PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Prospects for the Exploitation of Biological Nitrogen Fixation [and Discussion]

J. R. Postgate, M. O. Moss and S. P. S. Andrew

Phil. Trans. R. Soc. Lond. B 1980 **290**, 421-425
doi: 10.1098/rstb.1980.0105

Phil. Trans. R. Soc. Lond. B **290**, 421–425 (1980) [**421**]
Printed in Great Britain

Prospects for the exploitation of biological nitrogen fixation

By J. R. Postgate, F.R.S.
A.R.C. Unit of Nitrogen Fixation, University of Sussex,
Brighton, BN1 9RQ, U.K.

Agricultural productivity is largely determined by the availability of nitrogen, added as fertilizer or introduced by microbial nitrogen fixation. A doubling of the World's population early in the next century will require a doubling of the effective agricultural nitrogen input. Economic and environmental constraints will probably preclude multiplying the input of industrial nitrogen fertilizer appropriately; so biological fixation, which is an exclusively microbiological process, must be exploited more effectively.

Short term prospects include the expanded use of existing systems, such as exotic legumes, grasses and woody symbioses. In the medium term, plant breeding and genetic manipulation of the appropriate bacteria should yield more effective symbioses. In the long term, new nitrogen-fixing systems might be developed: somatic hybridization of plants might yield new symbiotic systems; manipulation of genetic information for nitrogen fixation into the plant genome might yield plants able to fix nitrogen independently of bacteria.

INTRODUCTION

The paramount importance of the nitrogen input in determining the productivity of the World's agriculture has excited, among scientists, widespread interest in nitrogen fixation. The numerous recent publications in the research area of nitrogen fixation have included many that discuss prospects for the exploitation of the biological process. Examples include: Brill (1977); Brown *et al.* (1975); Evans (1975); Gutschick (1977, 1978); Hardy (1976, 1977); Postgate (1977, 1980); Wittwer (1977); this list is far from exhaustive. Since discussion of this topic has been extensive in recent publications, and my own views have been given in detail, I shall restrict this presentation to a *précis* of the current position.

BACKGROUND

The information that lies at the root of present day activity in this research area is the knowledge that the productivity of World agriculture is determined by the input of nitrogen. This is not true of uncultivated land: in well established, natural 'climax' or 'equilibrium' eco-systems, other nutrients (phosphorus, potassium, sulphur etc.) may limit biomass production. But very soon after such areas are disturbed and, in particular, are brought under cultivation, or are exploited for forestry or pasture, the eco-systems become nitrogen-limited, and nitrogen-fertilizer must be added. Postgate (1980) surveyed nitrogen inputs into terrestrial soils and gave references, which will not be repeated in detail here; Svensson & Soderlund (1976) and a popular symposium report (Arrhenius 1977) gave concise quantitative data on the nitrogen cycle, including its role in agriculture. There is widespread agreement that the exponential growth of the World's population in the twentieth century has largely been supported by increased nitrogen-input into the World's agricultural soils, and an increasing proportion of that input is in the form of chemical nitrogen-fertilizer manufactured by the fertilizer industry.

As the 1980s approach, about a third of the World's population depends on chemical fertilizer for its food. Even if all projects for population control are successful, the World's population will double from the present figure of *ca.* 4×10^9 by the early decades of the next millenium. To feed that population, the nitrogen input into the World's agricultural products must also double.

CHEMICAL AND BIOLOGICAL NITROGEN INPUTS

Nitrogen inputs are part of the biological nitrogen cycle, which is described in many textbooks of microbiology or plant physiology. As in all the cycles of the major biological elements, microbes play a dominant role. Most of the nitrogen-input into the land and waters is in the form of recycled inorganic nitrogen, as nitrate or ammonia: nitrogen originating from the excretions or decomposition of living things. But a net loss of nitrogen to the atmosphere occurs, largely as a result of biological denitrification, the nitrogen becoming, as gaseous N_2, inaccessible to plants and animals. The compensating conversion of N_2 to forms available to the biosphere (the process called nitrogen fixation) takes place by three major routes.

Spontaneous nitrogen fixation is the formation of oxides of nitrogen in the atmosphere as a result of combustion (forest fires, the internal combustion engine), electric discharges (e.g. lightning) and u.v. irradiation. These nitrogen oxides become washed into soil and waters as nitrites and nitrates.

Industrial nitrogen fixation is the production of nitrogen fertilizer from N_2 by the chemical industry. The Haber–Bosch process, which is the reduction of N_2 to NH_3 with H_2, has effectively superseded earlier oxidative processes.

Biological nitrogen fixation is the conversion of N_2 to NH_3, and thence cell material, by prokaryotic microbes (bacteria, including actinomycetes and blue–green algae). Eukaryotes, from fungi to plants and animals, are unable to conduct the reaction. Ecologically and agriculturally, the most important systems are those in which nitrogen-fixing bacteria form symbiotic associations with plants such as legumes.

Quantitative estimates of the turnover of the nitrogen cycle, and the nitrogen input by nitrogen fixation, are subject to considerable error because estimates of the contributions of the various routes differ greatly in their precision. Recent estimates are converging on a global nitrogen input by nitrogen fixation approaching 2.4×10^8 t/a, distributed as two-thirds biological input, one quarter as industrial nitrogen fertilizer and the remainder supplied by spontaneous chemical processes. 'New' nitrogen introduced by fixation represents almost one tenth of the nitrogen incorporated into terrestrial biomass annually (*ca.* 3×10^9 t nitrogen per year).

CHEMICAL INPUTS IN THE FUTURE

In principle, it ought to be possible to increase the output of the fertilizer industry so as to provide a nitrogen content in the World's agricultural crops adequate for a population of *ca.* 8×10^9. It is important to emphasize that this action might require as much as a *quadrupling* of the global nitrogen fertilizer production because, under present day agricultural practices, only about 50 % of added fertilizer nitrogen reaches the crop and the rest leaches into subsoil or runs off into waterways and aquifers. The problems of increasing global nitrogen fertilizer production are immense and take four forms:

1. The capital cost and technical sophistication of a Haber plant requires an industrialized and highly developed society; the building and staffing of the number apparently required for the 21st century is a daunting prospect.

2. The major need for nitrogen fertilizer is in developing countries, so transport costs of fertilizer will be high.

3. The Haber process consumes fossil energy, being based on natural gas, and competition with other demands for energy will increase the real cost of nitrogen fertilizer and hence of food.

4. Environmental costs resulting from leaching and run off range from mild pollution problems to threats of carcinogenesis and damage to the ozone layer (see Arrhenius 1977). Some of these threats may be exaggerated, but the problem of nitrate contamination of drinking water supplies, with concomitant medical consequences, seems real.

Many of these problems could, in principle, be solved. Redistribution of fertilizer use, partly by changed agricultural practice, partly by inhibiting microbial nitrification, could minimize run-off; development of low technology, low energy, catalytic processes for nitrogen fixation (see, for example, Treharne *et al.* 1978) could overcome some of the problems arising from the technological sophistication of the Haber process, with its energy and transport costs. But new developments must arise in chemistry, biochemistry and, indeed, politics before enhanced chemical nitrogen fertilizer production is accepted as a complete solution to the World's immediate food problems.

Biological inputs in the future

A more realistic contribution to the global nitrogen input into agricultural soils ought to be possible through the increased exploitation of biological nitrogen-fixing systems. Legumes (e.g. clover in grassland, grain legumes as food or fodder) are already used in agriculture, and faster expansion of World production of grain legumes compared with World production of cereals was recommended as an immediately applicable policy by Hardy (1977). Means of exploiting the biological process to decrease the demand for chemical fertilizer range from short-term and reasonably practicable projects to long-term proposals that require extensive research; all involve microbiology and its applications; many involve plant science in various forms. The following are examples of means that have been discussed.

1. Increased use of unfamiliar legumes, for example, chickpeas or soya protein in human food, lupins in ruminant nutrition. Wider use of lucerne (alfalfa) as fodder and/or green manure. Exploitation of non-leguminous nitrogen-fixing symbioses (e.g. Purshia, scrub alders) as fodder or to up-grade land. Use of nitrogen-fixing microbes themselves as fodder.

2. Survey of lesser known natural nitrogen-fixing systems (e.g., grass associations, algal associations) for microbes that might become exploitable when coupled with appropriately bred crop plants.

3. Augmentation, by genetic manipulation, of the effectiveness of existing systems. For example, presence of the genes for hydrogen uptake (*hup*) in rhizobia increases the nitrogen-fixing efficiency of the symbioses (see, for example, Evans *et al.* 1978). Mutant bacteria that escape regulation of nitrogen fixation (*nif*) genes by ammonia exist; they continue to fix nitrogen

in the presence of fixed nitrogen. The *nif* gene cluster has been transferred from *Klebsiella pneumoniae* to various new hosts and might in time be transferred to useful plant or animal symbionts, thus generating new exploitable systems.

4. Somatic hybridization might be used to generate new types of plant that combine agricultural desirability with ability to form a nitrogen-fixing symbiosis.

5. A *nif* gene cluster, or relevant portions thereof, might be introduced into the genome of crop plants such as cereals, together with other genetic information necessary for expression, making them independent of microbes and of chemicals as far as nitrogen input is concerned. Numerous problems of localization, expression, regulation and stabilization of *nif* in the new host, not to mention problems of protection of the gene products from oxygen damage, would have to be overcome, but discussions of ways to do this have revealed no fundamental reasons why it should not be done.

Examples 1 and 2 above are short-term projects; those encompassed by item 3 involve more detailed research and are in the medium term range; examples 4 and 5 are clearly long-term projects the real potential of which cannot be fully evaluated with present knowledge.

Conclusions

Neither increased nitrogen fertilizer nor enhanced biological nitrogen fixation will provide the sole solution to mankind's escalating demand for agricultural nitrogen in the next few decades. Redistribution and more careful use of existing fertilizer production, together with increased production, will go some way to meeting World needs. However, environmental, economic and political constraints on fertilizer use compel the investigation and exploitation of existing and conjectural biological processes. These present challenging problems in microbiology and plant science at the levels of biochemistry, ecology, genetics and physiology.

References (Postgate)

Arrhenius, E. 1977 Nitrogen, a special issue. *Ambio* (*Stockholm*) **6**, 95–182.

Brill, W. J. 1977 Biological nitrogen fixation. *Scient. Am.* **236** (3), 68–81.

Brown, A. W. A., Byerly, T. C., Gibbs, M. & San Pietro, A. (ed.) 1975 *Crop productivity ... Research imperatives.* Yellow Springs, Ohio: Charles F. Kettering Foundation.

Evans, H. J. (ed.) 1975 *Enhancing biological nitrogen fixation.* Washington, D.C.: National Science Foundation.

Evans, H. J., Emerich, D. W., Ruiz-Argüeso, T., Albrecht, S. L., Maier, R. J., Simpson, F. & Russell, S. A. 1978 Hydrogen metabolism in legume nodules and rhizobia: some recent developments. In *Hydrogenases: their catalytic activity, structure and function* (ed. H. G. Schlegel & K. Schneider), pp. 287–306. Gottingen: Erich Goltze.

Gutschick, V. P. 1977 Long term strategies for supplying nitrogen to crops. *Los Alamos Sci. Lab. Rep.* LA-6700-MS. Los Alamos, U.S.A.

Gutschick, V. P. 1978 Energy and nitrogen fixation. *Bioscience* **28**, 571–575.

Hardy, R. W. F. 1976 In *Proceedings of the 1st international symposium on nitrogen fixation* (ed. W. E. Newton & C. J. Nyman), vol. 2, pp. 693–717. Pullman, Wisconsin: Washington State University Press.

Hardy, R. W. F. 1977 Increasing crop productivity: agronomic and economic considerations on the role of biological nitrogen fixation. In *Report of the public meeting on genetic engineering for nitrogen fixation* (ed. A. Hollaender), pp. 77–106. Washington, D.C.: National Science Foundation.

Postgate, J. R. 1977 Possibilities for the enhancement of biological nitrogen fixation. *Phil. Trans. R. Soc. Lond.* B **281**, 249–260.

Postgate, J. R. 1980 The nitrogen economy of marine and land environments. In *Food chains and human nutrition* (ed. Sir K. Blaxter), pp. 161–185. London: Applied Science Publishers.

Svensson, B. H. & Soderlund, R. 1976 Nitrogen, phosphorus and sulphur-global cycles. Scope report 7. *Ecol. Bull.* (*Stockholm*) **22**, 23-73.

Treharne, R. W., Bruce, M. R., Moles, D. R. & McKibben, C. K. 1978 A nitrogen fertilizer generator for farm use. *Technical note* 1. Yellow Springs, Ohio: Charles F. Kettering Research Laboratories.

Wittwer, S. W. 1977 Agricultural productivity and biological nitrogen fixation – an international view. In *Genetic engineering for nitrogen fixation* (ed. A. Hollaender), pp. 515–519. New York and London: Plenum Press.

Discussion

M. O. Moss (*Department of Microbiology, University of Surrey, Guildford, Surrey, U.K.*). Since nitrogen fixation has not evolved in higher plants, does this not suggest that the amount of fixed nitrogen might be a control element in the stability of the biosphere?

J. R. Postgate. I do not see a mechanism by which this could work. It is not the supply of fixed nitrogen, but other factors, such as phosphate or pH, that are growth-limiting for climax plant populations. Nitrogen only becomes limiting if the system is already disturbed, for example by agriculture. The use of the Haber process of nitrogen fixation will only speed up the nitrogen cycle and does not affect the overall nitrogen balance.

M. O. Moss. Can we really go on increasing agricultural productivity indefinitely without upsetting the biosphere?

J. R. Postgate. Of course we can not. We are all agreed that the population cannot go on increasing indefinitely. However, as the population is bound to increase in the next generation, we should, at least, try to provide food for them.

S. P. S. Andrew, F.R.S. (*I.C.I. Agricultural Division, Billingham, Teesside*). Can I suggest a different approach to the nitrogen problem, namely the use of poisons to kill the nitrifying bacteria that reoxidize ammonia. The ammonia would be provided by the Haber process, which is the most efficient method of nitrogen fixation.

J. R. Postgate. Yes, an inhibitor of nitrifying bacteria is commercially available for application with nitrogen fertilizers. However, I cannot agree that there is nothing more efficient than the Haber process; for instance, *Azotobacter* fixes nitrogen more efficiently.

S. P. S. Andrew. But the Haber process is 75% thermodynamically efficient!

J. R. Postgate. These comparisons of efficiency inevitably depend on how one does the sums. In any case, there is a need for improved biological nitrogen fixation for use in areas where the high technology of the Haber process is not feasible, given the high transport costs of fertilizer. The Haber process is also expensive on energy.