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RESEARCH ARTICLE

Organic and inorganic phosphorus uptake by bacteria in a plug-flow microcosm

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Abstract Phosphorus (P) is a vital nutrient for sustaining natural water productivity. Both particulate and dissolved forms of organic and inorganic P are potentially important sources of bioavailable P for primary and secondary producers. A microcosm system to imitate the bacterial community in Plym river sediment and pore water is described and bacterial uptake rates for inorganic and organic phosphorus are presented in this paper. The aim of this study was to investigate the uptake of two organic phosphorus compounds (phytic acid and D-glucose-6-phosphate) by freshwater bacteria. The bioreactors comprise glass columns packed with two types of small glass beads on which bacterial biofilm can develop. The glass beads with different porosity were introduced to simulate River SPM. The selected P compounds spiked into the inflow of the microcosm, and measured the step change of P concentration in the outflow to investigate the behavior of bacterial uptake of nutrients. The results showed that organic phosphorus was converted into inorganic phosphorus but the conversion rate depended on the type of phosphorus species. One experiment suggested that phytic acid (refractory) could displace phosphate from the biofilm surface; the other experiment showed that D-glucose-6-phosphate (labile) could be hydrolysed and utilized easily by the bacteria. The results also suggested that bacteria might break down the C-P bonds to utilize the carbon. Further experiments should investigate the effect of varying the C:N:P ratio in the microcosm system to determine which nutrient limits bacteria uptake.

Keywords organic phosphorus, bacteria, uptake

1 Introduction

Organic phosphorus is involved in many biologic processes, yet its role is the most poorly understood aspect of the global phosphorus cycle [1]. Organic phosphorus species represent a significant proportion of the total phosphorus pool in the aquatic environment and they are more abundant than inorganic phosphate in many water and sediment systems [2]. Dissolved organic phosphorus (DOP) is at least partially biologically available to bacterioplankton [3] and can support bacterial growth and metabolic activities, especially when the dissolved inorganic phosphorus (DIP) concentration becomes critically low [4,5]. Species such as heterotrophic bacteria can be inorganic phosphorus limited in natural environments. Studies have shown that bacteria can take up DOP via enzymatic hydrolysis and this is dependent on bacterial species competition, substrate concentrations, saturation, storage capacity and the availability of other nutrients such as organic carbon [3,6,7]. Based on the strength of the P bonds to carbon and/or oxygen, DOP can be defined as labile or refractory [8] and bacteria may take up P from labile DOP species when inorganic P is limited. Heath [9,10] reported that heterotrophic bacteria can also take up P following the photolytic breakdown of refractory DOP species. However the bacterial uptake of P from different DOP species is poorly understood [7].

Bacterial uptake of organic phosphorus in aquatic ecosystems was first studied in the 1970s, when attention was focused on eutrophication and the factors that influenced phytoplankton and bacterial growth. However, the potential role of organic phosphorus compounds in the environment has been largely ignored, partly because of the lack of widely available techniques for their measurement [11]. In contrast, bacterial uptake of inorganic P in sediment has been well studied as sediment is a major source for P. Addition of DIP to sediment may promote bacterial growth [12,13] or have no impact if carbon is the limiting nutrient [14].

Microcosms are suitable tools with which to measure bacterial utilization of nutrients in natural waters over a time scale of days. Lucena et al. [15] and Frias et al. [16]

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developed such an approach in order to measure dissolved organic carbon (DOC) degradation. Here, an indigenous bacterial community was allowed to colonize an inert and porous material placed in a glass column. Kaplan and Newbold [17] studied the utilization of DOC by bacterial biofilms grown on borosilicate glass beads packed in a glass column, and stream water was used as the inoculums. An increasing concentration gradient of DOC was then applied and DOC concentrations in the microcosm inflow and outflow were determined. Their results showed that the biodegraded DOC (BDOC) as a percentage of influent DOC did not change significantly as the DOC concentration increased, implying that the microcosms had an excess of metabolic potential; in contrast, other experiments found that the uptake of DOC was proportional to the influent concentration of DOC. Søndergaard and Worm [18] used the same approach and concluded that microcosms were not site-specific and required no acclimation to measure BDOC from three different waters. These studies showed that it is appropriate to use a plug-flow microcosm approach to investigate the bacterial uptake of nutrients but that the experimental design is crucial for addressing a particular hypothesis.

Badr et al. [19] were the first to apply the microcosm approach to investigate the ammonification and nitrification of dissolved organic nitrogen (DON) and its relationship to total dissolved nitrogen (TDN). Their microcosm experiments showed that 12% of the DON flux from the River Plym may be available to bacteria, indicating significant potential for DON removal during the residence time of the water in the estuary (several days). The bioavailable nature of some of the DON pool means that this N fraction significantly adds to the eutrophication burden of the receiving coastal waters and therefore cannot be ignored in environmental assessments.

The aim of the microcosm experiments undertaken in the present work was to examine the potential uptake of selected DOP and DIP by indigenous bacteria over periods similar to the freshwater residence time of the Plym Estuary. The key questions are: 1) Do estuarine bacteria hydrolyse DOP and if so, do they selectively hydrolyse particular DOP species, e.g. those containing C-P, P-O-P or C-O-P bonds? and 2) Do estuarine bacteria convert DIP to DOP in the water column?

Orthophosphate was selected as the inorganic phosphorus species and glucose-6-phosphate (labile species) and phytic acid (refractory species) were selected as the two organic phosphorus species. The specific objectives were to: calculate the microcosm water residence time; study the physico-chemical partitioning of DOP and DIP under abiotic conditions; investigate the bacterial uptake of added orthophosphate, glucose-6-phosphate and phytic acid; investigate whether bacteria convert inorganic P to organic P or vice versa; compare the extent of P uptake and partitioning in the biotic and abiotic microcosms.

2 Experiment

2.1 Model P compounds

The phosphorus compounds chosen for this study were:

- Potassium dihydrogen phosphate (KH_2PO_4). This is the simplest in a series of inorganic phosphates and forms orthophosphate ion (PO_4^{3-}) in solution at the pH of the water used for this study. This compound is referred to as DIP in the text.
- Glucose-6-phosphate ($C_6H_{13}O_9P$). This plays an important role in the carbohydrate metabolism of bacteria, plants and animals [20]. It is also one of the most easy hydrolysis organic P compounds. This compound is referred to as G-6-P in the text.
- Phytic acid salt ($C_6H_{18}O_{24}P_6$). Phytic acid is generated from dead plants, and it is one of the most chemically and biologically resistant organic P compounds. It is not normally bioavailable to animals and most microorganisms but faecal [21] and lactic acid (in animal blood) producing bacteria such as Bacteroides, Eubacterium, and Clostridium groups [22] produce an enzyme, phytase, which converts phytic acid to inorganic phosphate [23]. Monbet et al. [24] reported phytic acid in the Tamar Estuary in winter contributed up to 20% of total dissolved phosphorus (TDP). It has been shown that phytic acid is not extensionally hydrolysed in natural water under oxic conditions [24]. However, Suzumura and Kamatani [25] reported that phytic acid in marine sediments in Tokyo Bay was almost completely degraded by bacteria after 60 days under anoxic conditions. In contrast, only 50% was degraded after 60 days under oxic conditions. Phytic acid also acts as a strong ligand because of its high anionic charge [26]. Its propensity for complexing polyvalent cations is well known in soils and sediments, and on colloidal and particulate matter in aquatic systems [1,27] de Groot and Golterman [1] found that the addition of phytic acid to a suspension of orthophosphate adsorbed onto FeOOH and orthophosphate.

2.2 Plug-flow microcosm

2.2.1 Microcosm set up

The design and operation of the microcosms followed the approaches of Kaplan and Newbold [17], Badr [28] and Badr et al. [19]. The first microcosm system, termed 'Siran' was set up on the 7th November 2006. It was composed of four parts: a 100 L glass tank containing the river feed water, an 80 L glass tank for the collection of out flow water, a glass column microcosm containing Siran™ beads (Siran, QVF Engineering, Mainz, Germany) and a peristaltic pump (PD12P,Watson-Marlow Flexicon, Cornwall, UK). The microcosm was housed in a wooden box in the dark and the microcosm consisted of three 125 mL

glass columns filled with Siran beads and quartz wool bed supports (Fig. 1). A schematic diagram of the microcosm setup is shown in Fig. 2. Each column contained 45 g of beads. The Siran beads are borosilicate glass, with diameters of 2–3 mm and a porosity of 50%–65% (Fig. 3 (a)).

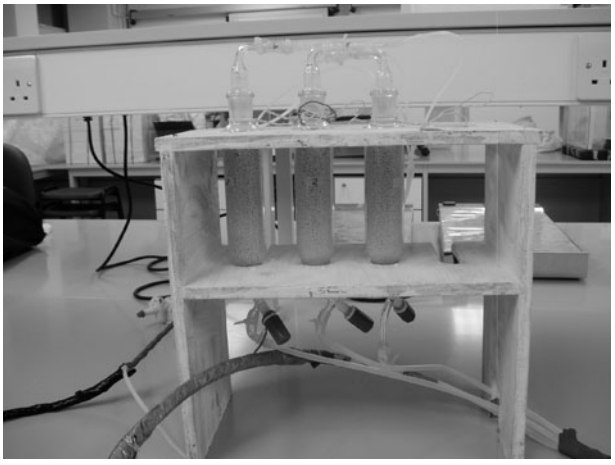

Fig. 1 Microcosm setup (microcosm array of three separate columns filled with Siran beads)

A second microcosm named 'Duran' was set up in December 2007. The design was the same as the 'Siran' microcosm except that the borosilicate beads were of the Duran type (type: TF7, QVF Engineering, Mainz, Germany); these have a diameter of 1 mm and zero porosity (Fig. 3(d)). Fifty seven grams of Duran beads were required to fill each glass column. The Siran and Duran beads were cleaned prior to use by soaking them in methanol for 24 h and then in 2% HCl (v/v) for 24 h, in order to remove attached organic matter and inorganic contamination. Then they were soaked in ultra high purity (UHP) water for 24 h, dried and then placed in a muffle furnace at 450°C for 1.5 h in order to burn off recalcitrant organic matter.

The incubations were carried out in the dark and at a constant temperature of 15°C, which is the average temperature of the year Reservoirs and microcosm columns in a wooden box were wrapped in black plastic film to inhibit photosynthetic activity. The feed water was kept oxygenated through the use of a 24 h operation air pump and the dissolved oxygen concentration remained in the range 10.2–11.8 $mg \cdot L^{-1}$, i.e., fully aerated, as measured by a dissolved oxygen meter (YSI 5700, YSI limited, Hampshire, UK). As a control, a third microcosm was set up as the abiotic microcosm in 2008. This microcosm had two glass columns, one filled with Siran beads and one with Duran beads. The abiotic microcosm was constantly supplied with UHP water.

Colonisation of the beads by a microbial community was achieved in three months by running Plym estuarine water through each column with a total flow rate of 1 $mL \cdot min^{-1}$. The prior to use water was filtered though a GF/F glass fiber filter (0.7 μm, Whatman, UK). This type of filter was selected to allow rapid filtration of the large volumes of water required. A 0.7 μm pore size removes all of the large organic and inorganic suspended particulate matter and plankton [29]. However, bacteria remain in the filtrate and bacteria are an integral part of the experiment. After 3 months the concentration of DIP in the outflow water was uniform, which indicated that the bacteria in the microcosm were in the stationary growth phase. The appearance of the beads before and after exposure to estuarine water is shown in Fig. 3. Transmission electron microscopy (TEM) (JEM1011, JEOL Ltd, Tokyo , Japan) was used to monitor bacterial growth on the beads [30]. The clean Siran and Duran beads can be seen in Figs. 3(b) and 3(e), whereas 1 month after incubation, bacteria and biofilms can be seen on the surface of Siran and Duran beads (Figs. 3(c) and 3(d) respectively).

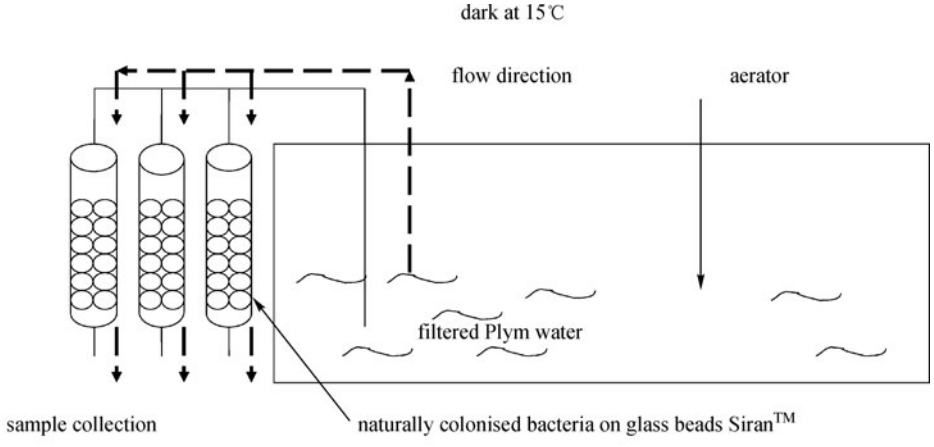

Fig. 2 Schematic diagram of the plug flow microcosm

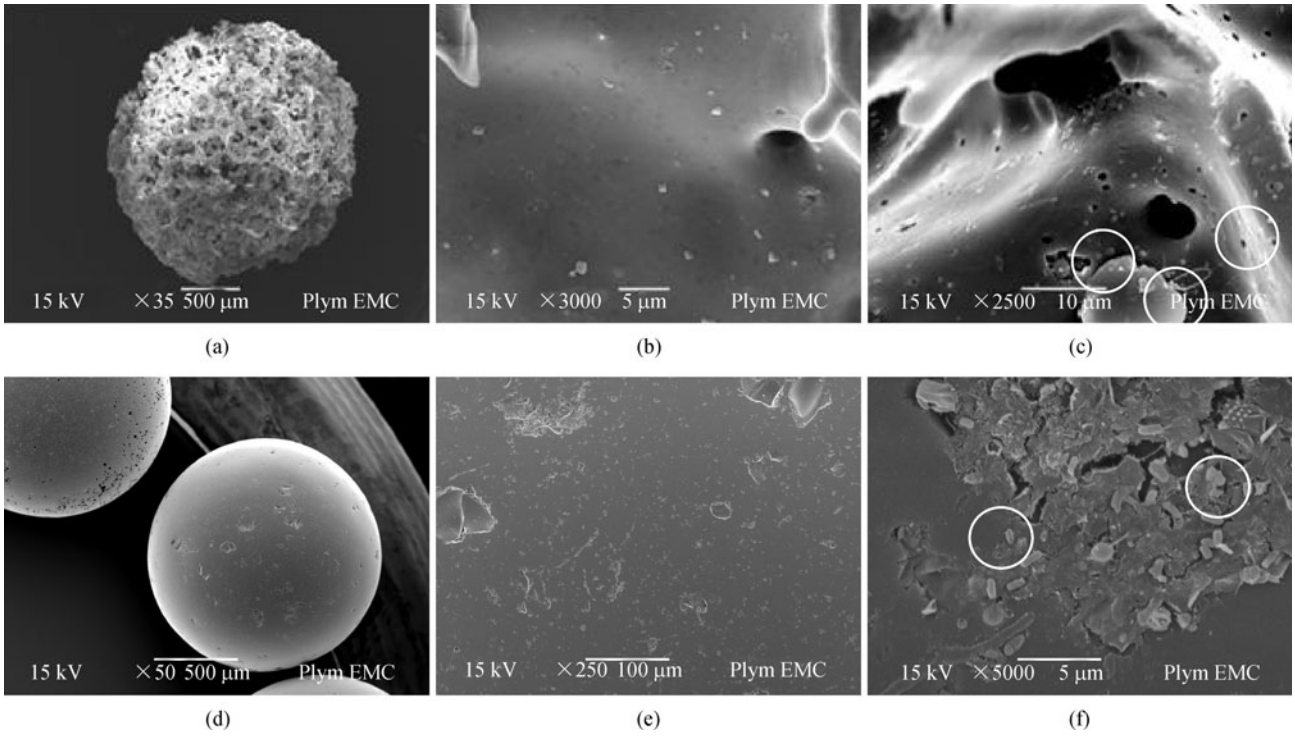

Fig. 3 Electron micrographs of the beads: (a) a single Siran bead (×35); (b) a clean Siran bead (×3000); (c) Siran bead following one month of continuous exposure to Plym estuarine water colonised by bacteria (×2500); (d) Duran beads (×50); (e) a clean Duran bead (×250); (f) Duran bead after one month of continuous expose to Plym river estuarine water colonised by bacteria (×5000). The circles highlight bacteria on the surface of beads

Water for the experiments was subsequently collected from the 4th November, 2006 to 11th April, 2009 from the freshwater tidal reach of the Plym Estuary (Devon, UK; Grid reference SX 519634). Concentrations of DIP and DOP during the initial 3 month colonisation period were monitored for the Siran microcosm. In the first few weeks of incubation, the outflow DIP and DOP concentrations varied in an irregular manner as the natural bacteria colonised the beads. There was no evidence of loss of DIP or DOP at this stage. The TEM images showed that the beads had a diversity of bacterial species as identified by morphology. After the first few weeks of incubation, concentrations of TDP (DIP and DOP) in the outflow water decreased from 20–15 $\mu g \cdot L^{-1}$ to below the detection limit of the segmented flow analyzer. The TEM images showed that a biofilm had formed on the beads and the bacteria population had qualitatively increased. In the last few weeks of the initial 3 month period the DIP and DOP concentrations in the outflow waters could be detected but were still low. The bacterial populations in the microcosms were estimated following the end of the step change experiments using the standard colony forming unit (CFU) method [31].The numbers of bacteria in the biofilms in the Siran and Duran microcosms were similar, at 1.4 × 105 (Duran) and 1.8 × 106 (Siran) $cfu \cdot cm^{-2}$ per g of beads, respectively.

Stock solutions ($10\,mg \cdot L^{-1}$) of the three phosphorus compounds were made up and stored in the dark at 4°C, and sub-samples taken as required. The orthophosphate and G-6-P solutions were stored for up to 2 weeks, and the phytic acid solution was stored for up to 6 months.

During the experiments, samples were taken at regular intervals from the inlet and outlet flows using low-density polyethylene (LDPE) bottles. 40 mL aliquots were collected each time and immediately filtered through 0.2 μm pore diameter cellulose acetate membranes. Separate 20 mL aliquots were taken for DIP and TDP determinations. The former aliquot was refrigerated and analyzed within 48 h, while the latter was frozen and analyzed within one month.

Water samples for the determination of TDP and DIP were analyzed using a segmented flow analyzer. DOP was estimated by the difference between TDP and DIP. This was only done once because of the impracticality of opening the columns on a frequent basis during the experiments. For this method 0.1 g of Duran and Siran beads were taken and carefully washed with 0.9 mL of balanced bacteriological saline solution water for 15 min. This water was then diluted 100 fold and 0.1 mL was then smeared onto an nutrient agar plate. The agar gel was prepared by dissolving 10 g glucose; 5 g $Ca_3(PO_4)_2$; 0.3 g NaCl; 0.5 g $(NH_4)_2SO_4$; 0.3 g $MgSO_4$; 0.3 g KCl; 0.03 g $MnSO_4$; and 0.03 g $FeSO_4$ in 1000 mL of distilled water. Plates were done in triplicate, and bacterial colonies were

counted after culturing at 15°C for seven days. The Siran microcosm operated for 15 months, and the Duran microcosm operated for seven months.

2.2.2 Water residence time

The water residence time is defined as the time taken for the sample solution to travel from the inlet to the outlet of the microcosm, while the refill time is defined as the time taken to completely replace the volume of estuarine water in the microcosm. If the flow rate is high and the residence time is low, the nutrients could pass though the columns too quickly for bacterial uptake or enzyme induction. Therefore the residence time is an important operational parameter for the experimental design. The determination of the water residence time was undertaken using the abiotic microcosm.

1 L of 1 mol·L^{-1} KCl was loaded onto the microcosm until the outflow KCl conductivity reached a steady-state, and then the water residence time of the microcosm was calculated from outflow plots of KCl conductivity versus time. The steady-state concentration was defined as the P or KCl concentration that showed $< 10\%$ variation over time.

The step change experiment can be divided into a step up phase and a step down phase. An outflow P concentration model was therefore designed and divided into five phases (Fig. 4). For the step up experiment, the inlet tube was transferred to a smaller 10 L reservoir containing 1 mol·L^{-1} KCl. When the microcosm outlet reached Phase III (or steady-state), the inlet tube was then switched back to the normal UHP water reservoir (step down experiment); the outflow KCl conductivity was investigated until it reached Phase V. The conductivity of the KCl in the inflow was measured once with the outflow KCl conductivity being measured at regular time intervals. After each experiment, the microcosm system was reconditioned with UHP water for two weeks. The same step up/step down approach was used for the P compounds and each P step change (spike) experiment was repeated twice in the same microcosm, expect abiotic control experiments and residence & refill time experiment was only done once.

While the physical characteristics of the beads were different (Table 1) the theoretical volume occupied by each bead type in the columns was similar. This was confirmed by measurements of the water volumes in the Siran and Duran columns, which were also similar (Table 1). As a consequence, the refill times for both column types were similar for the same inflow rates.

Figure 5 shows the transport of a 20 h step up addition of 1 mol·L^{-1} KCl solution through the two column types. Siran and Duran columns had residence times of 73 min and 68 min, which is in agreement with the residence time of 62 min reported by Badr [28] using the same microcosm

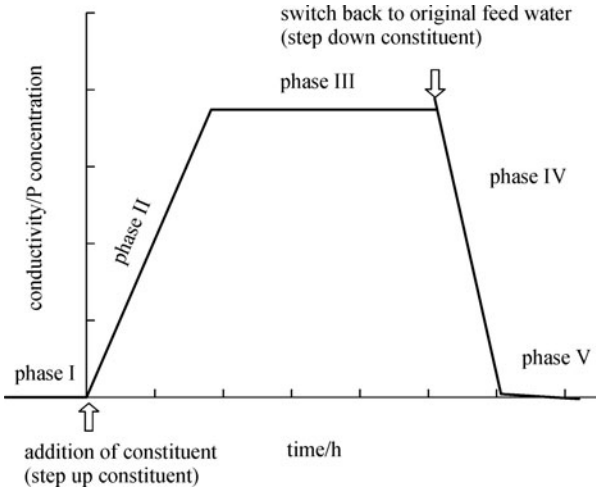

Fig. 4 Schematic diagram showing expected change in the conductivity or concentration of a conservative (non-reactive) dissolved constituent, with time, in the outflow water of the microcosm following a single addition of the constituent to the inflow water. Phase I represents the initial concentration in the y – axis property (steady baseline) before the addition of the dissolved constituent (KCl or P compounds); Phase II represents the increase after addition of the constituent; Phase III shows the y-axis property reaching a plateau; Phase IV shows the decrease y-axis property after switching back to the original water; Phase V is the steady-state concentration after switching back to the original water

Table 1 Physical and hydrodynamic characteristics of Siran and Duran bead filled columns

characteristics	Siran beads	Duran beads
glass column/mL	125	125
bead total weight/g	46	57
bead density/(g·cm^{-3})	0.80	1.07
bead diameter/mm	2–3	1
bead porosity	50%–65%	0%
bead volume/mL	57.1	53.4
water volume/mL	67.9	71.6
inlet flow rate/(mL·min^{-1})	0.33	0.33
outlet flow rate/(mL·min^{-1})	0.17	0.16
minimum refill time for each microcosm/h	6.6	7.7

set up. A more accurate assessment of residence time could have been obtained with a higher sampling frequency but was not necessary in the context of this work. Both the Siran and Duran columns reached a steady-state after 10 h, which is therefore the refill time. The refill time was confirmed by the step down results. At 20 h, the inflow was switched back to UHP water and both columns took about 10 h to replace all of the KCl with UHP. Although both the

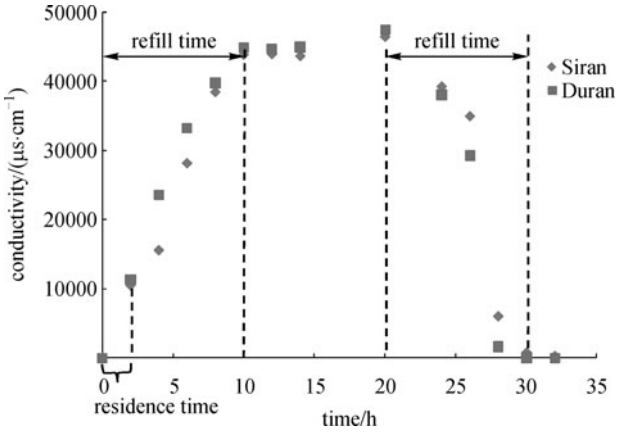

Fig. 5 Determination of the residence and refill time for a solution of KCl in the microcosms.

Siran and Duran columns reached a steady-state after approximately 10 h, there was a lag in response for the Siran column. This can be explained by the higher tortuosity (i.e., longer path length) in the Siran columns due to their porous nature, and hence longer flushing times.

2.2.3 Microcosm experiments

For the abiotic (control) experiments the step change experiments used 80 μg $P \cdot L^{-1}$ addition of orthophosphate, G-6-P and phytic acid respectively to the inlet UHP water. The reason UHP water was chosen as the abiotic matrix was because of the difficulty in sterilising a large volume of estuarine water. The concentration of 80 μg $P \cdot L^{-1}$ was chosen because it was at the upper end of the range of total dissolved phosphorus (TDP) concentrations typically found in Plym river water (see Table 2) and because it provided analytically robust measurements.

Table 2 Concentrations of DIP and DOP in the Plym Estuary water collected between November 2005 and April 2008

	mean	S.D[a)]	min	max
DIP/(μg $P \cdot L^{-1}$)	34	26	8	60
DOP/(μg $P \cdot L^{-1}$)	9	11	< 3	20

Note: a) represents±1 standard deviation of the results from 22 samples analyzed in triplicate

3 Results

3.1 Chemical characteristics of the Plym estuarine water used in the microcosm experiments

The average concentration of DIP and DOP in Plym estuarine water varied between November 2005 and April 2008, as shown in Table 2. The maximum TDP (DIP + DOP) concentration was 80 μg $P \cdot L^{-1}$; which was chosen as the step up concentration for each of the three P species (orthophosphate, G-6-P and phytic acid) in the experiments discussed below.

During periods of low river flow both DIP and DOP in the estuary were high, with a maximum of 60 μg $P \cdot L^{-1}$ DIP and 20 μg $P \cdot L^{-1}$ of DOP. In contrast during periods of high river flows, the DIP and DOP concentrations were lower, between 10–20 μg $P \cdot L^{-1}$ and less than 10 μg $P \cdot L^{-1}$, respectively. These patterns probably reflect the dilution of sewage effluent at high river flows. Each of the collected estuarine water samples lasted about 3 months, during which period a slight decrease in concentration of DIP occurred. However, there were no significant ($p < 0.05$) change of DOP concentration was observed during the experiment.

3.2 Phosphorus uptake in abiotic microcosm

The aim of abiotic microcosm experiments was to study any physico-chemical uptake of the model P compounds onto the Siran and Duran beads in the absence of the bacterial biofilms.

The orthophosphate step change experiments (Figs. 6(a) and 6(b)) gave similar concentration profiles in both the Siran and Duran columns. The DIP concentrations in both the Siran and Duran outflow water reached a steady-state at 22 h. Table 3 gives the changes in DIP concentrations in the outflow water during the experiment. Thus, before the step up the DIP concentration in the outflow was less than the detection limit. During phase III, the outflow DIP concentration increased to 86 (Siran) and 84 (Duran) μg $P \cdot L^{-1}$ (Table 3), which is equivalent to the step up concentration taking into account measurement error. Following the step down, the DIP concentration decreased to 1 μg $P \cdot L^{-1}$ during phase V, indicating that there was insignificant partitioning of DIP on to the beads and other internal surfaces. For the G-6-P step change experiments (Figs. 6(c) and (d)), DOP concentrations had increased in the outflow by 4 h in both the Siran and Duran columns. Concentrations of DOP increased with time and reached a steady-state at about 57 (Siran) and 53 (Duran) μg $P \cdot L^{-1}$ after 22 h of the step up experiment. DOP concentrations decreased back to the original concentrations in the step down experiment in both systems at 55 h. DIP concentrations also increased and reached steady-states at 24 (Siran) and 31 (Duran) μg $P \cdot L^{-1}$. Phytic acid reached a steady-state concentration in the outflow water after 22 h in both columns. The steady-state concentrations were 69 and 80 $\mu g \cdot L^{-1}$.

3.3 Orthophosphate spiked addition experiments

Dissolved inorganic orthophosphate is generally thought to be the main phosphorus species taken up by bacteria. The step change inorganic phosphorus experiments in the biotic microcosms were designed to investigate the uptake/

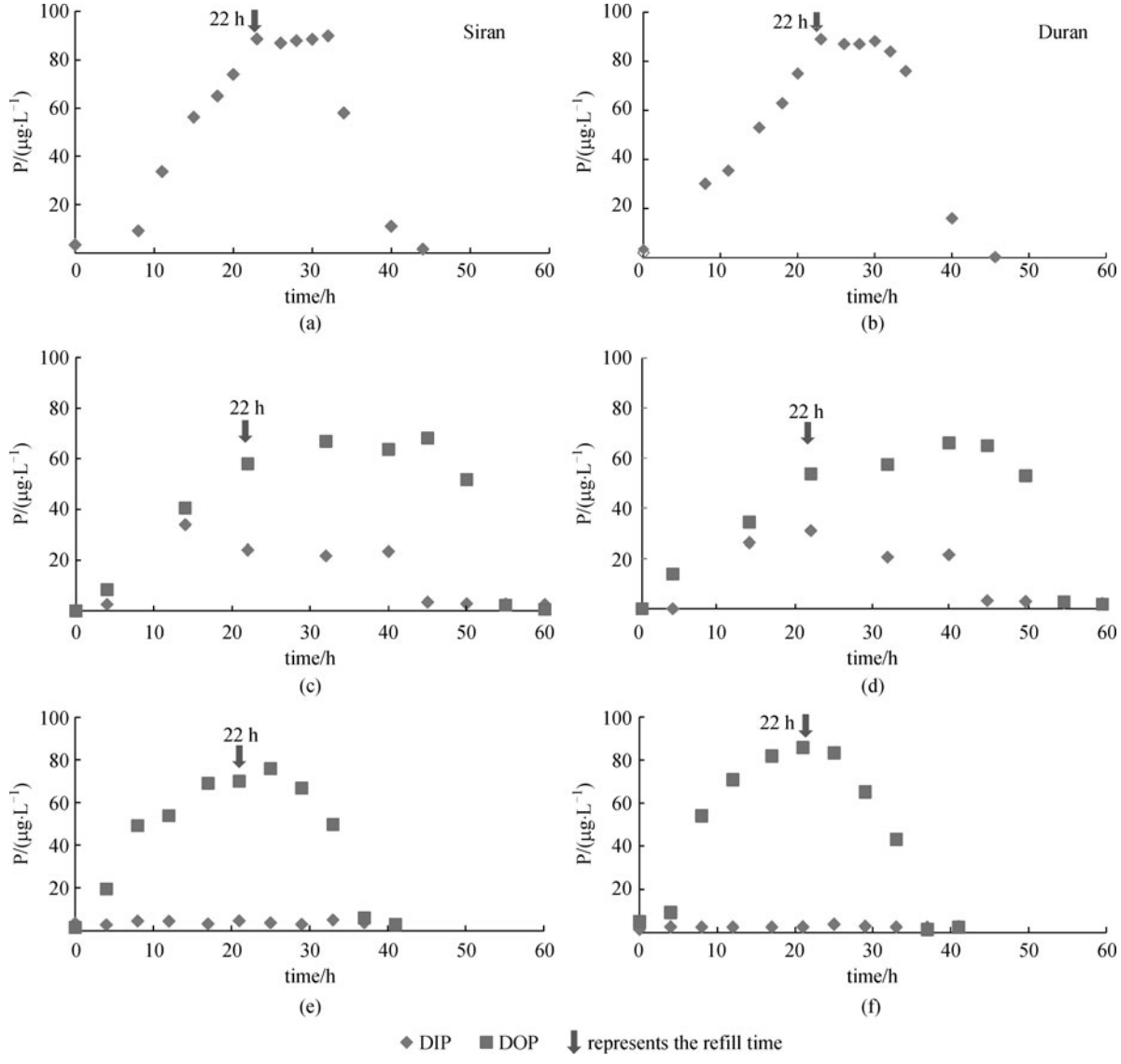

Fig. 6 DIP and DOP concentrations in the outflow water from the microcosm Siran and Duran during the abiotic experiment. (a) and (b) DIP concentration from spiking with 80 μg $P \cdot L^{-1}$ as orthophosphate; (c) and (d) DIP and DOP concentration from spiking with 80 μg $P \cdot L^{-1}$ as G-6-P; (e) and (f) DIP and DOP concentration spiking with 80 μg $P \cdot L^{-1}$ as phytic acid. (a), (c) and (e) are microcosm Siran; (b), (d) and (f) are microcosm Duran. Time 0 represents the DIP and DOP background concentrations and the time at which the inflow was spiked.

utilization of DIP in the two microcosms over a period of 80 h. The starting conditions for the Siran microcosm were 17±1 μg $P \cdot L^{-1}$ of DIP and 5±1 μg $P \cdot L^{-1}$ of DOP at the inflow and 3 μg $P \cdot L^{-1}$ and 2 μg $P \cdot L^{-1}$ DIP and DOP at the outflow. The Duran microcosm had similar inflow and outflow concentrations (see Table 4).

Figure 7 shows that the DIP profiles for the Siran and Duran microcosms were similar. The DIP concentrations in the outflow of the Siran and Duran microcosms increased from 3 μg $P \cdot L^{-1}$ and 5 μg $P \cdot L^{-1}$ to steady-state concentrations at phase III of 68 μg $P \cdot L^{-1}$ and 79 μg $P \cdot L^{-1}$ at 38 h and 30 h, i.e. net gains of 65 μg $P \cdot L^{-1}$ and 74 μg $P \cdot L^{-1}$, respectively, during the step up experiments (see Table 4). The DOP profiles for the two microcosms were also similar with net gains during phase III of 13 (Siran) and 8 (Duran) μg $P \cdot L^{-1}$, respectively. The DIP and DOP concentrations in the outflow decreased back to the original concentrations during the step down experiments.

3.4 Fed with glucose-6-phosphate experiments

Monbet et al. [24] suggested that in the summer the labile DOP pool in the Tamar Estuary can constitute up to 26% of dissolved total phosphorus (TDP) and therefore be a potentially significant source of bioavailable P. The G-6-P step change experiments were designed to evaluate whether this was the case for natural bacteria in the adjacent Plym Estuary.

Table 3 Mean DIP and DOP concentrations (μg $P \cdot L^{-1}$) in inflow and outflow waters following additions of 80 μg $P \cdot L^{-1}$ orthophosphate, G-6-P and phytic acid during separate abiotic experiments (ND = not determined; LDL = less than detection limit). For phase I, III and V refer to Fig. 4

		inflow before step up	outflow before step up (phase I)	outflow during phase III	gain /loss (phase III – phase I)	outflow during phase V	gain/loss (phase V – phase I)
orthophosphate (Siran)	DIP	LDL	3	86	+ 83	1	– 2
	DOP	ND	ND	ND	ND	ND	ND
orthophosphate (Duran)	DIP	LDL	3	84	+ 81	1	– 2
	DOP	ND	ND	ND	ND	ND	ND
G-6-P (Siran)	DIP	LDL	LDL	24	+ 24	2	+ 2
	DOP	LDL	LDL	57	+ 57	2	+ 2
G-6-P (Duran)	DIP	LDL	LDL	31	+ 31	2	+ 2
	DOP	LDL	LDL	53	+ 53	2	+ 2
phytic acid (Siran)	DIP	LDL	3	4	+ 1	2	– 2
	DOP	LDL	1	70	+ 69	2	+ 1
phytic acid (Duran)	DIP	LDL	1	2	+ 1	2	+ 1
	DOP	LDL	5	85	+ 80	2	– 3

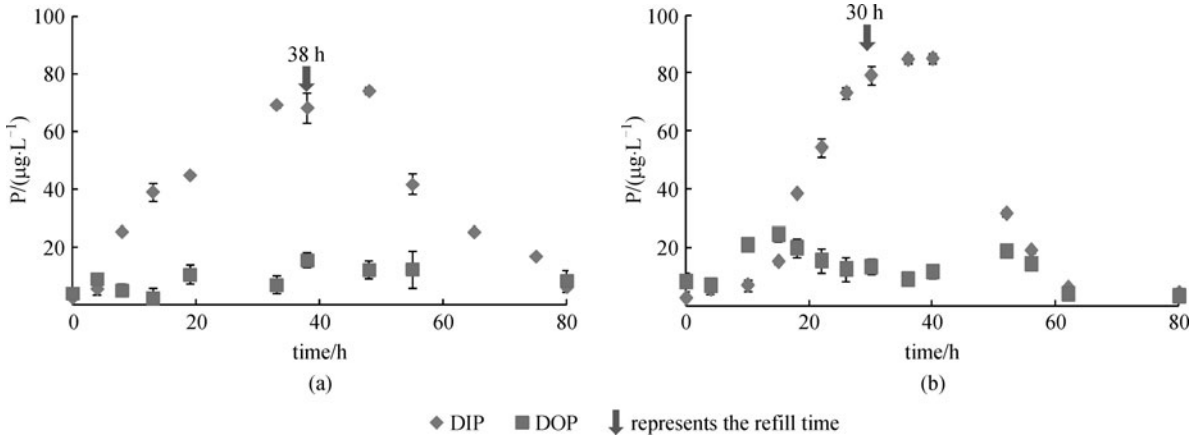

Fig. 7 DIP and DOP concentrations in the outflow water of the microcosms during the biotic experiment incorporating the addition of 80 μg $P \cdot L^{-1}$ orthophosphate. (a) microcosm Siran; (b) microcosm Duran. Time 0 shows the beginning of the step up phase. Error bars represent ±1 s.d. of the results from 3 columns with each sample analyzed in triplicate (n = 9)

Table 4 DIP and DOP concentrations (μg $P \cdot L^{-1}$, n = 9) in the inflow and outflow waters of the microcosms during biotic experiments incorporating the addition of 80 μg $P \cdot L^{-1}$ orthophosphate

microcosm		inflow at phase I	outflow at phase I	outflow at phase III	gain /loss (phase III – I)	outflow at phase V	gain/loss (phase V – I)
Siran	DIP	17±1	3±0	68±5	+ 65	6±1	+ 3
	DOP	5±1	2±1	15±2	+ 13	8±3	+ 5
Duran	DIP	22±2	5±1	79±3	+ 74	4±1	– 1
	DOP	9±2	5±4	13±1	+ 8	3±1	+ 3

Figure 8 shows that the DIP profiles in the two microcosms were similar. The Siran microcosm gained 50 μg $P \cdot L^{-1}$ of DIP, equivalent to 62% of the total available G-6-P and the Duran microcosm gained 62 μg $P \cdot L^{-1}$, equivalent to 77% of total available G-6-P at phase III (Fig. 8 and Table 5).

3.5 Adding addition phytic acid experiments

There was a relatively fast release of DIP during the first 6 h in the Siran microcosm, with the concentration increasing from 7 to 41 μg $P \cdot L^{-1}$ as shown in Fig. 9. In contrast there was very little DIP released during this

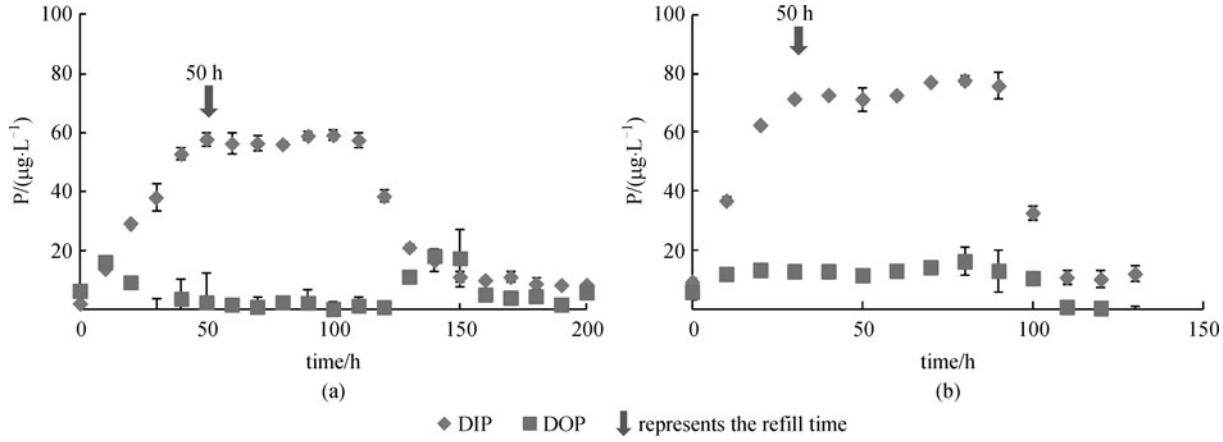

Fig. 8 DIP and DOP concentrations in the outflow water of microcosms during the biotic experiment incorporating the addition of 80 μg $P \cdot L^{-1}$ G-6-P. (a) microcosm Siran; (b) microcosm Duran. Time 0 shows the beginning of the step up phase. Error bars represent ±1 s.d. of the results from 3 columns with each sample analyzed in triplicate (n = 9)

Table 5 DIP and DOP concentrations (μg $P \cdot L^{-1}$, n = 9) in the inflow and outflow waters of the microcosms during biotic experiments incorporating the addition of 80 μg $P \cdot L^{-1}$ G-6-P

G-6-P		inflow at phase I	outflow at phase I	outflow at phase III	gain /loss (phase III – I)	outflow at phase V	gain/loss (phase V – I)
Siran	DIP	22±1	2±0	52±3	+ 50	10±2	+ 8
	DOP	6±2	10±0	4±2	− 6	5±2	− 5
Duran	DIP	26±2	9±0	71±3	+ 62	10±2	+ 1
	DOP	6±2	6±1	13±1	+ 7	2±2	− 4

period in the Duran microcosm. The DIP concentrations in the two microcosms reached steady-states of 48 μg $P \cdot L^{-1}$ and 76 μg $P \cdot L^{-1}$ at 40 and 43 h, respectively (Fig. 9 and Table 6). The DIP concentrations in both microcosms decreased to the starting concentrations after 100 h during the step down experiments. The DOP profiles were similar in both microcosms but the phase III concentration was higher in the Siran microcosm (30 μg $P \cdot L^{-1}$) than in the Duran microcosm (17 μg $P \cdot L^{-1}$).

4 Discussion

The abiotic experiments showed that physico-chemical interactions between Siran and Duran beads and inorganic P can be considered negligible when considering the results from the biotic experiments (Figs. 6(a) and 6(b)). For the G-6-P experiments, approximately 30% and 38% of the G-6-P was converted in the Siran and Duran columns respectively within 22 h. As the columns were

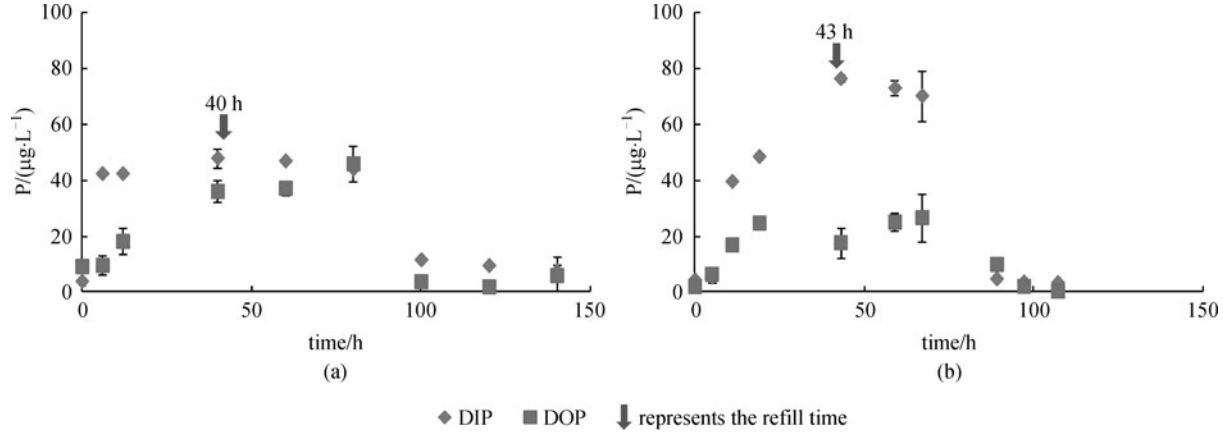

Fig. 9 DIP and DOP concentrations in the outflow water of the microcosms during the biotic experiment incorporating the addition of 80 μg $P \cdot L^{-1}$ phytic acid. (a) microcosm Siran; (b) microcosm Duran. Time 0 shows the beginning of the step up phase. Error bars represent ±1 s.d. of the results from 3 columns with each sample analyzed in triplicate (n = 9)

Table 6 DIP and DOP concentrations (μg $P \cdot L^{-1}$, $n = 9$) in the inflow and outflow waters of the microcosms during biotic experiments incorporating the addition of 80 μg $P \cdot L^{-1}$ phytic acid

phytic acid		inflow at phase I	outflow at phase I	outflow at phase III	gain /loss (phase III – I)	outflow at phase V	gain /loss (phase V – I)
Siran	DIP	29±4	7±0	48±3	+ 41	7±2	0
	DOP	11±2	6±0	36±4	+ 30	2±1	− 4
Duran	DIP	26±2	2±0	76±1	+ 74	3±0	+ 1
	DOP	14±1	1±0	18±5	+ 17	1±0	0

kept in the dark it is likely that G-6-P has undergone hydrolysis to release DIP. This is consistent with the additional experiments described that up to 17% of G-6-P were hydrolysed in 3 days at 4°C [32]. The difference in the extent of hydrolysis compared with the batch microcosm experiments indicates that either temperature or the dynamic movement of the water affected the rate of hydrolysis (Figs. 6(c) and 6(d)). There was no partitioning of the phytic acid onto the Duran beads, but a small uptake onto the Siran beads. In addition, the DIP data showed no conversion of phytic acid during the experiments consistent with the refractory nature of this compound. None of the species exhibited physico-chemical partitioning on to the Siran or Duran beads, except for a small amount of adsorption of phytic acid on the Siran microcosm due to the high porosity as show in Table 1. However, there was a significant (30%–38%) hydrolysis of G-6-P which was confirmed by increased DIP, as shown in Table 3.

The biotic orthophosphate experiments showed that P was not the limiting nutrient and that some DIP was converted to DOP in the water column. Over a period of 30–40 h, 15% of the DIP was taken up by the Siran and Duran microcosms, while the same amount of DOP was released. This could have been due to bacterial uptake of DIP followed by intracellular conversion to DOP and subsequent release as DOP into the surrounding water [33].

The biotic G-6-P experiments showed similar profiles of P exchange for both the Siran and Duran systems with 62%–77% of added G-6-P converted to DIP. There was no significant increase in DOP in either microcosm at phase III from G-6-P experiments (Fig. 8) implying almost complete conversion of G-6-P to DIP. Since the abiotic experiment found only 30% and 38% of G-6-P hydrolysis, it is significant evident to prove that bacteria are able toconvert labile organic P compounds such as G-6-P.

The biotic phytic acid experiments showed that there was no significant bacterial uptake but there was significant displacement of DIP from the biofilm surface in both microcosms releasing bioavailable DIP into solution. The release of DIP from both microcosms indicates some interplay between phytic acid and DIP (Fig. 9). Monbet et al. [24] reported that phytic acid is resistant to degradation in natural water for up to 6 months, and this experiment showed only for 5 days, suggesting that the release of DIP was not due to natural hydrolysis or bacterial conversion. The more likely process in these experiments is physical replacement of DIP by phytic acid on the biofilm surface [26]. The difference between the phase III concentrations of phytic acid and the step up concentration (80 μg $P \cdot L^{-1}$) would be sufficient to account for the increase in DIP. Hence, the role of refractory DOP species cannot be ignored when considering phosphorus cycling in particle – water systems. Bacteria do not directly or indirectly utilize refractory P species (represented by phytic acid in these experiments). However phytic acid can contribute to the bioavailable DIP pool by physical displacement of DIP from the surface of suspended and benthic particulate matter [26].

Refill time is higher than the refill time for KCl (10 h) which is attributed to changes in the physical packing of the beads in the columns and changes in the design of the hardware, particularly the pumping system. However, the flow rates were monitored throughout the abiotic experiments and remained constant over this period. There is an alternative explanation which is not excluded that the possibility of heterogeneous reactions is taking place between inflowing solved DIP and the surface of the glass beads, which could delay steady-state until a reversible, stable equilibrium is achieved.

The Siran and Duran microcosms showed generally similar trends, and none of the P species exhibited physical partitioning onto the Siran or Duran beads under abiotic conditions, except for 13.7% uptake of phytic acid onto the Siran beads. About 30%–38% of the G-6-P was hydrolysed under abiotic conditions in both the Siran and Duran microcosms. The porosity and the diameter were the major differences of the Siran and Duran beads and caused the difference of surface to water ratio. Bacteria should have more chance to attach on Siran beads and possible affect its utilization of P.

5 Conclusions

A microcosm system has been successfully used to investigate bacterial uptake of P. The microcosm system appears suitable to investigate the bacterial up take of nutrients over periods of days. River bacteria are able to fast hydrolyse labile DOP, whereas for the refractory

organic P, the utilization was not significant during a short-term (<150 h). Even in a P rich estuarine environment, this study demonstrates that bacteria still display DOP to DIP conversion characteristics.

In the wider estuarine context, the microcosm results showed that DIP and labile DOP can act as sources of P for bacteria but the direct uptake of refractory DOP is not significant. Therefore DOP concentration in the estuarine cannot be ignored. This paper introduced porosity of 50%–65% and 0% borosilicate beads to simulate SPM in the river suggested that estuarine SPM size and surface to water ratio did not affect the amount of bacteria and its behavior to P exchange.

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