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Cultivation of Microalgae Using Struvite and Human Urine for oil Production.

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ABSTRACT

Chemical Fertilizer industrial effluent rich with nutrients was used for struvite precipitation and evaluating cultivation of *Spirulina maxima S-platencess* microalgae. Human urine solar treated was evaluated and compared with struvite precipitated from synthetic human urine, authentic human urine, and struvite precipitated from chemical industrial effluents. Both struvite and solar treated human urine enhanced the biomass production as well as lipids accumulation by 11% - 12% compared to control. The dried microalgae were collected and subjected for Lipids extraction. Lipids were studied by Spectrophotometer. The increase in oil content was evaluated of the algae. This observations reflects the high adaptability of *S-platencess* microalgae towards struvite as nutrient and suitability to be grown under human urine solar treated as nutrient.

Key words: Solar energy, human urine, struvite, industrial effluents, micro algae.

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INTRODUCTION

Microalgae are considered the best organisms to produce oil, due to their ability to photo-synthetically fix atmosphere CO_2 producing biomass more efficiently and rapidly than terrestrial plants [1]. The major challenges in mass production of algal biomass are the costs of fertilizers, harvesting and the availability of a suitable source of water, besides the limitations imposed by lighting [2] and [3]. Biomass can be applied for energy production in two types of utilization (i) recovery of nutrients from industrial wastewater as struvite Mg $NH_4PO_4.6H_2O$ slow release fertilizer and (ii) Solar energy treated human urine as potassium di-hydrogen phosphate [4]. The costs of biomass production can be significantly reduced if struvite or human urine solar treated is used instead of the expensive artificial amendments [5] and [6].

Biodiesel production from microalgae biomass has been suggested to be promising as microalgae have improved characteristics over crops and plants sources, such as: increased photosynthetic efficiency, shorter reproduction cycle, higher nutrients absorption efficiency, lipid content and Biomass productivity [6] and[7] and [8]. Solar radiation, an abundant resource in most regions of the developing world, can be used as energy source. Treatment with solar UV for urine is effective and certain since temperature above 50 °C should be attained. The limitations of solar disinfection, depends on the type of solar photo-reactor and operation mode. The application of human urine in algal fertilization could establish new transmission routes for disease infection possible for persons who are involved in the application work. The number of micro-bacteria negatively correlated with the increasing pH (around 9) of the all urine samples had the least survival time [9] and [10]. Hence, for fertilization with recycled urine it is advisable to store urine at 15 °C more than five week in order to prevent the exposure route for pathogenic micro-bacteria [11].These precautions are of no need when using solar still [12]. This work focused on cultivation of microalgae with low cost nutrient recovered from human urine derived fertilizer using solar still and struvite precipitated from industrial effluents and that precipitated from human urine.

MATERIALS AND METHODS

Urine source

Undiluted urine was collected from male and females. Urine was mixed and stored in large plastic tanks. The urine was frozen to be hydrolyzed before introduction to solar reactor.

Solar reactor (Solar Still)

Undiluted urine was fed into both solar still design 5 liters for each. The glass photo reactor which has a surface area of 0.5 m^2 with black tiles as a base and side walls of mirrors, and was protected by a glass cover sloping one at 15◦ Fig. (1). The solar reactors were placed on balcony to receive adequate sun exposure during the day morning from sun rise to sun set, to allow for maximum exposure to direct sun light.

Hydrolyzed fresh urine was poured slowly from the top and covered tightly. The temperature of the urine increased by means of convection and the green- house effect, both caused by transmission of the solar radiation. The glass cover on the other hand was cooled by the wind, thus causing condensation of the vapors inside to form small droplets at the underside side of the cover. Under the influence of gravity, these droplets ran down the underside of the glass cover into a drainage and were evacuated from the unit by gravity flow.

Figure 1: Design for solar reactor (5 liter human urine) as lab-scale

Nutrient Recovery

Urine was collected from a dozen persons (6 mail and 6 females). The collected urine was stored in plastic tanks (10 liters) and was hydrolyzed by freezing.

Nutrient Recovery by solar reactor

Five liters of urine were fed into the solar reactor. The photo-reactor had surface area
0.2m2, was with black tiles as a base and mirrors (side walls), and was protected by glass cover sloping at15◦.

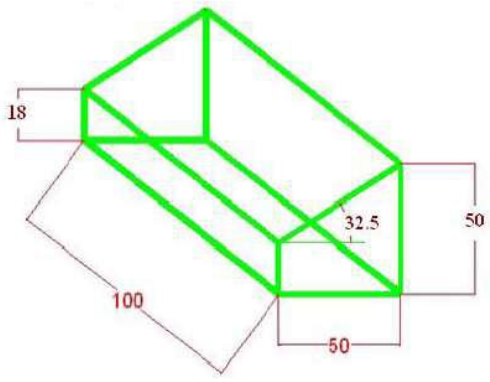

Figure 2: Design of the solar still concentrator

During 22 days the urine samples were taken and analyzed daily for phosphate, total nitrogen, pH and electric conductivity [APHA]. The volume of the generated condensate was measured by measuring cylinder. A second experiment was performed using a glass cover with sloping 15◦ with the aim to assess the nutrient content of the final products. The pH of

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urine was lowered to pH4 by adding phosphoric acid (H3PO4,89%) approximate addition 15.7 ml/5L. In both cases 5L of acidified urine was fed into the solar reactor. After 22 days of treatment, the liquid fraction had evaporated completely and the generated solids we recovered. Only the final products were analyzed for nutrient content, Table (1).

Table 1: Chemical composition of the bio fertilizer prepared by solar reactor

Parameter	Concentration in solid fertilizer from Authentic urine
pH	8.5
conductivity	2.4mS/cm
COD	1484.8 mg/L
Total P	21.324g/L
NH4	4.56 mg/L
TDS	1.2 g/L
Temp.	29 ºC
Mg	5.664 mg/L
Fe	64 mg/L
Mn	14.0mg/L
Ca	17.04 mg/L
Zn	0.80 mg/L
MO	ND
CO	ND
Cr	ND
Cd	ND
SO4	680 mg/L
Pb	1.4 mg/L
K	400 mg/L

Fertilizer Characterization

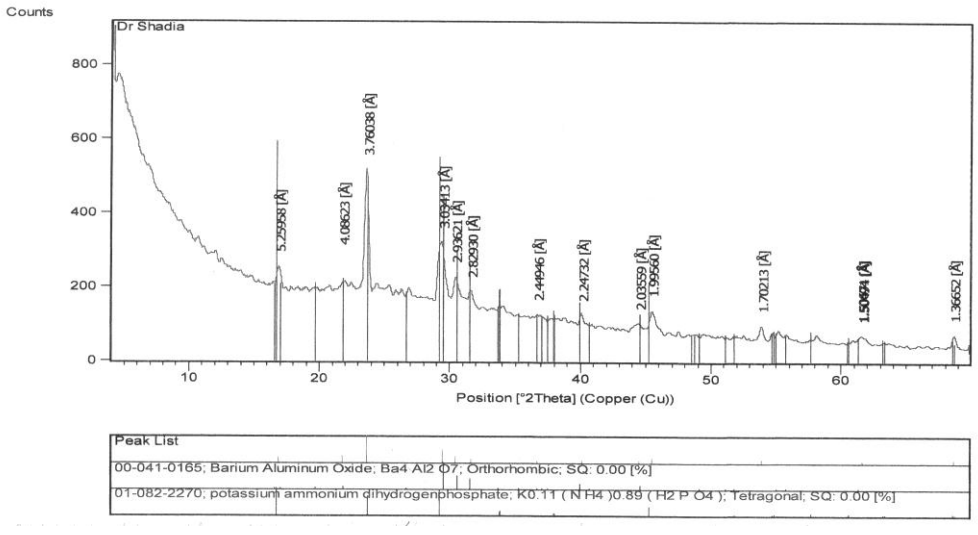

Figure 3: XRD of derived fertilizer prepared from urine in solar reactor 15◦ design showing chemical content more than 80% is potassium ammonium phosphate.

The urine- derived product recovered from solar reactors were subjected to XRD analysis. Eight grams of sample with 2% citric acid were diluted to 1 liter with distilled water,

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the samples were shacked at 40 rpm and then filtered. The filtrate characterization is illustrated in Table (1) and the chemical composition is illustrated

From the XRD analysis of the derived urine fertilizer produced from 15◦ sloping showing chemical composition of potassium ammonium phosphate Fig (3). The 15◦sloping derived urine fertilizer was selected for micro algae nutrient growth. This design Fig (2) gave 18.66 g yield as solid derived fertilizer.

Struvite preparation from human urine and bittern

Struvite precipitation represents a possibility to produce a solid fertilizer from urine.Struvite (Mg NH4 PO4.6H2O) is formed by addition of magnesium in the form of Mg Cl2.6H2O or (Bittern) the waste brine remaining after salt (NaCl) extraction from seawater, was obtained from EMISAL company-Egypt. A series of batch investigations was conducted to study the influence of species concentration and pH on srtuvite precipitation. Experiments were carried out at 20oC in 1000 ml stirred vessel. Stock solutions of synthetic urine were prepared as stated in section (2.1) Distilled water was used to prepare the synthetic urine, and pH was then adjusted at several pH values (8.5 – 10.5) by the gradual addition of 0.1N NaOH and kept constant all over the reaction period of (30 min). The homogeneity of the solution was performed by a magnetic stirrer. Prior to any analyses done, all samples were allowed to settle overnight in order to separate the crystallized precipitate from bulk mother liquor. The formed precipitate was collected by filtration through medium filler paper (Whatman, Maid stone, UK) and dried at ambient temperature. The pH of urine samples (synthetic and Authentic) was measured by pH meter (Hanna Instruments, HI 8314) and a probe (Hanna Instruments, HI 1230). Total dissolved solid (TDS) and conductivity were measured by (HACH\conductivity meter 44 600-00). Kjeldahl nitrogen, total phosphorus, ammonia nitrogen, Chemical oxygen demand were conducted by the procedure described in the Standard Methods using a portable HACH DR 2000 photo-spectrometer. Table (2) shows the properties of human urine under test. Residual concentrations of ions in solution after filtration were analyzed by atomic absorption (AA). X- Ray diffraction (XRD) and Electron microscope- was used to determine the composition of the precipitates and the crystal morphology.

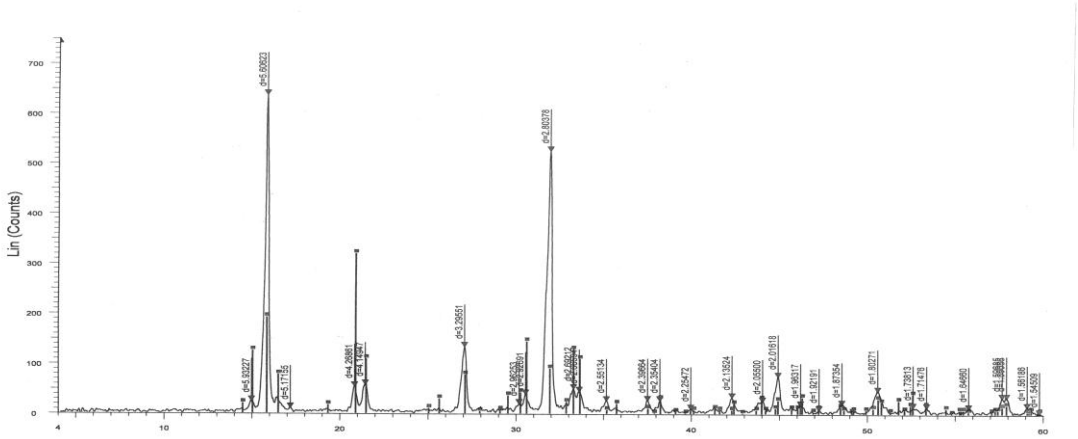

Figure 4: XRD chart of Struvite – Mg NH4PO4(H2O)6 precipitated from human urine

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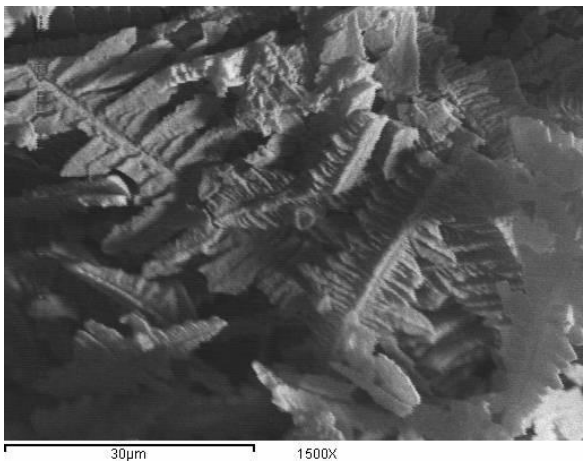

Figure 5: SEM of for Struvite – MgNH4PO4(H2O)6 precipitated from human urine crystals appeared in prismatic elongated shape.

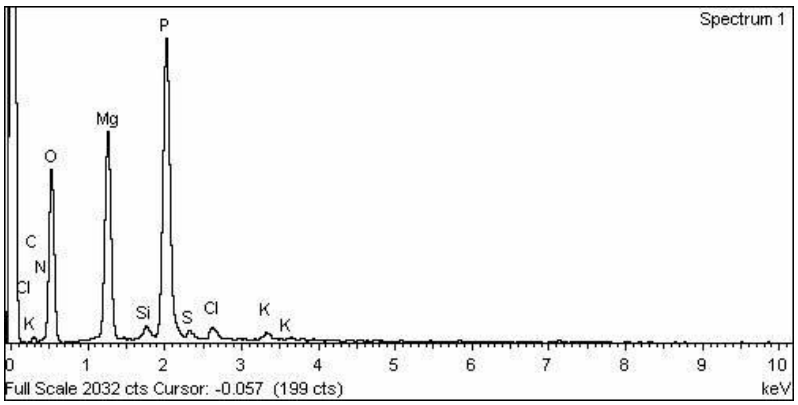

Figure 6: EDS curve of struvite - MgNH4PO4(H2O)6 precipitated from human urine showing Phosphorous, Magnesium, and nitrogen peaks.

Table 2: EDS showing Elemental % of struvite - MgNH4PO4(H2O)6 precipitated from human urine .

Element	Weight%	Atomic%
C K	11.54	16.55
N K	8.05	9.9
O K	51.85	55.84
Mg K	12.44	8.81
Si K	0.45	0.28
P K	14.3	7.95
S K	0.4	0.22
Cl K	0.61	0.29
K K	0.37	0.16

Table 3: Properties of human Urine under Test

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Elements	Concentration in synthetic urine	Concentration in Authentic urine
pH	6.48	5.38
conductivity	2.55 mS/cm	19.62 mS/cm
COD	69.2 mg/L	37216 mg/L
Total P	3.9634 g/L	3.769 g/L
NH4	57.12 mg/L	784 mg/L
TDS	1.27 g/L	9.8 mg/L
Temp.	25 °C	30 °C
Mg	15.57 mg/L	9.963 mg/L
Fe	0.114 mg/L	70 mg/L
Mn	0.038 mg/L	0.038 mg/L
B	ND	0.1 mg/l
Ca	H	H
Cu	0.006 mg/L	14 mg/L
Zn	0.016 mg/L	42 mg/L
MO	ND	ND
CO	ND	ND
Cr	ND	ND
Cd	ND	20 mg/L
SO4	1.55 g/L	0.0475 g/L
Pb	ND	34 mg/L
K	16.05 mg/L	2.48 mg

Cultivation of Microalgae

The *Spirulina maxima* was cultivated (during winter season 2013, in National Research Centre, Egypt) in Zarrouk's medium (Zarrouk 1966) containing normal concentrations of NaCl (0.02 M) and sodium nitrate as a nitrogen source (2.50 g L^{-1}). Aeration was accomplished using air pumps to achieve an air flow rate of 20 L/h. The cultures were gassed with 0.03% volume CO_2 in an air and temperature was maintained at 25ºC ±3. The pH of all media was adjusted to 9.5. The cultures were illuminated with continuous 10 cool white fluorescent lamps (Philips 40 W) provided an illumination of 2500 lux. In all cultivated flasks, conductivity, salinity, pH and temperature were daily measured with Hanna (HI 09812-5) conductivity meter. The purity of cultures was checked periodically by microscopic observation following taxonomy guidelines. All solutions and glassware were autoclaved at 121ºC for 15 min prior to use.

Assays of struvite Competiveness with Zarrouk's medium

Cultivation of Microalgae with synthetic and authentic struvite from urine and industrial effluents:

In this experiment, *Spirulina maxima* was cultured in 1 liter Erlenmeyer flask containing 750 ml of Zarrouk medium supplemented with struvite as **natural fertilizer** prepared from: (1) struvite from synthetic human urine, (2) struvite from authentic human urine precipitated and dried by air and (3) human urine (natural-fertilizer) prepared in solar reactor at different concentrations 1, 2, 4 and 8 g/L. The values of pH of all cultures were adjusted at 8.5 and regularly determined with Hanna pH meter.

Growth measurements and harvesting of micro algae

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The growth rate of *Spirulina maxima* was monitoring every three days through cultivation period by determining the dry weight (d.w.) and optical density at 670 nm methods [13]. The cells were harvested at the stationary phase, by centrifugation at 10,000 *g* (4°C) for 15 min and the cells masses were stored at -20°C until analysis Fig(7).

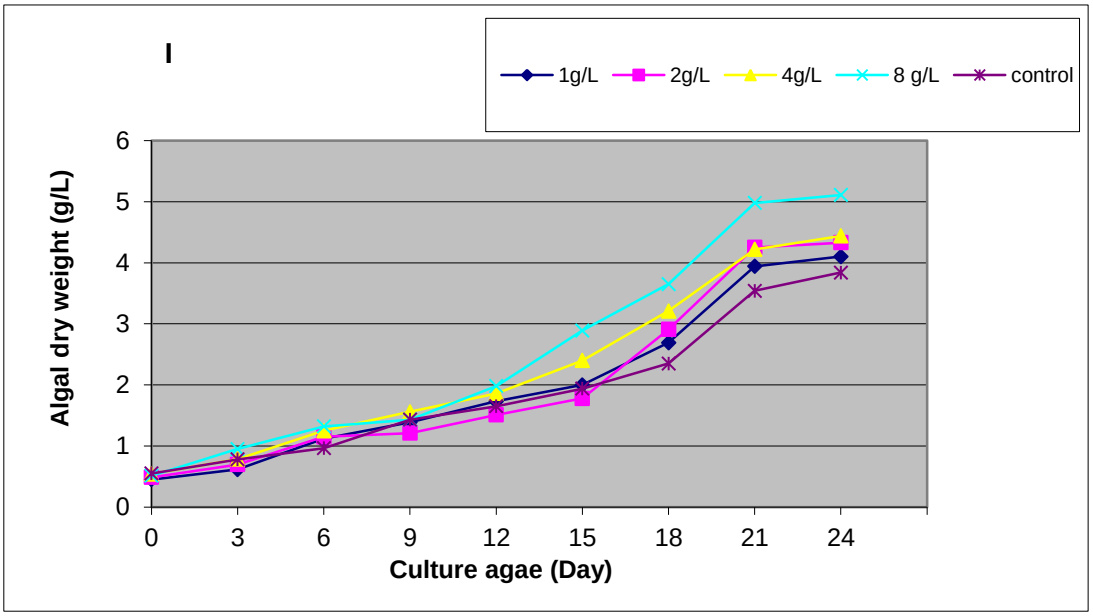

Figure 7: Increase in dry weight of micro algae through days of cultivation using synthetic human urine.

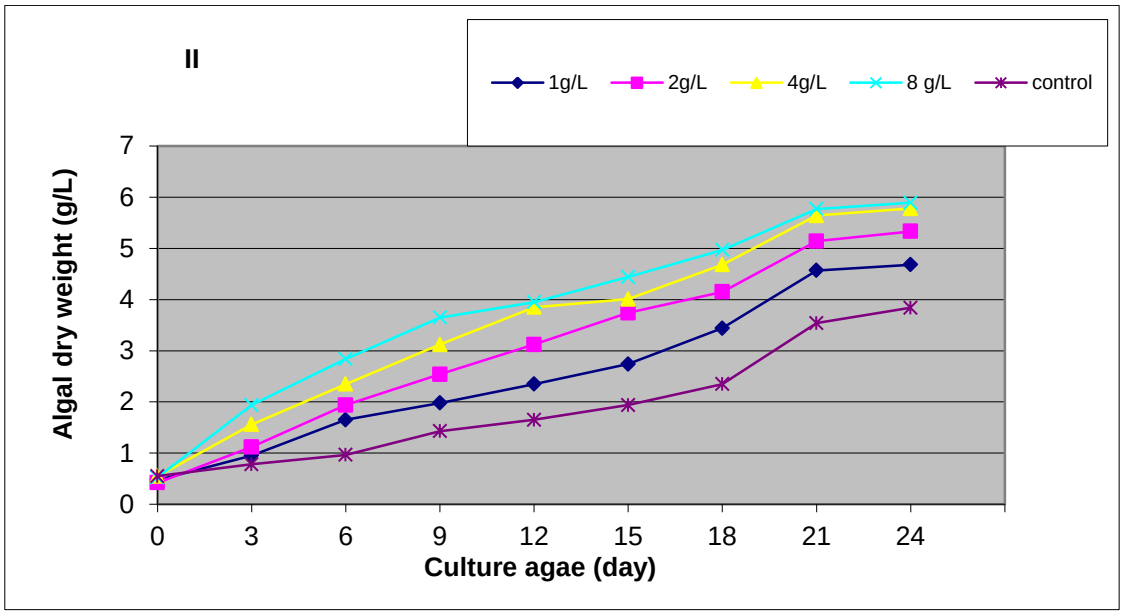

Figure 8: Increase in dry weight of micro algae through days of cultivation using struvite precipitated from original human urine.

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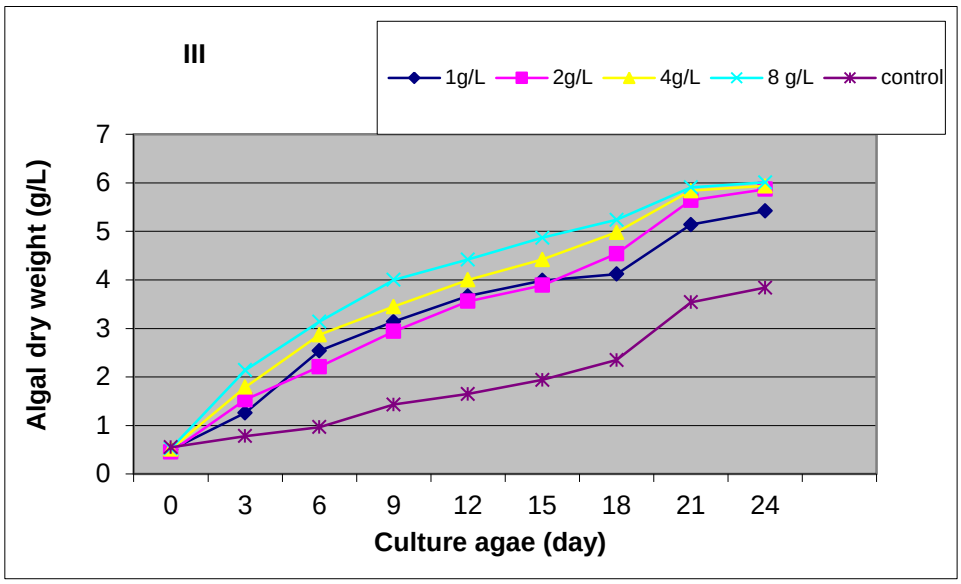

Figure 9: Increase in dry weight of micro algae through days of cultivation using human urine treated with solar still.

Extraction and determination of total Chlorophyll content

Chlorophyll was extracted with 90% acetone at 4C for 24 h and determined according to [14] and [15].

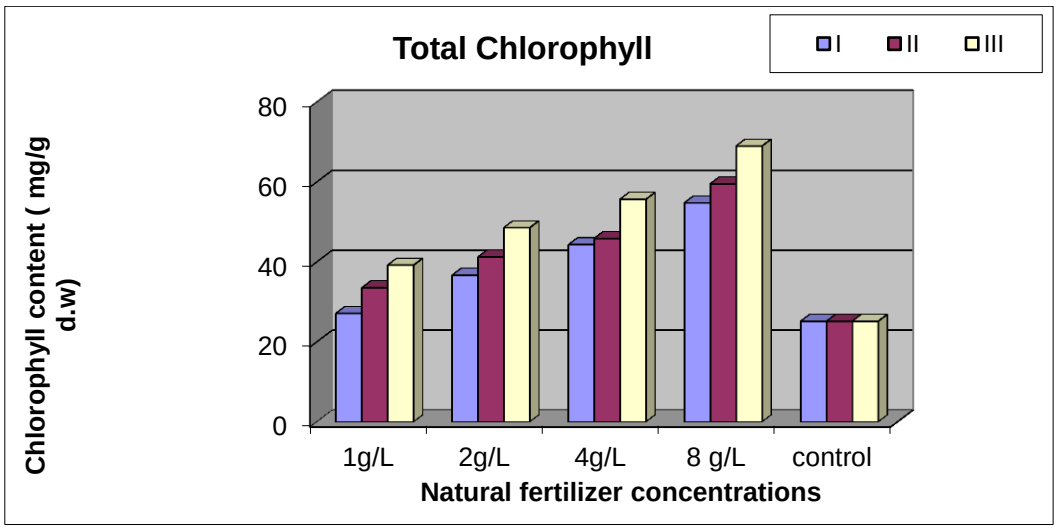

Figure 10: Total Chlorophyll of Microalgae grown in different natural fertilizer

I = struvite from synthetic urine, II = struvite from authentic urine, III = urine treated with solar reactor.

Lipid extraction of microalgae

After appropriate cell growth (30 days), the cells were harvested from culture by centrifugation at 10,000 xg for 10 min. Cell pellets were frozen at -20^{o}C for overnight and then 10 g of wet algae were disrupted with glass beads (Sigma grad) in a vortex mixer for 10 min. The lipids were extracted with chloroform/methanol mixture (2:1, v/v), then separated into chloroform (bottom layer) and aqueous methanol layers by adding methanol and water

to give a final solvent ratio of about chloroform: methanol: water of 1:1:1 (v/v/v). The upper layer (methanol/water layer) was removed and the chloroform layer including lipids were washed several times with 10% NaCl solution, and evaporated to dryness under reducing pressure to yield the algal lipids. The total lipids was gravimetrically determined and stored at -20^{o}C under nitrogen to prevent lipid auto-oxidation or used directly for subsequent analysis [16] and [17].

Table 4: Composition of control medium and natural fertilizer I,II and III using microalgae cultivation:

Control medium				Compositions of Organic fertilizer		
Stock Solutions	**Per Litre distilled water**	**ml/L**	**Elements**	**I**	**II**	**III**
1. NaNO3	25.0 g	10				
2. CaCl2.2H2O	2.5 g	10				
3. MgSO4.7H2O	7.5 g	10				
4. K2HPO4	7.5 g	10				
5. KH2PO4	17.5 g	10				
6. NaCl	2.5 g	10				
7. EDTA	50.0 g	1				
KOH	31.0g	1				
8. FeSO4.7H2O	4.98 g	1				
H2SO4	1.0 mL	1				
9. H3BO3	11.42 g					
10. Micronutrients	$g.L^{-1}$	1				
ZnSO4.7H2O	8.82 g					
MnCl2.4H2O	1.44 g					
MoO3	0.71 g					
CuSO4.5H2O	1.57 g					
Co(NO3)2.6H2O	0.49 g					

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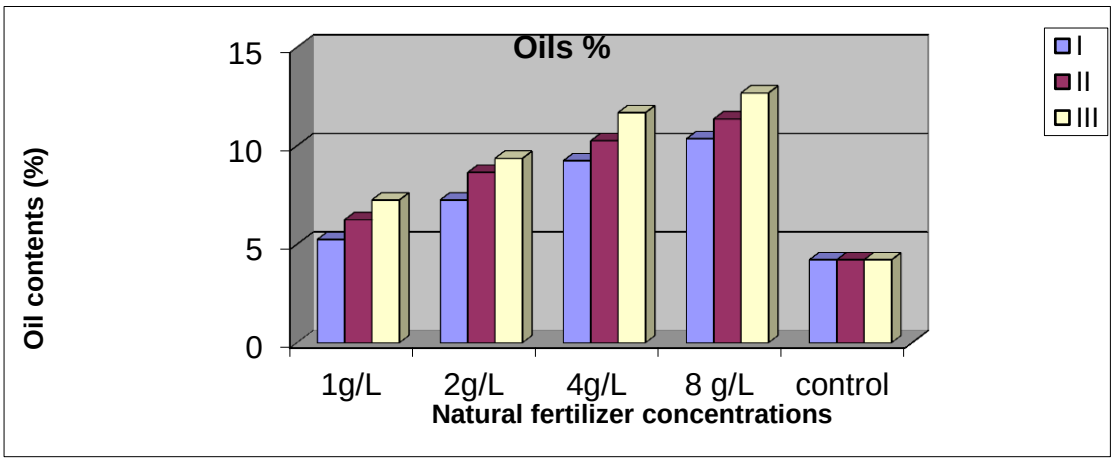

Figure 11: Oil content of Microalgae grown in different natural fertilizer

I = struvite from synthetic urine, II = struvite from authentic urine, III = urine treated with solar reactor.

RESULTS

Nutrient recovery by solar reactor

Temperature inside the solar reactor reached 67 ◦C. Through- out the treatment period, temperature maxima inside the reactor ranged 41◦C - 67◦C. On average the maximum temperature recorded inside the solar reactor at noon was 24◦C higher than the maximum ambient temperature, which reached 43◦C - 45◦C. During treatment, a total volume of 660 ml of urine samples were removed from the reactor for analytical purpose. The concentration of total phosphate increased gradually through -out the 22 days of treatment as shown in Fig(12) .

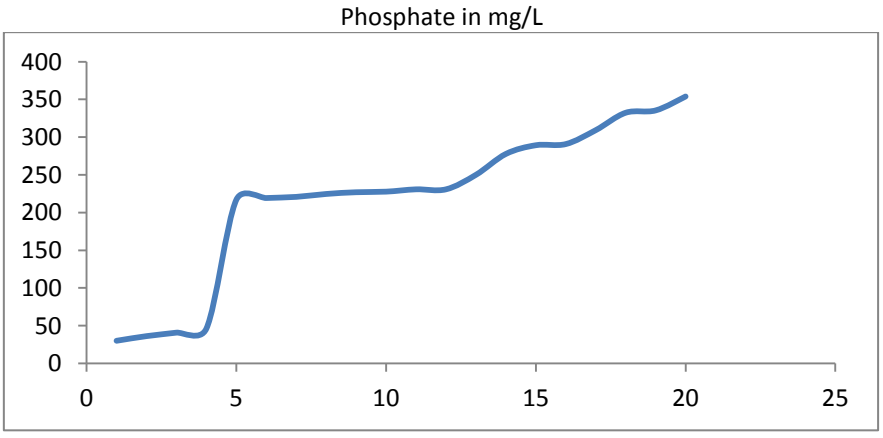

Figure 12: Effect of solar concentrator reactor (15 degree slope) on variation of phosphorous concentration during 22 days.

The urine that was fed into the unit initially had a phosphate concentration 46.15 mg/L, whereas the last urine sample taken on day -22 had a concentration of 273.8 5 mg/L.

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The urine nitrogen concentration first decreased from 0.08mg/L to 0.055 mg/L in day (11), then rose again up to 0.146 and increased up to 0.45mg/L on day (22) in the 15◦ slope design and the nitrogen increased from 0.045mg/L up to 0.94 mg/L after 22 days. Nitrogen concentration mg/L

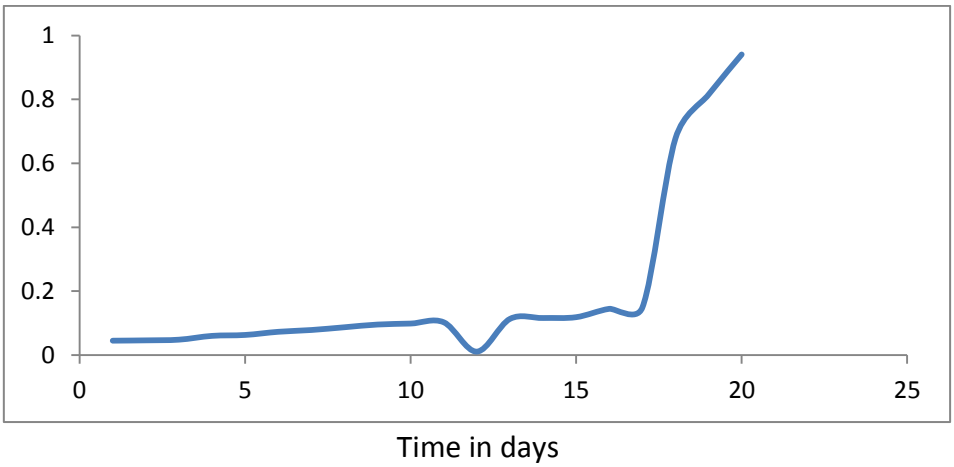

Figure 13: Effect of solar concentrator reactor (15 degree slope) on variation of nitrogen.

The electric conductivity showed a pattern which was very similar to that of total phosphate concentration. The conductivity gradually increased from 21.16 mS/cm on day one and reached 95 mS/cm on day 22 Fig(14). It was directly correlated to the phosphate concentration in Fig (12).

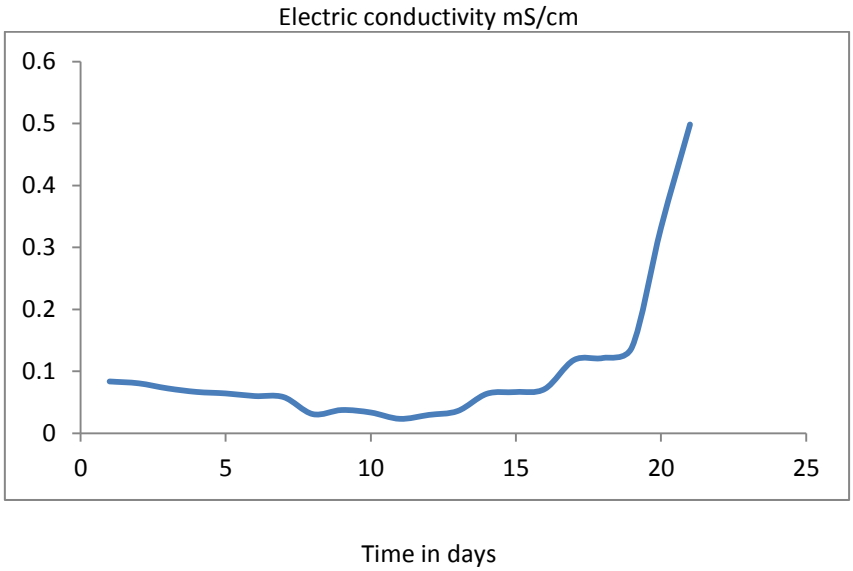

Figure 14: Effect of solar concentrator reactor (15 degree slope) on variation of electric conductivity during 22 days.

The pH measurements Fig(15) did not show a particular trend. The pH of urine varied from 3.56 to 6.7. After complete evaporation of the liquid fraction, about 18.7 g of solid material could be recovered from the solar reactor.

pH value

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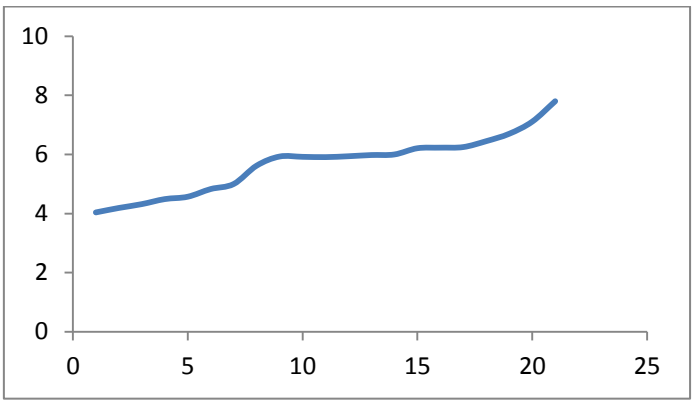

Figure 15: Effect of solar concentrator reactor (15 degree slope) on variation of pH value during 22 days.

Fertilizer Characterization

The results analysis of urine fed into solar reactor at the beginning showed 0.56 mg/L ammonia and 20 % were lost with the condensate. Analysis of XRD for 15◦ slope design yield showed almost that 80% of (derived fertilizer) ammonium hydrogen phosphate, sodium sulphate and sodium chloride Fig (4).

The struvite MgNH4 PO4.6H2O was also prepared by precipitation from human urine and showed elongated prismatic crystals by SEM analysis Fig (5) and DES Fig(6) and Table (2). Our results agree with [18]. The nutrition of algae showed good plantation Fig(9), Fig(10) and natural solar still fertilizer showed the best results Fig (11).

DISSCUSION

Data have shown that the solar thermal distillation of human urine by means of solar reactor was very practical with few resources and like expertise. With a surface area 0.2 m2 and 15 slope degree, it took only 22 days of treatment in order to reach the complete evaporation of the liquid fraction from 5 L of undiluted urine. Treatment was achieved by exposure to sun light and therefore no complex technologies, no qualified workforce, and no cost- intensive energy sources were required for operating the system. It was possible to concentrate nutrients. This was suggested by an increase of the electric conductivity in urine sample Fig (14), and confirmed by an increase of total phosphate concentrations by a factor of 7.7 % Fig (12). Nitrogen concentration increased to 0.94 mg/L Fig (13), and phosphate concentration rose up to 353.85 mg/LFig(12). Unlike phosphate the nitrogen concentration were not directly correlated to the electric conductivity. This is due to treatment at high pH and high temperatures promote the prevalence of nitrogen in the form of volatile ammonia, making this a considerable drawback of the evaporation method. In theory, the nitrogen, which was lost with the condensate, could be reclaimed by adding a further treatment stage for processing the condensate, which is known as gas stirring [19] and [20].

Although it was possible to concentrate the essential plant nutrients nitrogen and phosphorus in derived fertilizer and XRD analysis has shown that these were not only elements to accumulate.

The dry weight of all *S. platunes* cultured (DW-SP) in Zarrouk's medium supplemented with struvite precipitated from human urine was increased significantly with increase of concentration of struvite when compared with DW-SP of obtained from culture grow in standard Zarrouk's medium. Also, the levels of DW-SP were changed as results of process of struvite prepared from human urine. The high value of DW-SP grown in medium supplemented with natural fertilizer prepared from human urine in solar reactor, as compared with that struvite precipitated from authentic human urine 6.1g/L d.w. Fig. (10) show that increasing concentrations of struvite in Zarrouk's medium increased total photosynthetic pigments, *i.e*. chlorophyll. For instants, 65 mg/g d.w of struvite treatment was most effective in promoting the synthesis and accumulation of photosynthetic pigments over that in 1, 2, 4 and 8g/L. However, photo-synthetic pigments in *S. platensses* were greater for all of the natural fertilizer obtained by solar reactor processes. Total lipid content (TL) in *S. platencess* increased with increasing concentrations of struvite up to 8g/L in nutrient medium Fig (11).

At the 8g/L level of human urine treated by solar energy the TL content in *S. planeness* cells was 12.5% while TL content in *S. planeness* cells grow in arterial struvite supplement was 11%. For the similar comparison, the increase in levels of human urine treated with solar energy in Zarrik medium led to increase TL in the range of 7 % to 12.5%. In general, this study has demonstrated beneficial responses of *S. platensses* algae to increased concentrations of struvite in nutrient medium up to 8 g/L.

Although our results agreed with [21], further studies are recommended to evaluate the role of struvite supplement to Zarrouk's medium on the growth and photosynthetic total lipid, pigment and dry weight response of *S. paleness* in order to use that ingredient in food industrials [22].

Estimation of nutrient costs in cultivating Microalgae using struvite and natural struvite from urine drived by solar still:

Based on the optimum cultivation conditions of *S.planenesscell* using struvite precipitated from industrial effluent or human urine and struvite from natural human urine by solar still, a simple cost analysis was conducted. The cost of organic fertilizer purchased at local market was 1.2 USD per pack (400 g) whereas the overall cost to prepare the inorganic fertilizer (BBM) was 15 – 25 USD per liter. The cost of struvite prepared from industrial effluents is calculated as 0.430 USD /Kg [18]. In addition for the preparation of natural struvite from human urine showed that it was possible to concentrate nutrients derived from 5-liters of undiluted human urine to get 18.7 g of solid fertilizer material at 22 days of exposure to direct sunlight. Investment and operational costs were low as the photo reactor was characterized by a simple design with low maintenance and operational efforts, and entirely constructed of materials available at local hardware suppliers. No energy source other than solar radiation was required. Previous studies suggested that solar radiation may be an appropriate hygiene method that could, even when used with human urine, potentially produce a safe fertilizer by degrading pathogens and micro pollutants. Also the struvite prepared from human urine as natural fertilizer from solar still, show better fertility to chlorophyll contents 11% and 12.5 % with lipids uptake.

CONCLUSION

Hence, utilization of cheap nutrients source such as natural fertilizer from human urine will be more beneficial in large scale microalgae cultivation system, typically in terms of cost saving and better environmental protection. Therefore, more efficient and economic culture by natural fertilizer developed by solar reactor enhances commercial viability of microalgae biofuel industry.

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