Human Urine as an Alternative Source of nutrient for Biomass Production of *Scenedesmus dimorphus*

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Research Article

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Abstract

Microalgae are known to produce an array of value-added components like pigments. The nutrient medium used to cultivate microalgae is generally more expensive. The prominence of this work is on the practicality of human urine as a low-cost source of nutrients for microalgae culture, as it includes major nutrients and minor minerals and is cost-effective. Of course, it is a digressive approach from the aesthetic point of view. Nevertheless, the ultimate products can be purified, obviating pathogenicity. Human urine was collected, sterilized and different dilutions were prepared (5%,10%,15%,25%,50%,75%,100%). Cultures of *Scenedesmus dimorphus* were inoculated into a diluted human urine medium and incubated for 15 days at room temperature (around 29ºC) under window spread sunlight. The concentrations of 5%, 10%, and 15% have supported the production of microalgae. The maximum biomass was observed in 5% human urine concentration with a cell density of 1.74×10^6 cells/mL with 3.2 g/L biomass production. The biomass productivity was 0.257 g/L /day. Growth cessation could be perceived at more than 25% of human urine concentration. This study offers scope for considering human urine for algal production, targeting value-added products, aside from obnoxious liquid waste utilization.

1. Introduction

Microalgae are getting much attention because of their high-value compounds in food, feed, fuel, bioremediation, medicine, and cosmetics. (Levasseur et al. 2020). However, because of the high manufacturing costs and associated limitations, its viable production is still in its infancy. Nutrients are the most expensive part of growing microalgae (34% of total cost), followed by harvesting (20–30% of total cost) and processing for algal-based goods (Fasaei et al. 2018). For this problem, algae culture in the wastewater system reduces the cost of the nutrients. For a long time, wastewater has been utilized to produce biomass (Edward and Pullin,1988). *Scenedesmus acuminatus* flourished well in wastewater-contaminated stream water and dominated the principal ponds in a Norwegian experiment (Kallqvist et al. 1996). *Scenedesmus acuminatus* have proven suitable for culture in domestic wastewater (Adammson 2000). Recently, human urine has assumed more attention to culture the microalgae. The potential for human pee to be used as a fertilizer in agriculture is an age-old practice. In a designed food chain, urine may be used as a fertilizer source for green algae, which can then be given to zooplankton, which can then be fed to fish. Urine is a nutrient-dense, easily available kind of waste that is both sustainable and inexpensive, and it may help microalgae thrive, (Suresh et al. 2019; Tuantet et al. 2014) and grow. Human pee has a volume of only 1–1.5 liters per person and per day. It is now feasible to recycle the nutrients (N and P) from the urine (Hanaeus et al. 1997; Karrman 1997; Jonsson et al. 1997). Algae grown in urine will grow with nitrogen sources such as nitrate, nitrite, ammonium, or urea. Urea is the main source of most ammonia in human urine (80%). Growing microalgae in urine have the advantage of treating urine by eliminating nitrogen and phosphate while simultaneously producing a high biomass output (Jaatinene et al. 2015). Very few studies have employed human urine as a microalga growing medium (Adamsson 2000). This study investigates the growth potentials of

microalga (*Scenedesmus dimorphus*) in human urine and the dilution factor, demonstrating that human urine is a feasible growth medium for the generation of viable microalgae biomass. This exploratory study attempted to encourage the use of human urine for long-term biomass production by using nutrients in accordance with ecological sanitation standards.

2. Material And Methods

2.1 Microalgae culture

Scenedesmus dimorphous (NRMC-F 0155) was procured from National Repository for Microalgae and Cyanobacteria-Freshwater, NRMC-F (BHARATHIDASAN UNIVERSITY, Trichy). The culture was upscaled and maintained in a 15 mL test tube with 10 mL of Bold Basal Medium (BBM) for 10 days with fluorescent light illumination at a temperature of 25ºC to 28ºC (Lam and Lee,2012). Then the 15 mL culture was transferred to a 100 mL conical flask culture and maintained for 10 days.

2.2 Human urine collection

500 mL of fresh human urine was collected from a volunteer using a 500 mL water bottle. The urine was collected in the morning, and after collecting the urine, the bottle was mixed well. For sterilization, the urine was autoclaved at 120□C for 15 minutes. After the sterilization, the urine was diluted with distilled water immediately.

2.3 Experimental design

The sterilized urine was diluted with distilled water to arrive at 5%,10%,15%, 25%, 50%, 75% and 100%concentrations. For control, BBM (Bold Basal Medium) was used for the comparison study. In that diluted human urine 10 mL of BBM grown *Scenedesmus dimorphus* culture was inoculated. These 8 conical flasks were placed under sunlight for 15 days. The experiment flow chart is shown in Fig. 1. The flask was shaken manually thrice a day. Initial water quality parameters were noted (pH, salinity, cell density, and weight of algae). At 7 days of the interval, all the parameters were estimated. For biomass, algae were counted using the heamocytometer, and the 50 mL of algae was filtered to measure the weight of biomass. The experiment was performed in triplicate. The one-way analysis of variance (ANOVA: single factors) was performed using Microsoft Excel to determine the differences between the treatments

3. Results

The growth potential in the diluted human urine was tested using a pure *Scenedesmus dimorphus* culture. The *Scenedesmus dimorphus* was cultured in human urine at the concentration of 0%,5%,10%,15%,25%,50%.75% and 100% for 15 days and observed that the 5% of Human urine concentration has a potential to culture this microalga *Scenedesmus dimorphus (*Fig. 4*).* The pH of the initial culture was 6.7 ± 0.3 and it was observed to be 8.5 ± 0.3 at the end of the experiment (Fig. 2*).* The pH of the urine gradually increased during the experimental period. The salinity of the *Scenedesmus*

dimorphus culture was shown in Fig. 3. The remarkable variation in salinity could be observed at different concentrations. However, within the treatments there was only mild variation ($p < 0.05$), with little reduction was discovered which could be attributed to the usage of nutrients by algae, especially from dissolved fraction of urine. Up to 7th day 10% concentration had a favorable condition and thereafter sustained growth could be seen in 5% concentration, when the urine concentration increased the microalgae decreased, and at more than 25% the microalgae growth ceased in total (Fig. 4*).* At the end of the experiment (15th day), maximum growth (1.74×10^6 cells /mL) was observed in the 5% urine concentration.

The microalgae growth observed in BBM showed cell density 64.5×10^4 cells /mL. The dry weight of the *Scenedesmus dimorphus* also followed this trend, On the 7th day 10% concentration attained the maximum biomass 2.2 g/L compared to other concentrations. At the end of the experiment, 5% showed the maximum dry weight 3.2 g/L, and the control (BBM) showed 2.4 g/L. The algae could not tolerate a concentration of more than 10% as could be seen at higher concentrations in terms of survival and growth (Fig. 5). A dry weight biomass 0.186 g/L /d and 0.257 g/L /d could be obtained control (BBM) and 5% human urine, respectively. More than 25% of human urine has not supported microalgae growth. The microalgae survival and growth in the human urine medium significantly differed in different concentration levels ($p < 0.05$).The raw data are available in the form of table at the end of the manuscript.

4. Discussion

This study is highly comparable with previous studies (Adamsson 2000, Jaatinen et al. 2019), with appreciable production in 5% concentration in 15 days. Compared to earlier studies remarkably higher production could be seen in this investigation in *Scenedesmus dimorphus* at the usage of 5% human urine. Through 10% concentration showed elusive better production up to 7 days, sustainable production increase could be seen only at 5% level. Only very low concentration 0.6% (biomass 2.32 g/L in 7 days) and 0.8% (0.81 g/L in 7 days) in *Spirulina* sp. (Feng et al. 2006; Chang et al. 2013), 1% (biomass 0.6 g/L in 21 days) in *Chlorella* sp. (Jaatinen et al. 2019) and 2% (biomass 0.16 g/L in 10 days) in *Scenedesmus* sp. (Adamsson,2000) was considered to be of threshold dosage as nutrients. The algae utilized the nutrients from the human urine (P & N), which is explicit in the appreciable growth. Previous research has shown that a similar concentration of cow urine is effective in producing microalgae biomass (Suresh et al. 2019). Furthermore, this study rendered a clue that concentrated raw human urine could not be tolerated and supported the growth of this algae. Muluye et al. (2021) was endorsive that at more than 20% of human urine concentration microalgae could not grow. Further research is needed to optimize human urine as a nutrient in the culture of various strains of algae meant for value-added products like pigment or biomass as fertilizer along with cost analysis.

5. Conclusion

This study is indicative of the fact that microalgae *Scenedesmus dimorphus* could be comfortably produced at 5% human urine concentration with a dry biomass 0.257 g/L /day. Further, it is also corraborates that human urine can be used as a low-cost nutrient medium for long-term biomass production, lowering the cost of value-added products from algae. Further research on cost analysis and nutrient modification is required for better biomass production, and scaling up of this operation at the commercial level.

Declarations

Author's contribution

The study was created and planned by all authors. Guidance given from author Manikandavelu D and Aruna S. The experiment was carried out by authors Vennila M and Rajeshwari C. Data analysis and writing of the manuscript were done by Vennila M. The article was edited by the author Manikandavelu D and Aruna S. The revised manuscript included input from all authors. The final version of the text was authorised by all writers, who concur to be held responsible for its content.

References

1. Adamsson, M., 2000. Potential use of human urine by greenhouse culturing of microalgae (Scenedesmus acuminatus), zooplankton (Daphnia magna) and tomatoes (Lycopersicon). Ecological Engineering, *16*(2), pp.243–254.
2. Chang, Y., Wu, Z., Bian, L., Feng, D. and Leung, D.Y., 2013. Cultivation of Spirulina platensis for biomass production and nutrient removal from synthetic human urine. Applied energy, *102*, pp.427–431.
3. Edwards, P. and Pullin, R.S.V., 1988, December. Wastewater-fed aquaculture. In *Proceedings, International Seminar on Wastewater Reclamation and Reuse for Aquaculture, Calcutta, India* (pp. 6–9).
4. Fasaei, F., Bitter, J.H., Slegers, P.M. and Van Boxtel, A.J.B., 2018. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Research, *31*, pp.347–362.
5. Feng, D.L. and Wu, Z.C., 2006. Culture of Spirulina platensis in human urine for biomass production and O2 evolution. Journal of Zhejiang University Science B, *7*(1), pp.34–37.
6. Hellström, D., Johansson, E. and Grennberg, K., 1999. Storage of human urine: acidification as a method to inhibit decomposition of urea. Ecological Engineering, *12*(3–4), pp.253–269.
7. Jaatinen, S., Lakaniemi, A.M. and Rintala, J., 2016. Use of diluted urine for cultivation of Chlorella vulgaris. Environmental technology, *37*(9), pp.1159–1170.
8. Jönsson, H., Stenström, T.A., Svensson, J. and Sundin, A., 1997. Source separated urine-nutrient and heavy metal content, water saving and faecal contamination. Water science and technology, *35*(9), pp.145–152.

9. Källqvist, T., Johansen, S.S. and Löffler, H., 1996. Lake Victoria—limnological study of Murchison Bay and Napoleon Gulf, Uganda, with references to water supply and wasteater disposal at Kampala and Jinja. Republic of Uganda, The World Bank. *Norwegian Institute for Water Research, Department of Zoology, University of Vienna*.
10. Kärrman, E., 1997. *Analysis of Waste Water Systems, with Respect to Environmental Impact and the Use of Resources*. Chalmers Tekniska Hogskola (Sweden).
11. Lam, M.K. and Lee, K.T., 2012. Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnology advances, *30*(3), pp.673–690.
12. Levasseur, W., Perré, P. and Pozzobon, V., 2020. A review of high value-added molecules production by microalgae in light of the classification. Biotechnology advances, *41*, p.107545.
13. Muluye, A., Sali, B., Bahta, B., Melese, B., Girma, B., Kebede, M. and Suresh, A., 2021. Potential of Human Urine as a Nutrient Medium for the Biomass Production of Microalga Scenedesmus sp. East African Journal of Agriculture and Biotechnology, *3*(1), pp.35–41.
14. Suresh, A., Tamilvanan, S., Harini, K., Seventhi, H.V., Deepan Guna, R., Mahalakshmi, R., Suriyapriya, S., Sharmila, D. and Thenmozhi, M., 2019. Feasibility of cattle urine as nutrient medium for the microalgal biomass production. Global Journal of Environmental Science and Management, *5*(4), pp.441–448.
15. Tuantet, Kanjana, Marcel Janssen, Hardy Temmink, Grietje Zeeman, René H. Wijffels, and Cees JN Buisman. "Microalgae growth on concentrated human urine." Journal of applied phycology 26, no. 1 (2014): 287–297.

Tables

Table 1 pH variation in the culture of *Scenedesmus dimorphus* in seven-day intervals

Human urine concentration	pH		
	First day	Seventh day	Fifteenth day
Control (BBM)	6.9±0.04	7.12±0.07	8.7±0.1
5%	6.8±0.1	7.24±0.11	8.3±0.15
10%	6.4±0.21	7.22±0.02	8.5±0.08
15%	7.11±0.12	7.33±0.04	8.6±0.07
25%	7.06±0.07	7.14±0.04	8.7±0.06
50%	7.08±0.14	7.12±0.05	8.7±0.09
75 %	7.05±0.12	7.24±0.04	8.8±0.07
100%	7.03±0.10	7.31±0.09	8.8±0.08

Table 2. Salinity variation in the culture of *Scenedesmus dimorphus* in seven-day intervals

Human urine concentration	Salinity		
	First day	Seventh day	Fifteenth day
Control (BBM)	0ppt	0ppt	0 ppt
5%	2 ppt	2 ppt	0 ppt
10%	2ppt	2ppt	0 ppt
15%	5ppt	5ppt	2 ppt
25%	5 ppt	5 ppt	5ppt
50%	10 ppt	10 ppt	10 ppt
75 %	18 ppt	18 ppt	15 ppt
100%	24ppt	24ppt	20 ppt

Table 3. Cell density of *Scenedesmus dimorphus* in seven-day intervals

Human urine concentration	Cell density ×10^4 (Cells /mL)		
	First day	Seventh day	Fifteenth day
Control (BBM)	4.75	13.75±0.25^{d}	64.2±1.15^{b}
5%	4.75	22.91±0.8^{b}	174.2±2.72^{a}
10%	4.75	25.65±0.4^{a}	92.4±1.1^{b}
15%	4.75	16±0.76^{c}	-
25%	4.75	10.06±0.30^{d}	-
50%	4.75	1.63±0.07^{e}	-
75 %	4.75	1.31±0.05^{e}	-
100%	4.75	1.13±0.12^{e}	-

Table 4. Biomass of *Scenedesmus dimorphus* in seven-day intervals

Human urine concentration	Weight (g/l)		
	First day	Seventh day	Fifteenth day
Control (BBM)	0.3	1.03±0.15^{cd}	2.4±0.2^{b}
5%	0.3	1.73±0.2^{b}	3.2±0.4^{a}
10%	0.3	2.1±0.1^{a}	2.5±0.3^{b}
15%	0.3	1.2±0.3^{c}	-
25%	0.3	0.8±0.3^{d}	-
50%	0.3	-	-
75 %	0.3	-	-
100%	0.3	-	-

Figures

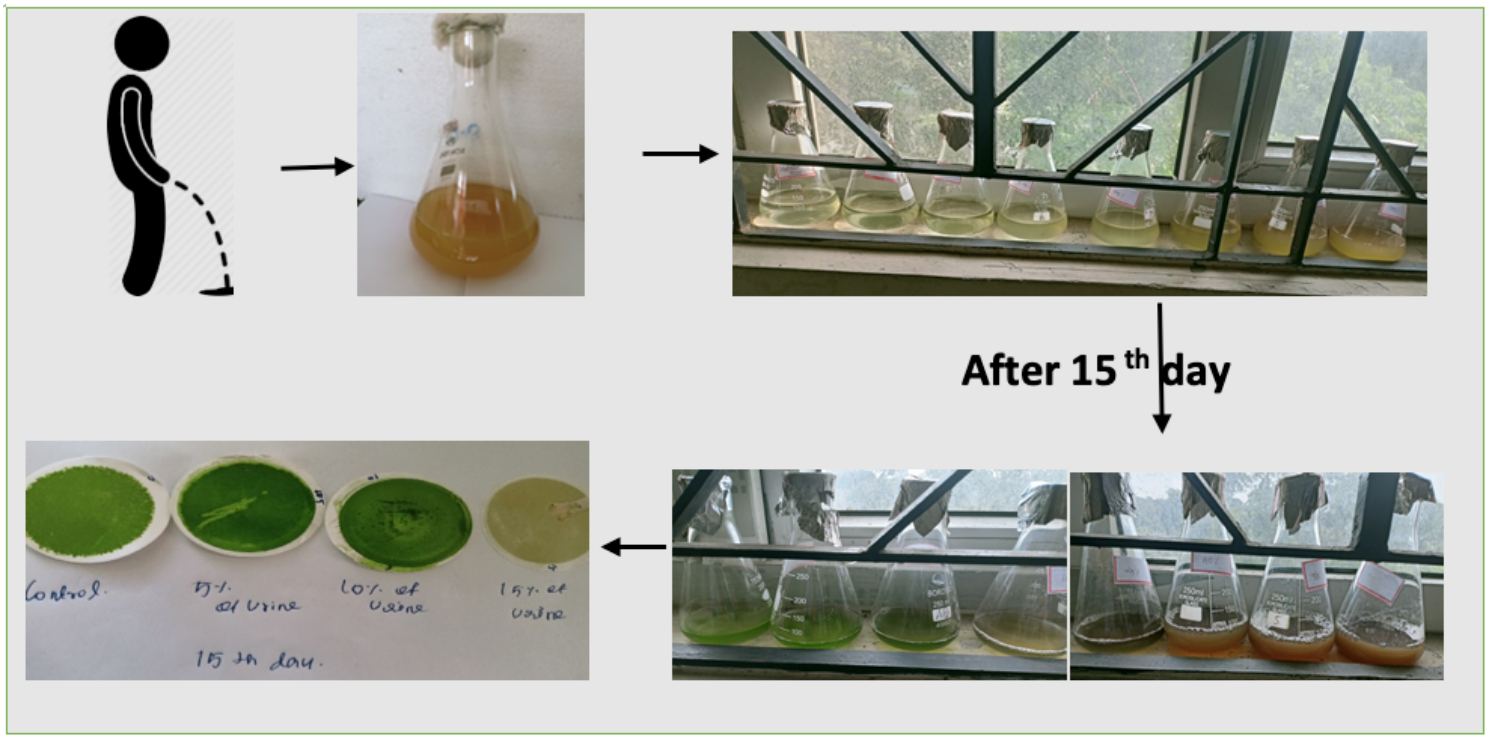

Figure 1

Flow chart of the experiment

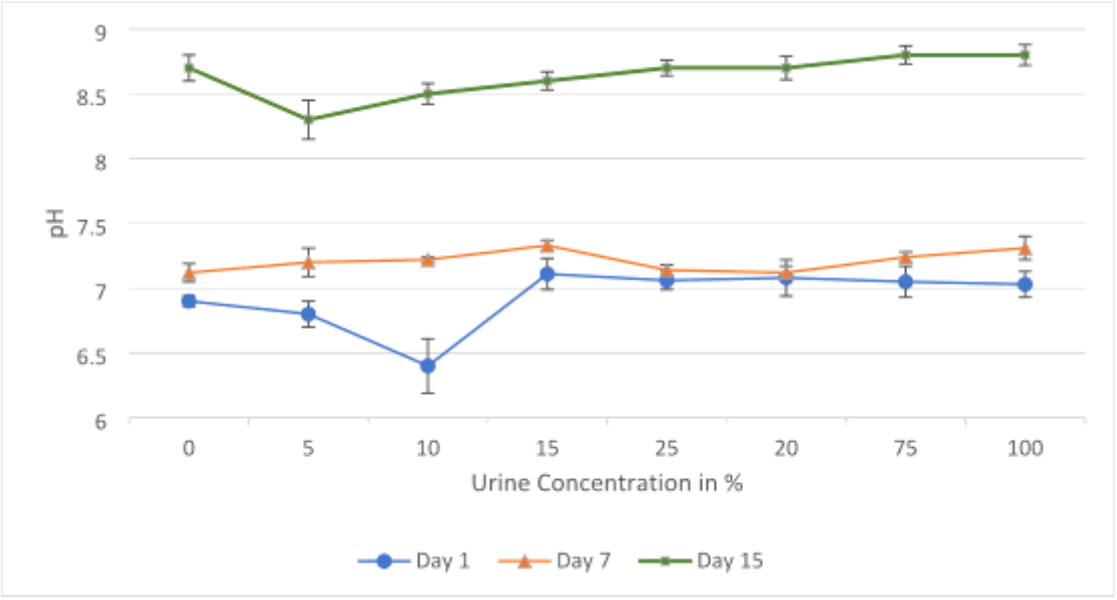

Figure 2

pH variation in the culture of *Scenedesmus dimorphus* in seven-day intervals

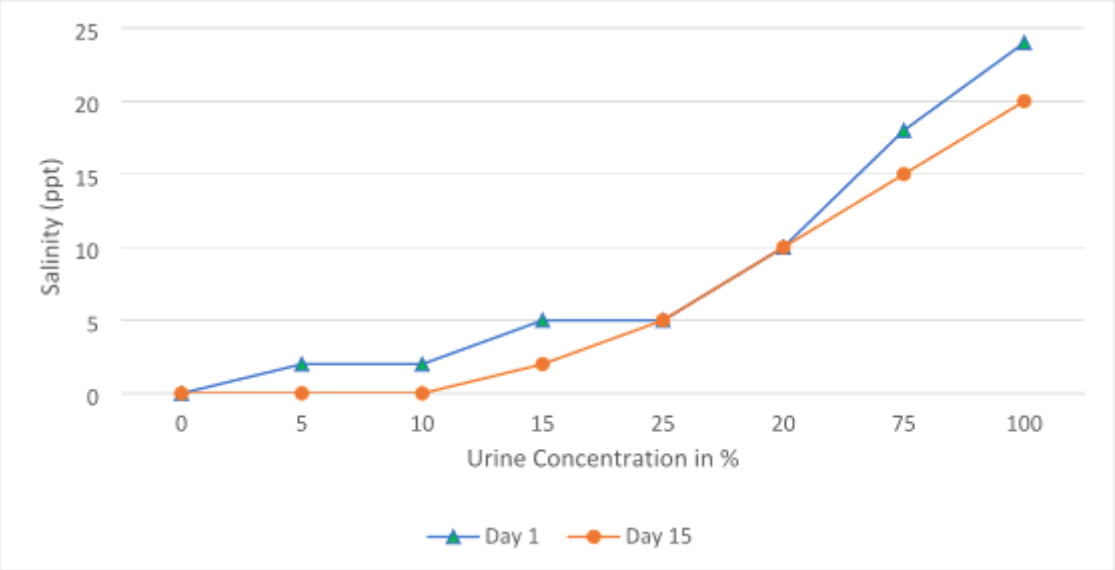

Figure 3

Salinity variation in the culture of *Scenedesmus dimorphus* in seven-day intervals

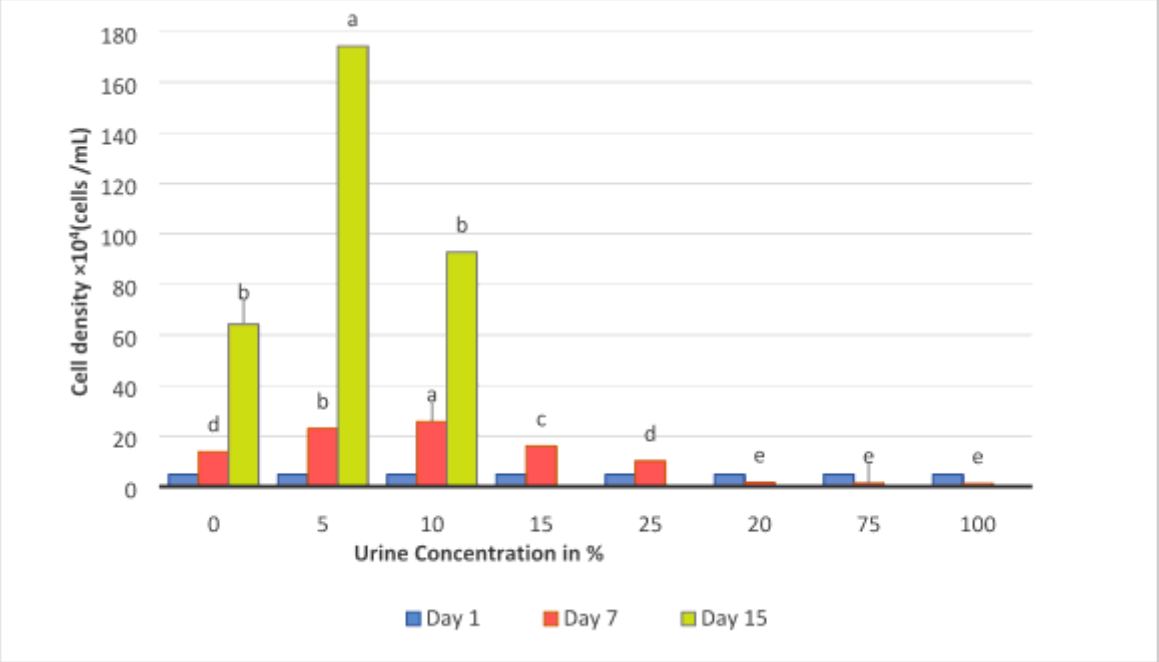

Figure 4

Cell density of *Scenedesmus dimorphus* in seven-day intervals

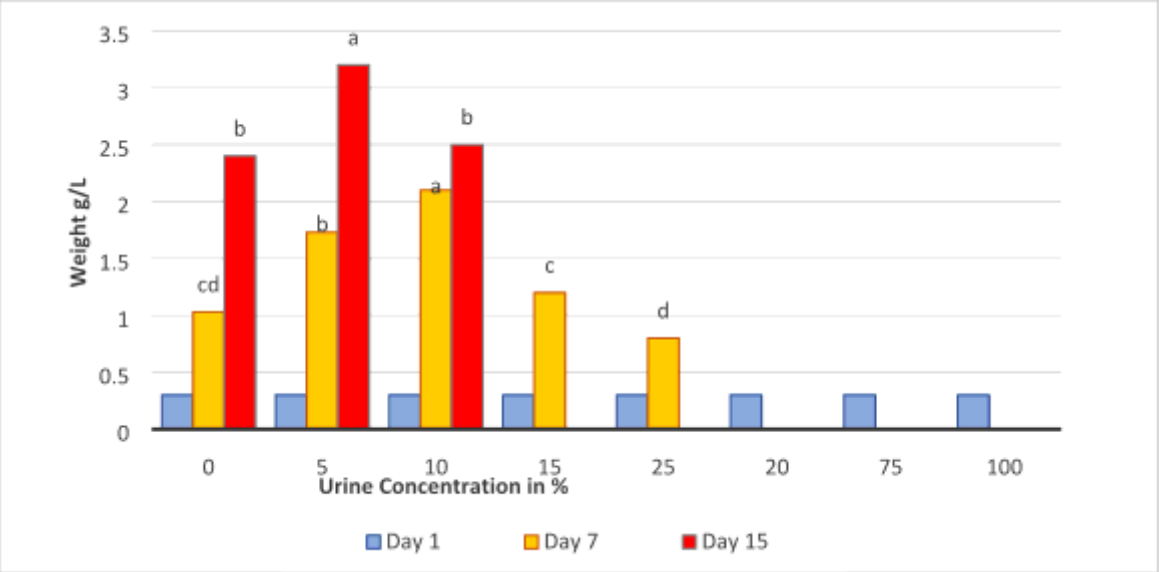

Figure 5

Biomass of *Scenedesmus dimorphus* in seven-day intervals