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AN INTEGRATED DUCKWEED AND ALGAE POND SYSTEM FOR NITROGEN REMOVAL AND RENOVATION

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ABSTRACT

An integrated pond system, consisting of duckweed and algae ponds, was investigated for duckweed production and for further treatment of anaerobically treated domestic wastewater. The system consisted of 10 ponds in series, arranged in 3 stages of 2 duckweed ponds, 3 algae ponds and 5 duckweed ponds, respectively. Production of duckweed ranged from 7.4-16.4 g/(m^2.day) (or 27-60 ton/(ha.year)) dry weight in the first pond, to 2.7-8.2 g/(m^2.day) (or 10-30 ton/(ha.year))in the last pond. Regression analysis suggested that the production was positively influenced by the concentration of organic compounds in the pond water. The ammonium concentration in the range of 20-60 mg/l NH_4-N did not affect duckweed production. Fifty-six percent of the pond influent nitrogen, mainly ammonium, was removed. Ammonium removal was due to uptake by the duckweed plants (18%), nitrification (3%), sedimentation (8%) and combined volatilization of NH_3 and denitrification (73%). © 1998 Published by Elsevier Science Ltd. All rights reserved

KEYWORDS

Duckweed ponds; integrated systems; *Lemna giba*; nutrient removal; stabilization ponds; wastewater treatment.

INTRODUCTION

Duckweed ponds have been shown to be a successful wastewater treatment method (Oron *et al.*, 1984, 1987; Alaerts *et al.*, 1996), as well as a biomass production system (Skillicorn *et al.*, 1993; Oron, 1994). The duckweed crop has been used as fish feed (Hassan and Edwards, 1992) and as chicken feed (Haustein *et al.*, 1990). The growth characteristics of the duckweed species, the biomass composition, the feed properties etc., have been extensively described.

Duckweed ponds as wastewater treatment method

Duckweed ponds are a modified type of stabilization ponds, covered with a floating mat of plants. The treatment process in duckweed ponds is based on settling of suspended solids and on bacterial activity. Since the floating duckweed mat prevents mixing of the pond by wind action, excellent conditions for settling prevail. Moreover, the duckweed mat reduces solar radiation penetration, suppressing algae growth, yielding a very clear effluent. The bacterial activity is either aerobic or anaerobic, depending on the organic load and oxygen input. The duckweed plants were shown to release oxygen to the pond water at a rate of 3-4 g/(m^2.day) (Alaerts *et al.*, 1996). Duckweed ponds that were loaded with 48-60 kg BOD/(ha.day) remained completely aerobic and could remove 95-99% of the influent BOD (Alaerts *et al.*, 1996). The duckweed plants in principle could also contribute to the treatment process by direct assimilation of simple organic compounds, such as simple carbohydrates and various amino-acids (Hillman, 1976). Körner *et al.* (in press) reported however that uptake of organics by duckweed did not significantly affect the COD removal. Nutrients are removed from the wastewater by several processes, including volatilization of NH_3 and sedimentation of suspended solids with organic nitrogen. Part of the nutrients are removed by conversion into plant proteins and by harvesting the biomass. The nutrient uptake by the duckweed plants was reported to be 60-80% of the nitrogen and phosphorus load (4.9 kg N-Kjeldahl/(ha.day) and 0.9 kg P-total/(ha.day), respectively) (Alaerts *et al.*, 1996).

Rapid removal of fecal coliforms (indicator of pathogenic bacteria) as observed in algae ponds, does not occur in duckweed ponds. This is due to reduced sunlight penetration into the duckweed ponds, because of shading by the duckweed mat (Van der Steen *et al.*, in press). If the effluent from the duckweed ponds must comply with microbiological guidelines for reuse for agricultural irrigation, then algae ponds should usually be included in the treatment system. A possible configuration could be a set of algae ponds followed by duckweed ponds. In the algae ponds, wastewater is partially treated and the fecal coliform count is sharply reduced due to the combined mechanisms of light penetration and algae activity (Curtis *et al.*, 1992). The algae pond effluent is then passed through duckweed ponds, where due to lack of light the algae decay, then settle and disintegrate. The nutrients from the algae pond effluent can be converted into proteins by the duckweed.

Duckweed ponds as a biomass production system

The aim of a biomass production system is to generate a valuable by-product. This could increase the economic feasibility of treatment schemes, especially in developing countries. It is therefore essential to optimize the duckweed production per unit area, rather than nutrient removal. Ponds with long retention times are expected to have low nutrient concentrations, and therefore reduced duckweed production (Whitehead *et al.*, 1987; Alaerts *et al.*, 1996). These ponds with extended retention times are therefore expected to be less efficient in biomass production. The duckweed biomass can be easily harvested by skimming the plants from the surface. In large systems this could be mechanized, but in smaller ponds it could be done manually by a simple kind of fork. The water body should remain undisturbed as much as possible and after harvesting the pond surface should still cover the whole pond surface. The treatment and agricultural objectives could be combined in an integrated pond system, consisting of algae ponds (for pathogen removal) and duckweed ponds. The duckweed ponds can be situated in the treatment scheme both before and after the algae pond treatment; in the former case, to benefit from high nutrient concentrations, and in the latter, to remove the algae from the algae pond effluent and for additional nutrient conversion.

This paper reports the performance and nutrient conversion efficiency of an integrated system of duckweed and algae ponds. The potential for pathogen removal is reported elsewhere (Van der Steen *et al.*, in press). The nutrient removal processes are investigated and their contribution quantified. The effects of several environmental factors on duckweed production are assessed and a mathematical expression to predict the duckweed production under certain environmental conditions is presented. This will help the design of duckweed pond systems for maximum biomass production and optimal wastewater treatment.

METHODS

The experimental pond system

A pilot-scale study of a pond system consisting of three stages is in progress (Fig. 1). The first stage consists of 2 duckweed ponds (DP). This stage is included to benefit from the high nutrient concentration in the UASB effluent for duckweed production as well as for settling of solids. The duckweed species used was *Lemna giba*. The second stage consists of 3 algae ponds (AP), especially for the removal of bacterial pathogens. The third stage consists of 5 DP for the removal of algae and for further nutrient conversion and effluent polishing. The system was designed with a large number of ponds in series because this gives the system a plug-flow configuration, known to be more effective for pathogen removal (Marais, 1974).

The influent to the pond system is effluent of an Upflow Anaerobic Sludge Blanket (UASB) reactor, fed with domestic sewage of the Sde Boker Campus (Negev Desert, Israel). This reactor has been shown to be a reliable technology for the preliminary treatment of domestic sewage in warm climates (Lettinga *et al.*, 1993). The pond system comprises 10 mini-ponds in series. The volume of each mini-pond is 63 litres, the depth 0.29 m and the surface area 0.24 m^2. The mean flow rate is 6.2 l/hr and the overall retention time 4.2 days. The ponds are located in an open field exposed to local weather conditions, characterized by high summer temperatures (30-35°C in the afternoon), mild winter temperatures (about 20°C in the afternoon), occasionally strong winds and continuous strong sunlight radiation. Annual precipitation is around 90 mm. Mean daily global radiation ranges from 450 to 600 W/m^2. The results presented in this paper were obtained during the period March 1996 to January 1997.

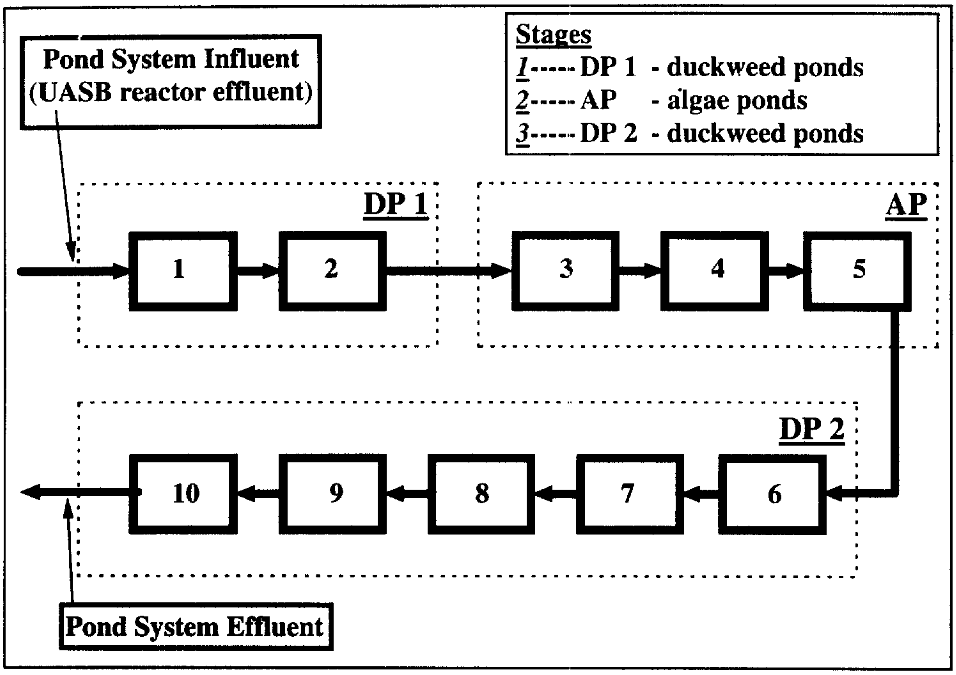

Figure 1. Schematic description of the integrated pond system consisting of duckweed and algae ponds for post-treatment of UASB-effluent.

Main characteristics of the UASB reactor effluent, used as the pond system influent, are given in Table 1. Once a week, a composite sample (24 hours) of the pond system influent and a grab sample of the pond system effluent were collected for chemical and microbiological analysis. Pond system effluent samples were taken 10 cm below the duckweed cover of the last pond, to prevent duckweed withdrawal. All samples were analyzed for Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), pH, NH_4-N, PO_4-P and Fecal Coliforms according to Standard Methods (APHA, 1995). The method for ammonium determination actually includes ammonia. At the range of pH in the ponds, ammonium is however the predominant form. The pH and oxygen concentration in each pond were monitored routinely several times during the day, from just after sunrise until sunset.

Once a week, 50% of the duckweed cover was harvested from each duckweed pond. It was expected that this harvesting frequency would result in a duckweed mat dense enough to prevent growth of algae. The duckweed harvested from ponds 1, 6 and 10 was used for the determination of duckweed production. Weekly grab samples of the same ponds were analyzed for NH_4 to study the relationship between ammonium concentration and duckweed production. When necessary, the duckweed was washed to remove attached suspended solids. It was first dried for two days on trays in a shaded area and afterwards dried overnight at 105°C in an oven. The density of the duckweed cover and the production could subsequently be calculated. All calculations were performed with the dry duckweed weight. The nitrogen content of the duckweed and of the sediment in the ponds was assessed using the Digesdahl digestion apparatus (Hach, 1992).

Table 1. Characteristics of pond system influent (UASB reactor effluent) and effluent, and removal efficiencies

Parameter	Concentration (mg/l, unless otherwise indicated)		Removal efficiency (%)	No. of samples
	Influent	Effluent		
COD-total	132 ± 87	49 ± 20	55 ± 26	30
COD-filtered	51 ± 30	29 ± 20	41 ± 38	30
COD-suspended solids	81 ±70	20 ± 20	70 ± 31	30
TSS	37 ±34	11 ± 4	57 ± 31	31
NH_4-N	48 ±20	24 ± 13	53 ± 30	29
NO_3-N	nd*	2 ± 1	-	7
N-organic	6 ± 9	nd*	100	7
PO_4-P	17 ±2	10 ± 3	39 ± 25	9
pH (-)	7.6 - 7.9	7.4 - 8.3	-	30
fecal coliforms (#/100 ml)	10^5 - 10^6	10^2 - 10^3	> 99	15

*nd - not detectable

RESULTS AND DISCUSSION

Removal of COD and TSS

Pond system effluent characteristics and removal efficiencies are given in Table 1. Removal of COD, TSS and fecal coliforms is discussed elsewhere (Van der Steen *et al.*, in press). The duckweed cover on ponds 6-10 caused the die-off of most of the algae present in the effluent of the AP stage, by reducing the penetration of sun-light into those ponds. It appears that the algae died, settled and disintegrated. Consequently, the mean pond system effluent TSS concentration was only 11 mg/l.

Nutrient assimilation by duckweed biomass

Duckweed growth is stimulated if the nitrogen is available as ammonium rather than nitrate (Skillicorn *et al.*, 1993). Pond system influent nitrogen was mainly (90%) ammonium, since organic nitrogen was hydrolyzed in the UASB reactor. The nitrate concentration in the ponds was only about 1.5 mg/l NO_3-N, therefore most of the nitrogen available to the duckweed was in the form of ammonium. Optimal growth rates could therefore be expected.

The highest duckweed production (8.2-16.4 g/(m^2.day)) was achieved in the first duckweed pond (Table 2). Similar results were reported previously by Oron and co-workers (1984;1987) for growth of *Lemma giba* on settled sewage. Edwards *et al.* (1992) achieved around 10 g/(m^2.day) production with *Lemma giba* grown in septage loaded ponds (6.4 mg/l nitrogen in pond water). Alaerts *et al.* (1996) observed duckweed production rates of 5.8-10.4 g/(m^2.day) in pond water with less than 8 mg/l NH_4-N.

Table 2. Mean monthly duckweed production, ammonium concentration, COD-filtered concentration and environmental parameters for duckweed ponds P1, P6 and P10 (2-4 samples per month if standard deviation is given)

Month	Ambient temp. (°C)	Solar radiation (W/m^2)	Production* (g/(m^2.day))			Ammonium concentration in pond water (mgN/l)			CODfiltered** (mg/l)	
			P1	P6	P10	P1	P6	P10	P1	P10
March '96	12.1	433	14.0±0.3	10.4±0.3	4.9±1.9	47±1	40±1	40	56	34
April	16.3	517	16.4±2.7	9.9±1.6	7.9±0.8	49±4	44±5	27±8	53±3	38±11
June	26.5	590	17.8±4.9	12.1±1.9	7.4±2.5	63±8	40±7	34±7	86±20	44±22
July	25.4	567	8.5±1.6	8.8±2.2	de***	54±3	39±6	33±5	63±30	37±19
August	25.4	550	7.9±2.7	6.8±2.5	8.8±3.8	55±0	19±8	12±8	32±10	16±24
September	23.9	524	8.2±2.2	4.4±1.6	7.4±1.9	48	33	28	40±15	23±11
October	19.6	455	9.0±2.2	3.6±3.8	6.0±1.6	16	9	2	24±15	20±18
November	16.3	349	7.4±1.6	4.1±0.5	5.2±1.1	16±2	6±2	2±1	22±25	12±11
December	12.0	335	7.7±9.9	nm***	4.1±0.3	22±14	nm	13±12	35±22	14±14
January '97	11.0	300	5.5±3.0	nm	nm	47	nm	nm	30±24	nm

* 1 g/(m^2.day) = 3.65 ton/(ha.year)
** COD-filtered concentration in the first pond was assumed to be equal to the influent concentration.
*** nm - not monitored, de - data excluded

The production of duckweed decreased along the pond system. Mean production in pond 10 was about 37% less than the production in the first pond. Multiple linear regression with the data of Table 2 was carried out in order to assess the factors that affect the production levels. A 95% confidence interval was used to determine if a factor affected the production significantly. Surprisingly, only the concentration of soluble COD affected the production in a significant way. The following equation explains 61% of the variation in the production ($R^2 = 0.61$):

$$y = 3.0 + 0.16 * COD\text{-}f \qquad (1)$$

where
y = duckweed production, g dry weight /(m^2.day).
COD-f = dissolved (filtered) Chemical Oxygen Demand, mg O_2/l.

The result of the regression analysis should be interpreted carefully and conclusions can only be preliminary, due to the large standard deviations of the data. However, it seems that the concentration of dissolved organic matter positively affects the production (Fig. 2). In previous experiments with raw and settled sewage it was suggested that the COD concentration either did not affect the production, or even reduced the production (Oron *et al.*, 1987). The different results of the present study could be due to the use of anaerobic effluent as pond influent. In the anaerobic reactor, complex organic molecules are fermented into small organic molecules and partly transformed into methane. Part of the COD-filtered that enters the pond system could therefore consist of simple organic molecules that, according to Hillman (1976), can be used directly in the metabolism of the duckweed. The regression analysis suggests that the availability of these compounds enhances production. Oron *et al.* (1987) could not observe this because no anaerobic pretreatment was applied. This hypothesis, although partly supported, should be further investigated in additional experiments.

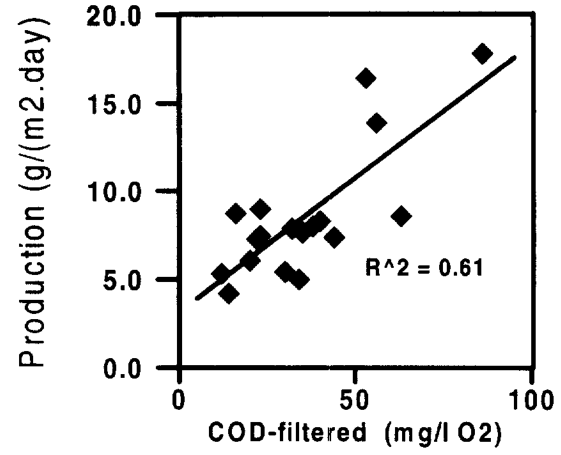

Figure 2. Duckweed production in ponds with wastewater as a function of COD-filtered.

A higher COD concentration might also result in a higher CO_2 concentration in the pond water. The effect of CO_2 concentration on duckweed growth also should be investigated. If, however, the provision of anaerobic degradation products could enhance duckweed production, then partial anaerobic pre-treatment might be recommended for duckweed pond systems. Such partial anaerobic degradation can be achieved in anaerobic reactors with very short retention times (2-3 hours) (Kaijun, 1994).

It seems that the ammonium concentration did not affect production. Other authors (Whitehead *et al.*, 1987; Alaerts *et al.*, 1996) reported that the ammonium concentration affects the production, for they found reduced production at the nutrient-poor end of duckweed pond systems. However, the ammonium concentrations in the ponds studied in this research are much higher. It therefore seems that the nitrogen supply in all ponds of the system was sufficient and not a limiting factor for growth (Fig. 3).

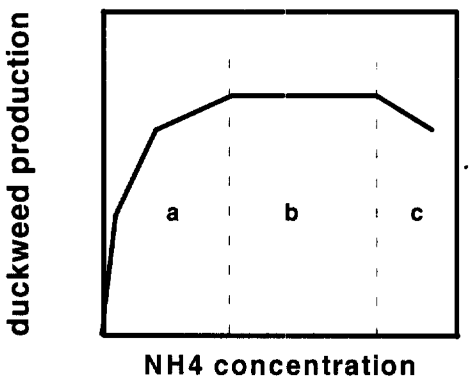

Figure 3. Possible relation between duckweed production and ammonium concentration. (a) ammonium supply is limiting factor; (b) ammonium supply is sufficient; (c) ammonium supply is inhibiting factor.

The very intense radiation of the Negev desert (300-600 W/m^2 average daily global radiation) did not reduce the duckweed production. Also, the mean ambient temperature in the range 11-27°C has no negative effect on the production. However, when the water temperature exceeded 30-34°C, the growth decreased and the duckweed became yellowish instead of fresh green. The pH of the growth medium was not monitored frequently enough to use it as a regression parameter. The range of the pH in the ponds 1, 6 and 10 was 7.0-8.0, 7.3-8.4 and 7.4-8.2, respectively. The optimal pH for duckweed growth is from 6.5 to 7.5 (Skillicorn *et* *al.*, 1993). The duckweed in the first pond after the algae-stage was not always fresh green, but sometimes rather yellowish. Algae material could be detected between the duckweed plants in this pond. However, they continued to grow, although probably at reduced rate due to algae competition and the raised pH.

The nitrogen content of the duckweed from ponds 1, 6 and 10 was virtually the same, 0.059 ± 0.006 g N per gram dry weight (n=9). In the literature it is reported that the nitrogen content of duckweed increases with increasing NH_4^+ concentration in the growth medium. Oron *et al.* (1984) reported 0.065-0.070 g N/g dry weight, with N-NH_4^+ concentrations higher than 25 mg/l. Edwards *et al.* (1992) found 0.05 g N/g dry weight, with 12 mg/l N-NH_4^+ in the pond medium. At higher ammonium concentrations, the nitrogen content of duckweed apparently does not surpass 0.06-0.07 g N/g dry weight. The maximum nitrogen content is probably reached in all ponds along the plug-flow, due to the relatively high NH_4^+ concentrations. A high nitrogen content is associated with a high crude-protein content and is therefore important for the economic value of the duckweed as fodder (Oron, 1994).

Nitrogen balance in the pond system

Nitrogen is removed from the pond water by uptake of ammonium by the duckweed, sedimentation of particles with organic nitrogen, volatilization of NH_3 and nitrification/denitrification. Results of the balance presented here are approximate figures that indicate the importance of each process rather than the exact contribution.

The nitrogen removal by duckweed growth can be calculated from the production and the nitrogen-content of the duckweed. The production in ponds 1, 6 and 10 was about 10.8, 7.4 and 6.6 g/(m^2.day) (Table 2). The production in ponds 2, 7, 8 and 9 was not determined, but estimated to be 10, 7, 7 and 7 g/(m^2.day), respectively. Therefore total N-uptake by duckweed averaged about 5.3 mg per litre of influent, equal to 18% of the total nitrogen removal.

Sedimentation was of minor importance because almost 90% of the nitrogen in the influent was in the NH_4^+ form. During the five months of operation the settling of solids in ponds 1,5 and 10 was 10.7, 5.5 and 5.5 g dry weight/(m^2. day), respectively. The nitrogen content was 0.038, 0.033 and 0.016 g N/g dry weight, respectively. It was assumed that the removal of nitrogen compounds by sedimentation in pond 2 was the same as in pond 1, in pond 3 and 4 the same as in 5 and in ponds 6 till 9 the same as in 10. The total contribution of sedimentation to the removal can be calculated as about 6%.

The nitrate concentration in the effluent was about 1.5 mg N /l, indicating that approximately 3% of the incoming nitrogen was nitrified without subsequently being denitrified. Most of the nitrogen removal (73%) in the pond system was therefore due to either volatilization of NH_3 or denitrification. Denitrification probably only played a minor role, due to consistent aerobic conditions in the ponds. Oron *et al.* (1987) found that more than 50% of the influent nitrogen could not be accounted for in the balance and was probably volatilized as NH_3. Reed *et al.* (1995) reports that the ammonia volatilization is considered the major pathway for nitrogen removal in stabilization ponds.

Volatilization from the algae ponds was probably increased, while the raised pH shifts the equilibrium between ammonium and NH_3 towards the latter. However, the importance of volatilization from the shallow duckweed ponds should not be neglected. In pond 1, about 66% of the removal was due to volatilization, while in ponds 6 to 10 it was around 59%. This shows that volatilization of NH_3 is probably the most important N-removal mechanism under the reported conditions.

CONCLUSIONS

The integrated pond system consisting of algae ponds and duckweed ponds is a promising system for further treatment of anaerobic effluent and biomass production. The final effluent had a high quality and satisfied the bacterial guidelines of the World Health Organization for unlimited irrigation (1000 fecal coliforms per 100ml) and is therefore suitable for irrigation from a health perspective. The integrated system combines duckweed production and wastewater treatment in one simple system. The concentration of COD and TSS in the final effluent is low, due to algae removal in the second duckweed stage.

The main conclusions derived from this study may be summarized as follows:

Production of duckweed varied from 4.1-16.4 g/(m^2.day). The highest production was achieved in the first pond, 7.4-16.4 g/(m^2.day). The regression analysis suggested that this might be due to a higher concentration of dissolved organic compounds (COD-filtered) in the pond water of the first pond.

Mean ambient temperature up to 27°C has no negative effect on duckweed production. When the pond water temperature exceeded 30-34°C, the duckweed plants became yellowish and the growth rate was reduced.

The integrated pond system removed 56% of influent nitrogen. Volatilization, duckweed growth, sedimentation and nitrification were responsible for approximately 73%, 18%, 6% and 3% of the ammonia removal, respectively. Volatilization was found to be the major mechanism for nitrogen removal, the pH in the ponds is therefore of great importance. A small part of the losses due to volatilization might have been in reality due to denitrification.

The system layout included a stage for algae removal, that consisted of a series of duckweed ponds. Passing the effluent from the algae ponds through this stage with reduced solar radiation penetration, with a retention time of 2 days, proved sufficient to remove practically all algae. Effluent quality with respect to TSS was excellent (11 mg/l).

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