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Biotechnology for the Environment in the Future

SCIENCE, TECHNOLOGY AND POLICY

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FOREWORD

This paper builds and draws on a workshop funded by the governments of Italy and Japan held on 16-17 September 2010 in Rimini (Italy), see Annex 1. This stream of work was developed by the Working Party on Biotechnology (WPB) following a proposal made by Japan to examine the links across the innovation cycle and how to make these links more effective and efficient, relating to the translation of research into products (and vice versa) in the field of environmental biotechnology.

The report was prepared by James Philp (OECD Secretariat). The report draws on earlier work carried out by other members of the OECD Secretariat who deserve special thanks, Iain Gillespie, Alexandre Bartsev and Robert Wells. Further support was given by the workshop facilitator, Professor Joyce Tait.

The Committee for Scientific and Technological Policy (CSTP) agreed to the declassification of this report in December 2012.

The report is published under the responsibility of the Secretary-General of the OECD.

INTRODUCTION

Growing concerns about the lack of environmental sustainability of past economic growth patterns, and increased awareness of a potential future climate crisis, have made it clear that the environment and the economy can no longer be considered in isolation from one another. At the same time, the financial and economic crisis has provided the stimulus for policy interventions aimed at encouraging recovery and renewed growth on more sustainable grounds.

A strategic vision is necessary to ensure that the policies that governments implement are the most appropriate from an economic efficiency, environmental integrity and social equity point of view, as well as being coherent at both a national and an international level. As noted in the OECD Innovation Strategy (OECD, 2010b), a return to sustained and sustainable growth will depend upon innovation that delivers a much greener growth model than hitherto. Environmental and industrial applications of biotechnology could underpin such innovation and there is a clear need now to ensure that the policy measures in place are appropriate for fostering developments that truly deliver on green growth.

So the question arises as to what – if anything – needs to be done to improve the ability of innovation systems to harness the potential of biotechnology for environmental and industrial applications in ways that deliver against the range of challenges facing our future sustainable growth?

There are at least two policy regimes to be considered, one for environmental biotechnology, and another for industrial biotechnology. Environmental biotechnology is focused on biotechnologies for environmental clean-up, and many of the policy tools used in this area are about compliance. The materials being treated are "waste" materials (contaminated soil and water) and have no intrinsic value as such. Therefore the industries that make them have no economic interest in treating them because the treatment does not give profit to the companies. On the contrary, treating wastes is a significant financial burden for companies and historically they would not treat them unless made to do so. When the companies are made to treat these wastes, as a result of the polluter pays principle and other legislative/regulatory mechanisms, their interest is in achieving compliance, and they will usually treat the wastes according to the level demanded by regulation.

Industrial biotechnology, on the other hand, has quite different policy objectives. Industrial biotechnology only started to grow as a field with the world-wide interest in biofuels. Much of the world now has targets for bioenergy and favourable policy regimes to stimulate production and use of biofuels. But sustainability is now a real issue for biofuels production, and an appropriate balance between promoting biofuels and ensuring sustainability is a target for many governments.

The other industrial biotechnology products, *i.e.* biobased chemicals and bioplastics, are less subject to policy intervention compared to biofuels. Given that the integrated biorefineries of the future will use a range of (sustainable) biomass sources, and will, however, be expected to produce fuels, chemicals and plastics, there is a strong case for biobased chemicals and bioplastics to have a supportive policy portfolio. When companies are involved in making products for environmental improvements using biobased plastics, biobased chemicals or biofuels, the set of policies is more to do with making those products attractive to the consumer and ensuring that the infrastructure is in place to make the production happen rather than based on compliance or regulation.

Whilst genetically modified (GM) crops should be seen strictly as the domain of agricultural biotechnology, at some point in the future, GM non-food crops will be considered as a feedstock for industrial biotechnology. Therefore this report also examines some of the high-level issues involved.

In order to examine these questions, the OECD held a workshop on "Biotechnology for the Environment in the Future: Science, Technology and Policy" on 16-17 September 2010 in Rimini (Italy). This report sets out some policy options that arose from the discussions at the workshop. It fulfils the obligation under intermediate output result 1.3 of the Programme of Work and Budget (PWB) 2011-2012. The workshop covered a range of different biotechnologies for environmental improvement that will result in at least two different sets of policies. Therefore the remit of the workshop was rather broad.

This report focuses mainly on two different categories of technologies, environmental biotechnology and industrial biotechnology. It starts with environmental clean-up technologies and the policies that have influenced their development, particularly landfill taxation and risk assessment. Bioremediation is then specifically dealt with as one of several competing contaminated land and water clean-up technologies.

The report progresses to look at industrial biotechnology: biofuels, biobased chemicals and bioplastics, and identifies the drivers and policies influencing their development. The policy regime affecting biobased chemicals and bioplastics is weak compared to biofuels and some suggestions are made to try to address this.

An emerging field is eco-genomics, which potentially offers a platform for research and development in environmental biotechnologies, such as bioremediation. For example, genomics applied to contaminated soil may be used to determine the presence of useful micro-organisms and/or enzymes present in the soil that might indicate treatability using bioremediation techniques. Therefore the report examines eco-genomics, with a particular focus on genetic modification (GM) issues for environmental and industrial biotechnology.

The report concludes with a suggested future "Rimini Agenda", whereby the OECD plays a role in identifying a more supportive policy and regulatory environment. The report is accompanied by the agenda for the workshop, and there are four Appendices containing supplementary material that expand on the content of the report whilst freeing the main body from detail.

AN OVERVIEW OF ENVIRONMENTAL CLEAN-UP TECHNOLOGIES

Industrial wastewater and toxicity biosensors

Toxicity is an issue in wastewater treatment for at least two reasons: toxicity of treated wastewaters to humans and the environment (eco-toxicity); and toxic materials in industrial wastewaters can poison the microorganisms that perform the treatment, thereby partially or totally destroying the treatment and causing regulatory compliance failure.

For at least two decades there has been a drive towards identifying routine and inexpensive methods for determining toxicity of industrial wastewater to move away from expensive and controversial animal testing. The biosensors that have had the greatest exposure are based on bioluminescence. Much of the following discussion on toxicity within the framework of wastewater treatment has been adapted from Philp *et al*. (2005).

Legislation on the toxicity monitoring of wastewater

In the European Union, under the Urban Wastewater Treatment Directive (91/271/EEC), the functioning of wastewater treatment works is considered important and reaching non-toxic levels is of primary concern (Farré & Barceló, 2003). In common with most legislation in the area, the Directive is directed towards regulation of discharges to the environment, rather than to the wastewater treatment plant (WWTP) itself. The more recent EU Water Framework Directive, 2000 (2000/60/EC) is mostly concerned with protecting receiving waters from pollution and toxic discharges.

The Integrated Pollution Prevention and Control Directive, adopted in 1996 (96/61/EC) is one of the cornerstones of the European Union's environmental legislation. It is likely that financial penalties based upon toxicity levels, in addition to the current physico-chemical parameters, will be presented to companies discharging industrial effluents at concentrations above guidelines (dos Santos *et al*., 2002).

Industrial effluents tend to contain more toxic substances than domestic wastewater, and numerical limits are set for discharges to ensure compliance with Environmental Quality Standards (EQSs). Compliance is then monitored by chemical analysis. There are several problems with this approach:

- There are many substances for which there is no EQS (over 99%);
- There are no eco-toxicological data available for many thousands of chemicals, and most EQS's are based on limited data;
- Difficulties are experienced predicting the interaction of chemicals with each other and the subsequent effect on the environment; and
- There are analytical difficulties for many chemicals and great cost in separating and identifying all the constituents.

Research into setting discharge consents based on toxicity began in the early 1990s. This became known as Direct Toxicity Assessment (DTA). DTA provides a direct measure of acute toxicity and it is not necessary to identify the substances causing effluent toxicity to treat or reduce it *i.e.* the property can be measured and treated directly. If toxicity is detected but considered acceptable, a toxicity consent can be derived and applied. If the toxicity of the effluent is unacceptably high, however, remedial action may be needed and then a toxicity consent can be derived. In the United States, a similar process is applied to discharges to receiving waters, and the process is called Whole Effluent Toxicity (WET).

Policy framework for contaminated land clean-up

To understand the position of bioremediation as a technology for clean-up of contaminated land and groundwater, it is necessary to place it within the larger context of contaminated land policy. Bioremediation faces policy challenges that can be broadly placed in two categories:

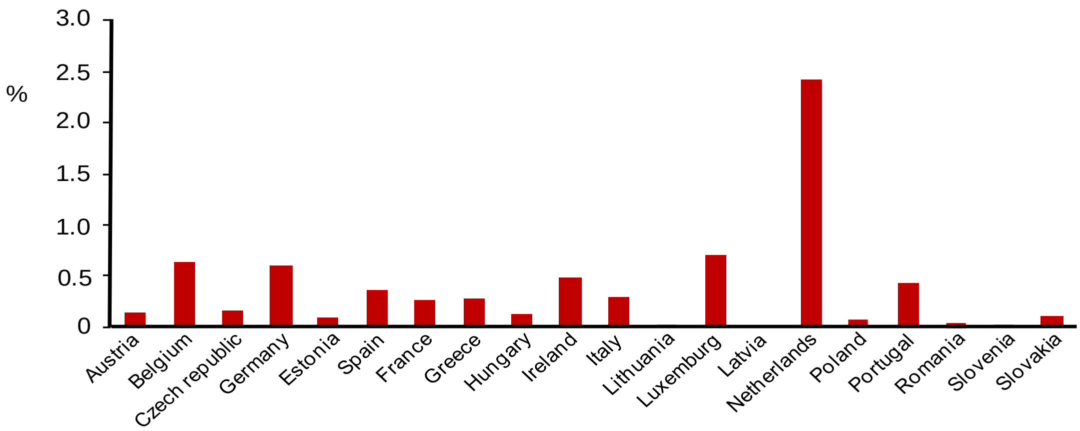
1. The general policy area for contaminated land and water identification and clean-up, and;
2. Bioremediation in competition with other remedial technologies.

In the European context there are at least three policy tools present to drive contaminated land clean-up:

1. Direct regulatory intervention;
2. The need for the development of urban industrial areas ("brownfield sites");
3. National - mainly state-funded - programmes ("orphan sites" *i.e.* those where the parties responsible for the contamination are either unknown - or are unable or unwilling - to pay for needed remedial actions).

It has been said that the most significant driver of the regeneration of contaminated sites in the United Kingdom is the development process, especially for brownfield sites (Luo *et al.*, 2009). This is likely to be the case in most relatively small, but densely populated countries with a high demand for housing provision. It has also been the case in the United States and Canada (De Sousa, 2003). Scarcity of land, particularly for house building, has raised the political profile of brownfield site redevelopment, and as a result contaminated land has gradually risen up the political agenda (Catney *et al.,* 2006). The development of brownfield sites helps prevent the use of green sites for housing (Figure 1), and also promotes economic growth in inner cities, and is therefore a potentially important component of sustainable growth.

Figure 1. Comparison of land cover data for 1990 and 2000 (% decrease in 10 years)



The figure indicates an estimated loss of 970 000 ha of agricultural land for 20 EU member states in this ten year period due to urbanisation The rate of change is not the same across all countries. It should be noted that non-agricultural land is also consumed by urbanisation. These trends continue in the period 2000-2006 as shown in the SOER 2010 land use assessment (EEA, 2010b).

Source: EEA, 2010a

Limit of existing policy tools

Technical barriers have been significant in the past, but most contaminated sites now are treatable, given sufficient funding to do so. More significant barriers now exist.

- Sites with high levels of stigma (*e.g.* where the contamination problems are known widely within the public and media) can deter volume house builders from developing in those areas.
- High remediation costs set against low land values can create an unacceptable level of risk for builders and investors (Alberini *et al.*, 2005). When land values are high the cost of remediation can often be met within the overall redevelopment budget.

Extent of the problem

To date there is a huge difference internationally in how countries approach the problem of contaminated land. Some countries have barely addressed the problem at all, and others are at an advanced stage in tackling it. However, there are two aspects that are widely agreed upon:

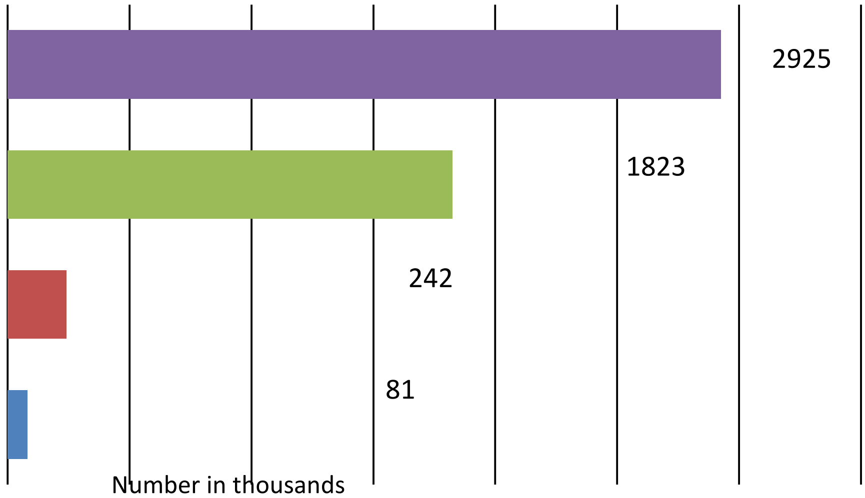
1. The extent of the problem is very large indeed, and by no means known in its entirety;

2. Regulation plays a fundamental role.

There are a very large number of contaminated sites, and the number of known sites is growing due to improvements in data collection (EEA, 2010a). The European situation illustrates some of the issues both driving and inhibiting the site remediation business generally. Other factors are at play more specifically for bioremediation.

According to the European Environmental Agency (EEA), it is estimated that, in Europe, potentially polluting activities have occurred at about three million sites (Figure 2), from which more than 8% (or nearly 250 000 sites) are highly contaminated and need to be remediated. Projections based on the analysis of the changes observed in the last five years, indicate that the total number of contaminated sites needing remediation may increase by more than 50% in 2025 (EEA, 2007).

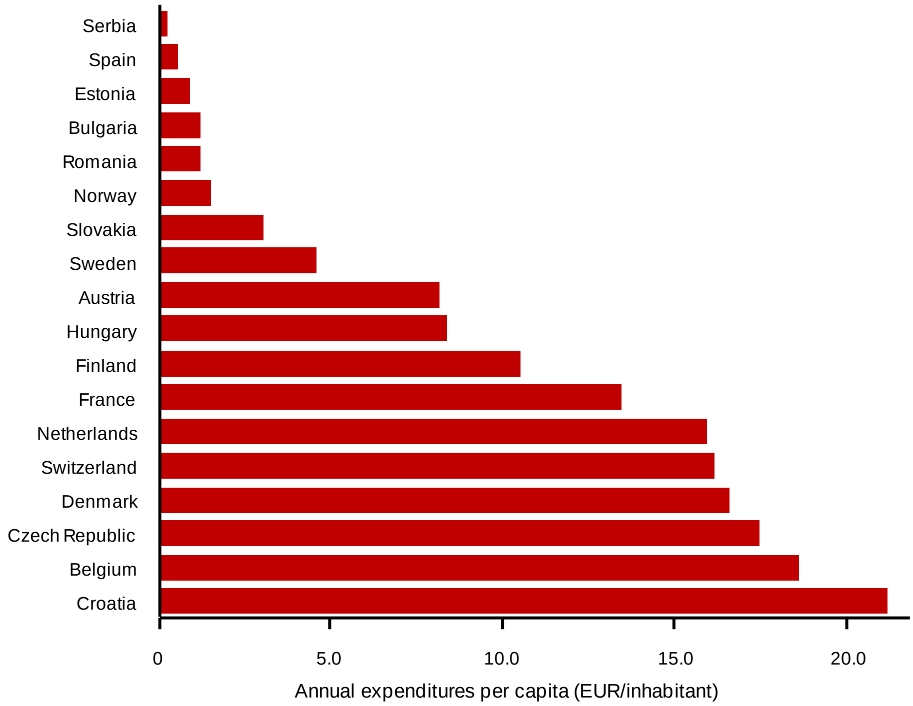
Figure 2. The number of contaminated sites in Europe, 2006, in thousands.



Source: Adapted from EEA, 2007

Remediation is progressing relatively slowly: in the last 30 years, only just over 80 000 sites have been cleaned up in the countries where data on remediated sites is available. Moreover, expenditure on site remediation is highly country-specific (Figure 3). Although annual expenditure on clean-up in the European Union member states for the period 1999–2002 have reached EUR 35 per capita per year in some countries and that a substantial sum of money has already been spent on soil remediation in Europe, this is still relatively small (up to 8%) when compared with the estimated total costs (EEA, 2007).

Figure 3. Annual site remediation expenditures in selected European countries as EUR per capita, January 2007



Source: Adapted from www.eea.europa.eu/

These differences reflect not just the varying degrees of awareness, but also the different environmental standards applied in each country, the different hydrological conditions and degree of industrialisation. International aspects of contaminated land policy are given in Appendix 1.

Remediation costs are on average 10 times higher than site investigations costs. Links between environmental merit and invested budgets are highly dependent on national standards, in terms of remediation targets and local site conditions. Detailed investigations and remediation activities are generally progressing slowly. More is being learned on the size of the problem but the speed of the clean-up is slow. An excellent resource for determining the situation in individual EU countries is CABERNET (www.cabernet.org.uk).

Often a major driver is public perception: in the case of contaminated land, public perception is generally low but is increased by serious accidents (Barlow & Philp, 2005), and by people simply knowing that they live on or close to contaminated soil. For example, in the Netherlands, which has some of the highest standards for contaminated land remediation globally, 30 to 40% of the population currently lives or works on, or nearby, seriously contaminated soil. Over 50% of the people become very concerned when they hear they are living on contaminated soil. Apparently, this concern relates not only to the potential effects on their health, but also on the value of their property (van de Griendt, 2007). It stands to reason that if an individual country has low awareness of the problem and has relatively poor environmental legislation and standards, then public perception will also be low.

Landfill tax as a driver for innovative environmental clean-up technologies

In many countries, by far the least expensive solution to contaminated soil problems has been to landfill it (the so-called "dig and dump" method of waste disposal). In this way the liability is removed from the landowner, and the problem is totally removed, so that development can move forward. However, there is a movement in many countries to use and build landfills more sustainably. Even in a country like Australia, with a large land mass and low population, there are good reasons to consider the available supply of landfill to be a scarce resource that should be used conservatively (Pickin, 2009). A country with quite the opposite conditions is Japan, where there is limited space and high population density. In Japan, it is becoming increasingly difficult to obtain public acceptance to install waste disposal facilities, such as landfill sites, due to a rising pressure on land use and growing public concern over environmental and health protection (Ishizaka & Tanaka, 2003).

Day *et al.* (1997) demonstrated in a comparative analysis of remediation techniques that landfill was the least expensive option, compared with bioremediation and soils washing. Contaminated sites undergoing redevelopment in Europe are often small sites, for example a former petrol station being developed for housing. In such situations, the complexity associated with the use of bioremediation can give rise to a number of costs. For example:

- Investigation costs are increased by the treatability study required;
- The employment of an expert consultant to advise on the design of the remediation is required;
- General and specialist labour is needed during the remediation itself, to monitor and manage the treatment process;
- Monitoring also requires the presence of a convenient laboratory;
- The timescale (with uncertainty), required for bioremediation may extend the timescale of the whole project, increasing time-related overhead costs, and the on-site land requirements needed for the process may make the site more complex to manage;
- External organisations may require additional monitoring, for example of groundwater after remediation is complete, adding more to costs.

In comparison, the costs associated with the removal of relatively small amounts of contaminated soil from small urban sites to external landfill sites are less onerous, though with the addition of a landfill tax, the costs of alternatives to landfill disposal become more comparable.

In the European Union, in order to comply with the Landfill Directive 1999/31/EC (CEC, 1999), waste producers are required to increase the biodegradable waste diversion from landfills by means of recycling, reusing and recovery (McMahon *et al*., 2008). Landfill Tax is a tax on the disposal of waste. It aims to encourage waste producers to produce less waste, recover more value from waste, for example through recycling or composting, and to use more environmentally friendly methods of waste disposal.

Over the years, landfill tax progressively increases to make it progressively more expensive to dispose this way. Besides, the problem is not solved, merely moved elsewhere. In the United Kingdom, however, there is a current landfill tax exemption on the disposal of contaminated soil, which has existed since landfill tax was introduced in 1996. The UK Government recently acted to discourage dig and dump by announcing that the exemption will be phased out by 2012. The exemption is not consistent with sustainability objectives, and it has been posited that government support would be better linked to remediation relief (a relief from Corporation Tax) than to a landfill tax exemption (HM Treasury, 2007).

It is apparent that the Landfill Directive will have profound implications for the contaminated land industry throughout the European Union (Hartman *et al.*, 2005). The requirement for treatment of most wastes, including contaminated soils, prior to disposal coupled with increasing landfill fees and haulage costs has driven the market for both *in-situ* and *ex-situ* treatments including bioremediation.

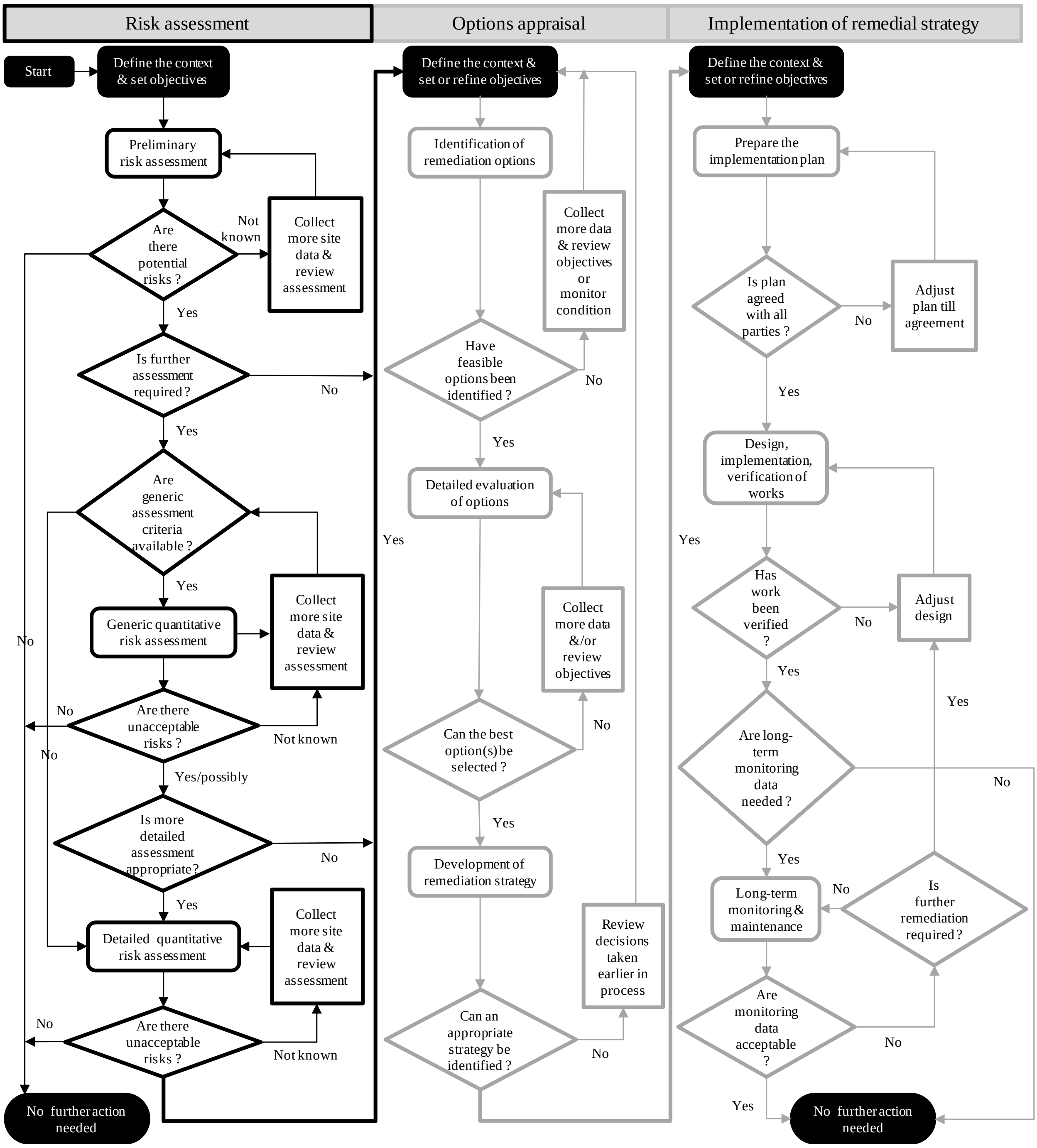
Risk assessment and a landmark change in contaminated land policy

Risk assessment is a central policy issue in its own right. Risk assessment was not designed as a policy to promote bioremediation, but it may incidentally have helped. The origins of the risk-based approach can be traced to the Netherlands experience where land was remediated to the highest possible standard, according to the "multi-functional" approach, whereby all contaminated soil was treated as the same, regardless of the end-use. Unfortunately, this approach also disregarded the potential costs of remediation. By 1997 these costs had become so high that the Netherlands was forced to change to an approach based on cost-effectiveness and risk-based assessment (CABERNET, 2003). This change of policy is intended to increase both the societal and environmental benefits, the intention being that the future remediation of contaminated land will be adapted to the future land-use.

Instead of the multi-functional approach, the "suitability for use" approach has taken root in many countries. The suitable-for-use doctrine is a fundamental component of, for example, UK land regulation that underpins the risk-based approach (DEFRA, 2006). The concept of suitability for use entails the identification and removal of unacceptable risks from contaminated land, the reclamation of land into beneficial use, and controlling cost burdens (Latawiec *et al.*, 2011).

Risk assessment should be seen as an integral part of the overall contaminated land management process (Figure 4).

Figure 4. The contaminated land management process



Source: Adapted from DEFRA and Environment Agency, 2004.

The risk assessment concept

Risk assessment with respect to contaminated land serves two main purposes:

1. It can be used to measure the degree of significance of contamination at a site;

2. The level of clean-up required in order to make a site suitable for its intended use may also be determined.

Several terms used in the field of contaminated land risk assessment require working definitions. Toxicity is the potential of a material to produce injury in biological systems. A hazard is the nature of the adverse effect posed by the toxic material. Risk is a combination of the hazardous properties of a material with the likelihood of it coming into contact with sensitive receptors under specific circumstances. This type of assessment also considers potential pathways by which hazards may reach receptors. Risk, then, is a statistical entity.

When applying the basic principles of risk assessment to a potentially contaminated site, a site should only be statutorily "Contaminated Land" if the Local Authority can establish the presence of a significant pollutant linkage, which must include a hazard, a pathway and a receptor (Rudland *et al*., 2001). A critical point about this UK legal definition of contaminated land is that if no pathway can be found to link a hazard to a receptor, then the site cannot constitute contaminated land (Clifton *et al*., 1999). In the example of domestic gardens, the receptors (sometimes referred to as targets) could be infants who may inadvertently ingest soil in gardens. Most risks to human health and the environment can be described in terms of single or multiple source-pathway-receptor scenarios.

This type of pollutant linkage analysis allows remediation strategies to be designed in a more realistic and cost-effective manner. The result on most sites is likely to be a less conservative remedial strategy than one based purely on multi-functionality, *i.e.* restoring a site to greenfield conditions.

There are four key steps in the process of assessing the risks associated with pollutant linkages.

1. *Hazard identification*. This is the stage at which the chemicals present on a site are anticipated, along with their characteristics, *e.g.* their concentrations, water solubility and toxicity. Due to the likelihood of many tens or even hundreds of potential contaminants being present at a site, the hazard identification stage usually focuses on known contaminants of concern.

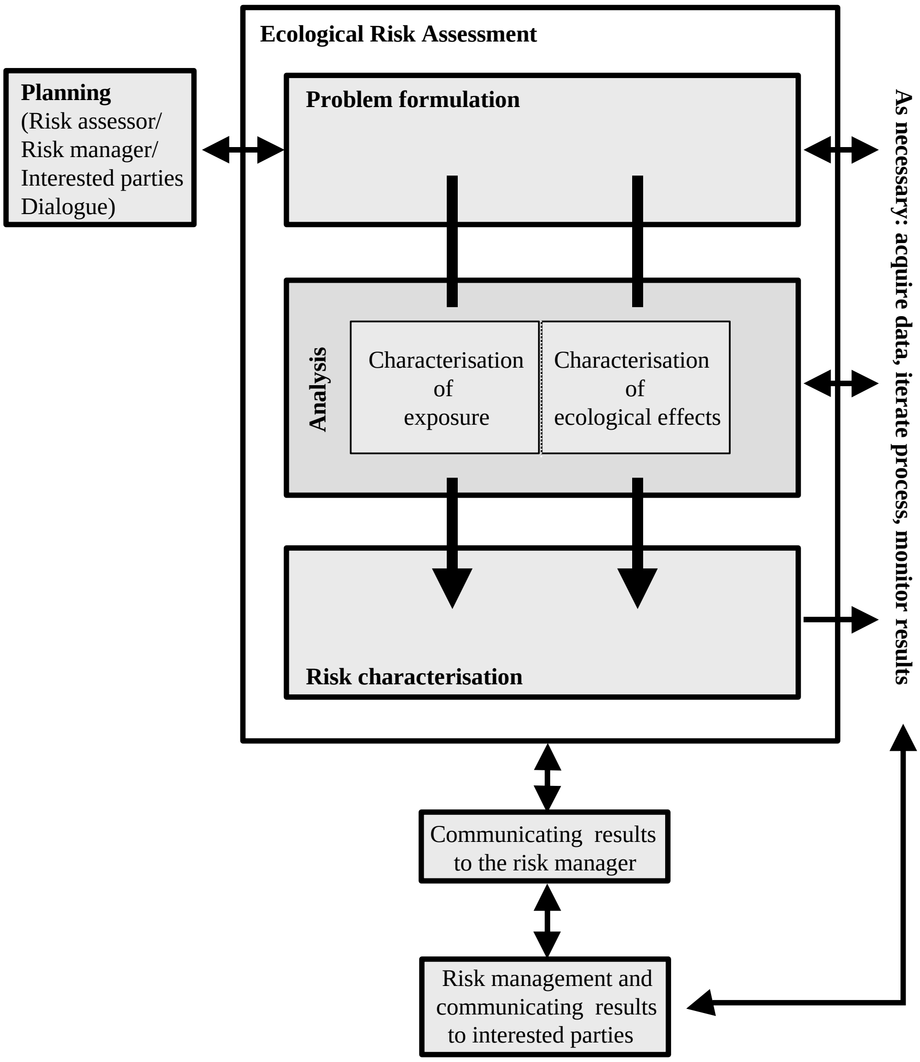
2. *Exposure assessment*. Exposure assessment is the estimation of pollutant dosages to receptors, based upon the use of the site and the conditions therein. There are multiple facets to these calculations. Among the factors to be considered are exposure duration and frequency, mean body weight and future population growth or decline.

3. *Toxicity assessment*. This is the acquisition of toxicity data, such as dose-response, and its evaluation for each contaminant for both carcinogens and non-carcinogens.

4. *Risk characterisation*. This is an assignment of the level of risk to each pollutant linkage. For many contaminated sites the best that can be reasonably expected at an initial desk-based stage is a qualitative risk estimate, such as insignificant, low, medium or high. The amount of data required for quantitative risk characterisation may be beyond all but the most rigorously characterised sites.

The US EPA has adopted detailed methodologies for achieving this process (US EPA, 1998). The overall procedure is summarised in Figure 5.

Figure 5. Framework for environmental risk assessment (US EPA, 1998)



Source: Adapted from US EPA, 1998.

Monitoring is a requirement to support the development and implementation of European Union environment policies, such as the Water Framework Directive (2000). The risk assessment approach, linked to suitability for use, results in the derivation of remedial target end-points as a means of protecting receptors, and they are applied regardless of the remedial technology chosen. Perhaps the greatest technical constraint on bioremediation is confidently predicting then meeting these end-points. The contractor must have confidence that the target threshold can be met through bioremediation. To do this, the contractor should have available the means to monitor the progress of clean-up. It is widely accepted that bioremediation cannot be effectively monitored with any single parameter. The likelihood of success is defined by the bioavailability of the contaminants (Diplock *et al*., 2009).

Bioavailability as a concept in contaminated land risk assessment

A definition of bioavailability that alludes to the risk assessment practice (Loehr & Webster, 1997) is "a measure of the potential of a chemical for entry into ecological or human receptors".

Bioavailability impinges upon contaminated land remediation in two respects:

1. It has implications in risk assessment related to the suitability-for-use approach to remediation;

2. Biosensors, both natural and GMO, that "measure" bioavailability could be used to monitor the progress of bioremediation of a contaminated site.

However, measuring bioavailability has remained elusive. Issues to be addressed include:

- Despite about 30 years having gone by since the early research, the work is still mostly in universities. Many companies have been spun out, very few have survived as a focussed environmental biosensor company (they have to diversify to survive);

- There is no method of accreditation, which gives the biosensors no legislative credibility compared to, say a standard chemical analysis. Without this, environmental biosensors cannot progress beyond interesting research tools or "back-up" to traditional chemical analysis. Accreditation must become easier;

- Lack of focus on markets *i.e.* the research effort has not been accompanied by diffusion effort;

- Industry has avoided the use of GMOs, even though, in the case of environmental biosensors, they are benign and the way that they are used is not conducive to environmental spread.

The bioavailability quandary has been accurately articulated by the International Union of Pure and Applied Chemistry (IUPAC).

> "*In the IUPAC Conference of Pesticide Chemistry in London in 1998, there was a controversial discussion on the topic whether agrochemicals in the environment are really hazardous. The conventional wisdom is that chemicals in soil are available to micro-organisms, plant roots, and soil fauna like earthworms and animals via dermal exposure. Then bioaccumulation through the food web may induce indirect exposure to higher organisms. National governments are simply reducing the threshold levels of chemicals in the environments for larger safety margins in their guidelines. However, the question raised at the Congress was that chemical residues in the environment are not always bioavailable, so that the actual exposure of biota to chemicals is rather different from the amount (concentration) present in the environment. In addition, the persistence and efficacy of agrochemicals are affected by their bioavailability in soil. There is a lack of comprehensive understanding of the bioavailability of chemicals with different chemical characteristics, such as non-polar organochlorines, polar agrochemicals, etc. Thus, there is a strong need to clarify the scientific basis for bioavailability: definition, estimation methods and affecting factors".*

Measuring bioavailability

Measuring or even estimating bioavailability is problematic as it is affected by many individual and interacting conditions relating to soil and water chemistry, pollutant chemistry and partitioning and biological transformation and concentration factors (Hund-Rinke & Kördel, 2003). Moreover, the "ageing" process brings about a time-dependent decrease in bioavailability as compounds become sequestered in soil over time by ill-understood mechanisms (Chung & Alexander, 2002). The techniques currently being researched can be categorised broadly as: direct or indirect; and biological or chemical (Lanno *et al.*, 2004), although, as pointed out by these authors, only organisms can determine whether a chemical is bioavailable, so direct chemical methods are not possible.

Direct biological methods

These are techniques that measure the actual amount of a chemical taken up by a target organism, and this may ultimately be the most accurate measure of bioavailability, although it is by no means routine. For example, the determination of chemical levels in earthworms is relatively expensive and time-consuming. The great strength of these direct techniques, however, is that they integrate all the biotic and abiotic modifying factors of chemical bioavailability (Lanno *et al*., 2004).

Indirect biological methods

It is possible to observe a response to a chemical in an organism without actually measuring the concentration of the chemical. A range of responses is possible, such as lethality, enzyme induction or inhibition, reproductive effects. These are regarded as indirect biological methods because the effect may be quantifiable in the organism, but the chemical concentration remains unknown.

A promising, novel approach is the use of genetically modified micro-organisms that can detect and quantify specific pollutants. This type of biosensor is based on the highly specific genetic control mechanisms used by micro-organisms to ensure that specific proteins are only expressed when they are needed; for example, for the detoxification of a particular toxic substance. Biosensors of this type are easily generated (Philp *et al.*, 2004). The most popular reporter is the bacterial *lux* system, which produces light, because light can be easily quantified and, due to its rarity in biology, there is no interference from the background biochemistry of the host organism.

Such biosensors can be both highly specific and responsive to very low concentrations and the presence of the microbial cell membrane makes this approach truly a measure of bioavailability. There are limitations to developing such biosensors for detection of organic pollutants as often the required specificity is lacking. With heavy metals, specificity may not be a problem. Such biosensors offer the prospect of a much more routine measure of bioavailability. Ingenious and very different designs have proven the key to the lab-scale development of reliable environmental monitoring devices.

Biosensors may never substitute for legislation but they are mature enough to be used in routine analysis where legislative compliance is not required. Diplock *et al*. (2009) stated that there is an urgent need to equip bioremediation practitioners with a suite of analytical devices to demonstrate the genuine scientific basis that underpins the process. But this can be countered by the assertion that the size of the market may be so small that it does not attract serious investment to enable full development of sensors to the point where they can actually be used in real field situations (Alcock, 2005).

Indirect chemical methods

Indirect chemical methods usually involve the extraction of a fraction of the chemical (metals or organics) from a soil, the extractability being defined by the chemical itself, the nature of the extractant(s) and the experimental conditions applied. The origins of this approach are in sequential extraction procedures (Tessier *et al.*, 1979), which attempt to quantify the speciation of toxicants into those weakly bound and those strongly bound to the soil matrix.

A number of methods are based around the human gastro-intestinal tract (the physiologically based extraction test, PBET, Ruby *et al.*, 1996). To mimic human conditions they incorporate gastric juices and enzymes with mixing at 37°C, and use soil residence times similar to those found in children after ingestion of food. Analysis of all solutions produced in the PBET extractions is completed by analytical chemistry techniques under matrix-matched conditions.

An ISO standard for bioavailability

The International Standards Organisation (ISO) has developed guidance for the selection and application of methods to measure bioavailability in soil (ISO 17402, 2008). This was created as a response to an increasing demand for a validated pool of methods to be used in soil assessments and promotes the development and the introduction of the bioavailability concept for a particular receptor in the context of specific site circumstances.

Other aspects of contaminated land remediation policy

Liability relief

Liability relief usually comes in the form of letters of no further action, certificates of cleanup completion or covenants not to sue. The United States has increasingly resorted to liability relief to promote environmental remediation of contaminated sites (Alberini *et al.,* 2005).

Direct subsidies as financial incentives

The attractiveness of subsidies seems to vary across types of site developers, and is influenced by prior experience with (and hence efficiency in taking advantage of) these incentives. Developers with prior experience with contaminated sites are more responsive than other developers, whereas the other economic incentives work better for developers without such prior experience. Subsidies may be a relatively inefficient way of soliciting cleanup and redevelopment at locales where virtually all prospective developers have not engaged in brownfield projects before.

Land remediation tax credits

In some countries a company that has a qualifying land remediation loss for an accounting period can make a claim to surrender that loss, or a part of that loss, in return for a payment of land remediation tax credit. In the United Kingdom this applies both to a loss arising from cleaning up land in a contaminated state and to a loss arising from bringing long term derelict land back into productive use. This Land Remediation Relief in the United Kingdom is a relief from corporation tax only. It provides a deduction of 100%, plus an additional deduction of 50% for qualifying expenditure incurred by companies in cleaning up land acquired from a third party in a contaminated state.

In considering an extension to Land Remediation Relief, respondents to a questionnaire suggested the following types of expenditure, among others, to be included (HM Treasury, 2007):

- Early stage professional fees;

- Feasibility studies for re-development;

- Site investigation study fees;
- Costs related to planning permission;
- Restoring sites as open spaces.

Bioremediation: an innovative, green technology for the clean-up of contaminated land and groundwater

Bioremediation is a good case example to use, as it is a relatively new technology that is not yet rolled out world-wide, and has certain controversies and advantages that have both limited its use, and in some circumstances made it an attractive technology. It is a technology applicable to both contaminated soil and groundwater. A good all-round reference textbook is Atlas & Philp (2005).

Drivers of bioremediation for the clean-up of contaminated sites

The drivers are the same as for contaminated land remediation generally, as already discussed. Bioremediation is one of a suite of potential remediation technologies and it is in competition with others at many sites. It is necessary to look at those other techniques to know the advantages and disadvantages of bioremediation in comparison.

The most frequently used established technologies in the United States are incineration (thermal), thermal desorption (thermal), solidification/stabilisation (physical) and soil vapour extraction (SVE) (physical), and, for groundwater, pump-and-treat technologies (see Technology Briefs boxes in Appendix 2, from Barlow & Philp, 2005). SVE and thermal desorption are interesting cases as they were until recently classed as innovative technologies, but they have crossed the barrier to implementation and are now established. The US EPA has defined innovative technologies as those whose use is limited by lack of data on cost and performance. They have only limited full-scale application and *in-situ* and *ex-situ* bioremediation thus remain classed as innovative.

In global terms, it appears that bioremediation technologies are used in around 10 to 15% of cases. There are several reasons why bioremediation may occupy this (rather low) fraction of the market;

- Bioremediation relies on either biodegradation (in most cases) or uptake of metals into plants (far fewer cases), so the market is strongly site-specific as it is strongly dependent on the biodegradability of contaminants;
- There have always been uncertainties about the rate of progress of treatment (there tends to be a fast rate of biodegradation early, which slows later);
- There are uncertainties about end-points. Probably due to lack of substrate, bioremediation usually stops before complete removal of contaminants. The point at which this stoppage occurs is variable, and if remediation stops at a point where contaminant concentrations are above the agreed end-point level, then further, non-biological treatment may be required;
- Site investigations tend to be more detailed, more intrusive and therefore more expensive when considering a bioremediation option;
- Many remediation contractors are unfamiliar with bioremediation.

Uncertainties are incompatible with the mind-set of site developers and often a safer technology will be selected. Risk assessment can have positive effects on bioremediation as end-points may be set higher than would have been under a strategy of multi-functionality.

But as experience with bioremediation has grown, treatment costs have dropped, and confidence has been gained by contractors. Therefore in those countries with experience of using bioremediation it may be that bioremediation has reached an equilibrium market share that will not significantly increase unless for external factors, such as a step-change in the technology, or a landmark change in policy that disfavours other remedial technologies. Whilst new bioremediation technologies have been described (*e.g.* Guimarães *et al.*, 2010), it is less likely that a step-change in the technology will occur as in other areas of biotechnology. Most bioremediation does not involve augmentation using cultures, but relies on biostimulation, using fertilisers and oxygen. The non-soluble, non-available nature of the typical contaminants means that microbes are limited in the rate at which they can utilise them, even in the laboratory. Field conditions make the task even more difficult. And in many countries research in bioremediation is not a high priority. The contractors tend to be small companies, or divisions within traditional civil engineering companies, and therefore the willingness of the industry to pay for R&D is limited.

In the United Kingdom the Biotechnology and Biological Sciences Research Council (BBSRC) is the main public funding body for Industrial Biotechnology and bioremediation. The BBSRC Industrial Biotechnology portfolio has been subdivided into a number of cross-disciplinary scientific areas for research, training, and knowledge exchange. These categories, with the associated annual research spend for 2008/09 (Industrial Biotechnology Industry Report, 2011) in brackets, were:

- Biocatalysis and metabolic engineering (GBP 7.8 million);
- Bioenergy (GBP 4.4 million);
- Non-food crop/non-food application (GBP 2.6 million);
- Bioremediation and waste treatment (GBP 0.24 million).

The United Kingdom had previously been engaged in bioremediation research at a reasonably high level. The spending on bioremediation was largely sustained by the Bioremediation LINK programme, but activity in this area has decreased to a very low level in recent years. Other areas of Industrial Biotechnology have assumed much higher importance.

A comparative advantage of bioremediation in respect to other remedial technologies is sustainability. Conversations with remediation contractors reveal that life cycle analysis (LCA) is hardly ever conducted when comparing and selecting remedial technologies for a site. In the context of contaminated site remediation, generically the goal of a LCA study is to assess the environmental burdens of a product or a process, identifying and quantifying raw material use, energy consumption and waste generation with the objective of evaluating impacts and to improve possible environmental issues. A LCA study can therefore be applied to selecting remedial technologies.

In this regard, the *in-situ* bioremediation technologies may compare well with other, non-biological technologies. One of the major advantages of these is that the primary impacts are low, since the processes do not demand any transport, excavation and landfill. On the other hand, as the technologies usually take a long time to reduce the contaminants level, secondary impacts can be high.

Morais & Delerue-Matos (2010) critically reviewed the challenges concerning the LCA application to site remediation services. They concluded that, in site remediation decision-making, LCA can help in choosing the best available technology to reduce the environmental burden of the remediation service or to improve the environmental performance of a given technology. However, this is a new approach with little or no legislative authority, and its application requires time, skill, and adds to the cost of a project. Also the standardisation and certification of remedial techniques has been discussed as a means of ensuring the quality of the "product", cleaned soil (van Hees *et al.,* 2008).

The application of eco-efficiency measures of remedial technologies is not common-place currently, but may become so in the future. Sending contaminated soil to landfill, for example, is incompatible with modern views on recycling, and is an inefficient way to use limited landfill availability. Some initial work on eco-efficiency of remedial technologies has been done by Sorvari *et al.*, (2009) (Table 1).

Table 1. Eco-efficiency of some selected contaminated land remediation technologies

Remediation method	Positive factors	Negative factors
Reactive barrier	Generally no need for removal of the barrier	Long-term operating costs, suitable only for some contaminants
Soil stabilisation, isolation	No need for soil removal; quick; can be economical	No removal of contaminants from environment; can be energy-intensive
Soil vapour extraction (SVE)	Generally cost-effective; low uncertainties in risk reduction	Suitable only for volatile contaminants; exhaust air needs to be treated
Incineration (mobile)	Effective contaminant removal	Flue gas treatment needed; energy-intensive; often needs fuel
Composting	Low cost; treated soil may be used for landscaping; no emissions requiring treatment	Suitable only for some organic contaminants; can be long duration; depends on contaminant concentrations
Landfill	Effective control of risks; soil can be used in daily cover	Not treatment; not suitable for re-use; becoming more expensive; not efficient use of landfill sites

Source: Modified from Sovari *et al*., 2009.

Phosphorus recycling: wastewater treatment meets resource conservation

The example of phosphorus recycling was used to demonstrate the potential role of biotechnology in resource conservation. Supplies of phosphorus, increasingly in demand in the automobile industry, computer manufacture, the manufacture of flame retardants, and perhaps most important, food production, are limited. In fact, if present trends continue, there will be a crisis in its availability in the near future.

The main sources of waste for recycling are steel-making sludge, sewage sludge, and food and fermentation industry wastes. These wastes are currently causing serious environmental contamination problems, for example through algal blooms. After removal of phosphorus from some of these waste materials, the residue can be used for cement production, where the phosphorus residues would have had a detrimental effect.

Again, GM technology is not seen as particularly useful in this area. Currently *E. coli* can accumulate polyphosphate, up to 50% dry weight as phosphorus; and an activated sludge microbial consortium is currently the best option for phosphorus removal from waste waters.

Remaining problems in adopting phosphorus recycling technology include the cost of construction of a recycling plant, though there are advanced systems currently in operation in Japan where four government ministries are involved in a co-ordinated national approach to phosphorus recycling.

Main messages on environmental clean-up technologies

A clear distinction has been made between the regulatory and policy background to the clean-up of contamination generally, and bioremediation specifically. Bioremediation operates as a business sub-sector within a regulatory framework that controls all remedial technologies. For the time being, there is no clear consensus established on the comparative advantage of bioremediation.

It is judged that the switch to risk-based assessment of contaminated land and groundwater has had the greatest influence in stimulating remedial technologies. The regime of multi-functionality had become prohibitively expensive, and risk assessment allows remedial end-points to be modified according to the future purpose of the land. This switch to risk-based assessment of contaminated land and groundwater may stimulate remedial technologies. In fact, more countries are switching to risk assessment and risk-based remedial design, and this should be encouraged. Landfill taxation has other goals, often the diversion of putrescible material from landfill. The over-arching concern is the decreasing availability of suitable new landfill sites as waste production increases. It is clear how the banning of contaminated soil dumping in landfill would (and has) stimulate(d) the remediation industry. Some nations have made specific exemptions for contaminated land from landfill taxation. The removal of exemptions should further stimulate the remediation industry, and thereby create other opportunities for bioremediation technologies.

Bioremediation has achieved a certain level of market penetration in the remediation industry. But there are several factors that limit the further expansion of bioremediation in competition with other remedial technologies. Some of these factors may be overcome through R&D. However, remedial technology R&D is not as high a priority as it has been previously. This is often country-specific. The expenditure on environmental remediation has varied enormously in different countries.

One of the central issues in bioremediation is how to monitor its progress. Stakeholders such as property developers need certainties about the time-scales involved. Bioremediation is less certain in this respect than other remedial technologies. A potential solution is the development of biosensors to monitor the bioremediation process. Much has been done in the R&D of toxicity and bioavailability biosensors, and yet they do not have any regulatory status. As such, they will not be treated seriously as tools within the industry. An encouraging development is the ISO standard for bioavailability testing. Another issue, however, is the market size for biosensors which may inhibit serious investment.

THE NEW INDUSTRIAL BIOTECHNOLOGY: BIOFUELS, BIOBASED CHEMICALS AND BIOPLASTICS

Environmental and industrial biotechnology overlaps

The Rimini workshop discussed biofuels and biobased chemicals only briefly. More time was dedicated to bioplastics (Narayan, 2010). There was also some brief discussion of Japan's ambitions in biofuels. The approach to this section is to examine the different types of supportive policies that have been used to support biofuels and to identify policy gaps concerning support for biobased chemicals and bioplastics. If these gaps are not acted upon, then the economics of integrated biorefineries may be sub-optimal.

There has long been a debate about the overlap between environmental and industrial biotechnology. Whilst not making specific definitions of these two sectors, this report makes the following distinction between the two:

- *Environmental biotechnologies* are those that are applied to environmental clean-up, decontamination or improvement. Specifically this distinction refers to wastewater treatment and bioremediation within the boundaries of the Rimini workshop, with some discussion also of enabling technologies such as environmental biosensors.

- *Industrial biotechnology* refers broadly to the production of biofuels, biobased chemicals and bioplastics. The latter group can now be realistically sub-divided into biodegradable plastics and biobased plastics.

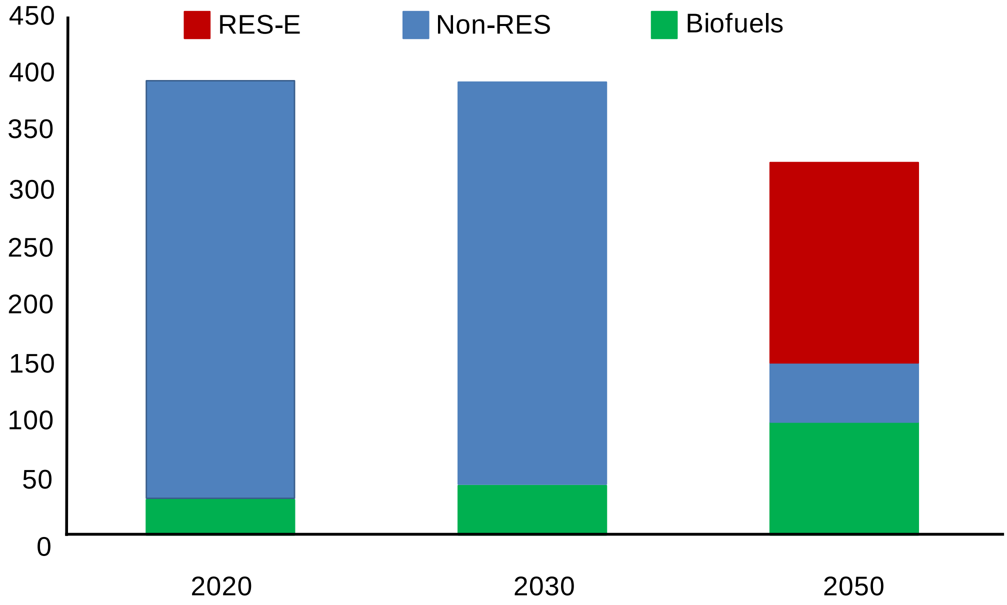
This distinction is a necessity from the point of view of policy description, as discussed earlier, but it is also an inexact distinction. One of the primary motivations for developing biofuels, biobased chemicals and bioplastics is to help mitigate climate change (OECD, 2011a), and therefore there is an inextricable link with environment issues.

Landmark innovations of global impact in wastewater treatment date back to the early 1900s, at a time when industrial biotechnology was unknown. Whilst bioremediation is a major full-scale environmental biotechnology developed in the late 1900s, it is fair to say that environmental biotechnology is languishing compared to industrial biotechnology. The latter can be described by slow but steady progress in fermentation technologies over a period of decades. The rush to Single Cell Protein (SCP) in the 1960s seemed to signify the start of a major industry, but for various reasons SCP failed to become accepted (Davidson, 1995). SCP seemed to be an answer to the so-called protein gap, and the "food from oil" idea became popular and facilities for growing yeast fed by *n*-paraffins were built in a number of countries.

The dawn of the biofuels era

However, industrial biotechnology came of age with the huge developments made in the global production of liquid biofuels. Between 2005 and 2010, fuel ethanol production worldwide more than doubled (FO Licht, 2010b), and biodiesel production more than quadrupled (FO Licht, 2010a). What is more, predictions see biofuels as a major and growing part of the transport fuels energy mix into the future (Figure 6).

Figure 6. Contribution of biofuels to global transport fuel demand (Mtoe)



Note: RES = renewable energy source.

Source: From European Renewal Energy council (www.erec.org/statistics/res-future.html)

This sudden growth in biofuels production was only possible as a result of major policy decisions, especially in the United States, following on earlier successful policy and ethanol production in Brazil. It is important to realise that policy in biofuels is by no means complete, and that to maintain predicted growth into the future will require constant policy attention *e.g.* future expansion may overwhelm transport infrastructure, which may necessitate financing of controversial pipeline construction.

Rationale for biofuels policy

There is no single critical factor driving biofuels development. Rather, there is a complex interplay of factors involved, some of which are country-specific. As of early 2009, 73 countries (many of them developing countries) had bioenergy targets (REN21, 2009).

Environmental and social drivers

Climate change

Climate change is now a widely accepted phenomenon, and is challenging the ways in which many activities are conducted, including the future ways in which energy will be generated and consumed. It could adversely affect water supplies and agricultural productivity, and the need to cut CO_2 emissions to avoid harmful environmental degradation has made the transition from conventional fossil fuels to alternative and renewable resources a global priority. The United Nations Environment Programme (UNEP, 2010) has noted that “doubling of wealth leads to 80% higher CO_2 emissions”. Therefore there is a need to break the link between growth and the expense of sustainability.

Population growth

Added to converging economic and environmental dilemmas are the problems associated with growing global population. It is estimated that the global population will reach approximately 8.3 billion in 2030, with 97% of the growth occurring in developing countries (OECD, 2009). There are obvious consequences of such a population rise: impact on land use and water resources, increased waste and wastewater production, impact on food prices through growing demand, increased demand for transportation fuels and climate change mitigation problems that result.

Waste production

We have become accustomed to living in a throw-away society. A recent analysis estimates that, globally, one million mobile phones, ten million plastic cups, one billion plastic bottles, and ten billion plastic bags are disposed of every day (Ravenstijn, 2010). The consequences can be seen in:

- A constant cycle of GHG emissions – more plastics are required to replace the ones thrown away, requiring more oil and energy;
- Human intellect and productivity is tied up, where it could be usefully engaged elsewhere;
- Exacerbation of the landfill dilemma – as geologically suitable sites for landfilling in densely populated countries dwindle, single-use, light and bulky plastics take up more landfill space;
- The materials intensity of a microchip is orders of magnitude higher than that of "traditional" goods, and the semiconductor industry uses hundreds, even thousands of chemicals, many in significant quantities, and many of them toxic (Williams *et al.,* 2002). A great many of the materials are returned to the biosphere as waste.

New jobs

Federal policy in the United States supporting biofuels has resulted in an additional 240 000 jobs and contributed USD 65 billion to GDP in 2008 (Carr *et al.,* 2010). The Brazilian ethanol programme provided nearly 1 million jobs in 2007, and cut 1975–2002 oil imports by a cumulative undiscounted total of USD 50 billion (Wonglimpiyarat, 2010). A further dimension to the driving force for industrial biotechnology is the regeneration of the rural environment, where a huge number of agricultural jobs have been lost due to increased efficiency (USDA, 2010).

Energy security

The International Monetary Fund (IMF) has calculated that a 10% increase in the price of crude oil shaves 0.2-0.3% off global GDP in one year. A large spike in the price of oil can do great damage. High prices and oil shocks have contributed significantly to historical recessions (Jones *et al.,* 2004). The same dynamic that drove oil prices skyward in 2008 is steadily re-emerging. Supply has not significantly increased, and demand has considerably increased (world demand grew by a huge 2.7 million barrels per day in 2010). Price volatility and supply uncertainty drive the need for biofuels development. This has been especially the case in the United States, where the Industrial Biotechnology drive has been characterised by a top-down approach (centrally, initiated by government and/or administration, with massive public research funding) (Lorenz & Zinke, 2005).

The European Union countries have few oil producers and exporters. Hungary, for example, imports 80% of its domestic crude oil requirements, and this percentage may increase (Republic of Hungary National Renewable Energy Action Plan/2010-2020, 2010). Oil production in the United

Kingdom and Norway has been falling steadily in recent years following peak production in 1999 and 2001 respectively. The United Kingdom has just turned net oil importer (OilPrice.com, 2010).

Some of the world's largest economies are located in the European Union, and there is an inherent energy insecurity. Hence it is no surprise that the European Union has developed a sophisticated biofuels policy portfolio, with a major landmark being the publication of the Renewable Energy Directive (Official Journal of the European Union, 2009).

Energy security is also a key driver in developing countries. The case of Thailand typifies the developing world dilemma of sustaining growth whilst in the grip of high dependency on crude oil imports (currently accounting for more than 10% of GDP) (Siriwardhana *et al.*, 2009).

Globalisation and feedstocks

An important aspect of globalisation is that biofuels feedstock costs vary across the globe: low cost sugar cane in Brazil, maize in the United States, high cost wheat in Europe, palm oil in Indonesia. Sub-Saharan Africa, India and parts of Southern Asia have huge potential for feedstock cultivation and export, with less oil-dependent growth. If managed correctly and sustainably, feedstock production in poor countries has the potential to alleviate poverty, or at least to rejuvenate farming communities, whilst developing their own capacity to work towards energy independence. In developed countries with developing bioeconomy strategies, biofuel production could be inhibited by lack of feedstock. International trade of feedstock is potentially a major revenue source for developing countries.

Examples of policies related to biofuels

It is not the intention to produce an exhaustive list of biofuels policies. Rather, it is more important to highlight the trends and provide some specific examples. A key message has to be that a balance of supply- and demand-side policies is essential (OECD, 2011b). Generic supply-and demand-side policies are shown in Table 2. International aspects of biofuels policy are given in Appendix 3.

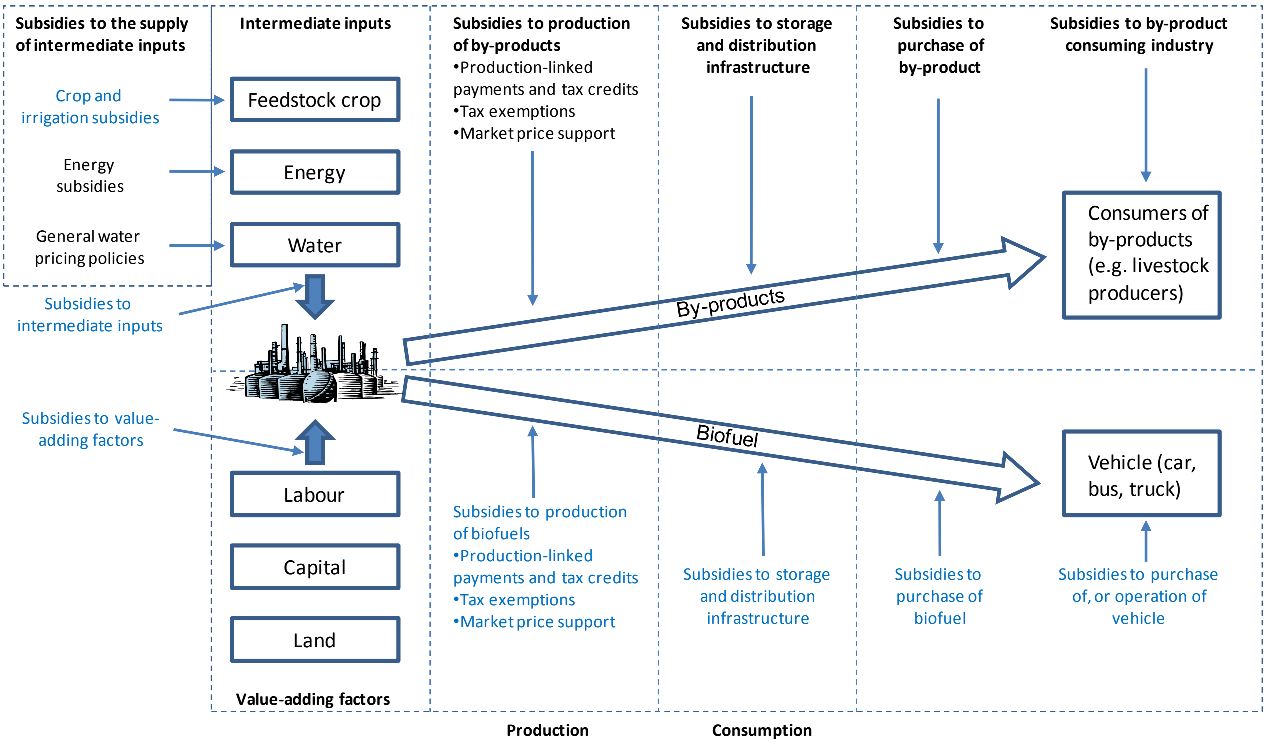
Table 2. Examples of supply- and demand-side policies important to the development of biofuels

Supply-side (supply-push)	• Government-sponsored R&D • R&D tax credits for companies to invest in R&D, particularly for SMEs • Enhanced capacity for knowledge exchange • Support for education and training • Infrastructure support (*e.g.* biorefineries and demonstrator facilities) through public and public-private finance • Supply volume mandates
Demand-side (demand-pull)	• Public procurement (*e.g.* transport fleets, flex-fuel vehicles) • Tax credits and rebates for consumers • Technology mandates • Innovation-specific regulations and standards (*e.g.* sustainability) • Certification and labelling • Public outreach

Subsidies throughout the biofuels supply chain

The Global Subsidies Initiative has developed a framework to examine support levels at different points in the supply chain for biofuels, spanning the production of feedstocks to final consumers (Figure 7). At the beginning of the supply chain are subsidies to intermediate inputs. In several countries, the largest of these are subsidies to producers of feedstock crops used to make biofuels. Further down the chain are subsidies directly linked to output, which includes the protection from foreign competition provided by import tariffs on ethanol and biodiesel; exemptions from fuel-excise taxes; and grants or tax credits based on the volume produced, sold or blended.

Figure 7. Different points in the biofuel supply chain to which subsidies can be applied



Source: Adapted from Steenblik, 2007.

Second-generation biofuels

Controversies associated with corn-derived ethanol, particularly the food versus fuel debate (*e.g.* Mueller *et al.,* 2011) and clarity over the true GHG emissions reductions associated with it, have driven many countries to invest in future second-generation biofuels based on lignocellulosic feedstocks, especially those not derived from food crops. But there are large economic repercussions – based on the current state of technology, second-generation biofuels plants have capital costs of the order of five times greater than starch ethanol plants (Wright & Brown, 2007).

For first generation bioethanol, the most significant cost was feedstock (Carryquiry *et al.*, 2011). There are several areas where cost reductions can be made, but not necessarily all of them should be policy targets. Out of feedstock, plant cost, and feedstock conversion cost, the most significant cost element for second-generation cellulosic biofuels is conversion cost. Therefore it would be advisable to shift policy to favour progress in reducing conversion costs.

Algal biofuels may provide disruptive breakthroughs

Theoretical yields of biofuels from algae are orders of magnitude higher than yields from other feedstocks. However, algal technology will take the longest to achieve commercial scale. The cost of biodiesel from algae is currently several times higher than the cost of oil-derived diesel. Nonetheless some companies claim that the first commercial plants will be available soon. Darzins (2008) indicated that, as of 2008, seven United States government laboratories, thirty United States universities, and around sixty biofuels companies were conducting research in this area. Intense efforts are also taking place in other parts of the world, including Australia, Europe, the Middle East, and New Zealand (Pienkos & Darzins, 2009). In particular, algae are attracting the attention of the oil majors. Investments are large: the huge theoretical yields and limited implications for land use are very attractive, and there are high probabilities of spill-over benefits in terms of chemical and plastics production. Monsanto recently acquired a stake in Sapphire Energy, a San Diego–based algae fuel company (Nature Biotechnology News, 2011). The partnership will initially focus on genes that increase yield in algae under optimal and sub-optimal growth conditions, which is of interest to Sapphire to help accelerate the commercial production of algal biofuels.

Sustainability should be a theme

Experiences with first-generation corn-derived bioethanol should alert policy makers to the fact that not all biofuels are equal. Policies should be tailored to picking the most favourable biofuels, and perhaps even to disadvantaging unfavourable ones. In this regard sustainability is a key issue. Life cycle analysis (LCA) may become the norm, and if it does, then there will be an urgent need for international harmonisation to ensure that standards are consistent and that trade barriers are not generated. Currently LCA outcomes depend enormously on variables such as boundary conditions, which prevents like-for-like comparisons.

A key area for the deployment of LCA in the future will be lignocellulosic ethanol production. Various authors have already applied LCA to lignocellulosic feedstock, and already there are discrepancies in approaches that lead to uncertainty and inaccuracy, which will diminish the credibility of LCA testing if not addressed (Singh *et al.,* 2010).

Ayoub *et al.* (2007) proposed a general bioenergy decision system (gBEDS) as an effective tool in planning for bioenergy production expansion. Their model, developed with Japan's specific conditions in mind, included environmental, economic and social decision support. Very recently, a decision support tool (DST) has been described that presents a basic framework to evaluate biofuel production pathways, with the purpose of providing decision makers with a structured methodology. The tool integrates the most important aspects along the entire value chain (*i.e.* from biomass production to biofuel end-use), namely the technical, economic, environmental and social aspects (Perimenis *et al.*, 2011).

Biosafety

In this context, biosecurity refers to protecting the integrity of each nation's biological resources (agricultural production, biodiversity and ecosystem services). As the leading sector in Industrial Biotechnology, biofuels crop production has largely ignored the biosecurity risks that could compromise current and future agricultural production and natural ecosystems. The debate is not simply about genetic modification and the accuracy of predicting which new plant species will become invasive, which is not always possible. Another major biosecurity concern is the threat from pests. The future threats posed by large-scale, non-food agricultural production may not have been fully thought through (Sheppard *et al*., 2011). As long as doubt exists, biosafety aspects need to be thoroughly investigated.

Sugarcane is the best example of a threatened feedstock due to its importance to the biofuels industry. Industrial Biotechnology can only increase the global demand for sugarcane, especially with expansion of the biofuels sector (Fisher *et al*., 2009). It is attacked by over 1 500 insect species worldwide, and is subject to over 80 diseases from bacteria, fungi, viruses and others. The larva of the giant cane borer *Telchin licus* Drury causes significant yield losses in both sugar and biomass, and its presence was recorded for the first time in 2008 in São Paulo, the largest Brazilian sugarcane growing state (Goebel & Sallam, 2011).

Despite the fact that Australia has one of the best biosecurity and quarantine systems in the world, several borer species have severe implications for the capacity of the Australian sugar industry to develop a viable biofuel and other bioproducts capacity (Sallam & Allsopp, 2005). As a result, Australian state and federal government agencies have worked with the sugarcane industry to develop risk assessment procedures that will enable a rapid response in the event of an emergency incursion.

Integrated biorefining

Integrated biorefineries have to be capable of efficiently converting a broad range of industrial biomass feedstocks simultaneously into affordable biofuels, energy, and a wide range of biochemicals and biomaterials. These goals are met simultaneously by integrating chemical and fuel production within a single operation (Bozell, 2008). In such an operation, high value products become an economic driver providing higher margins to support low value fuel, leading to a profitable biorefinery operation that also exhibits an energy impact. This is how petrochemical oil refineries are operated - the 7 to 8% of crude oil dedicated to chemical production results in 25 to 35% of the annual profits of integrated petrochemical refineries.

However, for integrated biorefineries to utilise a range of feedstocks efficiently, significant technology development and financial risk are implied. The best way to spread the financial risk is through public-private partnerships. This may also require new financial models to attract investors, but the lessons from integrated petrochemical refining remain the same.

The initial construction of biorefinery pilot and demonstration plants is not only a costly undertaking but it also involves bringing together market actors along new and highly complex value chains. This includes the diverse suppliers of biomass raw materials (*e.g.* farmers, forest owners, wood and paper producers, biological waste suppliers, producers of macro- and microalgae); the industrial plants that convert the raw materials and industries providing them with the necessary technologies; and the various end users of intermediate or final products. This necessitates integrative policy: ignoring one link in the chain will give sub-optimal results.

Critical messages for biofuels policy of the future

- Balance between supply and demand policies is essential.

- The transition to second generation biofuels will require special policy attention as they are currently unviable economically.

- Algal biofuels could change a great deal. But algal biofuels are still posing large technical challenges, and the large financial implications indicate that public-private partnerships are required.

- Policies could support only those biofuels that demonstrate the best characteristics *i.e.* that are supported by solid LCA data. Policy makers should thus offer different levels of support depending on the performance of the biofuel in economic, environmental and social terms.

- Integrated biorefineries that integrate production of biobased chemicals and bioplastics will be needed to maximise the chance of economics success. Brazil, for example, has an emerging biobased plastics industry that was enabled by bio-ethanol production.

Policy for biobased chemicals

One of the major messages from this section is that the supportive biofuels policy regimes that have been generated in many countries are not in place to support biobased chemicals (or indeed bioplastics). This is a conclusion drawn in the United States (Industrial Biotechnology Industry Report, 2010) as well as in the European Union (Carus *et al.*, 2011). And yet some biobased products are much closer to market, at scale, than are second-generation biofuels (Shaw *et al.*, 2011), and therefore their development is sub-optimal due to this lack of policy support. Some venture capitalists are now of the opinion that basic chemicals and simple polymers represent the "sweet spot" for funding (Hasler, 2010), on the basis that more people are convinced that fuel production from cellulosic biomass alone is not the most viable business strategy. There is an obvious need for greater dialogue between the investor community and public service: much greater value would be captured through public-private collaboration.

Biobased chemicals pose a major challenge for policy makers because of the need to address the complete value chain of intermediate products in a cradle-to-grave perspective (Hatti-Kaul *et al.*, 2007). Such chains are generally much longer then the equivalent ones based on fossil feedstocks, although the products containing such biochemicals are environmentally benign in comparison with the products made from fossil feedstocks.

Some suggested policy measures from the United States (based on the Industrial Biotechnology Industry Report, 2010)

Provide product parity and early-stage support in biorenewables tax policy via the following steps:

- Enact a production tax credit (PTC) for biobased products;
- Open the 48C advanced energy manufacturing credit to renewable chemical and biobased product biorefineries;
- Provide robust early-stage R&D credits to drive development of specialty biochemicals.

Increase funding through grants and other programmes for non-fuel biobased products via the following steps:

- Open existing loan guarantee programmes to biobased product projects;
- Ensure that existing and future grant programmes support biobased products;
- Establish grants and loans to help struggling biorefineries add high-value chemical production.

Ensure that biobased products are incentivised in climate change/carbon legislation via the following steps:

- Include production of biobased products in the list of eligible offset project types to drive investment in critical low-carbon biobased products.

Ensure timely implementation and eligibility of renewable chemical intermediates in USDA Biopreferred voluntary labelling and procurement programmes.

Some suggested policy measures for the European Union (from Carus* et al.*, 2011)

Quotas, bans, public procurement and emission trading

- Indicative/mandatory targets and quotas for biobased products by 2020 (similar to the quotas already in place in Japan).
- Open the European Union biofuel quota to biobased products (as the target for the share of renewables in transport was opened for electric cars in 2008).
- Bans on critical fossil based chemicals, plastics and additives which can easily be substituted by less hazardous biobased chemistry.
- Implementation of strong green public procurement programmes for biobased products
- Ensure that biobased products are incentivised in climate change/carbon legislation including carbon trading and credits (ETS – Emission Trading System).
- Open regulations, programmes, and subsidy systems supporting bioenergy and biofuels to biobased chemicals and materials.

Taxes

- Support taxation of non-renewable carbon as input for the chemical industry; at present, a paradox system is implemented with double disadvantages for industrial material use of biomass: In the energy sector there are high taxes on fossil carbon sources and high support for bioenergy – in the material sector there are no taxes on fossil carbon and no support for biomaterials.
- Allow member states to reduce taxes for sustainable biobased product categories (like the European Union framework for member states in the energy taxation directive).
- Comprehensive establishment of CO_2 taxes including all biobased sectors (energy and material).

Agriculture

- The Common Agriculture Policy (CAP) reform could be an option for rebalancing the support of bioenergy *vs.* industrial material use.

- Replace the former CAP "production refund" by an alternative incentive to support the use renewable raw materials for industrial uses.
- CAP should become an interface between agriculture and the biobased economy, including biobased chemicals and materials; this is a huge chance for the farmers.
- Integrate in the new CAP specific financial incentives for farmers to improve the logistical capabilities to collect biomass by-products and residues from agriculture and forestry.
- All programmes in structural funds and rural development that are being used to support and implement bioenergy and biofuels should be opened to biobased products – all criteria for funding should be handled equally.

Additional instruments

- Use regulations like the European Union End-of-Life Vehicle Directive for supporting biobased products through waste legislations (consider biobased materials as recycled regardless of how they are recovered) to make biobased products attractive for the industry.
- Ensure that biobased products can enter all waste collection and recovery systems, including composting, recycling and energy recovery. Biobased plastics certified compostable according to EN 13432 should gain unhindered access to bio waste collection.

Policy for bioplastics

The issues are very similar to the issues for biobased chemicals, but arguably the situation is more urgent. It is expected that overall plastics consumption will grow from the current 250 000 kilotonnes per year to about 1 million kilotonnes by the end of this century. The environmental concerns over conventional petrochemical plastics are well-described: they lack biodegradability, they have GHG emissions of concern, and they are often light and bulky, creating a dilemma for landfill disposal. Despite being inexpensive to produce, there is an economic concern over the projected consumption rates – the 1 million kilotonnes figure would require about 25% of current oil production to meet that market demand. With easy crude oil becoming difficult to find, there is great competition for its use. There are many reasons, not simply environmental, why a search for alternative polymers of is a high priority.

Some positive policy developments

The Japanese government's Biomass Nippon Strategy legislated in 2002 for 20% of all plastics consumed in the country to be sourced renewably by 2020 (prompting Toyota, NEC and others to accelerate R&D into bioplastics). In June 2009, after a review of the achievements of the Biomass Nippon Strategy, a basic law promoting the use of biomass was enacted in order for the government to take more comprehensive and concrete measures to promote biomass utilisation. The basic law established a committee that released the basic plan to promote biomass utilisation in December 2010. The basic plan was consistent with the New Growth Strategy and the Basic Energy Plan approved by the Cabinet in June 2010.

Among Asian countries, Japan is the biotechnology leader in biodegradable plastics. The Japan Bioplastics Association (JBPA) has estimated that demand for biomass-based plastics will reach 20% of total plastic consumption in 2020. Thus, the JBPA started the "Identification and Labelling System" in 2000 and has certified about 900 biodegradable plastic products in Japan. The system is based on a positive list system for all components, biodegradability specifications based on Japanese Industrial Standards, safety certification of all components, and proof of no hazardous effects to soil (Chanprateep, 2010).

New regulations in the German Packaging Directive cover packaging made from bioplastics. As such, bottles produced from at least 75% renewable resources are exempt from the compulsory deposit for single-use drink bottles. Exempting a single-use bottle from the deposit system gives the brand owner a EUR 0.25 pricing advantage over its deposit-carrying competitors on the supermarket shelf. The scheme is not expected to compromise the existing high level of recycling.

Belgium has established an eco-tax of EUR 3 kg^{-1} on packaging such as shopping bags, whereas compostable shopping bags that conform to the European Standard (EN) 13432 for compostable packaging material will be exempted.

The Netherlands has established a carbon-based packaging tax based on CO_2 emissions from the production of packaging material and the embedded carbon content of the packaging. France, Italy, and Spain are considering similar legislation.

At least on the regulatory side, progress has been made internationally in reducing the usage of non-degradable plastic bags, thereby promoting the use of biodegradable plastic bags (Table 3).

Table 3. Global policies and measures to limit the use of non-biodegradable plastic bags

Country	Policy
Germany	German Packaging Directive has been in force (2005). The compostable packaging will be exempt from the requirements in § 6 of the Directive
France	A law for the promotion of French agriculture has been in force (2006) stating a requirement for biodegradability of disposable retail carry bags by 2010
Italy	Markets in Florence had been charging €0.10–0.20 per plastic bag (2009)
Ireland, Scotland, Denmark, Sweden	These countries have already imposed levies and taxes on non-degradable plastic bags
United Kingdom	Since 2003, county Durham has been charging Ecotax per plastic bag
United States	San Francisco: in March 2007, the San Francisco Board of Supervisors approved first-in-the nation legislation that outlaws the use of non-biodegradable plastic bags in large supermarkets within 6 months and large chain pharmacies in about a year
Canada	Toronto City Council: retailers will be required to charge a minimum of 5 cents for each plastic retail shopping bag that customers take (2008)
Japan	Law on Promoting Green Purchasing and Law on Recycling have been in force in 2001
India	Plastic is officially banned in Ladakh
Australia	Thin non-biodegradable plastic shopping bags have been prohibited in South Australia from 4 May 2009, and in the Australian Capital Territory from 01 November 2011.
Bangladesh	From the beginning of January 2002, the Bangladesh government banned the use of plastic bags in Dhaka

Source: Chanprateep, 2010.

Other possible policy measures for bioplastics

Similarities to policy measures for biobased chemicals will be evident.

- Political objectives concerning future utilisation: % of market/consumption share at a given period of time, measures to support implementation *i.e.* tailoring support so that promising products are supported at the appropriate time.
- Tax legislation *e.g.* reduced value-added tax, environmental tax, investment support.
- Preferential treatment of products in public procurement programmes *e.g.* bioplastics used in office supplies.
- Simplified special regulations in waste legislation.
- Treatment of bioplastic secondary raw materials as sources of renewable energies (electrical and thermal energy recovery).
- Opening of community recovery systems for biowaste ("biobin") for certified compostable plastic products.
- Provision of equity and venture capital to small and medium-sized businesses.
- Government R&D programmes for the co-financing of industry and university projects.
- Activities related to communication and market introduction (Ghanadan & Long, 2011).
- Agricultural policy: Promotion of cultivation of renewable resources on fallow (set-aside) or other fields.
- Measurement of bio-based (carbon) content to determine true value in renewability.
- Disposal environment: biodegradability under composting conditions is very different from the conditions of an anaerobic landfill site, or a marine environment *i.e.* policy directed at end-of-life options.
- Degree of biodegradation is important. Partial biodegradation may result in intermediates more toxic than the original material. Therefore harmonisation of standards is desirable.
- All such criteria are necessary to be investigated to prevent misleading claims of biodegradability. Policy that prevents “greenwashing” is also desirable.

A set of measures to promote the uptake of bioplastics and other biobased polymers was presented by Crank *et al*. (2005) (Table 4).

Table 4. Suggested general policies and measures to promote wider use of renewable raw materials (RRM)

Potential policy measures	Objective
Medium and long term R&D and demonstration	Increase applications and economic performance, increase range of additives to improve engineering parameters
Standardisation	Harmonise standards (*e.g.* composting)
Public procurement	Enable commercialisation, create economies of scale
Limited fiscal and monetary support (*e.g.* reduced VAT rate)	Enable commercialisation, create economies of scale
Include in climate and product policy	CO_2 credits for manufacturers/users
Adaptation of waste legislation and waste management	Improve infrastructure for separate collection (financial incentives for consumers)
Inclusion in agricultural policy	Secure stable supply of biomass feedstocks
Public awareness	Widen understanding of benefits

Note: RRM refers to renewable raw materials, and RRM is a synonym for biobased materials. Apart from biobased polymers the group of RRMs comprises biobased lubricants, solvents and surfactants.

Source: Modified from Crank *et al.,* 2005.

Comparative biobased chemicals and bioplastics policy: a common regime?

OECD (2011b) suggested that with such similarity in thinking between biobased chemicals and bioplastics, there is potentially a justification for treating them in the same policy regime, for the following reasons.

- Some of the companies that make biobased chemicals and bioplastics might be expected to make both *e.g.* BioAmber – succinic acid and polybutylene succinate plastic.
- The supply and value chains are very similar. Essentially bioplastics extends the value chains of biobased chemicals as bioplastics may be durable and have quite different end-of-life options.
- They share the same feedstocks.
- Given recent advances in biobased plastics and biocomposites, it is inevitable that a significant proportion of biobased chemicals will become platforms for the production of plastics. Ultimately, in fact, many paths lead back to bioethanol.
- The national administrations in grants, loan guarantee programmes, tax offices would be simplified by having one common regime instead of two.
- The administrative burdens on companies would similarly be simplified – not more regulation but more streamlined regulation.
- Manufacturing incentives given to moulders (manufacturers) for the introduction of bioplastics would stimulate another step in the value chain: incentives only to the makers of the plastics would not guarantee uptake by the moulders.

Policy based on preferential treatment?

There may also be justification for preferential treatment of some biobased chemicals and plastics over others, based on economic, environmental and social benefits. This is similar to the thinking that some biofuels should be given greater support than others (IEA Bioenergy, 2008). Some examples are given here.

1. The production of bio-based polyethylene, despite the fact that it is not biodegradable, may accumulate policy advantages based on the following:
   - It would potentially be required in huge volumes, which would mean more jobs;
   - Large volume production would also mean greater GHG emissions savings as petrochemical polyethylene would be incrementally replaced;
   - There could be greater advantage based on the sustainability of the feedstock;
   - Being identical to petrochemical polyethylene, it would be able to enter standard, established recycling facilities.
2. Succinic acid as a platform biobased chemical could be stimulated as a result of:
   - The succinic acid fermentation process actually consumes CO_2, whilst ethanol fermentation produces CO_2 (Zeikus *et al*., 1999);
   - If the bioplastics that succinic acid are used in the production of prove to have high distinctive advantages (based on LCA), then this also may justify preferential policy treatment over other platform chemicals.
3. Lactic acid is another platform chemical, and it is used in the production of the high profile bioplastic polylactic acid (PLA). It may gain extra advantage based on economic benefits because the margins in its production are significantly more attractive than, say, ethanol production (Hasler, 2010). Incidentally, next-generation PLA, already potentially a CO_2 sink (Jamshidian *et al*., 2010), would further benefit from the attractiveness of the margins of the monomer, lactic acid.

ECOGENOMICS AND ENVIRONMENTAL BIOTECHNOLOGY

An overview of ecogenomics technologies

Ecogenomics is the application of genomics to ecological and environmental sciences; it defines biodiversity at the DNA, RNA and protein levels (Maphosa *et. al*., 2010). Functionally, the main use of ecogenomics in this context is as a rapid method for the identification of microbes present in various environments. Until the arrival of robust techniques in molecular biology, the identification of microbes in their natural habitats was complex, time-consuming and often inconclusive. The problem was amplified greatly by the inability to grow the vast majority of microbes, especially bacteria that are present in soil and water. The polymerase chain reaction (PCR) made possible a routine molecular biological tool that removed the guess work from microbial identification, and started a new era in microbial discovery. In the past decade, a large number of laboratory-based –omics technologies have been developed and described (*e.g.* genomics, proteomics, metabolomics: see Appendix 4). Several of these are applicable to the contaminated land and water remediation industry, but as yet they await a formal role in the remediation process for various reasons. One of the reasons might be exactly as a result of this explosion in technologies – perhaps there are too many -omics at this point to choose from.

At this stage the most applicable and familiar of the -omics technologies in Environmental Biotechnology is metagenomics (Appendix 4), which refers to the genomic analysis of entire populations *i.e.* of whole samples of soil and water without the inefficient culture techniques. One of the factors that has restrained the development of bacteriology is the inability to characterise the biodiversity of bacteria. The so-called "great plate count anomaly" refers to the difference in orders of magnitude between the numbers of cells from natural environments that can be grown in the laboratory and the numbers countable by microscopic examination (Connon & Giovannoni, 2002). It has been recently estimated that 10^{16} microbes inhabit a ton of soil (Curtis & Sloan, 2005), but culture techniques may miss 99% of this microbial biodiversity. This inability to grow microbes means that there is a great untapped resource available that, once mastered, will open up many new discoveries *e.g.* new medicines, industrial chemicals and enzymes.

Ecogenomics: A potential regulatory tool in the bioremediation industry?

To bring some of these techniques into regulation requires standardisation. Roelofsen *et al.* (2008) described ecological genomics (ecogenomics) as the application of genomics techniques in the field of (soil) ecology in order to enhance our understanding of ecosystem function. This gives a sound basis for academic understanding of the term but falls short of explaining an application to the remedial technologies industry.

What prevents the application of ecogenomics in regulation of contaminated land and soil bioremediation?

Proof of need

Academics may say that the advances in understanding of microbial communities would improve bioremediation project design, and therefore improve the efficiency of the process. That remains to be proven, and the added expense that the laboratory investigations would entail cannot yet be justified in the absence of proof.

Contaminated land remediation is not proceeding quickly enough

As explored elsewhere in this report, new contaminated sites are being discovered and made. The rate of clean-up of known contaminated sites has been slow, and the impetus to speed this up seems lacking. This would tend to disfavour the introduction of a new set of (expensive and non-standardised) technologies to monitor progress.

Buy-in by the regulator

Ecogenomics concepts and language are best understood at present by the academic community. The regulators by and large are not biotechnologists, and bridging the gap between academic theory and pragmatic regulation will be a major hurdle. A focus on biological effects of pollution is currently a bridge too far, as governmental rules and regulations have a strong focus on measuring concentrations of pollutants.

Lack of standardisation

Standardisation would make the task of the regulator much clearer. The difficulty here is similar to that experienced with bioavailability, but is even more acute due to both the advanced equipment requirements and the large choice of techniques encompassed by ecogenomics.

Technological limitations

Even once the technologies have been mastered by the leading laboratories and the equipment and techniques have been diffused outwards, the added costs of the analyses represent a hurdle to entering practice. Some of the remedial technologies, such as thermal techniques, do not have as high a burden in site investigation. When time and cost are determining factors in remediation projects, ecogenomics will not gain many allies.

Lack of subsidies/incentives

For analytical laboratories to become involved there may be R&D tax incentives to be exploited. Individual analysis laboratories would examine the cost/benefit analysis, and given the large financial investments required (the costs of consumables and maintenance is also high, which add to the Opex as well as there being substantial Capex implications), there is the possibility that only the larger analysis laboratories would find ecogenomics attractive.

Buy-in from the analytical laboratories

The obvious candidates as the professionals to carry out such analyses are the accredited laboratories that already undertake chemical analyses for contaminated land and water investigations. But the laboratory set-ups required in both genomics and proteomics are substantially different from the set-ups required for chemical analyses. This necessitates isolation of these facilities from chemical testing facilities, and careful attention to air-flow and HEPA filtration. Analytical chemists would even object strongly to having glassware washed in the same facility as bio-contaminated materials. Glassware washing procedures even have to be different (for biological analyses, destruction of DNA and sterilisation are more important than acid washing to remove trace metals to very low levels).

Therefore for the existing chemical analyses laboratories the implications of adding a suite of PCR, genomic or proteomic analysis have serious reverberations in terms of staff qualifications, equipment, ancillary facilities and even the civil engineering of facilities. Added to this the need for large-scale computing facilities and highly trained bioinformatics staff that would not necessarily fit well in a commercial environment. These laboratories would have to be given strong legal guarantees that the investments would be worthwhile. The alternative would be to set-up quite separate laboratories for contract-based ecogenomic analyses, and that, for reasons discussed above, seems unlikely in the near to mid-term.

Buy-in by other stakeholders

Stakeholders, such as companies that are confronted with the need for remediation, and faced with paying for it, would probably not be in favour of immediate and dramatic scale and rule changes. Dealing with current regulatory regimes, which in themselves change with time, has a comfort value in at least there is familiarity and infrastructure.

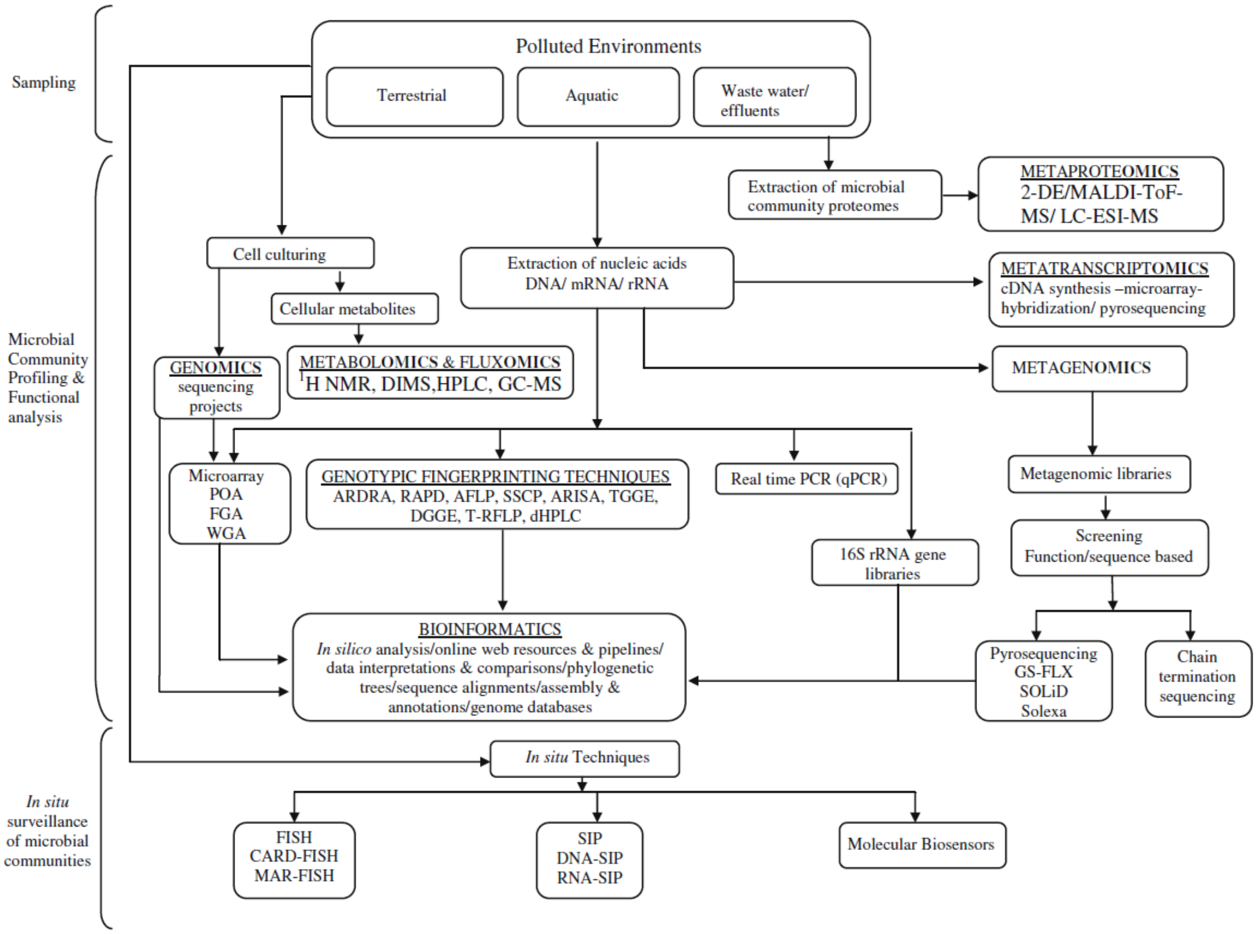
Could this situation be transformed?

A possible disruptive technology that would change this situation has been described (de Lorenzo, 2008). The availability of genes, genomes, and metagenomes of biodegradative micro-organisms make it possible to model and even predict the fate of chemicals through the global metabolic network that results from connecting all known biochemical transactions. As such, this technology is a hybrid of biotechnologies and computing. Were it to become suitably advanced, then one of the major objections to bioremediation – uncertainty in rates of destruction of chemicals and end-points – may be overcome. This would have the likely effect of stimulating the bioremediation industry as it would bring to the industry a level of engineering certainty not yet seen.

Integration of -omics technologies is the key

Contemporaneous analyses integrating the functional proteomics and metabolomics approaches would create a system-wide approach in studying site-specific micro-organisms underlying active xenobiotic mineralisation processes (*e.g.* Keum *et al.*, 2009). An overview of current integrative molecular and -omics technologies employed to survey intrinsic microbial communities underlying bioremediation at contaminated sites is depicted in Figure 8. The recent review by Desai *et al.* (2010) highlighted advances in the application of these technologies for studying microbial communities and their functional roles in environmental bioremediation.

Figure 8. An overview of molecular and -omics technologies employed to survey intrinsic microbial communities underlying bioremediation at contaminated sites



Source: Desai *et al.*, 2010.

A lesson from the recent past: public incentives for sequencing technologies

In 2004, the National Human Genome Research Institute of the National Institutes of Health (NIH– NHGRI) announced a total of USD 70 million in grant awards for the development of DNA sequencing technologies that would reduce the cost of sequencing the human genome from USD 3 billion, the amount spent on the public Human Genome Project, to USD 1 000 by 2014 (www.genome.gov/12513210). Complete Genomics (Mountain View, CA, United States) claims to have a technology that should become available in the near future that could bring the cost of the sequence of a human genome to USD 100.

Whilst a formidable array of barriers to the use of ecogenomics technologies within the bioremediation industry exists, the lesson is quite clearly that, given sufficient incentive then the possibility exists. However the ecogenomics tools are still very much research tools that are being used for the discovery of new genes, and also new products. Apart from public finance to fund research in ecogenomics, the use of these tools in discovery is an incentive for both private and public organisations to develop them. This is a greater incentive than for bioremediation applications.

Drug discovery and the marine environment

The most exciting and potentially lucrative (socially as well as financially) applications of ecogenomics technologies are in pharmaceuticals and industry. The environment that has received greatest attention so far is the oceans. Around the turn of the 21st century, the failure of combinatorial chemistry to deliver the anticipated wealth of new drug candidates drove a rekindling of interest in natural products as pharmaceuticals. As an illustration of the potential of this approach, by then approximately half of all anti-cancer discovery efforts were focused on marine organisms (Weissman, 2004).

Though a large number of marine bioactive substances have been identified, only recently have the first drugs from the oceans been approved. Quite astonishingly, the immense diversity of microbes in the marine environments and their almost untapped capacity to produce natural products was realised on a broad basis by the scientific communities only recently. Although culture-dependent studies continue to provide interesting new chemical structures with biological activities at a high rate, exploration and use of genomic and metagenomic resources are considered to further increase this potential.

The wealth of the marine pharmaceuticals pipeline is evidenced by at least three compounds in Phase III trials, seven compounds in Phase II trials, three compounds in Phase I trials and with numerous marine natural products being investigated in preclinical state representing the next possible clinical candidates (Mayer *et al.*, 2010).

The global market for marine biotechnology products and processes is currently estimated at EUR 2.8 billion (2010) with a cumulative annual growth rate of 4-5%. Less conservative estimates predict an annual growth in the sector of even up to 10-12% in the coming years, revealing the huge potential and high expectations for further development of the marine biotechnology sector at a global scale (Imhoff e*t al.*, 2011).

The impact of genomics and proteomics on the biotechnological exploitation of marine microbiota has hardly been felt yet. Given the overall potential of marine micro-organisms and the importance of marine environments, it is inevitable that a larger number of diverse marine micro-organisms will be brought into genome programmes (Borresen *et al.*, 2010).

Industrial biotechnology and ecogenomics

A large number of industrial biotechnology products have entered the market-place in the last decade: fuels, fabrics, specialty chemicals, bulk and platform chemicals, bioplastics in increasing types and applications (OECD, 2011a). This revolution in industrial biotechnology, allied to green growth strategies and the bioeconomy concept (OECD, 2009), is growing rapidly, largely started by top-down policy measures initiated in the United States for the development of biofuels about a decade ago. Current global revenues for goods produced using industrial biotechnology are estimated between EUR 50 and 60 billion annually, according to data released by industry trade publications. There are many predictions of future market values. For example, one estimate is that by 2030 the global market for industrial biotechnology could grow to roughly EUR 300 billion.

Ecogenomics could play a major role in the discovery phase work for new industrial biotechnology products, in a similar manner to mining the oceans to discover new drugs. The great genetic storehouses are soil, with perhaps 10^{10} live bacterial cells per gram dry weight, and the vast untapped and ancient oceanic habitats. Ecogenomics allied to synthetic biology is the tantalising combination of technologies that could make many break-through discoveries and products. Synthetic biology has already started to make an impact in industrial biotechnology (*e.g.* Jung *et al.*, 2010). Ecogenomics is yet to make its mark on the large scale, but researchers are active *e.g.* the concept of "intelligent screening" for the development of algal biofuels using genomics as a tool (Day *et al.*, 2011).

Policy issues: ecogenomics policy allied to synthetic biology?

There is policy work in existence in the OECD on Industrial Biotechnology (*e.g.* OECD, 2011a) and also marine biotechnology (OECD, 2011b). It is not intended to repeat that work here; rather this section will restrict itself to policy issues around ecogenomics applications to industrial and marine biotechnology, and will also touch on the interface with synthetic biology.

There is yet further on-going work within the OECD on synthetic biology policy (OECD, Royal Society, 2010). The ecogenomics technologies can be seen as enabling technologies for synthetic biology; that is, ecogenomics will provide many of the standard parts that will be used in the construction of new life forms. Indeed, the standard parts that ecogenomics can deliver vastly outnumber those that could be made with traditional genetic engineering techniques. However, there are many outstanding policy issues around synthetic biology that will not be discussed here, such as governance, knowledge management, ethics and education. These synthetic biology issues are being investigated separately in the OECD. At this stage, the ecogenomics technologies are producing results, but all of the technologies have technical problems that require to be resolved before they can be deployed on a large scale. Therefore the issues at the top of the policy agenda currently have to be public/private research funding and infrastructure issues, such as enabling education and training.

In the same way that the new sequencing technologies are dramatically decreasing the cost of sequencing, ecogenomics instrumentation and technology integration has to be funded to make the step changes required to lower cost and complexity. It should also be remembered that consumables for sequencing is an expensive part of the operation; many organisations that would be able to afford sequencing equipment would not be able to afford to run them on a semi-continuous basis due to consumables costs. The meta- technologies all generate vast quantities of information, and training and education in bioinformatics will remain high on the policy agenda. For example, a single Chinese institute in the field of commercialised DNA sequencing, (the Beijing Genomics Institute, BGI) hired 1 500 bioinformatics specialists recently (Pei *et al.*, 2011). By way of comparison, the entire workforce (2010) of the Bentley Motor Company is 3 726, Maserati 696, and Ferrari 2 721. Whilst ecogenomics constitutes a small part of the overall global sequencing capacity, nevertheless the technical challenges are very high, but the potential societal and scientific benefits should indicate that investment in technologies and education will reap rewards.

Ecogenomics should be ripe for the creation of spin-out companies. The open innovation business model would be attractive to pharmaceutical and industrial MNE's to work in joint ventures with small, nimble, ecogenomics-centred companies to carry out discovery phase research. This approach de-risks projects for MNE's whilst providing access to markets and customers, as well as research funding, for SMEs. There are already substantial venture capital-funded synthetic biology companies established, mostly in the United States that have gone through IPO. Such companies would be contenders for the uptake of reliable, mature ecogenomics technologies.

The Dutch Ecogenomics Consortium

The Ecogenomics Consortium was established in 2003 and co-funded by the Dutch government, with the important precondition that players from public research and industry are brought together. As a result, the Ecogenomics Consortium was organised as a large-scale public/private R&D consortium in which universities, national research institutes and companies with a focus on R&D participate. Its aim is to pursue genomics science and technologies for sustainable use of soil ecosystems for agriculture and other anthropogenic purposes and it comprises research across the disciplines of ecology, microbiology, soil sciences, environmental sciences, biotechnology and bioinformatics (Roelofsen *et al.,* 2011).

This is an interesting case for the policy-maker to follow. Cases such as the strong negative reaction to GM technology in Europe may partly result from the current R&D practice of involving non-scientific parties at a late stage of technology developments. As a result, the public remain dislocated from controls of science thereby generating a need for public accountability (Mayer, 2003). With this in mind, the Ecogenomics Consortium includes research projects on interdisciplinary collaboration and societal aspects of ecogenomics. The main assumption underlying these projects is that interdisciplinary collaboration and involvement of relevant stakeholders increases the scientific and societal value of research, and results in the development of products that effectively address complex societal problems.

Specifically, research has aimed at identifying and integrating a broad range of societal perspectives on (un)desirable future directions for ecogenomics. Roelofsen *et al.* (2011) have applied constructive technology assessment (CTA) as an interactive design process within the Ecogenomics Consortium. CTA activities aim to create a shared responsibility for the societal embedding of new science and technology between research institutes, businesses, end users, policy makers, and financial institutions. They concluded that a well-prepared dialogue, with a specific focus on learning between stakeholders, has the potential to bridge the gap between research and practice. One of the challenges they identify is elaboration of the role of policy-related participants, specifically the question of how to develop a mutually beneficial relationship between dialogue processes and policy-making processes.

GENETIC MODIFICATION ISSUES IN ENVIRONMENTAL AND INDUSTRIAL BIOTECHNOLOGY

"I haven't been able to find one example of a recombinant DNA species that has introduced problems: if anyone finds one, I'd love to have the reference".
Jack Newman,(Newman et al.*2010)*

For the most part, genetic modification (GM) is not a central concern of environmental and industrial biotechnology. GM microbes have long been used in fermentation systems for the production of a wide range of products. Within the fermentation systems, the genetically modified organisms (GMO) are contained. Modern fermenters can be sterilised automatically.

The main full-scale areas of environmental biotechnology are wastewater treatment and bioremediation. In the vast majority of circumstances there is no need for GMO in these areas. In fact, the use of GMO in wastewater treatment would probably prove prohibitively expensive and their effect in an open system such as activated sludge is likely to be negligible. The use of GM biosensors for bioremediation monitoring is a different issue in that the amounts of GM material in a sensor are extremely small. Also they are contained within a sensor housing, or the tests are performed in a laboratory.

In industrial biotechnology, the main source of concern over GMO will be the use of GM crops to provide biomass for the biorefineries. Handling GMO correctly in the refineries should be a condition of plant design. A far greater focus will be on the cultivation and export of GM crops. However, it is necessary to realise that worldwide research is focusing on appropriate, naturally-occurring non-food crops for Industrial Biotechnology, in particular for the burgeoning biofuels industry.

Second-generation lignocellulosic biofuels are becoming the focus of the industry (Carriquiry *et al.*, 2011) and forest and non-food agricultural crops are centrally important (Doherty *et al.*, 2011). Naturally occurring non-food crops such as Switchgrass (*Panicum virgatum* L.), Miscanthus (*Miscanthus giganteus*) and Jatropha (*Jatropha curcas*) are under intense study, and as yet most of the studies are focused on the natural plants, not GM variations. Generally there are many unanswered questions regarding yield levels in realistic field conditions for these plants: there is much to be done before considering GM. Another aspect of the use of these non-food crops is that their genetic systems are not well-characterised compared to many food crops, and therefore their genetic manipulation is more difficult.

Feedstock requirements for biobased chemicals and bioplastics are much lower and therefore land requirements are concomitantly lower. However, yields of some products are low, especially for pharmaceutical intermediates. Major incentives for an increased commercial interest in "plant biopharming" lie in its scalability and dramatically lower production costs for raw materials, relative to fermentation-type manufacturing procedures (Twyman *et al.*, 2005), and therefore future interest in GM plants in the biobased chemicals and bioplastics sectors may also be envisaged. Work has already begun on the use of GM plants to produce biobased chemicals, *e.g.* spider silk (Scheller *et al.*, 2001), and bioplastics *e.g.* PHB in Switchgrass (Somleva *et al.*, 2008). The use of GM technology in non-food plants to make new products, and implications for the bioeconomy, have been described recently (van Beilen, 2008).

One of the concerns of using GM crops for industrial biotechnology is that there are many potential routes to an admixture of food and non-food crops. For example, neither regular grain transport vehicles, nor grain milling facilities, used in food production are typically designed for complete clean-out. The use of the same equipment, therefore, constitutes a risk of admixture.

New agriculture

The notion of a 'new agriculture' based on the widespread use of GM and related technologies to deliver increased yields, to reduce fertiliser use (along with savings in energy requirements for fertiliser production), to improve plant defences and reduce pesticide use, and with better drought tolerance with reduced water use, has been regarded by some as an important component of a green growth based economy.

However, for industrial biotechnology's non-food and feed applications, particularly biofuels, naturally-occurring non-food crops with these same characteristics are being investigated. For example, Jatropha can be grown in semi-arid conditions and marginal land with few inputs like fertiliser and pesticides (Jongschaap *et al.,* 2007). Switchgrass can be grown in many locations: swamplands, plains, streams, and along shorelines and highways. It produces immense amounts of biomass and has a high cellulosic content. It is naturally resistant to many diseases and pests, can produce high yields with low applications of fertiliser and other chemicals, is tolerant of poor soils, flooding, and drought (Rinehart, 2006). With attributes like these, the need for genetic modification may not appear obvious. GM technologies may, however, be critical in reducing the lignin content of woody energy crops as this currently limits conversion (IEA Bioenergy, 2008). Algae for biofuels are also obvious targets for genetic modification to increase yields (*e.g.* Mascarelli, 2009).

New forestry

A new approach to forest development and exploitation, particularly regarding the sustainability of new forestry, is critical to second generation biofuels development. As well as the woody energy crops, some fast-growing tree species have also shown promise for biofuels production. Important attributes include the relatively high yield potential, wide geographical distribution, and relatively low levels of input needed when compared with annual crops (Smeets *et al.,* 2007). The forest products sector is looking for new opportunities to produce value-added products while securing access to emerging carbon capture markets (Sheppard *et al*., 2011).

Extending the limits of conventional breeding, which is a very slow and inefficient process in tree development, to give faster and more accurate trait improvement for application in plantation forests (including faster growth, improved pest and disease control) has the potential to allow easier and cheaper development of second generation biofuels. Public sentiment against GMOs has led researchers and companies to use alternative conventional and less efficient technologies (*e.g.* marker-assisted selection).

GM related constraints

The main GM-related constraints to consider are negative public reactions to the technology and very difficult regulatory systems nationally and internationally. This negative reaction to the technology is not gradually disappearing as was expected and excessively demanding regulatory systems are not being modified on the basis of experience.

Internationally, the *Codex Alimentarius* document on GM food safety assessment is used by all countries, and also the WTO. It was negotiated with NGOs and now works reasonably well. In contrast, for the environmental release of GMOs, Annex III of the Cartagena Protocol on Biosafety, which is the basis of risk assessment, is very difficult to implement. The Cartagena Protocol governs the trans-boundary movement of GMOs. It requires advance agreement for shipments containing living modified organisms (seeds) intended for release into the environment. Notification is also required for importers in the case of shipments of commodities, intended for use as food or feed, or for processing, that contain or may contain GMOs via the Biosafety Clearing-House, an information centre which tracks such movements (Clapp, 2008).

Most GMO exporting countries have not ratified the Cartagena Protocol. However, given that importing countries are increasingly putting restrictions on imports that are in line with the rules as set out in the Protocol, it may well be that the rules will have an impact on policies in exporting countries even if they have not ratified the agreement (Falkner, 2007). This could lead to trade barriers for the biofuels industry in future. There is a body of opinion that Annex III of the Cartagena Protocol should be modified to allow comparative safety assessments based on the properties of the introduced trait, rather than the current testing requirements.

At a national level, there is a need to create opportunities to adapt regulatory systems to support GM-related technologies that could deliver green growth with less risk and greater environmental and economic benefits than current technologies. However, a caveat is needed in the light of negative experiences with introductions of non-native species, for example in Australia. This is the purpose of well-organised and well-funded national scientific risk assessment.

There are many different publics with different perspectives on GM, with different and conflicting messages. For example, it has been reported that a majority of Americans support the use of biofuels, but favour some types of biofuels (corn-based and GMO-based) more than others (wood-based) (Delshad *et al.*, 2010). In Canada for the development of GM trees, each province has its own rules and there are also different public perceptions on how the forest should be managed, making it difficult to conduct GM trials. GMO are currently used in the biofuels sector in Latin America for soy bean production and at research level for sugarcane. GM soy is mainly cultivated in Argentina, accounting for 99% of the soy production (Yankelevich, 2008). In the long term it is expected that GMO applications will increase in Latin America. This is underlined by ongoing research on GM sugarcane in Brazil. In April 2008, the Brazilian Technical Biosafety Committee (CTNBio) approved the first field experiments on GM sugarcane with higher sucrose content (Janssen & Rutz, 2011).

Developments relevant to GM technology

Products based on GM crop biotechnology have been successful in those parts of the world where the technology is accepted. Restrictive regulatory systems have arisen as a result of negative public perceptions that have little to do with scientific evidence and objective risk assessments (Miller, 2006). Greater citizen acceptance of this technology is a necessary precursor to regulatory reform. The lasting consequences of the GM debate have clearly demonstrated the power of orchestrated public opposition and the importance of social interaction and involvement (Schuurbiers *et al.*, 2007). Possible ways of improving communication and transparency have been suggested by Tamis *et al.* (2009).

Policy and regulatory change

As a consequence of public opinion on GMOs, Europe has adopted particularly stringent provisions that apply also to GM biofuel crops. The legal framework in this sector is particularly complex, and is partially based, (Table 5), on the EC directives 90/220/CEE and 2001/18/EC. These instruments require an authorisation for the import, cultivation and processing of GM crops, based on a technical opinion by the European Food Safety Authority (EFSA) that has established a specific GMO Panel (EFSA, 2009). The risk assessment for GMO plants that are used for non-food or non-feed purposes includes, amongst other elements, assessments of persistence, invasiveness and selective advantage or disadvantage. Member states are required to designate a national authority responsible for complying with the legal requirements, and any notification must be accompanied by a risk assessment. So GMO policies cover the risks of causing new pest invasions much more comprehensively than the biofuel framework (Genovesi, 2011).

Table 5. The legal framework covering GMO in the European Union

Legal framework	Coverage
Directive 98/81/EC	On the contained use of GMOs. Applies to research stages of product development.
Directive 2001/18/EC	On the deliberate release into the environment of GMOs, repealing Council Directive 90/220/EEC.
Regulation (EC) No. 1829/2003	On GM food and feed. Specifies authorisation procedures and labelling requirements.
Regulation (EC) No. 1830/2003	Concerns the traceability and labelling of GMOs and derived food and feed products. Specifies amendments of Directive 2001/18/EC.
Regulation (EC) No. 1946/2003	On transboundary movements of GMOs. Implements the Cartagena protocol on biosafety.
Regulation (EC) No. 641/2003	On detailed rules for GMO authorisation with respect to documentation on detection and identification methodology.
Regulation (EC) No. 65/2004	On the development and assignment of unique identifiers for GMOs.

Source: Alderborn *et al.,* 2010.

The EFSA document suggests that principles and practices for the risk assessment of non-food/non-feed GM (NFGM) plants should be analogous to those of ordinary GM food and feed plants, and that the degree of their unsuitability in food or feed shall dictate the degree of agricultural containment and, where appropriate, other risk-reduction measures (Alderborn *et al.*, 2010). Three risk levels are suggested – one assigned as low/negligible and being devoid of requirement for confinement and two higher levels, each tied to a proportionate containment practice. Moreover, a two-step risk assessment for NFGM plants is suggested; one based on exposure assessment excluding confinement measures and one (whenever applicable) which incorporates segregation method(s) proposed by the applicant. However, for industrial biotechnology applications, the assignment of the lowest level of risk would be a step forward given the lack of confinement need. Figure 9 is a regulatory flowchart that gives a schematic overview of the interplay between the intended uses of a GM plant and the applicable European Union legislation.

Genetic use restriction technologies

In industrial biotechnology applications, physical containment works well and does not seem to give rise to any public concerns. For unconfined use of GM micro-organisms, plants and trees, genetic use restriction technologies (GURTs) could provide effective biological containment, particularly given new techniques being developed in synthetic biology. The UN Biodiversity Convention currently prohibits use of this technology. But a fast track regulatory approach could be set up for any GM organisms incorporating GURTs if they have the potential to deliver significant benefits in terms of green growth. Public understanding and support for such technological developments, however, would be crucial.

Screening and monitoring procedures

For NFGM plants, there is currently a timely opportunity to introduce a much simplified monitoring system, relative to that currently applied to food/feed GM plants. Alderborn *et al.* (2010) proposed a mandatory tagging of plants for surveillance of NFGM plants prior to cultivation or marketing in the European Union. The proposed molecular tag is envisaged to be a transgenic silent DNA identifier and is designed to enable simple, inexpensive, reliable, high through-put identification and characterisation of any NFGM. The introduction of such a system may help to alleviate public concerns by demonstrating that scrupulous monitoring is being conducted.

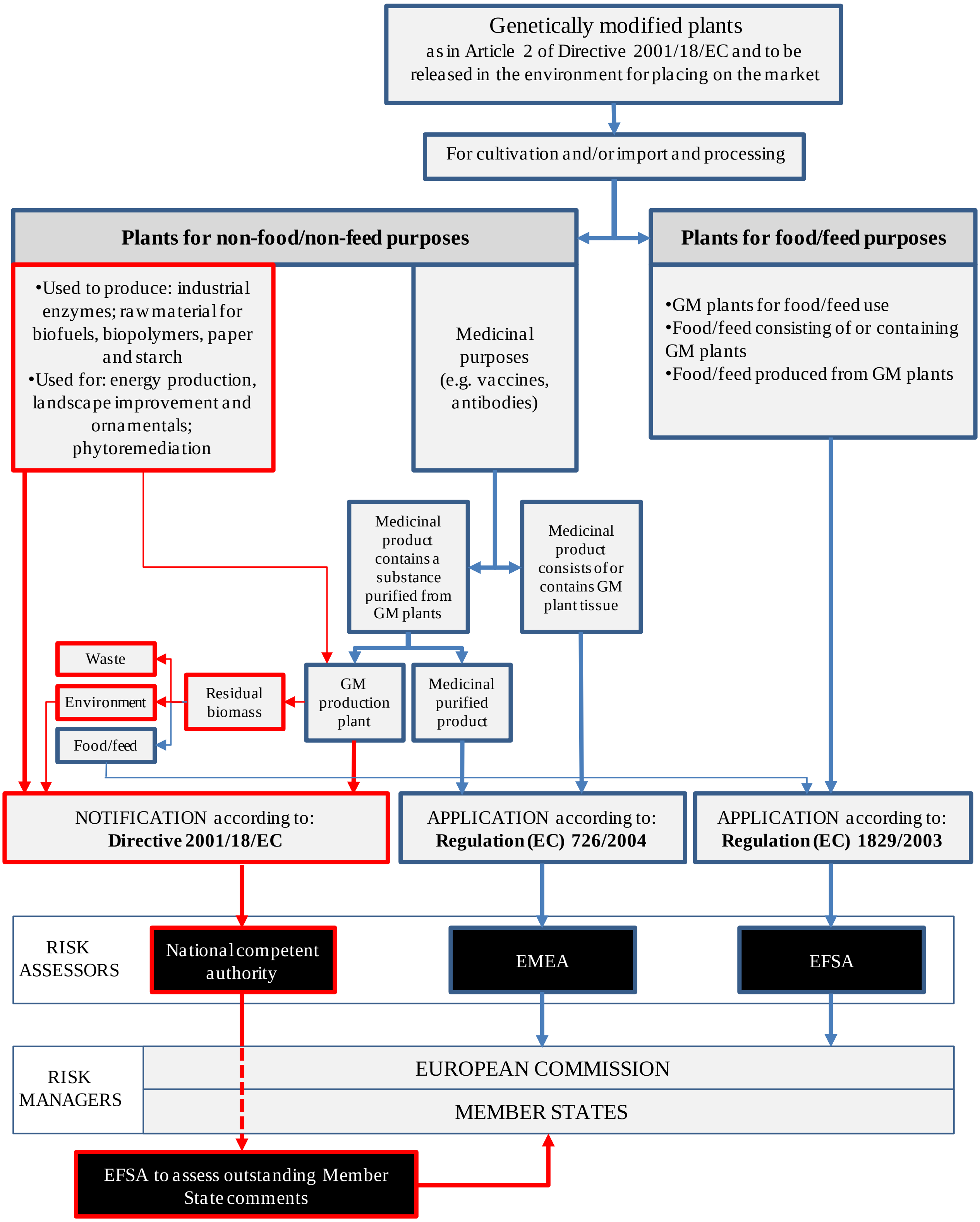
Main messages on GM issues in environmental and industrial biotechnology

In the vast majority of cases where bioremediation is applied in the field, there is no need for GM technology. The industry broadly applies the principles of biostimulation (the application of fertilisers and non-limiting aeration), and, less frequently, bioaugmentation with naturally occurring microbes. The most obvious need for GM would be to deal with the biodegradation of contaminants that are highly resistant to biodegradation, such as polychlorinated biphenyls (PCBs). Research on GM organisms capable of speeding up this biodegradation, however, is difficult to justify, since there are usually alternative remediation technologies that can be used that do not require research and are not associated with the public's negative perception of GM technologies. Nevertheless, as pressures on available land continue to grow, it would be advisable to at least remain open to the possibilities of GM technology and, in the future, synthetic biology, particularly in cases where suitable pollutant-using microbes are absent from a polluted site.

The implications within industrial biotechnology are quite different. Work has already begun on GM non-food crops for biofuels, biobased chemicals and bioplastics. At this early stage in the development of these products, however, the naturally occurring sources of biomass are yet to be determined. The need for GM versions of these non-food crops will be determined as more is known about the actual yields possible from natural crops. GM policy will need to be developed and implemented speedily if GM biomass sources are deemed necessary.

There is a widely held opinion in the scientific community that some countries and regions have GM regulatory regimes that are too strict, given the paucity of scientific evidence indicating that they are harmful. The effects are likely to be detrimental to trade. For example, the European Union is likely to be a net importer of biomass. If it bans the importation of GM biomass, then the European Union may lose out in competition with other regions. Since 1982 the European Commission has invested more than EUR 300 million in research projects examining the biosafety, environmental and health effects of genetically modified organisms (GMO). There is a view at the EC that, given that GMOs so far have not been proven to be more environmentally harmful than conventional crops, future research needs to include the potential benefits of GMOs as compared to baseline conditions (*e.g.* conventional agriculture and organic farming) (Cichocka *et al.*, 2011).

Figure 9. Regulatory flowchart showing interplay of intended use of a GM plant and the respective European Union legislation applicable



Source: Modified from EFSA, 2009.

TOWARDS A NEW POLICY AND REGULATORY FRAMEWORK – A FUTURE RIMINI AGENDA?

According to some descriptions, the world can currently be seen as composed of countries and major actors who either “think green” or “think growth”. There are very few who think “green growth”, which aims to decouple growth from unsustainable use of environmental resources. Nevertheless, the world is also moving away from an oil-based economy and into a bio-economy and there is a need for a paradigm shift across a range of functions as products move from discovery to development and from demonstration to application.

The suggested future “Rimini Agenda” would address two over-arching questions:

i. Can we change the dynamics of policy and regulation and implement change to enable environmentally beneficial biotechnologies to be developed more effectively than at present?

ii. How can we engage with civil society so as to allow free expression of divergent opinions and at the same time enable open choices among a range of biotechnology based products and processes for citizens and stakeholders?

The OECD is one of the few international organisations with the capacity and opportunity to address this challenge. Recent initiatives on which this process can build include the OECD Bioeconomy to 2030 Report (OECD, 2009) and the European Union 2020 Strategy (European Commission, 2010), which includes a series of initiatives designed to make the European Union “smart, sustainable and inclusive”.

A systemic challenge

A future Rimini Agenda thus needs to address a complex set of systemic issues and interacting problem areas. It is currently difficult to foresee a way forward in one direction that will not be stopped by a road-block somewhere else. However, there is an urgent need to find a new way of moving from discovery to application for biotechnology-based innovations of all kinds, and to begin to use policy constructively as an incentive to beneficial behaviour, rather than as an insurmountable hurdle.

There are signs that several international and national organisations, in addition to the OECD, increasingly see the need for such changes and are actively seeking mechanisms to bring them about. Indeed, this may be the cusp of an historic “tipping point”, where a relatively modest set of co-ordinated actions could bring about the much needed dramatic changes.

What role might the OECD play in addressing the issues identified?

Based on the outputs of the workshop discussions and the above reflection on the issues identified, the following suggestions for work have been identified. The specific actions proposed below need to be set in the context of an overall systemic approach that is capable of:

1. Achieving nationally and internationally co-ordinated change in policies and regulatory regimes;
2. Enabling green growth supported by innovative biotechnology to proceed on a level playing field alongside other innovative technologies, and;
3. Commanding broad public support.

The systemic context will need to take account of the optimum conditions for implementation of proposed actions and should be applicable to all novel environmental and industrial biotechnologies. It will be important to move to a situation where technologies are evaluated on the basis of their properties and not the method of their production.

ANNEX 1

WORKSHOP AGENDA

DAY 1 – 16 September 2010

Welcome and Introduction
Dr. Giuliana Gasparrini, Head of the "Division of Sustainable Development, Climate and Energy" of the Italian Ministry for the Environment

Robert Wells, Head of the Biotechnology Unit, OECD

Keynote Session

Addressing Global Challenges through Science and Technology
Michio Oishi, Executive Director, Kazusa DNA Research Institute, Japan

Setting Science and Technology Priorities for Green Growth, Agricultural Biotechnology to alleviate environmental problems (including hunger & poverty)
Wilhelm Gruissem : Professor, ETH Zurich, Switzerland

Main themes:

National and international science and technology policy agenda and mitigation of global challenges
Leverage of national strategies on green growth and innovation through biotechnology applicable to the environment

Workshop Chair: *Michio Oishi*, Executive Director, Kazusa DNA Research Institute, Japan (TBC)
Workshop Rapporteur: *Joyce Tait*, ESRC Innogen Centre, University of Edinburgh, United Kingdom

Session I: Biotechnology for Environmental Benefits: Current and Future Trends in Science and Technology

Session Chair: *Cameron Begley*, General Manager, Business Development and Commercialisation at CSIRO, Australia

Objectives of the session:

(i) To provide the current and future S&T trends of Environmental Biotechnology
(ii) To map emerging issues of Environmental Biotechnology that might impede further S&T advancements in this area.

Potential questions to be addressed:

- What are the main promising areas of biotechnology applicable to the environment?
- What are the current scientific and technological advancements in these areas? What is needed (should or could be done) in the future?
- Are the Environmental Biotechnology R&D phases optimal compared to other sectors of biotechnology?
- Are there knowledge gaps (in the basic and applied life sciences) that might impede further translational research on Environmental Biotechnology while applying leaving organisms to (in) the open environments?
- How could these gaps be filled? Are there any successful mechanisms in place?

Soil Bioremediation *Lenka Wimmerova*, Project Manager, Dept of Development and the Biotechnological Laboratory, DEKONTA, Czech Republic
In situ groundwater bioremediation: perspectives and barriers *Mauro Majone*, Professor, University of Rome La Sapienza and Member of the technical board of the Italian Ministry of Environment, Italy
Water Treatment and Technologies to valorise organic wastes *Emmanuel Trouvé*, Director, Dept Assessment Municipal WW & Sludge Dept. Manager, Veolia Water, France
Agricultural biotechnology, GM crops and trees *Armand Séguin*, Research scientist, Natural Resources Canada, Laurentian Forestry Centre, Canada
Questions & comments from the audience
DAY 2 – 17 September 2010
Session I (cont'd): Biotechnology for Environmental Benefits: Current and Future Trends in Science and Technology **Workshop Rapporteur:** *Joyce Tait*, ESRC Innogen Centre, University of Edinburgh, UK Session Chair to recall questions to be addressed and rapporteur to summarise
Biorecycle of Phosphorus Resource for Sustainable Agriculture and Industry *Hisao Ohtake*, Professor, Dept of Biotechnology, Graduate School of Engineering, Osaka University, Japan
Biotechnology for preventing environmental contamination (current and future trends and issues) *Ramani Narayan*, University Distinguished Professor, Michigan State University, Department of Chemical Engineering & Materials Science, United States (he has provided a case of industrial bio and its contribution to the environment).
Bio-detection protocols and tools (current and future trends and issues) *Davide Merulla*, University of Lausanne, Switzerland
Questions & comments from the audience
Speakers: *Martin Remondet*, Haut Conseil des Biotechnologies, France *Davide Viaggi*, Associate Professor, Dept of Agricultural Economics and Engineering, University of Bologna, Italy *Kazuo Watanabe*, Professor, Graduate School of Life and Environmental Science, Tsukuba University, Japan
Questions & comments from the audience

Session III: Supportive Policy Environment: Current and Future Policy Trends and Issues

Session Chair: Iain Gillespie, Head of Science and Technology Policy Division, OECD

Objectives of the session:
(i) To understand the national/international policy environment in which the Environmental Biotechnology currently evolves and the current policy imperfections as well as the ways to overcome these

(ii) To identify emerging policy issues and potential ways to address these.

Questions to be addressed:

- What national S&T policies (including regulatory frameworks) have been developed and implemented nationally to frame the development of Environmental Biotechnology R&D?
- How such policies have impacted (or may impact) the development of Environmental Biotechnology R&D?
- What successful examples could be reported?
- Are there any examples of failures caused by the current policy and regulatory frameworks?
- How the policy/regulatory inefficiencies may be overcome?
- What might be the emerging policy challenges in this sector?
- What are the core S&T policy issues that might impede further advancement of the Environmental Biotechnology innovation (science, technology, translational research, policy, financial, regulatory, public perception, etc.)?
- What might be the priority S&T policy goals to be addressed by governments to support the Environmental Biotechnology R&D?
- What role the OECD may play to address the core policy issues and to foster the development of Environmental Biotechnology R&D?

Speakers:

Sue Popple, Department for Environment, Food and Rural Affairs (Defra), United Kingdom

John Claxton, Deputy Head of Unit, Biotechnologies, DG Research, European Commission

Rapporteur's report on the main points from Sessions I and II

Roundtable discussion

Rapporteur's report on the main findings and messages

Closing remarks

APPENDIX 1: INTERNATIONAL ASPECTS OF CONTAMINATED LAND POLICY

Much use has been made of the United Kingdom regulatory framework for risk management of contaminated land. This is because there is a large literature trail available, and the measures implemented have commonalities with other countries. The United Kingdom was among the earlier adopters of a risk assessment framework. In this section, some specific international aspects are mentioned.

European Union

Much has changed in Europe since Christie and Teeuw (1998) recognised the following three groups of countries:

1. The "concerned" states, all with particular problems and all have contaminated land policy, all of which, except one, have traditionally had policy goals of multi-functionality.

2. The "less concerned states", which have environmental concerns, but where the perceived high cost of protection and remediation is seen as an important consideration. All of the states in this group have pursued the policy of suitability-for-use.

3. The "unconcerned" group with relatively weak environmental protection, and who await EU guidance. All have a policy goal of suitable for use for waste sites.

A plethora of environmental policies exist across the European Union. The main ones that impact soil contamination are listed in Table A1.1.

Table A1.1. Main European Union environmental policies that address soil contamination aspects

European Union Environmental policies	
Waste	Waste Framework Directive (2006/12/EC, codified version of Directive 75/442/EEC as amended) Directive 91/689/EEC on Hazardous Waste, amended in 1994 Directive on the Disposal of Waste Oils (75/439/EEC amended in 2000) Landfill Directive (1999/31/EC) Sewage Sludge Directive (86/278/EEC) Directive2006/21/EC on the management of waste from the extractive industries
Water	Water Framework Directive (2000/60/EC) Nitrates Directive (91/676/EEC) Urban Wastewater Treatment Directive (91/271/EEC) Bathing Water Directive (2006/7/EC)
Air	Air Quality Framework Directive (96/62/EC) and its Daughter Directives Directive on National Emissions Ceilings (2001/81/EC) Directive on Integrated Pollution Prevention and Control (96/61/EC) Directive on Large Combustion Plants (LCPD) (2001/80/EC)
Chemicals	Thematic strategy on the sustainable use of pesticides Directive on Biocidal Products (98/8/EC)62 Directive 91/414/EEC on plant protection products
Impact assessment	Environmental Impact Assessment Directive (85/337/EEC amended in 1997 and 2003) Strategic Environmental Assessment Directive (SEA) (2001/42/EC)
Environmental liability	Directive 2004/35/EC on environmental liability with regard to the prevention and remedying of environmental damage

Source: Adapted from Rodrigues *et al*., 2009a

Now the risk assessment approach is much more common and many countries have developed specific policy on contaminated land management (Table A1.2).

Table A1.2. How countries across Europe manage contaminated land (from Rodrigues *et al.*, 2009a)

Country	**Most common contaminated land management approach**	**Specific policy for contaminated land**
Austria	Site-specific risk assessment.	Yes
Belgium (Flanders)	Site-specific risk assessment (exposure assessment).	Yes
Bulgaria	Norms of max. admissible contents of hazardous substances in the soil.	No
Czech Republic	ABC limit values: A — background values; B — Possible adverse effects.	No
Denmark	Risk-based guideline values.	Yes
Estonia	Target values and guidance values (based on risk for human health).	Preliminary
Finland	Risk-based guideline values.	No
France	Site-specific risk assessment (tiered approach: preliminary site investigation; simplified risk assessment; detailed risk assessment).	No
Germany	Risk-based soil screening values (trigger values) and action values.	Yes
Hungary	Limit values for soil and groundwater: A: background values; B: Threshold values of contamination; C: Threshold values of measures; D: target values. (based on Dutch, German, US EPA and Canadian guidelines).	Preliminary
Italy	Original 'limit value' approach has been included into a 'risk-based' multi-tier approach: Tier 1 — screening values or contamination threshold values; Tier 2 — site-specific target levels or risk threshold values.	Yes
Latvia	Threshold values (Dutch threshold values used as reference).	No
Lithuania	Standards for contaminated soil and groundwater drafted (in line with Dutch threshold values). Site-specific simplified risk assessment.	No
Norway	Tiered approach: Tier 1 — generic target values (TVs based on existing Dutch and Danish guidelines); Tier 2 — site specific risk assessment (when TVs are exceeded); Tier 3 — Detailed investigation.	Yes
Poland	Standards for environmental protection are generally based on fixed regulatory limits, but still no generic values for contaminated land. US EPA methods often used in site-specific risk assessments.	No
Portugal	Guideline values — Ontario (Canada) guideline values used as reference.	In development
Slovakia	Target values or permissible levels (former Dutch threshold values list was adapted in 1994).	Yes
Slovenia	Limit, warning and critical concentration values of dangerous substances in soil.	Yes
Spain	Screening/guideline values and site-specific risk assessment.	Yes
Sweden	Site-specific risk assessment (exposure assessment). The Swedish EPA defined guideline values for levels in polluted soils, for the most sensitive types of land-uses.	No
Switzerland	Site-specific risk analysis. Intervention values for leachate and gaseous phase.	Yes
Netherlands	Risk-based norms (criteria): target values and intervention values.	Yes
United Kingdom	Site-specific risk assessment based on Source–Pathway–Receptor approach and on the definition of "pollutant linkages.	Yes

Source: Adapted from Rodrigues *et al*., 2009a

The Netherlands

The rigorous stance taken by the Netherlands produced some staggering statistics for that country: a likely number of contaminated sites of 110 000 and a likely number of suspect sites of 600 000. The corresponding figures for Germany are 50 000 and 200 000 (Soczo and Meeder, 1992).

Spain

In Spain, soil protection policy was established with the publication of the Royal Decree, 9/2005, which came into force on 15 January 2005. This Law develops the legal basis for wide soil protection and describes methods and standards for the characterisation of potentially contaminated soils. Ecosystem protection is based on the potential ecological risk of soils, and combines chemical and biological tools (Fernández *et al.,* 2006).

Finland

In Finland the number of potentially contaminated sites totals around 20 000. The annual costs of remediation are EUR 60–70 million. Excavation combined with disposal or off-site treatment is the most common soil remediation method. Almost 3 000 contaminated areas have been remediated during the last 15 years. This has cost around EUR 200 million. Within the last 20 years, the number of remedial decisions has increased from the two cases registered in 1988 to the current level of some 300–400 cases per year (Sorvari *et al.,* 2009).

Portugal

In Portugal, specific regulatory decisions for contaminated land management are still in the early stages of development and it has yet to implement a national soil policy (Rodrigues *et al.,* 2009b). A preliminary inventory of contaminated sites prepared in 2001 identified 3 256 sites for further priority interventions (1 765 petrol station areas; 1 491 industrial areas – petroleum refineries, chemical and steel/metal industry). A total of 6 315 sites were identified as second priority, and included mainly industries of electronics, components and explosives. A further 450 sites for potential intervention were identified. Potential contamination at these sites relates to both metals and hydrocarbons. Portugal is considering developing a number of aspects of the contaminated land management regimes from the United Kingdom, the Netherlands and Spain that can be most relevant for soil policy formulation.

United States

The awareness of human health problems associated with soil contamination in the United Sates led to the development of the Comprehensive Environmental Response and Liabilities Act (CERCLA) in 1980, also known as "Superfund" as this act introduced specific provisions for setting a fund for the remediation of contaminated sites. The Risk Assessment Guidance for Superfund, RAGS was published in 1989 and has been a major impetus to the application of risk assessment to the management of contaminated land in the United States.

The practice of human and ecological risk assessment became the primary decision-making tool to the management of contaminated sites, following the publication of the Risk Based Corrective Action (RBCA) standard by the American Society of Testing Materials (ASTM) in 1995. Other landmark publications such as the US EPA's RAGS (Part D) Preliminary Remediation Goals (1994), the Brownfields Action Agenda (1996), The US EPA's Draft Vapour Intrusion Guidance Document (2002), state-specific RBCA programmes and voluntary clean-up programmes, define the general framework for contaminated sites management in the United States (De Sousa, 2001; Rodrigues *et al.*, 2009a).

Contaminated site risk management in the United States has these main elements:

- Risk based site characterisation (that involves the collection of site specific data, the identification of exposure pathways and the quantification of risk for each pathways);
- Risk assessment (integrated and multidisciplinary analysis of risks);
- Risk management and communication (involves measures for risk reduction and post-risk management).

Canada

In Canada, environmental regulatory issues including contaminated sites are shared among the different levels of government. Relevant legislation and administrative policies at the federal level include the Canadian Environmental Protection Act from 1998, the Guidance Manual for Developing Site-specific Soil Quality Remediation Objectives for Contaminated Sites in Canada, 1996, and the Recommended Canadian Soil Quality Guidelines from 1997 (De Sousa, 2001).

Two types of criteria, risk-based guideline values and site-specific risk assessment, are used for the investigation of contaminated sites and the definition of clean-up goals in Canada. National guidelines comprise both generic soil quality criteria and guidance for developing site-specific criteria. Each Canadian province and territory is responsible for the development of their own remediation criteria, guidelines for use at contaminated sites and procedures for the implementation of site-specific risk assessments (Rodrigues *et al.,* 2009a).

Russia

Risk assessment techniques have been slow to develop in Russia, but progress has been made, sometimes in collaboration with the United States (Rubin *et al*., 2003). Risk assessments for air contamination in cities ranging from Perm to Volgograd have been completed (Larson *et al.,* 1999). For contaminated land, however, it appears that the risk assessment approach is still not commonly used. Literature searches indicate a much higher concern over radioactive contamination than organic pollutants.

China

In recent years the issue of contaminated land has become a major concern in China. Water and air pollution have had greater attention and, as a result, the regulatory system for contaminated land remains largely undeveloped. However, the 11th Five-Year-Plan of the Chinese government draws attention to the hazards associated with contaminated land (Li, 2006). China faces many problems, with large reported numbers of contaminated sites. The major challenges can be summarised (Luo *et al.,* 2009):

- A lack of a clear policy framework (such as overall policy objectives and principles) and an integrated legislative regime on contaminated land at a national level;
- An extremely limited supply of experienced administrators and dedicated organisations responsible for contaminated land at both central and local levels;
- Little technical expertise on management of contaminated land;
- No financial incentives for cleanup and re-use of contaminated land.

As an example of current developments, China is examining the possibility of using existing risk assessment models to develop Generic Assessment Criteria (GAC) as there are none in existence for human health based risk assessment for contaminated sites in China (Cheng & Nathanail, 2009). GAC provide nationally consistent guidance, thereby saving money and time.

Japan and South Korea

Japan is often considered to be at the forefront of the introduction and implementation of environmental policy. The culmination of various policies established Japan as one of the cleaner environments earlier than most OECD countries and demonstrated that a good environmental reputation is not only good for the environment but is also a valuable economic and cultural asset (Cole *et al.*, 2010).

In Japan the Soil Contamination Countermeasures Law was enforced in 2003, as described by Ogata & Murakawa (2008) and Japan is also currently involved in the development and implementation of regulatory decisions for risk-based management of contaminated land.

In South Korea, the Korean Soil Protection Act was established in 1995 and amended in 2002 and 2005 (Jeong *et al.*, 2008).

APPENDIX 2. TECHNOLOGY BRIEFS FOR REMEDIAL TECHNOLOGIES IN COMPETITION WITH BIOREMEDIATION

Technology brief - Soil vapour extraction

Vacuum pumps or blowers induce a pressure gradient in the subsurface, resulting in an air flow field about an extraction well. These systems can be combined with groundwater pumping wells to remediate soil previously beneath the water table.

Gas-phase contaminants are removed via advective air flow entering the extraction wells. Volatilization is induced. High vapour pressure contaminants are removed first, and the soil progressively becomes enriched in less volatile compounds.

Advantages. Few moving parts – low operating costs – rapid installation – rapid results – no excavation – not disruptive – remediate under buildings – no toxic reagents - enhances bioremediation if air flow is controlled.

Limitations. Contaminants with low vapour pressures have poor removal efficiencies - may require other techniques (treatment train) – constraints in soils with low air permeability – contaminants are not treated (toxicity unaltered).

Technology brief – Incineration

A typical incinerator system consists of several components: waste storage, preparation and feeding; combustion chamber(s); air pollution control; residue and ash handling; process monitoring.
Rotary kilns are the most common incinerators for waste materials. The rotary kiln is a cylindrical, refractory-lined reactor set at a slight angle (rake). As the kiln rotates, the waste moves through the reactor and is mixed by tumbling.

Advantages. Removal efficiencies of beyond 99% have been reported – detoxification – heat recycling.

Limitations. Very high initial capital investment – requires highly trained operatives – much more complex than other treatment technologies – emission of hazardous gases – de novo synthesis of dioxins and furans – adverse public perception – destroys soil.

Technology brief – Thermal desorption

Thermal desorption is a lower temperature thermal treatment (up to 600 °C) which involves two processes: transfer of contaminants from the soil into the vapour phase (volatilization); and off-gas treatment *e.g.* higher temperature treatment to destroy (up to 1400 °C).

Advantages. Can be used for very small scale projects – flexibility in operations *e.g.* variable temperature, use of catalysts – organic material of soil may not be destroyed.

Limitations. Clay soils can greatly increase treatment cost – emissions – volatile metals cause operational problems.

Technology brief – solidification

Cement and pozzolan-based solidification can be done both *in-situ* and *ex-situ*. *In- situ* can be performed using an auger system that drills into the soil, combined with blades that mix. Solidification agents can be injected into the mixing zone. *Ex-situ* treatments can be done in dedicated facilities using equipment borrowed from cement mixing. Soil and solidification agents can be added directly to a rotating drum.

In vitrification, soils are heated to melt them and produce a glass-like product. The high temperature results in the combustion of organic contaminants and non-combustible contaminants are immobilised in the glassy matrix.

Advantages. Inorganics permanently immobilised, especially with vitrification – vitrified material has long-term durability.

Limitations. Relatively high cost – vitrification is an energy-intensive process – soil structure is completely destroyed, especially during vitrification – organic contaminants not well fixed by inorganic binders – cement solidification results in large volume increase, which increases the cost of subsequent landfilling.

APPENDIX 3. INTERNATIONAL ASPECTS OF BIOFUELS POLICY

Brazil is the special case

In the 1970s oil shock, the Brazilian government introduced fuel ethanol to reduce oil consumption. In 1975 the Brazilian government launched the national alcohol programme PróÁlcool. Soaring oil prices put Brazil at the forefront of the biofuel movement. Brazil has subsidised biofuel during market development until economies of scale have allowed fair competition with oil products. By 2004, ethanol in Brazil had become economically competitive with gasoline based on international prices for oil (equivalent to USD 40 per barrel) (Goldemberg, 2008). At these costs, the production of ethanol from sugarcane is much cheaper than from other crops such as corn, wheat and sugar beet. It is an interesting historical note that energy security was the main driver at the time of the launch of the PróÁlcool programme. Brazil is today the world's second-largest producer of bioethanol and the world's largest exporter.

At the time, climate change had only just started to emerge as a global concern. However, GHG emissions savings has become an additional driver for bioethanol production in Brazil. In 2010 the US EPA calculated that sugarcane ethanol from Brazil reduces GHG emissions compared to gasoline by 61%, using a 30-year payback for indirect land use change (iLUC) emissions (GreenMomentum, 2010).

Since 1976 the government made it mandatory to blend anhydrous ethanol with gasoline, fluctuating between 10% to 22%. The Brazilian car manufacturing industry developed flex-fuel vehicles that can run on any proportion of gasoline and hydrous ethanol. Today there are no longer any light vehicles in Brazil running on pure gasoline. The first flex-fuel motorcycle was launched by Honda's Brazilian subsidiary Moto Honda da Amazônia in March 2009.

It is by no accident that the biobased plastics industry has taken root in Brazil, as bioethanol can be used as the basis for making bio-ethylene and bio-propylene, the monomers for the highest production volume thermoplastics. This is potentially a massive spill-over benefit from the original Brazilian biofuels policy. Brazil now also has federal policy on biodiesel, which is aimed at alleviating rural poverty (stimulating rural activities to increase employment in rural areas).

US biofuels policy

In the United States, the Energy Independence and Security Act (EISA) (2007), and the Farm Bill (2008), which between them set biofuels volume mandates, and created tax incentives, and provided funding for demonstration plants, will pave the way for very large investments in research and infrastructure, and create further rural regeneration whilst working towards the aim of energy security.

The United States Renewable Fuels Standard, RFS 2 (Federal Register, 2010) lays out the strategy and targets for the United States till 2022, and therefore covers current and near-term biofuels development, but also has a provision for the inclusion of new technologies. The congressionally mandated RFS2 goal is to use at least 36 billion gallons of biobased transportation fuels by 2022 (USDA, 2010). Fifteen billion gallons can come from conventional biofuel sources such as corn ethanol. Of the remaining 21 billion gallons of advanced biofuels needed to achieve the total 36 billion gallon goal, 16 billion gallons is required to come from advanced cellulosic biofuels (fuels made from cellulosic feedstocks that reduce greenhouse gas emissions by at least 60% relative to gasoline). The contribution of biomass-based diesel to the 21 billion gallons goal can be no less than 1 billion gallons. An additional 4 billion gallons is to come from advanced biofuels. In total the mandate will displace about 14% of the motor gasoline demand in 2022.

Assuming an average biorefinery size of 40 million gallons per year, the USDA estimates that meeting the RFS2 advanced biofuels goals will mean the building of 527 biorefineries, at a cost of USD168 billion. Whilst this and the infrastructural implications seem daunting, the USDA expects the market to react to this need (USDA, 2010).

An interesting policy aspect of the EISA, 2007 and the volume mandates is that it also contains targets on the life cycle analysis (LCA) of these different types of biofuels. Standardisation and LCA are areas of policy ripe for more detailed study. For each renewable fuel pathway, GHG emissions were evaluated over the full lifecycle, including production and transport of the feedstock; land use change; production, distribution, and blending of the renewable fuel; and end use of the renewable fuel. The GHG emissions were then compared to the lifecycle emissions of 2005 petroleum baseline fuels (base year established as 2005 by EISA) displaced by the renewable fuel, such as gasoline or diesel. This is significant because it is the first time that lifecycle emissions reduction has become a legal requirement.

The European Union

Today there are many political, environmental and scientific initiatives in Europe where biofuels are involved, but in a somewhat unco-ordinated manner. In January 2007, a radical energy and climate change package to cut greenhouse gas emissions by at least 20% by 2020 (largely through energy measures) was proposed.

In the early years of bioenergy policy in the European Union, biofuels were supported mainly through Directive 2003/30 (Official Journal of the European Union, 2003), with the main objective being to trigger both domestic production and consumption in the member countries through measures of fiscal stimulus and incentives (Ninni, 2010).

A major landmark in the European Union was the publication of the Renewable Energy Directive (Official Journal of the European Union, 2009). This Directive established a common framework for the promotion of energy from renewable sources. It set mandatory national targets for the overall share of energy from renewable sources in gross final consumption of energy and for the share of energy from renewable sources in transport. It also established sustainability criteria for biofuels and bioliquids. In the light of recent research addressing the risks of biofuels, the European Commission proposed further to favour the use of biofuels produced from wastes, residues, non-food cellulosic material, and ligno-cellulosic material over the use of first generation biofuels (Bringezu *et al.*, 2009).

Japan and energy resources

Japan is the world's third-largest oil consumer (after the United States and China). Since the oil crises of the 1970s, the Japanese government has embarked on national projects in developing alternative energy resources with the purpose of raising productivity of bioethanol production. Currently, the government allows oil companies to blend about 3% of ethanol into gasoline. In future, oil companies plan to introduce Ethyl Tertiary Butyl Ether (ETBE) mixed gasoline to meet the potential demand of approximate 1.8 million kl per year. Japan planned to replace about 500 000 kl (3.14 million barrels) per year of transportation fuels with bioethanol by 2010.

Japan is engaged in a mixture of public and private investment and development projects in other countries. In terms of development, in order to help reduce GHG emissions Japan will provide technical assistance to Southeast Asian nations, in particular to Thailand and Vietnam. Several Japanese trading companies have started to invest in Malaysia and Indonesia for producing biodiesel from palm oil and bioethanol from sugar cane and jatropha. Some Japanese trading companies have shown interest in Brazilian ethanol investments (USDA Foreign Agricultural Service, 2009).

Demand-side policies: the Swedish example

Economic theory has it that innovation requires the co-existence of demand-side policies to complement supply-side measures (and not replace them). It has been observed that countries vary in the level of priority they give to demand-side policies (OECD, 2011c). A very good example of the benefits of demand-side policies to biofuels development, and demand-side spill-overs, is the case of Sweden. The Swedish approach to using biofuels to reduce dependence on oil relies on using incentives to change the direction of fuel consumption, rather than setting mandates or benchmarks that may be un-attainable (Kroh, 2008). The Swedish government has set out an ambitious target to eliminate oil imports by 2020.

Sweden is reported to have the largest bioethanol bus fleet in the world, with over 600 ethanol-operated buses in service. In 1994 the first three flex-fuel cars (powered by both ethanol and petrol) were imported. At the same time, the BioAlcohol Fuel Foundation (BAFF), founded in 1983 under the name of The Swedish Ethanol Development Foundation (SSEU), began lobbying other municipalities to invest in ethanol. At present there are about 1400 E85 pumps found throughout Sweden, which is not much less than in the entire United States (SEKAB website).

Sweden has produced a range of other consumer-oriented, demand-side policies supportive of biofuels to complement the supply side:

- A SEK 10 000 bonus to flex-fuel vehicle buyers (over EUR 1 000, and over USD 1 500 as of July 2011);
- Exemption from Stockholm congestion tax;
- Discounted auto insurance;
- Free parking spaces in most of the largest Swedish cities;
- Lower annual registration taxes;
- A 20% tax reduction for flex-fuel company cars.

On the supply side, Ford Sweden and Saab have become leaders in flex-fuel ethanol cars, and Volvo currently markets several ethanol-operated models (SEKAB website). This is an example of supply- and demand- side policies operating simultaneously.

APPENDIX 4: GENOMICS TECHNOLOGIES

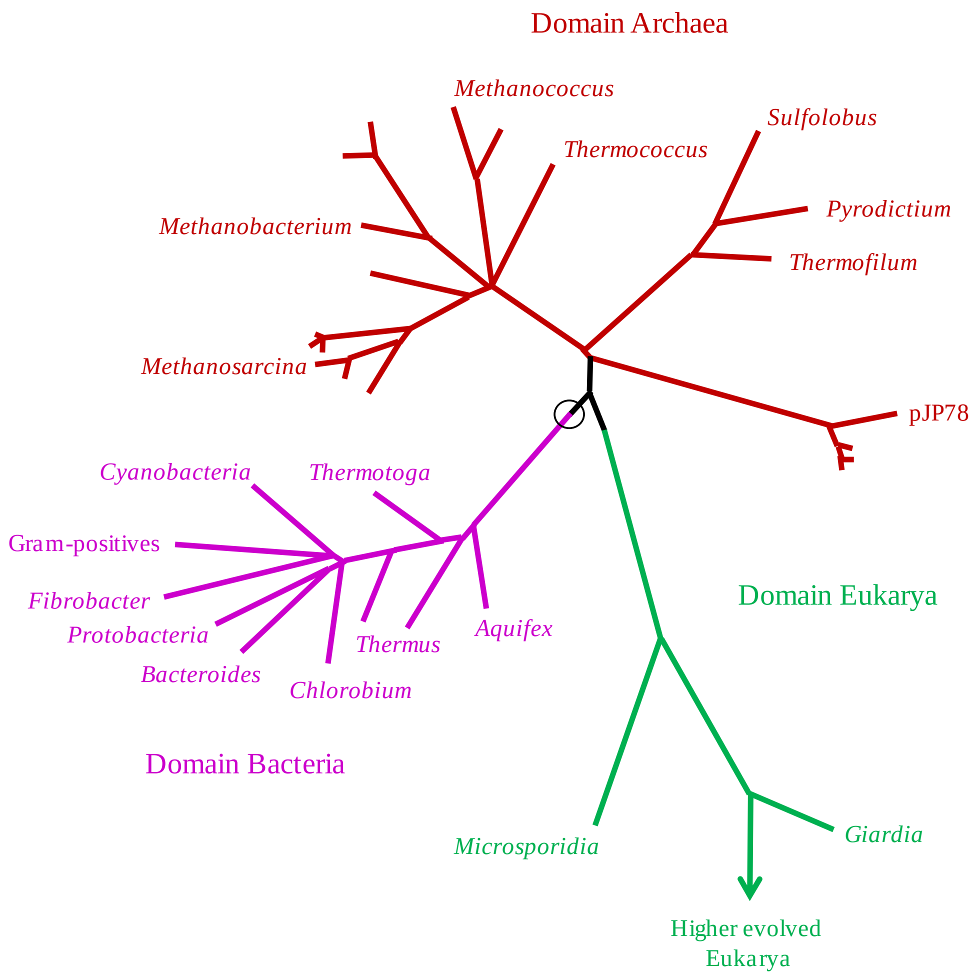
Metagenomics

Metagenomics is the application of modern genomics technologies to microbial communities in their natural environments, bypassing the need for culturing (Röling *et al.*, 2010) and potentially negating the great plate count anomaly. The field has its roots in the culture-independent retrieval of 16S rRNA genes (rDNA), pioneered more than two decades ago (Olsen *et al.*, 1986). These are the most useful molecular chronometers used today for the determination of bacterial phylogenetic relationships (Woese, 1987), for several reasons:

1. The function of ribosomes has not changed for about 3.8 billion years;
2. The 16S rDNA genes are universally present among all cellular life forms;
3. The size of 1540 nucleotides makes them easy to analyse;
4. The primary structure is an alternating sequence of invariant, more or less conserved to highly variable regions, and;
5. Lateral gene transfer is very infrequently observed among organisms (if at all).

Phylogenetic relationships can be assessed by pairwise similarities. One hundred per cent similarity found between a pair of 16S rDNA sequences using different methods indicates very high relatedness, if not identity of the investigated organisms. The lower the value, the more unrelated the compared organisms. If, however, the number of organisms is too large, the respective similarity matrix cannot be interpreted meaningfully. In this case phylogenetic relationships can be visualised graphically by using algorithms that transform the similarity values into dissimilarity values to compensate for superimposed (multiple) substitutions. These phylogenetic distances form the basis for phylogenetic trees (*e.g.* Figure A4.1).

Figure A4.1. The phylogenetic tripartation of living organisms based on the analysis of the gene coding for small subunit rRNA (16S and 18S rDNA)



Source: Author's diagram

The origin of the evolutionary lineages appears to be located close to the branching point of the lineage of the Bacteria (indicated by a circle). The current canonical phylogeny is that the domains bacteria and archaea diverged first. Each domain subsequently diverged to produce new branches as the earth and the biosphere evolved and produced new ecological niches: Bacteria to form lineages with such morphological, metabolic, and physiological diversity that they inhabit almost every niche on the Earth; and archaea to form hyperthermophiles, methanogens, extreme halophiles and more recently identified environmental lineages. Eukarya subsequently diverged from archaea, with mitochondria and chloroplasts evolving from endosymbionts.

Despite the breakthroughs of metagenomic analysis from clone libraries (Figure A4.2), the technique is somewhat cumbersome and has flaws that limit its ability to uncover all the microbial diversity in a sample. It requires PCR amplification, which is known to introduce bias (*e.g.* Sipos *et al.*, 2007). Additionally, many deficits exist in the expression of genes in *E. coli* and other expression vector libraries (Ferrer *et al.*, 2007). Metagenomic communities dominated by archaea may be seriously underestimated in terms of diversity. Hong *et al.* (2009) estimated that typical rRNA environmental gene surveys miss a significant amount – around 50% – of microbial diversity. The introduction of new sequencing technologies, such as pyrosequencing, removes some of the bias (Mardis, 2008). Pyrosequencing has resulted in several successful metagenomic studies (*e.g.* Petrosino *et al.,* 2009). Single molecule sequencing is a novel approach that simplifies the DNA sample preparation process and avoids many biases and errors. When metagenomics goes down to the single-molecule level, it will be possible to make more accurate assessments with poorer samples (Blow, 2008). For the metagenomics community this next-next-generation promises higher through-put, lower costs and better quantitation of genes. However, in the process of solving many issues, it will also introduce new ones. It requires more starting DNA. Error rates are also likely to remain high. Because of this, single molecule methods may need to be used in combination with existing shorter read methods.

Other –omics technologies relevant to Environmental Biotechnology

Future applications of –omics technologies to environmental applications are likely to depend on high through-putting ability. The following major techniques and technologies are currently available at least at research laboratory scale. This is a dynamic area, however, with new developments occurring continuously. Information in this section thus provides a summary only. Microfluidic technology can facilitate the progress of –omics technologies by enabling miniaturisation. Like all these technologies, microfluidics has its technical challenges, many relating to miniaturisation itself (Feng *et al.*, 2009).

Proteomics

Proteomics has progressed radically in recent years and is now on a par with most genomic technologies in throughput and comprehensiveness (Mann & Kelleher, 2008). Analysing peptide mixtures by liquid chromatography coupled to high-resolution mass spectrometry (LC-MS) has emerged as the main technology for in-depth proteome analysis. The latest generation of mass spectrometers combines extremely high resolving power, mass accuracy and very high sequencing speed in routine proteomic applications. Metaproteomics is the study of the entire protein content of a given habitat (Wilmes & Bond, 2006), and it has greater potential than genomics for the functional analysis of microbial communities. However, environmental metaproteomics is still in its infancy for various technical and bioinformatic reasons, despite the advances in mass spectrometry.

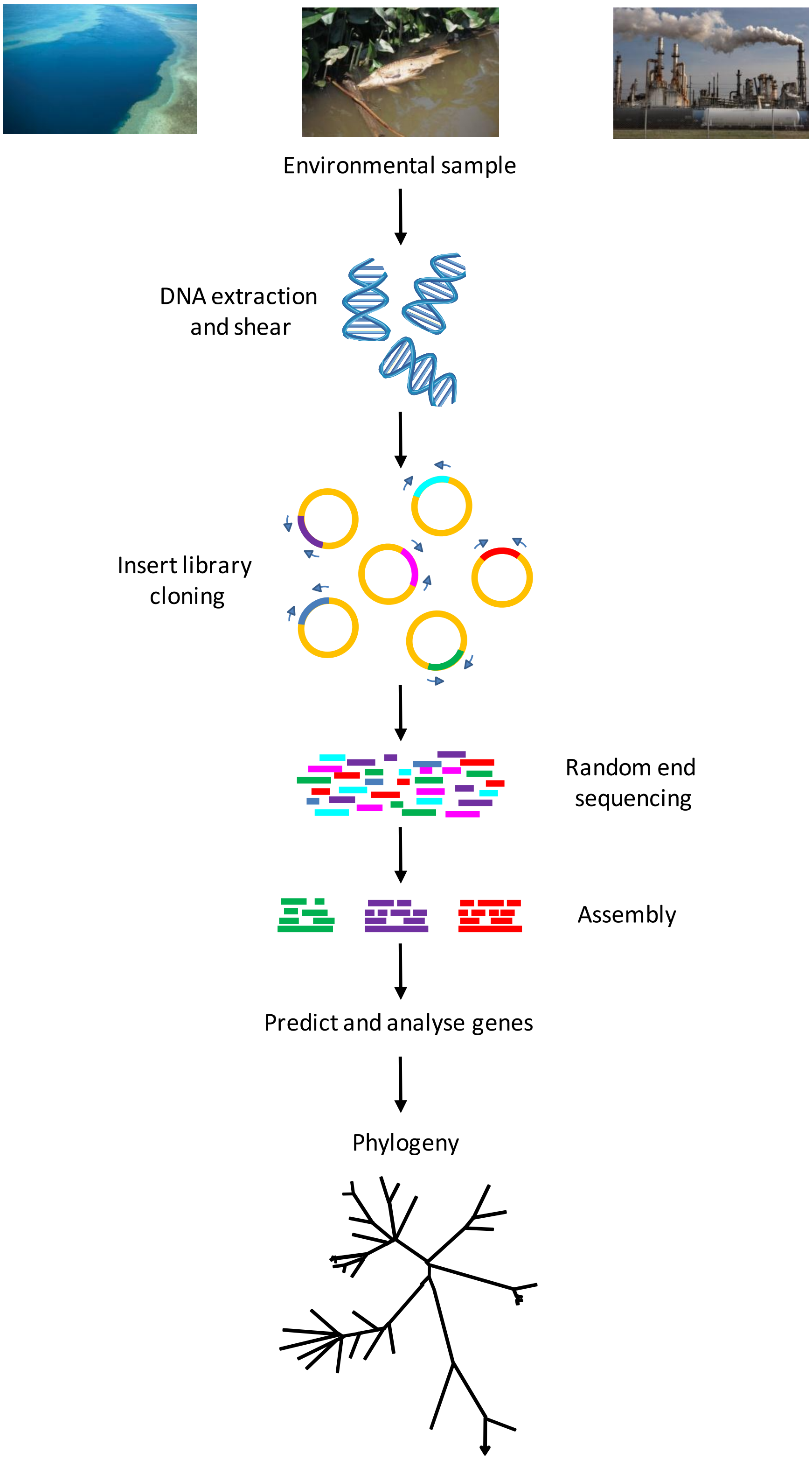
Metabolomics

This refers to the comprehensive analysis of all low-molecular-weight (<1000 Da) primary and secondary metabolites present in and around cells growing under defined physiological conditions (Mashego *et al.,* 2007). It is emerging as a rapidly developing field of research with the promise to speed up the functional analysis of genes with unknown function. It is still technically limited. Metabolite turnover is very fast. The interconnection of metabolic pathways makes it challenging to establish a direct link between metabolites and genes. And metabolite diversity is much higher than gene or protein diversity.

Metatranscriptomics

Metatranscriptomics refers to the analysis of the collective transcriptomes of a given habitat (Stenuit *et al.,* 2008). While metagenomics provides information on the possible activities of a microbial community, it cannot reveal the actual activities at a specific time and place, or how those activities change in response to environmental forces or biotic interactions. The challenge is to narrow down the suite of possible actions – the metagenome – to those that are ongoing at a particular time - the metatranscriptome - and ultimately to identify what is responsible for the difference. Like other -omics technologies, technical difficulties have hampered the progress of metatranscriptomics, not least of them being RNA half-life on the order of minutes, even under optimal conditions (Moran, 2009).

Figure A4.2. Metagenomics and its use in discovering bacteria without culture techniques.



Source: Author's diagram

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