content of *Spirulina* may vary depending on the medium composition. The cost for the biomass production of *Spirulina* may fluctuate depending on the medium nutrient composition (Habib et al., 2008), where the cost of nutrients is considered the second major factor influencing the cost of *Spirulina* biomass production after labour (Vonshak, 1997). Therefore, an alternative way to reduce the cost production of *Spirulina* is by using a low-cost medium.

The basic elements that are needed by the microalgae to conduct organic substance synthesis are carbon (C), nitrogen (N) and phosphorus (P) (Delrue et al., 2016). Devanathan and Ramanathan (2012) and Gantar et al. (1991) stated that wastewater from livestock farm has the potential to be used as low-cost nitrogen source. Animal wastewater is gaining more attention due to its high essential nutrients that can alternatively be used as a source of fertilizer proven to be beneficial for microalgae growth (Muhamad et al., 2015) and used as a low-cost nitrogen source (Cheunbarn & Peerapornpisal, 2010). Algae uses solar energy where it absorbs the nutrients from the wastewater to fix carbon substances to produce plant biomass that will become a valuable source of compound for animal feeding supplements and at the same time, help to decay organic pollutants, thus decreasing water pollution (Chaiklahan et al., 2010; Cheunbarn & Peerapornpisal, 2010).
Most of the research done previously focused mainly on the production of *Spirulina* from swine manure and waste (Devanathan & Ramanathan, 2012). Later research showed the potential of dry chicken manure that provided the necessary nutrient source for *Spirulina platensis* culture. Therefore, this study was conducted to determine the effects of four different types of wastewater: goat, poultry, seawater (30 ppt) and UPM farm supplied water at dilution rates of 25%, 50%, 75% and 100% on the cultivation of *Spirulina platensis TBSH1-5.*

Materials and Methods

Wastewater preparation

Four different types of wastewater, namely goat, poultry, seawater (30 ppt) and UPM farm supplied water were gathered from various sources. The goat wastewater, poultry wastewater and UPM Ladang 16 farm supplied water were collected from the Universiti Putra Malaysia farm and seawater (30 ppt) was collected from the research site of the International Institute of Aquaculture and Aquatic Science Universiti Putra Malaysia, Port Dickson, Negeri Sembilan. Approximately, about 20 g of the goat and poultry manure were weighed and mixed with 1 L of farm supplied water. Sodium metabisulfite (5 mg L^{-1}) was added into the medium to prevent microbial contamination (Cheunbarn & Peerapornpisal, 2010; Yilmaz & Sezgin, 2014) and was left for 7 d with aeration. Later, the medium was filtered through a cotton filter and the wastewater was sterilized using an autoclave at 121^0C for 15 min.

Nutritional composition of wastewater determination

The four types of wastewater: goat wastewater, poultry wastewater, seawater and UPM farm supplied water were sent for nutritional composition determination at UNIPEQ in Universiti Kebangsaan Malaysia, Bangi, Selangor.

Optimization of wastewater as nitrogen source for media

The wastewaters were diluted to produce dilution percentages of 25%, 50%, 75% and 100%. Zarrouk medium was used as the control. Each medium was prepared in a 500-mL conical flask with final volume of 250 mL (Table 1). *Spirulina platensis TBSH1-5* stock solution (OD_{560} at 1.0) with 10% dilution was added into the medium. The cultures were maintained for 30 d with sufficient aeration and pH 8.8-9.0.

Table 1: Dilution percentage treatments of wastewater

Treatments[1]	Media (mL)	Wastewater (mL)
25%	187.5	62.5
50%	15.0	125
75%	62.5	187.5
100%	-	250
Control (Zarrouk media)	250	-

[1]% dilution of wastewater in Zarrouk media

Monitoring growth and determination of dry weight

Each of the culture growth was monitored for 30 d. Every 5-day interval, around 1 mL of each culture was pipetted out and transferred to a multi-plate reader and read under a fluorescence spectrophotometer. After 30 d, the homogenous suspension of known quantity of *Spirulina* sp. sample was centrifuged for 10 min at 4000 rpm. The collected cells were washed with distilled water to remove any excess salt and dust attached to cell surface and filtered using Whatman's no 1 filter paper and followed by drying in a freeze dryer for 24 h.

Results and Discussion

Nutritional composition of wastewater

Table 2 shows that the animal wastewaters containing the essential nutrients that are valuable for the growth of *Spirulina* sp. such as sulphate, calcium, magnesium, potassium, iron and chloride. The most important basic elements especially sodium were found in considerably high amount mostly in all wastewaters. Sodium is one of the important minerals for carbon source as it will help in maintaining the alkaline condition of the medium. It plays an important role in the productivity of biomass as the low presence of sodium in the medium may reduce the biomass, chlorophyll and protein content (Raoof et al., 2006). Animal wastewater is known as a valuable source of nitrogen due to the presence of two components: nitrate and ammonia found in high level in poultry and goat wastewater. Ammonia is readily assimilated by *Spirulina* sp. (Converti et al., 2006).

Table 2: Nutritional content of goat wastewater, farm supplied water, seawater and poultry wastewater

Parameter	Goat wastewater	Farm supplied water	Seawater	Poultry wastewater
pH	8.15	8.2	7.7	8.42
Turbidity, NTU	583.3	1.17	1.41	715
Colour, Pt/CO (Hz)	>500	<0.2	2.2	>500
Ammonia as N, mg/L	2.16	<0.05	<0.05	2.7
Chromium hexavalent, mg/L	3.47	0.1	0.1	4.01
Total hardness ($CaCO_3$)	183	8	439	138
Total dissolved solids	4.6	134	20323	9458
Cyanide, mg/L	0.49	0.019	0.014	0.55
Sulphate, mg/L	13500	12300	12200	13500
Sulphide, mg/L	11	7	9	12
Chloride, mg/L	2500	370	12500	1010
Fluoride, mg/L	7.6	3.2	3.1	7.7
Nitrate, mg/L	11.4	6.4	1.7	53.1
Calcium, mg/L	212.3	2	54.6	448
Cadmium, mg/L	0.07	N.D.	N.D.	0.007
Copper, mg/L	1.17	N.D.	N.D.	7.4
Magnesium, mg/L	321.1	1.1	892.7	1220.8
Iron, mg/L	29.8	N.D.	N.D.	21.4
Sodium, mg/L	437.8	13.2	27835	432.6
Potassium, mg/L	1080	0.42	268.5	1600.7
Lead, mg/L	0.06	N.D.	N.D.	0.03
Antimony, mg/L	0.02	0.001	N.D.	0.007
Manganese, mg/L	12.2	0.005	N.D.	2.7
Zinc, mg/L	4.1	0.17	N.D.	2.8

N.D.= Not detected. The value is <0.001

However, there were traces of heavy metals found in the wastewater such as chromium hexavalent, cadmium, copper, lead and zinc. There was no presence of cadmium, copper and lead in the farm supplied water and seawater. The traces of heavy metal present in the wastewaters might not affect the culture because *Spirulina* has a unique quality to detoxify (neutralize) or chelate the toxic minerals - a characteristic that is not yet confirmed in any other microalgae. *Spirulina* may detoxify poisonous effect of heavy metals from water, food and environment (Habib et al., 2008).

Determination of monitoring growth, dry weight and pH

The growth curve was significantly affected by different dilutions of each wastewater (Figure 1), where for farm supplied water at 100% and poultry wastewater at 50%, 75% and 100% showed declining growth throughout the observed 30 d and slower growth for goat wastewater at 100%. The inhibition of growth noted in the poultry wastewater and goat wastewater might be due to the presence of high ammonia in the wastewaters. Even though ammonia can readily be assimilated by *Spirulina*, however, it may be toxic and cause inhibition in the growth of *Spirulina* when it appears in excess amount (Converti et al., 2006). Previous studies stated that the range of ammonia in the medium was 1.7-2.0 Mm which could cause an inhibitory effect, whereas level of 10 Mm may cause toxicity (Abeliovich & Azov, 1976; Belkin & Boussiba, 1991; Converti et al., 2006).

There were significant wastewater and treatment interaction for the dry weight (P = 0.0002) after 30 d of cultivation (Table 3). Dry weight was significantly affected by different dilution treatments; 25% (P<.0001), 50% (P<0.05), 75% (P<0.05), 100% (P<0.05) (Table 4). Between the wastewaters, the dry weight showed significant difference between poultry wastewater (P = <.0001) and farm supplied water (P = 0.022) and the control. While in poultry wastewater, the dry weight (P = <.0001) decreased linearly and quadratically with the increasing dilution. Table 4 shows that seawater had the highest dry weight; 25% (0.08 g), 50% (0.41 g), 75% (0.26 g) and 100% (0.29 g). The highest dry weight recorded in the seawater is due to the high amount of bicarbonate present in the seawater medium which helps in increasing biomass production. However, these ions were poorly soluble due to the presence of considerable amount of Ca^{2+} and Mg^{2+} in the seawater thus forming a precipitation. This led to the increase in the turbidity and induced nutritional deficiencies (Materassi et al., 1984). Thus, it allows lower sunlight penetration resulting in light limitation.

Table 3: The effect of wastewaters and dilution treatments on dry weight and pH of *Spirulina platensis TBSH1-5* after 30 days of cultivation

Parameter	Dry weight	pH
Dilution		
Control	0.179	10.203
25%	0.132	10.072
50%	0.187	9.957
75%	0.149	9.819
100%	0.104	9.502
Contrast, P-value		
Linear	0.462	0.0003
Quadratic	0.650	0.229
Wastewater type		
Control	0.179	10.203
Poultry wastewater	0.037	9.852
Goat wastewater	0.137	9.962
UPM farm supplied water	0.166	9.990
Seawater	0.244	9.547
SEM	0.017	0.064
Source of variation		
Dilution	<0.0001	<0.0001
Wastewater	0.182	<0.0001
Dilution x wastewater	0.0002	0.317

Significantly different at (P<0.05); SEM= Standard error of mean
Control= Zarrouk media

Table 4: Mean dry weight of Spirulina platensis TBSH1-5 where wastewater and dilution x treatment interactions were significant after 30 days of cultivation

Treatment	Poultry wastewater	Goat wastewater	Farm supplied water	Seawater	SEM	P-value
Control	0.18^{a}	0.18	0.18^{a}	0.18		
25%	0.15^{b}	0.15	0.22^{a}	0.08	0.016	<.0001
50%	0^{c}	0.13	0.33^{a}	0.41	0.063	<0.05
75%	0^{c}	0.14	0.17^{a}	0.26	0.033	<0.05
100%	0^{c}	0.13	0^{b}	0.29	0.043	<0.05
P-value	<.0001	0.267	0.022	0.122		
SEM	0.019	0.009	0.044	0.055		
Linear	<.0001	0.066	0.084	0.075		
Quadratic	<.0001	0.561	0.208	0.768		

Significantly different at (P<0.05); Sem= Standard error of mean; Control= Zarrouk media
[a, b and c:] Means with different letter within a column differed significantly

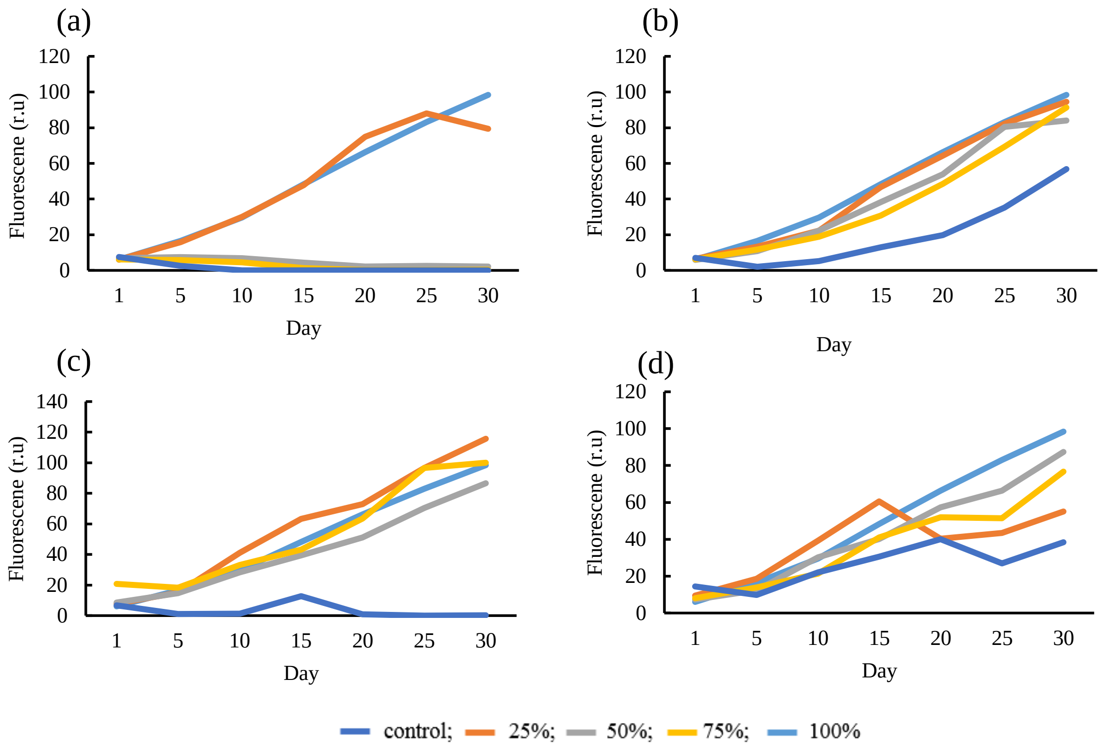

Figure 1. Growth of *Spirulina platensis* for 30 days in (a) poultry wastewater, (b) goat wastewater, (c) UPM farm supplied water and (d) seawater.

Farm supplied water at 25% and 75% showed the highest growth compared to the control. This finding agrees with Delrue et al. (2016) who stated that Zarrouk media could be diluted up to five times without impacting the biomass productivity.

During the cultivation of *Spirulina platensis TBSH1-5*, pH was reduced linearly with increase in dilution. But no significant difference on the interaction between wastewater and dilution rate on pH was detected (Table 3). Dry weight and growth performance were observed to be higher at lower dilution rate. This finding is supported by Binaghi et al. (2003), Rangel-Yagui et al. (2004) and Ungsethaphand et al. (2009) who demonstrated that when a cyanobacterium (*Spirulina*) could grow effectively in the medium leading to a progressive pH increase because of carbon-source consumption - a process when bicarbonate ions was assimilated by the cyanobacterium (*Spirulina*) and subsequently converted into CO_2 and carbonate. In the process CO_2 are utilized for photosynthesis and carbonate is excreted into the medium. Thus, the shift of the bicarbonate-carbonate equilibrium towards the carbonate resulted in increase in pH (Ungsethaphand et al., 2009).

Conclusion

The growth of *Spirulina platensis TBSH1-5* was found to be significantly affected by different types of wastewater, where farm supplied water shows to have the highest growth performance on the algae. The dilution of Zarrouk media with UPM farm supplied water at 25% may be a potential low-cost medium for *Spirulina* cultivation.

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