BACTERIA IMMOBILIZED ON NATURAL ZEOLITIZED TUFF ARE ABLE TO SURVIVE IN THE CONDITIONS OF EXTREME pH

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ABSTRACT

The bacteria in the form of biofilm are more adapted to survive environmental stresses. We prepared biofilm by immobilizing phosphate-accumulating bacterium *Acinetobacter junii*, a heterotrophic bacteria normally present in activated sludge, on the particles of natural zeolitized tuff. The obtained bioparticles had thick biofilm with number of immobilized bacteria $2.2\pm0.12\times10^9$ CFU g^{-1}. To test the survival of such bacteria in harsh conditions of wastewater treatment plants, such as unfavourable pH, the bioparticles were introduced in reactors containing simulated wastewater of extreme pH values, namely, 2, 3, 4 and 5 during 24h. The bacteria immobilized on bioparticles showed great resistance to unfavourable pH. The bacteria were viable in the biofilm, but also detaching from the biofilm and continued its growth in the media as planktonic cells. At pH 4 the survival of bacteria was 82% of starting number as opposed to the control reactors where survival was 0%. At pH 3, there were 38% of bacterial cells able to survive, while at pH 2 no viable cells were present in the reactors after the incubation. The results proved that bacteria immobilized on natural zeolitized tuff are able to survive the extreme conditions of wastewater treatment plants such as 24h disturbances of pH. This way the survival of bacterial population is enabled while the system upsets are fixed and bioparticles could serve as seeds for regeneration of bacterial population in the treatment plant.

Keywords: bacteria, immobilization, pH, wastewater, zeolite.

INTRODUCTION

Over the last 30 years the preferred process for phosphate (P) removal from wastewater has become the enhanced biological phosphorus removal (EBPR), a process based on the metabolism of P-accumulating bacteria present in the activated sludge. Currently much attention is on the technology of bacterial immobilization. The bacteria that are immobilized on suitable carriers are better adapted to survive periods of environmental stresses [1] and show enhanced metabolic activity in the bioreactors.

We tested the ability of P-accumulating bacterium *A. junii* immobilized on the natural zeolitized tuff (NZ) to survive in the conditions of extreme pH values. The immobilized bacteria form biofilm, a specific community of microorganisms which offer protection from environmental insults and assaults [2].

EXPERIMENTAL

Bacterium

The culture of P-accumulating bacterium *A. junii* (DSM 1532) was obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen GmbH.

Growth medium

The bacteria were incubated in a chemically defined water solution that simulated the real wastewater. The composition was (in mg L^{-1} of distilled water): Na-propionate 300; peptone 100; $MgSO_4$ 10; $CaCl_2$ 6; KCL 30; yeast extract 20; KH_2PO_4 88. Before autoclaving (121°C/15 min) the pH of wastewater was adjusted to desired values with 1 M NaOH and HCl.

Carrier of the bacteria

The NZ from Bigadic, Turkey [3] of particle size fraction 0.125-0.25 mm was used as a carrier of bacteria. Before the experiment the NZ samples were washed with demineralized water and sterilized at 105°C for 16h.

Design of the experiment

The experiments with immobilized bacteria were performed by adding 1 g of previously prepared bioparticles in bottles with 100 mL of simulated wastewater with pH values 2-5 and aerobically (1L min^{-1} of sterile air) incubating the bottles for 24h at a water bath with shaker (30°C/70 rpm). The numbers of planktonic and immobilized bacteria before and after the incubation were determined.

The bioparticles were prepared by aerobically incubating 1g of NZ in a suspension of *A. junii* in Nutrient Broth (Biolife, Italy) for 6h with aeration. After the incubation the bioparticles were washed with 100 mL of sterile saline solution to remove the cells that were not immobilized in the biofilm.

In preliminary experiment the optimal pH for growth of *A. junii* was determined by incubating the pure culture of the bacteria in simulated wastewater with pH values ranging from 3-10 during 24h, at the same conditions as mentioned above. Bottles from this experiment were marked as control reactors.

The numbers of viable planktonic and immobilized cells were respectively determined as CFUs (*Colony Forming Unit*) per L^{-1} and g^{-1}. The methodology of this procedure was explained in detail in [3]. Statistical analysis was done using the Statistica Software 8.0 (StatSoft, Tulsa). Statistical decisions were made at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

In preliminary experiments the pH 7 was determined as optimal for growth of *A. junii* since the number of bacteria after 24h of incubation in reactors pH 7 was significantly higher when compared to other reactors (Figure 1). The *A. junii* showed marked sensitivity to low pH. At pH 4 there were no viable cells after 24h of incubation (Figure 1). The die-off of bacterial biomass was noted at pH 5, clearly visible by reduction of the number of bacteria when compared to starting number. At pH 6 the bacteria were actively multiplying during 24h so this value could be set as limiting for *A. junii* growth. The number of bacteria in reactors with pH 8, 9 and 10 did not differ significantly after the incubation so high pH of wastewater was not toxic to *A. junii*.

After 24h of incubation the pH value in the control reactors differed from the start. In reactors pH 4 and 5 where there was no significant bacterial activity it was 4.27 and 5.12. However in the remaining reactors where the bacteria actively multiplied it differed significantly from the start and was 7.54±0.16 (mean±s.d. for the reactors pH 6,7,8,9 and 10). Where there was intensive metabolism of bacteria the pH value was shifted towards the neutral which is at the same time optimal for bacterial growth. The population of *A. junii* was able to self-regulate the pH value if it was not toxic towards the bacteria.

Several studies showed relationship between pH value and the successful EBPR process where higher pH values are preferred [4, 5]. Filipe et al. [6] state that in aerobic conditions the biomass growth and P-uptake was reduced at pH 6.5 and that higher pH values (7.0-7.5) were more adequate for P-accumulating organisms.

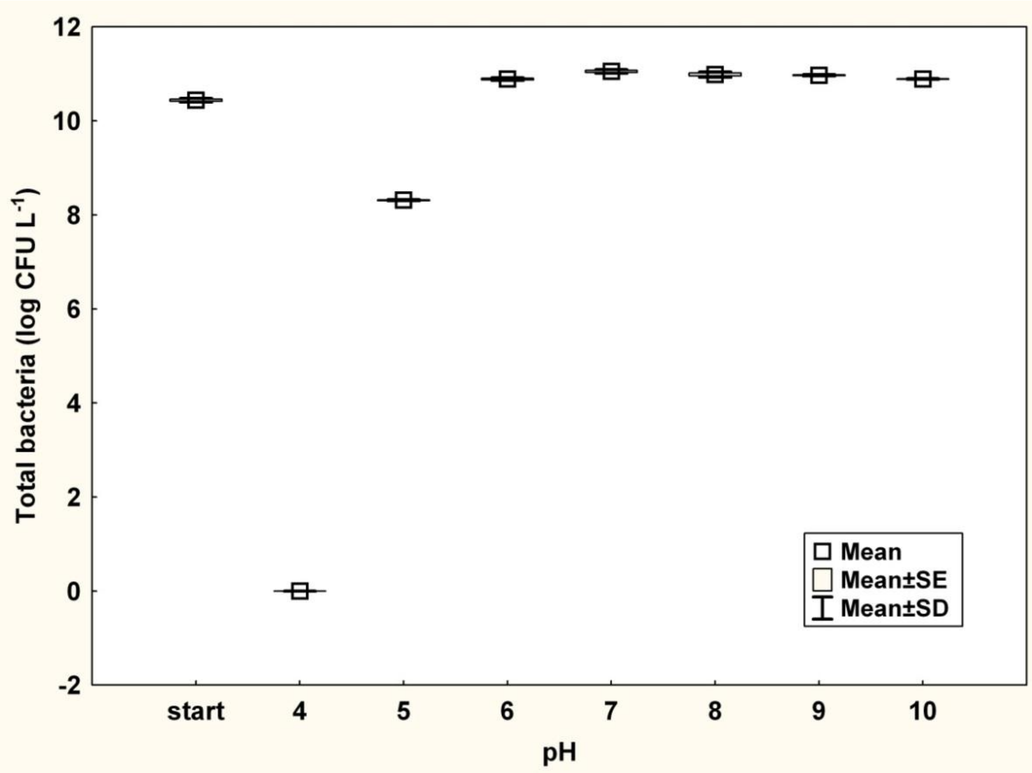

Figure 1. Number of planktonic *A. junii* cells in control reactors with different pH values of wastewater after 24h of incubation.

Since high pH values showed non-toxic toward the bacteria the bioparticles were introduced to the reactors where pH was set from 2-5, which are in every sense extreme conditions for the *A. junii*. The die-off of bacterial biomass was also noted at pH 5, visible from the reduction of the starting number of bacteria (Table 1).

Table 1. The number of viable *A. junii* cells in the reactors with different pH values of wastewater and added bioparticles after 24h of incubation.

	pH			
	2	3	4	5
c_0 total bacteria (CFU L^{-1})	$2.2\pm0.12\times10^{10}$			
pH	2.16	3.64	4.5	5.25
Immobilized bacteria (CFU g^{-1})	0	$1.03\pm0.17\times10^{4}$	$8.97\pm7.03\times10^{7}$	$7.80\pm0.6\times10^{8}$
Planktonic bacteria (CFU L^{-1})	0	$4.45\pm0.55\times10^{5}$	$6.03\pm0.32\times10^{7}$	$1.44\pm0.06\times10^{9}$
Total bacteria (CFU L^{-1})	0	$5.68\pm0.34\times10^{5}$	$1.13\pm0.83\times10^{9}$	$1.13\pm0.08\times10^{10}$
Survival of bacteria (%)	0	38	82	96

However, the bacteria in the form of bioparticles were significantly more resilient to negative influence of pH when compared to the planktonic bacteria in the control reactors. In the reactors with pH 3 and 4 there was still much of viable bacteria after 24h of incubation, as opposed to the control reactors where there was no viable cells after the incubation at pH 4 (Figure 2). Although the pH values were extremely low in the reactors during the incubation

(Table 1) the *A. junii* were present as immobilized bacteria in the biofilm but also as planktonic bacteria. This indicates that a stable biofilm on the bioparticles was achieved where bacteria were multiplying through time and detaching from the biofilm to continue the growth in the reactor as planktonic cells. This way the bioparticles could serve as a safe environment for bacterial survival through extremely unfavourable conditions.

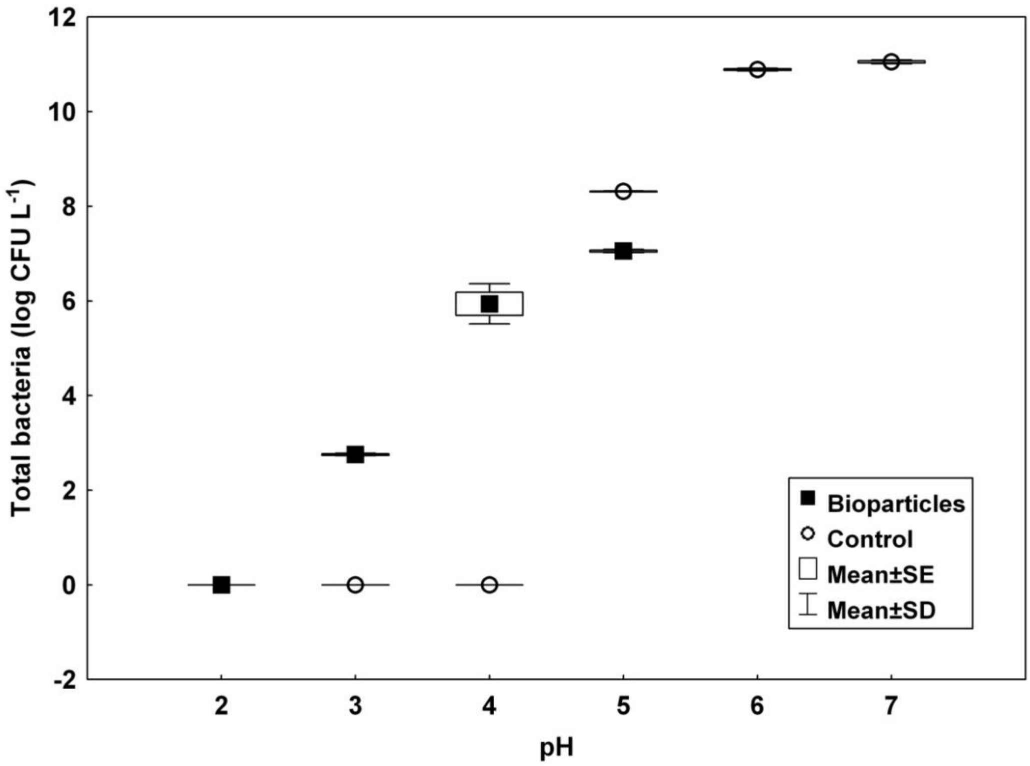

Figure 2. Number of *A. junii* cells in control reactors and in reactors with addition of bioparticles after 24h of incubation.

CONCLUSION

The results proved that bacteria immobilized on NZ are able to survive the extreme conditions of wastewater treatment plants such as 24h disturbances of pH. This way the survival of bacterial population is enabled while the system upsets are fixed and bioparticles could serve as seeds for regeneration of bacterial population in the treatment plant.

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