

THERMAL PROPERTIES OF BIOSTIMULANT-INFUSED POTATO FIBERS

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Crushing or otherwise processing potato and subsequent mild washing of the pulp produces mostly granular and fibrous starch. These fibers are experimental carrier material for microbes in bioremediation of polluted waters. This method offers the benefit of increasing the exposure of the microbes to the pollutant by increasing their residency within the site. Because of the physical nature of the material, it also offers the possibility of carrying, in addition to the microbes, essential macronutrients such as nitrogen and phosphorous that would be limited in availability in contaminated waters. We have previously reported on the physical nature of these fibers through thermal analysis and on their ability to bind/aggregate bacteria. We have extended that study in this report by infusing the fibers with a source of nitrogen and phosphorous, namely ammonium phosphate. The TG curves for ammonium phosphate-infused white and sweet potato fibers exhibited three main mass loss steps corresponding to the three exothermic DTG peaks. Infusion with the ammonium phosphate salt also affected the bacterial binding/aggregation capacities. The range of their binding capacities decreased to a range of 26.9–43.3% compared to untreated fibers.

Keywords: bacteria, bioremediation, DTG, sweet potato (*Ipomoea batatas*), TG, white potato (*Solanum tuberosum*)

Introduction

In bioremediation, one may inoculate a contaminated site with a mixture of microbes (bioaugmentation) or simply apply biostimulants to site (biostimulation) to assist resident microbes in removing or inactivating the pollutants [1–3]. In contaminated waters, indigenous microflora with significant activities against the pollutant will be limited. During the past twenty years or so, there have been significant developments in this area, with particular focus on the appropriate type of microorganisms that could be used and the identification of factors that could enhance the activity of those microorganisms on detoxification of the site. In tackling polluted waters, the factors that need to be considered to enhance the activities of the inoculum include the availability of macronutrients, such as nitrogen and phosphorus, as well as the increased residence time of the microbes within the site. In open waters, the latter becomes a significant factor as the inoculated microbes are ‘diluted’ away from the site and indigenous microbes may actually lack the ability to breakdown that pollutant. In such a situation biostimulants alone will not have an impact [4, 5].

Using a carrier material for the delivery of microbes onto the polluted site offers many obvious advantages. Recently, there have been a number of reports on the use of a variety of natural as well as

modified/synthetic material as microbial carriers. Microbes adsorb onto these materials or prepared so as the microbes form a biofilm and used for applications ranging from removal of inorganics (nitrates), xenobiotics, or crude oil from water or soil. Some of the materials used in other laboratories include a product from the thermal processing of wood and most are called Lessorb [6], silica or biosand [7], synthetic membranes [8], diatomite, clinoptilolite, silk zeolite and coal fly ash [9], porolon, claydite, glass broaches, maize stem and barley straw [10], biodegradable plastic made from starch [11], as well as alginate [12].

We have previously examined the physical properties of insoluble potato fibers with the objective of developing it as a bacterial carrier for bioremediation [13]. Our hypothesis is that the association of the microbes with the potato fiber would increase the residency time of the microbes within the site of the initial inoculum, and thus enhance and accelerate the detoxification process. We have also suggested that the potato fibers could be infused with macronutrients and biostimulants to further enhance and accelerate the bioremediation process. In this report, we present our findings of changes in the physical properties of potato fibers as a result of infusing the fibers with a mixture of potassium phosphate and ammonium sulfate. The effect of this treatment on bacterial binding capacity of the fibers was also investigated.

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Experimental

Materials

Preparation of insoluble potato fibers

White potatoes (*Solanum tuberosum*) and sweet potatoes (*Ipomoea batatas*) were purchased locally and processed as described previously [13]. Briefly, washed, peeled, and chopped potatoes were processed through a juicer, which allows for the separation of the fiber from the juice. The fiber portion was collected and mixed with water. Soluble starch was removed by transferring the suspension to a screen and mixing under running water until the flow through ran clear. The goal was to remove as much soluble starch as possible since soluble starch has been shown to inhibit bacterial attachment [14]. The remaining fiber was soaked in 50% ethanol for several days at 4°C. Ethanol was subsequently washed out with water and excess water removed by squeezing the fiber against the screen. The fiber was frozen at -86°C overnight and then moved to the freeze drier. Further drying was carried out in an oven at 80°C for 8 h. The dried fiber was weighed and then ground into smaller pieces. To get uniform sized pieces the fiber was passed through a larger screen onto a smaller screen. Only particles that remained on the second screen were used.

Bio-stimulant infusion of the potato pulp

The prepared potato pulp was infused with bio-stimulants by soaking the pulp in an aqueous solution of salt mixtures. Approximately 5.0 g of each of the white and sweet potato pulp, separately, were suspended in 200 mL of 50 mM potassium phosphate buffer adjusted to pH 6.8, and containing 100 mM of ammonium sulfate. Following an incubation period of 2 h at ambient temperature, excess solution was partially removed through application of the mixture onto a strainer. The expanded and still moist-rich pulp was further processed through a spinner to strain the remaining excess liquid and the pulp was finally dried in a 50°C oven for several hours.

Methods

TG and DTG measurements

Thermogravimetry (TG) and derivative thermogravimetry (DTG) on powder samples (~10 mg) were carried out using a TGA Q500 T.A.I. instrument at 10°C min⁻¹ from room temperature (*rT*) to 700°C under nitrogen atmosphere using a flowing rate 60 mL min⁻¹.

Binding assay

Binding of bacteria to starch particles was carried out according to our previous study [13] and as modified version of the protocols described in other reports [15–17]. Briefly, 3 mL of cell culture in midlog were mixed with 80 mg of either white potato starch or sweet potato starch granules. The tubes were incubated at 37°C for 1 h with regular mixing, 1 mL of cell suspension was removed, without disturbing the starch granules, and the turbidity measured ($A_{600\text{ nm}}$). Attachment efficiency was defined as:

$$[1-(C_f/C_i)] \cdot 100$$

where C_i is the initial cell concentration and C_f is the final cell concentration. Bacterial growth during the incubation period was accounted for with the appropriate controls.

Statistical tests

Un-paired t-tests were used to determine if the differences in binding capacity between starches were significant.

Results and discussion

TG and DTG curves of white potato fiber are given in Fig. 1. The TG curve exhibits the three main mass loss steps between *rT*-200, 200–280, 280–700°C corresponding to the elimination of water molecules and decomposition of ammonium phosphate salt and white potato fiber; respectively, accompanied by 56% total mass loss. The DTG curve showed three corresponding exothermic DTG peaks at 80, 255 and 330°C. The TG and DTG curves of sweet potato fiber are presented in Fig. 2. The TG curve of sweet potato fiber also shows three main mass loss steps between *rT*-180, 180–280, 280–700°C corresponding to the elimination of water molecules and decomposition of ammonium phosphate salt and sweet potato fiber; re-

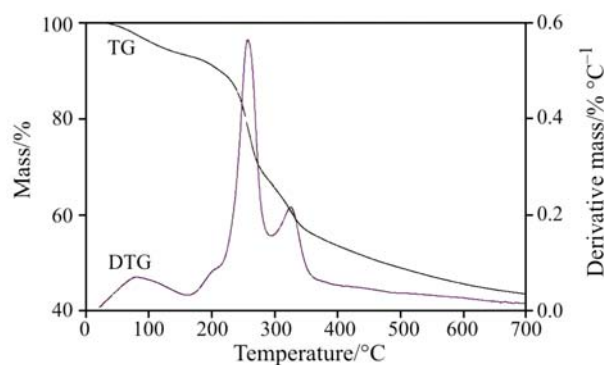
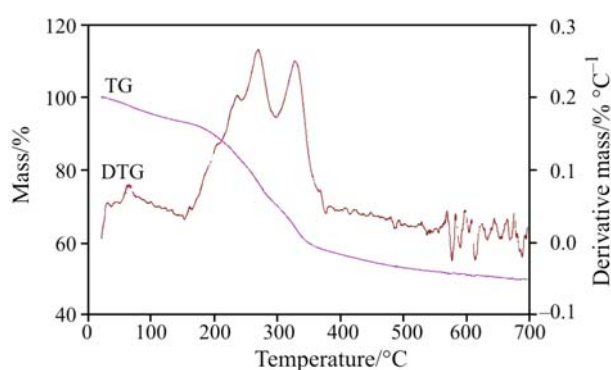


Fig. 1 TG and DTG curves of white potato fiber

Table 1 Effects of infusion with biostimulant salts on the bacteria-binding capacities of potato fibers

Potato type	Mass increase/% ¹	Binding capacities/cells g ⁻¹			
		<i>B. cepacia</i>	Change/% ²	<i>P. putida</i>	Change/% ²
White potato	12±3.3	9.8·10 ⁹ ±8.7	43.3	3.2·10 ⁹ ±6.8	29.9
Sweet potato	8±2.1	3.4·10 ⁹ ±17.3	36.7	6.2·10 ⁹ ±13.6	26.9

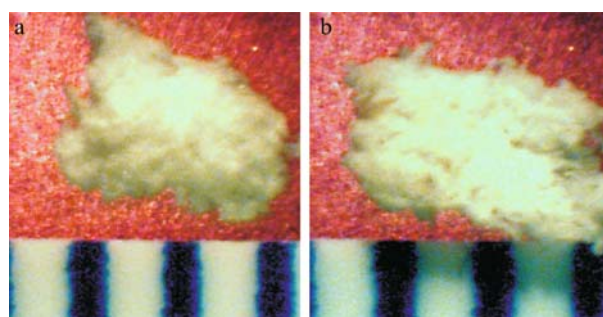
¹Increase in weight of potato fibers due to infusion with the ammonium phosphate. ²Change in binding capacities is compared to untreated fibers as reported previously [13]


Fig. 2 TG and DTG curves of sweet potato fiber

spectively, accompanied by 50% total mass loss. The DTG curve exhibited three corresponding exothermic DTG peaks at 70, 260 and 320°C [18, 19]. There are significant differences in thermal properties between treated and untreated potato fibers. The untreated potato fibers exhibited mainly one mass loss step with one corresponding DTG peak [13].

Binding assays showed that both bacterial species used in this study were able to attach to the starch particles, albeit with less efficiency than the untreated particles (Table 1). Attachment efficiency was determined by comparing the initial $A_{600\text{ nm}}$ to that of the cell solution after incubation with starch particles. There were significant differences in binding capacity between starch types and bacterial species, as observed previously [13]. Infusion of the granules with the biostimulant salts has decreased the binding capacities of both white and sweet potato pulp. Starch particles from white potato were able to bind *P. putida* significantly more than *B. cepacia* ($p < 0.01$). Sweet potato starch particles, on the other hand, were able to bind *B. cepacia* significantly more than *P. putida* ($p < 0.01$). For white potato starch particles, average binding capacities were $3.2 \cdot 10^9 \pm 6.8\%$ cells g⁻¹ for *P. putida* and $9.8 \cdot 10^9 \pm 8.7\%$ cells g⁻¹ for *B. cepacia*. Average binding capacities for the sweet potato starch were $6.2 \cdot 10^9 \pm 13.6\%$ cells g⁻¹ for *P. putida* and $3.4 \cdot 10^9 \pm 17.3\%$ cells g⁻¹ for *B. cepacia* (Table 1).

The most significant effect of the biostimulant infusion on the potato fibers was the change in the texture of the material (Fig. 3). Naturally, addition of the salts, followed by dehydration, has resulted in in-


Fig. 3 Physical appearance of the potato fibers following treatment with the biostimulant salts. a – white potato; b – sweet potato. Scale is 1 mm per division

crease in the mass of the carrier. Being a salt, dehydration would precipitate/crystallize the salt within the carrier granules. However, in the final preparation of the carrier and before use in the field, it would be mixed with a cocktail of microorganisms and the mixture dried and stored until needed. These preparatory steps should dissolve the biostimulant salts and recover the porosity and rehydration properties of the potato pulp. This system still offers several advantages over the use of other carrier material. Particularly carrier material containing agents such as CaO or other agents that may react with the ammonium phosphate and release either toxic products and/or change the physical parameters of the polluted site. In the case of CaO use, the carrier material produces Ca(OH)₂ and results in significant increase in the pH [8]. This high alkalinity would have deleterious effects on the microbes' physiology or even fatal. It would also significantly reduce any enzymatic activities elaborated by the microbes and which are required for the successful remediation of the site.

Conclusions

This work shows that potato pulp and/or fiber is a candidate carrier of microbes for bioremediation. This carrier material is low cost, somewhat porous, biodegradable, efficiently binds/aggregates microbes, and is amenable to carry biostimulants to give a complete package to transport and deliver to a polluted site. The TG curves for biostimulants-infused white

and sweet potato fibers show three main mass loss steps corresponding to the three exothermic DTG peaks, respectively.

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