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Removal of phosphate by the green seaweed *Ulva lactuca* in a small-scale sewage treatment plant (Ios Island, Aegean Sea, Greece)

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Abstract In the present study, the use of seaweeds for phosphate absorption was examined as a tertiary treatment in sewage treatment plants, to improve the water quality and reduce eutrophication risks. The data came from both laboratory and field experiments that took place on Ios Island sewage treatment plant. Three different macroalgae were tested and *Ulva lactuca* was finally chosen thanks to its high survivability in low salinity waters. Since the main restrictive factor was low salinity, we initially established the ratio of seawater:effluent that combined satisfactory viability with maximum phosphate absorption. The biomass growth under these conditions was also examined. Based on the above results, we designed a continuous-flow system with a 1/4 volume per hour water turnover, in a mixture of 60% sewage effluent: 40% sea water and 30 g L^{-1} initial biomass of *U. lactuca* that must be renewed every 10 days. Under these conditions and time frame, the phosphate content of the effluent was reduced by about 50%.

Keywords Biofilter · Chlorophytes · Phosphate absorption · Sewage effluent · *Ulva*

Introduction

Increasing human activities in coastal areas, especially agriculture, aquaculture, and sewage treatment, cause eutrophication in inshore waters by liberating nitrogen and phosphorus (Correll 1998). To counteract the undesirable effects of nutrients from secondarily treated sewage, tertiary treatment is sometimes applied. This usually involves the use of expensive or environmentally damaging chemicals (de-Bashan and Bashan 2004). An interesting alternative is the cultivation of algae in the effluent. Microalgae have long been used for treating sewage, particularly in developing tropical countries (Dunstan and Menzel 1971; Dunstan and Tenore 1972; reviews by Oswald 1988; de-Bashan et al. 2002, de-Bashan and Bashan 2004). The main problem with their use in such applications is that their small size makes it difficult to separate the algal mass from the treated effluent. In contrast, macroalgae show similar efficiency in nutrient uptake and they are much easier to harvest.

The suitability of seaweeds as biofilters in tertiary treatment of sewage depends on: (1) the ability of the species to utilize nutrients delivered by secondarily treated sewage; (2) the utilization rate of the major nutrients by the species; and (3) the salinity tolerance of the species. A number of studies have examined the feasibility of using different seaweed species as biofilters in tertiary sewage treatment using either sewage sludge or effluent (Prince 1974; Goldman et al. 1974a,b; Chan et al. 1979; Ryther et al. 1979, 1984; Wong and Lau 1979). More recently, the use of seaweeds as biofilters has been extended beyond the treatment of domestic sewage, and has focused on the removal of inorganic nutrients from the effluents of fish farms in integrated aquaculture (Krom et al. 1995; Chopin et al. 2001; Neori et al. 2004; Troell et al. 2003), or on the removal of heavy metals from industrial effluents (Davis et al. 2003).

Among macroalgae, *Ulva* is one of the most commonly used genera in commercial mass cultivation for the

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production of food, fertilizers, paper, etc. (for reviews see Critchley and Ohno 1998; Sahoo 2000; Sahoo and Yarish 2005; Andersen 2005). Because of its ability to absorb heavy metals and nutrients, and also to grow well in polluted waters, the relationship between *Ulva* growth and sewage pollution has been under scrutiny for a long time (see Steffensen 1976 and literature therein). More recently, *Ulva* has been used as a biofilter of fishponds effluents (Vandermeulen and Gordin 1990; Cohen and Neori 1991; Jimenez del Rio et al. 1994, 1996; Martinez-Aragón et al. 2002), as well as for the uptake of nitrogen from sewage in laboratory-scale experiments (Gil et al. 2005).

However, the only information on phosphate absorption by *Ulva lactuca* cultivated in domestic sewage effluent or in a mixture of effluent and seawater comes from the work of Wong and Lau (1979) and Chan et al. (1979). The former paper reported on the use of sludge extract and sewage effluent as a culture medium, and mainly focused on thallus growth and heavy metal absorption under different concentrations of sludge in the culture medium. The latter study dealt with thallus growth and nutrient absorption using effluent characterized by high salinity (16 ppt), compared to the usual effluents, since seawater was used for flushing in that treatment plant. The reported results are based on batch experiments with duration of 10 days. More recently, Lehnberg and Schramm (1984) studied productivity and nutrient accumulation by seaweeds adapted to brackish waters, and cultivated in sewage-enriched seawater. They examined the suitability of the green alga *Enteromorpha prolifera* (O.F. Müller) Ag. to remove total nitrogen and phosphorus in both batch cultures and continuous flow. As far as we know, there are only a few other continuous-flow experiments conducted mainly in waste waters from fish pond effluents (Vandermeulen and Gordin 1990; Neori et al. 1991; Cohen and Neori 1991; Martínez-Aragón et al. 2002: Hernandez et al. 2002). Also, Jimenez del Rio et al. (1994, 1996) used *Ulva rigida* Ag. to remove nitrogen from fishpond effluents, by applying different densities with direct and continuous inflow at different flow rates (2, 4, 8, and 12 volumes of effluent per day).

The aim of the present work was to study the use of the green alga *U. lactuca* as a biofilter for the removal of phosphate from sewage treatment plants, focusing primarily on phosphate absorption rather than on biomass growth. Therefore, it was critical to establish the optimum combination of algal mass density, effluent concentration and flow rate that resulted in a satisfactory removal of phosphate. Since the algae were cultivated in extremely low salinity (40% lower compared to that of seawater), growth was expected to be low.

The sewage treatment plant selected for carrying out this research is situated on the island of Ios in the Aegean Sea (Greece, 36°44 N, 25°15 E). The Ios sewage treatment plant started operating in 2001 and is able to process sewage for population equivalent of 22,500 people. During low season, the inflow to the plant on a daily basis is approximately 300 m^3 of sewage whereas in high season, 1,300 m^3. The population of the island is 1,500 people during winter (low season) becoming 15,000 or even more during summer. The treated effluent is released into the sea via a 550-m long pipe that extends from the seashore to the middle of a small gulf. The sewage treatment plant operates by the active sludge method using prolonged aeration. Two aeration tanks equipped with electric motors manage to reduce nitrogen levels to more than 80%. The sewage treatment facility is designed to have only one aeration tank and one settler in operation when the load is low during winter, but can upscale to full operation during summer when the load increases.

The advantages of using this plant is the relatively low daily production of effluent as well as the fact that it processes mainly domestic sewage, since there is no industrial activity on the island. Additional facts making this unit more interesting are the dramatic change in the population (and consequently in the sewage production) between winter and summer, since Ios is a very attractive tourist destination.

Materials and methods

Ulva lactuca L. was collected either from a coast south-east of Athens (Greece, Aegean Sea, Saronikos Gulf, 37°49 12 N 23°45 27 E) or from the shore near the port of Ios (Greece, Aegean Sea, Cyclades Islands, 36°43 31 N 25°16 16 E). For the laboratory experiments, the material was cultivated in a mixture of tap water and seawater collected from the Saronikos Gulf, whereas the field experiments were conducted in a mixture of seawater from the coast of Ios and the treated effluent from the sewage treatment plant. The brown algae *Halopteris scoparia* (Kütz.) Sauv. and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin were collected from the coast of Ios.

For small-scale laboratory experiments, 300-mL Erlenmeyer flasks or small aquaria (volume about 5 liters) were used. The aquaria were made of inert plastic material, safe for living organisms, white-colored to increase light reflection, and had a footprint of 32×36 cm. The mixture of seawater and tap water was supplied to the cultivation tanks from a 40 L header tank. Seawater collected from the experimental site had a concentration of phosphate ranging from 2 to 4 μmol L^{-1}. In order to measure phosphate uptake by the algae, sufficient K_2HPO_4 was added to the header tank to raise the concentration to approximately 130 μmol L^{-1}, similar to values commonly measured in the

effluent of the sewage treatment plant used for the field experiments. Both the header and the cultivation tanks were constantly aerated by an air pump. For laboratory incubations, the irradiance was 60 μmol photons $m^{-2}s^{-1}$ with a photoperiod of 12 h (Mediterranean mean conditions, Flagella et al. 2007), and the temperature was 22.5±2°C.

For field experiments, three plastic tanks with a capacity of 2,000 L were used for batch cultures. The continuous-flow field experiments were applied in two tanks with a raceway design, consisted of a metal frame and bed made from inert plastic material friendly to living organisms (PVC). The use of white tanks makes the data difficult to extrapolate to large ponds, where light only hits from the top of the ponds. The raceways had a footprint of 2 m×1 m and were 0.5 m deep giving a total volume of 1,000 L. However, the water volume used in the continuous-flow experiments was 130 L. Two header tanks with capacity 500 L each were placed above the raceways and were colored black in order to avoid unwanted algal growth. The liquid flow from these tanks into the raceways was achieved by gravity. One tank contained seawater and the other sewage effluent that was mixed in the raceway by agitation caused by air blowing from an air pump and diffused in the water by the use of ceramic diffusers (see Fig. 1).

The quality parameters of the released effluent, as provided by the sewage treatment plant laboratory, were: pH = 8, Conductivity = 3,140 μS/cm, B.O.D. = 4.4 $mgO_2\ L^{-1}$, C.O.D. = 16 $mgO_2\ L^{-1}$, Suspended solids = 2 $mg\ L^{-1}$, Total dissolved solids 180°C = 1,856 $mg\ L^{-1}$, Oil & grease= 0 mgL^{-1}, Surfactants anionic MBAS=0 mgL^{-1}.

Biomass density, medium concentration, and flow rate determination

To determine the optimum biomass density and flow rate, a series of experiments were carried out in the laboratory, using different mixtures of seawater and tap water, as well as different cultivation periods (4, 7, and 12 days). The flow rate was controlled by a Watson Marlow MHRE 22 peristaltic pump. The results of these tests were used in all field experiments.

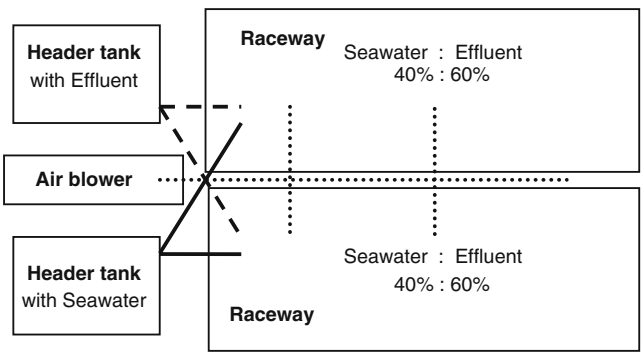

Fig. 1 Diagram showing the continuous-flow set up used for the field experiments

Chlorophyll measurement

Since it has been shown that plant health is related to chlorophyll levels (Force et al. 2003), the optimal seawater: tap water ratio for phosphate uptake was estimated by measuring the total chlorophyll content of the alga. All experiments were run in duplicate and were carried out in Petri dishes gently agitated by a Stuart Scientific Orbital Shaker SO3, at 40 rpm. The algal material used for this part of the experimental work was chosen from plants that had few or no epiphytes.

Photosynthetic pigments were extracted in 80 % acetone (McKinney, 1941; Arnon 1949; Bruinsma 1961). The extracted samples were filtered through Macherey-Nagel MN615 filter papers (diameter 125 mm) and their absorbance at 649 and 665 nm measured. The content of chlorophyll *a* and *b* was calculated using the following equations (Strain et al. 1971):

$$C_a\left(\mu g\,mL^{-1}\right) = 11.63(A_{665}) - 2.39(A_{649}),$$

$$C_b\left(\mu g\,mL^{-1}\right) = 20.11(A_{665}) - 5.18(A_{649}), \text{ and}$$

$$C_{a+b}\left(\mu g\,mL^{-1}\right) = 6.45(A_{665}) + 17.72(A_{649})$$

where C_a and C_b=concentrations of chlorophylls *a* and *b*, respectively, and *A*=absorbance.

Phosphate concentration-uptake

The concentration of phosphate in 10 mL samples of the seawater/effluent mixture from the culture tanks was determined by the standard molybdate method described by Murphy and Riley (1962, see also McKelvie et al. 1995).

The phosphate content of the plant material was measured using a method similar to that described by Corzo and Niell (1992). Samples of fresh algal blades were dried overnight (100°C) and ground to a homogeneous powder. Samples of this dry powder (0.1 g) were shaken in 50 mL of de-ionized water for 2 h before the mixture was filtered (Whatman GF/C) and the phosphate concentration was determined by the same method.

The results obtained from this method were also confirmed after wet digestion of the algal tissue using a mixture of sulfuric and nitric acids (APHA 1980).

In all the experiments, we use fresh weights by blotting the tissue in paper towel to remove excess water. The dry

weight to fresh weight ratio of the algae was estimated by measuring the fresh weight after blotting the thalli in paper tissue, and the dry weight after leaving the material overnight in a Gallenkamp Hotbox incubator at 100°C. Both fresh and dry weights were measured using a Chyo JL200, 0.1 mg balance. Whenever the term biomass is mentioned, it refers to fresh weight unless otherwise stated.

The net rate of phosphate uptake over a given time interval (i, i+1) was calculated using a modification of the formula proposed by Carmona et al. (1996) and Martinez-Aragón et al. (2002):

$$\mathrm{mmol}/\mathrm{PO_4^{3-}}\,\mathrm{g}^{-1}\,\mathrm{dry\,wt.\,d}^{-1} = \frac{C_{oi}V + QC_i\Delta t - \frac{Q(C_{oi}+C_{oi+1})}{2}\Delta t - C_{\mathrm{out}_{i+1}}V}{B\Delta t}$$

where C_i=mean inflow and C_o=outflow phosphate concentration (mmol L^{-1}) at times i and i+1; V=volume (L); Q=flow rate of the seawater/effluent mixture (L day^{-1}); B=algal biomass (g dry wt.) during the time interval (Δt, days) considered. The dry weight to fresh weight ratio was determined to be approximately 0.246 (N=10).

More specifically, each of the terms is explained below:

$C_{oi}V$	represents the total amount of PO_4^{3-} present in the tank at time=0
$QC_i\Delta t$	represents the total amount of PO_4^{3-} that entered the tank via the inflow during the time period (Δt)
$Q(C_{oi}+C_{oi+1})/2$	is the total amount of PO_4^{3-} that left the tank via the outflow during the time period (Δt)
$C_{\mathrm{outi+1}}$	is the total amount of PO_4^{3-} that remained in the tank at the end of the experiment.

Statistical analysis

For the statistical analysis of the results, we used post hoc LSD test, analysis of variance and Mann–Whitney non-parametric test using Minitab v15.

Results

Algal species selection—batch experiments

To identify the most suitable seaweed for nutrient absorption, three species selected from the local flora were examined in batch experiments. The species examined were the brown algae *Halopteris scoparia* and *C. compressa* and the green alga *U. lactuca*. The experiments lasted for up to 2 weeks without changing the medium, in order to test the plant tolerance to low salinity. Taking into account the limited survivability of seaweeds such as *Cystoseira* the salinity was lowered only by 25%. The first two species showed a relatively high nutrient absorption, but they could not survive in low salinity for long, and exhibited thallus deterioration after 14 days. In contrast, *U. lactuca* showed the best response (post hoc LSD error bars appear on graph) in terms of both nutrient absorption (Fig. 2) and growth in the experimental conditions used. It should be noted here that it is possible that other factors, e.g., nutrient limitation or pH changes could also cause problems in the survivability of the seaweeds. In all these factors, *U. lactuca* showed better tolerance.

Salinity, biomass density, and flow rate determination

Chlorophyll determination in *U. lactuca* plants growing in media with different salinities revealed that they could not survive when the percentage of freshwater or effluent exceeded 80% (salinity less than 8 ppt). At this salinity, plants exhibited high chlorophyll levels for 4 days, but there had been a large decline by 7 days. When effluent concentration was reduced to 60% (salinity 16 ppt), a high chlorophyll level (1,600 μg g^{-1}) was retained for about 7 days. Even after 12 days in such a mixture, chlorophyll levels had decreased by only 9% (from 1,600 to 1,400 μg g^{-1}) compared to material retrieved from the same dish after 4 days (Fig. 3, post hoc LSD error bars appear on graph). The final tap water (or effluent): seawater ratio selected for the continuous-flow experiments was 60%:40%.

As our aim was to find the optimum combination of mass density and flow rate in order to achieve maximal

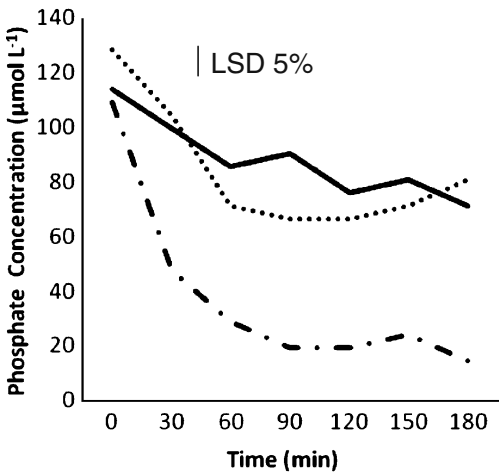

Fig. 2 Change in phosphate concentration during batch experiments using three different algal species cultivated in a mixture of 75% seawater and 25% tap water (salinity 30 ppt). Experiments were run in triplicate. *Solid line* represents *C. compressa*, *dotted line* represents *Halopteris scoparia* and *dash-dotted* line *U. lactuca*

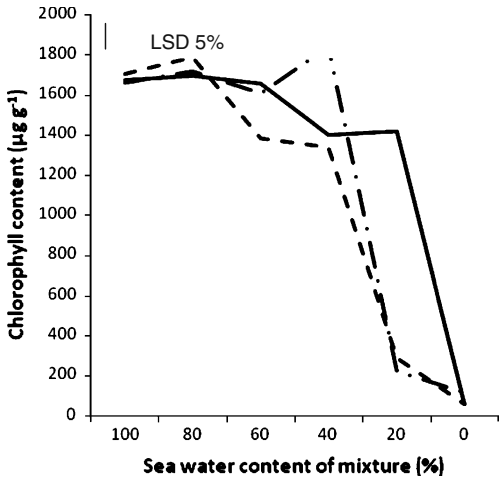

Fig. 3 Chlorophyll content (micrograms per gram dry weight) of *U. lactuca* grown in various salinities for different time periods. The experiment was run in duplicate in batch experiments. *Solid line* represents 4 days, *dash-dotted* line represents 7 days, and *dashed line* represents 12 days

uptake from the effluent after establishing the appropriate salinity, we used a biomass of 30±1 g fresh weight L^{-1}, which was the highest density that allowed the plants to be completely immersed in the medium. This biomass, expressed in units per surface area, gives 1,970 g fresh weight m^{-2}. It was expected that this value would cause limited growth due to self-limiting (low light penetration or restriction of nutrients). However, due to vigorous aeration–agitation and continuous flow of nutrients, growth was not collapsed, i.e., algal material was metabolically active.

The optimum flow rate was determined by measuring phosphate concentration in the outflow at different water flow rates. The percentage removal of phosphate increased as the flow rate decreased (Fig. 4, post hoc LSD error bars appear on graph). However, it was important to use the highest flow rate possible in order to treat as much effluent as possible. From these preliminary experiments, it was shown that the lowest flow rate giving a satisfactory reduction of the PO_4^{3-} in the outflow was 1/4 vol h^{-1}.

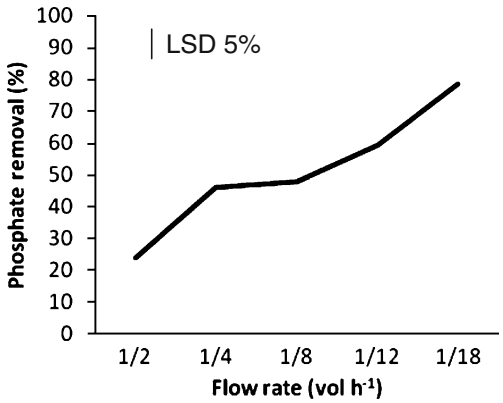

Fig. 4 Phosphate removal by *U. lactuca* from a 40% seawater:60% effluent mixture at different flow rates

Phosphate absorption

The salinity, biomass density, and flow rate established in the preliminary laboratory work were used for all subsequent field experiments. Continuous-flow field experiments lasted for three days (15–17 June 2007) and, during this period, phosphate concentrations were measured in the inflow and the outflow during daylight hours. On average, the phosphate concentration in the outflow was 35% lower than in the inflow ($p<0.05$; Fig. 5).

Based on our data and using the formula described in the materials and methods section, the phosphate uptake for daylight hours was: 2.13 µmol PO_4^{3-} g^{-1} dry wt. h^{-1}. This is equivalent to 0.1 g PO_4^{3-} $m^{-2}h^{-1}$. As it is shown in Fig. 5, it seems an initial burst of P uptake was followed by a much lower stable uptake rate. This is a typical pattern in seaweed nutrient uptake. Additional experiments revealed that phosphate uptake during the night was considerably lower ($p<0.05$; Fig. 6). Consequently, the expected daily absorbance would have been 0.042 mmol PO_4^{3-} g^{-1} dry wt. day^{-1} or 0.664 g P m^{-2} day^{-1} with a photoperiod of 16 h.

The estimated phosphate uptake by *U. lactuca* was confirmed by analysis of the phosphate content in the plant, after growing the plants for 1 h in laboratory conditions (60 µmol photons m^{-2} s^{-1} with a photoperiod of 12 h, and a temperature of 22.5±2°C). Two phosphate concentrations were used: one similar to that found in seawater (2.1 µmol L^{-1}) and one similar to that of the seawater:

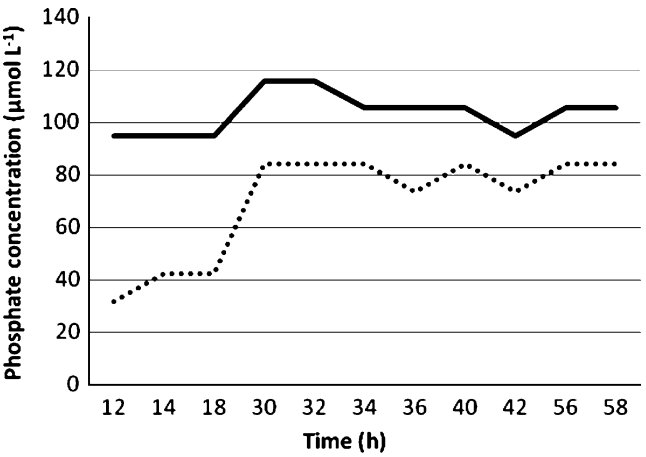

Fig. 5 Average phosphate concentration in the inflow and outflow of continuous-flow field experiments. The photoperiod in June was approximately 15 h. The temperature in the tank fluctuated from 19°C during the night to a maximum of about 25°C usually around 14.00 in the afternoon. *Solid line* represents the inflow and *dotted line* the outflow

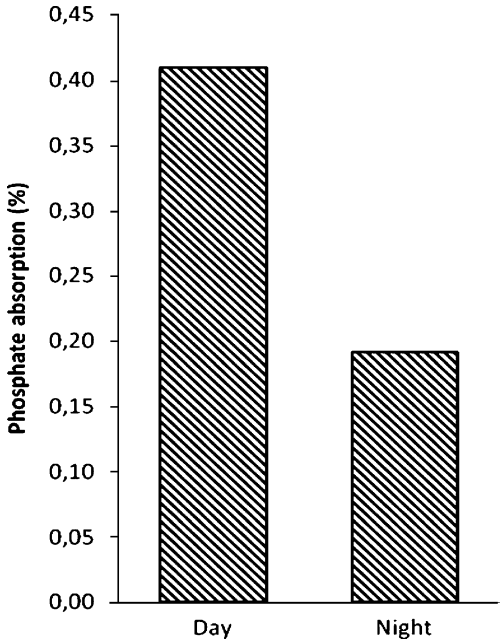

Fig. 6 Phosphate removal (%) during day and night by *U. lactuca* in continuous-flow laboratory experiments (flow rate, 1/4 vol h^{-1}; biomass ratio, 30 g fresh weight L^{-1}). Water was sampled at 30-min intervals over 5 h during daytime and during the night in a single 24-h cycle. Phosphate removal was calculated from inflow and outflow concentrations at each sampling time Wilcoxon non-parametric test showed a significant difference between day and night time measurements ($p<0.05$)

effluent (40%:60%) mixture (115.8 μmol L^{-1}). In the former, which was considered as the control, the phosphate content in the plant was 440 μmol g^{-1}, while in the latter it was 750 μmol g^{-1}. These experiments revealed that algae growing in medium rich in phosphate had a higher internal phosphate concentration than those growing in a lower phosphate environment ($p<0.05$; Fig. 7). The phosphate content ratio in high to low medium concentrations was 4.4. The ratio found by the alternative (APHA 424 C.I) method was 4.15, that is comparable to the above.

Algal growth

To examine algal growth under the conditions used for phosphate absorption in the laboratory, the field experiment was extended for five more days. The fresh weight of the material was measured at the beginning and at the end of this period of 8 days. In order to maintain the biomass density of 30 gL^{-1}, 3,940 g of material were added to 130 L of a mixture (40%:60%) of seawater and effluent. At the end of the experiment (after 8 days), the algal biomass was 4,090 g, exhibiting a small increase of 3.8%. This experiment was conducted in June, when the ambient temperature was between 20 and 25°C.

Discussion

The results presented here provide evidence for the ability of *Ulva* to be used as a biofilter in small-scale sewage treatment plants, and indicate for the first time the optimal combination of algal material density and flow rate for satisfactory nutrient uptake. Therefore, the present work provides further support to previous studies which have shown that *U. lactuca* is a suitable candidate for the development of a wastewater biofiltering treatment, because it is widely distributed, grows rapidly, absorbs and metabolizes nitrogen quickly, is resistant to environmental stresses, and is not readily colonized by epiphytes (Ryther et al. 1979; Vandermeulen and Gordin 1990; Cohen and Neori 1991; Jimenez del Rio et al. 1994, 1996; Martinez-Aragón et al. 2002; Gil et al. 2005).

A macroalga with a higher market value (for example species of *Gracilaria*) could provide a more valuable product but experiments have shown that many of these algae are susceptible to epiphytism (Friedlander et al. 2001) or breakage, so that their use as biofilters is not recommended (Neori et al., 2000, Schuenhoff et al. 2006).

Phosphate uptake

The present study is the first attempt to measure the uptake of PO_4^{3-} by *U. lactuca* from the effluent released by a sewage treatment plant in continuous-flow experiments. There have been previous attempts to cultivate *Ulva* in sludge extract, and it was shown that a concentration of 0.1–1% sludge gave good growth of *U. lactuca*, but the species could not survive in higher concentrations of sludge (Wong and Lau 1979). In addition, Chan et al. (1979) cultivated thallus disks of *U. lactuca* in laboratory

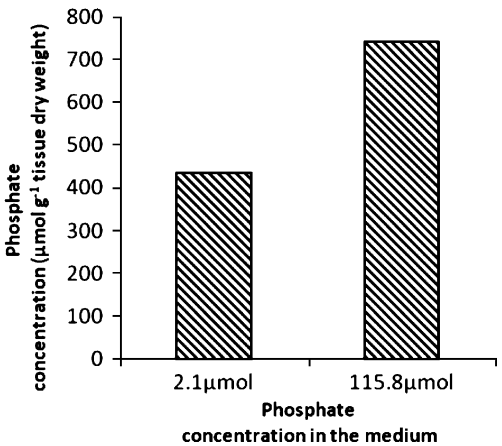

Fig. 7 Concentration of phosphate in tissue of *U. lactuca* grown for 1 h in two different media, with low (2.1 μmol L^{-1}) and high (115.8 μmol L^{-1}) phosphate concentrations. Mann–Whitney non-parametric one tailed test for independent samples showed that difference between samples was significant ($p<0.05$)

conditions using different dilutions of high-salinity effluent (16 ppt). They found a significant increase of the disk area (82%) in full-strength effluent but a smaller increase (50%) when 75% effluent was used. The initial uptake of PO_4^{3-} was high, but it decreased to non-detectable levels (less than 0.2 mg L^{-1}) after 10 days of cultivation. According to these authors, full-strength sewage effluent and 0.1% sludge extract contain appropriate concentrations of PO_4^{3-}, NH_3, and NO_3^- and could be employed for the cultivation of *U. lactuca.* The effect of nutrients on the development of *Ulva* was also studied by Waite and Mitchell (1972) and Steffensen (1976), who showed that addition of either NO_3-N or PO_4-P at a concentration of 7.89 μmol L^{-1} or 4.76 μmol L^{-1}, respectively, stimulated growth, while concentrations of nutrients above this optimum value, had no effect on, or decreased, growth. However, all these studies utilized batch experiments, and mainly focused on algal growth and not on nutrient uptake, so they cannot be directly compared with our results. There are also publications referred to nutrient uptake using continuous-flow experiments mainly in fish pond effluents (Vandermuelen and Gordin 1990; Neori et al. 1991; Cohen and Neori 1991; Jimenez del Rio et al. 1994, 1996; Martínez-Aragón et al. 2002; Hernandez et al. 2002;)

A more detailed examination of nutrient uptake by what is now regarded as another species of *Ulva*, *U.* (previously *Enteromorpha*) *prolifera* was conducted by Lehnberg and Schramm (1984). They cultivated *U. prolifera* in both outdoor batch cultures and in flow-through laboratory experiments and found that it was less effective in removing phosphates than nitrates. The maximum percentage of sewage effluent used in either batch or continuous-flow experiments by Lehnberg and Schramm (1984) was 49%, but they present results for mixtures of up to only 38% sewage effluent. The maximum P removal was 30% in the medium with 38% of sewage effluent.

In the present work, in order to exploit the natural capacity of *U. lactuca* to withstand low salinities, we increased effluent concentration to 60% without a marked effect on the chlorophyll content. The increase of the flow rates to approximately ten times those used by Lehnberg and Schramm (i.e., from 1/37 to 1/4 vol h^{-1}), together with the higher biomass density (30 gL^{-1} compared to 2.5 gL^{-1} used by Lehnberg and Schramm), resulted in a higher reduction of phosphate concentrations (35% in the 60% effluent, compared to 30% in 38% effluent found by Lehnberg and Schramm). This increase however had to sacrifice part of algal growth (see below).

Other researchers have conducted continuous-flow experiments to study the feasibility of using the red alga *Asparagopsis armata* as a biofilter for fish farm effluents (Schuenhoff et al. 2006). The stocking density they used was 5 g fresh weight L^{-1} and a water exchange rate of 2 vol h^{-1}. Comparing *A. armata* with *Ulva*, they suggested that the former was quite effective as a biofilter for fish farm effluents. In the present study, we used a much lower flow rate (1/4 vol h^{-1}), which is more effective for nutrient absorption. Moreover, *U. lactuca* can survive better in low salinity than *A. armata*, so that it will be more suitable for sewage treatment plants.

The biofiltering efficiency of three macroalgal species, including *Ulva rotundata* and *Ulva* (*Enteromorpha) intestinalis*, for removing phosphate from fish pond effluents were studied by Martinez-Aragón et al. (2002). The greatest rate of phosphate uptake was found to be 2.86 μmol PO_4^{3-} g^{-1} dry wt. h^{-1} for *U. rotundata,* which was similar to the uptake recorded in the present study (2.58 μmol PO_4^{3-} g^{-1} dry wt. h^{-1}). The main difference from the above study is that the fish farm effluent was seawater, so that *Ulva* was always growing in full salinity. Mata and Santos (2003) also cultivated *U. rotundata* in semi-intensive fishpond effluent, in order to determine the optimum density for maximum yield. In the present study, the main target was to achieve maximum P removal and not plant growth.

As it was expected, the PO_4^{3-} uptake during the dark period was reduced. As far as we know, there is limited information on PO_4^{3-} kinetics in *U. lactuca* during the dark:light cycle. Lavery and McComb (1991) studied kinetic parameters in a eutrophic estuary and found that *Ulva* and *Chaetomorpha* species showed higher values during the light period than in the dark. The observation of lower PO_4^{3-} uptake during the night by *U. lactuca* may be related to the photosynthetic activity of the plant as it is well known that phosphorus plays a significant role in energy transfer through ATP and other high-energy compounds in photosynthesis and respiration (Cembella et al. 1984, see also Lobban and Harrison 1997).

Algal growth

As mentioned above, the starting biomass used in the present study was the highest possible that allowed the plants to be submerged in the water during the experiment in order to acquire nutrients (Hurd and Dring 1990). Since this high biomass possibly has a negative effect on algal growth, due to self-limiting (low light penetration or restriction of nutrients), in order to facilitate light penetration and nutrient dispersion, vigorous agitation–aeration was applied inside the tank by air-blowers. Aeration was also used to increase the amount of C available to the algae. As a result, the algal biomass growth rate was limited but not stopped. An additional factor that has a negative effect on the growth rate is the low salinity of the medium. This is caused by the fact that we try to increase the amount of effluent passing through the system in a given time period.

For large-scale application of *Ulva* in bioremediation, therefore, mass cultures of *U. lactuca* in 100% seawater would need to be set up to provide the necessary biomass for the biofilter, because the low salinities in the treatment tanks do not support sustained algal growth. After the requirements of the treatment plant have been satisfied, excess biomass could be sold for other commercial applications.

Preliminary experiments with batch cultures of *U. lactuca* in a mixture of 25% effluent and 75% seawater showed a mass increase of about 165% within 1 month, between September 2 and September 28 (unpublished data). These results show that it would be possible to have a satisfactory algal growth and mass production by increasing the seawater concentration. This can be examined in combination with the daily production of effluent, which strongly depends on the season on the island of Ios, since the population changes dramatically between summer (around 15,000 inhabitants) and winter (1,500–2,000 inhabitants) due to tourist activities.

Conclusions—future perspectives

From the above, it can be concluded that *U. lactuca* is a valuable potential candidate for use as a biofilter for the removal of phosphate from the effluent that is currently discharged from sewage treatment plants. Taking into consideration the findings of the present study and the particular conditions on the island of Ios, a biofiltering installation using *U. lactuca* can be designed, which can be applied in this area as well as in similar small-scale sewage treatment plants. The seasonality of *U. lactuca* will not be a problem in the treatment of effluents in these sites, since the high sewage production coincides with the period of maximal vegetative growth of the alga.

A pilot plant should include a system of tanks through which the effluent—seawater mixture would flow continuously. The tank surface area necessary for treating the effluent produced during the high season on Ios (using the mass density and flow rate used in this study, and a water depth of 50 cm) is about 450 m^2, a size that could be easily accommodated within the sewage works on Ios. This is substantially less than that required if the flow rate used by Lehnberg and Schramm (1984) was used. In such a case, a tank surface at least 5,000 m^2 would be necessary for a similar treatment plant.

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