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# Production of biomass and lipids using a *Chlorella* consortium growing in human urine: Modeling the N and P removals

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**Abstract**
In recent years, the use of dry baths is becoming popular. These artifacts consist of urinals where urine is recollected without dilution or mixture with human wastes. This urine current can be employed with different purposes such as hydroponics, agricultural irrigation and others. Some authors (Torres *et al.* 2014 among them) [4] Have demonstrated that urine in water dilutions are very feasible to grow autotrophicaly microalgae, due to its high contents of total nitrogen and P. In this work, a consortium composed by *Chlorella, Scenedesmus* and some diatoms was grown in urine dilutions (1/50 to 1/200) and the removal of N and P were evaluated, as well as the biomass and lipids production. Using these data, P and N removal kinetics were established. From these assessments it was shown that for the 1/200 dilution it was achieved an N removal efficiency of 90 %, and the lowest efficiency occurred with the 1/150 dilution (42%). Regarding P, It was noted that for the 1/100 and 1/150 dilutions the best P removal efficiencies were obtained (61%). At the same time, biomass concentrations up to 1,300 mg/L were achieved. Final lipid concentrations were high, with an average of 178.28 mg/L for the different dilutions.

**Keywords:** biomass, heterotrophy, lipids, microalgae consortia, P and N removal

## Introduction
Tertiary treatment of wastewaters for elimination of P and N, prevents the eutrophication process in ponds, channels and natural reservoirs. This process can be carried out using physicochemical and biological process. The use of microalgae growing autotrophicaly has been reported because of its capacity of removing P and N, at the same time that an amount of biomass of microalgae is obtained. This biomass can be used for lipids extraction followed of the extraction of other interesting compounds (proteins, pigments, sugars, etc). Even residual biomass can be employed for energy production, by m means of dark anaerobic fermentation to produce methane. In that way and under the concept of biorefinery it is possible to obtain various chemicals and energy.
In recent years, the use of dry baths is becoming popular. These artifacts consist of urinals (at this stage only for gentleman) where urine is recollected without dilution or mixture with human wastes. This urine current can be employed with different purposes such as hydroponics, agricultural irrigation and others. Some authors (Torres *et al.* 2014 among them) [4] have demonstrated that urine in water dilutions are very feasible to grow autotrophicaly microalgae, due to its high contents of total nitrogen (1,100 mg/L) and P (170 mg/L as orthophosphates). In the same work, Torres *et al.* (2014) [4] identified the appropriate dilutions for the culture of *Chlorella vulgaris, Scenedesmus sp.* and *Chlorococcum humicola*. These dilutions were 1/50, 1/150 and 1/75, respectively.
Various groups have reported the growth of microalgae in wastewaters (Martinez *et al.* 1999; Xin *et al.* 2010; Mostafa *et al,* 2012; Sacristan-de-Alva *et al.* 2013, Zhang and Hong, 2014, to mention some) [1, 5, 2, 3, 6], but there is scarce information regarding a consortium of microalgae growing in urine dilutions.
Another aspect that it has not been reported deeply is the modeling of microalgae growth and N and P removals when growing in urine. The model of Verlhust describes the growth of microalga as a function of time:

$$X = \frac{XoXm\, e^{\mu t}}{Xm - Xo + Xo\, e^{\mu t}} \qquad \text{Eq 1.}$$

Where $X_o$ and $X_m$ are the initial and maximum biomass concentrations, t is the elapsed time and □□ is the microalgae growth rate.
Regarding the N and P consumption can be modeled though an equation of the form:

$$S = \frac{\left(\frac{Xo}{Y}\right)(So-Sna)-Sna\left(So-\left(\frac{Xo}{Y}+So\right)\right)e^{pt}}{(So-Sna)-(So-\left(\frac{Xo}{Y}+So\right))e^{pt}} \quad \text{Eq. 2}$$

Where $X_o$ is the biomass Initial concentration, Y is the yield biomass/substrate, $S_o$ is the initial nutrient concentration $S_o$, $S_{na}$ is the non-employed or residual nutrient concentration, p is a constant equivalent to □ (growth rate), and t is the evolved time.

## Materials and Methods
### Consortium
The inoculum was kindly donated by Universidad Mexiquense Del Bicentenario (Unidad Tultitlan, Edo. de Mexico, and MEXICO) and is composed by *Chlorella*, *Scenedesmus* and some diatoms including *Nitzia*. The inoculum was maintained by successive culture in BG11 medium.

**Baffled Flasks Assessments**

Experiments were carried out in baffled 500 mL flaks by quintuplicate. Flaks were agitated in a shaker day and night with an illumination equivalent to 75□E/$m^2$.s, with light/darkness of 12/12 hours. Temperature varied during the day cycle. During the process, biomass concentrations were measured by dry weight and absorbance, Total nitrogen and $P - PO_4^{-3}$ were monitoring by Total nitrogen Kjeldahl and colorimetric reaction with molivdo-vanate method, respectively. After the experiments in urine dilutions, experiments with real treated wastewaters were carried out in baffled flasks. Wastewaters were obtained from the San Juan Ixhuatepec, Edo de Mexico Treatment Plant. In this plant, wastewaters are treated by means of primary (sedimentation) and secondary treatment (activated sludge system) followed by a secondary settler. Wastewaters were obtained in a punt before the disinfection process (chlorine gas application). These wastewaters were characterized in terms of pH, conductivity, COD, Total solids, phosphates and total organic nitrogen in accord to Standard Methods (1985).

**Results and discussion**

Table 1 show the characteristics of the original batch of urine employed along this work. That batch has been kept under refrigeration by some months, so some of the nutrients concentrations have changed. This table is shown just as a reference of the level of nutrients and metals. Every time that the urine is employed the actual concentrations of N and P are measured and reported.

**Table 1:** Characterization of the human urine batch

| Parameter | Value(mg/L) | Parameter | Value(mg/L) |
|---|---|---|---|
| Total N | 1,110.8 | N/P | 6.48 |
| Nitrates | 0.065 | Na | 2.31 |
| Nitirites | 0.147 | K | 1.64 |
| $N\text{-}_{NH4}$ | 492.25 | Ca | 26.9 |
| $N_{Kjeldal}$ | 618.32 | Mg | 1.70 |
| $P_{tot}$ | 172.52 | Zn | 0.0442 |

Adapted from Torres *et al.* (2014) [4]

The real N and P initial concentrations as well as the N/P ratios are shown in table 2. As observed, the four assessments were carried out with N concentrations in the range 37.3-112.6 mg/L. On the other hand, the N concentrations are very similar (i.e., from 25.1 to 28.7 mg/L), giving N/P ratios from 1.48 to 3.9.

**Table 2:** Biomass and lipids production in15 days

| Test | $N_i$(mg/L) | $P_i$(mg/L) | N/P | $X_{max}$(mg/L) | μ max($day^{-1}$) | $P_x$ (mg/L day) | L(mg/L) | $P_L$(mg/L day) |
|---|---|---|---|---|---|---|---|---|
| 1/50. | 112.6 | 28.7 | 3.92 | 874.04 | 0.154 | 58.27 | 171.33 | 11.42 |
| 1/100 | 52.08 | 26.2 | 1.98 | 1,313.15 | 0.138 | 87.54 | 176.66 | 11.78 |
| 1/150 | 43.20 | 25.6 | 1.72 | 1,314.05 | 0.141 | 87.60 | 186.83 | 11.89 |
| 1/200 | 37.30 | 25.1 | 1.48 | 1,179.58 | 0.112 | 78.640 | 178.33 | 12.06 |

Mostafa *et al.* (2012) [2] reported N and P concentrations around 3.78 and 0.224 mg/L, respectively for a real wastewater while Sacristan de Alva *et al.* (2013) [3] used a wastewater containing 49.4 and 9.5 mg/L of N and P, respectively plus 13.4 mg/L of nitrates. Xin *et al.* (2010) [5] reported 2.5 mg/L of total N for the wastewaters employed in their work and Zhang and Hong (2014) [6] reported N and P concentration sin the initial wastewater around 10.8 and 0.93 mg/L, respectively. Note that all N and P concentrations mentioned are far below the concentrations used in this work.

In the same Table 2, the maximum biomass concentrations achieved at the end of the processes are shown. The higher the dilution (lower N and P concentrations), the higher the biomass concentrations. Up to 1.31 g/L of biomass were produced (for the 1/150 dilution). Regarding the growth rates, values of μ between 0.112 and 0.154 $day^{-1}$ were obtained. The lipid production was quite high, very similar for all the assessments (average of 178.28 mg/L), giving very similar values of $P_L$. The maximum values of $P_X$ and $P_L$ were of 87.06 and 12.06 mg/L.day, respectively (for 1/150 and 1/200 dilutions).

Zhang *et al.* (2014) [6] reported values of r (the equivalent to μ in $days^{-1}$) between 0.19-0.47 for sterile experiments with wastewater and 0.07-0.39 for non-sterile experiments. Martinez *et al.* (1999) [1] reported $\mu_{max}$ values of 0.852 for microalgae growing in wastewater at 20ºC, which is quite high.

Regarding the N and P removals, Table 3 shows the resume of the assessments. It is remarkable that maximum N removal was of 80% for the 1/200 dilution (N/P = 1.48), while maximum P removal was of 61.8% for the 1/150 dilution (N/P= 1.98). An interesting way of analyzing the N and P removals is by means of the N and P removal rates. Table 3 shows that N removal rates up to 3.6 mg N/L.day can be achieved for the 1/50 dilution. On the other hand, a maximum P removal rate of 1.10 mg P/L.day was achieved for the 1/150 dilution.

**Table 3:** Results of the N and P removals in 15 days

| Test | N/P | N Removal (%) | N removal rate (mg/L.day) | P removal (%) | P removal rate (mg/L.day) |
|---|---|---|---|---|---|
| 1/50. | 3.92 | 48.07 | 3.6084 | 43.90 | 0.8399 |
| 1/100 | 1.98 | 51.19 | 1.7773 | 61.83 | 1.1013 |
| 1/150 | 1.72 | 42.44 | 1.2222 | 61.72 | 1.0533 |
| 1/200 | 1.48 | 90.00 | 2.2380 | 50.20 | 0.8400 |

Sacristan de Alva *et al.* (2013) [3] found N and P removals of 93.6 and 66.2%, respectively while Xin *et al.* found N and P removals of <99 and <99% for N/P ratios between 2 and 8.Finally, Zhang *et al.* (2014) [6] found N removals between

13.3 and 88.5% for sterile treatments and 17.2-89.7% for non-sterile treatments. Regarding P, all removals were of 100%.

**Table 4:** Biomass growth adjusted to equation 1 (Velrust).

| Dilution | μ (days$^{-1}$) | $R^2$ |
|---|---|---|
| 1/50. | 0.261 | 0.9634 |
| 1/100 | 0.356 | 0.9451 |
| 1/200 | 0.292 | 0.9641 |
| 1/150 | 0.288 | 0.9755 |
| Average | 0.2992 | - |

Regarding the modelation of microalgae consortium growth, raw data were adjusted to the Verhulst equation and the values of μ and correlation coefficients calculated were those shown at table 4. As shown, the Velrust equation is capable of simulating the biomass growth along the whole process for the different dilution assessments. Values of m and $R^2$ are reported in the same Table. Growth rate  varied between 0.261 and 0.356 days$^{-1}$ and $R^2$ was always higher than 0.945. The average value for m was of 0.2992 days$^{-1}$, which represents the growth rate for the 5 dilutions assessed. Note that growth rates calculated with this equation are approximately 50% higher than those calculated using the traditional equation  = (Ln $X_2$- Ln $X_1/t_2$-$t_1$)

Regarding the modelation of N and P removals, Table 5 shows the adjustment of raw data to the Eq. 2. As well as the p and $R^2$ values obtained for every dilution assessment.

**Table 5:** Nitrogen and phosphorus removals adjusted to equation 2.

| Nitrogen removal | | | Phosphorus removal | |
|---|---|---|---|---|
| Dilution | -p (days$^{-1}$) | $R^2$ | -p (days$^{-1}$) | $R^2$ |
| 1/50. | 0.2933 | 0.9714 | 0.2450 | 0.9252 |
| 1/100 | 0.1164 | 0.9861 | 0.2809 | 0.9617 |
| 1/200 | 0.2940 | 0.8303 | 0.2488 | 0.9500 |
| 1/150 | 0.2330 | 0.8535 | 0.2863 | 0.9752 |
| Average | 0.2341 | - | 0.2652 | - |

N and P removals were modeled using equation 2 and the p and $R^2$ values obtained are summarized at Table 5, for every dilution assessment. It is remarkable that for N most of the dilutions assessments were well represented by Eq. 2, with exception of the 1/200 dilution assessment, where $R^2$ was below 0.8500. Values of p (the equivalent to μ) had values between 0.1164 to 0.2330 days$^{-1}$, always with a negative value, since N and P disappeared as a function of time. The average value for all dilutions was 0.2341 (days$^{-1}$).

In the case of P, all adjustments to Eq. 2 had $R^2$ values higher than 0.920. The p values ranged between 0.245 and 0.2863 days$^{-1}$. The average p value was of 0.2652 (days$^{-1}$), which represents all the dilutions assessed.

Lipids were measured at the end of the assessments. Final concentrations of 171.3, 176.6, 186.8, and 178.3 mg/L were found for dilutions of 1/50, 1/100, 1/150 and 1/200, respectively. These concentrations are very high if compared with the maximum concentrations reported by Mostafa *et al.* (2012) [2] for *Anabaena flos-aque* (5.5), *Oscillatoria sp.* (8.0), *Anabaena oryzae* (7.4), *Phormedium fragile* (12.2), *Nostoc humifusum* (14.8), *Chlorela vulgaris* (12.5), and *Nostoc muscorum (*16.8), all in mg/L.

## Conclusions

From the results of this work, it can be concluded the following. The higher the dilution (lower N and P concentrations), the higher the biomass concentrations. Up to 1.31 g/L of biomass were produced (for the 1/150 dilution). Regarding the growth rates, values of  between 0.0 and 0.154 day$^{-1}$ were obtained. The maximum values of $P_X$ and $P_L$ were of 87.06 and 12.06 mg/L.day, respectively (for 1/150 and 1/200 dilutions). Regarding the N and P removals, Table 3 shows the resume of the assessments. It is remarkable that maximum N removal was of 80% for the 1/200 dilution (N/P = 1.48), while maximum P removal was of 61.8% for the 1/150 dilution (N/P= 1.98). Regarding the modelation of microalgae consortium growth, raw data were adjusted to the Verhulst equation. Regarding the modelation of microalgae consortium growth, raw data were adjusted to the Velrust equation. The removal of N and P was well modeled for all dilutions by using equation 2, giving high $R^2$ values (>0.8303 for N and >0.9252 for P). Lipids were measured at the beginning and the end of the assessments. Final concentrations were high with an average of 178.28 mg/L for the different dilutions.

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