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Smart Culture of Spirulina Using Supernatant of Digested Rotten Tomato (*Solanum Lycopersicum*) to Produce Protein, Bio-Fuel and Bio-Electricity

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ABSTRACT: An experiment was conducted for the production of protein, bio-fuel and bio-electricity from the culture system of *Spirulina platensis* (Gomont) in supernatant of three different amount of digested rotten tomato (*Solanum lycopersicum*), and Kosaric Medium (KM) as control. Three different concentrations such as 25, 50 and 75% rotten tomato were allowed to digest under aeration. After 17 days, the colorless supernatant was screened and taken in 1.0 L conical flask with three replications. Then, *Spirulina platensis* was inoculated to grow in these three media (treatments) with the addition of 9.0 g/L $NaHCO_3$ and micronutrients, and also in KM as control for a period of 14 days. The cell weight, optical density, chlorophyll a and total biomass of spirulina was attained to the maximum values when grew in KM on the 10th day of culture followed by supernatant of 50% digested rotten tomato (DRT) than in 25 and 75% DRT culture. The chemical properties of the culture media such as pH, salinity, dissolved bio-oxygen, electric conductivity and bio-electricity were increased from first day up to 12th day of experiment. Total biomass of spirulina grown in these media had highly significant ($P < 0.01$) correlation with cell weight ($r = 0.825$) and chlorophyll a ($r = 0.866$) of spirulina. The results showed that the growth performances of *S. platensis* grown in supernatant of 50% DRT was significantly ($P < 0.01$) higher than that of spirulina grown in supernatant of 25 and 75% DRT. The percentage of crude protein (55.10 ± 0.45 to 59.90 ± 0.33%) of spirulina grown in supernatant of DRT was little bit higher than that of spirulina cultured in KM (58.40 ± 0.38%). But bio-fuel in terms of crude lipids (16.50 ± 0.31%) of spirulina cultured in supernatant of 50% DRT was almost two and half times higher than that of spirulina grown in KM (crude lipids, 6.30 ± 0.21%). Bio-electricity (300 ±10.20 mV) produced in culture of spirulina in supernatant of 50% DRT was higher than that recorded in KM (240 ±10.20 mV) followed by 75% DRT and other media. Bio-electricity had directly and strongly significant ($p < 0.001$) correlation with pH ($r = 0.812$), dissolved bio-oxygen ($r = 0.832$), salinity ($r = 0.788$) and electric conductivity ($r = 0.856$). Therefore, this procedure will produce huge amount of electricity in the world and will make a revolution in this field of bio-electricity production. Whole world will be benefited from the output (results) of this experiment.

KEY WORDS: Spirulina, protein, supernatant, rotten tomato, bio-fuel, bio-electricity

INTRODUCTION

Spirulina platensis is a filamentous, multicellular blue green microalgae and they are usually very small. Spirulina platensis is a cyanobacterium that has a high protein content and therefore, has high nutritional value. *Spirulina* contain 50-70% protein, 10-12% carbohydrate, 6-20% fat, 7% minerals and a lot of vitamins [1,2,3]. In Aquaculture, spirulina is used as feed ingredient to improve growth, feed efficiency, carcass quality, and physiological response to disease in several species of fish. Among microalgae, spirulina contains high protein (around 60-70%) and lipids (18-22%). But high protein and lipids were bioaccumulated in spirulina when grown in noodles factory waste and sago waste [1,4]. Other microalgae, *Chlorella*, *Ankistrodesmus*, *Scenedesmus* and marine algae contain less bio-energy (Protein 30%, lipids 10-15%) [5,6].

Microalgae can produce bio-fuel [7] and bio-diesel [8]in aquatic environment. Microalgal fuel cells (MFCs) can use bacteria to convert the chemical energy of a particular substrate contained in wastewater into electrical energy [9]. This actually occurs when bacteria transfer electrons to an electrode through electron transport system. Recently it is proved that MFCs could provide a source of green electricity or bio-electricity by using domestic and industrial wastes to generate power [10,11]. In photosynthesis, light energy splits water molecules into oxygen, protons and electrons [12]. Biophotovoltaic (BPV) platforms developed to harvest these electrons to generate bio-electricity using ITO anodes [12]. Spirulina provides all essential nutrients without excess calories and fats. It is recommended to control obesity & premenstrual stress, and chronic leukemia [13]. The beta carotene and other carotenoids are suggested to have role in the control of cancer in human, and enhancement of pigmentation of eggs, meats of hens

& birds [14,15], and coloration of ornamental fish & fish fillet [16]. Bio-fuel can be produced from lipids of spirulina [8] and bio-electricity may be produced in culture systems of spirulina in different media using biophotovoltaic device and measured by volta meter and HEQEP CP 6014 G2 [17,12].

The quality of about 12-15% vegetable and fruits become deteriorated during storage and marketing, and are sold for human consumption producing considerable amount of waste. These rotten materials contain high carbohydrate, protein, lipid, vitamin, mineral and phosphorus. This phosphorus might help to produce high phospho-lipids and ultimately increased the amount of total lipids [18,19]. These wastes are easily available nationwide all the time and can be collected from the market. Therefore, the supernatant of this inexpensive waste material may be used to produce spirulina (*Spirulina platensis*). The present study was conducted to culture spirulina in supernatant of digested rotten tomato(*Solanum lycopersicum*), to record the growth performances of spirulina and to produce of single celled protein, bio-fuel as lipids and bio-electricity with the following objectives:

a) To evaluate the culture and growth performance of *Spirulina platensis* in supernatant of digested rotten tomato (*Solanum lycopersicum*);

b) To analyze protein bioaccumulation and the proximate composition of spirulina grown in different media; and

c) To estimate bio-fuel as total lipids and bio-electricity from spirulina in laboratory condition.

MATERIALS AND METHODS

The experiment was conducted in the Live Food Culture Laboratory, Dept. of Aquaculture, Bangladesh Agricultural University (BAU), Mymensingh, Basgladesh. The rotten tomato (*Solanum lycopersicum*) was collected from KR market in BAU campus and 400 g of rotten tomato were digested in 5.0 L glass jars containing 4.0 L distilled water for 17 days. The chemical composition of rotten tomato and supernatant of digested rotten tomato were first screened using net of 30 µm mesh size to separate particles; It was filtered through Whatman filter paper of 0.45 µm mesh to isolate bacteria & suspended solids. Three different concentrations of supernatant of digested rotten tomato was used for the culture of *Spirulina platensis* (Table 3). Light intensity was determined by using lux-meter. Temperature was measured every day by using a Celsius thermometer and was recorded as degree Celsius. In the laboratory 12 h:12 h = light: dark was provided. Kosaric medium (KM) as control (Table 4) was prepared and used for spirulina culture. Cell weight, chlorophyll a and total biomass were recorded every alternate day [20]. pH, dissolved O_2 (mg/L), salinity (‰), electric conductivity (µhos/cm) and bio-electricity (mV) of culture media were recorded by digital meter (HEQEP CP 6014 G2). Growth of spirulina was reached at stationary phase on 12th day. Cultured spirulina was harvested before stationary phase on 10th day culture for the study of proximate composition.

Physico-chemical properties of wastes and supernatant were analysed using different equipments (HEQEP CP 6014 G2) following [20]. Proximate composition of spirulina was analysed following [21]. Spirulina was checked periodically for its purity [22,23,24]. Biofuel as total lipids was analysed using standard method following [8]. Bioelectricity was measured using (HEQEP CP 6014 G2) instead of biophotovoltaic device [12]. Data was analyzed using one way ANOVA followed by Duncan's New Multiple Range test (DNMRT) [25].

RESULTS AND DISCUSSION

Spirulina (*Spirulina platensis*) was cultured in supernatant of digested tomato (*Solanum lycopersicum*) in three different concentrations including Kosaric medium. The chemical composition of rotten tomato, physico-chemical properties of supernatant of digested rotten tomato are shown in Tables 1 and 2. Organic nutrients of rotten tomato were broken down in to inorganic forms (Table 2). During break down of organic nutrients into simple forms electron was discharged and transported through electron transport system (ETS) to the device [26,27]. Optical density of culture, chlorophyll a and total biomass of spirulina grown in supernatant of 50% DRT and Kosaric medium were significantly ($P < 0.01$) higher than those of spirulina cultured in other media (Table 5). Similar trend was followed in case of crude protein of spirulina grown in different media but crude lipids did not follow the trend (Table 6). High percentage of lipids (2.50 times) was bioaccumulated in spirulina during culture which grown in supernatant of 50% DRT than that of spirulina cultured in other media which helped more biofuel production (Table 6). Bio-electricity was produced from the microalgal (*Spirulina platensis*) culture system during the study and measured using digital meter (HEQEP CP 6014 G2). The production of bio-electricity was influenced by the production of spirulina, bio-oxygen and electrons due to splite of water molecules, electron transfer and microbial activities through electron transport system (ETS), and

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increase of pH, dissolved bio-oxygen, salinity and electric conductivity (Figs. 1, 2, 3, 4 and 5) [12]. Bio-electricity had directly and strongly significant ($p < 0.001$) correlation with pH ($r = 0.812$), oxygen ($r = 0.832$), salinity ($r = 0.788$) and electric conductivity ($r = 0.856$) (Table 7). Other growth parameters such as cell weight, chlorophyll a and total biomass had highly significant ($p < 0.01$) correlation with pH, dissolved bio-oxygen, salinity, and strongly significant ($p < 0.001$) correlation with electric conductivity (Table 7). The direct correlation values indicate that the production of bio-electricity was positively influenced by these parameters [12, 26, 27]. Bio-electricity was found to produce from microalgal culture using microalgae-microbial fuel cell technology [26]. Bio-electricity was produced from microalgal culture system in swine wastewater and measured using novel 3D electrode [9, 27]. Some researchers measured bioelectricity using the algal biophotovoltaic devices (BPV) and quantified using multimeter (Agilent U1251B) in terms of milivolts (mV) [12]. Maximum power generation (1236 mW/m3) was recorded when the microbe, *Klebsiella oxytoca* was cultured in complex substrate of palm oil mill effluent [28].
The present work was related with the production of bio-electricity from the culture system of spirulina in supernatant of digested rotten tomato which has the similarity with the findings of afore discussed findings of different authors. The rate of production of bio-electricity depends on the types of culture systems & agroindustrial wastewater, microalgal and microbial species cultured. Bio-fuel in terms of lipids production and bio-electricity are producing from the culture media of microalgae of these agro-industrial wastes in the laboratory. It is very promising that biofuel and electricity (as bioelectricity) can be produced from microalgal cultures of rotten agro-products and waste materials in the country in huge quantity [12]. There will be no scarcity of electricity even surplus electricity and bio-fuels can be generated from microalgal culture containing supernatant of digested rotten agro-products or agro-industrial wastes in different countries of the world. It will make a revolutionary change in the field for the production of electricity in the world and also surplus electricity will be produced.

CONCLUSION

Spirulina performed higher growth in 50% digested rotten tomato media than other concentrations. It might be used as feed ingredient for fish. It could be used to produce bio-fuel from crude lipids. Bio-electricity might be generated from the culture media of spirulina. Creating awareness among people about economic and health benefits of culturing *Spirulina platensis.* Bio-fuel and bio-electricity may be produced from spirulina grown in different agro-based products. Spirulina can be used as feed ingredient to replace fish meal for fish. Government and Nongovernment organization should come forward for nationwide production industrially.

Table 1. Characteristics of rotten tomato just after collection.

Sl. No.	Characteristics of past of rotten tomato	
1	Colour	Reddish
2	Odour	Little bid bad
3	Structure	Semi-solid
4	Temperature	28.30-28.60°C
5	PH	6.30-6.45
6	Total solids (TSS + TDS)	1954-2135 mg/L
7	Alkalinity	132-142 mg/L
8	Total N	1.55-1.76 mg/L
9	Available N (NO3-N)	1.10-1.15 mg/L
10	Available P (PO3-P)	2.90-3.30 mg/L

Table 2. Physico-chemical properties of supernatant of digested rotten tomato after digestion in aerobic condition

Sl. No.	Characteristics	
1	Temperature	29.60-29.90°C
2	Ph	6.80-6.90
3	Total solid (TSS + TDS)	156-162 mg/L

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4	Alkalinity	90-95 mg/L
5	Total N	0.90-1.10 mg/L
6	Available N (NO3-N)	0.60-0.70 mg/L
7	Available P (PO3-P)	0.80-2.85 mg/L

Table 3. Experimental design for *Spirulina platensis* culture using supernatant of three different concentrations of digested rotten tomato, and KM.

Types of medium	**Treatments**	Replications	Amounts of rotten tomato (%)	Duration of culture (days)
Supernatant of digested rotten tomato	1	3	25	14
	2	3	50	
	3	3	75	
Kosaric medium	4	3	-	14

Table 4. Composition of Kosaric medium (Modified after Zarrouk, 1996) for *Spirulina platensis* culture.

Sl. No.	Chemicals/compounds	Concentration in stock solution g/L
1.	$NaHCO_3$	9.0
2.	K_2HPO_4	0.250
3.	$NaNO_3$	1.250
4.	K_2SO_4	0.50
5.	NaCl	0.50
6.	MgSO4.7H2O	0.10
7.	$CaCl_2$	0.02
8.	FeSO4.2H2O	0.005
9.	A_5 micronutrient solution[a]	0.5ml/L
	a) A_5 micronutrient solution	G/L
	i) H_3BO_4	2.86
	ii) MnCl2.4H2O	1.81
	iii) ZnSO4.7H2O	0.22
	iv) $CuSO_4.5H_2O$	0.08
	v) MoO_3	0.01
	vi) $CoCl_2.6H_2O$	0.01

Table 5. Comparison of cell weight, Chlorophyll a and total biomass of *Spirulina platensis* grown in supernatant of three different concentrations of digested rotten tomato (DRT), and Kosaric medium on 10th day of culture before stationary phase.

Parameters	T1 (25% DRT)	T2 (50% DRT)	T3 (75% DRT)	T4 (KM)
Optical density	1.246 ± 0.018	2.026 ± 0.09	1.367 ± 0.007	2.640 ± 0.022
Cell weight (mg/L)	1.98 ± 0.011^{c}	18.859 ± 0.47^{a}	14.53 ± 0.95^{b}	16.44 ± 0.031^{ab}
Chlorophyll a (mg/L)	1.52 ± 0.11^{c}	16.95 ± 0.06^{a}	12.84 ± 0.54^{b}	14.96 ± 0.024^{ab}
Total biomass (mg/L)*	101.84 ± 1.05^{c}	1135.65 ± 9.56^{a}	860.28 ± 1.66^{b}	1002.32 ± 1.70^{a}

*Total biomass = Chlorophyll a x 67 (Vonshak and Richmond, 1988). Figures in common letters do not differ significantly at 1% level of probability.

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Table 6. Proximate composition (% in dry matter basis) of *Spirulina platensis*) cultured in supernatant of three different concentrations of digested rotten tomato (DRT), and control as Kosaric medium.

Treatments	T1 (25% DRTM)	T2 (50% DRTM)	T3 (75% DRTM)	T4 (KM)
Moisture	8.30 ± 0.06	8.35 ± 0.06	8.32 ± 0.06	8.33 ± 0.06
Crude protein	56.40 ± 0.32[b]	59.90 ± 0.33[a]	55.10 ± 0.45[b]	58.40 ± 0.38[a]
Crude lipids	12.70 ± 0.25[ac]	16.50 ± 0.31[a]	11.70 ± 0.15[bc]	6.30 ± 0.21[d]
Ash	8.50 ± 0.14	9.35 ± 0.15	9.10 ± 0.20	13.55 ± 0.12
NFE*	13.70 ± 0.30	5.55 ± 0.17	15.40 ± 0.24	12.57 ± 0.25
Crude fibre	0.30 ± 0.03	0.25 ± 0.04	0.28 ± 0.03	0.75 ± 0.04

*NFE (Nitrogen Free Extract) = 100 - (Moisture + Crude protein + Crude lipids + Ash). Figures in common letters in the same row do not differ significantly at 1% level of probability.

Table 7. Correlation coefficient (r) of cell weight, chlorophyll a & total biomass of *Spirulina platensis*, and bio-electricity with pH, dissolved bio-oxygen, salinity and electric conductivity during culture of spirulina in supernatant of digested rotten tomato.

Parameters	pH	Dissolved bio-oxygen	Salinity	Electric conductivity
Cell weight (mg/L)	0.721*	0.742*	0.702*	0.795**
Chlorophyll a (mg/L)	0.728*	0.735*	0.689*	0.780**
Total biomass (mg/L)	0.756*	0.760*	0.696*	0.788**
Bio-electricity (mV)	0.812**	0.832**	0.788**	0.856**

df = 30, *P < 0.01, **P < 0.001

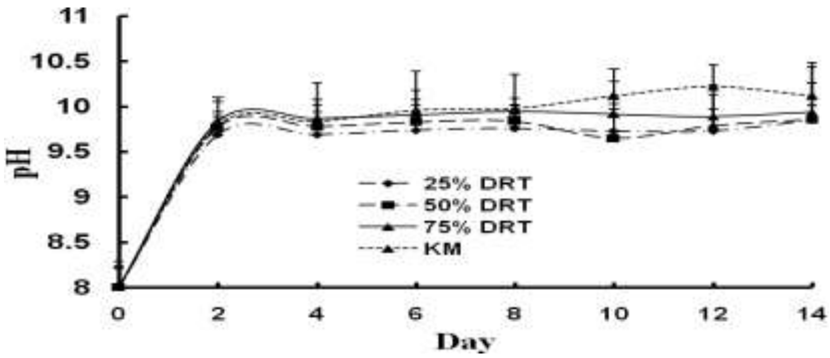

Fig.1. Mean values of pH during culture of *Spirulina platensis* in supernatant of three different digested rotten tomato (DRT), and Kosaric medium. Vertical bars represent standard errors.

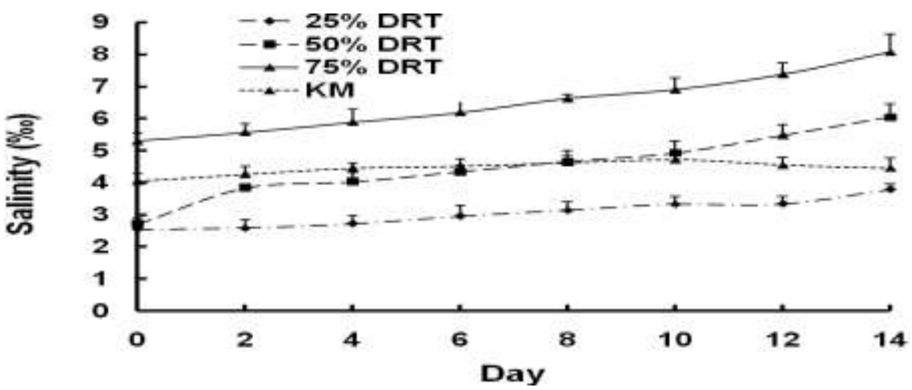

Fig. 2. Mean values of salinity during culture of *Spirulina platensis* in supernatant of three different digested rotten tomato (DRT), and Kosaric medium. Vertical bars represent standard errors.

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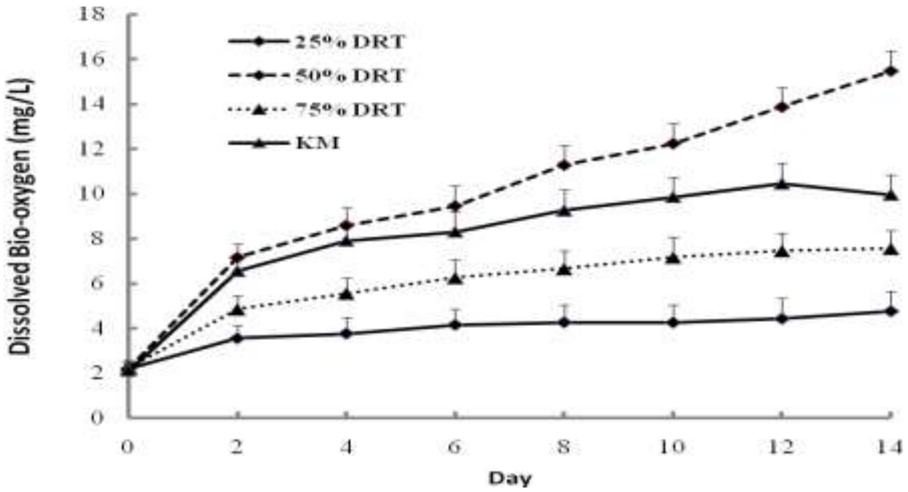

Fig.3. Mean values of dissolved oxygen (mg/L) during culture of *Spirulina platensis* in supernatant of three different digested rotten tomato (DRT), and Kosaric medium. Vertical bars represent standard errors.

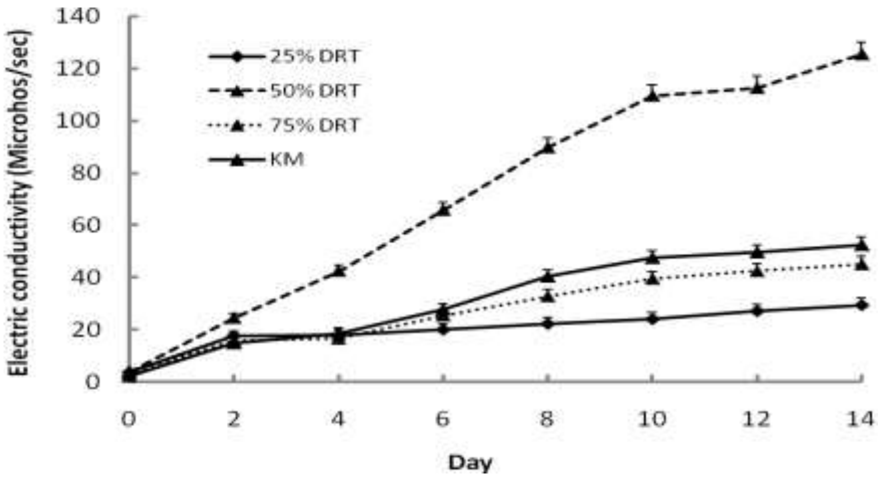

Fig. 4. Mean values of electric conductivity (μhos/sec) during culture of *Spirulina platensis* in supernatant of three different digested rotten tomato (DRT), and Kosaric medium. Vertical bars represent standard errors.

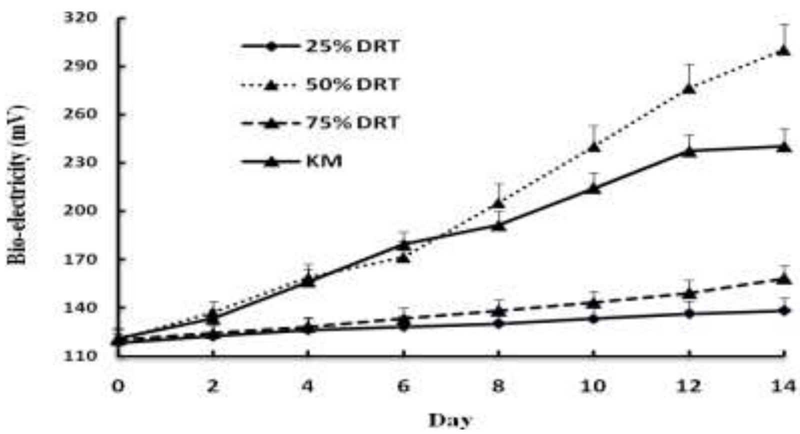

Fig. 5. Mean values of bioelectricity (mV)) during culture of *Spirulina platensis* in supernatant of three different digested rotten tomato (DRT), and Kosaric medium. Vertical bars represent standard errors.

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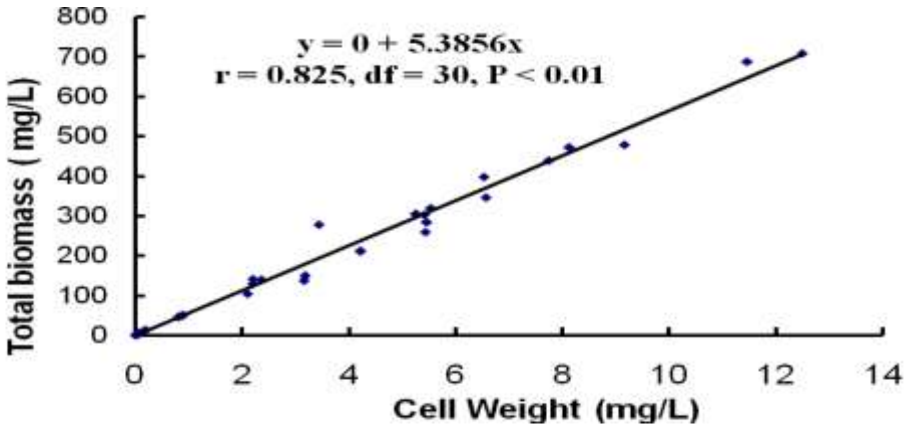

Fig.6. Correlation coefficient (r) of total biomass (mg/L) of *Spirulina platensis* with cell weight (mg/L) of *spirulina* grown in supernatant of three digested rotten tomato, and Kosaric medium.

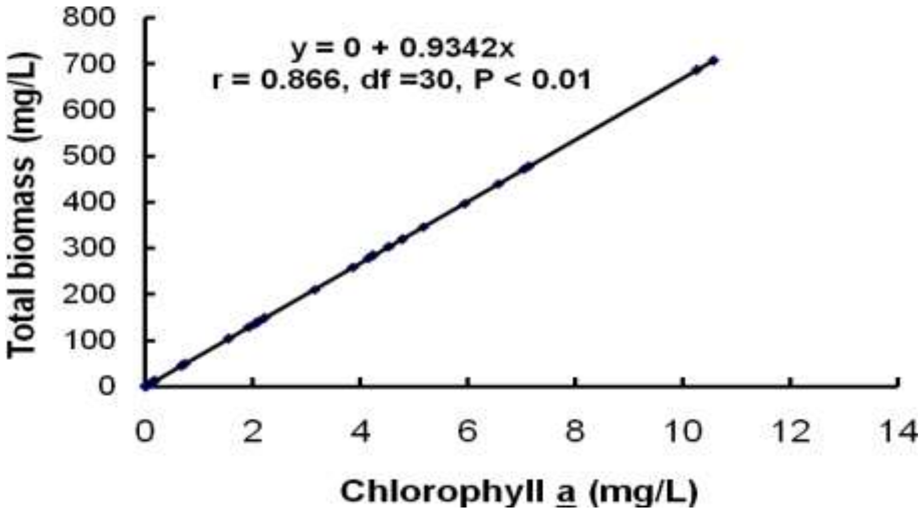

Fig. 7. Correlation coefficient (r) of total biomass (mg/L) of *Spirulina platensis* with chlorophyll a (mg/L) of *Spirulina* grown in supernatant of three digested rotten tomato and Kosaric medium.

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