

ORIGINAL RESEARCH PAPER

Removal of nitrate and phosphate from aqueous solutions by microalgae: An experimental study

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ABSTRACT: The aim of this study was to evaluate the ability of microalgae *Spirulina platensis* and *Chlorella vulgaris* to remove nitrate and phosphate in aqueous solutions. *Spirulina platensis* and *Chlorella vulgaris* microalgae was collected in 1000 ml of municipal water and KNO₃, K₂HPO₄ was added as sources of nitrate and phosphate in three different concentrations (0.25, 0.35 and 0.45g/L). During the growth period, the concentration of nitrate and phosphate was recorded at 1, 4, 6 and 8 days. The highest nitrate removal on the 8 day for *Chlorella vulgaris* was 89.80% at the treatment of 0.25g/L and for *Spirulina platensis* was 81.49% at the treatment of 0.25g/L. The highest phosphate removal for *Spirulina platensis* was 81.49% at the treatment of 0.45g/L and for *Chlorella vulgaris* was 88% at the treatment of 0.45g/L. The statistical results showed that the amount of phosphate and nitrate removal during different time periods by *Chlorella vulgaris* depicted a significant difference at P<0.01, while *Spirulina platensis* demonstrated a significant difference at P<0.05. Thus, *Spirulina platensis* and *Chlorella vulgaris* can be effectively used to remove nitrate and phosphate from effluent and waste water treatments, although it demands more research in different climatic conditions.

KEYWORDS: Aqueous solutions; *Chlorella vulgaris*; Microalgae; *Spirulina platensis*; Wastewater

INTRODUCTION

One of the essential needs of human life is clean water to create healthy conditions for human life. The rapid development of human activity is the result of increased nitrogen and phosphorus entering the groundwater and surface water resources (Sayadi *et al.*, 2012; Sayadi *et al.*, 2014; Abhijna, 2016). The access of this resource to water bodies on one hand aggravates the phenomena such as algal blooms and on the other hand affects the ecological balance of water bodies and subsequently declines the water quality (Rezaei *et al.*, 2015). Various physical and

chemical methods are used such as adsorption, chemical deposition, solvent extraction and ion exchange to separate these nutrients. Biological treatment of nitrogen and phosphorus wastewater has been widely studied, and organisms such as bacteria, fungi, protozoa and microalgae have been used for this purpose (Shahriari Moghadam *et al.*, 2016). Microalgae have the potential to remove mineral nutrients such as nitrogen and phosphorus from wastewater and therefore received considerable attention in the recent years (Khan and Yoshida, 2008). Wastewater treatment by algal cultures besides controlling additional pollution does not produce effective results in open-wheel food, and is a cheap and efficient tool for the removal of nutrients which cause ecological safety in

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aquatic ecosystems (Aslan and Kapdan, 2006). Many studies related to the viability and usefulness of microalgae in sewage has been reported (Caswell and Zilberman, 2002). Yang et al., in 2011, studied biomass production and nitrogen and phosphorus removal from municipal wastewater by green algae *Oleoabundans Neochloris*. The results indicated that the algae can completely remove the high nitrogen and phosphorus concentrations with subsequent increase of biomass production. *Chlorella vulgaris* and *Scenedesmus dimorphus* are unicellular microalgae that depicted an ability to eliminate 55% of phosphate from wastewater, dairy and pig farms in Colombia. Other studies related to evaluate the capability of nitrogen and phosphorus wastewater removal by two microalgae *Dunaliella viridis* and *Tetraselmis chuii* reported that the two species of algae are able to trace concentrations of dissolved salts in the 90% amount or less than that at the beginning of the decrease of biological tests (Koenig, and Demacedo, 2004). Accumulation of nutrients cause algae growth and at the same time separation phenomenon occurs in other nutrients such as ammonium evaporation and phosphate deposition produced from algae as a result of high pH (Ahmadpour et al., 2014).

The study was carried out in 2015 at Birjand city which is located in south of Khorasan Province and attempts to evaluate the rate of nitrate and phosphate removal from aquatic solutions using microalgae *Spirulina platensis* and *Chlorella vulgaris*.

MATERIALS AND METHODS

Medium of microalgae

Spirulina platensis is a cyanobacterium (blue-green algae) that can be growth in brackish water and consumed by humans and other animals. *Chlorella vulgaris* is a genus of single cell green algae belonging to the phylum Chlorophyta which can serve as a potential source of food and energy. Micro-algae used in this study viz, pure blue-green algae *Spirulina platensis* and *Chlorella vulgaris* (50ml) where was prepared from the Aquatic Research Institute of Bandar Anzali. Microalgae *Spirulina platensis* and *Chlorella vulgaris* were cultured to 1000ml in Zarrok's Medium and on the Bold's Basal Medium (BBM) respectively (De Moraes and Costa, 2007). Agitation and aeration were accomplished using air from a compressor (RESUN AC-9603-0.12MPa) and two fluorescent lamps light from the intensity of 3,500 lux in periods of 12 hours of darkness / brightness Erlenmeyer flask exposure levels was used to determine (Shabani et al., 2016).

Experiments

350 ml *Spirulina platensis* and *Chlorella vulgaris* microalgae were collected in 1000 ml of distilled water and due to the concentration of nitrate and phosphate in municipal waste water treatment plant, KNO_3 , K_2HPO_4 was added as sources of nitrate and phosphate in three different concentrations 0.25, 0.35 and 0.45 g/L, conversely in higher concentration of the nutrient algae growth is limited (Aslan and Kapdan, 2006). The experiment carried out for 8 days because the maximum growth rate of these types of algae occurs in 8 days (Shabani et al., 2016). Additionally the experiment was conducted in triplicate.

Chemical analysis methods

Primarily, in period of 1-4, 4-6 and 6-8 days, 50ml of the solution was separated via filter paper (0.45 micrometres) and later subjected to nitrate and phosphate analysis. All tests were performed at a constant temperature of 25°C and the pH was recorded daily. For determination of residual nitrate and phosphate, the microalgae was separated by centrifugation for 15 minutes at 5000 RPM, and the resultant was passed through a membrane filter with a pore size 0.45 μm and a diameter of 1 inch (Shabani et al., 2016). The concentration of nitrate and phosphate was measured by spectrophotometer Bio spec-1601 with wavelength 690nm for phosphate and 220-225nm for nitrate according standard methods (APHA, 1998).

Calculation of the specific growth rate

Specific growth rate was obtained, based on the dry weight using Eq. 1 (Tang et al., 2011):

$$\mu \text{ (1/day)} = \ln (x_t/x_0)/(t-t_0) \quad (1)$$

Where parameters μ represents the special rate of growth (1/day), X_0 primary biomass (g/L) and biomass amount of time t_1 is the X_t . For data analysis, the data obtained by Excel and SPSS were analyzed. Statistical analyzes based on three replicates per test was carried out via one way ANOVA and Tukey plan.

RESULTS AND DISCUSSION

Specific growth rate

According to the results of Tables (1, 2) which calculated by Eq.1, the growth rate increased with increasing concentrations of nitrate and phosphate in the treatments. As demonstrated, the highest growth

Table 1: Mean specific growth rate trend observed in the treatments of *Spirulina platensis*

Time (Day)	1-4	4-6	6-8
PO ₄ (0.25g/L)	0.029±0.123	0.072±0.288	0.046±0.233
PO ₄ (0.35g/L)	0.024±0.125	0.082±0.299	0.058±0.268
PO ₄ (0.45g/L)	0.033±0.167	0.038±0.169	0.017±0.571
NO ₃ (0.25g/L)	0.015±0.765	0.024±0.123	0.016±0.83
NO ₃ (0.35g/L)	0.024±0.816	0.026±0.134	0.019±0.097
NO ₃ (0.45g/L)	0.16±0.819	0.04±0.148	0.04±0.225

Table 2: Mean specific growth rate trend in the treatments of *Chlorella vulgaris*

Time (Day)	1-4	4-6	6-8
PO ₄ (0.25g/L)	0.029±0.127	0.072±0.291	0.046±0.221
PO ₄ (0.35g/L)	0.024±0.139	0.082±0.302	0.058±0.298
PO ₄ (0.45g/L)	0.033±0.172	0.038±0.238	0.017±0.602
NO ₃ (0.25g/L)	0.015±0.767	0.024±0.248	0.016±0.924
NO ₃ (0.35g/L)	0.024±0.924	0.026±0.365	0.019±0.137
NO ₃ (0.45g/L)	0.16±0.992	0.04±0.216	0.04±0.334

rate of *Spirulina platensis* and *Chlorella vulgaris* was in the treatments; 0.45 g/L nitrate viz. 0.503 (1/day) and 0.58 (1/day) and 0.45 g/L phosphate viz. 0.244 (1/day) and 0.354 (1/day), respectively. In a study conducted by Afshari *et al* (2011), on the ability of algae *Tetraselmis suecica* in urban secondary wastewater treatment to reduce nitrate and phosphate the similar trend in the different days was achieved. 78.1% and 70.3% nitrate in the aquatic solutions were removed by *Chlorella vulgaris* and *Scenedesmus* respectively (Kshyrsagr, 2013). Similarly, the results of the current study showed that the highest growth rate was in the concentration 0.45 g/L, with a higher nitrate density. In a study conducted by Mousavi *et al* (2009) on the growth of *Chlorella vulgaris* in different cell densities it was stated that less phytoplankton density resulted in low nitrogen removal efficiency which is in concurrence with the present study where is very high densities in the treatments reduce light penetration and increases shade effects which in turn limits the growth and metabolic activities in the phytoplankton cells. In this study, the growth rate of microalgae was effective for nitrate and phosphate removal of aqueous solutions of different treatments where by the highest growth rate and higher removal rate was obtained on the eight day. The average growth rate comparison showed no significant difference between the two microalgae ($P < 0.05$). The average growth rate of *Chlorella vulgaris* in both phosphate and nitrate treatments was more in relation to *Spirulina platensis*. However, with reference to time period, a significant difference was observed on the eighth day ($P < 0.05$) in different nitrate and phosphate concentrations.

pH changes

pH is an important phenomenon especially in the removal of nutrients that were assessed this study. Increased alkalinity and pH indicator phosphate and nitrate; in other terms it tends to reduce nutrients (Ji *et al*, 2014). However, the pH value in microalgae growth, showed an upward trend. Table 3 and 4 exhibit the pH changes in microalgae *Spirulina platensis* and *Chlorella vulgaris*, with increased cell growth, besides with an increase in nitrate and phosphate, the pH value increases (Mousavi *et al*, 2009). However, the highest pH value was indicated in the highest growth, and the highest cell density and growth was observed in the final days i.e. on the 8 day. In a study carried out by Zamani *et al.* (2010) on efficiency removal of nitrate, nitrogen and orthophosphate from wastewater by using several species of microalgae to check the pH changes during the 12 days culturing period, the results showed that for most of the studied species, this amount gradually increased from the initiation to the termination of the experimental period which was in concurrence with the results of this study. Increasing pH leads to phosphate precipitation in the medium. If the pH is reduced, it reduces the phosphate dissolved in the medium. Nitrogen absorption by microalgae influences the pH value. Absorption of nitrate ions lead to increased pH, but if ammonia is used as the nitrogen source then it reduces to very acidic pH of 3, which is good for growth. (Caswell and Zilberman, 2002). One of the negative effects of high pH in microalgae medium is that it reduces the removal of nutrients and therefore growth should be increased to

Table 3: pH changes in the culture of *Spirulina platensis* at various time intervals

Time (Day)	1	4	6	8
PO ₄ (0.25g/L)	6.73	6.67	7.4	9.76
PO ₄ (0.35g/L)	6.58	7.37	9.18	9.83
PO ₄ (0.45g/L)	7.32	7.52	9.74	9.99
NO ₃ (0.25g/L)	7.6	7.72	8.93	10.77
NO ₃ (0.35g/L)	7.39	7.94	8.46	10.87
NO ₃ (0.45g/L)	9.56	9.83	10.7	10.7

Table 4: pH changes in the culture of *Chlorella vulgaris* at various time intervals

Day	1	4	6	8
PO ₄ (0.25g/L)	7.36	7.40	7.69	9.55
PO ₄ (0.35g/L)	6.70	6.94	7.05	9.65
PO ₄ (0.45g/L)	6.49	6.72	6.81	9.63
NO ₃ (0.25g/L)	9.37	9.74	10.26	10.30
NO ₃ (0.35g/L)	9.61	9.58	9.39	9.43
NO ₃ (0.45g/L)	9.06	9.18	9.20	9.23

avoid turbulence for gas exchange between water and air and pH should be adjusted to a certain extent (Suva, 1999). Thus, in the present study, it can be suggested that the increase in pH is due to the photosynthetic uptake of carbon dioxide. In case of lack of carbon dioxide supply, the pH value of 10 is not uncommon and if carbon dioxide is limited and bicarbonate is used as a carbon source, then pH can be 11 or even higher (Oswald, 1988).

Nitrate removal

Removal of nutrients by microalgae cells is in accordance with their cell density and metabolic activity. In this study, the ability of microalgae to grow and remove nitrate at different concentrations of KNO₃, K₂HPO₄ were tested in the course of 8 days at three

different periods of 4, 6 and 8 days respectively. The results of this study showed that microalgae *Chlorella vulgaris* and *Spirulina platensis* grown in nitrogen-rich treatments, and as depicted in the Figs. 1 and 2, an optimal decrease in nitrate concentrations was shown in all the treatments on the 8 day. The highest rate pertained to 0.45 g/L where in a nitrate removal percentage of 89.97% and 89.90% were reported by microalgae *Spirulina platensis* and *Chlorella vulgaris* respectively. The removal process in both treatments increased with time. The lowest removal rate was observed in the treatment 0.25 g/L with 1-4 days viz. 28.71% and 20.08% for microalgae *Spirulina platensis* and *Chlorella vulgaris* respectively. Based on previous studies removed during the eight days of constant and numerous changes do not occur with the

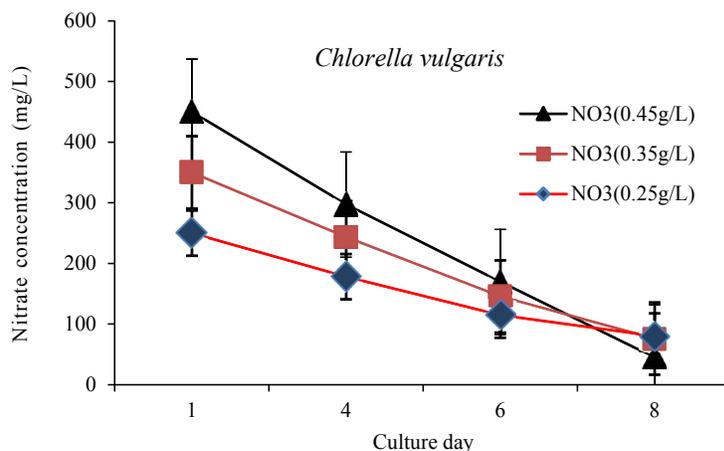


Fig. 1: Comparing the mean and standard deviation of remaining nitrate treatments

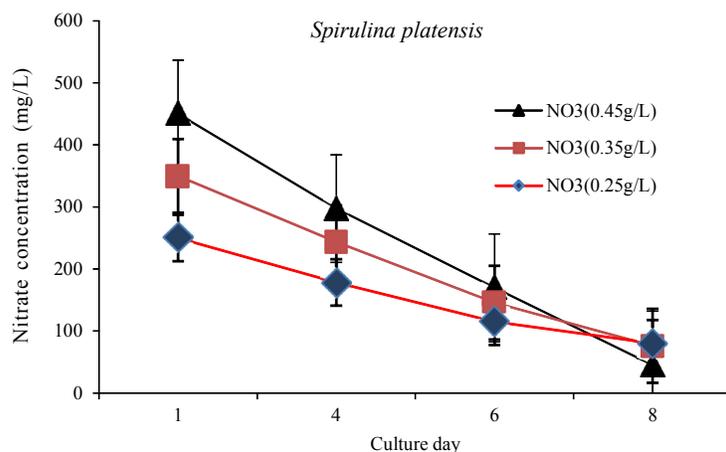


Fig. 2: Comparing the mean and standard deviation of remaining nitrate treatments

passage of time (Tam and Wong, 1994). Rasolamini *et al.* (2011) in a study conducted on the nitrate and phosphate removal from wastewater by microalgae reported that the percentage of nitrogen removal by *Chlorella* was 84.11% within 14 days and phosphorus removal rate by algae *Chlamydomonas* was 100%, which is a higher percentage in comparison to the results of the present study. Studies on the nitrogen and phosphorus removal from wastewater by microalgae *Scenedes musobliquus* under different temperature and mixing conditions showed that the highest nitrogen removal was at a temperature of 25 °C and vigorous mixing at a rate of 100% and the maximum phosphorus removal (98%) was obtained in the same conditions, relating a higher ability of microalgae *Spirulina* to remove nitrate and phosphate. In a similar study conducted by Dickinson *et al.* (2013) the ratio of nitrogen /phosphorus was reported 5: 1 to 12: 1 i.e. nitrogen and phosphorus removal rates were between 99-83% and 99% of phosphorus respectively. Tang *et al.* (2011) studied the reduction of phosphorus and nitrogen in wastewater using *Spirulina* algae and stated that *Spirulina* needs nitrate and phosphorus in the culture and reported that culturing of *Spirulina* in wastewater could reduce nitrate content from 16 ± 4.30 to 5.5 ± 1.50 ppm. In the present study, the highest nitrate and phosphate removal by the microalgae *Spirulina platensis* was on the eighth day, with a nitrate (115mg/L) and phosphate (54mg/L) reduction in the concentration of 0.45 g/L. Cheunbarn and Peerapornpisal, (2010) successfully cultured *Spirulina* in the wastewater, and after 12 days of

retention, measured the maximum efficiency, which was 67, 23, 45, 49, 92 percent for COD, BOD, NO₃ and NH₄⁺ respectively. In this study, the maximum efficiency of NO₃ and PO₄ within 8 days of microalgae culture was 65/78 and 49/81 respectively. Chngany *et al.* (2012) used micro-algae *Spirulina platensis* for wastewater treatment and showed that this microalga grows in nutrient-rich wastewater and can act as a substitute for appropriate secondary treatment. Nevertheless, in the present study, the maximum NO₃ and PO₄ removal were, 65.78% and 49.81%, respectively.

Phosphate removal

Phosphate ions, including chemical contaminants enter surface water and groundwater resources through municipal, industrial and agricultural wastewater. In this study, the ability of microalgae to grow and remove phosphate at different concentrations of K₂HPO₄ during the 8 days experimental course was tested at different time periods viz. 4, 6 and 8 days. The results showed that the highest percentage of phosphate removal was on the eighth day. The highest phosphate removal in the three different concentrations of PO₄ viz. 0.25, 0.35 and 0.45g/L on the 8 days for *Chlorella* was (29.12%), (40.88%) and (82.02%) and for *Spirulina* was (32.89%), (40.40%) and (88%) for days 1 to 4, 4 to 6, and 6 to 8, respectively. The lowest removal rates were observed in the treatment 0.25 g/L with 1-4 days viz. 24% and 20.06% for microalgae *Spirulina platensis* and *Chlorella vulgaris* respectively.

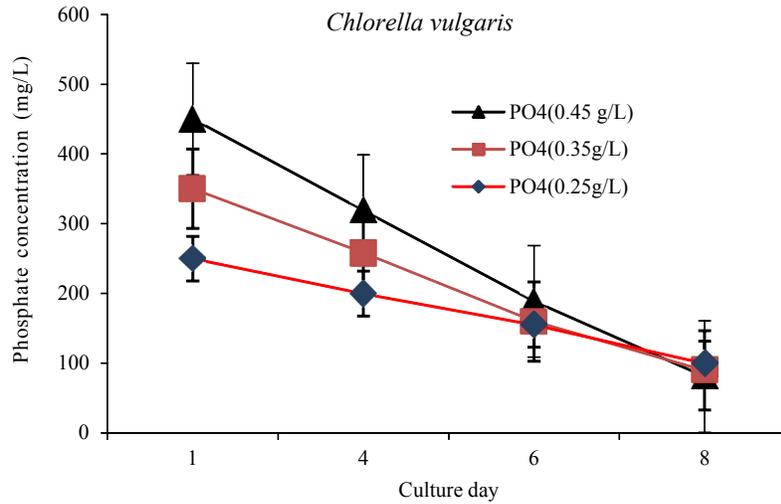


Fig. 3: Comparing the mean and standard deviation of remaining phosphate treatments

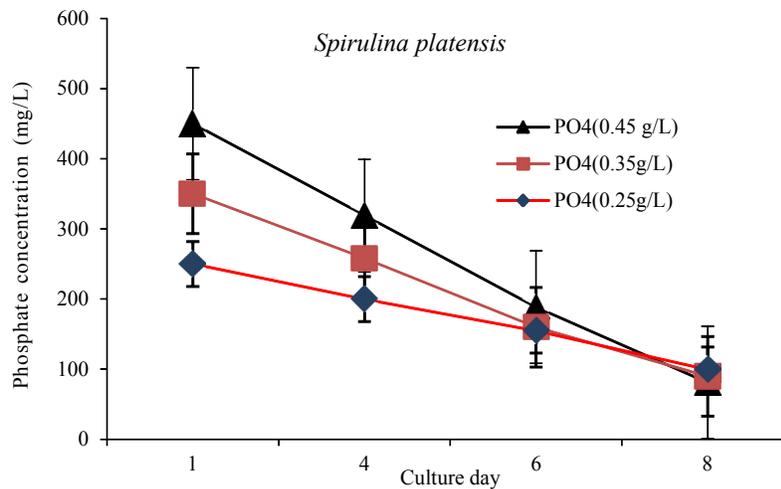


Fig. 4: Comparing the mean and standard deviation of remaining phosphate treatments

Figs. 3 and 4 represent the phosphate removal at various time intervals in *Chlorella vulgaris* and *Spirulina platensis*.

As the Figs. 3 and 4 indicate, with the passage of time, the phosphate concentration in the aqueous solution reduced and the lowest phosphate concentration was reported at (0.45 g/L) concentration of PO_4 i.e. (80.89 mg/L) in 8 days, thereby indicating 82.02% removal by microalgae *Chlorella vulgaris*. Dickinson et al. (2013) reported an optimal phosphate absorption rate for the microalgae *Scenedesmus* viz. PO_4 -P 0.104 mg/L/h.

However, in this study, the absorption rate of phosphate by the microalgae *Chlorella vulgaris* and *Spirulina platensis* within 8 days was 80.89 mg/L and 54 mg/L respectively. Moreover, the largest decline in eight days was reported in the (0.45 g/L) concentration of PO_4 . Wang and Lan in 2011 studied the biomass production besides nitrogen and phosphorus removal from municipal wastewater by green algae *Neochloris oleoabundans*. The results indicated that the algae had a potential to completely remove the high concentrations of nitrogen and phosphorus with an increase in biomass production, so it can be safely

considered as a viable option for the treatment of municipal wastewater. In this study, the micro-algae *Chlorella vulgaris* and *Spirulina platensis* could successfully remove nitrate and phosphate during the experiment. Likewise, Aravantinou *et al.* (2013) reported phosphate absorption rate of 0.475 mg/L for microalgae *Chlorococcum*. Statistical results based on the Tukey test showed that the amount of phosphate and nitrate removal during different time periods by microalgae *Chlorella vulgaris* exhibited a significant difference in $P < 0.01$, while microalgae *Spirulina platensis* showed significant differences at $P < 0.05$. Tukey test also showed that the removal efficiency of microalgae *Chlorella vulgaris* and *Spirulina platensis* are significantly different ($P < 0.01$) (Patel *et al.*, 2012). However, higher ability to remove phosphate and nitrate was shown by micro-algae *Spirulina platensis* in relation to *Chlorella vulgaris*.

CONCLUSION

Adsorptions of metal by bio-absorbents are considered as an effective process to remove metal ions from aqueous solution. In the present study, the ability of *Chlorella vulgaris* and *Spirulina platensis* absorbent to remove nitrate and phosphate ions influenced by different nitrate and phosphate concentrations was investigated. The results showed that micro-algae *Spirulina platensis* have a higher ability to remove nitrate and phosphate. Thus, it can be safely concluded that although some algae can remove phosphate and nitrate from the aqueous solution, but *Chlorella vulgaris* and *Spirulina platensis* have a high ability to reduce nitrate and phosphate from aqueous solutions, and thus can effectively remove phosphate and nitrate from the effluent at different climatic conditions, however, this finding demands more research.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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