DOI 10.1515/gps-2012-0041 — Green Process Synth 2012; 1: 427–437

Galip Akay* and Steven Fleming

Agro-process intensification: soilborne micro-bioreactors with nitrogen fixing bacterium *Azospirillum brasilense* as self-sustaining biofertiliser source for enhanced nitrogen uptake by plants

Abstract: A new application of agro-process intensification is described for nitrogen fixation by *Azospirillum brasilense* supported within the pores of sulphonated-neutralised polyHIPE polymers (PHPs) which are highly hydrophilic, elastic, crosslinked and ionic with nano-structured pore walls. These bioactive macroscopic polymer particles, when used as soil additives act as micro-bioreactors within the soil and facilitate the interactions between plant roots, root exudates, water, nutrients and bacteria (reactive components), because plant roots penetrate into these micro-bioreactors which simultaneously absorb water and nutrients while generating biofertiliser through the nitrogen fixing bacteria within them. Hence, these soil additives act as synthetic rhizosphere (SRS). In greenhouse experiments, it is shown that the presence of the bioactive SRS at 0.5 wt% level in the soil without any fertiliser addition increases the dry grass shoots by 9.6%, 9.5%, 40% and 145% after 3, 6, 9 and 12 weeks of growth, respectively, compared to grass grown with no SRS or bacteria. Progressive yield enhancement with *Azospirillum brasilense* supported on PHPs is due to reduction of soil nutrients thus switching nitrogen fixing bacterium from consumption to production of nitrogen. The environmental impact and sustainability of SRS media are also considered and compared with other soil additives: super absorbent polymers and biochar.

Keywords: agro-process intensification; *Azospirillum brasilense*; biofertilisers; nitrogen fixation; polyHIPE polymers.

***Corresponding author: Galip Akay**, Process Intensification and Miniaturisation Centre, Faculty of Science, Agriculture and Engineering, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK, e-mail: Galip.Akay@Newcastle.ac.uk
Steven Fleming: Process Intensification and Miniaturisation Centre, Faculty of Science, Agriculture and Engineering, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

1 Introduction

1.1 Need for agro-process intensification (A-PI)

The ultimate sustainable 'green processing and synthesis' is achieved through biological transformations. Agriculture thus represents a large-scale green synthesis and processing using solar energy, atmospheric gases, water and nutrients in soil which also acts as part of the reactor vessel. However, to enhance productivity, chemical fertilisers have been used while the demand for water increased. Global warming will increase water demand and reduce the availability of land for agriculture. Furthermore, combating climate change through the use of biomass as feedstock for energy, biofuels and chemicals such as ammonia will further impose stress on water, land and fertiliser use in agriculture. To address these emerging stresses in food, energy and water supply, new drought resistant and nitrogen fixing crops are being developed through genetic modification, with the knowledge that such developments have major scientific challenges.

However, the fundamental engineering aspect of plant growth and crop yield has not been evaluated in order to make the agricultural processes more efficient. This can be done by applying the principles of process intensification in general and micro-bioreactor philosophy in particular to plant growth by considering the biological processes that take place in the soil. Clearly, micro-bioreactor technology needs to be applied to plant growth at massive scale which amounts to ecosystem engineering. The advantages sought in plant photosynthesis through the use of micro-bioreactors in soil include water preservation, nutrient (fertiliser) generation and preservation and enhancement of biochemical reactions through the facilitation of interactions between plant roots, nutrients, water, soil microorganisms and plant root exudates which

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

act as messenger molecules. In this technology, micro-bioreactors put into the soil as soil additives are highly porous, hydrophilic, biologically active, elastic and ionic polymers which in fact deliver process intensification [1–4]. Here, we extend the scope of this A-PI by essentially generating fertilisers in the vicinity (tens of micrometres) of plant roots using microorganisms supported within the pores of the micro-bioreactors. The fertiliser generation is self-sustained through atmospheric nitrogen fixation, thus making the process highly efficient. The background review is given below.

1.2 Fertilisers and crop yield enhancement

One of the main reasons crop yields have increased in the past 50 years has been increased use of chemical fertilisers using expensive non-renewable feedstock (usually natural gas) and hence it contributes to global warming by emitting vast quantities of CO_2 estimated to be 1% of all greenhouse gases [5]. Furthermore, while the fertiliser efficiency falls [6, 7], additional stresses such as water and temperature appear. Because chemical fertilisers are produced from fossil fuels, diminishing feedstock availability is also a concern. The replacement of fossil fuel feedstock by renewables (biomass and biomass waste) is an alternative but this will also increase stress on biomass generation and land availability.

An alternative to artificial fertiliser is the use of biofertilisers which are more environmentally friendly, more economic to produce and sustainable over a longer timescale than artificial fertilisers. Biofertilisers are based on organisms that occur naturally in the soil which can utilise atmospheric nitrogen in particular but can also benefit plants in other ways including enhanced moisture uptake and phosphate uptake and production of plant growth hormones.

Soil is a living environment that contains a vast array of microorganisms. Most of them are harmless, but a few of these can have a big impact on plant productivity, either pathogenic organisms that have a detrimental effect, or beneficial organisms that stimulate plant productivity by supplying limited nutrients to the plant [8]. The relatively small numbers of beneficial organisms normally present in most soils mean that they do not have a significant effect on plant production, but if the number of these organisms could be increased and sustained within rhizosphere, their effect could then become more significant.

The two most important categories of beneficial organisms are mycorrhizal fungi and nitrogen fixing bacteria. Mycorrhizal fungi are present in most soils and form symbiotic associations with 80% of all terrestrial plant species [9]. The fungi obtain nutrients through their extensive fungal network by excreting a wide range of extracellular enzymes that can degrade organic matter [10]. They can therefore colonise areas with low nutrient availability but where organic matter is available in the form of litter and humus, their activity is limited. The presence of the fungus increases the surface area of the root system in contact with the soil by many hundreds-fold and therefore enhances water and nutrient absorption as well as increasing soil stability [11].

1.3 Biological nitrogen fixation by plants

Nitrogen fixing bacteria have evolved the ability to convert atmospheric nitrogen from the air into ammonium using the enzyme nitrogenase which can then be utilised by the plant and therefore does not require nitrogen to be available in the soil. There are two types of nitrogen fixing bacteria: symbiotic organisms which form nodules on the roots of leguminous plants [1, 4] and free living organisms which do not form associations with any specific plants. The nitrogen fixing activities of the bacteria make a significant contribution to the nitrogen requirements of the plants because of the high numbers of bacteria in the root nodules, intimately associated with the plants and therefore leguminous plants have an important and significant role to play in modern agriculture. The disadvantage is that they are species specific – so any specific bacteria will only form nodules with one specific legume so they have no use as a general biofertiliser. Free living bacteria, by contrast, do not form specific associations and therefore can potentially benefit any plant. However, they do not contribute significantly fixed nitrogen in agricultural crops because they are relatively few in numbers and have to compete for nutrients with all the other soil borne micro-organisms. From a mass transfer point of view, many bacterial colonies are remote from any plant roots. If the numbers of free living bacteria could be enhanced and brought into close proximity with the roots, then they may make a significant contribution to the nitrogen requirements of the plants.

Soil is a natural carrier of plant roots, water, nutrients and bacteria which can be considered to be the ‘reactant species’. However, these reactant species are widely distributed and inefficiently retained in the soil. As biochemical conversions take place in the rhizosphere, the root surface area per unit volume of soil (surface area density) should be large in order to facilitate the interactions between the roots, water, nutrients and bacteria. Furthermore, a large amount of fertiliser and bacteria are lost as a

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

result of water flow through the soil and they are therefore never of any benefit to growing plants. It has been estimated that 50–70% of applied fertiliser can be lost to the environment and never provides any benefit to the plants [12, 13]. Such antagonistic actions also restrict nutrient and water uptake by the plant.

1.4 Agro-process intensification

Recently, a novel method of enhancing the interactions between root, root exudates, water, nutrients and bacteria while eliminating antagonistic actions between them have been proposed [1–4]. This method uses the principle of process intensification [14, 15] in which micro-bioreactors are utilised as soil additives acting as synthetic rhizosphere (SRS). To extensively facilitate the multi-component (root, root exudates, water, nutrient and bacteria) interactions within a micro-bioreactor, it is necessary to use a highly hydrophilic (for water absorption), ionic (for metal ions and ammonia adsorption), elastic (to allow root penetration) and nano-structured microporous polymers (for bacterial support with no barrier for small molecules, including messenger molecules). Such materials are known as polyHIPE polymers (PHPs) which, together with their metallic or ceramic versions are used extensively in process intensification including bio-process intensification (B-PI) and 3D tissue engineering [16–18] and chemical process intensification (C-PI) [14, 19–22]. In particular, sulphonated version [22] of these materials is useful as SRS media for A-PI.

Recently, these materials have been used as solid acid absorbents to remove ammonia from the ammonia synthesis reaction mixture so that unreacted hydrogen and nitrogen can be recycled [21]. As a result, PHPs can also be produced with ammonia fertiliser already present within its pores and used as a slow release fertiliser. Once the fertiliser release is depleted, they then act as SRS media to enhance plant growth and crop yield enhancement. B-PI using PHPs as support for micro-organisms indicate that PHP provides a protective environment for the supported bacteria and it increases their growth rate as well as productivity [1–4, 18]. Bio-active PHPs with supported bacteria can therefore be used to enhance root bacterial infection [1, 2] in legumes or ammonia production within the vicinity of plant roots to promote fertiliser uptake by the plant.

Therefore, PHPs have the potential to act as a reservoir for these beneficial organisms, offering a protective environment for them to grow without competition from all other soil organisms. If the numbers of beneficial organisms could be increased, then they have the potential to make a significant contribution to nutrient requirements of the crop.

1.5 Driving forces for multiple interactions

For the present technique to be successful, it is necessary that the plant roots grow through the SRS media. Because PHPs are hydrophilic (they can absorb water 10–30 times of their own weight) they preferentially retain water which in turn allows roots to preferentially grow towards them through hydrotropism [23–25]. As the SRS media is also rich in nutrients (through cationic functionality and nutrient adsorption), root growth towards PHPs is also enhanced through chemotropism [26, 27]. Therefore, there are natural driving forces which make the multiple interactions selective and thus the proposed A-PI becomes feasible. Furthermore, in the case of biologically active SRS media containing bacteria, the root/bacterium interactions will be enhanced through high concentration of root exudates which provide plant/bacterium communication [28–30].

In this study, we investigated the effect of using PHP impregnated with the nitrogen fixing bacteria *Azospirillum brasilense* on grass growth over a 12-week period. We have chosen grass as our model plant because it is possible to harvest it repeatedly over a relatively short period. Furthermore, we have used grass previously in the demonstration of A-PI without the bioactivity of the PHP soil additive [1–4] and compared its performance with that of other polymeric soil additives [2].

2 Materials and methods

This study is presented in two sections: preparation of the SRS media and greenhouse experiments using grass.

2.1 Preparation of polyHIPE polymer (PHP)

2.1.1 Materials

All chemicals needed for the preparation of PHP were purchased from Sigma-Aldrich. They include styrene monomer, divinylbenzene crosslinking agent, sorbitan monooleate (Span 80) surfactant, potassium persulphate initiator for polymerisation and concentrated sulphuric acid (98 wt%) sulphonation agent as well as nano-structuring agent.

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

2.1.2 PolyHIPE polymer preparation

The polymer was prepared using the following method [31], which is conducted in three stages: emulsification, polymerisation and sulphonation. The aqueous phase composition in the emulsification stage consisted of 5 wt% conc. sulphuric acid, 94 wt% deionised water and 1 wt% potassium persulphate. The oil phase was made up of 76 wt% styrene, 14 wt% sorbitan monooleate (non-ionic surfactant, Span 80) and 10 wt% divinyl benzene as crosslinker. The two phases were mixed in a 12-cm diameter mixing vessel equipped with a stirrer with two flat paddles set at right angles close to the bottom of the vessel. The total emulsion volume of one batch was 250 ml of which 90 v% (225 ml) was aqueous phase and 10 v% (25 ml) was oil phase. The oil phase was added to the mixer which was set at 300 rpm. The aqueous phase was added with a peristaltic pump at the rate of 45 ml/min (i.e., dosing time of 5 min), followed by a mixing time of 1 min. The emulsion was then drained from the mixer into 5×50 ml plastic tubes, capped, inverted and placed in an oven at 60°C for 8 h for polymerisation to take place. The polymer was then cut into 4-mm thick discs and then dried overnight in an oven at 60°C.

2.1.3 Sulphonation of polyHIPE polymer

The resulting polymer is hydrophobic but for this application a hydrophilic product was required and this was achieved by sulphonation. Sulphonation was achieved by soaking the discs in 98% sulphuric acid for 2 h, then microwaving in a conventional 1-kW kitchen microwave oven for 30 s for total irradiation time of 150 s with five intervals in a fume cupboard. After 30 s, the door of the microwave oven was opened to allow fumes to escape and to cool and turning the discs over to help obtaining even sulphonation [22]. The sulphonated discs were washed with deionised water for 30 min twice, followed by 60 min soaking in 2.5 N ammonium hydroxide. The pH was then adjusted to 5–7 by adding acetic acid and washing with deionised water to remove any excess nitrogen. During microwave irradiation, PHP discs start swelling. The discs were then dried and cut into approximately 5-mm cubes, indicating that they did not fully reduce back to their original thickness of 4 mm. The resulting product was then ready for use.

2.2 Characterisation of polyHIPE polymer

Fracture surface of dry samples of PHP were examined under a scanning electron microscope (SEM) after gold coating using a Polaron e1500 Sputter Coater. PHP samples recovered at the end of greenhouse experiments were also examined by SEM. These samples contained roots and bacteria. PHP containing biological material were first washed in deionised water and then fixed using 2% glutaraldehyde/phosphate buffer solution, then dehydrated in progressively more concentrated ethanol (sequence of ethanol concentrations were 10%, 25%, 50%, 75% and 100% by weight). Samples were kept at each of the above concentrations for 10 min [16–18]. They were then critical point dried with liquid CO_2, followed by gold coating, the same as the dry polymer samples. Samples were then examined using a Cambridge S240 scanning electron microscope.

2.3 Greenhouse experiments

Grass was used in these experiments. There were four different treatments. Treatment 1, grass; treatment 2, grass+PHP; treatment 3, grass+*Azospirillum brasilense*; treatment 4, grass+PHP soaked with *A. brasilense*. There were four replicates of each treatment. PHP was added to the soil at a level of 0.5% by weight. The pots used held 250 g of soil and therefore 1.25 g of polymer was added to each pot.

2.3.1 Materials

Azospirillum brasilense was obtained from DSMZ Sales (Braunschweig, Germany). The grass was Johnsons Lawn Seed (Worcestershire, UK). The growth medium was nitrogen free nutrient solution. One litre of nutrient solution was made from 200 ml Hoaglands solution, 0.2 g sodium carbonate, 800 ml deionised water, 10 g mannitol and 1 g yeast. The soil mixture used was 75% John Innes No. 3 and 25% horticultural sand.

2.3.2 Inoculation of polyHIPE polymer

After the preparation of nitrogen free nutrient solution, 60 ml aliquots were autoclaved in 250 ml flasks. A starter solution was prepared by inoculating one flask with *A. brasilense* and incubated for 24 h in an orbital shaker at 26°C and 160 rpm. Six flasks were then inoculated with 500 µl from the starter solution. Two flasks of broth were for adding direct to the pots, with no PHP. Four 1.25-g aliquots of PHP were sterilised in universal bottles and added to the other four flasks of growing bacterial broth after 24 h, and then incubated for a further 8 h. At the time

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

of adding the PHP to the broth, the bacteria were approximately halfway through the exponential phase of growth and this proved to be the optimum time for addition of the PHP to get most bacteria into the polymer in the shortest time without damaging the PHP by prolonged shaking on the orbital shaker. The bacterial broth is absorbed into the polymer because of its hydrophilic nature, and then the bacteria continue to proliferate in the polymer for the remaining incubation time. The excess bacterial broth was then removed from the flasks containing PHP and the volume measured (30 ml), indicating that 30 ml of broth had been absorbed by the polymer. Therefore, the remaining broth (30 ml) was added to the pots with PHP, whereas for the pots without PHP 60 ml of broth was added. For the control pots any bacterial broth or PHP was not added.

2.3.3 Preparation for planting and harvesting

The PHP (with or without bacteria) was mixed into the soil then put in 10-cm tall pots with top diameter of 10 cm and bottom diameter of 7 cm. Pots were filled to a height of 7.5 cm with soil. In the case of treatment 3 (grass+*Azospirillum brasilense*), bacterial broth was added onto the surface before planting the seeds. Then, 0.5 g lawn grass seed was added to each pot and covered with a light covering of soil mixture. The experiment was conducted in a greenhouse from July to September and the minimum temperature was 10°C and the maximum 31°C. Plants were watered twice weekly using 50 ml water per pot for each watering session, and therefore there was no water stress. The shoots were harvested at 3-week intervals for 12 weeks after which the roots were washed and weighed.

2.4 Data analysis

Plant yield results were analysed by one-way analysis of variance at 95% confidence interval (a significant result if $p<0.05$) using Minitab statistical software. The standard error bars were added using Excel software.

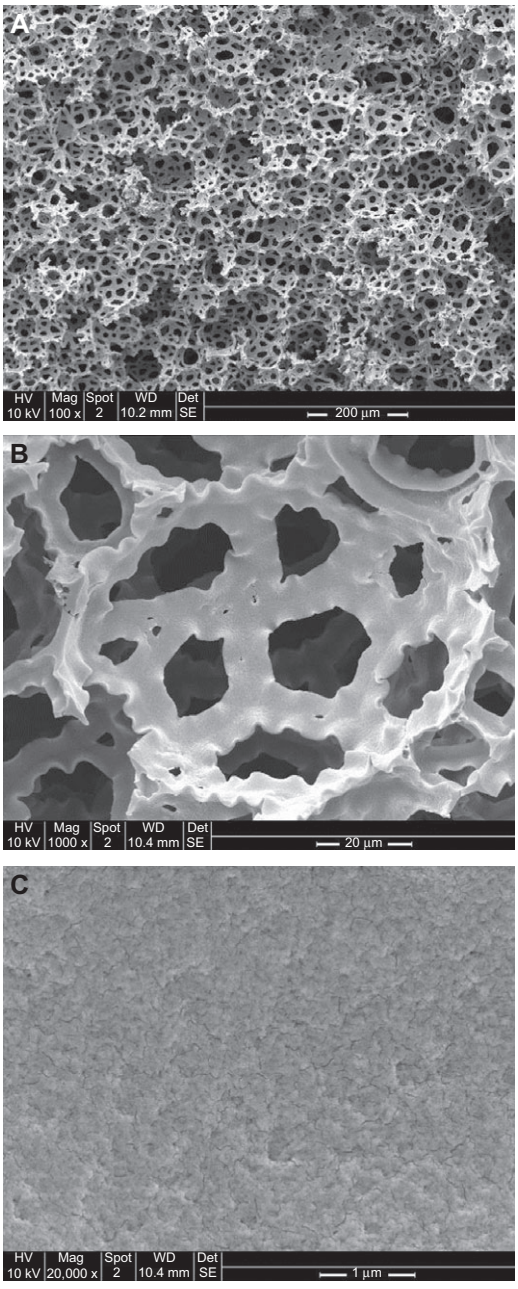

Figure 1 Scanning electron micrographs of the sulphonated-neutralised polyHIPE polymer used as synthetic rhizosphere soil additive in grass growth experiments.

3 Results and discussion

3.1 Characteristics of sulphonated polyHIPE polymer

Figure 1A–C illustrates the PHP used as SRS soil additive at different magnifications. This crosslinked hydrophilic elastic ionic micro-porous material had pore size (D) ranging from 30 to 100 µm with 90% void volume. Sulphonation causes the polymer to swell in water and become elastic, although before sulphonation PHP was not elastic or hydrophilic. Figure 1C illustrates the surface porosity at high magnification. These images will be compared with the images after 12 weeks of sulphonated PHP in the soil to show the changes in the SRS media when roots penetrate into them.

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

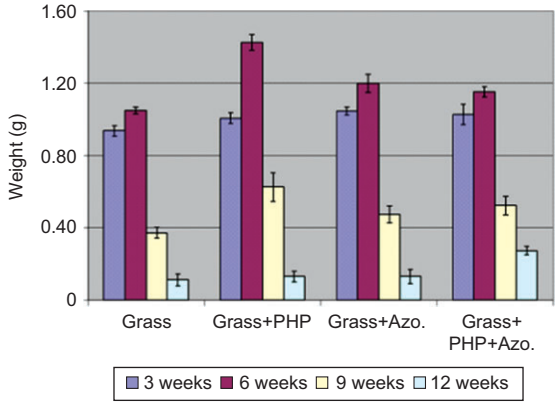

Figure 2 Grass yield under different soil treatment conditions with harvests carried out after 3, 6, 9 and 12 weeks. Treatment 1, grass only; treatment 2, grass+PHP; treatment 3, grass+*Azospirillum brasilense*; treatment 4, grass+PHP with *A. brasilense*.

3.2 Harvest results

The variation of average dry weight per pot for each treatment (T-1 to T-4) as a function of harvest time is shown in Figure 2. In all cases (including the control, T-1), grass yield increased after the first harvest compared with the control but then started falling for the third and fourth harvests. Enhancement of grass yield with respect to control sample (T-1) as a result of PHP or *A. brasilense* addition is clearly observable. The increase of yield in the first harvest for treatments 2, 3 and 4 is not significant compared with the control, treatment 1. The relative enhancements for treatments 2, 3 and 4 with respect to the control treatment as a function of harvest time are tabulated in Table 1.

3.2.1 First harvest of shoots (3 weeks)

After 3 weeks' growth, all treatments produced a positive increase in dry weight compared with the control plants but no results were significant.

3.2.2 Second harvest of shoots (6 weeks)

Plants plus PHP alone produced the biggest increase in dry weight at 36.2%. Other treatments also produced significant increases although plants with PHP+*A. brasilense* produced the lowest increase at 9.5%.

3.2.3 Third harvest of shoots (9 weeks)

The dry weights of the harvested shoots were reduced to less than half of the weights at 6 weeks, but the differences compared with the control treatment continued to increase. The biggest increases were still in the plants plus PHP alone, and plants with PHP plus *A. brasilense* again did not produce such a large increase compared with the control plants.

3.2.4 Fourth harvest of shoots (12 weeks)

After 12 weeks, the dry weights obtained again reduced compared with the first harvest, with some plants only yielding approximately one-tenth of the yield obtained in the first harvest. This could be attributed to the available nutrients in the soil becoming depleted, but a major factor would probably be the lateness of the season when all plant growth is slowing down because of reduced daylight hours and reduced temperature. The final harvest was on 30 September, 2010. Although the weights were much lower, the comparison between the different treatments changed compared with previous harvests. Grass plus PHP alone (T-2) increased by the lowest percentage, at 18.2% compared with the control plants, the same as plants plus *A. brasilense* broth (T-3). Grass plus PHP with *A. brasilense* (T-4) which were previously one of the lowest weights were now very much higher than the others, having increased by 145.4% compared with the control grass (T-1). Figure 3 shows the shoots immediately before

Time of harvesting	Grass+PHP (T-2)		Grass+*A. brasilense* (T-3)		Grass+PHP+*A. brasilense* (T-4)	
	% Increase	p-Value	% Increase	p-Value	% Increase	p-Value
3 weeks	7.4	0.138	11.7	0.024	9.6	0.211
6 weeks	36.2	0.000	14.3	0.032	9.5	0.021
9 weeks	70.3	0.025	29.7	0.110	40.5	0.041
12 weeks	18.2	0.708	18.2	0.744	145.4	0.007

Table 1 Percentage increase of shoot dry weights and their statistical significance levels (p-values) compared to controls which do not have any PHP or bacterium.
Significant increase is present when $p<0.05$.

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

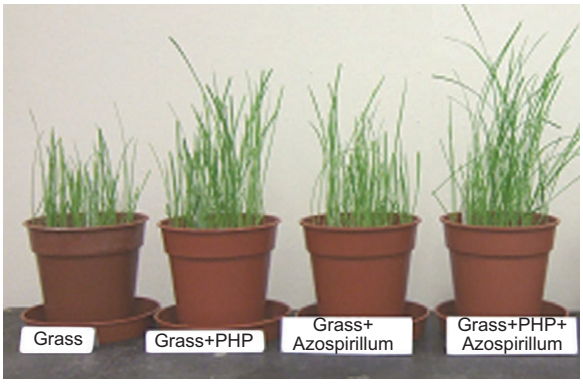

Figure 3 Appearance of grass yield after 12 weeks under different soil treatment. Treatment 1, grass only; treatment 2, grass+PHP; treatment 3, grass+*Azospirillum brasilense*; treatment 4, grass+PHP with *A. brasilense*.

the final harvest at 12 weeks. Grass with PHP+*A. brasilense* showed an obvious physical increase compared with all other treatments.

3.3 Root-SRS media interactions

Roots were tightly packed in the pots and it was difficult to wash all the soil out without also removing some roots. Therefore, an accurate measurement of root weights was not possible. There was extensive penetration into the SRS media by the roots as shown in Figure 4. The SEM image (Figure 4A) of the surface of the PHP shows the extent of root activity around the polymer. Figure 4B, C is an SEM image of the fracture surface which shows the extensive root penetration within the polymer illustrating root, root hair and SRS media interactions. Although the size of the interconnecting holes or the pores are smaller than the root, roots appear to penetrate into PHP. A comparison of the SEM images shown in Figures 1 and 4 (at the same magnification) indicate that the pore structure of the polymer is also modified.

3.4 Grass yield enhancement

In all experiments, there was no water stress (twice weekly watering) at any time. As no additional nutrient was administered during the 12-week growth period, we would expect increasing nutrient stress [1] due to nutrient consumption and removal by water described as antagonistic action previously. The results for all treatments for the first harvest suggest that during the first harvest, the effect of SRS media and *A. brasilense* are not significant due to

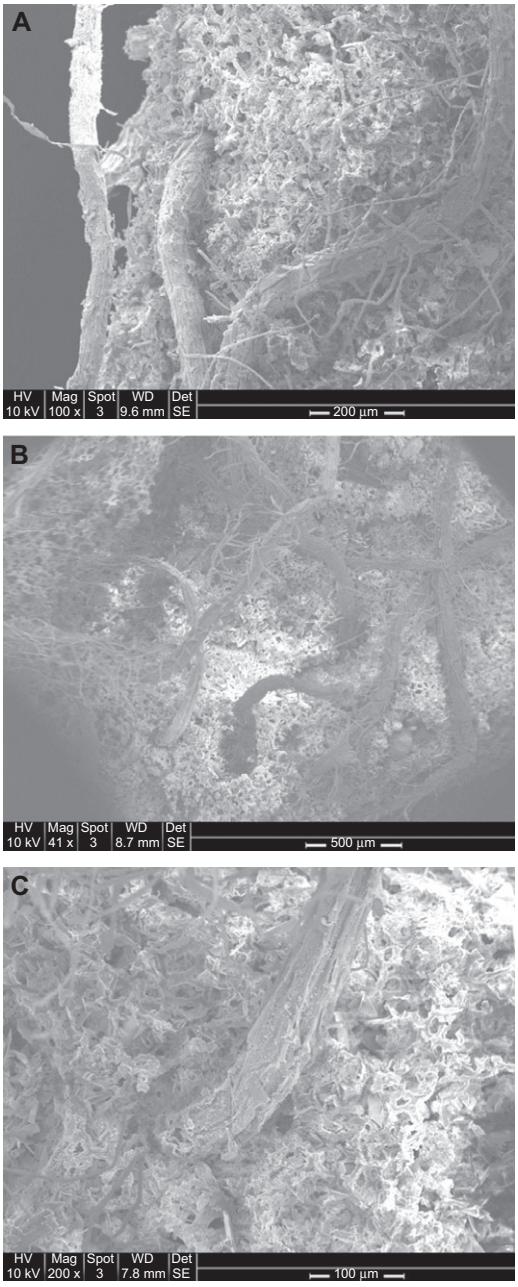

Figure 4 Scanning electron micrographs of the grass root system associated with SRS media in the form of sulphonated-neutralised polyHIPE polymer containing *Azospirillum brasilense* at different magnifications showing the extent of root and root hair penetration into the pores and changes made to the content of the pores when compared with Figure 1. (A) Surface appearance; (B) fracture surface showing the cross-section of the PHP; (C) same as in (B) at higher magnification.

lack of any water and nutrient stress. The effect of SRS media starts showing at the second harvest for treatment 2 (grass+PHP), which suggests that the PHP is acting as a slow release fertiliser capturing any runaway nutrients, similar to observations made previously [1–4]. However,

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

there is no corresponding enhancement in treatment 4 (grass+PHP+*A. brasilense*). This may be due to the fact that nutrients are consumed by bacteria within PHP and that the ionic species in the bacterial broth had already saturated the ionic sites when PHP was inoculated. However by the fourth harvest, in all four treatments, the effect of the SRS media was significantly reduced, suggesting that the nitrogen reserves in the soil and SRS media were becoming depleted and therefore the plant growth rate was reduced. In the case of treatment 4, reduction in yield with time is slower compared with the control or other treatments, and hence at the fourth harvest the yield enhancement was over 145% compared with the control treatment.

When nitrogen is available in the soil or in the polymer, nitrogen fixing bacteria use available nitrogen rather than fix nitrogen from the air, which is a more energy intensive process. The bacteria added in the polymer will compete with the plants for nutrients, including nitrogen, and therefore initially the grass yield was reduced compared with treatment 2. As available nitrogen is used up, bacteria then start to fix nitrogen from the air, some of which ultimately ends up being utilised by the plant following bacterial death and decomposition which results in the release of nitrogen fixed from the air and it becomes available to the plant. It is evident that bacterial nitrogen fixation became significant after the third harvest, which resulted in yield enhancement compared with the control and all other treatments.

3.5 Comparison of SRS media PHP with superabsorbent polymers and biochar

Apart from natural soil additives based on agricultural waste, there are also several other synthetic soil additives such as superabsorbent polymers (SAPs), which are hydrophilic polymers slightly crosslinked to prevent dissolution in water [32–34], coal combustion waste [35] and biochar, which are prepared through the pyrolysis of biomass [36–39].

SAPs can absorb over 500 times their own weight through swelling but this absorption capacity is reduced at least by a factor of 10 when electrolyte solutions (which are present in soil) are used. SAPs essentially form a viscous gel in the presence of water and therefore are mechanically weak and can be washed away from the soil. They are also biodegradable and hence have to be replenished. The greenhouse experiments carried out with a lightly crosslinked polyacrylamide polymer powder (manufactured by D1 Oils, Middlesbrough, UK) under the same conditions showed similar crop yield enhancements to PHP powder over a 6-week period [40].

Biochar is, by contrast, solid and can remain in soil indefinitely. Owing to its hydrophobicity, its function in soil is not water preservation but soil modification [38]. However, despite these claims, the techno-economics of biochar use as means of carbon capture and storage within soil is not sustainable and the claimed benefits for soil fertility enhancement are not universally accepted [39]. This sceptical view (see, for example, [39]) is enhanced because there are no long-term scientific field studies available to prove that biochar does indeed enhance soil productivity. Furthermore, there are no exergy studies [41, 42], which should consider the impact of carbon sequestration through biochar on food, energy and water and thus justifying the burial of a high energy density renewable energy source.

The immediate function of biochar appears to provide micro-nutrients to soil. Nevertheless, such micro-nutrients are also present in ash produced by gasification of biomass in which the energy of biochar is recovered as syngas and carryover carbon is recovered as part of ash [43–45].

Compared with SAPs and biochar, there are significant differences with SRS media PHPs. SRS media can stay in the soil indefinitely as they are non-biodegradable (although they can also be made from biodegradable polymers), their electrolyte uptake is similar to that of SAPs, and like biochar SRS media can contain and indeed capture nutrients from soil. However, the main and most important difference is the ability of SRS media to allow root penetration and enhancement of the interfacial area between the root system and the reactive components in soil. Furthermore, in the case of biologically active SRS media, useful soil bacteria are both protected and brought to close proximity of the root system. None of the other soil additives can provide all these functions simultaneously.

3.6 Environmental impact and sustainability of agro-process intensification

The introduction of A-PI is very recent [1–4] with limited available data. Nevertheless, the basic mechanism of A-PI and the underlying concepts are well understood and applied to various plants under different conditions. It is therefore likely that this technology has general validity based on enhancement of the mass transfer area density and micro-reactor technology using PHPs such as SRS media in the soil. In order to have a large impact on food production and water and fertiliser preservation, PHPs

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

must be used on a large scale leading to ecosystem engineering. Therefore, the environmental impact of the PHP needs to be assessed.

As reported in field trials for biochar [39], the level of biochar addition to soil is approximately 2 kg/m^2. Based on the volume occupied by the soil additive, the expected level of PHP in the soil will be in the range of 0.2–0.1 kg/m^2, which also corresponds to 0.5 wt% PHP in pots with 90–95% phase volume polymer, respectively. A recent review of biochar indicates that the effect of biochar on crop yield enhancement is temporary and reduces over time [39]. This situation is also true for SAPs which need to be replenished periodically. However, SRS media PHPs should not need replacing as their function for water and nutrient regulation as well as bacterial protection should continue for several years. These assumptions should of course be verified through long-term field trials. SRS media PHPs are therefore more useful for perennial plants including wheat which can be used in areas where there is water and nutrient stress [46]. Therefore, investment in SRS media PHPs should be seen as a capital investment rather than as operating cost of crop yield enhancement.

Biodegradation of styrene and polystyrene has been studied extensively. Styrene monomer is biodegradable and is consequently excreted in urine from the body as water soluble metabolites [47]. The metabolic pathways, genetic and physiological aspects of degradation have been well established [48–50]. Although several large epidemiological studies (see, for example, [51]) did not support a link between styrene and cancer, according to the US National Toxicology Programme styrene is reasonably anticipated to be a human carcinogen. Nevertheless, no country lists styrene as carcinogenic and the US NFPA 704 standard classifies (on a scale of 0–4; 0 being least dangerous; i.e., water) styrene as: health hazard=2; flammability=3; reactivity=2; and special notice=none.

Biodegradation rate of polystyrene, by contrast, is very low [52–55], although it is susceptible to catalytic biodegradation [56]. Any health concern for polystyrene and crosslinked and/or sulphonated polystyrene would stem from depolymerisation of the polymer for which there is no evidence. It must be indicated that expanded polystyrene beads are used in horticulture as a perlite substitute [57]. Furthermore, these PHP materials were also used as support for bacteria [18] and mammalian cells [16, 17, 31] in bioprocess intensification and were shown to have no adverse reaction in animals when they were used as bone graft [58]. Graft PHPs could not be distinguished from natural bone because bone cells migrated into the graft and even vascularised it [58]. In the absence of any discernible biodegradation of polystyrene, we can expect SRS media to remain in soil without being replenished periodically and therefore limiting the impact of any unforeseen adverse environmental impact.

4 Conclusions

Unlike artificial fertilisers, biofertilisers inoculated in a carrier such as SRS media PHP are self-sustaining – once they are established they will continue to benefit plants over a prolonged period, whereas artificial chemical fertilisers become depleted and must be continually replaced. Other forms of inoculations of beneficial bacteria must be re-inoculated regularly because they cannot survive in the soil against the competition from other soil bacteria. Because root, root exudates, water, nutrient and bacterial interactions occur within SRS media, fertiliser uptake by the plant is more direct and the diffusion path is in micrometres rather than centimetres. Bacteria responsible for nitrogen fixation and biofertiliser generation are further protected within SRS media and therefore potentially offer a viable sustainable alternative to expensive chemical fertilisers. In addition to acting as a support and protection for useful bacteria, SRS media PHP can deliver soil water and nutrient management simultaneously through a clearly understood mechanism. These attributes distinguish SRS media PHP from all other soil additives.

Acknowledgements: We are grateful for the support from the Engineering and Physical Science Research Council (EPSRC) UK for the PhD studentship to Dr. Steve Fleming and EU FP7 Project POLYCAT for the development and functionalisation of polyHIPE polymers.

Received May 31, 2012; accepted July 23, 2012; previously published online September 27, 2012

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

References

[1] Akay G, Burke DR. *International Patent Publication* PCT WO/2010/040996, 2010.
[2] Burke DR, Akay G, Bilsborrow P. *J. Appl. Polymer Sci.* 2010, 118, 3292–3299.
[3] Akay G, Fleming S. *WIT Trans. Ecol. Environ.* 2011, 144, 485–495.
[4] Akay G, Burke DR. *Am. J. Agric. Biol. Sci.* 2012, 7, 150–172.
[5] Aldhous P. *New Scientist* 2008, 197, 28–31.
[6] Conway G, Toenniessen G. *Nature* 1999, C55–C58.
[7] Tilma D, Cassman KG, Matson PA, Naylor R, Polasky S. *Nature* 2002, 418, 671–677.
[8] Van der Heijden MGA, Bardgett RD, VanStraalen NM. *Ecol. Lett.* 2008, 11, 296–310.
[9] Smith SE, Read DJ. *Mycorrhizal Symbiosis*, 2nd ed., Academic Press: London, 1997.
[10] Makoi JHJR, Ndakidemi PA. *Afr. J. Microbiol. Res.* 2009, 3, 664–675.
[11] Andrews M, Hodge S, Raven JA. *Ann. Appl. Biol.* 2010, 157, 317–320.
[12] Abraham J, Pillai VNR. *J. Appl. Polymer Sci.* 1996, 60, 2347–2351.
[13] Puoci F, Iemma F, Spizzirri UG, Cirillo G, Curcio M. *Am. J. Agric. Biol. Sci.* 2008, 3, 299–314.
[14] Akay G. In *Encyclopedia of Chemical Processing*, Lee S, Ed., Marcel Dekker: New York, 2006, pp. 183–199.
[15] Kumar V, Nigam KDP. *Green Process Synth.* 2012, 1, 79–107.
[16] Akay G, Birch MA, Bokhari MA. *Biomaterials* 2004, 25, 3991–4000.
[17] Bokhari MA, Akay G, Zhang S, Birch MA. *Biomaterials* 2005, 26, 5198–5208.
[18] Akay G, Erhan E, Keskinler B. *Biotechnol. Bioeng.* 2005, 90, 180–190.
[19] Akay G, Pekdemir T, Shokorfow AS, Vickers J. *Green Process. Synth.* 2012, 1, 109–127.
[20] Akay G, Vickers J. *US Patent* 7780854, 2011; *US Patent* 8177985, 2012.
[21] Akay G. *International Patent Publication* PCT WO/2012/025767, 2012.
[22] Akay G, Noor ZZ, Calkan OF, Ndlovu TM, Burke DR. *US Patent* 07820729, 2010.
[23] Takahashi N, Yamazaki Y, Kobayashi A, Higashitani A, Takahashi HH. *Plant Physiol.* 2003, 132, 805–810.
[24] Eapen D, Barrosso ML, Ponce G, Campos ME, Cassab GI. *Trends Plant Sci.* 2005, 10, 44–50.
[25] Miyazawa Y, Takahashi H. *Plant Signal Behav.* 2007, 2, 388–389.
[26] Sbrana C, Giovannetti M. *Mycorrhiza* 2005, 15, 539–545.
[27] Leitner D, Klepsch S, Bonder G, Schnepf A. *Plant Soil* 2010, 332, 177–192.
[28] Dakora FD, Phillips DA. *Plant Soil* 2002, 245, 35–47.
[29] Bais HP, Weir TL, Perry LG, Gilroy S, Vivaco JM. *Annu. Rev. Plant Biol.* 2006, 57, 233–266.
[30] Carvalhais LC, Dennis PG, Fedoseyenko D, Hajirezael MR, Borriss R, von Wiren N. *J. Plant Nutr. Soil Sci.* 2011, 174, 3–11.
[31] Akay G, Bokhari AM, Byron VJ, Dogru M. In *Chemical Engineering Trends and Developments*, Galan MA, Del Valle EM, Eds., Wiley: London, 2005.
[32] Xie J, Liu X, Liang J, Luo Y. *J. Appl. Polym. Sci.* 2009, 112, 602–608.
[33] Andry H, Yamamoto T, Irie T, Moritani S, Inoue M, Fujiyama H. *J. Hydrol.* 2009, 177–183.
[34] Dorraji SS, Golchin A, Ahmadi S. *Clean Soil Air Water* 2010, 38, 584–591.
[35] Dunker RM, Adriano DC, Koo BJ, Barton CD, Punshon T. In *Chemistry of Trace Elements in Fly Ash*, Sajwan KS, Alva AK, Keefer RF, Eds., Kluwer Academic/Plenum Publishers: New York, 2003, p. 319.
[36] Laird DA. *Agronomy J.* 2008, 100, 178.
[37] Cross A, Sohi SP. *Soil Biol. Biochem.* 2011, 43, 2127–2134.
[38] Lehmann J, Gaunt J, Rondon M. *Mitig. Adapt. Strat. Global Change* 2006, 11, 403–427.
[39] Anon. *Biochar: A Critical Review of Science and Policy* (funded by Wallace Global Fund). Available at: http://www.biofuelwatch.org.uk/biocharsciencereport.php, Biofuelwatch, November 2011.
[40] Burke DR. *PhD thesis* (supervisor: Akay G), Newcastle University: Newcastle upon Tyne, UK, 2009.
[41] Dincer I, Cengel YA. *Entropy* 2001, 3, 116–149.
[42] Dincer I, Rosen MA. *Exergy: Energy, Environment and Sustainable Development,* Elsevier, 2007.
[43] Akay G, Jordan AC. *Energy Fuel* 2011, 25, 2274–2283.
[44] Jordan CA, Akay G. *Fuel* 2012, 91, 253–263.
[45] Jordan CA, Akay G. *Biomass Bioenergy* 2012, 42, 51–58.
[46] Glover JD, Reganold JP, Bell LW, Borevitz J, Brummer EC, Buckler ES, Cox CM, Cox TS, Crews TE, Culman SW, DeHan LR, Eriksson D, Gill BS, Holland J, Hu F, Hulke BS, Ibrahim AMH, Jackson W, Jones SS, Murray SC, Paterson AH, Ploschuk E, Sacks EJ, Snapp S, Tao D, Van Tassel DL, Wade LJ, Wyse DL, Xu Y. *Science* 2010, 328, 1638–1639.
[47] Wigaeus E, Lof A, Nordqvist MB. *Br. J. Ind. Med.* 1984, 41, 539–546.
[48] O'Leary ND, O'Connor KE, Dobson ADW. *FEMS Microbiol. Rev.* 2002, 26, 403–417.
[49] Francesc X, Boldu P, Summerbell R, Hoog GSD. *FEMS Microbiol. Rev.* 2006, 30, 109–130.
[50] Gaszezak A, Bartelmus G, Gren I. *Appl. Microbiol. Biotechnol.* 2012, 93, 565–573.
[51] Anon. *CLH Report for Styrene*. Danish Environmental Protection Agency, EC No.: 202-851-5, CAS No.:100-42-5; Index No.: 601-026-00-0, September 2011.
[52] Kaplan DL, Hartenstein R, Sutter J. *Appl. Environ. Microbiol.* 1979, 38, 551–553.
[53] Otake Y, Kobayashi T, Ashabe H, Murakami N, Ono K. *J. Appl. Polym. Sci.* 1995, 56, 1789–1796.
[54] Shah AA, Hasan F, Hameed A, Ahmed S. *Biotechnol. Adv.* 2008, 26, 246–265.
[55] Atiq N, Ahmed S, Ali MI, Andleeb S, Ahmed B, Robson G. *Afr. J. Microbiol. Res.* 2010, 4, 1537–1541.
[56] Meekum U, Kenharaj R. *Suranaree J. Sci. Technol.* 2004, 11, 223–229.
[57] Cole JC, Dunn DE. *J. Environ. Hort.* 2002, 20, 7–10.
[58] Akay EM, Okumus Z, Yildirim OS, Bokhari MA, Akay G. *WIT Trans. Biomed. Health* 2011, 15, 383–394.

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM

Prof. Akay holds the established chair of Chemical and Process Engineering at Newcastle University since Jan 1998 and heads the 'Process Intensification and Miniaturisation' Research Group. He has a multidisciplinary research and education (all degrees from Manchester University) covering Chemical Engineering (BSc, MSc), Mathematics (PhD), Chemistry (Dozent). While at Unilever Research Laboratory (Port Sunlight) during 1983–1994, he pioneered the processing of PolyHIPE Polymers and after discovering 'flow induced phase inversion' and 'confinement' phenomena and applying them to Chemical Process Intensification which resulted in some 40 patents still utilised by Unilever. Later in academia, through collaboration with biologists, medical and agricultural scientists, he pioneered Process Intensification in energy conversion, as well as BioProcess and AgroProcess Intensifications based on the 'Confinement Phenomenon'. These intensified processes were underpinned with over 35 patents and patent applications in the last 12 years.

His work on Process Intensification in energy conversion processes resulted in the World's first intensified-integrated power generation plant from biomass proving that such plants do not have the burden of 'economies of scale'. This technology is now commercialized by the Newcastle University spin-out company ITI Energy. More recently, following the demonstration of the confinement phenomenon through tissue engineering, he developed intensified processes in biomass based energy/chemicals processing, catalyst synthesis, biotechnology and agriculture partly based on the use of his nano-structured micro-porous materials. In the last 4 years, he has been conducting full time research and technology transfer activities via two Newcastle University spin-out companies, ITI Energy and GAP Technologies which are based on the IPR generated in his research group. His interest in medicine led him to the formation of the medical research charity UK Children's Neurological Research Campaign with several medical academics and hospital consultants. He is a founding trustee of UKCNRC.

Dr Steven Fleming recently completed a PhD at Newcastle University entitled 'Agroprocess Intensification: the use of Poly High Internal Phase Emulsion Polymer to enhance crop production', and is now employed by GAP Technologies Ltd. His first degree was in microbiology at Edinburgh University after which he became an arable farmer in southern Scotland. Recently he completed a Masters in Brewing and Distilling at Heriot-Watt University, then a Masters in Biotechnology at Abertay University. He continues to maintain an interest in the farm, growing barley, wheat, beans, potatoes and grass.

Brought to you by | Brown University Rockefeller Library
Authenticated | 128.148.252.35
Download Date | 9/18/13 9:35 PM